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Annual Meeting. . .

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Milwaukee, WI  
July 26-31, 1980

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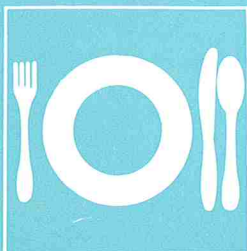
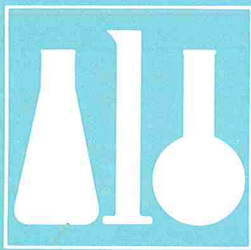
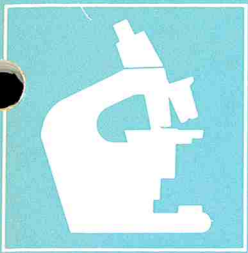


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## Bacteriological Quality of Infant Milk Foods

R. S. SINGH\*, SUKHBIR SINGH, V. K. BATISH and B. RANGANATHAN

National Dairy Research Institute, Karnal-132001, India

(Received for publication January 25, 1979)

### ABSTRACT

Ten samples of baby foods comprising seven brands of infant milk foods and three brands of milk-cereal weaning foods were examined for incidence of different types of microorganisms. One brand of infant milk food with  $91 \times 10^2$  organisms also exhibited the maximum number of staphylococci and some of these were coagulase positive. Some staphylococcal isolates showed thermostable deoxyribonuclease (DNase) activity and also produced enterotoxins A or B. One sample of weaning food showed high counts of *Bacillus cereus*. In one of the reconstituted baby food samples when held at ambient temp. (37.5 C), the *Staphylococcus aureus* and *B. cereus* counts increased 10-fold in 3 h.

Infant milk foods in India include either spray-dried or roller dried products and are manufactured from cow's or buffalo's milk or from a mixture of both types of milk. Milk-cereal based weaning foods constitute another category of baby foods which are fed to infants above 3 months old. Very little information is available in India on the bacteriological quality of such infant milk foods as well as processed cereal weaning foods. The problem is of serious public health concern since food poisoning outbreaks have often been traced to consumption of these products, which are known to contain staphylococci and their enterotoxins. Although pathogenic staphylococci are destroyed during processing of milk, these organisms, when present as contaminants from the personnel and equipment, may grow and produce heat-stable enterotoxins in these products.

The present study deals with the incidence of different types of microorganisms in some samples of baby foods and processed milk-cereal based foods available in the Indian market.

### MATERIALS AND METHODS

Ten tinned samples of dried baby foods, comprising seven brands of infant milk food and three of weaning milk-cereal based foods were collected from the local market. Samples were examined for total bacterial count, coliform count, staphylococcal count, *Bacillus cereus* and yeast and mold count. Sterilized tap water and Ringer's solution were used for reconstitution of baby foods and making serial dilutions of the samples, respectively.

Total bacterial count was determined by using the peptone yeast extract agar as per Indian Standard Institution specification (13). MacConkey agar medium (1) was used for enumeration of coliforms, while staphylococcus medium S-110 (7) was used for enumeration and isolation of staphylococci. Mannitol-egg yolk-polymyxin agar (MYP),

as recommended by Mossel et al. (18), was used for enumeration of *B. cereus*. Yeast and mold count was determined, using acidified potato dextrose agar (1).

After examining the 10 samples, two representative samples of baby food were further examined for microbial quality after reconstitution and storage at different temperatures and time intervals. For the purpose, the reconstituted sample was divided into two portions. One portion was stored at 37.5 C (room temperature), while the other was stored at 7 C. Both these samples were examined at intervals of 0, 1, 2 and 3 h by plating on selective media for determination of total bacterial count, coliforms, staphylococci, *B. cereus* and yeast and mold count.

Staphylococcal isolates were characterized on the basis of growth on S-110 medium, hemolysis on blood agar plates, mannitol fermentation, gelatin liquefaction, coagulase and DNase activity and enterotoxin production. The staphylococcal enterotoxins were detected by the microslide gel-double diffusion technique according to the procedures of Crowle (8) and Casman et al. (6). Standard enterotoxins A, B and C ( $C_1$  and  $C_2$ ) and their corresponding antisera were obtained from Prof. M. S. Bergdoll, U.S.A. for the tests carried out in the present study.

The isolates of *B. cereus* were characterized on the basis of their morphology and biochemical characteristics according to the methods suggested by Lord (16) and MacFaddin (17).

### RESULTS

The total bacterial count in the samples of different lots of infant milk foods and milk-cereal weaning foods are recorded in Table 1. The maximum bacterial count observed was  $9.0 \times 10^3$  per gram while the minimum was  $2.0 \times 10^3$  per gram. The sample showing highest number of bacteria also had the maximum number of staphylococci ( $6.0 \times 10^3$  per gram). However, a sample of milk-cereal weaning food showed the highest number of *B. cereus* ( $2.0 \times 10^3$  per gram). All brands of infant foods, including weaning foods, examined were free from coliforms (Table 1). Yeasts and molds were observed in only three samples.

Among 16 strains of staphylococci isolated from samples of baby foods and weaning cereal-milk based foods, three were positive for coagulase production in rabbit blood plasma while eight were deoxyribonuclease (DNase) positive (Table 2). Of DNase positive strains, only three produced thermostable DNase, and they also exhibited coagulase activity and enterotoxin A or B production. All the three strains of *S. aureus* designated as "Toxigenic" came from the sample showing highest total bacterial count as well as maximum staphylococcal count (Table 2). Table 3 records the distribution of hemolytic *B. cereus* strains in infant milk and milk-cereal weaning foods. *B. cereus* occurred in most of

the samples in the range of 250/g to 2500/g. Most *B. cereus* strains, i.e. 9 of 15 were strongly beta hemolytic. Five of the hemolytic strains came from samples having a range of 250 to 500 *B. cereus* cells/g.

Changes in counts of different types of bacteria were observed in the reconstituted baby foods after different intervals of storage at two temperatures (7 and 37.5 C). The total bacterial counts, staphylococcus counts as well as *B. cereus* counts in a reconstituted sample of one brand of baby food increased by 10-fold at 37.5 C after storage for 3 h. However, at 7 C little or no change in count occurred in the same reconstituted baby food sample held for 3 h. Yeasts and molds also followed the same pattern in the reconstituted baby food sample when held for 3 h at 37.5 and 7 C.

However, increase in counts of different bacterial types was not appreciable in another reconstituted baby food sample when held at both the temperatures for 3 h.

### DISCUSSION

Information on the microbiological quality of baby foods manufactured in India is scanty. Although a few sporadic cases of food poisoning and gastroenteritis due to consumption of reconstituted baby foods have been reported in the press, there are no scientific reports on such cases, presumably due to lack of follow-up action on the part of public health authorities.

The high incidence of staphylococci and *B. cereus* in nine out of the 10 brands of baby foods, including weaning foods (Table 1), indicates lack of adequate precautions taken either during production and processing of raw milk or during subsequent handling and distribution of the products.

TABLE 3. Distribution of *B. cereus* in infant milk and milk-cereal weaning foods.

Number of <i>B. cereus</i> per g (range)	Number of samples	Number of <i>B. cereus</i> strains tested	Number of hemolysin producers
0 - 250	3	4	1
251 - 500	5	7	5
501 - 1000	1	3	2
1001 - 5000	1	1	1
Total	10	15	9

Although the total number of bacteria in baby foods examined in the present study is much less than those prescribed for infant foods (13), the types of organisms present are significant from the public health point of view, especially since no standards for staphylococcal and *B. cereus* counts in milk products are available at present in India. It is in this context that the occurrence of staphylococci and *B. cereus* in nearly 90% of the baby food samples must be viewed with concern. In reconstituted samples of baby foods there was an appreciable increase in total bacterial count, staphylococci and *B. cereus*. In some samples, the total number of bacteria increased by 10-fold after reconstitution. Even at refrigeration temperature (7 C), samples of reconstituted baby food exhibited marginal increase in total bacterial count within 3 h. Ghodeker et al. (11) observed survival of staphylococci and enterococci in milk powders even after prolonged storage.

A point of particular epidemiological interest in the present study is the occurrence of the enterotoxigenic strains of staphylococci producing enterotoxins A or B among the 16 strains isolated from baby foods. Two of the isolates were positive for enterotoxin A while one was

TABLE 1. Incidence of different types of microorganisms in dried baby foods and milk-cereal weaning foods.<sup>a</sup>

Sample No.	Total bacterial count/g	Staphylococcal count per g	<i>B. cereus</i> count per g	Coliform count/g	Yeast & mold count per/g
1.	$5.0 \times 10^3$	$2.0 \times 10^3$	$0.5 \times 10^3$	< 1	< 1
2.	$3.0 \times 10^3$	$0.7 \times 10^3$	$0.2 \times 10^3$	< 1	$0.2 \times 10^3$
3.	$9.0 \times 10^3$	$6.0 \times 10^3$	$0.4 \times 10^3$	< 1	$0.1 \times 10^3$
4.	$3.0 \times 10^3$	< 1	< 1	< 1	< 1
5.	$6.0 \times 10^3$	$0.2 \times 10^3$	$0.3 \times 10^3$	< 1	< 1
6.	$6.0 \times 10^3$	$0.9 \times 10^3$	$0.4 \times 10^3$	< 1	< 1
7.	$7.0 \times 10^3$	$3.0 \times 10^3$	$0.6 \times 10^3$	< 1	< 1
8.	$3.0 \times 10^3$	$0.2 \times 10^3$	$0.2 \times 10^3$	< 1	< 1
9.	$8.0 \times 10^3$	$0.2 \times 10^3$	$2.0 \times 10^3$	< 1	$0.2 \times 10^3$
10.	$2.0 \times 10^3$	$0.5 \times 10^3$	$0.5 \times 10^3$	< 1	< 1

<sup>a</sup>The counts recorded are an average of each replicate.

TABLE 2. Distribution of staphylococci in infant milk foods and milk-cereal weaning foods.

Number of staphylococci per g (range)	Number of samples	Number of <i>S. aureus</i> tested strains	Characteristics of staphylococcal strains				
			Coagulase production	DNase production	Enterotoxin production		
					A	B	C
0 to 250	4	5	0	4	0	0	0
251 - 500	1	2	0	0	0	0	0
501 - 1000	2	3	0	0	0	0	0
1001 - 5000	2	3	0	1	0	0	0
5000	1	3	3	3 <sup>a</sup>	2	1	0
Total	10	16	3	8	2	1	0

<sup>a</sup>Thermostable DNase (resisted boiling for 15 min)

positive for enterotoxin B. All the three isolates also exhibited thermostable DNase activity which can be generally correlated with enterotoxigenicity in staphylococci (3,20,21). It may be noted that such enterotoxigenic strains producing enterotoxin A and B were isolated from only one brand of baby food which exhibited maximum total number of bacteria as well as staphylococci. According to Casman (5) enterotoxin A was most predominant among the toxins liberated from strains of staphylococci isolated from food poisoning cases in Britain and Canada. Donnelly et al. (9) also observed that enterotoxin A was the most common among the enterotoxins. Ghosh and Laxminarayana (10) reported that most milk and milk products examined for staphylococci in Karnal showed a predominance of enterotoxin A producers among the isolates. Batish et al. (3) also observed that production of enterotoxins A or B or both was most common in most staphylococcal isolates from milk and milk products.

The incidence of coagulase-positive and enterotoxigenic strains of staphylococci in milk-based baby foods may have to be ascribed to unhygienic practices during manufacture of the baby foods. The possible sources of entry of staphylococci in baby foods may be from infected air or human carriers handling the finished product.

The high incidence of *B. cereus* in baby foods reported in the present study (Table 1) should also be viewed with concern, since in recent years, food poisoning outbreaks have also been traced to contamination of food products with *B. cereus* (4,12,14,15,19). *B. cereus* has been reported to be present in large number in raw milk samples in India (2). Further, *B. cereus* in milk is known to survive pasteurization and boiling and hence the bacterium would be expected to persist during the manufacture of baby foods.

The present findings are limited in scope due to the availability of only a few brands of such foods in India. However, the results provide useful and pertinent data on the microbiological quality of Indian baby foods with particular reference to the occurrence of enterotoxigenic staphylococci and *B. cereus*, which are public health hazards.

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## Detection of *Salmonella* in Refrigerated Preenrichment and Enrichment Broth Cultures

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### ABSTRACT

Refrigeration (4 C) of non-selective and selective enrichment broth cultures for 72 h did not markedly affect detection of *Salmonella* in 160 contaminated high and low moisture foods. Detection in refrigerated preenrichment (non-selective) broth cultures of poultry and high and low moisture foods was 90, 95 and 100%, respectively; homologous results for refrigerated selective enrichment broth cultures were 90, 100 and 100%. All but one of the 22 negative results were obtained with poultry and two of the six laboratories participating in poultry analysis contributed 19 of the 21 negative results. Refrigeration of broth cultures provides greater operational flexibility by increasing the number of days on which analyses can be initiated without engendering work outside a normal work week.

Standard cultural procedures require 3-4 days for isolation of presumptive *Salmonella* colonies on selective agar media (8,11,12). Initiation of analyses that include non-selective preenrichment is restricted to Monday and Tuesday if analytical work is to be limited to the normal 5-day week. Departures from this work schedule result in weekend work because analyses can only be interrupted after incubation of selective agar plating media. The present work investigates the reliability of a 72-h refrigeration of non-selective preenrichment and selective enrichment broth cultures as a means of increasing the number of days on which standard cultural analyses could be initiated.

### MATERIALS AND METHODS

Contaminated low and high moisture food samples (Table 1) were obtained from retail outlets and processing plants by monitoring or investigational activities of Canadian federal agencies. Artificially contaminated pasta, milk powder, sesame seeds and chili powder were prepared as described previously (3). Contamination levels in selected samples were estimated by the three-tube Most Probable Number (MPN) technique and ranged from 0.9 to 1100 salmonellae/100 g.

In the standard cultural procedure (Fig. 1), high and low moisture food samples (100 g) were preenriched 18-24 h at 35 C in 0.9 liter of nutrient broth; samples of chocolate and candy bars were preenriched in 10% (w/v) reconstituted skim milk powder with added brilliant green (0.002% w/v) and artificially contaminated skim milk powder was preenriched in 0.002% (w/v) brilliant green water. A total of six laboratories collaborated in the analysis of the 105 poultry samples (Table 1). Poultry carcasses were thoroughly rinsed in 1 liter of nutrient broth and the entire rinse solution was incubated overnight at 35 C. One-ml portions of preenrichment broth cultures were transferred into

9.0 ml of tetrathionate brilliant green (TBG) and selenite cystine (SC) broths and incubated overnight at 43 and 35 C, respectively; the remaining preenrichment broth cultures were refrigerated at 4 C for 72 h and analyzed as outlined in Fig. 1. Selectively enriched samples were streaked on brilliant green sulfa (BGS) and bismuth sulfite (BiS) agar plates previously refrigerated for 24 h (2); the remaining selective enrichment broth cultures were refrigerated for 72 h at 4 C and then plated on BGS and BiS (Fig. 1). Presumptive *Salmonella* isolates obtained under standard and refrigerated conditions were screened biochemically on triple sugar iron and lysine iron agars and confirmed serologically with somatic and flagellar antisera.

Growth of non-salmonellae on BiS and BGS was scored according to the following scale: 1 = 0-25% incidence; 2 = 26-50%; 3 = 51-75%; 4 = 76-100%.

### RESULTS AND DISCUSSION

Recovery of *Salmonella* from refrigerated broth cultures using two selective enrichment broths and two plating media varied with different food categories (Table 2). Approximately 90% of the positive poultry samples were identified from refrigerated preenrichment and enrichment broth cultures; negative results were not found to be serotype-dependent, but 19 of the 21 negative results obtained with poultry samples were contributed by only two of the six participating laboratories (Table 3). *Salmonella* recoveries from high and low moisture foods under standard and refrigerated conditions were identical, except for a single contaminated pork liver sample which could not be identified from the refrigerated preenrichment culture. Recovery also varied with different enrichment-plating conditions (Table 4). TBG (43 C) in combination with one or two plating media was consistently more reliable than SC (35 C) under both standard and refrigerated conditions for detection of salmonellae in poultry and high moisture foods, but not in low moisture foods where differences were less striking. These findings concur with earlier reports on the superiority of selective enrichment of meats and other high moisture foods at elevated temperatures (4,5,9,10) and absence of a temperature-dependent *Salmonella* recovery in dried foods (6,7). Productivity of the BiS and BGS media was similar under standard and refrigerated conditions (Table 5), but the superiority of BiS over several *Salmonella* plating media, including brilliant green agar has been noted previously (1,7). Direct plating of refrigerated selective enrichment broths, presumably containing

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<sup>2</sup>Field Operations Directorate.

TABLE 1. *Salmonella* in contaminated food samples.

Food	Number of positive samples	Serotypes <sup>1</sup>	Level of contamination (cells/100 g)
<i>Poultry</i>			
Chicken	62	<i>infantis</i> (16); <i>schwarzengrund</i> (9); <i>haardt</i> (8); <i>typhimurium</i> (6); <i>manhattan</i> (6); <i>agona</i> (5); <i>albany</i> (3); <i>eimsbuettel</i> (3); <i>montevideo</i> (2); <i>drypool</i> (1); <i>cerro</i> (1); <i>heidelberg</i> (1); <i>blockley</i> (1).	0.6 - 21 <sup>3</sup>
Turkey	37	<i>saint-paul</i> (14); <i>infantis</i> (8); <i>schwarzengrund</i> (3); <i>cerro</i> (3); <i>typhimurium</i> (3); <i>niedstedten</i> (2); <i>haardt</i> (2); <i>agona</i> (1); <i>worthington</i> (1).	N.A. <sup>4</sup>
Chicken giblets	6	<i>bredeney</i> (3); <i>heidelberg</i> (2); <i>infantis</i> (1).	0.4 - 9.3
Sub Total	105		
<i>High moisture</i>			
Whole eggs	2	<i>thompson</i> (2)	4 - 460
Raw meats			
Pork	11	<i>typhimurium</i> (2); <i>brandenburg</i> (2); <i>london</i> (2); <i>anatum</i> (2); <i>haardt</i> (2); <i>muenster</i> (1); <i>panama</i> (1).	0.4 - 24
Beef	1	<i>typhimurium</i> (1)	0.4
Frog legs	2	<i>stanley</i> (2)	2.3
Shellfish	5	<i>manhattan</i> (1); <i>lexington</i> (1); <i>virchow</i> (1); <i>abaetetuba</i> (1); <i>miami</i> (1); <i>senftenberg</i> (1).	0.9 - 15
Sub Total	21		
<i>Low moisture</i>			
Egg powder	1	<i>senftenberg</i> (1)	15
Pasta <sup>2</sup>	4	<i>infantis</i> (2); <i>typhimurium</i> (2)	9 - 460
Dry yeast	4	<i>schwarzengrund</i> (2); <i>newington</i> (1); <i>infantis</i> (1).	0.4
Gelatin	1	<i>anatum</i> (1)	0.4
Milk chocolate	3	<i>eastbourne</i> (2); <i>senftenberg</i> (1)	0.4 - 230
Candy bar	3	<i>meleagridis</i> (3)	0.4 - 0.9
Milk powder <sup>2</sup>	1	<i>senftenberg</i> (1)	20
Sesame seeds <sup>2</sup>	1	<i>typhimurium</i> (1)	50
Chili powder	1	<i>chandans</i> (1); <i>toronto</i> (1)	24
Animal feed and feed ingredients	15	<i>montevideo</i> (7); <i>senftenberg</i> (2); <i>worthington</i> (1); <i>bareilly</i> (1); <i>cerro</i> (1); <i>infantis</i> (1); <i>saint-paul</i> (1); <i>kentucky</i> (1).	0.9 - 1100
Sub Total	34		

<sup>1</sup>Numbers in brackets denote frequency of isolation.

<sup>2</sup>Artificially contaminated product.

<sup>3</sup>Only 5 carcasses examined.

<sup>4</sup>Quantitative results not available.

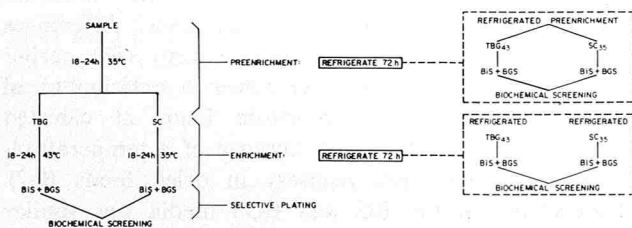


Figure 1. Analytical scheme for detection of *Salmonella* in refrigerated preenrichment and enrichment broth cultures.

stressed salmonellae, produced colonies with typical morphology on both agar media. BiS was generally more selective than BGS against background flora, parti-

cularly in low moisture foods (Table 6). Except for poultry refrigerated enrichment broths which yielded greater numbers of non-salmonellae compared to standard enrichment broths, results suggest that populations of background flora did not increase during refrigeration of preenrichment or enrichment broths as a result of growth or differential death rate kinetics.

The present study indicates that refrigeration of preenrichment and enrichment broth cultures is a valid approach to increase significantly the analytical capabilities of laboratories and provide for a more rapid identification of positive samples.

TABLE 2. Recovery of *Salmonella* from refrigerated broth cultures.

Food	Salmonella-positive samples <sup>1</sup>		
	Standard cultural	Refrigerated preenrichment	Refrigerated enrichments <sup>2</sup>
Poultry <sup>3</sup>	105	93 (90)	96 (91)
High moisture <sup>4</sup>	21	20 (95)	21 (100)
Low moisture <sup>4</sup>	34	34 (100)	34 (100)
Total	160 (100)	147 (92)	151 (94)

<sup>1</sup>Based on 4 different enrichment-plating conditions; percentage values are in brackets.

<sup>2</sup>Productivity of refrigerated TBG + SC broths.

<sup>3</sup>Results from six laboratories (see Table 3).

<sup>4</sup>Examined in a single laboratory.

TABLE 3. Negative results in poultry by reporting laboratory.

Laboratory	Samples positive	Negative refrigerated broth <sup>1</sup>	
		Preenrichment	Enrichment
A	14	0	0
B	12	0	0
C	17	1	0
D	14	1	0
E	25	5	2
F	23	5	7

<sup>1</sup>Found to contain *Salmonella* by standard but not refrigerated procedure.

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TABLE 4. Recovery of *Salmonella* under different enrichment-plating conditions.

Food	Total positive samples	Selective enrichment	Salmonella positive samples								
			Standard cultural			Refrigerated preenrichment			Refrigerated enrichment		
			BiS	BGS	BiS + BGS	BiS	BGS	BiS	BGS	BiS + BGS	
Poultry	105	TBG	82	96	103	82	78	87	82	84	93
		SC	42	42	54	50	44	58	36	37	40
High moisture	21	TBG	20	17	20	19	17	19	19	16	19
		SC	18	15	19	17	14	18	12	13	14
Low moisture	34	TBG	34	31	34	34	31	34	34	31	34
		SC	34	27	34	34	28	34	33	30	33

TABLE 5. Productivity of plating media with refrigerated broth cultures.

Food	Total positive samples	Selective enrichment	Salmonella positive samples					
			Standard cultural		Refrigerated preenrichment		Refrigerated enrichment	
			BiS	BGS	BiS	BGS	BiS	BGS
Poultry	105	TBG + SC	91	100	88	83	89	90
High moisture	21	TBG + SC	21	18	20	18	21	19
Low moisture	34	TBG + SC	34	31	34	31	34	31

TABLE 6. Background flora in refrigerated broth cultures.

Food	Total positive samples	Selective enrichment	Mean background flora <sup>1</sup>					
			Standard cultural		Refrigerated preenrichment		Refrigerated enrichment	
			BiS	BGS	BiS	BGS	BiS	BGS
Poultry	14	TBG	2.0	2.0	2.2	1.8	2.8	2.8
		SC	2.5	3.1	2.6	2.9	3.5	3.5
High moisture	21	TBG	2.4	2.5	2.0	2.3	2.4	2.8
		SC	2.7	2.8	2.7	2.9	3.0	3.0
Low moisture	34	TBG	1.2	2.0	1.1	1.8	1.2	1.7
		SC	1.4	2.3	1.2	2.4	1.3	2.3

<sup>1</sup>Background flora scored using scale: 1 = 0-25% incidence; 2 = 26-50%; 3 = 51-75%; 4 = 76-100%; all data provided by laboratory A (see Table 3).

## Time-Temperature Conditions of Gyros

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### ABSTRACT

Four *gyro* operations in foodservice establishments were examined for the possibility that pathogenic foodborne bacteria could survive and/or grow during each step of these operations. *Gyros* cooked on broilers attained temperatures lethal to vegetative pathogenic bacteria on the surface of the meat and in the thin layer just below the surface, but nowhere else. However, only meat sliced from the surface was normally put in *gyro* sandwiches or otherwise served. The temperatures of *gyros* as they cooled were such that bacterial growth could occur, both on the surfaces and within the mass. After *gyros* had been cooked and cooled, as many as 10,000 *Clostridium perfringens* per gram were recovered from samples taken just under the surface. Temperatures of *gyro* meat during reheating varied with the method of reheating, and they were in safe ranges when slices of meat were reheated in microwave ovens and steam chambers. When *gyros* were reheated on broilers, however, temperatures lethal to vegetative pathogenic bacteria occurred at and near the surfaces only. Recommendations for procedures to use for cooking, slicing, hot holding, cooling, and reheating *gyros* to prevent this product from becoming a vehicle of foodborne illness are given. Emphasis is on using the entire *gyro* the day it is originally cooked, rapid cooling of any leftover portions, and thorough reheating of leftover *gyros*.

A "gyro" is a meat dish or sandwich of either beef or lamb, or both, and often seasoned with onions, garlic, and parsley. The meat is either ground or sliced, and molded or stacked, often to form a frustum. The moisture and fat content of the mass causes the particles to cohere, particularly when frozen. Upon cooking, coagulation and other heat-induced processes provide even greater cohesion. The weight of *gyros* varies, usually within a 4.5- to 45-kg (10- to 100-lb) range; weights of 9 kg to 14 kg (20 to 30 lb) are common.

The frustum-shaped mass is impaled on a spit inserted along the vertical axis of symmetry. The spit is held vertically in an open broiler and rotates slowly as the *gyro* is heated. As slices of cooked meat are carved from the surface of the *gyro*, less-done surfaces are exposed which are in turn cooked more fully. The slices are usually characterized by a crusty exterior surface over a moist interior. They are frequently served as sandwiches in *pita* (bread) with a garnish of onion, parsley, lettuce and/or tomato and with a dressing of yogurt, sour cream or mustard.

*Gyros* or similar products are also called *yeros*, *dona kebabs*, *doner-kebobs*, *durno kebabs*, *dönnars*, *spelt dona*, *donah*, *chawarma*, *shawirma* and *souvlaki*. (The

term *souvlaki* also refers to cubes of meat that have been soaked in a marinade of oil, lemon juice and spices, put on a skewer and cooked on a grille or over an open flame.)

Various public health authorities have expressed concern about the microbiology of *gyros* during cooking, cooling and reheating (1,9). *Souvlaki* (*gyro*) has been alleged to be the vehicle of two small outbreaks in the United States (5,6). Meat products that are inadequately heated or reheated, improperly held hot or improperly cooled are often identified as vehicles of *Clostridium perfringens* gastroenteritis, salmonellosis or staphylococcal food poisoning (2).

The purpose of this investigation was to determine whether the temperatures of *gyros* during cooking, cooling and reheating in foodservice establishments constituted a potential for survival and multiplication of certain common foodborne pathogens.

### METHODS

Temperatures of the surface and internal regions of *gyros* were determined during four routine operations in three foodservice establishments. In one of the establishments, temperatures were determined as a *gyro* was cooked, cooled and reheated in a manner to simulate certain conditions observed and reported by foodservice managers and health department officials, as well as their usual operation.

#### Operations evaluated

1. Ground-beef *gyros*, approximately 46 cm (11.5 inches) in diameter and 8 cm (3 inches) high, weighing approximately 4.5 kg (10 lb), were purchased frozen from a commercial source. The *gyros* were thawed by holding at room temperature for about 4 h and then put in a walk-in refrigerator. Thawed *gyros* were cooked in an oven and then tempered for a few hours at room temperature before cooling in a walk-in refrigerator. The next day the chilled *gyro* loaf was sliced in approximately 10-cm (4-inch) squares, and the slices were reheated in a hooded compartment of a steam table. Leftovers were stacked to a height of 10 cm (4 inches) in a pan, cooled in a one-door, reach-in refrigerator and on the next day again reheated in the steam table.
2. *Gyros*, approximately 14 cm (5.5 inches) in diameter and 23 cm (9 inches) long, weighing about 4.5 kg (10 lb), were molded of ground beef in the establishment and were not frozen. They were cooked by an open broiler by an electrically heated metal coil. A short time after cooking, they were cooled in a two-door, reach-in refrigerator. Slices were heated in a microwave oven when an order for a *gyro* was given.
3. *Gyros*, approximately 20 cm (8 inches) in diameter and 33 cm (13 inches) long, weighing about 11 kg (25 lb) were molded of ground beef in the establishment. The spit was inserted, and they were frozen in a walk-in freezer. Frozen *gyros* were cooked by a gas-heated, open broiler. At closing time, the remaining portion of the *gyro* was left on the spit and cooled in a 3-door, reach-in refrigerator. It was heated

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<sup>2</sup>Pinellas County Health Department.

again by the broiler where it remained until sold. The last portion of a pound or two either fell or was cut from the spit. This was kept on a pan on a grille, and slices were heated on the grille when an order for a gyro was given.

4. A gyro, approximately 18 to 28 cm (7 to 11 inches) in diameter and 37 cm (14.5 inches) long, weighing 14 kg (31 lb), was purchased from the commercial source. It was cooked by a 3-element, electrically heated broiler. Leftovers were left on the spit and cooled in a walk-in refrigerator. The next day, the gyro was again heated by the broiler. Leftovers were again cooled in the refrigerator and reheated by the broiler the next day. During heating, one or more (and on some occasions all) of the heating units were turned off for various periods to prevent overheating the meat.

#### Temperature determinations

Type-T thermocouples were inserted into one of several 0.3-cm (1/8-inch) holes drilled vertically from the top to the middle of frozen gyros so that meat temperatures could be made near the geometric center and other internal locations. The distance down from the top is referred to in the figures as "deep," and the distance from the surface is referred to as "into." These thermocouples were thrust into the same region of unfrozen gyros or into cuts or cooked meat. Button-type probes were attached to external surfaces with wires or held against the surface by hand. Specific equipment and procedures were as previously described (4).

#### Laboratory procedures

Samples were aseptically collected with a sterile fork, after slicing the gyro with a sterile knife. They were put in sterile plastic bags, immediately refrigerated, and later iced and held in insulated containers for personal delivery to the laboratory.

The Tampa Branch Laboratory, Florida Department of Health and Rehabilitative Services, analyzed samples of raw and cooked gyro meat collected during this investigation by procedures that, other than where specified, followed the *Bacteriological Analytical Manual* (7).

*Staphylococcus*. Appropriate amounts of the homogenate and dilutions for a 3-tube MPN were enriched at 35 C for 48 h in tripticase soy broth (Difco) containing 10% NaCl. Broth from tubes showing evidence of microbial growth spread on tellurite polymyxin egg yolk (Difco) plates and incubated at 35 C for 48 h. Typical-appearing *S. aureus* colonies were picked and tested for coagulase.

*Clostridium perfringens*. One ml of the homogenate and appropriate dilutions to  $10^{-4}$  were plated in sulfite polymyxin sulfadiazine agar (BBL) and incubated anaerobically at 35 C for 48 h. Suspected colonies were picked to tubes of fluid thioglycolate (Difco) which were incubated at 35 C for 24 h and then in a water bath at 46 C for 4 h. The culture was confirmed by streaking on liver-veal egg yolk agar (35 C for 24 h, anaerobic incubation), by tests for gelatinase motility and nitrate reduction and by staining.

*Salmonella*. Twenty-five grams of sample were added to 225 ml of lactose broth (Difco) and incubated at 37 C for 24 h. A loop of this culture was transferred to tetrathionate brilliant green broth (Difco) and incubated in the same fashion. The culture was streaked onto *Salmonella-Shigella* agar (Difco), MacConkey agar (Difco), brilliant green agar (BBL) and bismuth salt agar (Difco). The first three agars were incubated at 37 C for 24 h and the latter at the same temperature for 48 h. Suspect colonies were stabbed and streaked to triple sugar iron urea agar slant, which was incubated at 37 C for 24 h. Isolates were confirmed by testing for the presence of indole, fermentation of lactose and sucrose and decarboxylation of lysine. Poly 0 and group 0 slide agglutinations were also performed.

## RESULTS

### Thawing

Gyros were subjected to thawing procedures in only Operation 1. The day before cooking, the frozen meat was taken from a walk-in refrigerator and kept at room temperature for 4 h and then transferred back to the

refrigerator. When cooking commenced, the gyro had not yet thawed (Fig. 1).

### Cooking

The gyro cooked in an oven like a meat loaf (Operation 1) reached temperatures throughout that would be lethal to vegetative pathogenic foodborne bacteria (Table 1, Fig. 2). The temperature of central internal regions increased after cooking during holding at room temperature before refrigeration.

The gyros cooked on broilers attained temperatures lethal to vegetative pathogenic foodborne bacteria only at the surface of the meat and in a thin layer just below the surface. Temperature data are presented in Table 1 and illustrated for Operation 2 in Fig. 3, for Operation 3 in Fig. 4 and for Operation 4 in Fig. 5.

### Cooling

Temperatures of gyros during cooling were such that some bacterial growth could have occurred on surfaces and within the meat (Table 2, Fig. 6 and 7). Temperature data are presented in Table 2 and illustrated for Operation 1 in Fig. 6, for Operation 2 in Fig. 7, and for Operation 5 in Fig. 8 and 9. Cuts of meat, 10 cm (4 inches) or less in thickness, or slices stacked to that height, cooled rapidly enough to avoid the possibility of bacterial multiplication of an order great enough to cause foodborne disease (Table 2; Fig. 10).

### Reheating

When a 10-cm (4-inch) high stack of slices of meat was reheated in a hooded steam-table chamber (Operation 1), the geometric center of the meat reached a temperature of 73.9 C (165 F) within 3 h (Fig. 11). During prolonged hot storage, the temperature of the meat varied with the temperature of the water in the steam table (Fig. 12). These data and data from other trials are summarized in Table 3.

The interior of a sandwich-size portion of this meat

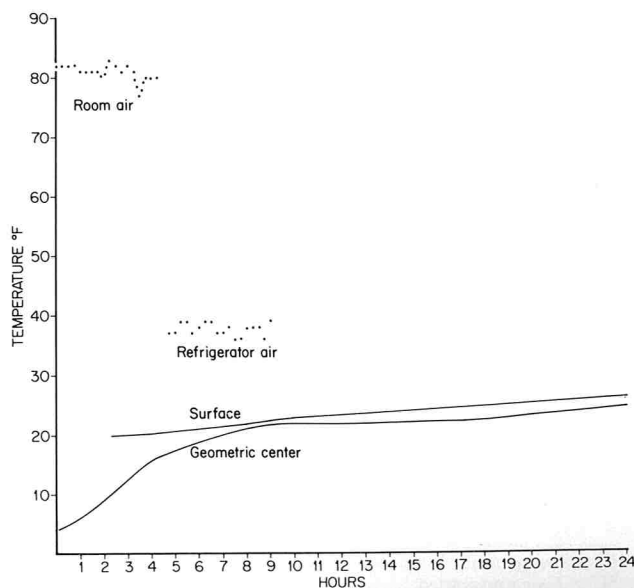


Figure 1. Temperatures of 9.5-pound gyro during thawing while held at room and refrigerator temperature (Operation 1).

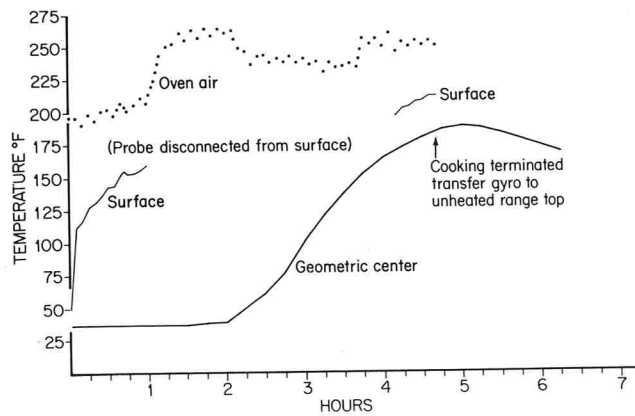


Figure 2. Temperatures of 9.5-pound gyro during heating in an oven (Operation 1).

reached a temperature greater than 93.3 C (200 F) during heating in a microwave oven (Operation 2; Fig. 13). During a second heating in gas or electrically heated broilers, the surfaces of gyros and a shallow layer below the surfaces attained temperatures that would be lethal to vegetative pathogenic foodborne bacteria. Temperature data are presented in Table 3 and illustrated for Operation 3 in Fig. 14 and for Operation 4 in Fig. 15 and 16. Other portions of the meat, however, did not attain lethal temperatures. Sandwich-size portions of meat, which were removed from the spit or carved from surfaces during times when the broiler was turned off, attained temperatures of 73.9 C (165 F) or higher when the meat was either heated on a grille (Fig. 17) or immersed in hot water (Fig. 18) long enough (Table 3).

**Bacteriological analyses**

Results of analyses of samples from three of the operations for the presence of *Salmonella* and the numbers of *C. perfringens* and *S. aureus* are given in Table 4.

TABLE 1. Times gyros were above certain temperatures during initial cooking.

Operation/weight (pounds)/heat source	Probe location	Time in minutes at or above (F)								Expected survival of vegetative cells
		180	135	140	145	150	155	160	165	
1/9.5 pounds/oven	Surface	R <sup>1</sup> /U <sup>2</sup>	R/U	R/U	R/U	R/U	R/U	R/U	R/U	-
	Geometric center	77	72	68	63	58	52	46	40 <sup>3*</sup>	-
2/10 pounds/hand-turned spit	Surface <sup>4</sup>	R/U	R/U	R/U	0	0	0	0	0	+ / -
	1-1/2-inch beneath surface	0	0	0	0	0	0	0	0	+
electrically heated gyro machine	Geometric center	0	0	0	0	0	0	0	0	-
	Surface	R/U	R/U	R/U	R/U	R/U	R/U	R/U	R/U	-
3/25 pounds/rotating spit gas-heated gyro machine	1/4-inch beneath surface	95	92	88	83	60	51	36	31	-
	Midway between surface and center	0	0	0	0	0	0	0	0	+
	Geometric center	0	0	0	0	0	0	0	0	+
4/31 pounds/rotating spit, gas-heated gyro machine	Surface	240	230	215	189	156	113	59	27*	-
	Midway between surface and middle probes, moved to near surface after 135 min	160	142	129	80	44	20	10	R	-
	Midway between surface and center	0	0	0	0	0	0	0	0	+
	Geometric center	0	0	0	0	0	0	0	0	+

<sup>1</sup>R = Temperature attained.

<sup>2</sup>U = Time unrecorded.

<sup>3\*</sup> = Time of storage at room temperature and post-oven temperature rise not included.

<sup>4</sup>Surface temperature recording not continuous.

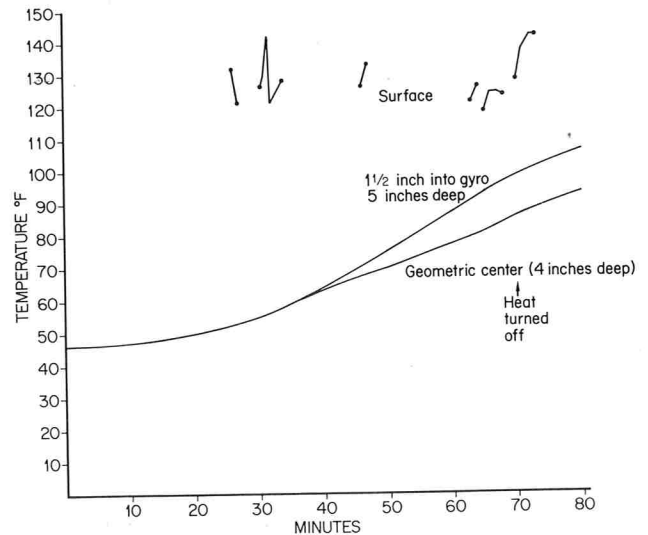


Figure 3. Temperatures of 10-pound gyro during cooking on an electrically heated gyro machine (Operation 2).

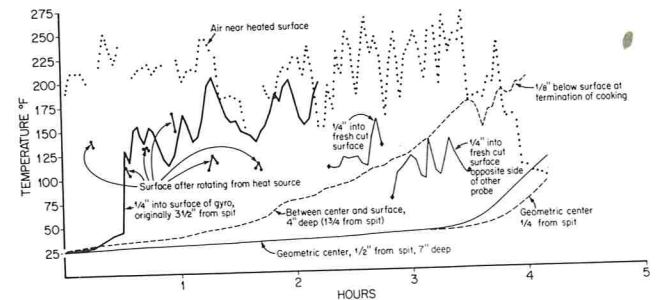


Figure 4. Temperatures of 25-pound, 5-ounce gyro during cooking on a gas-heated gyro machine (Operation 3).

**DISCUSSION**

Hazards attributed to temperatures of gyros during cooking, cooling and reheating differed somewhat among the operations. No significant growth of

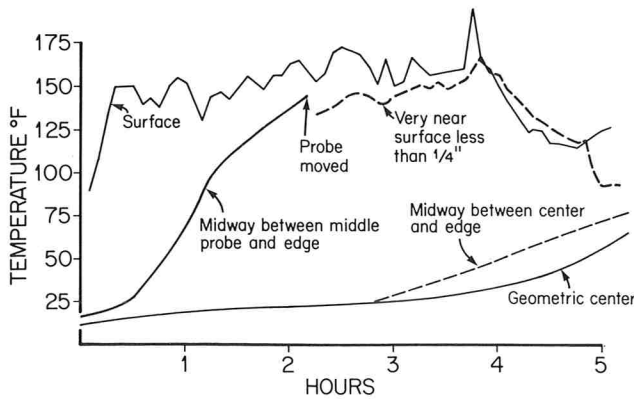


Figure 5. Temperatures of 31-pound gyro during heating on an electrically heated gyro machine (Operation 4).

pathogenic foodborne bacteria would be expected during the combination of room- and refrigerated-thawing used in Operation 1. In this operation, temperatures attained during cooking would have killed any vegetative pathogenic foodborne bacteria that were in the meat, but bacterial spores that survived cooking, and any bacteria that contaminated the meat as a result of carving or subsequent handling, could have multiplied during cooling. Subsequent reheating as observed (if continued long enough) would kill vegetative bacteria. Temperatures of the geometric centers of gyros during cooking, cooling and reheating are shown in Fig. 19.

In Operation 2, temperatures attained during cooking would have permitted any pathogenic foodborne bacteria present in most internal regions of the gyro to survive, and these organisms could have multiplied during cooling. These bacteria probably would have been killed during reheating in the microwave oven.

Temperatures reached in Operation 3 were sufficient to kill vegetative pathogenic foodborne bacteria on surfaces but not in most internal regions. All of the meat cooked in one trial was sold during cooking, so there was insufficient time for problems to occur. Although cooling was not evaluated it can be inferred from data on the other operations that certain bacteria present could have

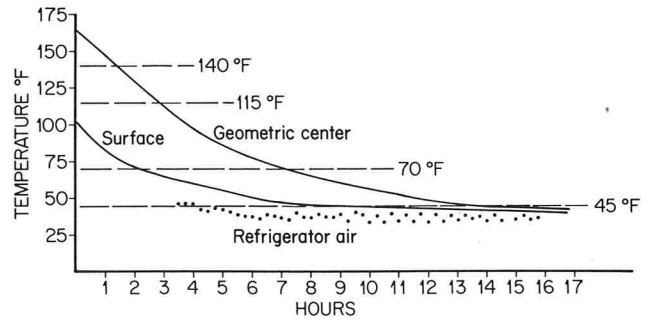


Figure 6. Temperatures of 9.5-pound cooked gyro during storage in a walk-in refrigerator (Operation 1).

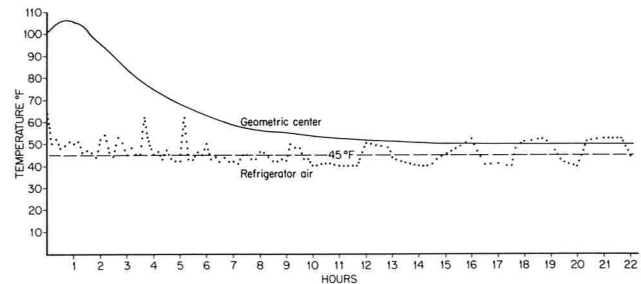


Figure 7. Temperatures of recently cooked 10-pound gyro during storage in reach-in refrigerator (Operation 2).

multiplied during storage in the refrigerator. Surfaces were reheated adequately.

The numerous problems that were observed in Operation 4 are indicated in Fig. 20. Temperature problems were seen in cooking during prolonged periods during which either the heat was turned down or off, or the gyro was moved away from the heat source. Considerable bacterial growth could also have occurred during the cooling period. Reheating was inadequate to kill vegetative bacteria in regions other than at or in shallow layers below the surface. Repeated cooling and reheating led to prolonged periods during which the temperatures of the interior regions were in a range within which bacteria could have multiplied rapidly.

Foodborne pathogens are present in raw or frozen gyro meat, as shown by Matossian and Kingcott (9) and data

TABLE 2. Times gyros were within certain temperature ranges during cooling.

Operation/weight or size	Probe location	Time in minutes between (F)				Expected growth
		85-115	70-115	60-122	45-140	
1/9.5 pounds	Surface	58	130	240	485	+
	Geometric center	130	260	395	755	++
1/4-inch pile	Geometric center <sup>1</sup>	45	65	105	215	-
2/10 pounds	Geometric center <sup>1</sup>	185	260	400	1320(+) <sup>2</sup>	++
	Geometric center <sup>1</sup>	0 <sup>2</sup>	35	105	900	(+) <sup>3</sup>
4/21.5 pounds	Surface	58	143	267	660	+
	Midway between surface and middle probe	188	345	480	720(+)	+++
	Midway between surface and center	188	345	480	720(+)	+++
4/10 pounds, 13 ounces	Surface	88	174	240	540	++
	Geometric center	120	210	289	540	++
4/5.5 x 5 x 4-inch cut of meat	Surface	45	120	180	271	+
	1-1/2-inch beneath surface	88	153	215	283	+
	Geometric center	115	174	238	299	++

<sup>1</sup>Multiple trials.

<sup>2</sup>(+) = Lower temperature of range was not reached at time food was removed from cooling unit.

<sup>3</sup>Cooling initiated before probe was inserted.

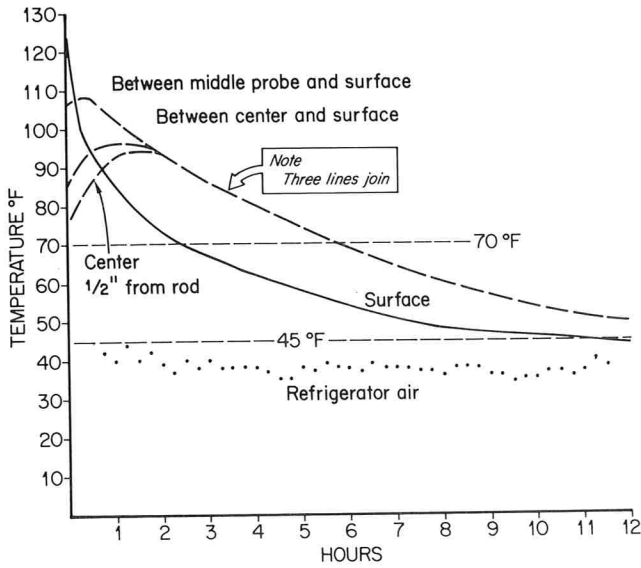


Figure 8. Temperatures of 21.5-pound previously heated gyro during storage in a walk-in refrigerator (Operation 4).

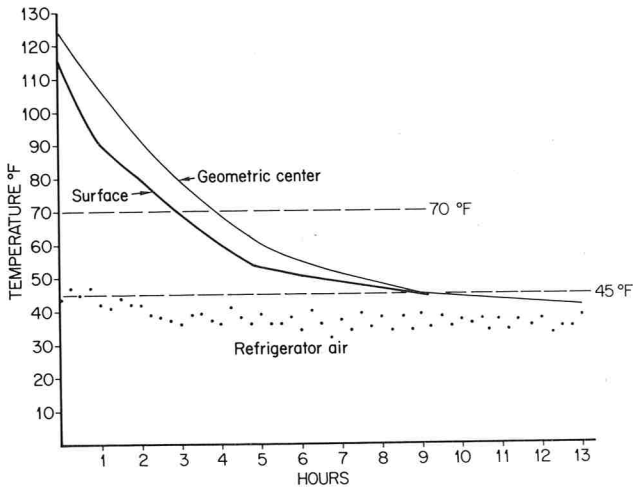


Figure 9. Temperatures of 10-pound, 13-ounce cooked gyro during storage in a walk-in refrigerator (Operation 4).

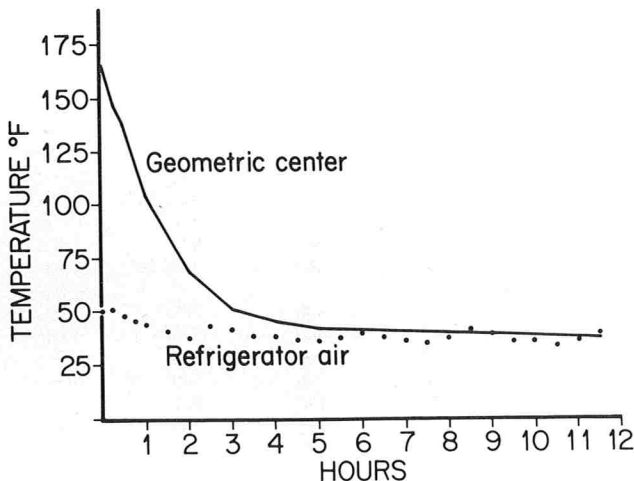


Figure 10. Temperatures of 4-inch pile of sliced cooked gyro meat during storage in a reach-in refrigerator (Operation 1).

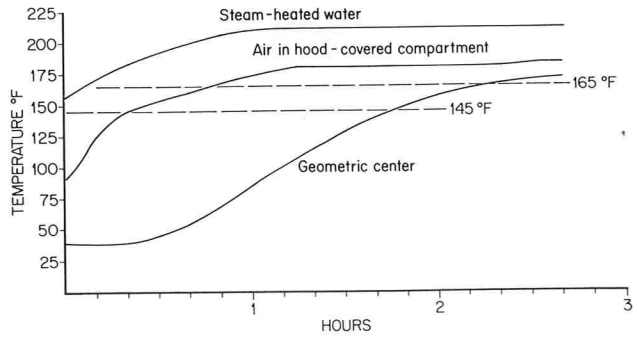


Figure 11. Temperatures of 4-inch pile of sliced cooked gyro meat during reheating in a hood-covered compartment of a steam table (Operation 1).

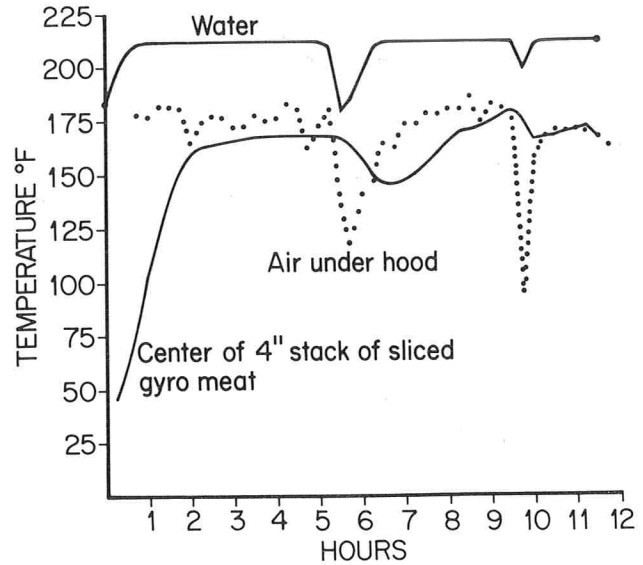


Figure 12. Temperatures of 4-inch pile of sliced cooked gyro meat during reheating and hot holding in a hood-covered compartment of a steam table (Operation 1).

in Table 4. During cooking, surfaces and shallow layers, approximately 0.6 to 1.3 cm (1/4 to 1/2 inch) in thickness, reached temperatures that were high enough and lasted long enough to kill pathogenic foodborne bacteria. According to Goodfellow and Brown (8) salmonellae would succumb in the geometric center of 4.5-kg (10-lb) or larger cuts of beef that were cooked in ovens at certain temperatures for sufficient time. If this conclusion is related to data observed during this investigation, salmonellae and organisms of similar ability to resist heat would be expected to survive in the geometric center and frequently in most other interior regions of the gyros during cooking, until these regions reached the surface as a result of carving off the outer portions. This hypothesis is supported by the observation of decreased staphylococcal counts on surfaces after cooking (Table 4).

Although sometimes there were long periods during which heat was turned off, surface temperatures increased again when the heat was turned on. The duration of the heating before serving, in conjunction with the temperature reached, would, however, not always have been sufficient to kill bacteria.



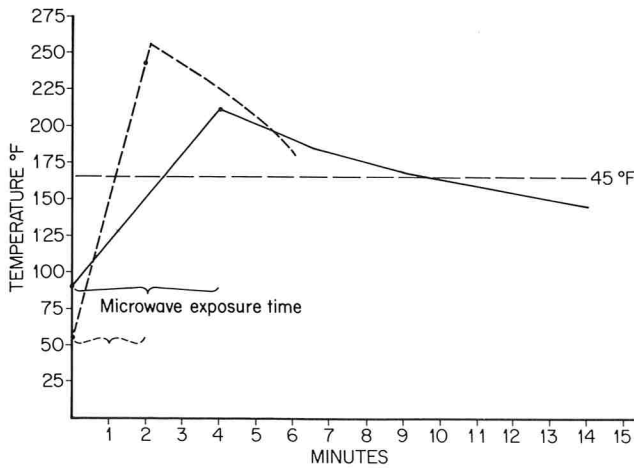


Figure 13. Temperature of slice of cooked gyro meat during reheating in a microwave oven. [Two trials presented by different line symbols; heating time and post-oven hold period also shown (Operation 2)].

There was considerable opportunity for bacterial growth in the interior regions of leftover gyro meat that was left on the spit during storage in refrigerators. Evidence of this happening was shown by the large numbers of *C. perfringens* found in chilled, cooked meat (Operation 4, Table 4). Also, Matossian and Kingcott (9) isolated heat-resistant strains of *C. perfringens* from 11 of 25 samples of cooked chawarma.

Although surfaces cooled more rapidly than interior regions, some bacterial growth could have occurred even on the surfaces. Cooling was more rapid when cooked

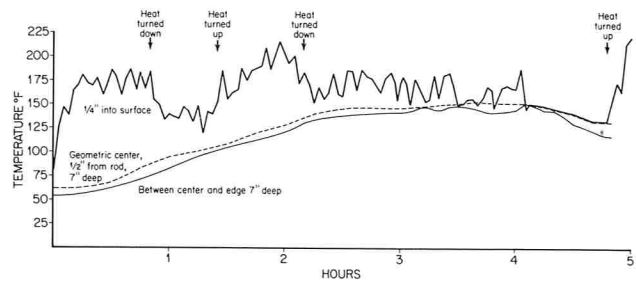


Figure 14. Temperatures of 14.5-pound, ground beef gyro during reheating on a gas-heated gyro machine after overnight refrigeration following initial cooking (Operation 3).

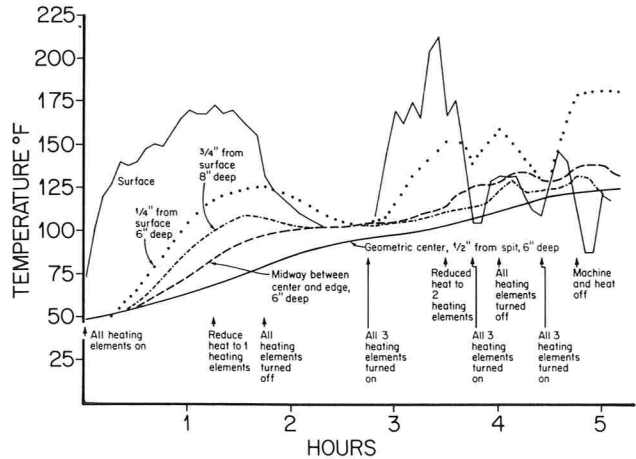


Figure 15. Temperatures of 21.5-pound previously heated and cooled gyro during reheating on an electrically heated gyro machine (Operation 4).

TABLE 3. Times gyros were above certain temperatures during reheating.

Operation/weight or size/heat source	Probe location	Time in minutes at or above (F)								Expected survival of vegetative cells
		130	135	140	145	150	155	160	165	
1/4-inch pile/ hood-enclosed compartment of steam table	Geometric center <sup>1</sup>	95	87	80	71	62	49	33	18(+) <sup>2</sup>	—
	Geometric center <sup>1</sup>	158	149	141	135	121	103	83	60(+)	—
	Geometric center <sup>1</sup>	69	61	60	54	49	43	34	23(+)	—
	Geometric center <sup>1</sup>	610	603	597	590	525	495	455	380(+)	—
2/slice of cooked gyro meat/microwave oven	Geometric center <sup>1</sup>	5.2 <sup>3</sup>	5.2 <sup>3</sup>	5.1 <sup>3</sup>	5.0 <sup>3</sup>	5.0 <sup>3</sup>	4.9 <sup>3</sup>	4.9 <sup>3</sup>	4.8 <sup>3</sup>	—
	Geometric center <sup>1</sup>	12.7 <sup>3</sup>	12.6 <sup>3</sup>	12.4 <sup>3</sup>	12.2 <sup>3</sup>	12.0 <sup>3</sup>	9.8 <sup>3</sup>	8.5 <sup>3</sup>	7.1 <sup>3</sup>	—
3/14.5 pounds/rotating spit, gas-heated gyro machine	Surface	294	279	264	238	218	195	174	161	—
	Midway between surface and center	140	126	100	45	2	0	0	0	—
	Geometric center	168	148	135	110	50	0	0	0	—
	Surface	157	144	128	116	110	94	83	70(+)	—
4/12.5 pounds/rotating spit, electrically heated gyro machine	1/4-inch beneath surface	124	108	98	86	66	47	32	30(+)	—
	3/4-inch beneath surface	18	0	0	0	0	0	0	0	+
	Midway between surface and center	70	30	0	0	0	0	0	0	+/-
	Geometric center	0	0	0	0	0	0	0	0	+
4/10 pounds, 13 ounces/rotating spit, electrically heated gyro machine	Surface	62	57	55	53	38	28	15	12(+)	—
	1/4 inch beneath surface	63	59	56	51	49	44	36	32(+)	—
	1/2 inch beneath surface	55	50	42	37	32	30	19	3(+)	—
	3/4 inch beneath surface	12	10	8	7	3	R <sup>4</sup>	0	0	—
	1 inch beneath surface	0	0	0	0	0	0	0	0	+
	2 inches beneath surface	0	0	0	0	0	0	0	0	+
4/slice/dipped into hot water	Geometric center <sup>1</sup>	0	0	0	0	0	0	0	0	+
	Geometric center <sup>1</sup>	2.7	2.5	2.3	2.1	2.0	1.7	1.3	0.5	—
4/slice/grille	Geometric center <sup>1</sup>	2.5	2.3	2.1	2.0	1.8	1.5	1.2	0.9	—
	Geometric center	1.9	0.9	0.8	0.7	0.6	0.5	0.4	0.3	—
3/slice/grille	Geometric center	1.9	1.6	1.4	1.3	1.1	0.9	0.6	0.3	—

<sup>1</sup>Multiple trials.

<sup>2</sup>(+) = Time of storage of portions of meat sometimes exceeded this period.

<sup>3</sup>Data include post-oven temperature rise.

<sup>4</sup>R = Temperature reached.

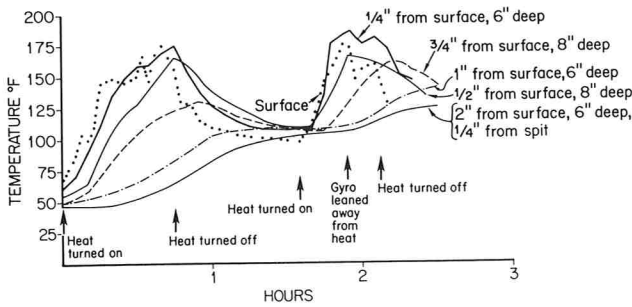


Figure 16. Temperatures of 10-pound, 13-ounce previously heated and cooled gyro during reheating in an electrically heated gyro machine (Operation 4).

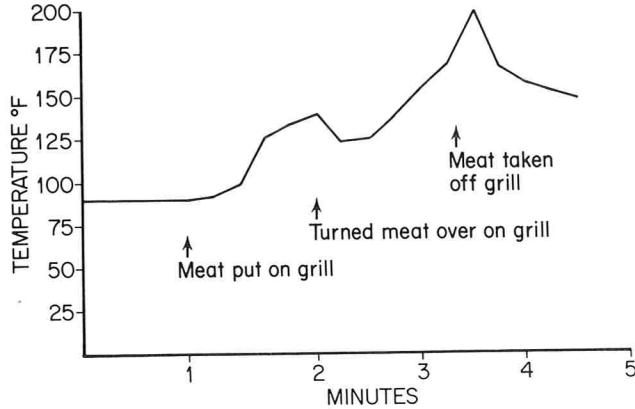


Figure 17. Internal temperatures of sandwich-size portion of gyro meat during heating on a grill after meat removed from gyro machine (Operation 3).

meat was cut or sliced from the spit and stored either in 10-cm (4-inch) high stacks or in cuts not larger than 10 cm (4 inches) in any dimension.

It is presumed that the temperatures reached at and near the surface during reheating of cooled gyro meat would have killed any vegetative pathogenic foodborne bacteria present in these regions. The temperatures would not, however, rise to levels lethal to this type of bacteria in the interior regions, such as that which fell or were removed from the spit at the termination of heating. The isolation of large numbers of *C. perfringens* and *S. aureus* from the interior of gyros after the cooking-cooling cycle, or subsequent reheating, cooling and reheating

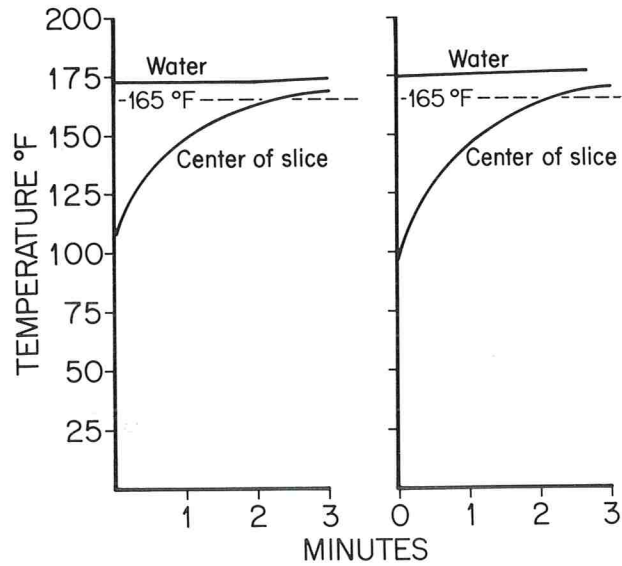


Figure 18. Temperatures of geometric center of slice of gyro meat during reheating in hot water on a range (two trials, Operation 4).

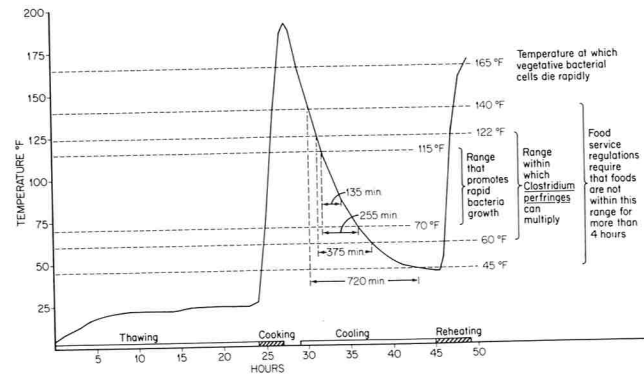


Figure 19. Time-temperature curve for geometric center of gyro during cooking, cooling, and reheating (Operation 2).

(Operation 4; Table 4), is compatible with this hypothesis.

Reheating slices of meat in a microwave oven, on grilles, and in either hot water or broth, for a sufficiently long time, caused the interiors to reach temperatures that would be lethal for vegetative pathogenic foodborne bacteria, but which would not destroy staphyloenterotoxin.

TABLE 4. Isolation of certain foodborne pathogens from gyros in three foodservice establishments.

Operation	Organisms	Treatment/Location of sample			
		Raw/surface	Cooked/just under surface	Cooked, cooled, and reheated/just under surface	Cooked, cooled, and reheated for second time/center
2	<i>C. perfringens</i>	10	10	*	
	<i>S. aureus</i> (MPN)	3	3		
	<i>Salmonella</i>	Neg	Neg		
3	<i>C. perfringens</i>	10	10		
	<i>S. aureus</i> (MPN)	430	3		
	<i>Salmonella</i>	Neg	Neg		
4	<i>C. perfringens</i>	10	10	10,000	1,000
	<i>S. aureus</i> (MPN)	230	3	230	2,400
		750	3		
	<i>Salmonella</i>	Neg	Neg		

\*Blank spaces indicate that no samples were taken.

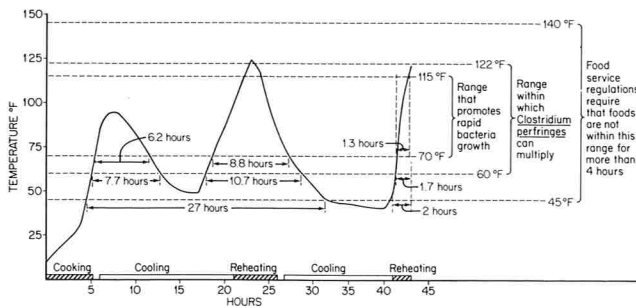


Figure 20. Time-temperature curve for geometric center for gyro during cooking, repeated cooling and reheating (Operation 4).

## RECOMMENDATIONS

### Cooking

Gyros should be as small as practicable to meet anticipated serving needs. It is better to cook several small gyros throughout the day rather than one large gyro. The outside surfaces of gyros should be thoroughly cooked before carving.

### Slicing

Knives and carved-meat catching pans should be cleaned and disinfected before use and after cuts are made that are deep enough to penetrate or slice into raw or undercooked regions of a gyro.

### Hot holding

Whenever meat is sliced from the gyro and held warm until served, internal temperatures at the center of the stack of slices or cuts of meat should stay above 54.4 C (130 F), preferably at or above 60 C (140 F).

### Cooling

It is best not to keep leftover gyros overnight. If they are kept, however, they should be either small in diameter or reduced in size. Never leave the gyros on the broiler overnight.

Leftover gyros that are 10 cm (4 inches) or less in diameter can be cooled by leaving them on the spit and putting them into a walk-in refrigerator that maintains temperatures below 4.4 C (40 F).

Leftover gyros that are larger than 10 cm (4 inches) in diameter should be trimmed to that size, and the trimmed cuts of meat should also be 10 cm (4 inches) or less in any dimension. The gyro should be stored as above. Alternatively, the spit should be pulled out and the gyro sliced or cut into sections not more than 10 cm (4 inches) in any dimension. The cuts should be put either into pans to form a single layer only or wrapped in foil or plastic and put on a shelf in a single layer with air space around them. (These procedures are only practicable if there are satisfactory reheating arrange-

ments for the slices or cuts.)

If practicable, rapidly cool the gyro or portions cut from it by ice or water baths, cold plates or pans or blasts of cold air as recommended by Bryan and McKinley (3,4) before putting them in the refrigerator.

### Reheating

Gyros of 10 cm (4 inches) or less in diameter can be reheated once on the open broiler, but any leftovers from this second heating should be discarded. Take particular care (even more so than during cooking) that the outside surfaces and other regions to be cut off are thoroughly cooked before carving.

Reheat chilled, leftover cuts of gyro meat to an internal temperature of 71.1 C (160 F) or higher. A cleaned and disinfected thermometer should be inserted so that the sensing portion is in the geometric center of the cut of meat or the stack of slices, so that the operator can see if a temperature of at least 71.1 C (160 F) is reached before the meat is served.

Sandwich-size portions of sliced gyro meat should be reheated to 73.8 C (165 F) or higher. These temperatures can be obtained by heating the meat on grilles, in boiling water or broth, in microwave ovens, in pressure cookers or in other steam-heated chambers, if enough time is provided.

## ACKNOWLEDGMENTS

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## Proteolytic and Lipolytic Activities of some Toxigenic and Nontoxigenic Aspergilli and Penicillia

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### ABSTRACT

Eighteen strains of *Aspergillus flavus* or *Aspergillus parasiticus*, one of *Aspergillus ochraceus* and 12 strains or species of *Penicillium*, many of them isolated from cheese, were evaluated for their proteolytic and lipolytic activities. Strains of *A. flavus* exhibited considerable proteolytic and little lipolytic activity, whereas the reverse was true for strains of *A. parasiticus*. Of the *Penicillium* cultures tested, 10 exhibited considerable lipolytic activity, but only five had marked proteolytic activity. Two cultures, *Penicillium patulum* M59, and *Penicillium cyclopium* No. 8, were markedly lipolytic and proteolytic. Of the other cultures, greatest lipolytic activity was associated with *Penicillium roqueforti* 849, *Penicillium puberulum* No. 33, *A. parasiticus* NRRL 3145 and NRRL 465 and *A. ochraceus* NRRL 3174, whereas greatest proteolytic activity of all the cultures was associated with *P. patulum* M59, *P. cyclopium* No. 25 and *A. flavus* WB500, 4018, 4098 and NRRL 5565.

Molds are of special importance in the food industry because of their usefulness in producing some foods, the spoilage they sometimes cause and the capability of many to produce toxins. Some molds in the genus *Aspergillus*, namely *Aspergillus flavus* and *Aspergillus parasiticus*, can produce aflatoxin which is acutely toxic or carcinogenic to experimental animals. Because of its biological effects and its occasional appearance as a contaminant of agricultural commodities, including foods, this fungal metabolite has been widely investigated (7,13). However little information has accumulated on some physiological characteristics of the toxigenic aspergilli.

Therefore, the major objective of this study was to develop information on two characteristics, proteolytic and lipolytic activities, of numerous strains of *A. flavus* and *A. parasiticus*. These characteristics were selected for study because they are commonly involved in the ability of these molds to grow on foods and to bring about their spoilage. For comparative purposes, similar information was obtained for *Aspergillus ochraceus* and numerous penicillia, many of which were isolated from cheese.

### MATERIALS AND METHODS

#### Cultures

All cultures of *A. flavus*, *A. parasiticus* and *A. ochraceus* listed in Table 1 and having NRRL numbers were obtained from the Northern Regional Research Center, U.S.D.A., Peoria, IL. Cultures with the CMI designation came from the Commonwealth Mycological Institute, Kew, Surrey, England, and those with the WB designation from the Department of Bacteriology, University of Wisconsin, Madison.

TABLE 1. Proteolytic and lipolytic activity of some *Aspergillus* species.

Species and strains	Proteolysis	Lipolysis
<i>Aspergillus flavus</i> (Toxigenic strains)		
NRRL 4018	++ <sup>a</sup>	—
NRRL 4098	++	Trace
NRRL 3161	— <sup>b</sup>	—
NRRL 3494	Slight	—
NRRL 3353	—	—
CMI 3251	—	—
CMI 93080	—	—
WB 500	++	—
<i>Aspergillus flavus</i> (Nontoxigenic strains)		
NRRL 5917	—	Trace
NRRL 5565	++	Trace
NRRLA 13668	+	Trace
NRRL 5918	Slight	—
WB 1957	+ <sup>c</sup>	—
<i>Aspergillus parasiticus</i> (Toxigenic strains)		
NRRL 3145	Slight	++
NRRL 3000	—	Trace
NRRL 465	—	++
NRRL 2999	Slight	—
CMI 15957	—	++
<i>Aspergillus ochraceus</i> (Toxigenic strain)		
NRRL 3174	+	++

a++ = Strong positive reaction.

b— = Negative.

c+ = Positive.

The culture of *Penicillium expansum* (Table 2) was obtained from the Northern Regional Research Center, whereas the other penicillia came from the culture collection of the Food Microbiology Laboratory in the Department of Food Science, University of Wisconsin-Madison. Finol (5) recently isolated from cheese the three strains of *Penicillium cyclopium*, *Penicillium roqueforti* 22, the two strains of *Penicillium puberulum*, *Penicillium viridicatum*, *Penicillium crustosum* and *Penicillium lanso-virde*. All of the penicillia isolated by Finol (5) can grow in the presence of high (> 3000 ppm) concentrations of sorbic acid. All molds were grown on slants of mycological agar (Difco) at 28 C for 7 days before use.

#### Tests for proteolysis and lipolysis

Proteolytic activity of molds was determined using a casein substrate as described by El-Gendy et al. (4). Each mold culture was inoculated onto the surface of mycological agar (Difco) to which sterile skim milk (2 ml/10 ml of agar medium) was added just before pouring the medium into petri plates. Plates were incubated for 7 days at 28 C.

TABLE 2. Proteolytic and lipolytic activity of some *Penicillium* species.

Species and strains	Proteolysis	Lipolysis
<i>Penicillium cyclopium</i> 8	++ <sup>b</sup>	++
25	++	Slight
40	- <sup>c</sup>	-
<i>roqueforti</i> 22	-	Slight
849	-	++
<i>puberulum</i> 33	-	++
37	-	-
<i>expansum</i> NRRL 2304 <sup>a</sup>	Slight	-
<i>patulum</i> M 59 <sup>a</sup>	++	+ <sup>d</sup>
<i>viridicatum</i> 34	-	Trace
<i>crustosum</i> 42	Slight	Slight
<i>lanso-viride</i> 44	-	Slight

a = Produces patulin.  
 b ++ = Strong positive reaction.  
 c - = Negative.  
 d + = Positive.

Before recording results, plates were flooded with a solution of 10% hydrochloric acid.

Lipolytic activity was measured using the method of Berry (1) that involves copper soap formation. Ten ml of mycological agar and 1 ml of sterile milkfat were added per petri plate. After solidification, a mold was inoculated onto the surface of the medium. Plates were incubated at 28 C for 7 days. Copper soap formation (using saturated copper sulfate solution) was employed as an indication of positive fat hydrolysis by the molds. Tests for proteolysis and lipolysis were repeated four times.

RESULTS AND DISCUSSION

Eighteen strains of toxigenic and nontoxigenic *A. flavus* or *A. parasiticus* and one strain of *A. ochraceus* were evaluated for their proteolytic and lipolytic activities. Strains of *A. flavus* and *A. parasiticus* are grouped as toxigenic or nontoxigenic (Table 1) on the basis of reports by several investigators (2,3,6,8,9,10, 11,12). Proteolytic and lipolytic activity of these *Aspergillus* strains and of *A. ochraceus* are shown in Table 1. Strains of the aspergilli varied in their proteolytic and lipolytic activity. Strains of *A. flavus* exhibited considerable proteolytic and little lipolytic activity, whereas the opposite was observed with strains of *A. parasiticus*. *A. flavus* WB500, 4098 and NRRL 5565 had most proteolytic activity of all strains tested, whereas greatest lipolytic activity was associated with *A. parasiticus* NRRL 3145 and NRRL 465 and *A. ochraceus* NRRL 3174. Four of the toxigenic strains of *A. flavus* were neither proteolytic nor lipolytic, whereas this was not observed with the nontoxigenic strains.

Table 2 gives the proteolytic and lipolytic activity of 12 *Penicillium* species or strains, most of them were isolated from cheese and some of them were considered to be the normal fungus used for blue-cheese manufacture. *P. expansum* NRRL 2304 and *P. patulum* M59 can produce patulin, whereas it has been suggested that *P. puberulum* may produce penicillic acid, another mycotoxin. Some strains of *P. cyclopium* can produce patulin or penicillic acid; however, we did not test any of our cultures for their ability to produce these compounds. Two of the test cultures, *P. patulum* M59 and *P. cyclopium* 8, were

markedly lipolytic and proteolytic. Of the other cultures, greatest proteolytic activity was associated with *P. patulum* M59 and *P. cyclopium* 25, whereas greatest lipolytic activity was associated with *P. roqueforti* 849 and *P. puberulum* 33.

From our results it is evident that some toxigenic and nontoxigenic aspergilli and penicillia have considerable proteolytic and/or lipolytic activities. This observation suggests that some toxigenic molds, if present on cheese, could, in addition to producing a toxin, cause spoilage of cheese or even contribute to ripening of certain types of cheese.

It is, of course, also possible to extract proteolytic and/or lipolytic enzymes from nontoxigenic molds and use such enzymes to accelerate ripening of certain types of cheese. Further work is needed to determine if the enzymes produced by molds in this study are worthy candidates for use in ripening of cheese.

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## Depletion of Brilliant Blue F.C.F., Penicillin G and Dihydrostreptomycin in Milk from Treated Cows with Experimentally Induced Mastitis

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### ABSTRACT

A field trial was set up to compare depletion of Brilliant Blue F.C.F., penicillin and dihydrostreptomycin following three infusions of Streptopen cerate 100 to six cows with experimentally induced staphylococcal mastitis. The presence of dye was noted by milk color at the time of milking, and by the ion exchange resin method in the laboratory. Penicillin residues were determined by a diffusion agar plate microbiological method using *Sarcina lutea* and by Delvotest P. Dihydrostreptomycin residues were measured by the diffusion agar plate method (*Bacillus subtilis*). Dye extinction times were found to be significantly longer than penicillin and dihydrostreptomycin extinction times in both the infected sides and the healthy sides. No significant differences were observed between healthy and infected sides for dye, penicillin or dihydrostreptomycin extinction.

A search of the literature indicated that previous trials with mastitis preparations containing Brilliant Blue F.C.F. as a dye marker used healthy cows only (1). Although quite extensive studies have been conducted to determine the effect of mastitis on drug excretion, reports are contradictory. Jacobs (2) studied elimination of penicillin from normal and mastitic udders after intramammary treatment and concluded that the drug was excreted more slowly from healthy than infected quarters. The difference had little practical importance and therefore they recommended that milk-out studies use only healthy cows with normal udders. Mercer (5) studied the active involvement of secretory tissue in clinical mastitis on milk-out rates of penicillin and dihydrostreptomycin administered by intramammary infusion. Duration of excretion of penicillin was significantly increased in the infected quarters, while dihydrostreptomycin excretions were not significantly different between the infected and normal quarters.

In a previous study from our laboratory (8), we compared depletion of Brilliant Blue F.C.F. and penicillin following three consecutive infusions at 24-h intervals to six healthy lactating cows. In this paper, we report the results from a trial using cows with experimentally induced mastitis. Both right quarters of the cows were infected with penicillin-sensitive *Staphylococcus aureus* 4 days before treatment. All quarters were then infused with dye-marked mastitis cream containing penicillin G and dihydrostreptomycin. The limits of detection for the analytical methods, used in this

study were as follows: (a) ion exchange resin method - Brilliant Blue F.C.F. 0.02 mg/l, (b) penicillin cylinder plate method - 0.01 I.U./ml, (c) dihydrostreptomycin cylinder plate method - 0.1 µg/ml and (d) Delvotest P - penicillin 0.006 I.U./ml.

### MATERIALS AND METHODS

#### Animals

Six Holstein cows with normal udders, which had not received any antibiotic therapy in the preceding 3 weeks, were used. Pre-treatment clinical and bacteriological examinations and cell counts were carried out for each quarter.

#### Infection of animals

Both right quarters of the cows were infected with penicillin-sensitive *S. aureus* 305 4 days before treatment, and the left side was left uninfected as a control. The organism for infection was prepared as described by Newbould and Neave (6), using 3,800 colony-forming units (CFU) in 0.2 ml of sterile milk. Clinical mastitis was confirmed in each animal by bacteriological cultural examination and by somatic cell count before infusion with the mastitis cream.

#### Mastitis preparation

One-tenth gram of procaine penicillin G (100,000 I.U.), 0.1 g of dihydrostreptomycin as sulfate (100,000 I.U.) and 250 mg of Brilliant Blue F.C.F. (C.I. 42090) in a cerate base were used (Streptopen cerate 100, Glaxovet, Australia). With this product a dye concentration of 0.02 mg/l corresponds to 0.004 I.U. of penicillin per ml of milk.

#### Method of administration

All four quarters were treated by three consecutive infusions of Streptopen cerate 100 after the evening milking, commencing 62 h before the first collection of milk samples. Routine procedure for infusion has been described previously (8).

#### Sample collection

The cows were milked at approximately 7 A.M. and 5 P.M. (10- and 14-h intervals). A quarter milker was used daily during the trial period, beginning the morning after the last treatment of the cows with the mastitis preparation. The yield was measured for each quarter. Milk from both infected quarters was emptied into a pail, mixed well and a 500-ml sample taken. The procedure was repeated with milk from uninfected quarters. The milker and pails were washed before milking the next cow. After taking portions for the microbiological assays, the remainder of the samples was immediately refrigerated (4°C). The portions for microbiological assays were kept frozen until assay. Sample collection continued for 120 h after the last mastitis cream treatment.

#### Tracer dye assay

The ion exchange resin column method developed in our laboratory was used to estimate the concentration of dye in the samples (4).

*Microbiological assays for residues*

*Cylinder plate method:* Quantitative penicillin G and dihydrostreptomycin residue assays followed the method of the U.S. Food and Drug Administration (3). However, the dihydrostreptomycin assay was modified using only one seeded layer of agar (6.0 ml) to improve the sensitivity of the method.

*Delvotest P method:* The method developed by van Os and co-workers (7) was used for qualitative analysis.

*Cell count and bacteriological examination*

Pre-treatment cell counts and bacteriological examinations were done by the Microbiological Section staff at the Veterinary College, University of Guelph, Guelph, Ontario. These counts and examinations were also made daily during the sampling period.

**RESULTS AND DISCUSSION**

In our previous paper (8), we reported results from a field trial using dye-marked mastitis products in six healthy cows. Dye extinction times were significantly longer than penicillin extinction times at the sensitivities used. The present trial was set up to determine if milk-out times of dye and antibiotics were the same when infection is present.

Before infection, the cell counts were normal for all quarters of the cows except the right hind quarter of cow no. 32, where the count was high. Bacteriological examination showed all quarters of the six cows were free of infection except for the right hind quarter of cow no. 32. The presence of nonagalactiae streptococci, indicating a latent infection, was confirmed for this quarter. However, since no replacement animal was available, cow no. 32 was retained for the experiment.

The cows were infected in the right side of the udder 4 days before commencement of three infusions of dye-marked mastitis preparations in all quarters at 24-h intervals. After inducing the infection, there was a sharp increase in somatic cell counts in milk from the infected quarters, and staphylococci were recovered from this milk but the milk production remained about the same.

Penicillin residues (cylinder plate method) were detected on the average for 48 h after the last infusion (Table 1). In four of six cases, penicillin could not be detected in milk from the infected side one milking sooner than in milk from the uninfected side. In the remaining two cases, penicillin was detected for an equal number of milkings from both sides. Essentially the same results were obtained by the Delvotest P method.

Dihydrostreptomycin residues were also detected, on the average, for 48 h after the last infusion (Table 2). However, in three of six cases, the uninfected side milked out one or more milkings sooner than the infected side, and in one case the infected side milked out one milking sooner than the uninfected side. Cow no. 32 retained the drug for at least 120 h on the infected side and 62 h on the uninfected side.

The amount of dye in this preparation was double that of all previous preparations used in our laboratory (8), since there were two antibiotics present. The color of the milk was much darker blue for the early milkings (Table 3), but was visible for 38 h on the average, which is similar to the previous trial (8). Cow no. 32 had very low production on the infected side (Table 4), and as a result the dye was visible for 72 h. On the whole, of six cows, the color was visually detected in milk from two cows for an equal length of time on both sides, the color in milk from two cows was detected longer on the uninfected side, and in two cows the color was detected longer on the infected side (Table 3).

The column resin method detected dye for an average of 72 h after the last infusion (Table 5). Four cows milked out the dye on both sides at the same time and two cows required one more milking for the infected side. Detection of dye for a longer time than in the previous trial (8) may have been due to the larger amount (double) present in the preparation. The theoretical penicillin concentration at the limit of detection of the dye, when using the resin method (0.02 mg/l), is

TABLE 1. *Levels of penicillin residues detected in milk.*

Cow No.	Quarters	Time of milking after the last infusion (hours)															
		+ 14		+ 24		+ 38		+ 48		+ 62		+ 72		+ 86		+ 96	
		C.P. <sup>a</sup>	D.P. <sup>b</sup>	C.P.	D.P.	C.P.	D.P.	C.P.	D.P.	C.P.	D.P.	C.P.	D.P.	C.P.	D.P.	C.P.	D.P.
25	R.S. <sup>c</sup>	6.6	ND <sup>d</sup>	0.4	ND	trace	+	neg	-	neg	-	neg	-	neg	-	neg	-
	L.S.	10.4	ND	0.9	ND	0.04	+	neg	+/-	neg	-	neg	-	neg	-	neg	-
32	R.S.	38.4	ND	2.8	ND	0.20	+	0.02	+	neg	+/-	neg	+/-	neg	+/-	neg	-
	L.S.	15.2	ND	1.9	ND	0.10	+	0.03	+	0.02	+/-	neg	-	neg	-	neg	-
65	R.S.	6.8	ND	1.0	ND	0.05	+	0.02	+	neg	-	neg	-	neg	-	neg	-
	L.S.	8.3	ND	0.9	ND	0.10	+	0.02	+	neg	+/-	neg	-	neg	-	neg	-
71	R.S.	4.5	ND	0.3	ND	neg	+	neg	+/-	neg	-	neg	-	neg	-	neg	-
	L.S.	7.8	ND	0.7	ND	0.04	+	neg	+	neg	-	neg	-	neg	-	neg	-
805	R.S.	12.2	ND	2.1	ND	0.09	+	0.02	+	neg	-	neg	-	neg	-	neg	-
	L.S.	11.6	ND	1.3	ND	0.10	+	0.02	+	neg	+/-	neg	-	neg	-	neg	-
4566	R.S.	4.6	ND	0.5	ND	0.03	+	neg	+	neg	-	neg	-	neg	-	neg	-
	L.S.	4.5	ND	1.0	ND	0.05	+	0.01	+	neg	-	neg	-	neg	-	neg	-

<sup>a</sup>C.P. - Cylinder plate ( $\mu\text{g/ml}$ ).

<sup>b</sup>D.P. - Delvotest P.

<sup>c</sup>R.S. - right side (infected). L.S. - left side (uninfected).

<sup>d</sup>ND - Not done.

0.004 I.U./ml. This is below the sensitivity level of both the cylinder plate and Delvotest P methods.

There was no definite pattern indicating that infected quarters retain drugs or dye for a longer time than healthy quarters (Fig. 1). Cow no. 32 did retain dihydrostreptomycin longer, but was milked with difficulty because of the secondary infection (*nonagalactiae streptococcus*). The yield on the infected side was so low (Table 4) that it would be more comparable to that of a dry cow than a lactating one. Because of the interference of the streptococcal infection (not an

experimentally induced infection) the results from this animal were not included in statistical analysis. The observed number of milkings for dye, penicillin and dihydrostreptomycin extinction are given in Table 6. Dye extinction times were significantly longer than penicillin and dihydrostreptomycin extinction times in both the infected sides and the healthy sides (individual one-tailed randomization tests at the 0.05 level of significance). The estimated proportion of cows requiring more milkings for penicillin extinction than for dye extinction was 0.0 with upper 95% confidence limit of 0.45. No significant

TABLE 2. Levels of dihydrostreptomycin residues ( $\mu\text{g/ml}$ ) detected in milk.

Cow No.	Quarters	Time of milking after the last infusion (hours)									
		+ 14	+ 24	+ 38	+ 48	+ 62	+ 72	+ 86	+ 96	+ 110	+ 120
25	R.S. <sup>a</sup>	14.5	2.5	0.4	neg	neg	neg	neg	neg		
	L.S.	27.0	3.3	0.4	neg	neg	neg	neg	neg		
32	R.S.	66.0	8.0	1.0	0.4	0.3	0.3	0.15	trace	trace	0.14
	L.S.	33.0	4.2	1.1	0.5	0.1	neg	neg	neg		
65	R.S.	12.4	3.9	0.5	0.2	neg	neg	neg	neg		
	L.S.	21.5	3.0	1.1	0.5	0.1	neg	neg	neg		
71	R.S.	11.4	1.6	0.2	0.2	neg	neg	neg	neg		
	L.S.	13.2	2.3	0.4	neg	neg	neg	neg	neg		
805	R.S.	22.5	5.7	1.2	0.5	0.1	neg	neg	neg		
	L.S.	20.5	4.3	1.1	0.4	neg	neg	neg	neg		
4566	R.S.	10.2	2.3	0.5	0.2	neg	neg	neg	neg		
	L.S.	10.6	3.7	0.7	0.2	neg	neg	neg	neg		

<sup>a</sup>R.S. - right side (infected), L.S. - left side (uninfected).

TABLE 3. Visual detection of color in milk at the time of milking.

Cow No.	Quarters	Time of milking after the last infusion (hours)						
		+ 14	+ 24	+ 38	+ 48	+ 62	+ 72	+ 86
25	R.S. <sup>a</sup>	blue	blue	lt. blue	—	—	—	—
	L.S.	blue	blue	lt. blue	—	—	—	—
32	R.S.	blue	blue	blue	lt. blue	lt. blue	lt. blue	—
	L.S.	blue	blue	blue	lt. blue	—	—	—
65	R.S.	blue	blue	lt. blue	—	—	—	—
	L.S.	blue	blue	blue	lt. blue	—	—	—
71	R.S.	blue	blue	lt. blue	—	—	—	—
	L.S.	blue	blue	lt. blue	—	—	—	—
805	R.S.	blue	blue	lt. blue	lt. blue	—	—	—
	L.S.	blue	blue	lt. blue	—	—	—	—
4566	R.S.	blue	blue	lt. blue	—	—	—	—
	L.S.	blue	blue	lt. blue	lt. blue	—	—	—

<sup>a</sup>R.S. - right side (infected), L.S. - left side (uninfected).

TABLE 4. Daily milk yield (kg).

Cow No.	March 13/78 <sup>a</sup>		Mar. 14		Mar. 15		Mar. 16		Mar. 17		Mean	
	R.S. <sup>b</sup>	L.S.	R.S.	L.S.	R.S.	L.S.	R.S.	L.S.	R.S.	L.S.	R.S.	L.S.
25	9.34	8.89	8.44	9.35	8.75	9.39	8.57	9.15	8.94	8.89	8.81	9.13
32	2.09	7.58	1.18	6.76	2.13	7.12	1.63	6.94	1.73	7.12	1.75	7.10
65	9.84	10.39	10.29	10.30	10.21	9.98	9.98	10.53	9.30	9.52	9.91	10.14
71	12.93	13.29	11.66	12.43	12.47	12.88	11.79	12.02	12.20	12.34	12.20	12.59
805	5.06	9.66	5.35	8.58	5.31	8.52	5.67	9.16	5.99	8.80	5.48	8.94
4566	16.51	14.56	14.70	12.70	16.06	13.84	15.01	12.83	14.38	12.61	15.33	13.31

<sup>a</sup>March 13, 1978 -- first day of milk sample collection.

<sup>b</sup>R.S. - right side (infected), L.S. - left side (uninfected).



differences were observed between healthy and infected sides for dye, penicillin or dihydrostreptomycin extinction. However, because only a small number of cows was used in this study, this does not necessarily preclude the need to use mastitic cows in further experiments. Also, more acute cases of mastitis were reported by Mercer (5) to result in increased duration of penicillin excretion due to tissue involvement. How the active involvement of secretory tissue would affect dye excretion is not known. An experimentally induced mastitis should be considered as a possible model for such studies.

ACKNOWLEDGMENTS

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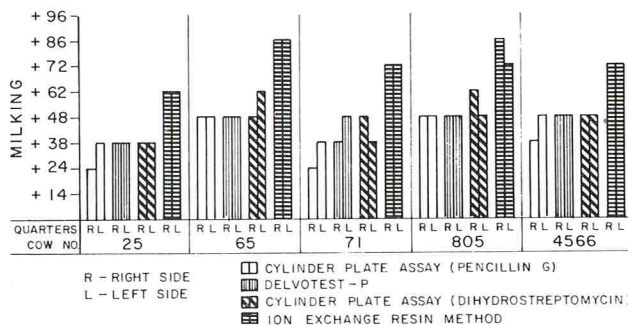


Figure 1. Excretion patterns for penicillin G, dihydrostreptomycin and dye marker.

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TABLE 5. Excretion of brilliant blue F.C.F. (µg/ml) in milk.

Cow No.	Quarters	Time of milking after the last infusion (hours)									
		+14	+24	+38	+48	+62	+72	+86	+96	+110	+120
25	R.S. <sup>a</sup>	240	24	0.8	0.1	0.03	—	—	—	—	—
	L.S.	320	24	1.6	0.2	0.03	—	—	—	—	
32	R.S.	960	96	4.8	1.6	0.96	0.48	0.12	0.06	—	—
	L.S.	480	32	4.8	1.2	0.24	0.08	0.03	—	—	
65	R.S.	240	24	2.4	0.4	0.08	0.04	0.02	—	—	
	L.S.	240	24	4.8	0.6	0.16	0.06	0.02	—	—	
71	R.S.	240	16	0.8	0.2	0.12	0.04	—	—	—	
	L.S.	480	24	1.6	0.2	0.04	0.02	—	—	—	
805	R.S.	320	48	4.8	2.4	0.32	0.24	0.04	—	—	
	L.S.	160	24	4.8	0.6	0.16	0.06	—	—	—	
4566	R.S.	160	24	1.2	0.3	0.06	0.03	—	—	—	
	L.S.	160	48	2.4	0.6	0.16	0.04	—	—	—	

<sup>a</sup>R.S. - right side (infected), L.S.- left side (uninfected).

TABLE 6. Number of milkings for extinction of dye, penicillin and dihydrostreptomycin.

Cow No.	Quarter	Dye	Penicillin	Dihydrostreptomycin	Differences		
					Dye-Penicillin	Dye-Dihydrostreptomycin	
25	R.S. <sup>a</sup>	6	4	4	2	2	
	L.S.	6	4	4	2	2	
65	R.S.	8	5	5	3	3	
	L.S.	8	5	6	3	2	
71	R.S.	7	4	5	3	2	
	L.S.	7	5	4	2	3	
805	R.S.	8	5	6	3	2	
	L.S.	7	5	5	2	2	
4566	R.S.	7	5	5	2	2	
	L.S.	7	5	5	2	2	
					Average R.S.	2.6	2.2
					Average L.S.	2.2	2.2

<sup>a</sup>R.S. - right side (infected), L.S. - left side (uninfected).

## Isolation of Toxigenic *Yersinia enterocolitica* from Retail Pork Products

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### ABSTRACT

Five of 69 (7%) processed and 63 of 128 (49%) raw retail pork products contained *Yersinia enterocolitica*. Thirty-two of 80 (40%) isolates were serotypable, with serotypes 0:3 and 0:5 occurring most frequently. All except one isolate of serotype 0:3 came from fresh pork tongues. Twenty-five isolates (32%) produced a heat-stable enterotoxin detected by the infant mouse and rabbit ileal loop assays. Live cultures in the rabbit ileal loop produced no response. Most of the serotype 0:3 (92%) and 0:5 (90%) isolates were mouse-positive, while most rhamnase-positive (89%) and citrate-positive (96%) isolates were mouse-negative.

When *Yersinia enterocolitica* is isolated from humans in many countries, including Canada (27, S. Tomá, Canadian National Reference Service for *Yersinia*, personal communication) (Table 1), Europe (1,16,25), Japan (33) and Israel (24), it is commonly serotype 0:3. In the United States, however, serotype 0:8 occurs more often (3,32). Swine have been identified as reservoirs of *Y. enterocolitica* serotype 0:3 (12,21,26,28). Large outbreaks of yersiniosis have occurred with serotype 0:3, but no common vehicle of transmission was ever identified (2,18,34). Although yersiniosis has often been suggested to be a foodborne disease, the only documented foodborne outbreak involved serotype 0:8 transmitted through chocolate milk (4). The transmission link between swine reservoir and human infection with serotype 0:3 remains unclear.

Pai and Mors (19) first reported formation of a heat-stable enterotoxin by *Y. enterocolitica* which was detectable in culture filtrates by the infant mouse assay

(10) and the rabbit ileal loop (9). Pai et al. (20) subsequently examined a larger number of strains of *Y. enterocolitica* from both human and environmental sources for enterotoxigenicity.

The study reported here was undertaken to determine the incidence of *Y. enterocolitica* in retail pork products, representing potential vehicles for transmission, particularly of the common human serotype 0:3. Enterotoxigenicity was evaluated in selected pork isolates by the infant mouse assay and the rabbit ileal loop technique.

### MATERIALS AND METHODS

#### Samples of pork products

All pork products were purchased from retail outlets located in western suburbs of Toronto. Samples were held under refrigeration and prepared for testing within 2 days after purchase.

#### Isolation methods

Twenty-five-gram portions of each product were examined for *Y. enterocolitica* by two methods, using 225 ml of broth for enrichment (1:10 dilution): (a) Modified Rappaport broth (15,30) incubated at room temperature ( $22 \pm 1$  C) for 7 days; and (b) M/16 phosphate buffered saline solution pH 7.6 incubated at 4 C for 21 days followed by transfer of 1.0 ml to 10 ml of modified Rappaport broth (MRB) which was incubated at room temperature (RT) for 2 days. Enrichments were streaked after incubation onto MacConkey agar which was incubated at RT for 2 days. Suspect colonies were fished to Kligler iron agar, and, if typical at 35 C (K/A<sup>-</sup>), tested on urea agar at 35 C. Urea-positive isolates were confirmed and characterized by a 30-reaction biochemical profile, and then forwarded to the Canadian National Reference Service for *Yersinia* for serotyping.

#### Infant mouse assay

Stock cultures of *Y. enterocolitica* were examined for production of enterotoxin by streaking a plate of trypticase soy agar containing 0.6% yeast extract, pH 7.6 (TSYE) and incubating overnight at 32 C. At least 10 colonies were used to prepare a suspension in phosphate buffered saline (PBS) solution, which was further diluted to provide a final inoculum of  $10^2$ - $10^3$  cells per ml in 25 ml of TSYE broth. The inoculated broth was incubated at 22 C for 48 h on a shaker (Psychrotherm Model G-26, New Brunswick Scientific) providing 240 rpm. Cultures were centrifuged at 10,000 rpm for 30 min and the supernatant fluid was passed through a 0.45- $\mu$ m membrane filter and then frozen at -20 C until testing.

Two additional broth media were evaluated for production of enterotoxin, brain heart infusion broth, pH 7.4 (BHI) and casamino acids-yeast extract broth pH 8.5 (CAYE) (13,19). Inoculations and recovery of culture filtrates were identical to those used with TSYE broth filtrates.

The infant mouse assay (10) was used to determine the presence of enterotoxin in culture filtrates. All initial tests were made with unheated filtrates. Infant mice 2-4 days old were injected with 0.1 ml of filtrate containing 2 drops of 2% Evans blue per ml. The material was

TABLE 1. Human isolates of *Yersinia enterocolitica* in Canada 1966 - August 1978<sup>a</sup>.

Serotype 0:	Isolations	
	Number	Percent
3	1128	76.0
5,27	69	4.6
6,30	51	3.4
8	51	3.4
5	27	1.8
4,32	13	0.9
Others <sup>b</sup>	72	4.8
Nontypable	74	5.0
Total	1485	99.9

<sup>a</sup>From S. Tomá, Canadian National Reference Service for *Yersinia*, Toronto.

<sup>b</sup>6,31 (11 ×); 7,8 (10 ×); 7,13 (10 ×); 16 (7 ×); 21 (7 ×); 34 (7 ×); 9 (4 ×); 16,29 (3 ×); 4,33 (2 ×); 8,19 (2 ×); 1 (1 ×); 2 (1 ×); 1,2,3 (1 ×); 11 (1 ×); 11,24 (1 ×); 12,25 (1 ×); 12,25 (1 ×); 15 (1 ×); 17 (1 ×); 20 (1 ×).

introduced into the stomach through the abdominal wall. Mice from at least two or more different litters were mixed for testing. At least three individuals and usually four were combined for each test. The injected mice were held 4 h at 22 C and then sacrificed with chloroform. The intestines were removed and consolidated for weighing as were the remaining carcasses. The test results were expressed as the ratio of combined intestinal weight to the combined body weight. A ratio of  $\geq 0.083$  was taken as a positive test for enterotoxin (22).

#### Rabbit ileal loop technique

Culture filtrates prepared for the infant mouse assay were used for examination in the rabbit ileal loop (9), using New Zealand white rabbits with an average weight of 1.6 kg (range 1.3 to 2.0 kg). The intestine was washed with 10 ml of 0.1 M PBS pH 7.4 and tied off into alternating 10- and 5-cm segments. Ten-cm loops were used for injection of 2 ml of culture filtrate, and 5-cm loops for injection of 1.0 ml of sterile broth. No test loop was accepted as positive if the fluid accumulation was not at least  $3\times$  greater than the adjoining broth control loop. Animals were held for 6 h and then sacrificed by an overdose of the anaesthetic, sodium pentobarbital. The lengths of excised loops were measured and the fluid accumulation was determined by change in weight when full and empty and by the volume collected in a graduated cylinder. Final results were expressed as the ratio of accumulated fluid to the length of the intestinal loop in cm. The agreement between ratios obtained by volume and by weight was very close, therefore only results based on weight differences, which have the higher precision, are reported herein.

Live cultures for testing in the rabbit ileal loop were prepared in TSYE broth incubated in roll tubes at 22 C for 48 h. Cells were recovered by centrifugation and resuspended in either PBS or TSYE broth for injection. These animals were held for 20-22 h before sacrificing and removal of intestinal loops.

## RESULTS

### Isolation from pork products

*Y. enterocolitica* occurred more frequently in raw than in processed pork products (Table 2), as might be

TABLE 2. Incidence of *Yersinia enterocolitica* in retail pork products.

Samples	Processed			Raw		
	R <sup>a</sup>	PR	Total	R	PR	Total
No. samples examined	69	69	69	128	128	128
No. samples positive	0	5	5	54	16	63
Percent samples positive	0	7.2	7.2	42.2	12.5	49.2

<sup>a</sup>R = modified Rappaport broth at 22 C for 7 days; PR = PBS pH 7.6 at 4 C for 21 days followed by R at 22 C for 2 days.

TABLE 4. Incidence and serotypes of *Yersinia enterocolitica* in raw pork products.

Type of product	No. of samples		Percent positive	No. of serotype 0:							
	Examined	Positive		3	5	8	16	17	21	13,7	NT
Chops & cutlets	20	7	35		2					1	5
Ear	2	1	50	1							
Fat	1	1	100								1
Feet	5	2	40								2
Ground pork	15	9	60		2	1				1	8
Heart	2	1	50								1
Hocks	5	2	40								2
Kidney	8	2	25		1						1
Liver	6	0	0								
Loin	1	1	100								1
Neck bones	4	2	50						1		2
Pieces	4	2	50		1						2
Ribs	8	6	75		4		1				3
Shoulder	1	0	0								
Side pork	3	2	67		1						1
Snout	2	0	0								
Tail	3	1	33								1
Tenderloin	1	0	0								
Tongue	37	24	65	11				1			16
Totals	128	63	49	12	11	1	1	1	1	2	46

expected. Furthermore, two of the positive processed products, fresh bratwurst, represent uncooked meat (Table 3). Use of MRB directly without prior cold enrichment was more productive for isolation from raw than from processed products. The better performance of MRB without cold enrichment was in contradiction with results previously obtained for raw milk (23).

TABLE 3. Processed pork products yielding *Yersinia enterocolitica*.

Type of product	No. positive samples	Serotypes isolated
Bratwurst, fresh	2	0:17, NT <sup>a</sup>
Frankfurters	1	NT
Head cheese	1	0:13,7
Luncheon meat	1	0:17

<sup>a</sup>NT = nontypable.

Most of the isolates (58%) were not typeable. The typeable isolates represented seven different serotypes with 0:3 occurring most frequently ( $12\times$ ) followed by 0:5 ( $11\times$ ). Eleven (44%) of the positive isolates were recovered from fresh pork tongue, while the 0:5 isolates were distributed among a number of different raw pork products (Table 4).

### Infant mouse assay

Seventy-nine isolates were examined for production of enterotoxin by the infant mouse assay and 25 (32%) were found positive (i.e. intestine: body weight ratio  $\geq 0.083$ ) (Fig. 1). Eleven (44%) of the positive isolates were serotype 0:3 and 9 (36%) were 0:5 (Table 5), making up 80% of the mouse-positive isolates. Four of the remaining five positive isolates were either 0:13,7 or 0:16. One of the

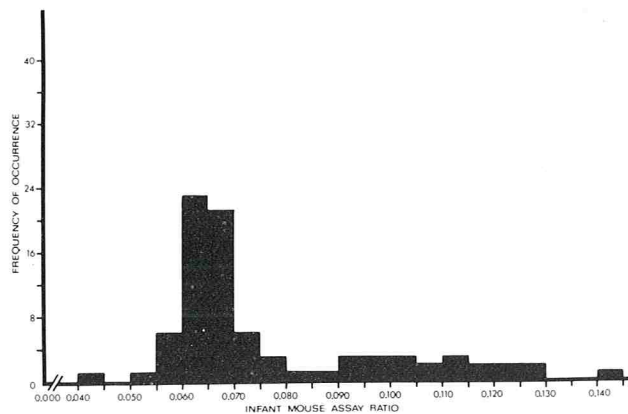


Figure 1. Distribution of infant mouse assay ratios on TSYE broth filtrates for 80 isolates of *Yersinia enterocolitica* obtained from pork products.

TABLE 5. Correlation between enterotoxin production and serotype of *Yersinia enterocolitica*.

Serotype 0:	Number tested	Infant mouse positive	
		Number	Percent
3	12	11	92
5	10	9	90
8	1	0	0
13,7	3	2	67
16	2	2	100
17	3	0	0
21	1	0	0
Nontypeable	47	1	2
Total	79	25	32

positive 0:13,7 isolates was obtained from head cheese, a cooked ready-to-eat meat product. Only one of the 47 nontypeable isolates was positive for enterotoxin, and only

TABLE 7. Stability of *Yersinia enterocolitica* enterotoxin<sup>a</sup> during frozen storage and to heat.

Culture no.	1st test result <sup>b</sup>	Storage time at -20 C (days)	2nd test result <sup>b</sup>		
			No treatment	80 C 30 min	100 C 15 min
E119	0.101	28	0.130	0.124	0.127
E123	0.091	20	0.098	0.083	0.080
E162	0.101	20	0.090	0.098	0.092
E163	0.090	21	0.079	0.073	0.091
E201	0.126	21	0.130	0.122	0.143
E215	0.119	49	0.091	0.073	0.108
E228	0.112	41	0.114	0.124	0.123
E285	0.087	49	0.114	0.113	0.122
E202	0.129	21	0.124	0.125	0.115
E222	0.099	48	0.109	0.099	0.073
E224	0.093	37	0.096	0.091	0.073
E226	0.116	37	0.110	0.095	0.086
E235	0.099	36	0.107	0.091	0.096
E239	0.101	37	0.083	0.081	0.081
E240	0.107	37	0.093	0.094	0.083
E253	0.140	36	0.104	0.096	0.109
E254	0.090	42	0.083	0.084	0.079
E256 <sup>c</sup>	0.101	42	0.102	0.090	0.073
E256 <sup>c</sup>	0.106	20	0.090	0.073	0.079
E258 <sup>c</sup>	0.076	42	0.096	0.094	0.099
E258 <sup>c</sup>	0.092	20	0.096	0.088	0.090
E260	0.111	42	0.103	0.119	0.134
E270	0.120	21	0.119	0.118	0.121
E292	0.121	29	0.120	0.119	0.115
$\bar{X}$	0.105	33	0.103	0.099	0.100

<sup>a</sup>Produced in trypticase soy broth with 0.6% yeast extract pH 7.6 incubated at 22 C for 48 h.

<sup>b</sup>Ratio for infant mouse assay.

<sup>c</sup>Culture filtrates prepared on different days.

TABLE 6. Enterotoxin production by selected isolates of *Yersinia enterocolitica* obtained from raw milk.

Serotype 0:	Number tested	Infant mouse positive	
		Number	Percent
5	7	6	86
Nontypeable	10	7	70

one of the 0:3 and 0:5 isolates was negative. These results were verified by retesting both the serotype and toxigenicity with new broth filtrates.

The association between enterotoxin production and serotype 0:5 and the absence of enterotoxin in nontypeable strains was compared with cultures previously isolated from raw milk (23). The first association was reproducible; however, 7 of 10 nontypeable isolates from raw milk were positive for enterotoxin (Table 6), which disagreed with the results for pork isolates.

*Enterotoxin stability*

The stability of the enterotoxin during frozen storage and to heat was examined with 24 isolates found positive on the first test (Table 7). All culture filtrates for these isolates except one showed a ratio in the infant mouse assay of  $\geq 0.083$  on retesting of the frozen (-20 C) filtrate after a storage time averaging 33 days. Only four filtrates gave a ratio of  $< 0.083$  after heating at 80 C for 30 min, and six after boiling for 15 min. The average ratios were comparable and the few lower results likely derive only from the variability in the test procedure rather than destruction of enterotoxin.

*Enterotoxigenicity and biotype*

*Y. enterocolitica* is a bacterial species with a high

degree of biochemical variability. Four different biotyping schemes have been proposed, three of which are presented in Table 8. Certain subtypes designated by letters have been inserted by the author to describe biochemical varieties observed among the pork isolates which do not exactly fit the biotype as originally proposed.

The demonstration of any correlation between enterotoxin production by the pork isolates and biotype of *Y. enterocolitica* is weakened by the limited number of isolates obtained, especially of certain biotypes. Nevertheless, there are a few correlations which are strong enough to recognize (Table 9). The first is in lecithinase-negative organisms which otherwise resemble Wauters biotype 1 in being indole- and xylose-positive. All of the 26 isolates of this type (1A) were negative for enterotoxin. Stronger correlations exist with Brenner's biotypes, which are based on DNA relatedness groups (7). Fourteen of 15 biotype 1 (rhamnose-negative) isolates were enterotoxin-positive, and all of 44 biotype 2 (rhamnose-positive) isolates were enterotoxin-negative. Types 1A and 1B represent rhamnose-negative isolates found enterotoxin-negative, while types 2A, 2B, 3 and 3A represent rhamnose-positive isolates which include some enterotoxin producers (6/11 = 55%).

The correlation of enterotoxin production and inability to ferment rhamnose for all isolates was high (76%), but 11% of the rhamnose-positive isolates produced enterotoxin. A much better correlation was observed with citrate, where only two of 53 (4%) citrate-positive isolates were enterotoxin-positive, and both of these isolates were unusual in being sucrose-negative. However, not all citrate-negative isolates produced enterotoxin (16/21 = 76% positive).

*Media for enterotoxin production*

Three different media were evaluated for production of enterotoxin with 12 isolates of *Y. enterocolitica* by titration of the culture filtrates, using sterile broth for dilution. Twelve of the filtrates were diluted to 1:4 (25%) and nine to 1:8 (12.5%) and then tested in duplicate in the infant mouse. The averages of the duplicate tests for each medium are shown in Fig. 2. The bottom line in the figure represents the average of four tests on each of the

TABLE 9. Correlation between enterotoxin production and biotype of *Yersinia enterocolitica*.

Biotype	Number tested	Enterotoxin positive <sup>a</sup>	
		Number	Percent
<b>Wauters:</b>			
1	38	12	32
1A	26	0	0
3	3	2	67
4	9	8	89
<b>Nilehn:</b>			
1	63	12	19
3A	2	2	100
3B	1	0	0
4	10	8	80
<b>Brenner:</b>			
1	15	14	93
1A	1	0	0
1B	3	0	0
2	44	0	0
2A	2	1	50
2B	3	2	67
3	5	2	40
3A	1	1	100
4	2	2	100
<b>Rhamnose:</b>			
Positive	55	6	11
Negative	21	16	76
<b>Citrate:</b>			
Positive	53	2 <sup>b</sup>	4
Negative	21	16	76

<sup>a</sup>By infant mouse assay.

<sup>b</sup>Both strains sucrose negative.

three sterile media, representing a negative control. The middle line at 0.083 represents the criterion accepted for a positive test, and the upper line at 0.090 represents a strong positive test (10).

The results in Fig. 2 indicate that there is great variability among isolates in the amount of enterotoxin produced, with some filtrates still showing positive reactions at a 1:8 dilution. Although there are no great differences in enterotoxin levels among the three media, CAYE broth seemed the less productive. There are no apparent differences in the amounts of enterotoxin produced in TYSE and BHI broths. Enterotoxin production by a few strains was too weak to allow pooling of culture filtrates for testing, as has been suggested for *Escherichia coli* to conserve materials (8).

TABLE 8. *Biotypes of Yersinia enterocolitica*.

Reaction <sup>b</sup>	Nilehn (17)					Wauters (29)					Brenner (14)													
	1	2	3	3A <sup>a</sup>	3B <sup>a</sup>	4	5	1	1A <sup>a</sup>	2	3	3A <sup>a</sup>	4	5	1	1A <sup>a</sup>	1B <sup>a</sup>	2	2A <sup>a</sup>	2B <sup>a</sup>	3	3A <sup>a</sup>	4	
Indole	+	+	-	-	-	-	-	+	+	(+) <sup>c</sup>	-	-	-	-	-	-	-	+	+	+	+	+	+	-
Xylose	+	+	+	+	+	-	-	+	+		+	+	-	-	-	-	-	-	-	-	-	-	-	-
Salicin	+	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Esculin	+	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Lactose	+	+	+	-	-	-	-	+	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-
Sucrose	+	+	+	-	+	+	-	-	-	-	-	+	-	-	+	+	+	+	+	+	+	+	+	-
Lecithinase	-	-	-	-	-	-	-	+	-	-	-	+	-	-	-	-	-	+	+	+	+	+	+	-
Rhamnose	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	-	-	-	-
Raffinose	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	-	-	-	-
Melibiose	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	-	-	-	-
Alpha-methyl-glucoside	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	-	-	-	-

<sup>a</sup>Varieties not recognized by originator of this biotyping scheme.

<sup>b</sup>Not all reactions for Nilehn and Wauters schemes included.

<sup>c</sup>( ) = delayed after 48 h.

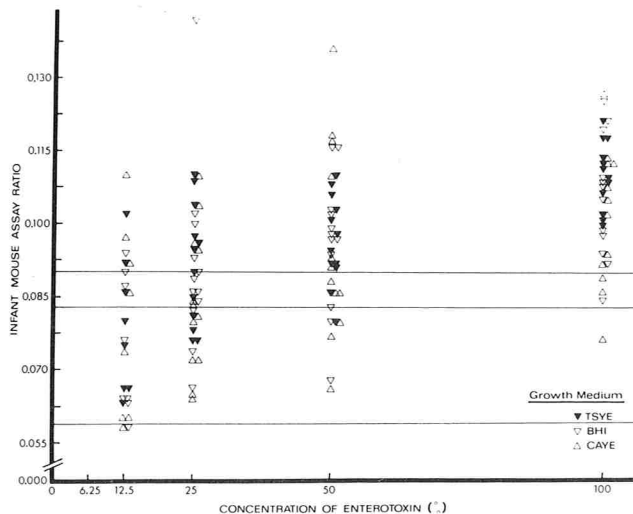


Figure 2. Titration of enterotoxin produced by twelve strains of *Yersinia enterocolitica* in three different media. Each point represents the mean of duplicate infant mouse assays.

### Rabbit ileal loop

Results from the rabbit ileal loop assay for four isolates positive in the infant mouse confirmed the presence of an enterotoxin in the culture broth filtrate (Table 10). The response to this enterotoxin in the rabbit was often erratic and the limited fluid accumulation is similar to that observed with the heat-stable toxin produced by *E. coli* (14).

Five live cultures were examined in the rabbit ileal loop, including one mouse-positive pork isolate of serotypes 0:13,7, 0:5, and 0:3, and one mouse-negative pork isolate which was nontypable. The fifth culture was a human mouse-positive isolate of serotype 0:3. Cells suspended in PBS were tested in four different animals, and cells suspended in TSYE broth in one animal, with completely negative responses.

TABLE 10. Rabbit ileal loop response to *Yersinia enterocolitica* enterotoxin.

Isolate serotype 0:	No. of test animals	Mean fluid accumulation (g/cm)		
		TSYE	BHI	CAYE
13,7	5	0.580	0.556	0.468
16	3	0.573	0.693	0.310
3	3	0.465	0.483	0.590
Nontypable	2	0.635	0.375	0.450
Medium control	10	0.054	0.043	0.053

### DISCUSSION

Any description of the distribution of a microorganism is only relative to the methodology, and the methodology presently available for recovery of *Y. enterocolitica* from foods is not reliable for all types of this organism. Serotype 0:8, for example, is very difficult to recover from foods, and only one isolate of this type was obtained during this survey. Nevertheless, the isolation methodology selected for this study is very reliable for recovery of serotype 0:3 (30). This methodology was chosen because the primary purpose of this study was to determine

whether retail pork products were an important link between swine, the only recognized reservoir of serotype 0:3, and humans, where it represents the most common type isolated.

This survey showed that retail pork products contain types of *Y. enterocolitica* which are identical with those associated with human disease. These types are accompanied by many others which are infrequently isolated from humans and probably are of little or no significance in human disease. The restriction of the most common human serotype 0:3 to pork tongues (with the exception of one isolation from an ear) is puzzling since it would seem that the organism could easily be disseminated during slaughter and processing from this apparent niche to other pork products. Wauters (31) also reported a high incidence of serotype 0:3 on market pork tongues in Belgium. Pork tongues do not seem to present a reasonable link between the reservoir of *Y. enterocolitica* serotype 0:3 and humans. (Just as this survey was completed, our laboratory isolated serotype 0:3 from raw pork sausage links implicated in a single case of gastroenteritis. We were unable, however, to isolate the organism from the patient's feces or find an antibody titer to 0:3.)

The role of heat-stable enterotoxin in the pathogenicity of *Y. enterocolitica* is not clear. Pai et al. (20) point out that because this enterotoxin is produced in vitro only at temperatures below 30 C, its role in human gastroenteritis is even more questionable. The inability to obtain enterotoxin in vitro at higher temperatures could, however, be related to growth factors or conditions not yet identified. We could not produce a response in the rabbit ileal loop with live cells of mouse-positive *Y. enterocolitica* strains, even when they were suspended in a good growth medium (i.e. TSYE broth), suggesting that the toxin is not produced in vivo. The enterotoxin produced by *Y. enterocolitica* resembles the heat-stable toxin produced by some strains of enteropathogenic *E. coli*; however, the toxin of *E. coli* is produced in identical media at body temperature and in vivo.

On the other hand, the correlation between enterotoxin production by *Y. enterocolitica* and serotypes and biotypes which are common in human disease, and the fact that most human isolates are mouse-positive and environmental isolates are negative (20), is too strong to completely dismiss the significance of in vitro enterotoxin production. For example, only one of 12 isolates from pork products of serotype 0:3 was negative for heat-stable enterotoxin. Pai and Mors (19) reported that all human isolates of 0:3 were enterotoxigenic, and confirmed this correlation with a larger number of strains (130 positive/131 tested) in a later report (20). At the same time, only one of 47 (2%) nontypable isolates from pork products was enterotoxigenic. Nontypable strains of *Y. enterocolitica* are infrequently associated with human disease (Table 1). The infrequent production of enterotoxin by rhamnose-positive and citrate-positive isolates also correlates with the lower incidence of these

biotypes in human disease. The more common human serotypes 0:3, 0:8 and 0:9 are always rhamnose-negative. We found that 11% of the rhamnose-positive isolates from pork produced enterotoxin, which correlates well with the 10% rate observed by Pai et al. with a larger number of strains (20). The only two citrate-positive enterotoxigenic isolates from pork were sucrose-negative, an unusual type of *Y. enterocolitica* which Bottone (5) has isolated from non-gastroenteric human infections.

It is apparent that our food and water supplies frequently contain *Y. enterocolitica*, and that mere isolation of an organism identifiable as *Y. enterocolitica* is not sufficient basis for condemning nor implicating any vehicle in an outbreak of gastroenteritis without accompanying human culture or serological evidence (11). The ubiquitous distribution of organisms which are biochemically variable but accepted as the same species further complicates a judgement regarding the significance of isolation from the environment. A clear separation of pathogenic and non-pathogenic types on the basis of serotype or biotype is also complicated by the occurrence of a wide variety of types in human infections, albeit many (e.g. rhamnose-positive or nontypable strains) occur with a low frequency.

The production of heat-stable enterotoxin is obviously not an adequate basis for judging the pathogenic potential of *Y. enterocolitica*. Lack of complete correlation with human types may be because this property is plasmid-mediated (35) and, therefore, not stable. However, production of enterotoxin, even though it may serve only as a marker and plays no direct role in pathogenicity, might be more significant if it could be related to other laboratory test systems (e.g. cell culture and animal infections) for evaluating the pathogenic potential of enteric bacteria.

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## pH, Acidity, and Vitamin C Content of Fresh and Canned Homegrown Washington Tomatoes

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### ABSTRACT

The safety of home canned tomato products in the Pacific Northwest has been questioned because of publicity surrounding low-acid tomato varieties recommended for gardens. One hundred fifty-two samples of home grown tomatoes collected from 19 counties in Washington were subjectively ranked into maturity categories by appearance. They were found to decrease in acidity and increase in pH with maturity, while the vitamin C content increased to a maximum in slightly overripe tomatoes. Ripe tomatoes grown in eastern Washington under low relative humidity and high temperatures may have greater pH values than tomatoes grown under high relative humidity and lower temperatures in western Washington. Ninety-one of the fresh tomato samples were canned with salt in a boiling water bath and stored 4 months at 4 C. The pH of the canned tomatoes decreased by 0.10 unit from the fresh values, while acidity did not change; vitamin C decreased by 30%. Overripe tomatoes had the lowest acidity and greatest pH. Thirty-one jars of tomatoes canned by home canners were collected and analyzed. The pH was sufficient in all samples to inhibit growth of *Clostridium botulinum*; however, two jars canned with no acidulant added had a pH > 4.6. Vacuum in jars ranged from 10.6 to 22.9 inches of mercury. All jars submitted were adequate for the home preservation of tomatoes.

Home canning involves use of heat to inactivate spoilage microorganisms such as molds, yeasts and acid-tolerant bacteria. The acid of the tomato prohibits germination of bacterial spores. If the pH is great enough, canned tomato products can support germination of spores of *Clostridium botulinum* at pH 4.7 and production of toxin at pH 4.8 (23). Concern has been expressed that tomatoes having pH values great enough to permit toxin production by *C. botulinum* may be used by home canners (14). An acid food is safe from *C. botulinum* if the heat process kills all organisms capable of growth at a pH ≤ 4.6 and there is no post-process contamination (11).

With respect to pH, the Food and Drug Administration has issued regulations for acidified foods (3). Tomatoes and their juices that have a finished equilibrium pH of less than 4.7 are not classified as low acid foods; therefore, the pH can be as high as 4.7 before the Good Manufacturing Practices for low-acid thermally processed foods apply to tomatoes.

Fields et al. (4) reported that many cold-packed, water-bath treated jars of tomatoes did not meet USDA processing recommendations, that improper containers

were used and that home canners used faulty canning procedures such as an open kettle method. Anderson and Mendenhall (1) collected 276 jars of tomato products in Utah, and, based on consumer information, found 85% of cold-packed samples and 30% of the hot-packed samples to be underprocessed. Powers and Godwin (15) found of 387 jars of tomatoes or tomato juice canned at home in Georgia, seven showed microbial growth indicative of underprocessing or seal failure. Mundt et al. (9) analyzed 378 jars of tomatoes and 382 jars of tomato juice collected from home canners in Tennessee and reported an apparent lack of attention to recommended procedures.

According to the USDA (24), a few tomato varieties are low in acid and home canning without the use of a pressure canner may present the hazard of botulism poisoning. Tomato varieties commonly termed small, fruited, light colored and/or low acid have not been shown to be low in acid (17).

Sapers et al. (19) found Ace and Garden State tomato cultivars to be low in acidity; samples of Garden State had 25% of individual ripe fruits exceeding pH 4.8. Overripe tomatoes, tomatoes infected with *Alternaria tenuis* or *Colletotrichum coccodes* (anthracnose) and fruits harvested from dead vines have been found to be abnormally high in pH and contain less acid than ripe tomatoes (19). Malewski and Markakis (8) reported the greatest vitamin C content of four tomato varieties was exhibited just before development of full red color or full maturation on the vine.

The USDA recommends addition of one-fourth teaspoon of citric acid per pint of tomatoes canned in a boiling water bath to increase acidity. Since citric acid is the predominant acid in tomatoes (16), it may be reasoned that acidification with citric acid would have the least effect on acceptability.

The purpose of this study was to determine the pH, acidity and vitamin C content of tomatoes grown in the Pacific Northwest and used for home canning, and to determine the safety of home canning Washington tomatoes with a boiling water bath process.

### EXPERIMENTAL

#### Materials

One hundred fifty-two home grown tomatoes were collected from 19 counties in Washington. The samples collected were voluntarily

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submitted by home gardeners. Each sample comprised 3-8 tomatoes, and was subjectively ranked in maturity categories by appearance. The tomatoes were ranked as green (green), slight green (pink with some green), ripe (all red with no green apparent and firm), slightly overripe (all red and soft) and overripe (all red and very soft).

In addition, thirty-one jars of home canned tomatoes were voluntarily submitted and divided into two groups as to whether or not they were acidified. All the home canners indicated that the tomatoes were processed in a boiling water bath, using a raw packed procedure and that the tomatoes canned were either slightly green or ripe. Cultivar validation was difficult to do for contributed fresh or home-canned tomatoes, and thus is not reported.

#### Sample preparation and analysis

Each sample was washed and quartered. Randomly selected quarters were juiced with a Champion Juicer (Lodi, California) and the pH was determined directly from the juiced tomato sample using a Chemtrix, Inc., digital pH meter type 60A with a standard glass electrode. Titratable acidity (TA) was determined by titrating 10 ml of tomato juice to pH 8.1 with 0.1 N NaOH and expressed as millequivalents of acid per liter of tomato juice. Vitamin C was determined by the microfluorometric method (2). Ninety-one samples comprising six or more tomatoes were quartered and randomly packed into pint mason jars closed with metal lids. One-half teaspoon (4.30 g) of reagent grade NaCl was added to the top of each of the filled jars. The pint jars were canned in a boiling water bath for 40 min using the USDA raw pack procedure (24), except the tomatoes were not peeled before canning. The canned tomatoes were stored 4 months at 4 C before juicing and analysis.

The home-canned tomatoes submitted by home canners were analyzed for pH, titratable acidity and vitamin C as described above. The partial vacuum was measured with an American Can Co. vacuum gauge.

All jars of tomatoes were inverted five times to mix the contents before samples for analysis were taken.

#### Statistical analysis

Analysis of variance was used to determine the significance of differences between maturity categories and differences between ripe tomatoes grown east or west of the Cascade mountains of Washington (22).

## RESULTS AND DISCUSSION

### pH, titratable acidity and vitamin C content of fresh tomatoes

The pH of fresh tomatoes grown in Washington increased with maturity (Table 1). Overripe tomatoes

had a significantly greater pH ( $P < 0.01$ ) than ripe tomatoes. Two firm ripe tomatoes had a pH  $> 4.6$  and the pH of one soft overripe tomato was 4.74.

The acidity of fresh tomatoes decreased with maturity. Overripe tomatoes had significantly less acidity ( $P < 0.01$ ) than ripe tomatoes. Overripe tomatoes may have a low acidity and present a hazard to home canners.

No significant correlation was found between tomato acidity and pH, a result in agreement with that of Lower et al. (7). Paulson and Stevens (12) determined that the relationship between  $[H^+]$  and TA is due to the buffering capacity among individual buffers and changes in buffering capacities due to pH.

The vitamin C content of fresh tomatoes grown in Washington increased with maturity until slightly overripe. Slightly overripe tomatoes had the greatest vitamin C content, while overripe tomatoes were lower. Observations of vitamin C concentration of developing tomato fruit is dependent on method of defining maturity and conflicting results have been reported previously (8). Selecting ripe tomatoes as opposed to slightly overripe for the safest pH and acidity for canning may sacrifice vitamin C content.

### Canning and storage

After 4 months of storage at 4 C, the pH of the canned tomatoes decreased 0.10 pH unit ( $P < 0.01$ ) from the fresh values (Table 2). No significant change was found in the titratable acidity due to canning or storage at 4 C. Schoenemann et al. (20) found the pH changes in tomatoes canned without salt after 1 year of storage were no greater than 0.10. Mundt et al. (10) reported the pH of home-canned tomatoes and tomato juice decreased slightly as the salt content was increased from 0 to 0.25%, 0.50% and 0.75%. One-half teaspoon salt is equivalent to 0.50% salt per pint of tomatoes. Sapers et al. (18) reported that added salt depressed the pH of canned tomatoes and attributed the pH depression to the Debye-Hückel effect (5) which predicts an increase in the dissociation of an acid with increasing ionic strength. It

TABLE 1. pH, titratable acidity and vitamin C content of fresh homegrown Washington tomatoes.

Ripeness	Samples (No.)	pH	TA (meq/l)	Vitamin C (mg/100 g)
Green	15	4.22 ± 0.17a <sup>1</sup>	89.0 ± 15.4a	16.1 ± 8.0a
Slightly green	7	4.30 ± 0.12ab	74.4 ± 14.4ab	22.7 ± 4.1ab
Ripe	85	4.39 ± 0.12bc	63.6 ± 14.8b	24.2 ± 8.2bc
Slightly overripe	35	4.43 ± 0.10c	56.8 ± 11.4c	28.3 ± 8.6c
Overripe	10	4.55 ± 0.22d	52.5 ± 12.2c	23.3 ± 12.4b

<sup>1</sup>Mean ± standard deviation. Column values not followed by common letters are significantly different ( $P < 0.05$ )

TABLE 2. pH, titratable acidity, and vitamin C content of thermally processed Washington tomatoes.

Ripeness	Samples	pH			TA (meq/liter)			Vitamin C (mg/100 g)		
		Fresh	Canned	Diff.	Fresh	Canned	Diff.	Fresh	Canned	% Decrease
Green	8	4.28	4.24	.04	89.6	76.1	13.5	17.1	12.8	25.1*
Slightly green	3	4.30	4.25	.05	7.77	72.2	5.5	25.7	18.8	26.8*
Ripe	54	4.42	4.30	.12**	60.5	63.2	2.7	24.7	16.9	31.6**
Slightly overripe	22	4.43	4.35	.08*	56.4	57.8	2.4	27.3	19.3	29.3**
Overripe	4	4.50	4.33	.17*	46.8	49.2	2.4	26.3	16.8	36.1*
All samples	91	4.41	4.31	.10**	62.0	62.7	0.7	24.8	17.2	30.6**

Significant difference between fresh and canned tomatoes \*( $P < 0.05$ ) \*\*( $P < 0.01$ )

is recommended that firm red ripe tomatoes be chosen for canning and one-half teaspoon of salt plus one-fourth teaspoon of citric acid per pint be added to enhance acidity and safety.

The mean vitamin C content of the canned tomatoes was 17.2 mg/100 g, which represented a 30.6% decrease from fresh values (Table 2); Skelton and Craig (21) found the mean vitamin C content of canned tomatoes was 18 mg/100 g. The vitamin C content was lowest in the jars which contained overripe tomatoes. There was no significant difference in the vitamin C loss with canning between ripe or slightly overripe tomatoes although the amount of vitamin C remaining was greatest when slightly overripe tomatoes were canned. Canning of slightly overripe tomatoes would yield the greatest amount of vitamin C, but due to safety considerations, ripe tomatoes should be selected for canning.

Lee et al. (6) observed that ascorbic acid destruction in tomato juice under anaerobic conditions was a first order reaction and that the reaction rate was influenced by the pH, reaching a maximum near the  $pK_a$  of ascorbic acid. The  $pK_a$  of ascorbic acid was determined to be 4.09. This would indicate that the greatest loss of ascorbic acid would occur at a pH of 4.09; however, the data in Table 1 indicate that the greatest loss of ascorbic acid in tomatoes occurred with greater maturity. Since pH increases with maturity, other factors may be involved. Lee et al. (6) noted that when the pH of a food system was changed to high or low pH values, the chemical activities of food components, which may directly or indirectly affect the rate of ascorbic acid destruction, may be significantly changed. Thus the rate constants may change.

Paulson and Stevens (12) have shown that the concentration of 14 buffers found in tomatoes influences the pH. A decrease of pH which changes the buffer composition and influences rate constants, may be a factor in the rate of ascorbic acid loss. More research is needed to determine the effect of changing buffer compositions on ascorbic acid loss with respect to pH.

#### Climatic differences

The Cascade Mountains represent a topographic and climatic barrier separating eastern and western Washington. In eastern Washington, air from the continent results in low relative humidity (30-65%) and high temperatures, while in western Washington a greater relative humidity (50-85%) and cooler temperatures prevail during the summer growing season (13).

Analysis of ripe tomatoes (Table 3) obtained from eastern Washington had a significantly greater pH ( $P < 0.01$ ) than ripe tomatoes obtained from western Washington. There was no significant difference in titratable acidity. These results indicate that ripe tomatoes produced in eastern Washington under low relative humidity and high temperatures may have greater pH values than tomatoes grown under high relative humidity and lower temperatures in western Washington. Canning tomatoes grown in dry climatic

TABLE 3. pH, titratable acidity, and vitamin C content of ripe tomatoes grown East and West of the Cascade Mountains of Washington.

Location	Samples	pH	TA (meq/l)	Vitamin C (mg/100 g)
East	47	4.43 ± 0.10**	64.0 ± 15.2	26.2 ± 9.2**
West	38	4.34 ± 0.12	63.0 ± 14.5	21.4 ± 5.6

\*\*Significant difference between fresh tomatoes grown east and west of the Cascade Mountains ( $P < 0.01$ )

areas may favor a high pH and require caution when canning.

A significantly greater amount ( $P < 0.01$ ) of vitamin C found in ripe tomatoes grown in eastern Washington may have resulted from a dehydrated product due to the drier growing conditions resulting in an increased concentration of vitamin C per tomato.

#### Home-canned tomatoes

The home-canned tomatoes submitted by home canners were divided into two groups corresponding to whether they were acidified or not (Table 4). If any organic acids were added such as vinegar, lemon juice and/or citric acid, the tomatoes were considered acidified. The amounts and combinations of acids, sugar and/or salt that the home canners reported adding varied greatly. This could partially explain the broad pH and titratable acidity ranges observed.

Jars of tomatoes submitted that had been acidified by home canners had a low pH, higher titratable acidity and lower vitamin C content than nonacidified samples. The vacuum of all jars of home canned tomatoes ranged from 10.6 to 22.9 inches of mercury. All jars submitted were mason-type jars with a two-part lid closure and were considered adequate for the home preservation of tomatoes.

TABLE 4. pH, TA, vitamin C and vacuum of thirty-one jars of homegrown and home-canned tomatoes.

Treatment	pH	TA (meq/l)	Vitamin C (mg/100 g)	Vacuum (in. Hg)
Acidified	4.08 ± .34 <sup>a</sup>	103.6 ± 60.2	15.0 ± 6.5	18.5 ± 3.6
No Acidulation	4.37 ± .17	64.8 ± 12.3	16.6 ± 6.2	17.0 ± 5.9

<sup>a</sup>Mean ± standard deviation. Various amounts and combinations of acids were added by home canners.

## CONCLUSIONS

Firm red ripe tomatoes should be selected for canning. A home canner who processes overripe tomatoes in a water bath may produce a product with not enough acidity to insure microbiological safety. This is in agreement with Sapers et al. (19) who reported that overripe tomatoes were low in acidity and should not be canned. Tomatoes grown under low relative humidity and high temperature conditions may have a greater pH and require greater caution when canning than tomatoes grown under high relative humidity and low temperatures. Even though slightly overripe tomatoes may contain more vitamin C than ripe tomatoes, overripe tomatoes should not be canned for safety reasons. It is recommended that home-canners add one-fourth tea-

spoon of citric acid and, if desired, one-half teaspoon of salt to water-bath canned tomatoes for increased safety.

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## Effect of Water Activity and Temperature on *Staphylococcus aureus* Growth and Thermonuclease Production in Smoked Snoek

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### ABSTRACT

Smoked snoek at different water activity ( $a_w$ ) values (0.966, 0.956 and 0.944) were inoculated with *Staphylococcus aureus* at a concentration of approximately  $\log 4.7$  colony forming units per gram (CFU/g) and incubated at different temperatures (4, 12, 24 and 37 C) for various periods. Samples of inoculated and control snoek were taken at different intervals to determine the microbial populations (*S. aureus*, total organisms and enterococci), pH and presence of thermonuclease. No growth of *S. aureus* nor thermonuclease production occurred at 4 C. At 12 C, notwithstanding high *S. aureus* log counts of 6.75/g, 9.74/g and 9.69/g in the inoculated 0.966, 0.956 and 0.944  $a_w$  samples, respectively, thermonuclease was absent after 21 days of incubation. At 24 and 37 C at all three  $a_w$  levels, very high *S. aureus* counts were recorded and were accompanied by thermonuclease production before the end of the relative incubation periods (5 and 3 days, respectively). A reduction of the  $a_w$  of the snoek resulted in a slightly slower *S. aureus* growth rate at 37 C and also delayed thermonuclease production at 24 and 37 C. The thermonuclease test was an effective means of detecting *S. aureus* growth except in samples incubated at 12 C. Enterococcus numbers increased in all control samples during storage, but none were found to produce thermonuclease.

Snoek (*Thyrssites atun*) is a type of fish which is widely found in the waters of the Southern Hemisphere. In South Africa, snoek is often smoked and eaten as a delicacy. The preparation of smoked snoek consists of cleaning and flaying the fish, followed by curing with NaCl (3.5-5.5% w/v) or by immersion in a saturated brine (NaCl) solution for a fixed period. The cured fish are then cooked for 30 min in a kiln at 100 C and at least 50% relative humidity (RH), followed by 2 h of smoking at 60 C and about 80% RH (20). This hot smoking process renders the smoked snoek almost sterile (21).

Only one case of staphylococcal food poisoning after consumption of smoked snoek has previously been reported (19), although the processing characteristics of the food would make it ideal for *S. aureus* growth, provided the temperature is suitable. Similar products such as cured meats are frequently implicated in food poisoning outbreaks by *S. aureus* apparently due to the ability of this microorganism to tolerate the reduced  $a_w$  of these products (8).

The purpose of this investigation was to determine the effects of  $a_w$  and temperature on growth of *S. aureus* in smoked snoek, as well as the effectiveness of thermonuclease as an indicator of *S. aureus* growth in this

product. Examination of foods for thermonuclease as an indicator of *S. aureus* growth (and likely presence of enterotoxin) has been reported to be a rapid, reliable and inexpensive screening method (22). The results of this study show that 4 C was necessary to stop *S. aureus* growth. Reduction of the  $a_w$  of the product delayed thermonuclease production at 24 and 37 C without any real effect on the final *S. aureus* counts. Thermonuclease was not detected when *S. aureus* grew at 12 C.

### MATERIALS AND METHODS

#### *S. aureus* strains

The *S. aureus* strain used in all the experiments was originally isolated from smoked snoek implicated in a case of food poisoning (19). This organism produced acid in the double tube oxidation/fermentation test; it also produced thermonuclease, protein A and gave a positive coagulase test; however, it did not produce any of the known enterotoxins (3). *S. aureus* 196E kindly supplied by M.S. Bergdoll was used as a comparative control in some experiments.

#### Preparation of samples

Different amounts of NaCl were used during the curing process to prepare smoked snoek with 0.966, 0.956 and 0.944  $a_w$  values (these values fall within the limits of those for commercially available smoked snoek). The whole snoek were cut into pieces, minced and mixed to obtain an equal distribution of the bacterial flora before 10-g portions were weighed out in 9-cm petri dishes ready for inoculation.

#### Inoculation

The minced snoek (10-g portions) were inoculated with 0.1 ml of a washed cell suspension (ca.  $\log 4.7$  CFU/ml) in 0.1% (w/v) peptone water (Oxoid) containing NaCl so that the suspending fluid had the same  $a_w$  as that of the sample to be inoculated.

#### Storage conditions

Petri dishes containing the inoculated and control samples were placed in desiccators along with a solution of NaCl which had the same  $a_w$  as that of the samples (9). The desiccators were incubated at 4, 12, 24 and 37 C for 30, 21, 5 and 3 days, respectively. Temperatures were checked regularly and found to be precise, except at 12 C where slight variations were noticed (standard deviation = 0.35 C). Samples were removed at appropriate intervals for evaluation.

#### Enumeration of microorganisms

Ten grams of minced snoek were added to 90 ml of 0.1% (w/v) peptone water and blended (2) before making appropriate decimal dilutions. Staphylococci were enumerated on Baird-Parker agar (Oxoid), and total viable counts on Plate Count Agar (Oxoid) by using a surface plate method. Plates were incubated at 37 C for 48 h before enumeration. Enterococci were enumerated in all samples as a control since Tatini et al. (22) had reported that some *Streptococcus faecalis* strains are able to produce thermonuclease, and this could lead to false interpretation of the thermonuclease test. Enterococci were enumer-

ated on m Enterococcus Agar (Difco) using a membrane filtration technique. Plates were incubated at 37 C for 3 days before counting.

**Determination of pH**

A 10<sup>-1</sup> homogenate in distilled water was prepared and pH was measured on a Metrohm E520 pH-meter (glass electrode).

**Measurement of a<sub>w</sub>**

The psychrometric method of Prior et al. (18) was used by equilibrating the sample at room temperature for 1 h before measurement.

**Thermonuclease determination**

The metachromatic agar-diffusion (MAD) microslide technique of Lachica et al. (11) was used to detect thermonuclease except that 3-mm wells were filled with approximately 10 µl samples (previously boiled for 15 min) of the 10<sup>-1</sup> homogenate as prepared for the bacterial counts. The slide was incubated at 37 C for 4 h before being examined for nuclease activity. Sensitivity for this procedure was found to be at least 10 ng/g when smoked snoek was spiked with pure nuclease from *S. aureus* (Sigma Chemical Co.), giving a zone of activity of approximately 6.5 mm.

**RESULTS**

**Influence of temperature and a<sub>w</sub>**

No thermonuclease was produced and a steady decline in *S. aureus* numbers in all the inoculated samples was observed at 4 C (Fig. 1). The absence of growth and thermonuclease production was expected at this temperature since it is less than the minimum growth temperature for *S. aureus* of 6.5 C. However, good growth of other microorganisms was recorded in the 0.966-a<sub>w</sub> control and inoculated samples at 4 C (log 7.69/g and 7.95/g, respectively) (Table 1).

The total viable counts in the 0.956- and 0.944-a<sub>w</sub> inoculated and control samples at 4 C remained constant or declined slightly. This suggests that the lowering of a<sub>w</sub> prevented large numbers of microorganisms sensitive to the lower a<sub>w</sub> from multiplying in the snoek.

Inoculated snoek held at 12 C resulted in growth of *S. aureus* and log counts of 6.75/g, 9.74/g and 9.69/g were reached after 21 days of incubation of the 0.966, 0.956 and 0.944-a<sub>w</sub> samples, respectively (Fig. 2). These counts represented 1, 97 and 98% of the total growth respectively (Table 1). A delay in reaching maximum *S. aureus* counts was noticed in the two lower a<sub>w</sub> samples incubated at 12 C. For example, 21 days were required before *S. aureus* reached its maximum number in 0.944-a<sub>w</sub> snoek, 18 days for the 0.956-a<sub>w</sub> sample, while near maximum was reached after only 6 days of incubation of the 0.966-a<sub>w</sub> sample (Fig. 2). However, a

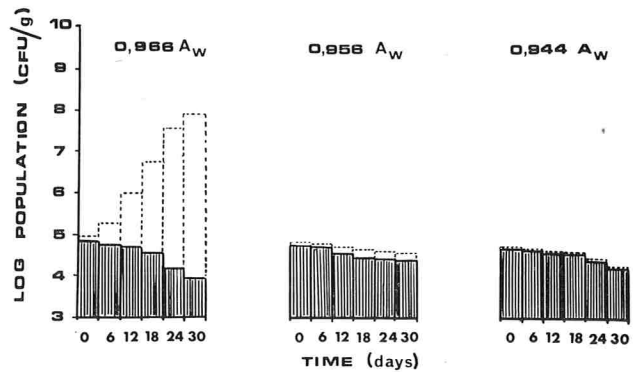


Figure 1. Effect of a<sub>w</sub> on growth of *S. aureus* at 4 C in smoked snoek. Legend:  $\square$  thermonuclease absent;  $\square$  thermonuclease present; — *S. aureus* CFU; - - - total CFU.

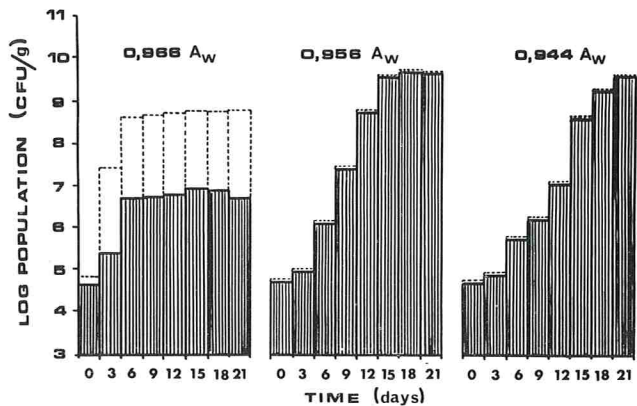


Figure 2. Effect of a<sub>w</sub> on growth of *S. aureus* at 12 C in smoked snoek. Legend:  $\square$  thermonuclease absent;  $\square$  thermonuclease present; — *S. aureus* CFU; - - - total CFU.

lower maximum count was recorded in the 0.966-a<sub>w</sub> snoek compared to the 0.956 and 0.944 samples suggesting that competition by other microorganisms was more successful at the higher a<sub>w</sub> (0.966) and suppressed staphylococcal growth to a greater extent in this sample. Notwithstanding the high staphylococcal counts, thermonuclease was not detected at any of the a<sub>w</sub> levels at 12 C (Fig. 2).

Very high *S. aureus* counts (> log 9/g in all instances) were observed in all inoculated 0.966, 0.956 and 0.944-a<sub>w</sub> snoek samples when incubated at 24 and 37 C for 5 and 3 days, respectively (Fig. 3 and 4).

Higher growth rate of *S. aureus* occurred at 37 C than at 24 C, which was expected since the optimum growth temperature lies between 30 and 37 C (4). However, at

TABLE 1. Changes in total viable counts (log population (CFU/g)) after incubation of smoked snoek at various temperatures and a<sub>w</sub> values<sup>a</sup>.

a <sub>w</sub>	Sample	4 C		12 C		24 C		37 C	
		0 h	30 days	0 h	21 days	0 h	5 days	0 h	3 days
0.966	Control	4.70	7.69	4.71	8.79	4.53	9.49	4.79	8.93
	Inoculated	4.97	7.95	4.85	8.85	5.00	9.82	4.98	10.27
0.956	Control	3.17	3.47	3.39	8.50	3.47	9.30	3.04	9.60
	Inoculated	4.76	4.59	4.81	9.76	4.74	9.97	4.79	9.81
0.944	Control	3.00	2.95	2.77	8.04	2.69	9.25	3.00	9.36
	Inoculated	4.68	4.17	4.74	9.70	4.87	10.04	4.82	9.91

<sup>a</sup>Each value is the mean of three determinations.

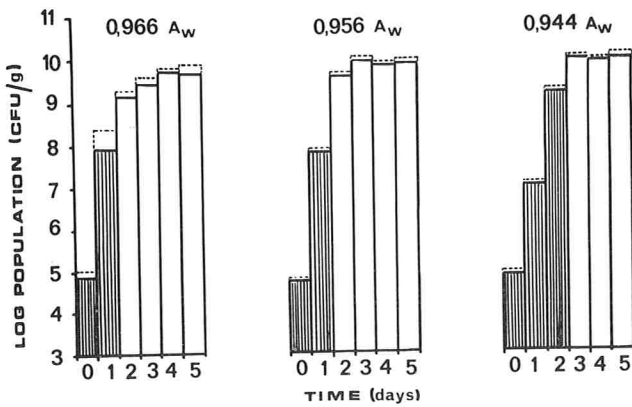


Figure 3. Effect of  $a_w$  on growth of *S. aureus* at 24 C in smoked snoek. Legend: ▨ thermonuclease absent; □ thermonuclease present; — *S. aureus* CFU; - - - total CFU.

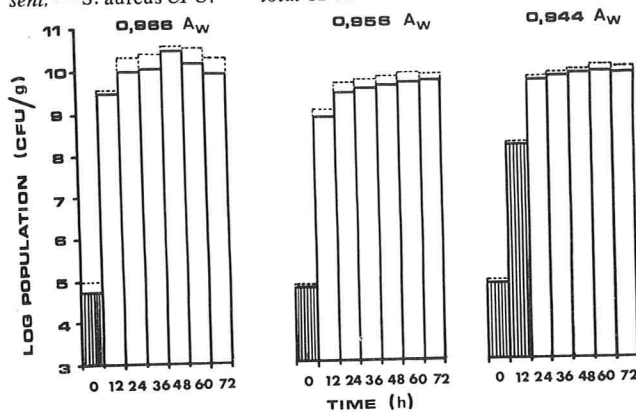


Figure 4. Effect of  $a_w$  on growth of *S. aureus* at 37 C in smoked snoek. Legend: ▨ thermonuclease absent; □ thermonuclease present; — *S. aureus* CFU; - - - total CFU.

both temperatures (24 and 37 C), the final *S. aureus* counts were approximately the same. At 37 C a slightly higher *S. aureus* growth rate was recorded in the snoek with the highest  $a_w$  value (0.966). The total viable counts of the inoculated samples after completion of the incubation periods at 24 and 37 C were largely *S. aureus* (Fig. 3 and 4). *S. aureus* is generally a very poor competitor at or below ambient temperatures (16); however, our results at 37 and 24 C are in accordance with findings of Peterson et al. (17) that staphylococci could multiply appreciably in the presence of other saprophytic bacterial populations provided the temperature is above 20 C and the pH range between 6 and 8.

Uninoculated control samples showed also high final total viable counts at these temperatures (Table 1). At 37 C thermonuclease was detected after 12, 12 and 24 h of incubation for the 0.966, 0.956 and 0.944- $a_w$  samples, respectively, while 2, 2 and 3 days were required for the corresponding samples to produce this enzyme when the temperature was lowered to 24 C.

*S. aureus* 196E, a producer of enterotoxins A and D, was inoculated into 0.966- $a_w$  snoek and incubated at 4, 12 and 37 C. The growth pattern and thermonuclease production were almost identical in all respects to that of the *S. aureus* strain used in all our experiments, suggesting that the behavior of other enterotoxigenic

staphylococci might follow a similar pattern in this particular foodstuff.

No ( $< \log 2.00$  CFU/g) *S. aureus* were detected in any of the uninoculated control samples.

#### pH changes during incubation

During incubation of samples at all temperatures, a marked increase in pH was noticed in the inoculated and control samples with the highest  $a_w$  value (0.966). In contrast, no pH changes occurred in the 0.956 and 0.944- $a_w$  samples during incubation at 4 C. A lack of microbial growth was observed at that temperature and those  $a_w$  levels. However, the pH values rose as the incubation temperatures increased. The biggest increases in pH occurred at 37 C while the lowest increases were recorded in samples which had the lowest  $a_w$  value of (0.944) (Table 2).

TABLE 2. Final pH after incubation of smoked snoek at various temperatures and  $a_w$  values<sup>a</sup>.

$a_w$	Sample	30 days at 4 c	21 days at 12 C	5 days at 24 C	3 days at 37 C
0.966	Control	7.2	7.7	6.6	7.5
	Inoculated	7.2	7.7	6.8	8.0
0.956	Control	6.1	6.4	6.5	7.9
	Inoculated	6.1	6.7	6.6	8.0
0.944	Control	6.1	6.2	6.2	6.5
	Inoculated	6.1	6.5	6.5	6.9

<sup>a</sup>Average initial pH of smoked snoek of 24 samples was 6.13 (standard deviation = 0.08).

#### Growth of enterococci and influence on thermonuclease detection

Initial counts of enterococci varied from  $< \log 2.00$  CFU/g to a count of  $\log 3.69$ /g, while the highest counts obtained after completion of incubation periods of control and inoculated samples were  $\log 8.47$ /g and  $\log 8.59$ /g, respectively. Highest counts of enterococci were found in the 0.966- $a_w$  samples incubated at 24 and 37 C. Reductions in the  $a_w$  of the samples and lowering of incubation temperatures reduced the final counts of enterococci.

However, notwithstanding high counts obtained after incubation, thermonuclease synthesis by any of these organisms could not be demonstrated in any of the control samples. Even under optimal conditions, when pure cultures of enterococci (isolated from the snoek) were incubated at 37 C in Brain Heart Infusion broth (BHI) (0.993  $a_w$ ), the thermonuclease test was negative, showing that the strains present in the smoked snoek were incapable of producing this enzyme and could not interfere with this test.

#### DISCUSSION

The results show that storage temperature is the most effective means of inhibiting growth of *S. aureus* in smoked snoek while  $a_w$  values within palatable limits will have no major effect on its growth. At 4 C, no *S. aureus*

growth was observed (Fig. 1) while abundant growth of other microorganisms occurred in the 0.966- $a_w$  snoek sample. Reduction of the  $a_w$  of the snoek to 0.956 and 0.944 resulted in growth of other microorganisms being prevented at this temperature (4 C) (Table 1). By ensuring that the  $a_w$  of snoek is below a suitable value such as 0.95, manufacturers may be able to substantially extend the shelf-life of their product.

Thermonuclease was shown to be a rapid means of detecting *S. aureus* growth in samples (at all three  $a_w$  values) stored at 24 and 37 C. The absence of thermonuclease in samples stored at 12 C for 21 days appeared to be as a result of the  $a_w$  of the snoek, and perhaps the food itself. When *S. aureus* was grown in BHI broth (0.993  $a_w$ ) at 12 C, a positive thermonuclease reaction was obtained after 3 days. Kamman et al. (10) reported that thermonuclease production is repressed by reductions in  $a_w$ . This effect combined with reduced temperature may be the reason for the lack of production of detectable levels of thermonuclease under these conditions. The fact that *S. aureus* growth occurs but no thermonuclease was produced suggests that the thermonuclease test is not always a reliable indicator of *S. aureus* growth. Whether enterotoxin synthesis would occur under these conditions is not known, but a number of workers have reported that conditions determining thermonuclease synthesis have similar effects on the synthesis of enterotoxin (23).

There did not appear to be a relationship between numbers of *S. aureus* in the various snoek samples and the presence of thermonuclease. In the 0.944- $a_w$  snoek samples incubated at 24 C for 2 days, a *S. aureus* count as high as log 9.21/g was observed without thermonuclease being detected, whereas in the 0.966- $a_w$  sample incubated at the same temperature for the same period (2 days at 24 C), thermonuclease was detected at a slightly lower count of log 9.17/g (Fig. 3). Niskanen and Nurmi (15) also reported a poor correlation between thermonuclease level and *S. aureus* count in dry sausage with  $a_w$  values between 0.96 and 0.875. Tatini et al. (23) found thermonuclease in high- $a_w$  foods such as milk, cream, skim milk and whey held at 37 C whenever *S. aureus* had attained log counts between 5.69 and 6.00/ml. Evidently temperature and  $a_w$  affect growth of *S. aureus* and thermonuclease production differently.

Although thermonuclease was produced in all the inoculated snoek samples (0.966, 0.956 and 0.944  $a_w$ ) at 24 and 37 C, its production was delayed by the lower  $a_w$  values (Fig. 3 and 4). A similar effect on enterotoxin synthesis caused by lowering the  $a_w$  and temperature in culture media has been reported (12,13,14), further suggesting a close correlation between enterotoxin and thermonuclease synthesis.

The initial and final pH values of the smoked snoek measured here had little effect on thermonuclease synthesis and growth of *S. aureus*. Optimum growth pH was 7.3 and ceased at 9.8 (5,7,21), while the highest pH measured during these experiments (Table 2) was only

slightly below (0.3 unit) the optimum pH of 8.3 for thermonuclease production (6). Thus the maximum and minimum pH readings (8.0 and 5.9) measured during incubation should not inhibit *S. aureus* growth and thermonuclease production.

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# Repeatability and Accuracy of Dye-Binding and Infra-Red Methods for Analyzing Protein and Other Milk Components<sup>1</sup>

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## ABSTRACT

Udy and Pro-Milk Mark II dye-binding methods and the Milko-Scan 104 infra-red device were evaluated for accuracy and repeatability in the analysis of protein in raw milk supplies. The infra-red device was also evaluated for accuracy in determination of milkfat, lactose and solids-not-fat, and was compared with the Milko-Tester for fat analysis. Repeatability of the three methods was, in all instances, less than 0.05% for protein. Standard deviation of accuracy ( $\sigma_{y,x}$ ) for protein (total nitrogen  $\times$  6.38) for the Udy Analyzer, Pro-Milk, and Milko-Scan units was 0.063, 0.062, and 0.067, respectively. Standard deviation of accuracy of the Milko-Scan rated against Mojonnier and Milko-Tester methods for milkfat was, respectively, 0.054 and 0.050. Compared with liquid chromatography lactose determination and Mojonnier solids-not-fat determination, the Milko-Scan showed a standard deviation of accuracy of 0.083 and 0.073, respectively.

Renewed interest in component pricing of milk has resulted in more serious attention being given to various methods that might be applied in analysis of these components. For routine work, both dye-binding and infra-red methods are available. Dye-binding procedures measure protein only; infra-red units may measure milkfat, protein, lactose, and/or solids-not-fat. In evaluating such methods, it is helpful to know how well they repeat themselves on the same milk sample, and also how well they compare with reference procedures. This last is usually referred to as accuracy. In this work, the Udy Analyzer, Pro-Milk Mark II dye-binding methods and the Milko-Scan 104 infra-red device were evaluated for repeatability and accuracy in protein testing of raw milk. The Milko-Scan 104 was also evaluated for accuracy of measurement of milkfat, lactose, and solids-not-fat.

## MATERIALS AND METHODS

### Milk samples

Pooled herd milk was collected for this work on two separate occasions. The first set of samples, 99 in all and representing a wide geographic area of Minnesota and western Wisconsin, were obtained in November, 1978. These samples were used primarily to evaluate protein testing devices. Herd size averaged 41 cows, and samples were purposely selected to reflect different breeds of cattle. Most were Holstein herds, but also included were six Jersey, eight Guernsey, five

Brown Swiss, and nine mixed herds. Samples were split into several sublots and preserved with 0.1% potassium dichromate for Pro-Milk and Milko-Scan analyses, and 0.07% mercuric chloride for Udy analysis. For milkfat and solids-not-fat analyses, a second set of 51 pooled herd milk samples covering the same geographic area, was secured in March, 1979.

### Reference methods

Total nitrogen (TN) and non-protein nitrogen (NPN) were determined on fresh (non-preserved) milk by the method of Rowland (8). Results were expressed in percent protein ( $N \times 6.38$ ) or percent true protein  $[(TN - NPN) \times 6.38]$ .

Milkfat and total solids were determined by Mojonnier, according to *Standard Methods for the Examination of Dairy Products* (1). Solids-not-fat was obtained by subtracting milkfat from total solids results. Lactose was determined by liquid chromatography (3). Samples for Kjeldahl and Mojonnier analyses were done in duplicate, lactose singly.

### Routine methods

Protein content of samples was measured by Udy Analyzer (Tecator-Udy Co., Boulder, CO 80301), Pro-Milk Mark II (Foss America, Inc., Fishkill, NY 12524) and Milko-Scan 104 (Foss America, Inc., Fishkill, NY 12524).

Analyses by these routine methods were made over as short a duration as possible to avoid errors which might have arisen due to variation in instrument settings. In addition, instrument response was checked regularly, using standard milk samples. No attempt was made to calibrate the equipment very accurately, since bias from this source does not affect statistical evaluation of accuracy. All analyses were made according to manufacturers' instructions, and were made in duplicate.

### Statistical analysis

The standard deviation of repeatability ( $\sigma_r$ ) was calculated from the absolute difference ( $d_j$ ) between duplicates of  $n$  results, using the equation:  $\sigma_r = \frac{\sqrt{1}{n}}{2n} \sum_{j=1}^n d_j^2$ . This expression is equivalent to the

standard deviation within samples obtained from a one-way analysis of variance of the data.

Accuracy of the mean (or accuracy) of an indirect method is best described by the standard deviation from the regression ( $\sigma_{y,x}$ ) of the reference method (dependent variable) on the routine method (independent variable). The estimated component value of 95% of the individual samples is assumed to be approximately within  $\pm 1.96 \sigma_{y,x}$ . Use of this method of statistical evaluation discounts bias resulting from inaccurate calibration of instruments and provides a valid analysis of the potential of a given instrument.

## RESULTS AND DISCUSSION

Repeatability of the three test methods is shown by data provided in Table 1. In all instances and for all components measured, the devices were able to repeat a test with an absolute difference of less than 0.05%. This was true for measurements made over a short period, as prescribed for this kind of evaluation. Larger differences

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TABLE 1. Repeatability of the Pro-Milk Mark II, Udy Analyzer, and Milko-Scan 104 in the analysis of various milk components.

Component	Method	n	Standard deviation of repeatability ( $\sigma_r$ )	Repeatability ( $r_{2.83 \sigma_r}$ )
Protein	Pro Milk	34	0.014	0.04
	Udy	36	0.010	0.03
	Milko-Scan	99	0.011	0.03
Milkfat	Milko-Scan	51	0.007	0.02
SNF	Milko-Scan	51	0.015	0.04

between duplicates might be expected, at least for dye-binding methods, when duplicate analyses are not made one immediately following the other, but rather spaced out over a period of hours.

Standard deviation of accuracy and confidence limits (95% probability) for the three protein test methods are shown in Table 2. Data in Table 3 give a comparative evaluation of the methods rated against each other. There was little or no difference in accuracy of the methods, whether for protein ( $TN \times 6.38$ ) or true protein [ $(TN - NPN) \times 6.38$ ]. Standard deviations for protein testing were 0.062, 0.063 and 0.067 for the Pro-Milk, Udy, and Milko-Scan methods, respectively. These values agree quite well with similar data reported in the literature (2,4,5,6,7,9,10). At 95% confidence limits of the estimated reference value, results for the three methods ranged from 0.12 to 0.13.

TABLE 2. Accuracy of the Pro-Milk Mark II, Udy Analyzer, Milko-Scan 104 in analysis of protein ( $TN \approx 6.38$ ), and true protein [ $(TN - NPN) \approx 6.38$ ] of milk ( $n = 99$ ).

Component	Method	Standard deviation of accuracy ( $\sigma_{y,x}$ )	95% Confidence limits of the estimated reference value ( $\pm 1.96 \sigma_{y,x}$ )
Protein ( $TN \times 6.38$ )	Pro-Milk	0.062	$\pm 0.12$
	Udy	0.063	$\pm 0.12$
	Milko-Scan	0.067	$\pm 0.13$
True protein [ $(TN - NPN) \times 6.38$ ]	Pro-Milk	0.062	$\pm 0.12$
	Udy	0.064	$\pm 0.13$
	Milko-Scan	0.067	$\pm 0.13$

TABLE 3. Comparison of the Pro-Milk Mark II, Udy Analyzer, and Milko-Scan 104 in the analysis of protein of milk.

Component	Method used as reference	Standard deviation of accuracy ( $\sigma_{y,x}$ )	95% Confidence limits of the estimated reference value ( $\pm 1.96 \sigma_{y,x}$ )
Pro Milk	Udy	0.024	$\pm 0.05$
Pro Milk	Milko-Scan	0.028	$\pm 0.05$
Udy	Milko-Scan	0.033	$\pm 0.06$

Not uncommonly, dye binding methods provide slightly better accuracy than infra-red devices in protein testing (4,5,6). In this work, standard deviations of accuracy between dye-binding and infra-red methods were lower than those found for each of these methods rated against the Kjeldahl. This fact suggests a relative

inaccuracy in the Kjeldahl procedure itself. A normal, good correlation was found between the dye-binding methods when compared to each other.

Theoretically, NPN is not measured by either infra-red or dye-binding procedures. For this reason, better accuracy might be expected when the instrument readings are related to true protein ( $TN - NPN$ ) instead of protein ( $TN$ ). That this was not observed in this study can perhaps be explained by the relatively low variability of the proportion of NPN to total nitrogen in the population of milk samples analyzed. NPN varied from total nitrogen in this study by a standard deviation of only 0.66. Table 4 gives data related to the accuracy of the Milko-Scan 104 in the analysis of milkfat, lactose, and solids-not-fat. Standard deviation of the accuracy for these three measurements was 0.054, 0.083, and 0.073, respectively. Rated against the Milko-Tester for milkfat analysis, this statistic was 0.050. Since infra-red and Milko-Tester fat tests respond differently to variations in the proportion of various fatty acids, the good correlation observed between both instruments would suggest a fairly homogeneous fatty acid composition for the samples tested.

TABLE 4. Accuracy of the Milko-Scan 104 in the analysis of milkfat, lactose, and solids-not-fat of milk.

Component	Reference method	n	Standard deviation of accuracy ( $\sigma_{y,x}$ )	95% Confidence limits of the estimated reference value ( $\pm 1.96 \sigma_{y,x}$ )
Milkfat	Mojonnier	51	0.054	$\pm 0.10$
	Milko-Tester	51	0.050	$\pm 0.10$
Lactose	Chromatography	99	0.083	$\pm 0.16$
SNF	Mojonnier	51	0.073	$\pm 0.14$

In summary, both dye-binding and infra-red methods, as evaluated in this study, would appear to be acceptable procedures for making analyses of protein in a component pricing system. In addition, the Milko-Scan could serve to accurately measure solids-not-fat and/or other milk constituents. Accuracy values reported herein, based upon analyses made in duplicate, reflect the discrepancy between reference procedures and routine tests as caused by differences in principle of measurement. It is also worth noting that the data of this study are characteristic of the sample population and might vary somewhat from area to area. However, data reported herein fall well within the rather large range of values found in the literature. Quite likely, such differences arise mainly from differences in statistical methods of assessing accuracy and from variations in precision of testing by the reference method. Major sources of error in dye-binding and infra-red methods tend to originate in improper or inaccurate calibration. For this reason special attention must be given to routine monitoring efforts. In addition, as noted by Biggs (2), infra-red devices should be calibrated on the same type of milk as will be routinely tested.

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## Heat Resistance of *Escherichia coli* in Cow and Buffalo Milk

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### ABSTRACT

Three *Escherichia coli* cultures (O111:B4, O127:B8 and NP) were selected to study their heat-resistant characteristics when in cow skim, cow whole and buffalo whole milk. The temperatures of heat-treatment included in this study were 50, 55, 60 and 63 C. The time interval during heat-treatment was 10 min at 50 and 55 C and 5 min at 60 and 63 C. Marked differences in heat-resistance were observed in the three *E. coli* cultures. The z-values obtained for strain O111:B4 were 8.3, 9.0 and 10.2 when tested in cow skim milk, cow whole milk and buffalo milk, respectively. The z-values for O127:B8 and NP were 17.5, 18.0 and 19.2 and 18.8, 19.0 and 20.3, respectively, for the three types of milk.

There is a considerable divergence of opinion regarding the thermal destruction pattern of *Escherichia coli* in milk and milk products. Little information is available on the comparative heat destruction pattern of pathogenic and nonpathogenic strains of *E. coli*. Most studies on this topic were limited to cow milk only. Such information is lacking for buffalo milk. Because of compositional differences between cow and buffalo milk, the data on thermal destruction of microorganisms may show some deviations in the two types of milk. In view of these considerations, the present investigation was undertaken to determine the heat resistance characteristics of some pathogenic and non-pathogenic strains of *E. coli* in cow and buffalo milks.

### MATERIALS AND METHODS

#### Cultures used

Three cultures of *E. coli* (O111:B4, O127:B8 and NP) were used for these studies. Strains O127:B8 and NP had been isolated from pasteurized milk and cream, respectively, while O111:B8 was procured from the International *E. coli* Reference Laboratory, Copenhagen, Denmark.

#### Temperature-time combinations during heat-treatment

The three cultures were subjected to heat-treatment at various time-temperature combinations. The temperatures of heat-treatment were 50, 55, 60 and 63 C. The exposure times at 50 and 55 C were 10, 20, 30, 40, 50 and 60 min, while 5, 10, 15, 20, 25 and 30 min were selected when the other temperatures were used.

#### Heat-treatment of *E. coli*

For each strain five glass ampoules of 5 ml capacity were used, as suggested by Pflug and Schmidt (10). Two ml of the cell suspensions in sterilized cow's skim milk, cow's whole milk and buffalo's whole milk were dispersed into each ampoule by means of a sterile syringe. The final concentration of cells in the suspensions was approximately

100,000 organisms per ml. After transferring the cell suspension into the ampoules, they were immediately heat-sealed under flame and immersed completely in a constant temperature water bath and held there at pasteurization temperature (63 C/30 min). The come-up time for 63 C was 1½ min. After exposure to the desired temperature-time combination, the ampoules were immediately removed from the water bath and chilled in cold water (8-10 C).

#### Determination of viability of heat-treated cells

After heat-treatment, the number of viable cells in the inoculated suspending medium was determined by withdrawing samples after breaking the neck portion of one of the ampoules. Appropriate dilutions of heated cell suspensions were plated in duplicate on yeast extract agar with 1% dextrose and incubated at 37 C for 48 h before counting the colonies.

#### Evaluation of thermal destruction of *E. coli*

Two methods of analysis of heat-destruction data were adopted for plotting the survival curve. The experimental (graphic method) was used, while for calculating the thermal death rate value (D values), graphic as well as regression coefficients based on statistical procedures were adopted. The thermal death time values (z values) were determined by the graphic method only. When the survival curves were linear, a straight line regression equation was fitted. In the event of survival exhibiting deviation from linearity, second degree curves were fitted. The methods recommended by Collins and Dunkley (3) were adopted D and z value determinations by the graphic method. The destruction rate curves (D values) were constructed by plotting data on semi-log paper. A line of usual best fit was drawn which satisfied the resulting points and the D values were calculated as the slope of this line. The thermal death time curve (z values) for each organism was determined by plotting the log of D values against the temperature of exposure. For purpose of comparison, standard pasteurization with reference to milk was also fitted.

### RESULTS

#### Thermal destruction of *E. coli* cultures in milk

The data pertaining to the number of survivors of *E. coli* strains exposed to different time-temperature combinations in three types of milk are in Tables 1 to 3. The geometric mean of three replicate trials for each time-temperature combination has also been included. Buffalo's milk offered the greatest protection against heat-destruction when compared to cow's skim milk or cow's whole milk. Marked differences were observed in different milk substrates in regard to the heat-resistance characteristics of three strains of *E. coli*. (Tables 1 to 3). Strain O111:B4 was the most sensitive to heat-treatment when compared to strains O127:B8 and NP. For instance, O111:B4 survived heat treatments of 30 min at 55 C and 5 min at 60 C in cow's milk (both skim and whole). When buffalo's milk was used as the substrate, that culture exhibited greater heat resistance of 40 min at 55 C and

10 min at 60 C. The culture failed to survive heat treatment at 63 C for 5 min irrespective of the type of milk used. Strains 0127:B8 and NP exhibited greater heat resistance in both cow's and buffalo's milk. Of the three cultures examined in this study, the non-pathogenic strain (NP) was most heat-resistant in both cow's and buffalo's milk.

The thermal destruction curves for the three *E. coli* strains exposed to different time-temperature combination in cow's and buffalo's milk are in Fig. 1 to 3. Exposure of cells to heat treatment at 50, 55 and 60 C resulted in a logarithmic order of death for the three cultures. However, with exposure at 63 C a deviation from linearity was noticed for two cultures, 0127:B8 and NP, [Fig. 1(a) to 3(d)] which are relatively more heat

resistant than the third. Variation in the slope of the curves between individual cultures was also observed. For example, 0111:B4 exhibited steep survivor curves, thereby indicating the heat-sensitive nature of the culture. The other two cultures, 0127:B8 and NP, gave flat survivor curves, thereby indicating the high heat-resistance characteristics of these organisms.

Table 4 (a to c) contains D values for three types of milk (graphically as well as those based on statistical calculation). Fair agreement was found between the D values obtained by the graphic method and those calculated by fitting regression equations for all these cultures in cow's skim milk. A similar trend was noticed when the cultures were heat-treated in cow's whole milk and buffalo's whole milk, although substantial differ-

TABLE 1. Resistance of *E. coli* to different time-temperature combinations of heat-treatment in cow's skim milk.

Time of exposure (min)	50 C			55 C		
	<i>E. coli</i> strains					
	0111:B4	0127:B8	NP	0111:B4	0127:B8	NP
	Number of survivors (viable counts/ml) <sup>a</sup> × 10 <sup>3</sup>					
10	23.50	57.00	62.00	1.17	43.20	47.00
20	9.20	43.00	49.10	0.265	15.00	21.00
30	6.50	32.00	35.50	0.017	9.20	14.20
40	17.80	18.40	21.20	0	4.30	7.40
50	0.92	8.50	9.20	0	2.80	4.40
60	0.47	6.20	6.50	0	1.87	2.17
	60 C			63 C		
5	0.03	16.00	27.00	0	0.262	0.38
10	0	6.90	7.30	0	0.049	0.07
15	0	1.77	2.30	0	0.019	0.027
20	0	0.168	0.35	0	0	0
25	0	0.072	0.097	0	0	0
30	0	0.015	0.032	0	0	0

<sup>a</sup>Initial viable cell count before heat-treatment was 100,000/ml.

NP = Non-pathogenic strain.

O = No growth.

TABLE 2. Resistance of *E. coli* to different time-temperature combinations of heat-treatment in cow's whole milk.

Time of exposure (min)	50 C			55 C		
	<i>E. coli</i> strains					
	0111:B4	0127:B8	NP	0111:B4	0127:B8	NP
	Number of survivors (viable counts/ml) <sup>a</sup> × 10 <sup>3</sup>					
10	31.00	65.00	77.00	2.25	55.00	59.00
20	12.70	49.00	56.00	0.29	23.00	25.00
30	7.00	37.00	43.00	0.013	14.00	19.50
40	2.42	22.70	29.30	0	5.50	9.50
50	1.41	12.00	15.70	0	3.90	6.70
60	0.59	7.70	9.10	0	2.30	3.30
	60 C			63 C		
5	0.25	19.50	31.70	0	0.372	0.55
10	0	7.20	9.90	0	0.092	0.107
15	0	1.90	2.70	0	0.027	0.039
20	0	0.40	0.45	0	0	0
25	0	0.085	0.10	0	0	0
30	0	0.012	0.037	0	0	0

<sup>a</sup>Initial viable cell count before heat-treatment was 100,000/ml.

NP = Non-pathogenic

O = No growth.

TABLE 3. Resistance of *E. coli* strains to different time-temperature combinations of heat-treatment in buffalo's whole milk.

Time of exposure (min)	50 C			55 C		
	<i>E. coli</i> strains					
	0111:B4	0127:B8	NP	0111:B4	0127:B8	NP
	Number of survivors (viable counts/ml) <sup>a</sup> × 10 <sup>3</sup>					
10	41.70	70.00	80.00	5.30	59.00	62.00
20	20.50	50.00	61.00	0.78	27.00	29.00
30	10.00	41.50	46.00	0.041	16.30	23.00
40	5.90	23.60	30.33	0.005	7.50	11.60
50	2.35	13.00	19.00	0	4.70	8.30
60	0.97	9.10	9.60	0	3.50	4.80
	60 C			63 C		
5	0.175	27.50	43.00	0	0.540	0.950
10	0.007	11.00	17.50	0	0.100	0.215
15	0	0.30	5.90	0	0.022	0.045
20	0	0.98	1.10	0	0.003	0.007
25	0	0.205	0.317	0	0	0
30	0	0.06	0.10	0	0	0

<sup>a</sup>Initial viable cell count before heat-treatment was 100,000/ml.

NP= Non-pathogenic.

O = No growth.

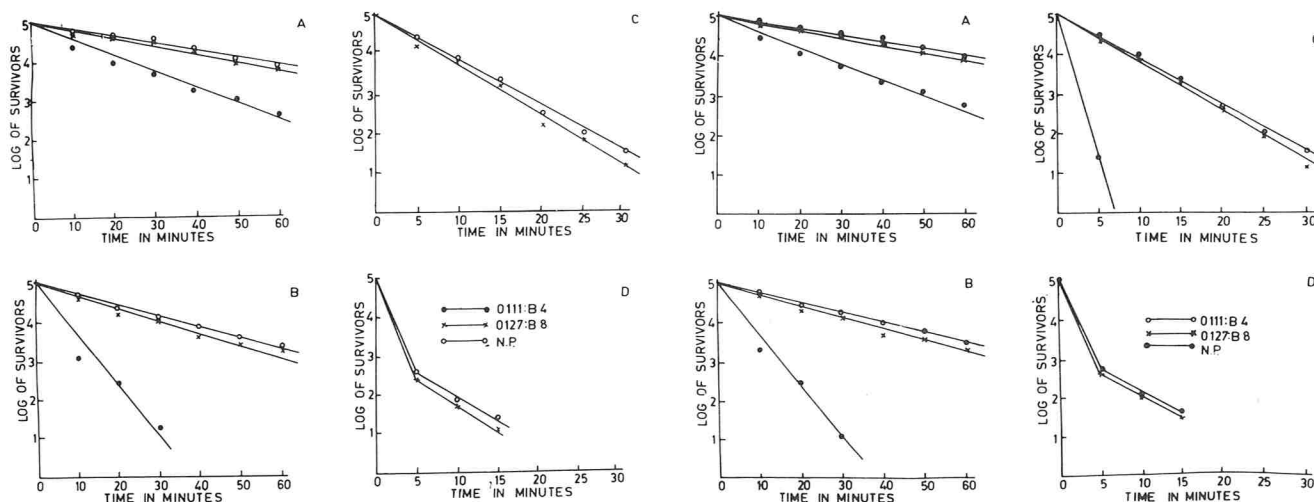


Figure 1. Thermal death rate curves of *E. coli* strains in cow's skim milk exposed to (A) 50 C, (B) 55 C, (C) 60 C and (D) 63 C.

Figure 2. Thermal death rate curves of *E. coli* strains in cow's whole milk exposed to (A) 50 C, (B) 55 C, (C) 60 C and (D) 63 C.

ences in D values were observed between the two types of milk.

A comparison of the D values obtained for the three *E. coli* cultures indicated that these values were much greater for 0127:B8 and NP than for 0111:B4. However, such differences in D values were more marked at lower than at higher temperatures of heat treatment (Table 4). Further, it was also observed that D values for buffalo's milk were higher than for cow's milk.

The thermal death time curves (z-values) of the three *E. coli* cultures in cow and buffalo milks are in Fig. 4 (a to c). These curves have been fitted by plotting the log of D values (obtained by graphic method only) against the temperature of heat treatment. On the above basis, a straight line of best fit was drawn. For purposes of comparison, a standard milk pasteurization curve has been plotted using the two time-temperature combinations normally used to pasteurize milk (145 F for 30 min

and 160 F for 15 sec). Results recorded in Table 4 (a to c) and Fig. 4 (a to c) show that the z-values for 0111:B4 were 8.3, 9.0 and 10.2 for cow's skim milk, cow's whole milk and buffalo's whole milk, respectively.

The z-values for 0127:B8 and NP were 17.5, 18.0 and 19.2 and 18.8, 19.0 and 20.3 F in the three milk substrates. The two pathogenic strains, 0111:B4 and 0127:B8, exhibited wide variations in their thermal resistance as evidenced by z-values. The non-pathogenic *E. coli* strain (NP) displayed greatest heat resistance among the three examined in this study.

## DISCUSSION

A comparative appraisal of the position and slope of the thermal death time curves of the three *E. coli* cultures with that of the standard pasteurization curve (Fig. 4) indicates that the curves of the two relatively

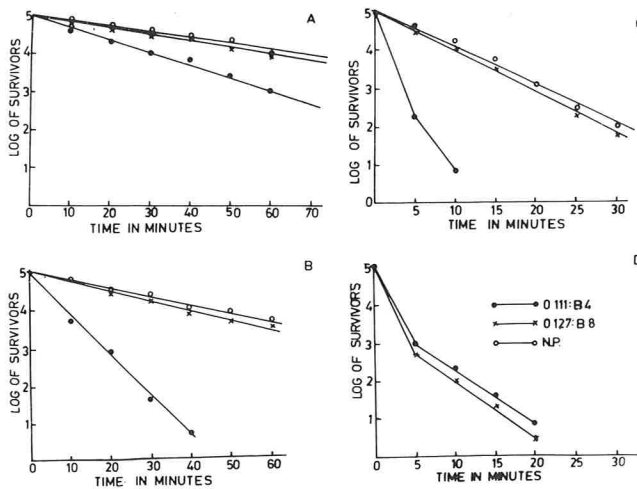


Figure 3. Thermal death rate curves of *E. coli* strains in buffalo whole milk exposed to (A) 50 C, (B) 55 C, (C) 60 C, and (D) 63 C.

heat-resistant *E. coli* cultures, 0127:B8 and NP, intersect the standard pasteurization curve, thereby indicating the possibility of their survival in buffalo's whole milk during the HTST process. In contrast to the behavior of those two cultures, the pathogenic *E. coli* culture 0111:B4 did not withstand pasteurization either in cow's or buffalo's milk. The results of the present study show that both pathogenic and non-pathogenic *E. coli* cultures are killed at 145 F for 30 min, but may survive 160 F for 15 sec. It should be pointed out in this connection that the two highly heat-resistant *E. coli* cultures 0127:B8 and NP, which had been isolated from pasteurized milk and cream samples, respectively, survived laboratory pasteur-

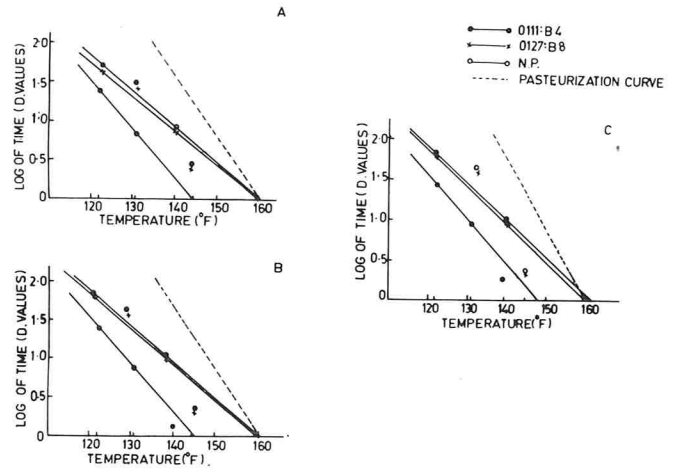


Figure 4. Thermal death time curves of *E. coli* strains in (A) cow's skim milk, (B) cow's whole milk and (C) buffalo's whole milk.

ization treatment both in the test tube and in the ampoule (12). These findings can be compared to similar observations made on the thermal destruction of *E. coli* strains by several workers (9,11,14). There are also some reports (4,13,14) on the greater heat resistance of organisms isolated from pasteurized dairy products, as compared to those isolated from raw dairy products. Some workers (1,5) have found loss in heat resistance of *E. coli* cultures on subsequent subculture. In the present study, we also found that the two *E. coli* cultures, 0127:B8 and NP, which had initially survived pasteurization at 63 C for 30 min were able to withstand heat treatment at 63 C for only 20 min on repeated subculture. This observation suggests the necessity of

TABLE 4. *D* and *z* values of *E. coli* strains suspended in different types of milk.

Temperature of heating (C)	Strains of <i>E. coli</i>					
	0111:B4		0127:B8		NP	
	Graphic method	Calculation method	Graphic method	Calculation method	Graphic method	Calculation method
a) Cow's skim milk						
50	24.00	20.58	47.00	50.37	54.00	51.87
55	7.00	5.53	30.00	27.32	35.00	34.49
60	0.00	0.00	7.80	7.79	8.80	8.37
63	0.00	0.00	2.00	1.78	2.10	1.91
<i>z</i> -value	8.30		17.50		18.80	
b) Cow's whole milk						
50	25.50	24.32	52.50	55.24	59.50	58.52
55	7.50	6.56	33.75	33.28	39.50	40.13
60	1.25	0.00	8.25	8.28	9.00	9.40
63	0.00	0.00	2.00	1.93	2.25	2.05
<i>z</i> -value	9.00		18.00		19.00	
c) Buffalo's whole milk						
50	30.00	30.74	58.00	57.81	65.00	62.22
55	9.00	8.54	36.50	36.87	41.50	44.71
60	1.75	1.55	9.25	9.76	10.50	11.12
63	0.00	0.00	2.20	2.28	2.50	2.67
<i>z</i> -value	10.20		19.20		20.30	

re-examining freshly isolated cultures for their heat resistance characteristics.

The results for the current study may be of interest to the dairy industry, since the variations in heat resistance of *E. coli* strains in cow and buffalo milks may significantly affect the subsequent keeping quality of milk and milk products. In view of the compositional differences between cow's and buffalo's milk (8,12), it is likely that the higher milk solids and fat content in buffalo's milk may be responsible for the greater protective effect offered by that substrate against destruction of *E. coli* cells. The importance of varying the amount of milk solids in influencing the heat-resistance characteristics of *Staphylococcus aureus* was recognized in milk products by earlier workers (7). In the light of observations reported in the present study as well as by earlier workers, it seems necessary to examine the adequacy of present time-temperature combinations of heat-treatment given to cow's milk for pasteurization of buffalo's milk.

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## Inhibition of Aflatoxin Biosynthesis by Organophosphorus Compounds

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### ABSTRACT

The effect of a range of organophosphorus and various other compounds on production of aflatoxin by *Aspergillus flavus* was investigated. Five organophosphorus compounds - Chlormephos, Ciodrin, Naled, Phosdrin and Trichlorphon- at concentrations of 20 and 100 µg/ml of culture fluid were found to have activity similar to Dichlorvos, in that they lowered the level of aflatoxin produced and caused formation of several anthraquinone pigments. Two of these pigments have not previously been described, one was named Versicol and a suggested structure is presented, whilst the other compound was shown to be its acetate derivative. A rationale is suggested for the required elements of structure, which are necessary for an organophosphorus compound to have Dichlorvos-type activity. Two unrelated compounds, ammonium nitrate and Tridecanone were also found to elicit Dichlorvos-type activity. It is likely that tridecanone or its breakdown products competitively inhibit enzymes involved in aflatoxin biosynthesis. It is possible that this inhibition effect explains the lowering of aflatoxin production in lipid-rich commodities infected by *A. flavus*.

Results from several studies have shown that the biosynthesis of aflatoxin B<sub>1</sub> (I) by *Aspergillus* species may be inhibited by a number of compounds, e.g. Dichlorvos (10), dimethyl sulfoxide (4) and sulfite (8). Dichlorvos (VIB) and the related compound Naled (VIC), at a concentration of 20 µg/ml of culture fluid reduced aflatoxin production with a concomitant appearance of versiconal hemiacetal acetate (II), suggesting that a specific step in the biosynthetic pathway had been blocked (12). This effect thus provided a powerful tool for further metabolic studies and also suggested a possible method for inhibiting aflatoxin biosynthesis in agricultural commodities infected with aflatoxin-producing strains of fungi.

As both Dichlorvos and Naled are organophosphorus insecticides capable of inhibiting acetyl choline esterase activity in insects, it was initially suspected that they inhibited esterase activity in aflatoxin-producing organisms and thus caused accumulation of the ester versiconal hemiacetal acetate (9). This hypothesis was supported by the observation that Dichlorvos does inhibit general esterase activity in cell-free extracts derived from *Aspergillus flavus*, although in similar cell-free extracts it was also noted that oxygenase activity was also inhibited (1). This latter observation lent credence to the view that oxygenase inhibition is principally responsible for the specific metabolic block in aflatoxin biosynthesis.

The investigation reported here explores the ability of various types of organophosphorus compounds to specifically inhibit aflatoxin biosynthesis. It was envisaged that the structural features required to be present in organophosphorus compounds for their specific inhibitor action upon aflatoxin biosynthesis would be identified. Predictions with regard to the specific inhibitory power of various organophosphorus compounds could then be deduced and this in turn may lead to a more effective control of aflatoxin formation in contaminated stores.

### MATERIALS AND METHODS

We are indebted to the companies who supplied the following pesticides: Chlormephos (Murphy Chemical Co.), Cyanthoate, Phen-thoate (Montedison) Ciodrin, Gardona, Phosdrin (Shell Chemical Co.), Dichlorvos, Trichlorphon (Bayer UK Ltd.), Dicrotophos, Monocrotophos, Phosphamidon (Ciba-Geigy), Durbusan, Nellite, Plondrel, Ronnel (Dow Chemical Co.) and Naled (Chevron Chemical Co.). Other chemicals were purchased as indicated: ammonium nitrate Analar (B.D.H.), 2- and 3- tridecanone (Koch Light) tributyl phosphate (Koch Light), trimethyl phosphate (Sigma). (See Fig. 1).

#### Cultures and inhibition studies

An aflatoxin-producing strain of *A. flavus* (N1) was maintained on potato dextrose agar and used throughout this study. The chemically defined medium of Reddy et al. (11) was used as the standard liquid culture medium. A 1-ml volume of spore suspension (approximately  $1 \times 10^6$  spores) was used to inoculate 70 ml of medium in a 250-ml conical flask, which was then incubated on a rotary shaker (150 revs/minute, 25 C). To avoid sporocidal action, the required potential inhibitor was added, (dissolved in a maximum of 0.4 ml of acetone), after 48 h of incubation, and at a range of concentrations, (for the purpose of this report only those concentrations at 20 µg/ml and 100 µg/ml are included). Usually there were at least four separate tests done for each inhibitor concentration. Control flasks were set up in the same manner but without addition of the potential inhibitor. Following a further 4-day incubation period the mycelium was filtered off and washed with acetone (10 ml), chloroform (20 ml) and ethyl acetate (10 ml) in sequence. These washings were then shaken with the culture filtrate and the organic layer was separated and dried over a minimum amount of anhydrous sodium sulfate. The culture filtrate was further extracted with ethyl acetate (20 ml) and this extract was used to wash the sodium sulfate which had dried the main extraction. Both extracts were combined and evaporated to dryness on a rotary film evaporator. The residue was dissolved in methanol and made up to 10 ml in a volumetric flask.

#### Analysis of extracts

The ultra-violet and visible absorption spectrum of the methanol solution of the extract was recorded on a Pye-Unicam 1800 spectrophotometer, (dilutions being made with methanol where necessary). A 50-µl sample of the methanol solution was spotted onto a silica gel-G thin-layer plate (0.25 mm thick) and this was developed

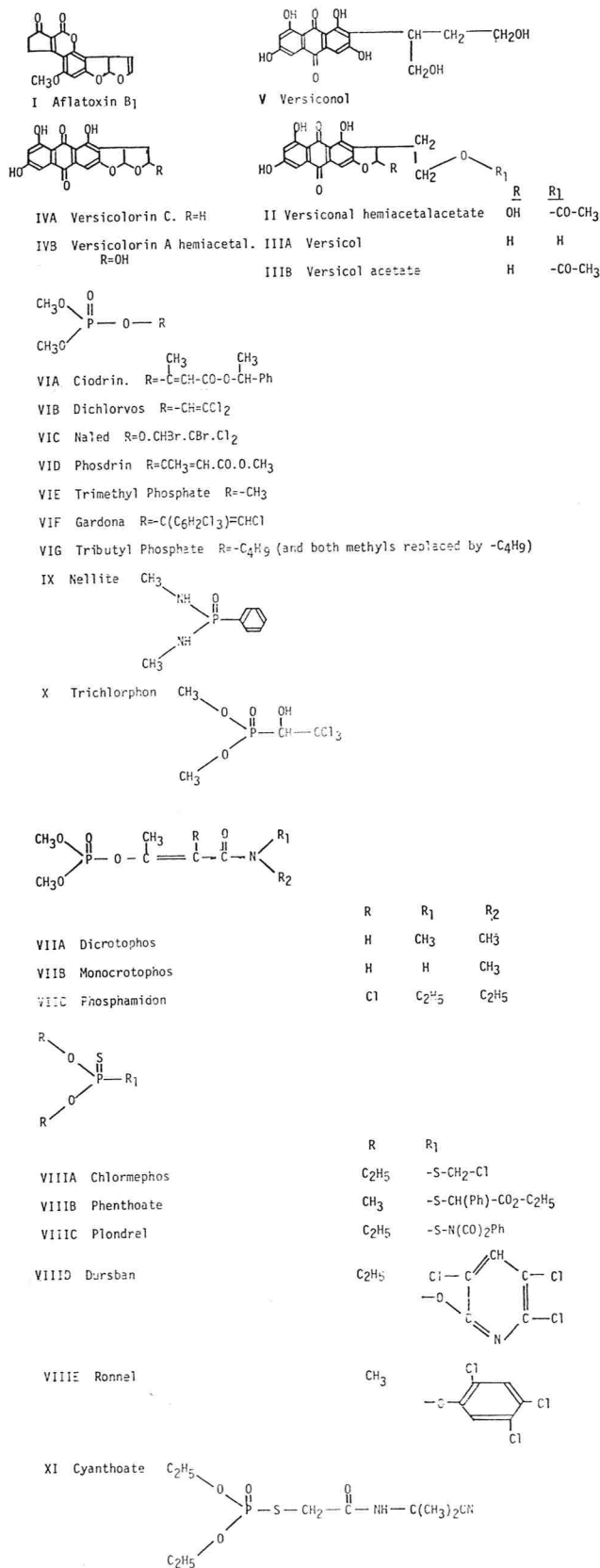


Figure 1. Identity of chemical compounds mentioned in the text. using toluene-ethyl acetate-acetone-acetic acid 50:35:15:2 v/v (TEAA) as the solvent system. Standard quantities of versiconal hemiacetal acetate, versicolorin C and aflatoxin B<sub>1</sub> were also chromatographed, and thus by comparison it was possible to ascertain the approximate

concentration levels of these metabolites in the original extracts. More accurate assessments of concentrations were made from the absorbance maxima in absorption spectra, aflatoxin being calculated in terms of aflatoxin B<sub>1</sub> ( $\lambda_{\max} = 363\text{nm}$ ,  $\epsilon = 21,000$ ) and anthraquinones in terms of versiconal hemiacetal acetate ( $\lambda_{\max} = 450\text{nm}$ ,  $\epsilon = 7,400$ ). In cases where larger amounts of anthraquinones were present, it was necessary to quantitatively isolate the aflatoxins by thin-layer chromatography and determine their concentrations separately since anthraquinones absorb to a moderate degree at 363 nm. Compounds were prepared for mass spectrometry by preparative thin-layer chromatography using silica gel G layers (0.5 mm) and various solvent systems depending upon the polarity of the compound to be isolated. Mass spectrometry was carried out on an A.E.I. MS9 mass spectrometer. Metabolites were identified by comparing their behavior on chromatography and mass spectrometry with that of authentic compounds.

## RESULTS AND DISCUSSION

The results presented in Table 1 are those obtained for organophosphorus compounds which exhibited similar inhibition of aflatoxin biosynthesis in *A. flavus* to that of Dichlorvos while those in Table 2 are the results for compounds which showed no action or had a general inhibitory effect on *A. flavus* metabolism. The addition of Dichlorvos to shake cultures of *A. flavus* resulted in formation of a group of seven related anthraquinone pigments. Three of these compounds were produced in relatively large quantities, i.e. versiconal hemiacetal acetate (II), versicolorin C (IVA) and an unknown pigment ( $R_f$  0.3 in the solvent system described; TEAA). The structure of this unknown compound, from mass spectral data (Table 3), is consistent with that of IIIA and the name versicol is suggested for the compound. The four other anthraquinones were all produced in smaller quantities; these included: averufin (14), averufanin (3), versiconol (V) and a fourth unknown compound ( $R_f$  0.4). On hydrolysis this unknown compound yielded versicol. Mass spectral and chromatographic properties were consistent with this compound being versicol acetate (IIIB). Versicol acetate may arise from versiconal hemiacetal acetate by direct reduction and thence to versicol by hydrolysis.

Examination of the structure of the pesticides capable

TABLE 1. Compounds shown to have similar inhibitory activity to Dichlorvos.

Compound added	Concentration added ( $\mu\text{g}/\text{ml}$ )	Aflatoxin concentration ( $\mu\text{mol}/\text{flask}$ )	Anthraquinone concentration ( $\mu\text{mol}/\text{flask}$ )	Mycelial dry weight (g)
Control	—	8.4	0.1	2.0
Dichlorvos control	20	2.5	1.8	2.0
Chloromphos	20	5.2	4.1	2.1
	100	2.5	6.2	1.9
Ciodrin	20	4.4	6.4	1.6
	100	3.0	9.4	1.9
Naled	20	2.2	2.7	1.8
	100	2.1	0.7	1.6
Phosdrin	20	3.1	3.6	2.0
	100	2.0	3.2	1.8
Trichlorphon	20	4.4	2.9	1.9
	100	5.0	8.0	1.8
Ammonium nitrate	150 mg/ml	2.5	5.3	0.9
2-Tridecanone	1.5 mg/ml	7.0	2.8	2.0
	7.5 mg/ml	2.5	5.0	2.5
3-Tridecanone	1.5 mg/ml	7.5	3.2	2.9
	7.5 mg/ml	3.1	6.4	3.0



TABLE 2. Compounds shown not to have similar inhibitory activity to Dichlorvos.

Compound added	Concentration added ( $\mu$ /ml)	Aflatoxin concent. ( $\mu$ mol/flask)	Anthraquinone concentration ( $\mu$ mol/flask)	Mycelial dry weight (g/flask)
Control	—	9.4	.1	2.1
Dichlorvos control	20	3.5	4.7	2.4
Cyanthoate	20	6.3	0.1	2.1
	100	3.0	0.1	2.8
Dicrotophos	20	9.3	0.2	3.0
	100	9.2	0.3	2.9
Durbusan	20	10.2	0.1	1.6
	100	10.1	0.1	1.4
Gardona	20	9.1	0.0	2.0
	100	9.1	0.0	2.0
Monocrotophos	20	1.4	0.4	1.2
	100	1.3	0.4	1.1
Nellite	20	10.5	0.1	1.4
	100	3.8	0.1	1.3
Phosphamidon	20	6.6	0.1	2.0
	100	3.8	0.1	1.8
Plondrel	20	6.8	0.1	1.9
	100	6.0	0.1	1.5
Ronnel	20	5.5	0.1	1.4
	100	3.3	0.1	0.9
Trimethyl phosphate	20	7.8	0.1	2.4
	100	11.9	0.1	2.9
Tributyl phosphate	20	10.0	3.2	2.1
	100	10.2	5.5	1.8

of eliciting pigment formation similar to Dichlorvos revealed that in general they possess two small alkyl groups, usually methyl, esterified to a phosphorus atom. Dichlorvos (VIB), Ciodrin (VIA), Phosdrin (VID) and Naled (VIC) have a third more complex group esterified to the phosphorus atom while Trichlorphon (X) has a carbon attached directly to this atom (i.e. it is a phosphonate compound). Finally Chlormephos (VIIIA) differed in that it has two oxygens replaced by sulfurs (i.e. it is a phosphorothiolate). A common feature in these compounds, however, is that they all possess an electron-deficient carbon atom one removed from the phosphorus atom. It is this character which presumably makes the phosphate ester bonds somewhat labile and therefore much more reactive towards target enzymes. Thus if an organophosphorus compound is to have Dichlorvos-like activity it must have at least this latter feature in its structure. Naled (VIC) is similar in

structure to Dichlorvos (VIB); however, its lower specific activity is probably due to the presence of a bulky bromine atom on the carbon adjacent to the ester oxygen. In other examples, bulky groups also appear to abolish Dichlorvos-type activity, e.g. a phenyl group as in Phenthoate (VIIIB), Gardona (VIF), Ronnel (VIIIE) and Nellite (IX) or as chloropyridine in Durbusan (VIIID). The presence of nitrogen also appears to affect activity, this is reflected in the lack of activity of Phosphamidon (VIIC), Monocrotophos (VIIB) and Dicrotophos (VIIA) in spite of the fact that they are very similar in structure to the active compound Phosdrin (VID).

From the evidence it appears that a phosphate ester group is involved in inhibition and therefore it seems likely that a group such as a serinyl hydroxyl is present in one part of the active site of the enzyme which is specifically inhibited. It seems therefore that the inhibition process is at least in part similar to that found in the inhibition of acetylcholine esterase by a phosphorylation process (7).

Simple alkyl phosphates, e.g. trimethyl phosphate (VIE), lack the ability to inhibit aflatoxin biosynthesis and this may be explained by the absence of the electron-deficient carbon atom present in the active compounds. However, there was one exception to the lack of activity in simple phosphate esters and this was tributyl phosphate (VIG). This compound did elicit pigment formation in *A. flavus* but the pigments formed did not correspond chromatographically to those produced by addition of Dichlorvos. Four compounds were isolated: versicolorin A, versicolorin C, versicolorin A hemiacetal (IVB) (6) [a compound which has been identified in cultures of *Aspergillus parasiticus* (1-11-105 Whl) in our laboratories], and a fourth compound ( $R_f$  0.76) which has not yet been identified.

When ammonium nitrate was added to the culture fluid (10-15% w/v), pigments similar to those produced by Dichlorvos treatment were extracted. This effect may be due to the simultaneous action of both ions since neither ammonium nor nitrate added individually produced the same effect. The addition of 2- or 3-tridecanone as solid (1 g) also resulted in Dichlorvos type

TABLE 3. Mass spectral data for various metabolites.

Peak heights quoted as percentage									
Versicolorin A		Versicolorin C		Versicolorin A hemiacetal		Versicol		Versicol acetate	
m/e	%	m/e	%	m/e	%	m/e	%	m/e	%
338	70	340	65	356	55	342	56	384	60
325	48	325	60	339	28	240	12	356	16
310	77	311	82	338	88	330	4	349	22
309	72	297	100	323	20	324	24	342	22
281	100			313	36	311	76	341	22
				311	85	309	26	340	20
				310	88	298	88	325	100
				309	100	297	100	324	100
						386	16	323	100
						385	12	311	60
								310	60
								309	100
								298	60
								297	100

action. At the temperature of the incubator (25 C) they melted into an oily layer which became visibly orange in color as the incubation period progressed. Eventually the tridecanone is metabolized, leaving behind an orange residue. It is possible that these compounds act by solvating the anthraquinones and hence allowing them to pass through the cell membrane which is normally impermeable, thus denying them as intermediates in aflatoxin biosynthesis. In addition it is also possible that tridecanones compete for oxygenases present in the hydrophobic regions of the fungal cell (cf. 5) and hence by a saturation effect limit the rate of anthraquinone metabolism. Such mechanisms are in accordance with other investigations which suggest that aflatoxin formation may be inhibited by naturally occurring long-chain compounds such as fatty acids (2).

It must be stressed that some of these conclusions are tentative and that compounds found to lack inhibitory activity may do so for a number of reasons, e.g. lack of penetration of the cell membranes, fungal detoxification (13) etc. Thus it is evident that further investigations are required before the specific inhibitory processes in aflatoxin biosynthesis are to be fully understood and it seems likely that this may only be achieved by utilizing cell-free systems.

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## Relationships of Microbial Quality of Retail Meat Samples and Sanitary Conditions<sup>1</sup>

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### ABSTRACT

A sanitation profile scoring form for evaluating sanitation in retail food stores was designed. The profile was tested in 10 Oregon retail markets to evaluate its ability to reflect sanitary conditions. At the time of inspection, samples of meat processed in-store were purchased for microbiological analysis to explore the feasibility of bacterial quality as a measurement of sanitary conditions. Microbiological tests performed included total aerobic plate count (APC), coliform, *Escherichia coli*, *Staphylococcus aureus*, *Clostridium perfringens*, and *Salmonella*. Certain deficiencies were noted in the profile designed; however, it provides a means for objective, uniform measurement of sanitary conditions. Data show no correlation exists between microbiological quality of products processed in the store and total store profile sanitary conditions. Fifty percent of the products sampled exceeded bacterial load guidelines currently enforced in Oregon. These "high" counts appear to be directly related to poor temperature control.

Food shoppers are concerned about cleanliness and food quality in selecting a food store. A recent food shopping survey (6) showed that food quality and store cleanliness were important considerations to shoppers in selecting a store. Terms used to describe these considerations by the shopper included "good food", "dependable quality", "clean store appearance", "neat and cleanliness", and "an orderly store that smells clean". Shoppers also indicated they felt the stores they patronized were doing a good job in keeping their stores clean and neat.

Practicing good sanitation and safe food handling procedures are ways the food market can reduce spoilage and loss. A good sanitation program prevents food contamination, protects quality and increases consumer acceptability. Customers are impressed with the aesthetic considerations and the more obvious aspects of a sanitation program - that is, a clean store and personnel in the store giving an impression of neatness and cleanliness as well as improved food safety and product quality.

Many government regulatory agencies are concerned about enforcing sanitation regulations. A Federal ordinance proposed by the U.S. Food and Drug Administration establishes a model sanitation program for retail stores to be adopted uniformly across the country (4). A sanitation program may include identification and recording of the source and condition

of foods received, the temperature at which potentially hazardous foods are held, facilities for hand washing, insect and rodent control, specifications for floor and wall materials and personal hygiene.

It is generally agreed that clean and sanitary implies a piece of equipment or room is free from dirt, soil and harmful microorganisms. However, individual judgments on cleanliness and sanitation do vary and uniformity is not always achieved. In some instances, regulatory agencies attempt to perform objective measurements rather than subjective. Analyses of food products for microbial quality are used in an attempt to establish a quantitative measurement of the sanitary conditions. Regulatory personnel are unable to inspect retail markets at intervals sufficiently frequent to provide them with evidence that good sanitation practices are being followed. Therefore, these personnel are inclined to look to microbial quality analyses as a substitute (9).

The question arises-do these microbiological analyses provide information on the sanitary conditions of the store? Counts above certain limits have been used by regulatory agencies as "a means of identifying poor sanitation practices" (9). Many questions have been raised in regard to such assumptions, but certain ideas are lacking in this area. What microbial quality of product should we expect under good manufacturing practices coupled with suitable retail case storage? How can the inspector communicate his findings to management? What aspects of sanitation inspection reflect sanitary conditions and what relationship do these factors have to product quality? What is important to public health?

The objectives of this research were: (a) to develop a model scoring system or profile that could be used by regulatory personnel and store employees alike to give a uniform reflection of the sanitary conditions in the retail store, and (b) to determine if a correlation exists between sanitary conditions and the bacterial quality of meat products.

### MATERIALS AND METHODS

#### *Profile development*

A model sanitation profile was designed taking into consideration the state sanitation requirements (8), score sheets used in FDA inspections in food service establishments (11) and requirements included in the proposed FDA model ordinance for food store sanitation (4). The factors delineated in the profile were; cleanliness of

<sup>1</sup>Oregon Agricultural Experiment Station Technical Paper Number 5235.

facility inside and out, water source, plumbing, lighting, restrooms, sewage, ventilation, evidence of rodents/insects, condition of foods, temperature controls and sanitation procedures. All departments of the store (meat, bakery, produce, delicatessen, dairy and general grocery) were scored individually. For each department, the above factors were evaluated for the display, preparation and storage areas, temperatures, load lines, code dates, cleanliness of equipment, food contact surfaces, proper separation of food, food protection, personal hygiene, hand washing facilities and garbage disposal. A scoring system was devised. A maximum score of 100 was possible and the factors considered most significant from a public health point of view were weighted with a range of 1 to 3. The "sanitation index" is the actual score obtained out of 100 maximum points possible.

#### Selection of stores

Ten stores were selected to represent a cross-section of the major supermarket chains and independents in the state. The stores selected for inspection were located within 1-h driving time from the laboratory to facilitate prompt delivery of samples. Temperatures of the products were measured and recorded at the time of purchase and at time of receipt in the laboratory.

The store manager was always contacted before proceeding with the survey. The purpose and intent of the activity were carefully explained and discussed with store personnel. In most instances, the store manager accompanied the survey team during the inspection. A copy of the recorded data, observations and sample analyses was sent to the store.

#### Temperature

Temperatures of product storage room and display cases were taken with a YSI model 42SF Telethermometer. The use of a flat surface probe readily permits temperature measurement of the outer surface of the container without product destruction as well as measurement of room or air stream temperatures. The temperature was observed on the direct reading dial of the electronic thermometer in approximately 20 sec. The temperature was then recorded on the sanitation profile form.

#### Collection of samples

At least two samples, as offered for sale, were obtained directly from the retail case at the completion of an inspection tour. One of these was ground meat and the other chopped cubed steak or pork sausage depending upon which product was prepared and available in the store. The samples were transported to the laboratory in insulated chests containing ice packs. Samples were prepared for microbial analyses immediately upon receipt in the laboratory.

#### Microbiological methods

A 50-g sample of each test product was weighed into a sterile blender jar containing 450 ml of sterile 0.1% peptone water. A second 50-g portion of the product was weighed into a blender jar containing 450 ml of lactose broth. The product was blended at high speed for 2 min. Subsequent 10-fold dilutions were prepared with 0.1% peptone water.

The procedures for total aerobic plate count (APC), coliforms, *Escherichia coli* and *Staphylococcus aureus* were according to those published in the Food and Drug Administration *Bacteriological Analytical Manual* (3).

TABLE 1. Sanitation indices<sup>1</sup> of ten retail food markets in Oregon.

Store number	Total score	General	Meat	Bakery	Produce	Dairy	Grocery
1	74.8	80.0	76.5	N/A <sup>2</sup>	87.0	57.9	62.5
2	85.6	88.6	89.7	N/A	87.0	80.0	75.0
3	75.8	84.8	79.4	N/A	69.0	73.7	64.7
4	89.9	91.4	100.0	64.5	98.2	94.7	94.1
5	92.2	94.3	95.6	93.5	100.0	89.5	72.5
6	84.4	88.6	95.6	N/A	82.4	76.3	67.5
7	85.5	87.1	98.5	N/A	75.9	86.8	72.5
8	85.5	90.3	92.6	N/A	87.0	78.9	70.0
9	85.3	90.0	91.2	80.6	96.4	76.3	67.3
10	77.2	80.0	86.8	67.7	91.1	68.4	60.0

<sup>1</sup>Sanitation index = actual score obtained out of 100 possible.

<sup>2</sup>N/A - not applicable.

#### Salmonellae

A 3-ml volume of 1 N NaOH was added to the original 10<sup>-1</sup> dilution of product in lactose broth. After incubation for 24 h at 35 C, a 1-ml portion was transferred to tubes containing 10 ml of selenite cystine broth and 10 ml of tetrathionate (TT) broth. These tubes were incubated 24 h at 35 C after which time loopfuls of culture were streaked onto brilliant green agar and XLD agar. Selective differential agar plates were incubated 24 h at 35 C; colonies typical of salmonellae (10) were subjected to confirmatory testing using the Analytab Products API 20 E system (1).

#### Clostridium perfringens

Ten ml of the 10<sup>-1</sup> peptone water dilution were divided among 3 plates which were poured with tryptose-sulfite-cycloserine (TSC) agar. The plates were incubated at 35 C for 24 h in a BBL anaerobic Gas Pak system. Typical colonies were counted and transferred to tubes of fluid thioglycollate broth which were incubated 4-6 h in a 45.5-C water bath. Portions of the growth from gassing tubes were streaked onto plates of trypticase-sucrose-egg-yolk-agar (TSEY). These were incubated anaerobically at 35 C overnight. A second purification was effected by transferring typical *C. perfringens* colonies to tubes of fluid thioglycollate medium for incubation at 45.5 C for 4-6 h. A loopful of inoculum from the gassing tubes was transferred to lactose-gelatin medium, sucrose broth, motility-nitrate medium and Bactotryptone broth for confirmation of *C. perfringens* (10).

#### Data analyses

The data were subjected to statistical analysis. Pearson coefficients were calculated comparing each profile score to total microbiological quality and absence or presence of pathogens (7).

## RESULTS

### Inspections

The profile was tested in 10 retail markets for its ability to measure objectively sanitary conditions in retail food markets. Table 1 shows the findings of the profile for the stores inspected. Not all stores inspected had all the various departments provided for in the profile. If the store did not have certain sections, it was considered not applicable (N/A) and was disregarded in determining the over all score possible. Each inspection required an average of 2 h. The total scores ranged from a low of 74.8 to a high of 92.9 out of a possible 100. No attempt was made to determine what should be an acceptable or unacceptable score. Further work and refinement of the profile form will be needed before such a judgement can be made.

No particular problem was noted in the areas of construction, water source, plumbing or sewage water disposal among the stores inspected. Failure to have proper shields on lighting fixtures was noted occasion-

ally. In the various sections, particularly warehouses, rodent and insect infestations were observed. Proper measures were not taken to prevent the infestation.

Most of the stores surveyed employ exterminators who use bait stations, particularly in warehouse sections. However, it is felt more could be done to prevent the entrance of rodents into the retail store, instead of simply trying to take care of the problem once they have it.

Lack of proper temperature control was mainly responsible for low sanitation index scores. In most instances, thermometers were present and visible in retail display cases and storage rooms, as required by regulations, but were often inaccurate. In scoring, points were allotted if thermometers were present and visible as required by law; however, the emphasis was on product temperatures and accurate and reliable thermometers.

Results of each inspection were made available to store management. The form was useful as a means of communicating results of an inspection. One of the criticisms of the present regulatory inspections in Oregon is the lack of uniformity among inspectors and quantitative measurements. A scoring sheet achieves uniformity and helps management know exactly how their operational procedures are evaluated.

Table 2 shows the relationship of temperature control and sanitation indices. "A" reflects "average" where conditions of foods held under refrigeration met minimum requirements of Oregon law, that is, products and storage conditions in the range of 45 and 0 F. "S" was assigned to a store if its temperature control was regarded as being "superior", in that all observed temperatures were 35 F or below and -10 F or below. "U" was assigned to the store if its temperature control program was "unsatisfactory" or if product or areas were above 45 or 0 F. It was an all or none assignment. For instance, many stores had good conditions for unfrozen, refrigerated foods but the frozen foods would be above 0 F. The temperatures checked were for potentially hazardous foods only (i.e., frozen foods, meat, dairy

products and delicatessen items). The ability of a store to achieve a good temperature control program is reflected in the total "sanitation index". For example, stores having an "unsatisfactory" temperature control program, had a "sanitation index" of 81.3. Similarly, in the meat area the stores rated as superior had an average sanitation index of 95.6.

#### Microbiological analyses

Table 3 shows the results of the microbiological examination of the meat samples. Oregon guidelines for fresh raw meat products consider a total aerobic plate count in excess of  $5 \times 10^6$  organisms/g to be a violation. Five of 10 samples of ground beef failed to meet these guidelines. Two of 6 pork sausage samples failed and 2 of 5 chopped beef samples.

Table 4 shows the results of the items sampled for *E. coli* levels. The state's guidelines consider a product in violation if the number of *E. coli* is in excess of 50 MPN/g. Only 2 of the 10 samples of ground beef exceeded the guidelines, and all samples of pork sausage and chopped beef were in compliance with the guidelines.

Table 5 shows the results of the microbiological examinations of the products for the presence of pathogens. It is generally agreed that large numbers of *S.*

TABLE 3. Microbial quality of retail meat samples.

Store number	Ground beef (APC/g)	Pork sausage (APC/g)	Chopped beef (APC/g)
1	$3.4 \times 10^6$	$32.0 \times 10^6$ *	$5.4 \times 10^6$ *
2	$1.2 \times 10^6$	—	$9.5 \times 10^3$
3	$5.3 \times 10^5$	—	$9.7 \times 10^5$
4	$7.0 \times 10^6$ *	—	$1.5 \times 10^5$
5	$12.0 \times 10^6$ *	$1.3 \times 10^6$	—
6	$16.0 \times 10^6$ *	$45.0 \times 10^6$ *	—
7	$5.0 \times 10^5$	$5.6 \times 10^5$	—
8	$11.0 \times 10^6$ *	$4.3 \times 10^6$	—
9	$5.0 \times 10^5$	$1.4 \times 10^6$	—
10	$17.0 \times 10^6$ *	—	$13.0 \times 10^6$ *
*Exceeded guidelines (8):	5/10	2/6	2/5

TABLE 2. Relationship of sanitation index<sup>1</sup> and temperature control.

Total store			Meat department		
Store number	Sanitation index	Temperature rating <sup>2</sup>	Store number	Sanitation index	Temperature rating <sup>2</sup>
1	74.8	U	2	89.7	U
2	85.6	U	3	79.4	U
3	75.8	U	7	98.5	U
6	84.4	U	10	86.8	U
7	85.5	U	Av.	88.6	
8	85.5	U	1	76.5	A
10	77.2	U	8	92.6	A
Av.	81.3		Av.	84.6	
4	89.9	A	4	100.0	S
9	85.3	A	5	95.6	S
Av.	87.6		6	95.6	S
5	92.2	S	9	91.2	S
Av.	92.2		Av.	95.6	

<sup>1</sup>See Table 1.

<sup>2</sup>U = unsatisfactory, above 45 F, above 0 F.

A = average, 45 F, 0 F.

S = superior all areas, 35 F or below, -10 F or below.

TABLE 4. Levels of *E. coli* in retail meat samples.

Store number	Ground beef (MPN/g)	Pork sausage (MPN/g)	Chopped beef (MPN/g)
1	21	43	15
2	21	—	15
3	7	—	< 3
4	4	—	< 3
5	93*	< 3	—
6	43	3	—
7	43	43	—
8	23	43	—
9	93*	< 3	—
10	4	—	4
*Exceeded guidelines (8):	2/10	0/6	0/6

*aureus* in foods signify a potential food hazard. According to Zaki et al. (12), foods containing numbers in excess of 100/g would be considered contaminated. We chose a level of 10 MPN/g for ranking samples since this is the minimum accuracy level of the method and MPN tables. Five of the 10 samples of ground beef contained numbers greater than 10. One of 6 pork sausage samples and none of the chopped beef samples contained counts of *S. aureus* greater than 10 MPN/g.

The presence of any pathogen in a food product is considered to be a potential health hazard (2). Therefore, salmonellae and *Clostridium perfringens* were reported as being present or absent in the samples. Only 2 of the pork sausage samples contained salmonellae (see Table 5); both of these samples were obtained from the same major food chain within a week. The meat market managers were immediately notified and the product resampled. It was determined that pork for both stores came from the same supplier. Several weeks later subsequent samples were found to be negative for salmonellae. *C. perfringens* was detected in 2 ground beef samples, 1 pork sausage sample and 1 chopped beef sample.

Table 6 shows the relationship of total aerobic plate count (APC) compliance and temperature ratings at the time of sampling for ground beef. The products were listed as to whether they exceeded the standard of

TABLE 6. Relationship of APC and temperature ratings of meat storage and display areas.

Store Number	Ground beef	
	APC (exceeded guidelines) <sup>1</sup>	Temperature rating <sup>2</sup>
1	NO	A
2	NO	U
4	YES	S
5	YES	S
6	YES	S
8	YES	A
10	YES	U
3	NO	U
7	NO	U
9	NO	S

<sup>1</sup>Reference No. 8.

<sup>2</sup>S = Superior all areas, 35 F or below, -10 F or below.

A = Average, 45 F, 0 F.

U = Unsatisfactory, above 45 F, above 0 F.

5 million organisms/g or not, and if the temperature was rated as superior (S), average (A) or unsatisfactory (U) based upon previous established criteria.

The bacterial findings were studied in relation to various determinants, namely the storage or display temperatures and sanitary conditions at the time of sampling. The bacterial counts and the sanitation index were subjected to regression coefficient analyses. Attempts to correlate the sanitation index with total aerobic plate count, coliform, *E. coli*, and presence or absence of pathogens for all products (ground beef, pork sausage, and chopped beef) were conducted.

## DISCUSSION

There is considerable interest in establishing microbiological standards for perishable foods. Regulatory agencies suggest high microbial levels in foods are associated with low-quality raw materials and/or flaws in processing, storage or distribution. The State of Oregon enforced bacterial standards and now has guidelines on maximum bacterial levels in raw and processed meat products. The results of these analyses and sampling program are considered to be "tools" for sanitation inspections (9).

TABLE 5. Presence of pathogens in retail meat samples.

Store number	Ground beef			Pork sausage			Chopped beef		
	<i>S. aureus</i> (MPN/g)	Salmonellae	<i>C. perfringens</i>	<i>S. aureus</i> (MPN/g)	Salmonellae	<i>C. perfringens</i>	<i>S. aureus</i> (MPN/g)	Salmonellae	<i>C. perfringens</i>
1	100*	—	—	< 10	—	—	< 10	—	—
2	< 10	—	—	< 10	—	—	< 10	—	—
3	< 10	—	—	< 10	—	—	< 10	—	—
4	10*	—	—	< 10	—	—	< 10	—	+
5	100*	—	+	< 10	—	—	< 10	—	—
6	< 10	—	—	< 10	—	—	< 10	—	—
7	10*	—	—	< 10	+	+	< 10	—	—
8	< 10	—	+	< 10	+	—	< 10	—	—
9	10*	—	—	10*	—	—	< 10	—	+
10	< 10	—	—	< 10	—	—	< 10	—	—
*Exceeded guidelines (8):	5/10	0/10	2/10	1/6	2/6	1/6	0/5	0/5	1/5

Results of this study indicate there is no correlation between microbial quality of retail meat products and the sanitary conditions of the store as reflected by a sanitation profile for a limited number of stores. All of the *r* values calculated were below the 5 and 1% levels of significance. Observations of the survey team indicate attitudes of personnel and careful attention to product temperatures appear to have a more significant influence on product quality. The model sanitation profile provides a means for objective measurements and adequately communicates the information, even though certain deficiencies were discovered in the model sanitation profile used in this study.

Sanitation deficiencies were noted in several bakery sections. Continued work is needed to relate the significance of these observations to public health.

No clear relationships between sanitary conditions and product quality were established. Many factors other than sanitary conditions of the store affect meat quality, such as conditions of slaughtering, processing, storage and distribution. The study was designed to model as closely as possible current inspection practices. Therefore, without complete knowledge of the whole chain of pre-retail conditions, it is difficult to determine the influences of retail level conditions on meat quality.

Refrigeration equipment in use today goes on "defrost" regularly and in some stores this occurs 3-4 times over a 24-h period. Recommendations have been put forth to hold frozen foods at -18 C (0 F) for maximum retention of their quality and nutrients (5). In some instances in this study, where refrigeration cases were set to meet minimum requirements of the law (i.e. 45 and 0 F), product temperature would rise quickly during the defrost cycle and be above required

temperatures. However, the total significance of this temperature fluctuation on safety, nutritive value and quality is not known.

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## Flavor and Texture of Cottage Cheese Made by Direct-Acid-Set and Culture Methods<sup>1</sup>

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### ABSTRACT

Consumers were asked to indicate a flavor and texture preference for one of two creamed and salted small-curd cottage cheeses made by direct-acid-set or conventional short-set culture methods. When a commercial starter distillate flavor was added to direct-acid-set cheese, its flavor and texture were preferred over cheese made by the culture method. Conversely, when flavor was not added to the direct-acid-set cheese, consumers preferred the flavor and texture of the cultured product. Differences among mean flavor or texture scores of three age groups were not significant (< 20, 20-40, > 40). Preferences for texture appeared to be biased by flavor.

The direct-acid-set (DAS) method of manufacturing cottage cheese, a relatively new process, is gaining rapid acceptance by the industry (2). Several advantages for it are claimed over the conventional culture method. White and Ray (6) reported reductions of 67% in cutting time and 60% in cooking time compared with the culture method. Sharma et al. (4) reported a significantly greater yield ( $P < .001$ ) for direct-acidification. Satterness et al. (3) also reported yield increases but not significant ( $P < .05$ ) differences.

Questions have been raised regarding flavor and texture of DAS cottage cheese. Gerson (2) reported that cheese made by the DAS process was judged 1st in flavor and texture in Michigan over 14 cultured cheeses. But two others (1,6) reported DAS cheese less desirable in flavor than cultured cottage cheese. The opposite reports may stem from differences in dressings used in the products. Starter distillate flavor was added to the Michigan cheese dressing, but apparently not to the cheese judged less desirable (1,6). We attempted to determine if consumers preferred cottage cheese made either by the DAS or by the conventional short-set culture method. We used direct-acid-set cheese made both with and without commercial starter distillate flavor added to the dressing.

### MATERIALS AND METHODS

We made small-curd cottage cheese by two methods: the conventional short-set culture and the Vitex/American DAS method (5) as described by Sharma et al. (4). In Experiment 1, we made 14 batches of cottage cheese, seven by each method, using skim milk with

$3.1 \pm .15\%$  protein and dressed with 44.2 kg of cream and 1.44 kg of salt per 100 kg of curd. The cream contained 13.5 to 14% fat and 0.3% stabilizer. In Experiment 2, we again made 14 batches, seven per method and used skim milk with  $3.9 \pm .15\%$  protein and with 3.52% protein for one batch by each method. All cheeses were dressed as in Experiment 1, except the recommended 3.2 ml of starter distillate flavor (supplied by Vitex/American) was added per 100 kg of the DAS curd. Distillate flavor was thoroughly mixed with the cream before dressing the curds.

Cartons were packed with 450 g of creamed cottage cheese. One carton each of DAS and cultured cheese were paired. In each trial, one of the two types of cheese in the pair was randomly assigned the code A or B (or 1 or 2); the other type received the other letter or number. Approximately 30 pairs from each trial were sold to consumers through the Kansas State University Dairy Bar at half the prevailing retail price; consumers completed an evaluation form (Fig. 1) and returned it to the researchers. The number of responses varied, depending on family size, from trial to trial. For every trial, all responses in each age group were added and percentages for culture, DAS, and no preference were computed for age groups.

The data were analyzed statistically by comparing the consumer response frequency in each category with the expected frequency using the chi-square test. The expected frequencies were obtained under the null hypothesis of random selection of categories. Tests were conducted: (a) by consumer age groups, (b) summed over age groups, (c) for the three response groups (Culture, DAS, and NP) and (d) for the culture and DAS response groups only, i.e. if a person preferred Culture or DAS was he or she equally likely to select Culture or DAS? and (e) for texture and flavor responses for each of the two experiments.

Cottage Cheese Evaluation Form

In the circle write the number of persons in each age group in the family preferring that sample.

Flavor	A	B	No Preference
I prefer sample			
a. Age group less than 20 years	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
b. Age group 20 - 40 years.	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
c. Age group over 40 years.	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
<b>Texture</b>			
I prefer sample			
a. Age group less than 20 years.	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
b. Age group 20 - 40 years	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
c. Age group over 40 years.	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>

Note: The code will be changed randomly from week to week.

Figure 1. Form used to evaluate cottage cheese.

### RESULTS AND DISCUSSION

Responses totaled 541 in Experiment 1 and 702 in Experiment 2. Table 1 presents flavor and texture preferences of each of the three age groups.

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TABLE 1. Consumer responses (%) by age groups to cultured and DAS cottage cheese<sup>1</sup>.

Product	Customer age groups, years				Customer age group, years			
	< 20	20 - 40	> 40	Total	< 20	20 - 40	> 40	Total
<b>Experiment I</b>								
	Flavor preference, %				Texture preference, %			
Culture	49	43	44	45	40	40	45	42
DAS	32	31	32	32	29	33	32	32
NP <sup>2</sup>	19	26	24	23	31	35	24	27
Total	158	209	174	541	158	209	174	541
<b>Experiment II<sup>3</sup></b>								
Culture	27	30	28	29	27	30	28	28
DAS	55	62	61	59	47	53	54	51
NP	18	8	11	12	26	18	18	21
Total	242	238	222	702	242	238	222	702

<sup>1</sup>The totals are frequencies, values in the table are percentages.

<sup>2</sup>NP = no preference

<sup>3</sup>Commercial starter distillate flavor was added to DAS cheese in Experiment II.

A significantly higher proportion of consumers preferred the flavor and texture of cheese made by the culture method when no flavor was added to DAS cottage cheese (Experiment 1). However, when flavor was added to DAS cheese (Experiment 2), a dramatic reversal was observed; a significantly larger proportion of consumers ( $P < .001$ ) preferred DAS cheese. The proportion of consumers choosing the no preference (NP) category decreased significantly,  $P < .001$ , when flavor was added to direct-acid-set cheese (Experiment 1 = 23%, Experiment 2 = 12%, Table 1). The increased confidence of consumers showed that differences between cultured and direct-acid-set cheese were better defined in Experiment 2.

Consumer trends for texture closely followed those for flavor. As the culture and DAS cheese were manufactured the same in Experiment 1 as in Experiment 2, differences in response to texture must have been influenced by the added flavoring in Experiment 2, so it appears that the average consumer is not a reliable judge of cottage cheese texture.

Consumers preferred DAS cheese over cultured

cottage cheese when starter distillate flavor was added to the DAS cheese; when flavor was not added to DAS cheese, consumers preferred cultured cottage cheese. Data for each group gave the same conclusions.

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## Effects of Carton Material and Storage Temperature on the Flavor of UHT-Sterilized Milk<sup>1</sup>

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### ABSTRACT

Ultra-high-temperature sterilized milk packaged in aluminum foil-lined or plain polyethylene-lined cartons was stored 1.5 months at 4 C in the dark or 22 C under fluorescent light. Five trained judges found milk at 4 C was not as stale and thus had a more acceptable flavor than milk at 22 C. Flavor of milk in aluminum foil-lined cartons was not as good as freshly pasteurized milk, but superior to milk in polyethylene-lined cartons. Increases in off-flavor intensity paralleled increases in concentrations of n-pentanal and an unidentified neutral volatile compound. A 24-member, untrained consumer taste-panel preferred the flavor of freshly pasteurized milk to 3-month-old commercial or 6-month-old experimental ultra-high-temperature sterilized milks in aluminum foil-lined cartons, which in turn were preferred over 6-month-old experimental ultra-high-temperature sterilized milk in polyethylene-lined cartons.

Ultra-high-temperature (UHT) sterilized milk has made strong inroads into European fluid milk markets (7), and it is gaining popularity in Canada (1). In Italy and Germany, an estimated 50% of the fluid milk consumed in 1976 was UHT (7). The reluctance of American dairymen to follow the Europeans and Canadians stems from concern that U.S. consumers will not accept the flavor of UHT milk. The problem likely to limit acceptance of UHT milk in the U.S. is the off-flavor it develops when stored for prolonged periods. Packaging the UHT milk in aluminum foil-lined cartons (AC) rather than polyethylene-lined cartons (PC) (3,6) and storing at refrigeration temperatures (2,4,5,8) helped control the rate that off-flavor developed. However, effects of combining different storage temperatures and different types of carton materials (with and without aluminum foil-lining) on the flavor of UHT milk have not been determined.

This research was to establish effects of carton material and simulated commercial storage conditions on the flavor of UHT milk and to relate changes in neutral volatile compounds in stored milk to the changes.

### MATERIALS AND METHODS

The cheapest and most practical method of storing and displaying UHT milk in grocery stores would be in PC at room temperature under fluorescent light (approximately 2000 lx). However, "ideal" storage conditions most likely involve refrigerating AC milk in the dark.

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We compared properties of UHT milk stored under "ideal" conditions with the same milk under simulated grocery-storage conditions.

Three batches of raw milk were UHT-sterilized (140 C for 3.5 sec) and each was packaged aseptically in Pure Pak AC or PC as described previously (6). Half the cartons were stored at  $22 \pm 2$  C and exposed to 2150 lx (200 ft candles) of cool-white fluorescent light; the other half, at  $4 \pm 2$  C in the dark; both light exposure and storage temperature are known to affect chemical and flavor changes in stored milk. Reference milk (R) was freshly pasteurized at 77 C for 15 sec either on the day of testing or the day before. Limitations in capacity to handle samples necessitated testing the 1½-month-old milks at different times (42 days at 22 C and 50 days at 4 C). Since different reference milks were required for the 42-day (22 C) and 50-day (4 C) samples, there are two entries for R-milk in Table 1. In Fig. 1, those bars designated "Untreated a" depict results for freshly pasteurized milks tasted and compared to UHT milks stored at 22 C. Those designated as "Untreated b" represent freshly pasteurized milks compared to UHT milks stored at 4 C.

Microbial, organoleptic and gas chromatographic (GLC) analyses were conducted as described by Mehta and Bassette (6). Five trained judges used a modification of the National Collegiate Student Judging Contest procedure to assign flavor scores to the milks; stale and cooked flavor intensities were scored on a scale of 0 = none to 9 = pronounced (6). GLC was used to quantify neutral volatile compounds (6). The model used for variance analysis of the randomized complete block design was:

$$Y_{tb} = \mu + \alpha_t + \beta_b + E_{tb}$$

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

$\mu$  = mean

$\alpha_t$  = treatment effects (AC 4C = AC stored at 4 C; AC 22C = AC stored at 22 C; PC 4C = PC stored at 4 C; PC 22C = PC stored at 22 C; R 4C = reference milk examined with UHT milk at 4 C; and R 22C = reference milk examined with UHT milk at 22 C)

$\beta_b$  = block effects (blocks 1-3)

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

In the second phase of the study, a taste-panel of untrained consumers compared the flavors of the last batch of our AC- and PC-milks to those of a Canadian commercial UHT milk and a freshly-pasteurized reference milk. The Canadian milk contained 2% milkfat, was sterilized by an indirect process and aseptically packaged in Tetra Brik AC carton. When presented to the panel, it had been stored at room temperature 90 days. Our UHT experimental milks were stored for 170 days at room temperature. The 24-member consumer taste panel consisted of almost equal numbers of men and women (students and faculty at Kansas State University). Panel members were asked to grade each coded sample of cold milk (4 C) as: like extremely, like moderately, like slightly, neither like nor dislike, dislike slightly, dislike moderately, or dislike extremely. Milks judged by the consumer panel were tasted cold, whereas the previous samples were analyzed at room temperature. The responses were rated on a scale of 1 = like extremely to 7 = dislike extremely and analyzed for variance with the same model as in the first phase of the study. Block

effects were represented by panelists, and treatments were R = reference milk, AC = 170-day-old experimental AC milk, PC = 170-day-old experimental PC milk and Commercial = 90-day-old commercial Canadian UHT milk.

**RESULTS AND DISCUSSION**

Microbiological results confirmed that all UHT milks were sterile. Organoleptic and GLC results of the first phase are presented in Table 1 and Fig. 1. Staleness appeared to be a major contributor to flavor of stored UHT milks. As expected, freshly pasteurized, untreated reference milks were superior in flavor to the UHT milks. Flavor scores and stale intensities indicated that among UHT milks, those stored at 22 C in PC had the least desirable flavor; it was beneficial to refrigerate the PC milks. The two AC-milks (4 and 22 C) and the PC-milk stored at 4 C had the same intensity of stale off-flavor; however, overall flavor scores of the AC-milks stored at 4 C were better than those of the refrigerated PC-milk. The absence of a statistical difference (P = .05) among cooked flavor intensities in milk stored 1½ months may be misleading because significant differences were observed in cooked flavor intensities at the 10% probability level. Average mean differences among cooked flavors in Table 1 are similar to those among stale intensity scores.

Statistical differences in the GLC data were observed only for concentrations on n-pentanal and an unidentified compound with a retention time of 28 min. These increases paralleled deterioration in flavor and the increase in intensity of a stale off-flavor. In a companion study on sterile milk (6), we observed statistically higher concentrations of propanal, n-pentanal, a 13.8-min peak, and n-hexanal in milk stored at room temperature in plastic-wrapped polyethylene-lined pint cartons. In the current study, propanal, n-pentanal, the 13.8-min peak, n-hexanal and the 28-min peak were all higher in PC

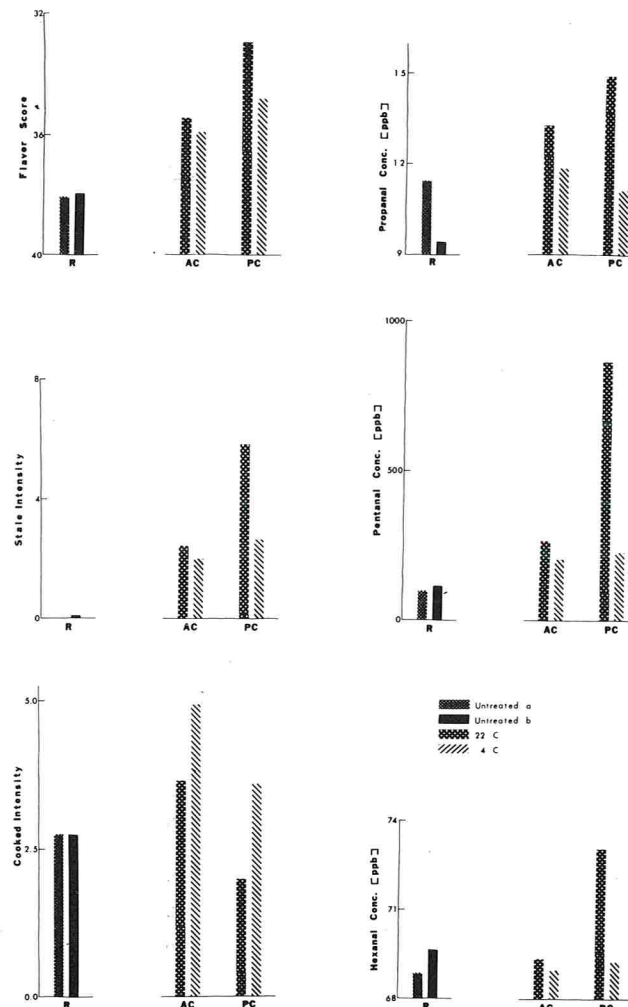


Figure 1. Changes in reference (R) milks and UHT milks from cartons (AC = Aluminum foil-lined and PC = polyethylene-lined) half exposed to fluorescent light at 22 C for 42 days and half stored in the dark at 4 C for 50 days. Untreated R milks (a) were freshly pasteurized and evaluated with 22 C milk samples and R milks (b) with 4 C samples.

TABLE 1. Comparison of UHT milks stored at 4 and 22 C for one and one half months.

Item	Organoleptic analyses		Component and/or its retention time on the chromatogram							
	Flavor score	Stale flavor	Cooked flavor	3.1 <sup>1</sup> min methyl sulfide	3.6 <sup>1</sup> min propanal	9.5 <sup>1</sup> min n-pentanal	13.8 <sup>2</sup> min	16.6 <sup>1</sup> min n-hexanal	28.0 <sup>2</sup> min	
Treatments <sup>3</sup>	.0000	.0001	.0684	.1675	.5744	.0053	.7475	.3641	.0110	
Blocks <sup>3</sup>	.1652	.7802	.6015	.1902	.2928	.4406	.4106	.0953	.3242	
Treatments <sup>4</sup>					Probabilities					
AC 4C	35.92a	2.00a	4.92a	(x)	24.43a	11.9a	207a	23a	68.95a	74ab
AC 22C	35.50ab	2.43a	3.63a	(y)	25.46a	13.3a	268a	34a	69.37a	124a
PC 4C	34.84b	2.63a	3.58a	(y)	23.76a	11.2a	229a	53a	69.26a	47b
PC 22C	33.02	5.81	1.98a	(z)	24.29a	14.9a	869	82a	73.06a	125a
R 4C	37.96c	0.04b	2.75a	(yz)	25.14a	9.4a	114a	20a	69.63a	0b
R 22C	38.22c	0.00b	2.76a	(yz)	26.31a	11.5a	96a	38a	68.82a	0b
						Means <sup>5</sup>				

<sup>1</sup>Concentrations in ppb calculated from the regression equation of the standard curve.

<sup>2</sup>Peak heights relative to the peak of 1 ppm acetone = 1000 units (% full-scale deflection peak height × attenuation).

<sup>3</sup>Degrees of freedom for treatments = 5, for blocks = 2.

<sup>4</sup>AC 4C = aluminum foil-lined carton at 4 C, AC 22C = aluminum foil-lined carton at 22 C, PC 4C = polyethylene-lined carton at 4 C, PC 22 = polyethylene-lined carton at 22 C, R 4C = reference milk examined with UHT milk at 4 C, and R 22 C = reference milk examined with UHT milk at 22 C.

<sup>5</sup>Means not significantly different at 0.05 are indicated with a common letter of the alphabet (a,b,c). For cooked flavor, means not significantly different at 0.10 are indicated with a common letter of the alphabet in brackets (x,y,z).

milk at 22 C (see Table 1) but only n-pentanal and the 28-min peak-component were statistically different. The concentration of methyl sulfide in this milk was not affected by carton material (see Table 1). Refrigerating both AC and PC milks resulted in lowered concentrations of all the observed compounds.

Results of the 24-member consumer taste panel (Table 2) and of our 5-member experienced panel (organoleptic analysis, Table 1) agree. The flavor acceptability of the two AC (our experimental and the Canadian) milks was equal; however, both had better flavors than the milk in PC. Freshly pasteurized milk had the best flavor. It is significant that when the average scores were rounded to the nearest whole number, average comment of the consumer panel was "like moderately" for the freshly pasteurized milk, "neither like or dislike" AC milks and "dislike slightly" the PC milk, which indicates that American consumers may accept the flavor of UHT milk. But a more comprehensive study needs to be conducted to confirm that.

TABLE 2. Probabilities and separation of means for consumer taste-panel scores

Item	Scores
Treatments	Probabilities
Panelists	.0000
	.0011
Treatments <sup>2</sup>	Means <sup>1</sup>
R	2.000
AC	4.250a
Commercial (Canadian)	4.333a
PC	5.458

<sup>1</sup>Means not significantly different at 0.05 are indicated with a common letter of the alphabet. Scores are on a 7-point scale with 1 = like extremely, 2 = like moderately, 3 = like slightly, 4 = neither like nor dislike, 5 = dislike slightly, 6 = dislike moderately, and 7 = dislike extremely.

<sup>2</sup>R = reference milk, AC = Al foil lined cartons, and PC = plain polyethylene-lined cartons.

## CONCLUSION

Refrigerating either AC or PC milk and storing it in the dark helped limit off-flavor development during 1½ months of storage; according to a 5-member trained panel, AC milk was superior to PC milk in maintaining acceptable flavor. Increases in off-flavor were accompanied by increases of several compounds, but the increases of only n-pentanal and an unidentified component (28-min peak) were statistically significant.

A consumer panel found that AC milks (3-month-old commercial and 6-month-old experimental) were superior in flavor to 6-month-old experimental PC milk. Freshly pasteurized milk was preferred over all the UHT milks.

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## Survey of Commercially Available Cheese for Enterotoxigenic *Escherichia coli*<sup>1</sup>

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### ABSTRACT

Seventy-eight commercial cheese samples were tested for the presence of *Escherichia coli*. None of the 136 *E. coli* isolates obtained produced either heat-labile or heat-stable enterotoxin, as measured in standard assays. None agglutinated in polyvalent antisera used to screen for classical enteropathogenic serotypes. None of the 47 *E. coli* isolates obtained from six raw milk samples produced enterotoxin, but eight agglutinated in one or more of the polyvalent antisera.

*Escherichia coli* was suspected as early as 1923 of being associated with infantile diarrhea (1). In the 1940s, proof of this association was obtained for certain serotypes of *E. coli* which became known as the classical enteropathogenic types (2,14). Severe diarrheal diseases in animals also have been shown to be caused by certain serotypes of *E. coli* that are different from those associated with human illness and that seem to be somewhat host-specific (30).

Since 1968 *E. coli* has been shown to cause diarrheal disease in humans of all age groups, with symptoms ranging from severe cholera-like diarrhea to a relatively mild food poisoning (27). The first documented outbreak in the United States of foodborne disease caused by *E. coli* occurred in 1971 and was traced to consumption of imported French Camembert and Brie cheeses (22). Enteropathogenic *E. coli* frequently has been associated with travelers' diarrhea in Mexico (16). The mode of action was the same in these cases. The organism colonizes the anterior region of the small intestine and produces a heat-labile (LT) and/or a heat-stable (ST) enterotoxin, which induces a net secretion of fluid into the intestine (27). Several investigators have shown that the ability to produce enterotoxin does not correlate with the classical enteropathogenic serotypes (13,15,28).

The classical enteropathogenic serotypes of *E. coli* have been found in many foods: beefsteak, pork liver, and frozen gravy (18), pasteurized dairy products (21), imported and domestic cheeses (8), and market milk and dairy products (29). Frank and Marth (10) found no classical types in 106 soft and semisoft cheese samples. None of these studies included tests for enterotoxin production.

A few surveys of foods for enterotoxin-producing (enterotoxigenic) strains of *E. coli* have been conducted. Sack et al. (28) found that 19 (8%) and 11 (5%) of *E. coli* isolates from foods of animal origin produced LT and/or ST, respectively. Foods from which enterotoxigenic *E. coli* were isolated included three different soft cheeses. None of the enterotoxigenic isolates belonged to the classical serotypes. Mehlman et al. (23) found one enterotoxigenic *E. coli* in 40 isolates from soft fermented cheeses, and one in 10 isolates from miscellaneous food items. Echeverria et al. (5) found no enterotoxigenic isolates in pork, leafy vegetable, and contaminated surface-water samples in a community in the Philippines.

The common occurrence of coliform organisms in dairy products, the implication of dairy products in at least two outbreaks of gastroenteritis caused by *E. coli* (20,22), and the attribution of most foodborne disease outbreaks in the United States to "agent unknown" together suggest that enterotoxigenic *E. coli* may be significant foodborne pathogens and that dairy products may be important vehicles for their transmission. This study was undertaken to determine the incidence of enterotoxigenic *E. coli* in commercially available cheeses.

### MATERIALS AND METHODS

#### *Bacterial strains*

Two *E. coli* strains of porcine origin were used as positive controls; strain 263 (produces LT only) and strain 1261 (produces ST only). They were obtained from Harley Moon, National Animal Disease Center, Ames, Iowa. The negative control strain, *E. coli* K12, was obtained from the culture collection of the Department of Food Technology, Iowa State University. All strains were maintained on 2% peptone agar slants sealed with wax and stored in the dark at room temperature.

#### *Isolation scheme*

Isolation of *E. coli* was according to the procedure for enteropathogenic *E. coli* in the fourth edition of the *Bacteriological Analytical Manual for Foods* (9), except that serotyping with polyvalent antisera (Difco, Detroit, MI) was performed at the end of the isolation scheme, and the Voges-Proskauer and cytochrome oxidase tests were performed as described by Edwards and Ewing (6).

#### *Preparation of culture filtrates*

Bacterial strains were grown in Casamino acids - yeast extract (CAYE) medium adjusted to pH 8.5, as described by Evans et al. (7). This medium supports good enterotoxin production. Shake flasks were inoculated with 2.5% of an overnight culture and were incubated 24 h at 37 C. The cells were removed by centrifugation at 12,000 × g for 15 min at 4 C, and the supernatant fluid was filter-sterilized. Filtrates were stored at -20 C and were tested in the labile toxin assay within 24 h and in the stable toxin assay within 3 months.

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*Stable toxin assay*

The suckling mouse assay of Dean et al. (3) was used to test for production of ST. Pregnant Swiss albino mice were obtained from Bio-Lab, Inc., St. Paul, MN. Suckling mice were 2-4 days old when used, and litters were randomly mixed. Three mice were used to test the culture filtrate of each *E. coli* isolate. A 2% Evans blue dye solution was mixed with each sample before 0.1 ml was administered to each mouse via stomach tube, and data were recorded only for those mice with blue color localized within the gastrointestinal tract. After a 4-h incubation period at room temperature, mice were killed, the intestinal tract from stomach to anus was removed, and a gut weight to remaining body weight ratio was obtained. A ratio > 0.085 was considered a positive response of fluid accumulation induced by ST.

*Labile toxin assay*

The adrenal cell assay of Donta et al. (4) was used to test for production of LT. Mouse Y-1 adrenal tumor cells (CCL 79, American Type Culture Collection) were grown in Ham's F-10 medium (GIBCO, Grand Island, NY) supplemented with 15% fetal calf serum (GIBCO) and 25 mM Hepes buffer (Calbiochem, LaJolla, CA), which enabled the cells to be cultured in a humidified atmosphere without CO<sub>2</sub> enrichment. Cells were grown on 24-well dishes (Costar, Cambridge, MA). When a confluent monolayer had formed, 1 ml of fresh medium containing 0.1 ml of culture filtrate was added to each well. Cells were incubated 20-24 h, and characteristic rounding of the cells in responses to LT was observed by using a light microscope. Rounding of > 50% of the cells was considered a positive test. Negative control wells typically contained 10-20% rounded cells. All samples were tested in duplicate.

*Coliform counts*

Coliform counts on Violet Red Bile Agar (Difco) were made according to standard procedures (19).

**RESULTS AND DISCUSSION**

The cheese samples surveyed were obtained from Ames, Iowa, retail food stores over a 1-year period. They are listed in Table 1. Seventy-eight cheese samples were tested for the presence of *E. coli*. Twenty-two (28%) of the cheeses contained viable *E. coli*, and 136 isolates (no more than 12 per sample) were obtained for enterotoxin testing.

Only one of the 20 process cheese samples contained recoverable *E. coli*. Evidently post-process contamination is relatively rare. No *E. coli* were isolated from the two low-moisture cheeses tested. The low water activity in these products limits survival and growth of *E. coli*. The minimum water activity for growth of *Escherichia* species is 0.95, well above the water activity of these cheeses (31).

Most of the *E. coli* isolated were obtained from the intermediate-moisture cheeses. Forty-one percent of these cheeses contained *E. coli*. Although this type of cheese has not been implicated in *E. coli*-associated food poisoning outbreaks, the high incidence of *E. coli* seen here suggests that it could be a vehicle for enterotoxigenic strains.

Only two of the 10 soft cheeses contained recoverable *E. coli*. This incidence was lower than expected because this type of cheese would seem to provide a good environment for *E. coli* survival and growth. It is difficult to draw conclusions from such a small number of samples.

The isolation procedure used in this study is designed to recover both typical and atypical (delayed lactose-fermenting) *E. coli* strains that would not be recovered by

TABLE 1. Cheeses tested for the presence of *E. coli*.

Type of cheese	Number of samples tested	Number of samples containing <i>E. coli</i>	Number of <i>E. coli</i> isolates obtained
Pasteurized process cheese	17	0	—
Cold-pack cheese food	3	1	4
Low-moisture cheese	2	0	—
Intermediate-moisture cheese	46	19	113
High-moisture cheese	10	2	19

using conventional techniques, and therefore should detect all viable *E. coli*. Determination of fecal coliform levels does not accurately count *E. coli*. Hall et al. (18) found *E. coli* in only 40.4% of fecal coliform-positive tubes in most-probable-number analyses of market foods.

No attempt was made here to enumerate *E. coli* or fecal coliforms in the cheese samples. Frank and Marth (10) have found that coliform levels in market cheese often are high enough to be a potential health hazard should these organisms be capable of causing disease.

None of the *E. coli* isolates agglutinated in the antisera used to detect the classical enteropathogenic serotypes. Although the pooled antisera do not give complete serotyping information, agglutination should occur if the isolate being tested belongs to any of the serotypes represented. These pooled antisera are used solely as a screening procedure for the presence of possible enteropathogenic types. If an agglutination reaction is obtained, the O, K and H antigens of the test organism must all be characterized to state with certainty that the organism is of an enteropathogenic serotype.

These results agree with those reported by other investigators. Frank and Marth (10) found no enteropathogenic serogroups in 106 market cheese samples surveyed. Jones et al. (21) found only 2% of the *E. coli* isolates were of enteropathogenic serotype among the coliforms found in Canadian pasteurized dairy products. Similar low levels of enteropathogenic serotypes were found by Murray (24) and Papavassiliou (25). The lack of correlation between serotype and enterotoxigenicity makes identification of enteropathogenic serotypes of questionable value in predicting the pathogenicity of food contaminants.

None of the culture filtrates contained enterotoxin activity, as measured in the suckling mouse assay for ST or in the adrenal cell assay for LT. Culture filtrates of the positive control strains elicited strongly positive responses in the appropriate assays. A weakly toxigenic isolate may have produced enterotoxin at a level below the limits of detection of the assays. Culture filtrates were not concentrated before testing.

Inasmuch as the plasmid(s) that carry the enterotoxin genes can be lost spontaneously upon storage of a strain (17), it is possible that some of the isolates lost their enterotoxigenicity before they could be tested. Culture

filtrates of most of the isolates were prepared and tested within 1 month of isolation.

These results suggest that enterotoxigenic *E. coli* are not common contaminants in cheese at the retail level. Dairy products have been sampled for enterotoxigenic *E. coli* in two other surveys. The incidence of enterotoxigenic isolates was 8% (28) and 2.1% (23). It is likely that these organisms are not found routinely in foods of high bacteriological quality, but may occur when there is a breakdown in sanitation procedures, as was the case in the gastroenteritis outbreak traced to imported cheese (22).

The absence of enterotoxigenic *E. coli* from the cheese samples tested raised the question of whether they were present in raw milk and were destroyed during processing, or were simply absent from raw milk. In an effort to answer this question, six raw milk samples from five different farms were obtained and tested for the presence of enterotoxigenic *E. coli*. The results are presented in Table 2.

The coliform counts of these samples varied over a wide range. Two of the samples showed high levels of coliform contamination, which indicates a probable sanitation problem on the farm. Although these organisms would not be expected to survive pasteurization, raw milk cheeses made from this milk could represent a health hazard if enterotoxigenic *E. coli* were present and survived the cheese-making process. Known enterotoxigenic strains have been shown to survive during manufacture of brick and Camembert cheeses (11,12,26).

Forty-seven *E. coli* isolates were recovered from the raw milk. None of the isolates produced detectable levels of either LT or ST. Although the number of samples tested was limited, the absence of enterotoxigenic strains suggests that these organisms are not commonly found in the environment of the dairy farm. More samples need to be tested to draw firmer conclusions.

Eight (17%) of the isolates agglutinated in one or more of the polyvalent antisera used to screen for classical enteropathogenic serotypes. These isolates all were obtained from milk samples from the two farms with the highest coliform counts.

TABLE 2. Raw milk samples tested for enterotoxigenic *E. coli*.

Sample <sup>1</sup>	Coliform count/ml <sup>2</sup>	Number of <i>E. coli</i> isolates obtained	Number of toxigenic isolates	Number of isolates agglutinated by pooled antisera <sup>3</sup>
1	< 2.00	9	0	0
2a	6.48	8	0	2
2b	2.80	9	0	5
3	< 2.00	4	0	0
4	2.00	15	0	0
5	3.48	2	0	1

<sup>1</sup>Sample number indicates the farm from which the milk was obtained. Farm 2 was sampled twice.

<sup>2</sup>Log<sub>10</sub> typical coliform colonies on violet red bile agar, average of duplicate counts.

<sup>3</sup>Agglutination in any one of the pooled antisera against the classical enteropathogenic serotypes was noted as positive.

The indication of classical enteropathogenic serotypes among raw milk isolates and the failure of these isolates to produce enterotoxin emphasize the need for the inclusion of enterotoxin assays when testing foods for enteropathogenic *E. coli*. In this case, failure to test for enterotoxin production would have resulted in the erroneous conclusion that potentially pathogenic *E. coli* were a significant proportion of the raw milk isolates, when in fact none of the isolates produced enterotoxin.

CONCLUSIONS

Because *E. coli* causes diarrheal disease by producing an enterotoxin, and because enterotoxin production does not correlate with serotype, it is necessary to screen food isolates for enterotoxin synthesis and not just for classical enteropathogenic serotype. Using standard assays for heat-stable and heat-labile enterotoxins, we found that enterotoxigenic *E. coli* are not common contaminants in commercial cheeses or in raw milk. They most likely enter the food supply and present a hazard to health through breakdowns in sanitation.

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## Effect of Incubation Temperature and Heat Treatments of Milk from Cow and Buffalo on Acid and Flavor Production by *Streptococcus thermophilus* and *Lactobacillus bulgaricus*

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### ABSTRACT

Variations in production of acid and acetaldehyde and proteolytic activity by pure and mixed cultures of *Streptococcus thermophilus* and *Lactobacillus bulgaricus* in response to incubation temperature were determined using cow and buffalo milk. *S. thermophilus* and the mixed culture produced more acid and acetaldehyde at 37 than at 42 C, while the reverse was true for *L. bulgaricus* in both types of milk. The largest amount of acid was produced by *S. thermophilus* in both types of milks heated at 65 C for 30 min, while *L. bulgaricus* produced the maximum amount of acid in milks heated at 85 C for 10 min. In the mixed culture, increased acid and acetaldehyde production were noted in milk sample steamed for 30 min.

were used. Fresh cow and buffalo milk samples were individually sterilized at 100 C for 30 min. For heat treatments studies, the samples were exposed to 65 C/30 min, 85 C/10 min or 100 C/30 min. Samples were inoculated (1%) with pure and mixed milk cultures and examined after 24 h for acid and flavor production. Titratable acidity was determined by titrating 10 g of sample with 0.1 N NaOH to the phenolphthalein end point. Results are expressed as percent lactic acid. Volatile acidity was determined by the method of Hemenius and Liska (6). Acetaldehyde was estimated by the procedure of Lindsay and Day (8), while proteolytic activity was determined according to the method of Hull (7).

### RESULTS AND DISCUSSION

The importance of the role of lactic cultures in cultured milk products is well recognized (2,4,9,13). Several investigations have been reported on growth and biochemical activity of *Streptococcus thermophilus* and *Lactobacillus bulgaricus* in skim milk or fortified skim or whole milk from the cow; however, research on the role of these cultures in milk from different animal species (e.g. buffalo) has been done only recently. Dutta et al. (3) and Singh and Ranganathan (12) reported considerable variation in the biochemical performance of lactic cultures grown in milk of different species. The present investigation was undertaken to study the effect of different heat treatments of milk and incubation temperature on acid and flavor production and proteolytic activity by *S. thermophilus* and *L. bulgaricus* in cow and buffalo milk.

Incubation temperature is one of the most important environmental factors that influence the biochemical performance of lactic cultures. Although the role of incubation temperature on acid and flavor production and proteolytic activity by lactic starters has been studied by several workers (1,2,11), a wide range of incubation temperatures has been reported as optimum for acid development by starters. Variations in acid and acetaldehyde production, and proteolytic activity by pure and mixed cultures of *S. thermophilus* and *L. bulgaricus* in cow and buffalo milk, in response to incubation temperature are evident from the data in Table 1. *S. thermophilus* and the mixed culture produced more acid and acetaldehyde at 37 than 42 C, while with *L. bulgaricus* 42 C was better than 37 C in both types of milk. No significant differences were noted in proteolytic activity of *S. thermophilus* and mixed cultures regardless of incubation temperature. Increased acid and acetaldehyde production and proteolytic activity by *L. bulgaricus* and higher volatile acidity production by pure and mixed

### MATERIALS AND METHODS

Pure and mixed-strain cultures of *S. thermophilus* (Hst) and *L. bulgaricus* (RTS) maintained at the National Dairy Research Institute,

TABLE 1. Effect of different incubation temperature on biochemical activity of pure and mixed strain cultures of *Streptococcus thermophilus* and *Lactobacillus bulgaricus*.<sup>a</sup>

Culture activity	Type of milk	<i>S. thermophilus</i> (Hst)		<i>L. bulgaricus</i> (RTS)		<i>S. thermophilus</i> (Hst) <i>L. bulgaricus</i> (RTS)	
		37 C	42 C	37 C	42 C	37 C	42 C
Titratable acidity	Cow	0.64 (4.42) <sup>b</sup>	0.59 (4.45)	1.40 (3.80)	1.85 (3.68)	2.05 (3.45)	1.64 (3.80)
	Buffalo	0.82	0.62	1.96	2.50	2.85	1.95
Volatile acidity (ml of 0.1 N NaOH/ 50 g of curd)	Cow	1.50	2.25	4.25	6.42	7.70	8.25
	Buffalo	2.20	2.85	5.15	9.86	8.45	10.40
Acetaldehyde (ppm)	Cow	7.20	7.00	10.60	12.80	17.95	16.50
	Buffalo	8.30	8.20	12.50	14.65	24.50	20.56
Proteolytic activity (mg of tyrosine)	Cow	0.30	0.32	0.35	0.54	0.32	0.35
	Buffalo	0.35	0.33	0.42	0.61	0.42	0.39

<sup>a</sup>Cultures were examined at the end of 24 h of incubation.

<sup>b</sup>Figures in parenthesis represent pH values. Results represent average of three trials.

TABLE 2. Effect of different heat treatments on the biochemical activity of pure and mixed strain cultures on *Streptococcus thermophilus* and *Lactobacillus bulgaricus*.<sup>a</sup>

Culture activity	Type of milk	<i>S. thermophilus</i> (Hst)			<i>L. bulgaricus</i> (RTS)			<i>S. thermophilus</i> (Hst) + <i>L. bulgaricus</i> (RTS)		
		65 C (30 min)	85 C (10 min)	steam (30 min)	65 C (30 min)	85 C (10 min)	steam (30 min)	65 C (30 min)	85 C (10 min)	steam (30 min)
Titratable acidity (% L.A.)	Cow	1.02 (4.25) <sup>b</sup>	0.86 (4.30)	0.64 (4.32)	1.05 (3.60)	1.25 (3.80)	1.43 (3.80)	1.45 (3.54)	1.58 (3.65)	1.64 (3.80)**
	Buffalo	1.95 (4.00)	0.95 (4.15)	0.82 (4.20)	1.75 (3.45)	2.10 (3.50)	1.96 (3.59)	1.50 (3.25)	1.65 (3.30)	1.95 (3.55)
Volatile acidity (ml of 0.1 N NaOH/50 g curd)	Cow	1.90	1.85	1.00	6.50	4.60	3.85	8.50	6.60	5.10
	Buffalo	2.05	2.00	1.45	7.60	4.85	4.20	9.65	7.85	7.32
Acetaldehyde (ppm)	Cow	7.65	7.35	7.20	7.00	8.80	10.60	15.30	16.45	16.50
	Buffalo	10.20	8.55	8.30	10.50	12.85	12.50	16.55	19.20	20.58
Proteolytic activity (mg of tyrosine)	Cow	0.52	0.38	0.30	0.62	0.52	0.35	0.45	0.35	0.37
	Buffalo	0.56	0.39	0.35	0.75	0.55	0.42	0.52	0.39	0.38

<sup>a</sup>Cultures were examined at the end of 24 h of incubation at 37 C.

<sup>b</sup>Figures in parentheses represent pH values. Results represent average of three trials.

cultures of *S. thermophilus* and *L. bulgaricus* at 42 C is noteworthy since that incubation temperature is normally used for manufacture of yogurt.

It is well known that heat treatment of milk affects growth and activity of lactic cultures (4,9). From data in Table 2, it is evident that some heat treatments of milk are favorable for acid and acetaldehyde production and proteolytic activity as compared to others. Higher titratable acidity was noted for *S. thermophilus* and *L. bulgaricus* in milk heated at 65 C/30 min and 85 C/10 min than for the other heat treatment. For the mixed culture, increased acid production occurred in milk samples steamed for 30 min. These results are not consistent with earlier reports (5,13), where an increase in temperature and holding time improved acid production by starter cultures. The increase in acid production by the mixed culture as a result of heat treatment of milk sample was ascribed to partial hydrolysis of casein, which provided the organisms with increased amounts of usable nitrogen. According to Nielson (10), heat treatment of milk liberates certain amino acids from milk proteins and these amino acids support growth of *L. bulgaricus*, which subsequently liberates more amino acids for enhancing the growth of *S. thermophilus* in yogurt.

As regards acetaldehyde production in cow and buffalo milk, *L. bulgaricus* and the mixed culture produced most acetaldehyde in steam sterilized milk, while *S. thermophilus* produced most acetaldehyde in milk heated at 65 C for 30 min. These results should be useful when different kinds of milk are used for preparation of a variety of cultured milk products.

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## Education is the Key to Solving Sanitation Problems

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### ABSTRACT

Although there is some justification in claiming that an effective sanitation program extends product shelf-life, reduces spoilage, provides protection from disease and infection and improves company productivity, the primary motivating force for management to promote sanitation is its direct impact on corporate planning, corporate marketing and the corporate relationship with the law. Management's commitment to corporate security — its entity and financial security — correlates directly with market share. Since market share is directly related to product acceptability, then effective sanitation becomes an economically viable program. Once a sanitation program has received support by management, and the tools of sanitation have been provided via the sanitarian and QC laboratory, the sanitation message must be taught and followed without exception by management supervisors, employees and inspectors. Industry education may occur through trade associations which prepare Codes of Practice for manufacture and sanitation, universities which offer correspondence courses, professional associations which are committed to improving the professional status of the sanitarian and federal and local regulatory agencies which provide educational materials.

It is still possible to find managements which believe that good sanitation is not absolutely necessary in their food plants. In many instances, such reasoning is easily justified upon recognizing that sanitation is a long term affair, and that companies operating on such policies frequently come into existence and vanish into oblivion long before the benefits of good sanitation can be realized.

In some instances, where there is likelihood that a food company will carry on to become an old established firm, some managements have been able to justify sanitation programs as being typical food industry investments which (a) cost a lot of money, (b) never pay a dividend and (c) can't be sold when the management tires of the concept.

In the instances where food companies have installed sophisticated sanitation programs, some managements have identified the following advantages. First, shareholders, visitors and inspectors are impressed with the quality control laboratory filled with petri dishes, Quebec counters, glassware, bottles and busy microbiologists counting bacteria. In addition, hundreds of feet of hoses, drums of quats and hypochlorites, hand-dip stations . . . strategically placed throughout the plant confirm the visitors' belief that the plant is operated by a sanitation-conscious management. Finally, some food company managements have discovered that sophisticated sanitation programs do no harm, and in fact can

effect corporation improvements, such as extended shelf-life and increased sales.

### MANAGEMENT — A SANITATION PROBLEM

By now the first and most important sanitation problem has been identified -- management. It is of utmost importance that management be educated as to the real value of a sanitation program - what it can or cannot accomplish. Without the total commitment of management, the effectiveness of a sanitation program is reduced.

Several benefits have been attributed to good sanitation, the fundamental one being protection from disease and infection. Economic benefits including improved employee morale through congenial surroundings and thus improved productivity, extended product shelf-life and extended best-before-date, reduced product returns due to premature off-condition, reduced spoilage, reduced water usage and energy consumption, reduced sewage load and subsequently reduced municipal surcharge, increased product sales and improved competitive position in the retail market have been correlated with sanitation efficacy.

Although there is some justification for claiming all of these benefits, the primary motivating force for management to promote sanitation is its direct impact on corporate planning, corporate marketing, and corporate relationship with the law. Management's commitment is to corporate security, its entity and financial security. Simply stated, "If there is no profit there is no existence." Corporate security correlates directly with market share, and market share is directly related to product acceptability, thus the motivating force for sanitation is identified.

As previously noted, sanitation programs impact directly on the corporate/regulatory interface. Both the U.S. Food and Drug Administration in sections 402 (a) (3) and 402 a(4) of the Food Act, and the Canadian Health Protection Branch under sections 4, and 7 of the Food and Drugs Act have the mandate to prohibit the production, preservation, packaging, storage or sale of any food under unsanitary conditions.

A thorough sanitation program will reduce the potential for product seizure, product recall, food poisoning outbreaks, and thus minimize the associated damaging publicity.

In Canada an additional motivation-factor for sanitation is the alpha-numeric durable shelf-life regulation. The extension of product shelf-life through sanitation is directly reflected in the extension of the Best-Before-Date. Extended durable shelf-life permits not only an extended sell-through period which is necessary for national distribution from centralized plants, but also extended consumer shelf-life.

#### THE SANITARIAN — A SANITATION PROBLEM

The second sanitation problem can be the sanitarian if he is not educated in the basics of sanitation. The sanitarian must be a serious, concerned professional who understands clearly the corporate policy, and his role in the organization. The sanitarian must have the full support of management and an effective direct communication mechanism with management. The sanitarian's responsibility is directly to management, employees, regulatory agencies and the consumers. In addition the sanitarian must have access to the laboratory, a vital tool for the quantitative, precise and objective assessment of ongoing sanitary practices.

To effectively fulfill the requirements of a sanitarian, he must be educated in microbiology, since bacterial counts can reflect the sanitary history of the food process--incoming raw materials, process failure, storage and packaging conditions. To have an effective sanitation program the sanitarian must be educated in specific surface conditions such as hardness, porosity, resistance to oxidation and corrosion and ease of sanitation for wood, galvanized metals, black metals, stainless steel, plastics, ceramics, concrete, paint; soil types and characteristics such as inorganic, organic, water-soluble, base-soluble, acid-soluble; plant layout and construction and equipment design.

The sanitarian must be educated in the safety and efficacy of detergents, functions of detergent auxiliaries and sanitizers and the need for protective equipment. Through comprehensive understanding of the nature of cleaners and sanitizers (a) employee injury can be prevented, (b) cleaner/sanitizer waste can be reduced and (c) cleaning efficiency can be optimized. Through proper cleaning procedures water consumption often can be reduced, sewage load may be reduced and labor intensity can be optimized. Recognizing that disease-producing microorganisms can be transmitted throughout a food plant by rodents, insects, flies and birds, the sanitarian must be educated in their control and eradication.

All of this information must be conveyed to the sanitation crew in a form that is easily understood. Sanitation crews tend to have rapid turn-over, thus an effective sanitation program must provide a simple, easily accessible, visual instruction manual covering briefly each piece of equipment or area to be cleaned, choice of cleaner, mixing instructions and method of application. There must be a continuing ongoing educational program of awareness provided through

posters, management and the sanitarian. Management must endorse and support the work of the sanitation crew and demonstrate to these employees the importance of cleaning and sanitation to the integrity of the company.

#### THE EMPLOYEE — A SANITATION PROBLEM

The third potential sanitation problem is the employee. Again education of employees must be simple and continuing - posters are an effective vehicle. The sanitation message must be consistent and followed by management, supervisors and inspectors. There must be no exceptions to the use of hand dips, hairnets, white coats, etc.

As a result of extensive education of these three industrial sectors the application of the sanitation program will optimize human resources and available technology, in attaining quality throughout the plant -- in raw material, in process and in finished product.

The basic sanitation principles include an adequate sanitation plan, operational methods and personal practices, pest control, good cleaning practice and preventive maintenance. The application of these principles will impact on the operational appearance, practices and performance, and will reflect directly on the corporate security.

Many products have been implicated in food poisoning outbreaks which have been caused not by negligence during production nor processing, but by poor handling practices in homes or in restaurants. The education of the consumer and the food handlers is a priority to complete the sanitation cycle. Having identified the four fundamental sources of sanitation problems, vehicles of education must be identified

#### EDUCATION

Trade associations are one effective means of industrial education. As an example, the Meat Packers Council of Canada prepares and distributes several sanitation-oriented documents. As part of industry response to the regulatory proposals by the Canadian Health Protection Branch regarding microbiological standards vs. guidelines, the Council in 1978 prepared a Statement of Good Manufacturing Practices for Ground Beef and Related Products. The Code of Practice contains 21 pages color-coded according to general personnel hygiene, cleaning procedures, economic benefits and technology evaluation.

In addition to other topics, this Statement of Good Manufacturing Practices for Ground Beef and Related Products deals with raw materials, tempering, final product condition, frozen storage, retail display and cleaning procedures.

Recently *Salmonella* serotypes which have been associated with human illness have been traced back through the poultry chain and ultimately isolated from feedstuffs. In response to this issue, the Meat Packers Council prepared and distributed a Statement of

Awareness for the Reduction of *Salmonella* Contamination in Rendered Meats. The Statement lists over 45 potential sources of *Salmonella* contamination, including air-borne contamination, water supplies, personnel, pests, raw materials, plant construction, process failure, cleaning procedures and transport vehicles.

The Council also distributes a monthly technology bulletin dealing with research and quality control. In past issues, topics such as refrigeration and incidence of sticky beef; microbiological implications of slaughter, dressing and processing; retail shelf-life of prepackaged beef; modified atmospheres; product contamination and industrial detergency have been reported. In addition to a technical library/library loans program which is heavily oriented to processing sanitary and environmental aspects, the Council also distributes temperature guides and sanitation film strips.

The Meat Packers Council of Canada cosponsors with the University of Guelph, a correspondence course "Plant Sanitation for the Meat Packing Industry." This correspondence course covers the significance of sanitation as well as microorganisms, personnel hygiene, construction, equipment, cleaning materials, cleaning procedures, pest control and water disposal.

The Council also sponsors, in conjunction with the Canadian Food Processors Association and St. Clair College in Windsor, Ontario, a course on retort - canning operations, closure evaluation and sanitation.

Professional associations such as the International Association of Milk, Food and Environmental Sanitarians and its Affiliates are committed to improve the professional status of the sanitarian and to increase food industry awareness of the need for sanitation. Thus professional associations have an extremely important role to play in sanitation education.

Establishment of Industry/Government committees in Canada has been a particularly successful method of education for not only industry but also government. These committees provide a forum of mutual information exchange which assesses regulatory options (e.g. regulations vs. guidelines), status of technology, research

priorities, ongoing research and identifies centers of expertise.

In addition to Industry/Government committees on human nutrition and plant protein, there are several committees directly involved with sanitation and food protection, including the Coordinating Committee on the Microbiological Quality of Ground Beef, Poultry Industry -- Agriculture Canada Salmonella Committee, Expert Committee on Meats, Expert Committee on Food Safety and Canada Committee on Foods.

Federal, provincial and municipal regulatory agencies in Canada have educational services which provide sanitation and food protection information to industry, schools and consumers. The Health Protection Branch of Canada distributes films, slides and cassettes dealing with food protection. In addition, the Health Protection Branch prepares consumer education dispatches on microbial food poisoning, poultry safety, botulism and home canning, health protection and food laws, kitchen care, food poisoning and food safety.

Finally, many companies have intensive, formal in-house training programs where the human resource has been identified as the key to food sanitation. These companies have detailed sanitation strategies based on their Quality Assurance needs which include (a) quantitative, precise, objective assessment; (b) accurate manpower requirement and designation; (c) simplicity and ease of communications and (d) effective management information system, including effective communication to non-technical persons. Companies with the in-house sanitation programs have understood the limitations of sanitation programmes, and thus have been able to optimize productivity and perfect the organizational effectiveness.

"Sanitation is a way of life. It is the quality of living that is expressed in the clean house, the clean farm, the clean business and industry, the clean neighborhood, the clean community. Being a way of life it must come from within the people; it is nourished by knowledge, and grows as an obligation and an ideal in human relations." (From a poster credited to the National Association of Sanitarians).

## Application of Enzyme-Linked Immunosorbent Assay for Detection of Staphylococcal Enterotoxins in Food

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### ABSTRACT

The enzyme-linked immunospecific assay (ELISA) is equally as sensitive as the radioimmunoassay (RIA) for detecting staphylococcal enterotoxins (SE). The substitution of an enzyme in ELISA for I-125 in RIA results in a more stable reagent and enables quantitation spectrophotometrically or, with appropriate enzymes, semi-quantitation by visual estimation. Assay procedures identical in principle to RIA are employed with, of course, the necessity to avoid the presence of enzyme inhibitors. To date, nanogram quantities of SEA, SEB and SEC have been successfully measured in food extracts. In common with RIA, sensitivity is decreased by the presence of food materials.

Application of affinity chromatography utilizing antibody-antigen reactivity to assay for staphylococcal enterotoxins has enabled quantitation in the nanogram to picogram range. By separating the desired antigen (toxin) immunologically rather than through physical techniques, specificity and sensitivity are increased dramatically. Two of these assays, radioimmunoassay (RIA) and enzyme-linked-immunospecific-assay (ELISA) or, as the latter is also designated by some, enzyme immunoassay (EIA) are widely employed in clinical and metabolic studies. They are both specific and relatively rapid assays and, while they are closely related in principle, both possess a number of distinct attributes due to the conjugate marker used for quantitation - iodine-125 (I-125) for RIA and an enzyme for ELISA.

Substitution of an enzyme for radioactive iodine, though, has increased the versatility for detecting or assaying staphylococcal enterotoxins. In fact, ELISA has already demonstrated its applicability for assaying agents either causing or resulting from infections, and identifying serum antibodies, antigens, drugs and metabolites.

Both RIA and ELISA are sensitive, specific and rapid, but both suffer from the problem of decreased sensitivity due to non-specific adsorption (Table 1). Food constituents can significantly contribute to this problem and in certain instances should be removed or neutralized (1,4,5,7,9).

The ELISA assay, by eliminating the need for I-125, utilizes equipment common to most laboratories by replacing a gamma or scintillation counter with a spectrophotometer. Moreover, if desired, some substrate-enzyme reactions can be evaluated visually. The enzyme conjugate, unlike I-125, is relatively stable and can be stored at -20 C for over 1 year.

TABLE 1. *A comparison of RIA and ELISA.*

<i>IN COMMON WITH RIA</i>	<i>ADVANTAGES OF ELISA</i>
Specificity	No radioactive compounds
Sensitivity	Only commonly used laboratory equipment needed
Rapidity	Stable enzyme conjugates
Non-specific adsorption	Capability for visually evaluating a positive reaction

For both techniques, highly purified homologous antisera and antigen must be employed to achieve maximal sensitivity. The usual range of sensitivity for assaying enterotoxins in foods by either RIA or ELISA varies from 100 pg for enterotoxin C (SEC); to less than 1 ng per g of enterotoxins A(SEA), B(SEB), D(SED) or E(SEE) (6,12,13,14). In the authors' laboratory, sensitivity for detecting SEA and SEB by RIA increased from 0.5 ng per ml of extract to less than 0.1 ng per ml of extract by substituting a more highly purified IgG immunoglobulin fraction for a relatively crude antibody preparation.

The principles for ELISA are identical with those of RIA allowing, of course, for the presence in ELISA of the enzyme conjugate. A number of applied and theoretical treatments of RIA (15,16) are also applicable to ELISA and can serve as a useful guide for developing assays.

### PROCEDURES FOR ELISA

The sequential and competitive procedures for the direct ELISA assay are illustrated in Fig. 1. Although not essential (7), antibodies are generally attached to a support material, usually by direct adsorption onto a plastic surface although other procedures, such as first adsorbing bovine serum albumin (BSA) to a surface, fixing the BSA with glutaraldehyde and then absorbing the antibody to the BSA, could be used (13). Various plastics including polystyrene, polypropylene and polyvinyl chloride have been evaluated and the most suitable plastic appears to be polystyrene (2). Successful assays, as seen below, have been achieved with such varied forms as test tubes, microtitration plates and plastic beads.

To minimize non-specific adsorption by sites not covered by the antibody, either or both BSA or/and Tween 20 is/are added as blocking agents. The antigen (enterotoxin) is added and incubated at some specific temperature range. When extended incubations are used to achieve maximum sensitivity, it might be necessary to

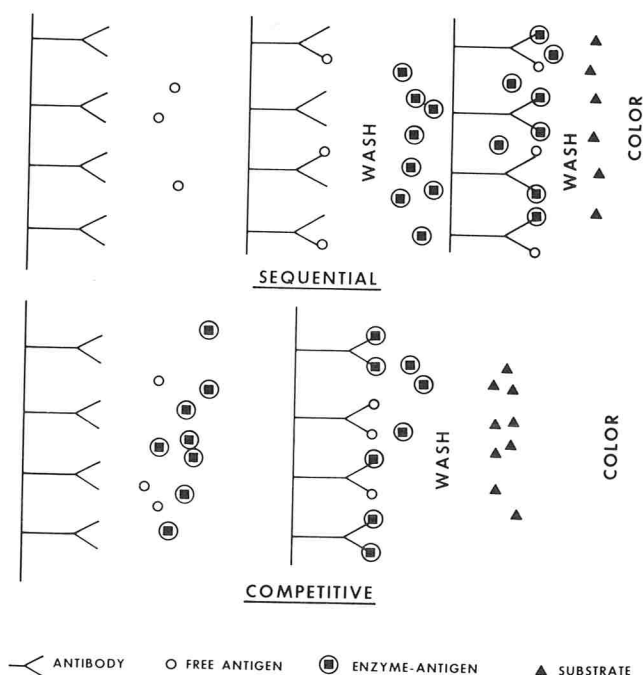


Figure 1. Direct ELISA assays.

add a preservative such as sodium azide. This can present a problem for peroxidase is inhibited by sodium azide, and we have found that merthiolate is also inhibitory. Stiffler-Rosenberg and Fey (14), using 0.02% azide in BSA buffer, apparently found that sodium azide did not seriously interfere with their peroxidase activity. Sodium azide does not interfere with the activity of alkaline phosphatase.

After antigen-antiserum binding and washing, the complex is exposed to an enzyme-homologous-antigen conjugate. The enzymes of choice are usually either peroxidase or alkaline phosphatase, although Morita and Woodburn (7) have employed  $\beta$ -amylase. Procedures for conjugation of these enzymes are usually those of Engvall and Perlmann (3) or Nakane and Kawaoi (8). Peroxidase is an inexpensive enzyme and more stable than alkaline phosphatase, but as seen below, both can be equally sensitive for assaying staphylococcal enterotoxins.

In the direct ELISA, the conjugate will associate with the unoccupied sites. This reaction is relatively rapid, as short as 1 h, but greater sensitivity has been noted when longer incubation times - as much as 18 h-are used (13). Unlike its use in RIA, BSA is usually omitted in ELISA since it was found to interfere with the rate of binding of the conjugate to the antibody site. After washing, the substrate is added, the chromogen allowed to develop and its intensity is read in a spectrophotometer. The substrates commonly used for peroxidase are 5-aminosalicylic acid (purple-brown) or z, z azino-di (3-ethyl benzthi azoline-6-sulfonate) and for alkaline phosphatase it is p-nitro-phenylphosphate. A terminator, usually sodium hydroxide, is used to stop the enzymatic reaction.

In the direct competitive method, both the free and conjugate antigens are allowed to compete at the same

time for the available antibody binding sites. It is apparent that the competitive method eliminates one binding step and a washing, but it is as accurate as the sequential procedure.

The use of the sandwiching technique is also possible with ELISA (Fig. 2). Either the antibody or the antigen can be assayed in the double antibody sandwich. The antigen, capable of binding two homologous antibodies, can be assayed after binding on the adsorbed antibody by, in turn, binding a conjugated homologous antibody.

In the indirect sandwich technique, homologous antibodies can be assayed by the use of a conjugated antibody. For example, if enterotoxin rabbit antisera was to be titered, it could be adsorbed onto homologous enterotoxin molecules and in turn assayed by the use of conjugated anti-rabbit goat antisera.

In our laboratory at NARADCOM, we have employed both the direct competitive or sequential procedures for both RIA and ELISA. Some typical assay curves are presented in Fig. 3 to demonstrate the sensitivity that can be obtained using either phosphatase or peroxidase labeled antigen for SEA or SEB. The sensitivity of the assay for these toxins in buffer was less than 0.5 ng per ml.

Recent publications on the application of ELISA to assaying staphylococcal enterotoxins in foods reflect the versatility of this method (7,10,13,14). Saunders and Bartlett (13) used a simple extraction procedure to assay for SEA by the sandwich technique, using spiked extracts of hot dog, milk and mayonnaise. They employed microtitre plates and reacted them with gluteraldehyde to increase their capacity for adsorbing anti-SEA. The conjugated anti-SEA enzyme was peroxidase and the range for their assay was 2.5 to 100 ng per ml. Their data indicate a sensitivity for detecting SEA varying from 3.2 ng per ml in a 1 to 3-h test to 0.4 ng per ml with a 20-h test period. They used

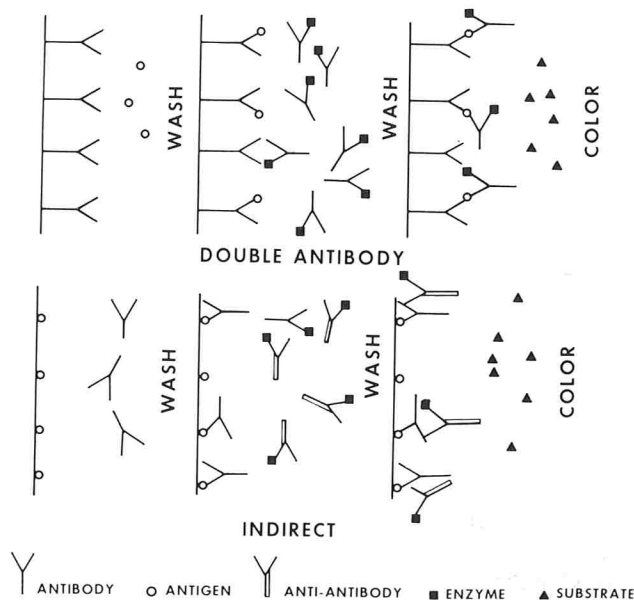


Figure 2. Sandwich techniques used in the ELISA.

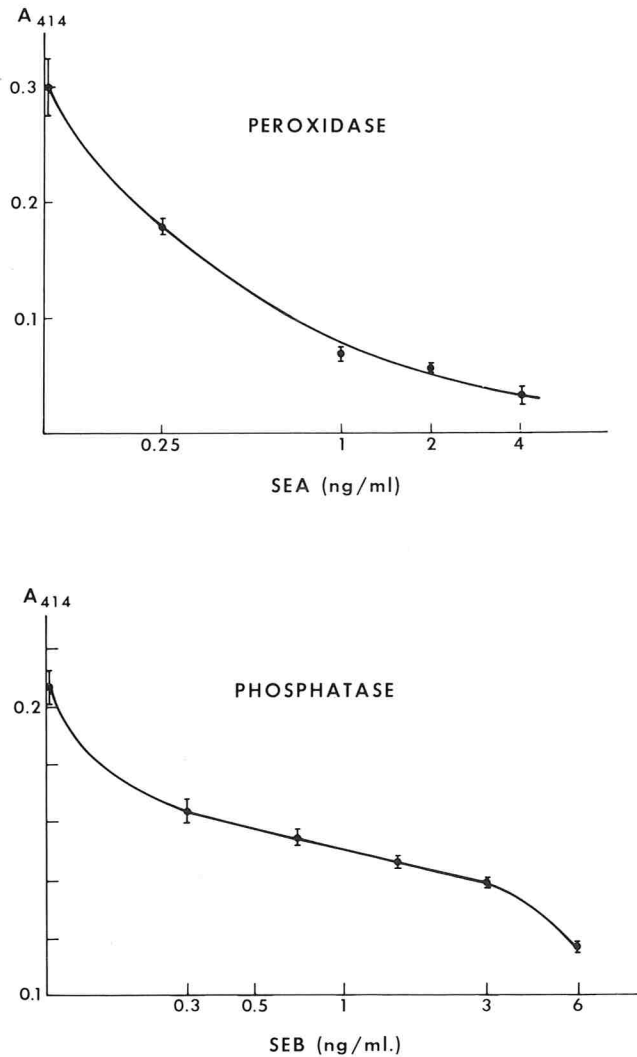


Figure 3. The use of peroxidase and phosphatase in the ELISA of SEA and SEB.

sodium azide in the 20-h test, apparently with no ill effect on peroxidase activity. Their sensitivity, assuming a sample size in each well of the microtiter plate of 0.05 ml, corresponds to 20 ng per well - a remarkable demonstration of the sensitivity of this technique. They did experience difficulty, shared by both RIA and ELISA - a decrease in percentage recovery as the concentration decreased. For a mayonnaise extract spiked with 10 ng per g, 94% of the added SEA was recovered but recovery decreased to 84% for a sample containing 2.5 ng per g. With a spiked hot dog extract, recovery decreased from 94% for a 10-ng-per-g extract to 72% for a 2.5-ng-per-g-extract.

Stiffler-Rosenberg and Fey (14) have taken a different approach in assaying for enterotoxins. Using polystyrene balls coated with enterotoxin SEA, SEB or SEC, they could assay sample volumes of up to 20 ml. The enzyme used in their conjugate was alkaline phosphatase and they employed the direct assay technique. Their procedure for assaying cheese extracts required approximately 24 h for completion. They employed 0.02% sodium azide as a preservative and used both BSA and Tween 20 as

blocking agents. A sensitivity of 1 ng per ml was achieved with a 1 ml sample and 0.1-0.05 ng per ml when a 20-ml sample was used (Table 2). No cross reactions were noted between SEA, SEB or SEC although they did note some interference by cheese extracts - most likely due to non-specific adsorption (11).

TABLE 2. Sensitivity of the ELISA using polystyrene balls.<sup>a</sup>

Enterotoxin	Concentration of enterotoxin detected (ng per ml)	
	1 ml Extract	20 ml Extract
A	1	0.1
B	1	0.1
C	1	0.05

<sup>a</sup>From the data of Stiffler-Rosenberg and Fey (14).

Morita and Woodburn (7) adapted the "homogenous enzyme immune assay" to foods. In this technique, the free antigen (enterotoxin) competes with the enzyme-linked antigen for the homologous antibody. Presence of the antibody adjacent to the antigen-enzyme conjugate interferes with enzyme activity and is a measure of the presence of free toxin in the sample. The enzyme of choice in this study was  $\beta$ -amylase, which is not produced by *Staphylococcus aureus*. Their data indicate a sensitivity in excess of 10 ng per ml. Interference due to food constituents, resulting in high blank values, were found.

#### LIMITATIONS

One should not assume that the sensitivity for spiked food extracts encountered in the literature can be indiscriminately applied to any food sample. As stated above, when analyzing particular classes of foods, one can expect, and many have noted, an appreciable variation in non-specific adsorbance. The suggestion has been made that standard curves be performed on representative food items at the same time as the unknown has been made, but this adds greatly to an assay effort. Moreover, there are few or no data to indicate that this approach, when applied to foods obtained from a wide variety of sources, will result in the required accuracy. It is also not sufficient to derive a value for sensitivity obtained by comparing the results from a spiked extract to those of a buffer control. Pober and Silverman (11) discussed this problem for RIA and attempted to minimize it by pretreating the antibodies with a food extract. Notermans et al. (10), using the ELISA sandwich technique, enjoyed only limited success in minimizing interference from food extracts due to non-specific adsorbance by reacting the homologous antibody with culture filtrates from nontoxic strains of *S. aureus*.

In the authors' laboratory, we have taken a somewhat different approach in an attempt to eliminate, as much as possible, nonspecific adsorbance and derive an analytical technique capable of acting as a standard procedure. The procedure we use (4) in this effort is shown in Fig. 4, and uses affinity chromatography to separate the toxin from the food extract. The toxin in the supernatant fluid was recovered on an immunosorbent



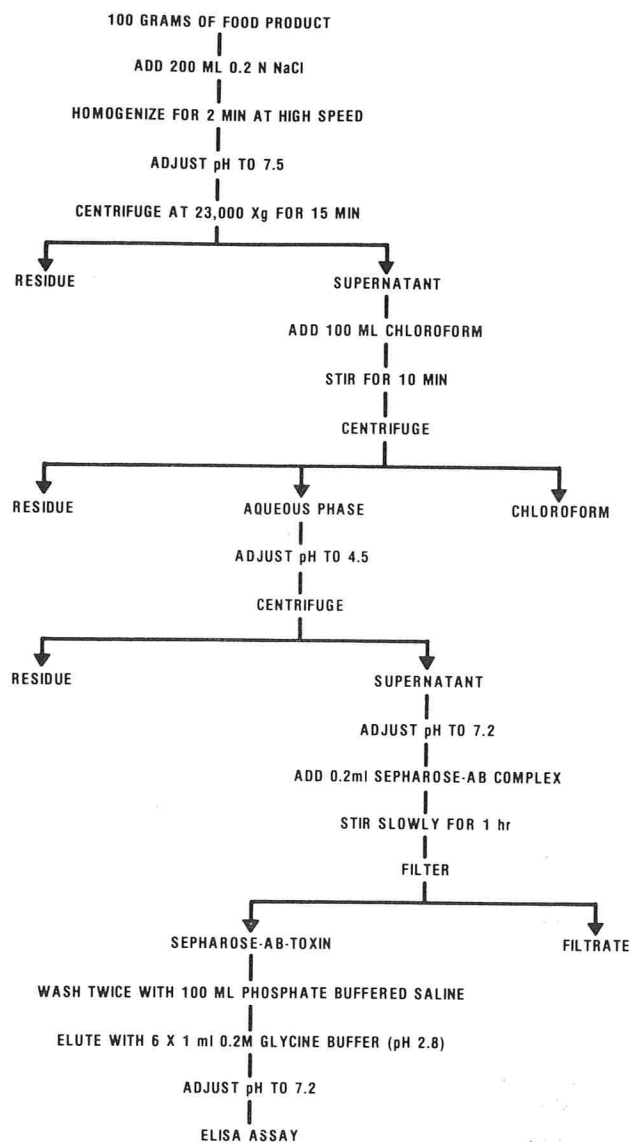


Figure 4. Schematic for the removal of compounds in food capable of interfering with the ELISA.

affinity gel consisting of sepharose conjugated to a homologous antibody. After shaking with the gel for 1 h at room temperature, the gel was separated from the supernatant fluid and washed with phosphate buffered saline solution (0.1 M phosphate, pH 7.4). The toxin was then eluted from the gel with glycine buffer (0.2 M + 0.5 M NaCl, pH 2.8) and recovered by the ELISA technique.

A direct sequential ELISA, using a polystyrene tube as the solid matrix is used. The antibody adsorbed into the polystyrene surface is highly purified homologous rabbit IgG anti-SEA or anti-SEB suspended in 0.1 M carbonate buffer (pH 9.6). Adsorption occurs overnight at ambient temperature and the tubes are then stored at 0-2 C until needed at which time the tubes are drained of their antibody solution. The sample (1 ml) is then incubated at room temperature for 18 h and the enzyme-labeled toxin reacted with its substrate for 4 h.

In Fig. 5, the results of ELISA assay of spiked extracts

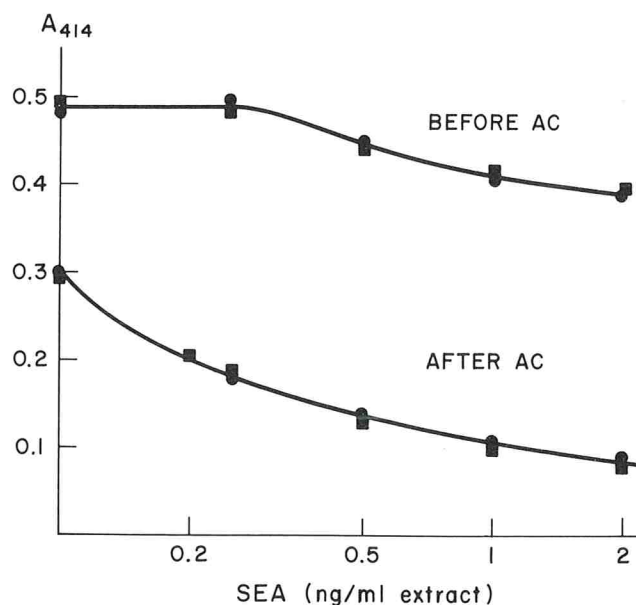


Figure 5. The effect of removing interfering compounds from food on the ELISA.

of milk and cheese both before and after extraction by affinity chromatography are shown. The non-specific interference due to food constituents has been drastically reduced, and a sensitivity of 0.25 ng SEA per ml of extract was obtained. The standard curves derived from milk and cheese were the same, indicating that immunological techniques are more efficient than physical techniques, and can, if verified, simplify the requirements for deriving standard curves for quantification.

This technique, though, is time-consuming and tedious and studies are underway to shorten the procedure. Recovery, presently near 30%, must also be increased, but this recovery level is considerably higher than that reported by Niskanen and Lendroth (9) for the extraction techniques required for the micro-slide assay.

#### ACKNOWLEDGMENT

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## Improved Inspection Scheduling for Foodservice Establishments

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### ABSTRACT

Theoretical and experimental considerations indicate that overall sanitation scores for a community's foodservice establishments would improve if the frequency of sanitation inspections were made to vary according to perceived risks attributable to sanitation deficiencies.

Sanitarians inspect and issue sanitation scores to foodservice establishments to promote sanitation and prevent foodborne illness. Usually the establishments are inspected the same number of times per year. There is increasing theoretical and experimental evidence that providing different inspection frequencies for establishments with different risks of sanitation deficiencies would more effectively reduce demerit points per establishment for a given total number of inspections within a jurisdiction.

Sanitation is, at least in part, a culture-dependent concept. Disease-provoking and "unclean" conditions are both reflected negatively in measurements of sanitation. Attempts to quantify combinations of such conditions will involve subjective judgments as to their relative importance under a variety of situations. Under these circumstances, a major challenge to administrators remains the lack of a good operational definition of sanitation. The parameters currently used to measure it and the way such parameters are combined into an aggregate sanitation rating, lead to too tenuous a connection between sanitation ratings and the risk of illness and uncleanliness.

Nevertheless, we make the assumption that sanitation ratings (or demerit-point sanitation scales), as currently used, at least roughly correlate with the public health and uncleanliness risks determined by sanitarians. Thus if a change in sanitation scheduling yields higher sanitation scores (i.e., fewer demerit points), then we assume that sanitation has improved, on the average.

### FACTORS INFLUENCING SANITATION

There are many factors that influence the sanitation (and the sanitation score) of a foodservice establishment at any point in time. We do not know what all these

factors are, nor how they interrelate; but we can assume that for various establishments different factors have various levels of importance in the sanitation score assigned during periodic inspections. We believe that a reasonable case can be made that in spite of imperfect understanding of establishment characteristics, variable inspection schedules can be structured which improve overall sanitation levels in a jurisdiction. To illustrate this point, suppose that factors influencing sanitation level at a point in time include (but are not limited to): (a) physical nature of the establishment (e.g. liquor store with no in-house food preparation, restaurant with "from scratch" food preparation, etc.) (b) nature of the food sold by the establishment (e.g. prepackaged food heated in a microwave oven, griddle cooking, etc.), (c) management and employee attitudes toward sanitation (e.g., cleanliness is good for business, uncleanliness won't be noticed, etc.), (d) management and employee expectation regarding next sanitation inspection (e.g. an inspection is imminent, etc.) and (e) historical sanitation scores received (e.g., always high, etc.).

Establishments that are strong with regard to (c) and (e) or have minimal food handling are likely to remain sanitary even if their inspection frequency is reduced from current levels and the establishment personnel are aware of such reduction. On the other hand, the sanitation of establishments that are weak in (c) and (e) might be greatly improved if the frequency of inspections is increased, thus affecting (d) and possibly (c). That is, establishments that have been historically "good" are probably "good" for reasons other than the threat of inspections. Conversely, personnel in establishments that have been historically "poor" may have low self-motivation or capability to maintain high standards, and the expectation of more frequent inspections could be a strong factor for sanitation improvement.

### NUMBER OF INSPECTIONS

No absolute number of inspections can be recommended for establishments with high or low sanitation levels. Minimum inspection frequencies, or the average inspection frequencies for all the establishments, must be determined in part on the basis of the public's willingness to pay for the assumed benefits accruing

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from inspections. However, if different establishments benefit from inspections to a different degree, then different inspection schedules seem appropriate.

The clustering of establishments by sanitation types has been studied recently by several investigators (1,4,5). Kaplan and El-Ahraf (4) showed that clustering based on physical characteristics alone (i.e., establishment type) may yield dramatic differences in rate of reported food-poisoning outbreaks.

Table 1 is a reworking of the data presented in Bader et al. (1). They randomly selected matched experimental and control food establishments. The control group was inspected the usual four times per year. The experimental group was inspected once with additional visits only subsequent to complaints from the public in accordance with health department policy. The Table shows that natural clusters of establishments that have historically good scores suffered less of a decline in scores during the experiment than establishments with historically lower scores, when the inspection frequency for both was reduced.

TABLE 1. Historical demerit points and net increase in demerit points induced by experimentally reduced inspection frequency.<sup>a</sup>

Establishment type	Historical demerit points before reduced inspections	Demerit points under reduced inspections	Net increase in demerit points under reduced inspections
School	3.6	6.8	3.2
Simple menu (small)	5.8	9.4	3.6
Drive-in	7.2	12.1	4.9
Complex menu (small)	8.6	17.4	8.8
Large	9.9	17.4	7.5
Oriental	10.6	25.2	14.6

<sup>a</sup>From: Bader et al. (1).

Furthermore, in a 6-month study, Zaki et al. (5) clustered establishments by sanitation scores and noted that establishments with historically high scores generally maintained them even under reduced inspection schedules, whereas establishments with historically low scores appeared to require more frequent inspections to maintain or improve their scores.

Our intuitive argument that establishment characteristics that affect sanitation can be used to institute variable inspection schedules to increase sanitation scores in a jurisdiction is complemented by the following simple mathematical illustration.

Suppose an inspector must divide a total of 10-day-long inspection visits between two restaurants. On any given day when there is no inspection, restaurant A is estimated (by some method) to have a 0.02 probability of causing foodborne illness. Similarly, restaurant B has a 0.04 probability. On a day when either restaurant is inspected, the probability for that restaurant drops to 0.0. If the inspector carries out  $x$  inspections at restaurant A and  $y$  inspections at restaurant B, where  $x + y = 10$ , he will have prevented (on the average)  $(0.02)x + (0.04)y$  illnesses. Thus, if he uses an inspection schedule with  $x = y = 5$ , he will have

prevented an average of 0.3 illness. However, if he inspects restaurant A zero times and restaurant B ten times, on the average he will have prevented 0.4 illness, which is a 33% improvement.

Naturally, our example is too simplistic to be directly implemented. It suggests an extreme solution, and the original premise of a constant rate of outbreaks per day regardless of inspection frequency is probably unrealistic. However, it is not unreasonable to assume that if establishments can be identified and clustered according to their expected rate of undesirable occurrences (as reflected in historical sanitation scores), then an adjustment of inspection schedules so that "riskier" establishments are inspected more frequently relative to less "risky" establishments will result in more efficient use of inspection manpower.

A basic economic principle for resource allocation (2, p. 383) is: "Optimal allocation of an input. If an input  $i$  can be used in producing both commodities  $x$  and  $y$ , then the marginal social product of  $i$  in the manufacture of  $x$  must equal the marginal social product of  $i$  in the manufacture of  $y$ ." An alternative statement of this principle is (2, p. 386): "If resources are to be allocated optimally between any two outputs  $x$  and  $y$ , then the ratio of the marginal social utility of  $x$  ( $MSU_x$ ) to the marginal social cost of  $x$  ( $MSC_x$ ) must equal the corresponding ratio for commodity  $y$ ; i.e., we must have  $MSU_x/MSC_x = MSU_y/MSC_y$ . (Here, marginal cost of  $x$  may be interpreted to mean the quantity of resources needed to produce an additional unit of  $x$ , etc.)"

In our case, the input is foodservice establishment inspections (or the cost of those inspections) and the social product is, for example, number of outbreaks of food poisoning prevented. The above economic principle may not apply if there are constraints on the use of resources, such as requirements for at least a minimum annual number of inspections per establishment. Also, we do not possess a sophisticated model for measuring the marginal effect of an additional inspection (or expenditure of an inspection dollar) on the social product. However, in searching for a solution that is an improvement over a constant inspection frequency regardless of expected benefits, one can see that it is reasonable to infer that inspection frequency should be somewhat proportional to presumed potential changes in sanitation risk and inversely proportional to inspection costs.

A practical test of clustering by sanitation score was made in Arlington County, Virginia (3). In general, the lower the historical sanitation score of an establishment, the more frequently it was inspected and vice-versa. The result was a continuous and substantial increase in average sanitation scores, in spite of reduced manpower levels.

In the mathematical sense of the word, "optimal" *con't on p. 413*

## Public Participation: An Introduction

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### ABSTRACT

Public participation has become an increasingly important activity within the Food and Drug Administration (FDA). Translating a concept such as public participation into an operational reality, however, presents a constant challenge to federal as well as state and local governments. FDA has developed a model Public Participation Program that provides a variety of ways for consumers to become involved in the Agency's decisionmaking process. The FDA program is policy oriented and strives to achieve five basic objectives. The ultimate goal of this program is to provide consumers with the opportunity to participate in and impact on the Agency's decisions concerning important health issues. Realizing this goal will enable the Agency to ensure that its policies and regulations are responsive to expressed consumer concerns.

I am very pleased to share with you a rather new and exciting facet of the Food and Drug Administration's many activities. The one activity that I would like to target today is the Agency's Public Participation Program. Public participation more than likely sounds like just another bureaucratic "buzz" word. The principles and philosophy underlying the notion of public participation, however, have provided the foundation for American democracy. It is a concept that has been used to establish a form of government that recognizes the important role of the people in determining national policies and practices.

At times, this role is played out at the voting booth — where people elect representatives to know their constituency's concerns and ensure that these concerns are reflected in legislation. At other times, there is a more direct and individual involvement through such activities as referenda, recalls and initiatives — where people consolidate their efforts, educate themselves about an issue and voice a concerted view towards a proposed or an already established governmental action.

Through citizen involvement, government becomes a reality for the public, a structure for which they are responsible and a system that can be questioned and held accountable. Government was never meant to be a cold machine, beyond the public's control, that automatically cranks out rules and regulations. It is, and I think it was originally envisioned, to be a dynamic and evolutionary structure that makes decisions about complex issues that directly or indirectly affect the lives of the American public. Public participation is as American as mom, the flag, apple pie — and milk!

### A NEW APPROACH

Although historically rooted in the beginnings of our country, public participation is considered to be by many a new and exciting activity in 1979. For it has only been within the past decade that the federal government has made a deliberate effort to recapture the essence of this concept in its programs and policies. Today, many federal agencies — the U.S. Department of Agriculture, the Department of Commerce, the Federal Trade Commission, and the Department of Transportation — are establishing public participation programs to include consumers in their respective activities.

Throughout the years, the need for a greater degree of citizen involvement in government decisionmaking has been recognized and addressed by many presidential administrations. In April 1978, President Carter formally advised all federal departments and agencies that one of his administration's ultimate goals was "...to ensure that each Agency of the Federal Government adequately responds to consumer needs in its development of policy and provides adequate opportunity for consumer participation in the decisionmaking process."

### FDA OFFICE OF CONSUMER AFFAIRS

Within the Food and Drug Administration, the Office of Consumer Affairs has been given the lead responsibility for involving consumers in FDA's decision-making process. It is the office that designs and carries out the Agency's Public Participation Program.

At the beginning of this paper, I described public participation within FDA as a "rather new and exciting activity." I will now complete this description by adding that it is also a significant activity. Public participation is regarded by the Agency as a policy-oriented activity. The Office of Consumer Affairs is located within the Commissioner's office and is included as part of the Agency's Policy Board. With the Agency's support, the Office of Consumer Affairs has received an increased staff and budget for "institutionalizing" public participation and representing the "consumer position" in major policy decisions.

For the past 8 years, FDA's Office of Consumer Affairs has been attempting to develop a variety of methods for making public participation a working concept within the Agency. As a result, the Agency has been able to take a strong lead in following the direction set by President Carter. FDA has developed a model

Public Participation Program — a program that is today both an organizational and operational reality.

### THE FDA GOAL

FDA's ultimate goal in public participation is to ensure that the public is provided with the opportunity to participate in and impact on the policy, regulations and administrative decisions established by the Agency. To a large degree, this goal is being achieved through a variety of activities that operate under the Public Participation Program. At this point, the Agency has established a basis from which to work and is now in the process of refining and expanding its basic program activities so that they become an integral part of institutional operations and consumer behavior.

Before describing FDA's Public Participation Program in more detail, I would like to make you aware of the monumental challenge that is involved in translating a concept such as public participation into an operational program. With a potential constituency of approximately 220 million citizens within the United States, let me ask what activities would you design to ensure the greatest participation opportunities for as many individuals and groups within this constituency as possible? What would you develop to additionally ensure that such widescale public participation consistently generated a substantial contribution to the Agency's decisionmaking process? These are questions that FDA continues to ask itself — always testing and exploring the direction that public participation should be, or could be, taking in the Agency.

There is no one best way to achieve effective public participation. T. S. Elliot forcefully ended his poem, "The Hollow Men," with the insight:

“...Between the idea  
And the reality  
Between the motion  
And the act  
Falls the shadow...”  
“...Between the conception  
And the creation  
Between the emotion  
And the response  
Falls the shadow...”

So too with public participation; it will always present an element of the unknown — it requires a much too dynamic and evolutionary approach to be contained once and for all in a single, static program. Each activity possesses the inherent capacity for extending itself into yet another innovative way for reaching the public and encouraging participation.

### THE OBJECTIVES

I would now like to turn your attention to the basic objectives that comprise the FDA's Public Participation Program. The Agency, both at Headquarters and its Field offices, has directed a concerted effort towards

involving the public in FDA's decisionmaking and regulatory process. We have essentially aimed this effort to achieve five basic objectives. These objectives, which I will discuss shortly, are the guiding principles for all activities undertaken to establish a substantial relationship with the public.

The first objective is *to inform the public about upcoming issues and activities early enough in the decisionmaking process to allow for their timely participation*. Surprising as it may seem, there are some administrative procedures within the government that do come to an end, even within what is now considered to be the expected life span of the average man. The comment period for proposed regulations usually lasts for about 30 days; open public hearings are usually announced in the *Federal Register* about 30 days before they are held; and nominations for membership on advisory committees are usually requested by a certain cut-off date.

There is nothing more frustrating than to miss out on a good thing; there is nothing more irritating, however, than to be invited to participate in a good thing after it's already happened. The Agency attempts to place all of its communications announcing public participation opportunities on a *timely* schedule.

The *Consumer Update* and "Dear Consumer" letters are two avenues that the Office of Consumer Affairs uses to advise the public of forthcoming participation opportunities within the Agency. The *Update* is sent to about 20,000 consumers and advises them of proposed regulations, final regulations, how to petition the Agency, vacancies on advisory committees, and open public hearings. "Dear Consumer" letters single out the significant events within the Agency's day-to-day operations and encourage consumers to become more informed and involved in specific issues.

The second objective of FDA's Public Participation Program is *to actively seek and assess diverse public views on important health issues*. One of the important lessons learned from the Agency's past experience with consumers is that "the public" — no matter how unconcerned, vague or anonymous it may appear — possesses the unique ability to regroup, gain a tremendous momentum and exert a powerful influence on the course of decisionmaking.

The Agency has initiated a number of activities that are specifically designed to create an "open door" to the public. By establishing a variety of these "open door" activities, the Agency is able to be more aware of the public's perspectives towards its regulatory activities. The dialogue which develops as a result of many of these activities enables the Agency and consumers to informally discuss issues and to explore reasonable courses of FDA action.

These activities include National Consumer Exchange Meetings, District Consumer Exchange Meetings, monitoring the type of consumer interests and opinions that are submitted to the Agency and reviewing the consumer concerns expressed to the Agency's Consumer

Affairs Officers. All such "open door" activities are intended to either directly or indirectly bring Agency decisionmakers closer to the public by identifying potential health issues, trends and shifts in consumer concerns, and topics of high consumer interest.

The third objective of the FDA Public Participation Program is to provide educational and training opportunities to strengthen the quality of public involvement in the Agency's activities. As I have previously mentioned, there is a two-fold challenge involved in developing a public participation program: (a) to encourage the public's involvement in Agency activity and (b) to foster a more thoughtful and substantive public response to complex health and regulatory problems.

Two of the more significant activities that have been established to meet this challenge involve contracts to provide training and education for interested members of the public. One of these contracts, which is known as the National Consumer Access and Awareness Project, is essentially a law course designed to teach consumers about FDA's administrative practices and procedures. The second contract, the Consumer Advocacy Skill Training Project, provides an indepth course on the various techniques that can be used to advocate a specific point-of-view. This course is primarily directed to present as well as potential consumer representatives on FDA Advisory Committees.

The Agency recognizes that the public may need access to supplementary information about the issues for which the Agency is seeking comments. It is unreasonable to expect consumers to submit substantive comments on FDA's activities if they are not aware of the various issues, alternatives, differing positions, and other considerations involved in a particular action. Thoughtful reaction, just as thoughtful decisionmaking, requires access to a broad diversity of opinion and views; it cannot be achieved in a vacuum.

For this reason, the Agency prepares background information to assist consumers in developing their comments on certain important issues. For instance, the Office of Consumer Affairs recently prepared a "Dear Consumer" letter advising consumers of a proposed regulation on Patient Package Inserts and asking for their comments. This was one proposal that required background information to help consumers be more aware of issues for which the Agency was seeking comments. This background material was developed and sent to consumers as an accompaniment to the "Dear Consumer" letter.

The Food Labeling Hearings are another example of how the Agency has met the two-fold challenge of effective public participation. These hearings were a special project that the Agency initiated to find out what consumers thought of the information that was provided with the foods they purchased.

From August to October of 1978, public Food Labeling Hearings were held in Wichita, Little Rock,

Washington, D.C., San Francisco, and Boston. In an effort to augment public involvement, an advance team went to each city approximately 2 weeks before the scheduled hearing date and conducted a series of meetings for "grassroot" consumers. These prehearing meetings were intended to encourage consumers to attend and participate in the formal Food Labeling Hearings. More importantly, however, they were designed to inform consumers of the food labeling issues on which the Agency was focusing its attention.

"Grassroot" consumers who attended these meetings represented a broad spectrum of community organizations including senior citizen groups, YMCA, Red Cross, farm wives collectives, church groups, Hispanic organizations, the Urban League, and homemakers. The advance teams planned and attended a total of 94 prehearing meetings, with an average of 20 meetings being held in each city. Over 1300 consumers attended these meetings and took advantage of the opportunity to meet with government representatives and discuss food labeling issues before the formal hearings.

Stimulated by at least a cursory knowledge of the issues, a large number of consumers were willing to present their views at the formal hearings. Consumers who were not able to attend the formal hearings were encouraged to write their views and send them to the Agency. Altogether, FDA received approximately 10,000 written and oral comments from consumers.

For many consumers, this was their first opportunity to gain access to FDA in a way that would guarantee that their views would be considered by Agency decisionmakers. These prehearing meetings helped consumers to have a basic understanding of food labeling issues. Given this basic understanding, consumers were then able to modify the issues and make whatever personal observations they believed relevant to these issues.

A significant number of consumers participated in both the prehearing meetings and the formal Food Labeling Hearings. Consumers had the opportunity to express their opinions about food labeling and the Agency received a variety of perspectives from which to view the issue. Although this is a good start, it is only a part of the total process necessary to achieve effective public participation.

The basic principles of good communication are inherent within the concept of public participation. If the Agency limited its scope of public participation to providing the opportunity to participate and receiving consumers' views, the essence or nature of good communication would be lost. Consumers would attend activities and express their views on issues highlighted by the Agency, but they would have no indication as to how their views impacted on the Agency's decisions or what was the FDA's final decision.

The Agency believes that to recapture the spirit of public participation, it is essential to strive for a program that provides a basis for good communication with consumers. The final two objectives of FDA's Public

Participation reflect this goal. These objectives are: to direct the public's views to Agency decisionmakers so that their views can impact on administrative and regulatory policies and to advise the public of how their views assisted the Agency in reaching final decisions and policies.

Following the close of the Food Labeling Hearings, the Office of Consumer Affairs assisted the Agency in formulating a Food Labeling Plan. This plan reflects the consumer views that were submitted to FDA during these hearings. Part of this plan will provide the basis for a proposed regulation that is scheduled to be published in the *Federal Register* sometime early this fall (1979).

Consumer participation at the Food Labeling Hearings provided the Agency with the support and guidance necessary to establish revised policies in numerous important areas. In addition, public views enabled FDA to state its intentions to support legislation in areas that are presently beyond the scope of its authority.

The Food Labeling Hearings did not provide all of the answers necessary for development of improved food labeling. The consumer response, however, was sufficient in some areas to indicate that a revision in policy was desirable and for FDA to be able to state its intention to consider alternatives to current policies. The Agency plans to address several areas of concern raised at these hearings through studies, experimentation and other means.

Public participation at the Food Labeling Hearings exerted a significant impact on the Agency's food labeling policies — and it is important that consumers know this. This is the final stage of good communication and effective public participation. As soon as the proposed food labeling regulation is published in the *Federal Register*, the Office of Consumer Affairs plans to send the proposal to all of the consumers who testified at the hearings or sent written comments to the Agency. Approximately 10,000 consumers will receive the food labeling proposal.

#### IN CONCLUSION

Regardless of whether it is the industry, health professionals or consumers, the ultimate goal of any group that interacts with FDA is to have its particular point of view reflected in Agency policy or final regulations. Heretofore consumers have not consistently expressed their views to FDA during the decisionmaking process. As a result, the Agency did not have access to consumer views when attempting to resolve an issue or establish a certain regulatory policy. When such a situation occurs, the courtroom usually becomes the first rather than the last forum for discussing differences and considering alternative approaches to a problem. One of the benefits of public participation is that diversity and controversy are actively sought and openly addressed at the early stages of the decisionmaking process.

#### Keo and Silverman, *con't from p. 407*

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#### Kaplan and Zalkind, *con't from p. 409*

inspection schedules cannot be derived. Too much is unknown or unmeasurable about establishment characteristics and sanitation. However, our arguments strongly support the case that substantial improvements can be made by changing the current widespread practice of constant inspection frequencies. By incrementally varying inspection frequencies in proportion to sanitation deficiencies (and in inverse proportion to inspection costs if the differences are significant enough to warrant it), observing subsequent changes in sanitation scores, and again varying the inspection frequencies, a superior inspection schedule can be successively approached.

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## A Field Topic

### Pinpointing Post-Pasteurization Contamination

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3525 East Hanna Avenue, Indianapolis, Indiana 46227

(Received for publication October 15, 1979)

#### ABSTRACT

Post-pasteurization recontamination is the main factor in determining shelf-life. The chief source of such contamination is a neglected filler, which can be readily pinpointed by holding a sample from each filler each day at 7 C (45 F) for 7 days before plating.

Nowadays, when milk is often many days old when consumed, shelf-life is of cardinal importance. Since microbial spoilage at refrigeration temperatures results from growth of psychrotrophic organisms, post-pasteurization recontamination with these organisms must be reduced to a minimum. Experience has shown that the filler is the chief source of contamination. This is clear from the data in Table 1, where Samples 3, 4 and 11 came from the same filler, while the remaining samples from other fillers all had counts below 1,000/ml after being held at 7 C (45 F) for 7 days. This plant had previously been holding samples for 5 days (1) before plating, and the management was satisfied with the results. But when persuaded to re-plate samples after 7 days they

<sup>1</sup>Deceased, February, 1980.

TABLE 1. Standard Plate Counts on pasteurized products after 7 days at 7 C.

Product	SPC/ml
Gallon Homo.	500
Half gallon	400
Quart	370,000
10 oz	390,000
Half-pint	600
" "	400
Half gallon 3.5%	700
Gallon 2.0%	600
Half-gallon 2%	700
" " low fat	400
Quart, half and half	TNTC
Half-pint	900

got quite a different picture! Unfortunately, the records of these comparative tests were discarded 5 or 6 years ago. However, experience with a number of other plants has convinced the writer of the necessity of extending the pre-incubation period from 5 to 7 days. The latest edition of *Standard Methods* (2) allows 5 or 7 days for the Keeping Quality Test. It is believed that the next edition should call for 7 days.

As LaGrange has recently reported, considerable multiplication of psychrotrophs occurs between the farm bulk tank and the pasteurizer (4). This points to the importance of keeping the numbers of psychrotrophs to the minimum in the raw milk, since their heat-resistant en-

zymes survive pasteurization and continue their attack on the proteins and fat, thus shortening shelf-life and adversely affecting quality and quantity of product. This is best done by emphasizing the vital importance of *thorough cleaning and sanitizing of all milk-handling surfaces immediately after every milking.*

For the benefit of those still doing routine coliform tests on 'fresh' products in the belief they will adequately reflect post-pasteurization contamination, it should be mentioned that none of the products shown in Table 1, nor in numerous others tested by the writer, gave a positive test for coliforms in 1-ml portions. Which makes one wonder about the value of a standard which allows 0.1/ml (6) for detecting post-pasteurization recontamination.

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## PROGRAM

# Sixty-Seventh Annual Meeting International Association of Milk, Food and Environmental Sanitarians, Inc.

in conjunction with  
Forty-Fourth Annual Meeting  
National Environmental Health Association

*In cooperation with*  
*Wisconsin Association of Milk and Food Sanitarians, Inc.*  
*Wisconsin Dairy Plant Fieldman's Association*  
July 27-31, 1980

Red Carpet Hotel [IAMFES]  
Marc Plaza Hotel [NEHA]

Milwaukee, Wisconsin  
Milwaukee, Wisconsin

*William Kempa*  
*President*  
IAMFES, Inc.

### REGISTRATION

Sunday, July 27 - 1:00 P.M. - 5:00 P.M.  
Monday, July 28 - 8:00 A.M. - 5:00 P.M.  
Tuesday, July 29 - 8:00 A.M. - 5:00 P.M.  
Wednesday, July 30 - 8:00 A.M. - 5:00 P.M.  
Thursday, July 31 - 8:00 A.M. - 12:00 Noon

*Junior Past President:* Howard E. Hutchings, Pierre,  
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*Senior Past President:* David D. Fry, Orlando, Florida  
*Executive Secretary:* Earl O. Wright, Ames, Iowa

### REGISTRATION FEES

	<i>Member,* Advance</i>	<i>Member,* At Door</i>	<i>Non- Member</i>
Registration Fee	\$20.00	\$25.00	\$30.00
Spouse	\$ 8.00	\$11.00	\$16.00
Student	No Charge		
Banquet	\$15.00	\$17.00	\$22.50
Gemütlichkeit Abendgesell- schaft	\$ 2.00	\$ 3.00	\$ 5.00
*Member: IAMFES and/or Wisconsin Dairy Plant Fieldmen Association			

### JOURNAL OF FOOD PROTECTION

*Editor:* Elmer H. Marth, Madison Wisconsin  
*Managing Editor:* Earl O. Wright, Ames, Iowa  
*Associate Managing Editor:* Jan Richards, Ames, Iowa

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Wisconsin Student Affiliate  
 Executive Board  
 Executive Board

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*Co-Chairman* ..... John Collier  
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*Speakers' Hospitality* ..... Clarence Williams  
*Banquet and Entertainment* ..... Roger Hulbert  
*Housing* ..... Robert Parker  
*Publicity* ..... John Collier  
*Registration* ..... Richard Rowley  
*Arrangements/Visual Aids* ..... Russ Churchward  
*Ladies' Entertainment (Social Functions)* Alma M. Pace  
*Door Prizes* ..... Justin Drake  
*Milk Breaks* ..... Boyd Cuff  
*WAMFS Coordinator* ..... Don Raffel  
*WDPFA Coordinator* ..... Glaris Hams

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*Secretary:* Archie Holiday

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Janis Bush	North Texas
Marshall Cooper	Virginia
Llody Luedecke	Washington
Donald G. Raffel	Wisconsin

**SATURDAY, JULY 26**

12:00 - 10:00 p.m.                      Local Arrangements - Cajun  
 4:30 - 7:30 p.m.                        Executive Board - Bisque

**SUNDAY, JULY 27**

9:00 a.m. - Noon                        Executive Board - Bisque  
 10:00 a.m. - 5:00 p.m.                Registration - Jackson Square  
 8:00 a.m. - 5:00 p.m.                Local Arrangements - Cajun  
 1:00 - 5:00 p.m.                        *Journal* Management Committee - Directors 4  
 1:00 - 5:00 p.m.                        Committee on Communicable Diseases Affecting Man - Directors 3  
 1:00 - 5:00 p.m.                        Milking Machine Manufacturers - Directors 1, 2  
 2:00 - 4:00 p.m.                        Council of Affiliates - Directors 10  
 3:00 - 5:00 p.m.                        Executive Board (Executive Board should schedule part of this time to attend part of Council meeting.) - Bisque  
 4:00 - 6:00 p.m.                        *Journal* Sanitarians and Fieldmen's Committee - Directors 6  
 6:00 - 9:00 p.m.                        Early Bird Reception - Grand Ballroom

**MONDAY, JULY 28**

10:00 a.m.                                IAMFES Members are invited to attend the NEHA Opening Session, featuring IAMFES Member Bailus Walker, Director, Health Standards Programs, OSHA, "Occupational and Environmental Health Priorities of the 1980's" - Marc Plaza Hotel  
 7:00 - 9:00 a.m.                        Executive Board - Bisque  
 8:00 a.m. - 5:00 p.m.                Ladies' Hospitality - Creole  
 8:00 a.m. - 5:00 p.m.                Registration - Jackson Square  
 8:00 a.m. - 5:00 p.m.                Local Arrangements - Cajun  
 8:00 a.m. - 5:00 p.m.                Committee on Communicable Diseases Affecting Man - Directors 3  
 1:00 - 2:30 p.m.                        Farm Methods Committee (Sub. Com.) - Directors 1, 2  
 2:30 - 5:00 p.m.                        Farm Methods Committee - Directors 1, 2  
 1:00 - 5:00 p.m.                        Food Equipment/Sanitary Standards Committee - Directors 4  
 1:00 - 5:00 p.m.                        Council of State Sanitarians Registration Agencies - Directors 10  
 1:00 - 5:00 p.m.                        *Journal* Foodservice Committee - Directors 5

- 1:00 - 5:00 p.m. Sanitarians Joint Council - Directors 6  
 1:00 - 5:00 p.m. Food Equipment/Sanitary Standards Committee - Directors 7  
 1:00 - 5:00 p.m. Applied Laboratory Methods Committee - Directors 8  
 4:00 - 6:00 p.m. Executive Board - Bisque

### TUESDAY, JULY 29

- 6:30 - 8:00 a.m. Breakfast, Sponsors: Wisconsin Egg Producers Assn.; Wisconsin Poultry Improvement Assn.; Wisconsin Turkey Federation; Wisconsin Dept. of Agriculture, Trade, and Consumer Protection; Wisconsin Assn. of Milk and Food Sanitarians - Poolside

#### Morning — General Session — New Orleans William Arledge, Presiding

- 8:30 a.m. DOOR PRIZE (Admission by Registration Badge)  
 8:35 a.m. INVOCATION — Clarence Luchterhand  
 8:40 a.m. ADDRESS OF WELCOME — Gary E. Rohde, Wisconsin Dept. of Agriculture, Trade and Consumer Protection, Madison, Wisconsin  
 9:00 a.m. PRESIDENTIAL ADDRESS — William Kempa  
 9:15 a.m. "PREPAREDNESS FOR RADIOLOGICAL CATASTROPHE (MILK/FOOD)" — Lawrence McDonnell, Dept. of Health and Social Service, Madison, Wisconsin  
 9:45 a.m. MILK BREAK  
 10:00 a.m. DOOR PRIZE  
 10:05 a.m. ANNUAL BUSINESS MEETING:  
 1. Report of Executive Secretary  
 2. Report of Secretary-Treasurer  
 3. Report of Editor  
 4. Committee Reports  
 5. 3-A Symbol Council Report  
 6. Report of Resolutions Committee  
 7. Report of Affiliate Council  
 8. Old Business  
 9. New Business  
 10. Election of Officers

#### Afternoon — General Session — New Orleans William Kempa, Presiding

- 1:25 p.m. DOOR PRIZE  
 1:30 p.m. "YOU ARE WHAT YOU EAT" — Nancy Topp, St. Mary's Hospital Medical Center, Madison, Wisconsin

- 2:30 p.m. "CORPORATE COMMITMENT TO QUALITY ASSURANCE" — C. Dee Clingman, Red Lobster Inns, Orlando, Florida  
 2:45 p.m. MILK BREAK  
 3:00 p.m. DOOR PRIZE  
 3:05 p.m. "ROLE OF MASS MEDIA IN FOOD PROTECTION" — Paula Brookmire, The Milwaukee Journal =Newspapers, Inc., Milwaukee, Wisconsin  
 3:45 p.m. "SALMONELLA DETECTION IN FOODS USING AN ENZYME IMMUNOASSAY" — Scott A Minnich and Paul A. Hartman, Dept. of Bacteriology, Iowa State University, Ames, Iowa  
 4:00 p.m. "INDUSTRY INVOLVEMENT IN FOOD REGULATIONS" — Harvey Ebert, Land O'Lakes, Inc., Minneapolis, Minnesota  
 Adjourn  
 4:30 - 5:30 p.m. AFFILIATE COUNCIL MEETING — Directors 10

#### Evening — "Cracker Barrel" Sessions — Directors 9, 10

- 7:00 - 9:00 p.m. Milk Sanitation Service — "NEW ANTIBIOTICS TESTS AND THE S.T.O.P. PROGRAM" — Directors 10, John Adams, National Milk Producers Federation, Washington, DC, Chairman; Dennis Kwider, Yankee Milk, Inc., North Andover, Massachusetts; William Menz, DRINC, Rosemont, Illinois; Eugene McGarrahan, FDA, Washington, DC; John Spalding, USDA, Washington, DC  
 7:00 - 9:00 p.m. "15TH ED./STANDARD METHODS FOR THE EXAMINATION OF DAIRY PRODUCTS" — Directors 9, Elmer H. Marth, Dept. of Food Science, University of Wisconsin, Madison, Wisconsin, Chairman

### WEDNESDAY, JULY 30

- 8:00 a.m. - 5:00 p.m. WISCONSIN DAIRY PLANT FIELDMAN'S ASSOC., ANNUAL MEETING — New Orleans South (see program at end of IAMFES-program)  
 12:00 Noon - 1:30 p.m. WISCONSIN DAIRY PLANT FIELDMAN'S BANQUET — Grand Ballroom East  
 12:00 Noon - 1:30 p.m. WISCONSIN ASSOCIATION OF MILK AND FOOD SANITARIANS, INC., ANNUAL MEETING AND LUNCHEON — Grand Ballroom West

**Morning — Milk Sanitation Section —  
New Orleans North  
Harry Haverland, Presiding**

- 8:55 a.m. DOOR PRIZE  
9:00 a.m. "INACTIVATION OF AFLATOXIN M<sub>1</sub>  
IN MILK BY HYDROGEN PEROXIDE"  
— R. S. Applebaum\* and E. H. Marth,  
Dept. of Food Science, University of Wis-  
consin, Madison, Wisconsin  
9:20 a.m. "CHANGES IN THE 1978 P.M.O." —  
Eugene McGarrahan, FDA, Washington,  
DC  
9:50 a.m. "COMPARISON OF TESTS OF MILK  
SAMPLES TAKEN CONVENTIONALLY  
AND WITH AN AUTOMATIC IN-LINE  
SAMPLER" — R. T. Marshall\* and D. S.  
Shelley, Dept. of Food Science and Nutrition  
University of Missouri, Columbia, Missouri  
10:05 a.m. MILK BREAK  
10:20 a.m. "UPDATE ON THE STATUS OF MASTI-  
TIS" L. H. Schultz, Dept. of Dairy  
Science, University of Wisconsin, Madison,  
Wisconsin  
10:50 a.m. "CAMPYLOBACTERIOSIS AND YERSI-  
NIOSIS: FOOD-ASSOCIATED ILL-  
NESSES OF RECENT CONCERN" — M.  
P. Doyle, Food Research Institute, Mad-  
ison, Wisconsin  
11:20 a.m. "RESULTS OF DHIA SOMATIC CELL  
PROGRAM" - Alan Bringe, Dept. of Dairy  
Science, Univ. of Wisconsin, Madison,  
Wisconsin

**Morning — Food Sanitation Section —  
New Orleans Center  
Paul J. Pace, Presiding**

- 8:55 a.m. DOOR PRIZE  
9:00 a.m. "TEMPERATURE AND HANDLING  
PRACTICES OF PERISHABLE PRO-  
DUCE IN RETAIL FOOD STORES" — F.  
N. Bodyfelt\*, D. M. Lomnicki and C. J.  
Wyatt, Dept. of Food Science and Techno-  
logy, Oregon State Univ., Corvallis, Oregon  
9:20 a.m. "INSECT AND RODENT CONTROL  
IN FOOD ESTABLISHMENTS" — Rich-  
ard Gillespie, FDA, Cincinnati, Ohio.  
10:00 a.m. "RECOVERY OF FECAL COLIFORMS  
AT 44.5, 45.0, AND 45.5 C, and THEIR  
RESPECTIVE RATIOS TO *ESCHERI-  
CHIA COLI*" — K. F. Weiss\*, G. Riedel  
and S. Charbonneau, Health Protection  
Branch, Health and Welfare Canada,  
Ottawa, Ontario, Canada  
10:15 a.m. MILK BREAK  
10:30 a.m. DOOR PRIZE

- 10:35 a.m. "PCBs IN THE FOOD CHAIN AND  
REGULATORY ACTIVITIES" — Rich-  
ard Dees, FDA, Milwaukee, Wisconsin  
11:05 a.m. "PROTECTION OF *STAPHYLOCOCCUS  
AUREUS* 196E FROM HEAT INJURY"  
— James L. Smith, USDA, Eastern Re-  
search Center, Philadelphia, Pennsylvania  
11:20 a.m. "NITROSAMINES IN BEER" — David H.  
Hickman, Covington & Burling, Washing-  
ton, DC

**Afternoon — Milk Sanitation Section —  
New Orleans North  
Clarence Luchterhand, Presiding**

- 1:25 p.m. DOOR PRIZE  
1:30 p.m. "EFFICIENT CLEANING OF RAW MILK  
EQUIPMENT WITH COLDER WATER"  
— R. L. Bradley, Jr., Dept. of Food Science,  
University of Wisconsin, Madison, Wisconsin  
1:45 p.m. "UHT PASTEURIZATION SYSTEMS" —  
Roger Dickerson, FDA, Cincinnati, Ohio  
2:15 p.m. "MICROBIOLOGICAL EVALUATION OF  
PAPERBOARD CARTONS FOR MILK  
PACKAGING" — Sai Farahnik, Ex-Cell-O  
Corp., Walled Lake, Michigan  
2:30 p.m. "QUALITY ASSURANCE: NATIONAL  
AND INTERNATIONAL" — Thomas W.  
Holzinger, Borden Inc., Columbus, Ohio  
3:00 p.m. MILK BREAK  
3:15 p.m. DOOR PRIZE  
3:20 p.m. "EFFECTS OF DG, TBHQ, AND BAT ON  
GROWTH OF SELECTED BACTERIA"  
— Mohammed B. Gailani\* and Daniel Y. C.  
Fung, Dept. of Animal Science, Kansas State  
University, Manhattan, Kansas  
3:40 p.m. "IODINE RESIDUAL RESEARCH" — Roger  
W. Hempken, Dept. of Animal Science,  
Univ. of Kentucky, Lexington, Kentucky

**Afternoon — Food Sanitation Section —  
New Orleans Center  
John Gerberich, Presiding**

- 1:25 p.m. DOOR PRIZE  
1:30 p.m. "GROWTH AND ISOLATION OF *BACIL-  
LUS CEREUS* IN RETAIL PUMPKIN  
PIES" — C. Jane Wyatt\* and V. Guy, Dept.  
of Food Science and Technology, Oregon  
State Univ., Corvallis, Oregon.  
1:45 p.m. "GROWTH OF *SALMONELLA TYPHI-  
MURIUM* AND *STAPHYLOCOCCUS  
AUREUS* IN RETAIL PUMPKIN PIES" —  
C. Jane Wyatt\* and V. Guy, Dept. of Food  
Science and Technology, Oregon State Univ.  
Corvallis, Oregon  
2:00 p.m. "COOK/CHILL FOOD SERVICE SYS-  
TEM WITH A MICROWAVE OVEN:  
SURVIVAL OF *STREPTOCOCCUS FAE-  
CIUM* IN BEEF LOAF AND POTATOES

\*Asterisk indicates person presenting paper.

AFTER MICROWAVE-HEATING" C. A. Dahl\*, M. E. Matthews and E. H. Marth, Dept. of Food Science, Univ. of Wisconsin, Madison, Wisconsin

2:15 p.m.

BREAK

2:30 p.m.

"RAPID MICROBIAL IDENTIFICATION SYSTEMS IN THE FOOD INDUSTRY — PRESENT & FUTURE," Panel: N. A. Cox, Chairman, USDA, Athens, Georgia; Paul Poelma, FDA, Washington, DC; Milton Albert, Morton Frozen Foods, Russellville, Arkansas; Mike Politte, Ralson Purina, St. Louis, Missouri; Irving J. Weintraub, General Diagnostics, Buffalo Grove, Illinois; James A. Buring, Inolex Corp., Owing Mills, Maryland; John Round, Roche Diagnostics, Nutley, New Jersey; Ken Hauser, Analytab Products, Plainview, New York.

3:00 p.m. - 5:00 p.m. N.M.C. Board of Directors Meeting — Directors 10

#### WEDNESDAY EVENING, JULY 30

6:00 p.m. - 7:00 p.m. RECEPTION — Foyer, Grand Ballroom

7:00 p.m. - 8:15 p.m. BANQUET, Entertainment — Grand Ballroom

8:30 p.m. - 9:30 p.m. AWARDS PROGRAM AND INSTALLATION OF OFFICERS

9:30 p.m. - 10:30 p.m. ENTERTAINMENT

#### THURSDAY, JULY 31

7:30 a.m. Executive Board, Breakfast Meeting — Creole

7:30 a.m. - 12:00 Noon National Mastitis Council, Registration — Foyer/Jackson Square

8:00 a.m. National Conference on Interstate Milk Shipments — Directors 9

8:30 a.m. - 5:00 p.m. National Mastitis Council, 1980 Summer Meeting — see program and details at end of IAMFES program — Grand Ballroom

#### ENTERTAINMENT, MEMBERS AND SPOUSES

##### SUNDAY, JULY 27

6:00 - 9:00 p.m. Early Bird Reception — Grand Ballroom

##### MONDAY, JULY 28

6:00 - 10:00 p.m. IAMFES Evening Social, Gemütlichkeit Abendgesellschaft" — Summerfest Grounds at Lakefront

#### TUESDAY, JULY 29

6:30 - 8:00 a.m. Breakfast — Red Carpet/Poolside  
5:30 - 7:00 p.m. Wine and Cheese Party — Grand Ballroom

#### WEDNESDAY, JULY 30

6:00 - 7:00 p.m. Reception — Foyer, Grand Ballroom  
7:00 - 10:30 p.m. Banquet and Entertainment — Grand Ballroom

#### ENTERTAINMENT — SPOUSES

##### MONDAY, JULY 28

7:30 a.m. - 5:00 p.m. Ladies' Hospitality — Creole  
9:30 a.m. - 3:15 p.m. "Magic of Milwaukee" Tour, Luncheon at 'Pieces of Eight' (\$6.00)

#### TUESDAY, JULY 29

7:30 a.m. - 5:00 p.m. Ladies' Hospitality — Creole  
10:00 a.m. - 2:00 p.m. "Unique Boutique Tour," Luncheon at 'Proud Popover', Stonewood Village, (\$6.00)

#### WEDNESDAY, JULY 30

7:30 a.m. - 5:00 p.m. Ladies' Hospitality — Creole  
MCTS (city buses) to South Ridge Mall. Travel time, approximately 15 minutes.

#### WISCONSIN DAIRY PLANT FIELDMAN'S ASSOCIATION July 30, 1980

Morning — Chairman — Glaris Hams  
New Orleans North and South

8:00 - 8:55 REGISTRATION  
8:55 DOOR PRIZE  
9:00 "HAVE OUR REGULATIONS GONE TOO FAR?" M. P. Dean, University of Wisconsin Food Science Cheese Research Institute — New Orleans South  
9:30 "DAIRY FARMING AUTOMATION — FUTURE DIRECTION" Winston Ingalls, DeLaval Separator Co., — New Orleans South  
10:05 MILK BREAK  
10:20 ATTEND IAMFES MILK SANITATION SECTION — New Orleans North  
10:50 "WHAT TO LOOK FOR IN A PROPER MILKING SYSTEM INSTALLATION" Joe Scolaro, Babson Bros. — New Orleans South  
11:20 ATTEND IAMFES MILK SANITATION SECTION — New Orleans North  
12:00 - 1:30 WDPF BANQUET — Grand Ballroom East

\*Asterisk indicates person presenting paper.

**Afternoon — Chairman — Ron Bladl**

- 1:25 DOOR PRIZE  
 1:30 ATTEND IAMFES MILK SANITATION SECTION — New Orleans North  
 1:45 "TROUBLE SHOOTING MILK FLAVOR PROBLEMS" John Bundler, Cornell — New Orleans South  
 2:30 ATTEND IAMFES MILK SANITATION SECTION — New Orleans North  
 3:00 MILK BREAK  
 3:15 DOOR PRIZE  
 3:20 "RESPONSIBILITIES OF A FIELD REPRESENTATIVE" Earl Brancel, Procurement Director, Wis. Dairies, Baraboo, Wis. — New Orleans South  
 3:40 ATTEND IAMFES MILK SANITATION SECTION — New Orleans North  
 4:15 "HELP YOUR PATRON HELP HIMSELF" — T. J. Tooley, President, Ag Systems International — New Orleans South

**NATIONAL MASTITIS COUNCIL  
 1980 SUMMER MEETING PROGRAM  
 July 31, 1980**

**Red Carpet Hotel  
 Milwaukee, WI  
 Grand Ballroom**

- 8:00 REGISTRATION  
 8:20 GREETINGS — James J. Jezeski, President, National Mastitis Council, University of Florida, Gainesville, Florida  
 8:30 WELCOME TO WISCONSIN — "Alice in Dairyland"  
 8:45 WHAT'S AHEAD IN THE NEXT FIVE YEARS — Truman Graf, Dept. of Agricultural

- Economics, University of Wisconsin-Madison  
 9:15 TROUBLE SHOOTING MILKING SYSTEMS — Roy Podolak, Dairy Equipment Company, Madison, Wisconsin  
 9:45 BREAK  
 10:00 PANEL — THERE IS ON FARM HELP AVAILABLE — Don Bosman, Wisconsin Department of Agriculture: Panel Members — Charles Kutzt, Golden Guernsey, Waukesha, Wisconsin; Darrell Johnson, Weyauwega, Wisconsin; Ray Biller, Balsam Lake, Wisconsin  
 11:00 QUESTIONS  
 11:15 THE NEW NATIONAL MASTITIS SLIDE SETS — W. Nelson Philpot, Louisiana State University, Hill Farm Experiment Station, Homer, Louisiana  
 11:45 LUNCH BREAK  
 12:45 USING THE WISCONSIN DHIA PROGRAM OVERVIEW — Bill Battista, Wisconsin Dairy Herd Improvement Co-op  
 1:15 HOW I USE SOMATIC CELL COUNT REPORTS — Jim Battist, Battist Farms Inc., Waterloo, Wisconsin  
 1:45 GERMANY'S APPROACH TO PROMOTION OF SOMATIC CELL COUNTING — Rolf Reisgies, Germania Dairy Automation Inc.  
 2:15 BREAK  
 2:30 PANEL — EXPERIENCES WITH QUALITY BONUSES — Earl Brancel, Wisconsin Dairies, Baraboo, Wisconsin; Panel Members — Don Berg, Land O'Lakes Inc.; Tom Lemke, Consolidated Badger Co-op; Dan Jindra, Lake to Lake Co-op  
 3:30 QUESTIONS  
 3:45 ADJOURNMENT

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## WILKOMMEN!

The Wisconsin Association of Milk and Food Sanitarians, Inc. and the Wisconsin Dairy Plant Fieldman's Association invite you to the following social activities, part of the 1980 IAMFES Annual Meeting.

Monday, July 28

9:30 A.M. - 3:15 P.M.

"Magic of Milwaukee". A comfortable coach tour of Milwaukee's city and lakefront highlights. The tour will include:

- 1) Mitchell Park Conservatory Domes - 3 separate displays ranging from lush tropics to an oasis, featuring seasonal floral arrangements.
- 2) St. Josephat's Basilica - the only Polish basilica in North America. A place of worship, an art gallery, and museum all in one.
- 3) Milwaukee Public Museum - stroll along the streets of "Old Milwaukee", explore the museum's new "European Village".
- 4) Luncheon at the "Pieces of Eight" - a panoramic view of Milwaukee's harbor.

Tuesday, July 29

10:00 A.M. - 2:00 P.M.

"Unique Boutique Excursion". A comfortable coach ride to "Stonewood Village" in Brookfield, WI. A New England style village/shopping center with 24 intimate stores. Luncheon will be in the "Proud Popover Restaurant", a colonial-theme eating house.

Spouses, members, and children are welcome on the tours. A modest fee of \$6.00, per person, has been set for each tour. Tickets will be one sale at the reservation desk Sunday, July 27.

---

**Don't be disappointed, reserve your tickets by mail!**

Mail to: *Richard Rowley*  
*PO Box 92156*  
*Milwaukee, WI 53202*

Make checks payable to:  
 IAMFES 1980 Meeting Fund

Magic of Milwaukee  
 Monday, July 28, 1980

Unique Boutique Excursion  
 Tuesday, July 29, 1980

No. of Tickets at \$6.00 \_\_\_\_\_

No. of Tickets at \$6.00 \_\_\_\_\_

## Coming Events/News and Events

May 15-16--ENVIRONMENTAL LAWS & REGULATIONS COURSES, Masters Course. Hotel Washington, Washington, DC. Contact: Margeurite Leishman, Government Institutes, Inc., PO Box 5918, Washington, DC 20014, 301-656-1090.

May 17-21--61st ANNUAL NRA RESTAURANT HOTEL-MOTEL SHOW. McCormick Place, Chicago, IL. Contact: National Restaurant Association, One IBM Plaza, Suite 2600, Chicago, IL 60611, 312-787-2525.

May 18-20--MIDWEST DAIRY PRODUCTS ASSOCIATION, Annual Convention. Pheasant Run Lodge, St. Charles, IL. Contact: MDPA, 5610 Crawfordsville Road, Suite 1104, Indianapolis, IN 46224, 317-243-9341.

June 2-4--ANIMAL PRODUCTS ON HUMAN NUTRITION. Conference made possible by a grant from the Nutrition Foundation, Inc. Scheman Continuing Education Building, Iowa State University, Ames, IA. To register, contact: Room 102, Scheman Continuing Education Building, Iowa State University, Ames, IA 50011.

June 2-5--PENNSYLVANIA DAIRY FIELDMEN'S CONFERENCE. Keller Conference Center, The Pennsylvania State University, University Park, PA 16802. Contact: William Killough, RD 1, Box 393, Conestoga, PA 17516.

June 9-10--HAZARDOUS WASTES REGULATIONS/RCRA SEMINAR. Sponsored by Government Institutes, Inc., Fee: \$395. Contact: Government Institutes, Inc. PO Box 5918, Washington, DC 20014, 301-656-1090.

June 11-13--INTERNATIONAL HEALTH: MEASURING PROGRESS. Sponsored by the National Council for International Health. George Washington University, Washington, DC. Contact: NCIH, 1980 International Health Conference, 2121 Virginia Avenue, NW, Suite 303, Washington, DC 20037, 202-338-1142.

June 15-18--ASSOCIATION OF FOOD AND DRUG OFFICIALS, 84th Annual Conference. Kirkwood Motor Inn, Bismarck, ND. Contact: Association of Food and Drug Officials, PO Box 3, Barrington, RI 02806.

June 15-18--75th ANNUAL MEETING, AMERICAN DAIRY SCIENCE ASSOCIATION. Virginia Polytechnic Institute and State University, Blacksburg, VA. Further details will be available once the program is finalized.

June 15-18--AMERICAN SOCIETY FOR AGRICULTURAL ENGINEERS, Summer Meeting. Convention Center, San Antonio, TX. Theme, "Managing Resources in Transition: Agriculture's Challenge for the 80's." Contact: Roger R. Castenson, ASAE, 2950 Niles Road, Box 410, St. Joseph, MI 49085, 616-429-0300.

June 19-20--CLEAN AIR ACT SEMINAR. Washington, DC. Sponsored by Government Institutes, Inc. Fee: \$395. Contact: Government Institutes, Inc., PO Box 5918, Washington, DC 20014.

June 24-25, 26-27--SEPARATION WORKSHOPS. Rockville, MD. Sponsored by LKB Instruments, Inc. Fee: \$150. Contact: Workshop Director, LKB Instruments, Inc., 12221 Parklawn Drive, Rockville, MD 20852, 301-881-2510.

June 29-July 3--WORLD CONGRESS ON FOODBORNE INFECTIONS AND INTOXICATIONS, Berlin, West Germany. Sponsored by the Institute of Veterinary Medicine, Robert Von Ostertag-Institute of the FAO/WHO Collaborating Centre for Research and Training in Food Hygiene and Zoonoses. Contact: Dr. K. Gerigk, Secretary General, World Congress on Foodborne Infections and Intoxications, D-1000 Berlin 33, Thielallee 88-92, Postfach 33 00 13.

July 6-11--XI INTERNATIONAL SYMPOSIUM ON PSYCHROTROPIC MICROORGANISMS IN SPOILAGE AND PATHOGENICITY. Aalborg Universitetscenter, Sohngaardsholmsvej 57, Aalborg, Denmark. Sponsored by International Association of Microbiological Societies, Committee on Food Microbiology and Hygiene. Contact: Secretariat, IAMS, XI International Symposium, Institute of Hygiene and Microbiology, Royal Veterinary and Agricultural University, Bulowsvej 13, DK-1870, Copenhagen V, Denmark.

July 7-11--SANITATION ASPECTS OF FOOD FACILITY PLAN PREPARATION & REVIEW. Stark Technical College, Canton, OH. Sponsored by the National Sanitation Foundation. For more information, see Aug. 11-15 entry.

July 7-25--DAIRY LABORATORY WORKSHOP, University Park campus, The Pennsylvania State University, State College, PA. Workshop will consist of two weeks of lectures and laboratories, followed by a third week of reviewing and completing regulatory tests, examinations. Fee for first two weeks,

\$250. For those wishing to take certification examination in third week, fee is an additional \$100. Contact: Agricultural Conference Coordinator, 409 J. O. Keller Building, University Park, PA 16802, 814-865-9547.

July 14-15, 16-17--SEPARATION WORKSHOPS. Research Triangle, NC. Sponsored by LKB Instruments, Inc. For more information, see November 18-19 entry.

July 26-31--IAMFES ANNUAL MEETING, Red Carpet Hotel, Milwaukee, WI. Contact: Earl Wright or Jan Richards, IAMFES, PO Box 701, Ames, IA 50010, 515-232-6699.

July 28-29, 30-31--SEPARATION WORKSHOPS. St. Louis, MO. Sponsored by LKB Instruments, Inc. For more information, see Nov. 18-19 entry.

July 28-Aug. 1--SANITATION ASPECTS OF FOOD FACILITY PLAN PREPARATION & REVIEW. Harvard University, Cambridge, MA. Sponsored by the National Sanitation Foundation. For more information, see Aug. 11-15 entry.

Aug. 4-8--ADVANCES IN FOOD AND APPLIED MICROBIOLOGY. Summer course, Massachusetts Institute of Technology, Cambridge, MA 02139. Contact: Director of Summer Session, RM E19-356, MIT, Cambridge, MA 02139.

Aug. 4-8--SANITATION ASPECTS OF FOOD FACILITY PLAN PREPARATION & REVIEW. Highline Community College, Midway, WA. Sponsored by the National Sanitation Foundation. For more information, see Aug. 11-15 entry.

Aug. 11-15--SANITATION ASPECTS OF FOOD FACILITY PLAN PREPARATION & REVIEW. State Technical Institute, Memphis, TN. Sponsored by the National Sanitation Foundation. Contact: NSF Education Service, National Sanitation Foundation, PO Box 1468, Ann Arbor, MI 48106, 313-769-8010.

Aug. 18-19, 20-21--SEPARATION WORKSHOPS. Houston, TX. Sponsored by LKB Instruments, Inc. For more information, see Nov. 18-19 entry.

Sept. 8-9, 10-11, SEPARATION WORKSHOPS. Los Angeles, CA. Sponsored by LKB Instruments, Inc. For more information, see Nov. 18-19 entry.

Sept. 15-16, 17-18--SEPARATION WORKSHOPS. San Francisco, CA. Sponsored by LKB Instruments, Inc. For more information, see Nov. 18-19 entry.

Sept. 15-19--CANADIAN INSTITUTE OF PUBLIC HEALTH INSPECTORS, 41st Annual Conference. Holiday Inn, Kingston, Ontario. Contact: William D. Vaughan, RR 1 Napanee, Ontario, Canada K7R 3K6.

Sept. 26--FOOD DEHYDRATION SYMPOSIUM, part of Kansas State University's Focus on Food Science Symposium series. Contact: Dr. D. Y. C. Fung, 913-532-5654, or Dr. D. S. Chung, 913-532-5580, Co-chairmen, Call Hall, KSU, Manhattan, KS 66506.

Sept. 29-30--CALIFORNIA ASSOCIATION OF DAIRY, MILK SANITARIANS, ANNUAL MEETING. Sacramento Inn, Sacramento, CA. Contact: John C. Bruhn, Extension Food Technologist, 101 Cruess Hall, Univ. of California, Davis, CA 95616, 916-752-2192.

Sept. 29-Oct. 1--ASAE NATIONAL ENERGY SYMPOSIUM. Co-sponsored by USDA, DOE, and 19 other organizations. Radisson Muehlebach Hotel, Kansas City, MO. Contact: Mark A. Purschwitz, American Society of Agricultural Engineers, 2950 Niles Road, Box 410, St. Joseph, MI 49085, 616-429-0300.

Oct. 6-7, 8-9--SEPARATION WORKSHOPS. Chicago, IL. Sponsored by LKB Instruments, Inc. For more information, see Nov. 18-19 entry.

Oct. 6-8--ENERGY AND THE FOOD INDUSTRY. International Symposium sponsored by Commission Internationale des Industries Agricoles et Alimentaires (CIIA), Madrid, Spain. Contact: CIIA, B.P. 470-08, 75366 Paris Cedex 08.

Oct. 8-9--NEBRASKA DAIRY INDUSTRIES ASSOCIATION, 26th ANNUAL CONVENTION. Regency West, I-680 and Pacific Street, Omaha, NE. Contact: T. A. Evans, Exec. Secretary, 116 Filley Hall, East Campus, University of Nebraska-Lincoln, Lincoln, NE 68583.

Oct. 20-23--11th INTERNATIONAL CONGRESS ON DISEASES OF CATTLE and Small Animal Satellite Symposium. Hilton Hotel and Dan Hotel, Tel Aviv, Israel. Sponsored by World Assoc. for Buiatrics, Israel Veterinary Medical Assoc., Israel Assoc. for Buiatrics. Contact: Dr. E. Mayer, PO Box 9610, Haifa, Israel.

Oct. 21-22, 23-24--SEPARATION WORKSHOPS. New York, NY. Sponsored by LKB Instruments, Inc. For more information, see Nov. 18-19 entry.

Nov. 3-4, 5-6--SEPARATION WORKSHOPS. Philadelphia, PA. Sponsored by LKB Instruments, Inc. For more information, see Nov. 18-19 entry.

Nov. 18-19, 20-21--SEPARATION WORKSHOPS. Rockville, MD. Sponsored by LKB Instruments, Inc. Registration for each workshop: \$150. Contact: Workshop Director, LKB Instruments, Inc., 12221 Parklawn Drive, Rockville, MD 20852.

## New IAMFES Magazine "In the Works"

Interested in being one of the first authors in the new IAMFES magazine? We can use your help writing or soliciting practical articles for the

first 1981 issues of the publication, *Food and Fieldmen*. A sample issue will be sent to all *Journal* readers in June.

Send ideas of articles you'd like to see. Better yet, send the article itself,

or "raw material" from which Jan Richards and other IAMFES staff members can prepare an article.

Think about it — it's an excellent professional opportunity!

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## 1980 Directory of Labs Available

A directory describing the capabilities and expertise areas of almost 400 food testing laboratories and consulting organizations is just available from the Institute of Food Technologists (IFT).

The 1980 IFT "Regional Guide to Food Testing Laboratories and Con-

sultants" is organized according to the six U.S. Census regions. It contains a section listing non-U.S. organizations, for those with testing requirements abroad.

The 56-page directory is available from: IFT Regional Guide, Lockbox 94332, Chicago, IL 60690 for \$10.00 per copy, postpaid.



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## Classified Ad

Single service milk sample tubes. For further information and a catalog, write: Dairy Technology, Inc., PO Box 101, Eugene, OR 97401.



### William K. Moseley, 1898-1980

William K. (Bill) Moseley, a pioneer in dairy and food plant sanitation, died February 3, 1980, in Indianapolis, IN.

He was born in Manes, Missouri on April 25, 1898 and was a graduate of the University of Missouri, receiving the Bachelor of Science degree in Dairy Science in 1923. Following graduation, he worked for Swift & Co. at Portland, OR. He returned for graduate study to the University of Missouri and was awarded the Master's Degree in 1926.

Bill then accepted a position with the Dairy Division of the Indiana State Board of Health. Soon, people in industry, appreciating his efforts, sought his advice so he started work with Schlosser Brothers Creamery, Indianapolis in 1927. He began his own commercial laboratory, W. K. Moseley Laboratory, in Indianapolis in 1928. The primary objective of the laboratory was to assist manufacturers of dairy products with problems, improving product quality and keeping quality through sanitation. Bill sold his laboratory and retired in 1972.

The Moseley Keeping Quality Test is used extensively by dairy plants to predict the keeping quality of refrigerated pasteurized milk and cream and to determine the growth rate of psychrotrophic bacteria. The test is an important aid to a plant having trouble with poor shelf life.

A charter member of the Indianapolis Dairy Technology Society, Bill was instrumental in its inauguration as the second Dairy Technology Society in the United States. In 1964, IAMFES presented him its Citation Award, and in 1966 he was the first recipient of the Indiana Distinguished Dairyman Award; an award presented annually since 1966 by the Dairy Technology Societies in Indiana.

### Fred H. Jolly Dies

Fred H. Jolly, R. S., Director of the Division of Housing and Environmental Health, Nebraska Department of Health died on February 26, 1980.

Fred was a dedicated environmentalist and realized early in his career the urgency of incorporating good environmental practices with health programs.

Starting as a Milk and Food Sanitarian for the South Dakota State Health Department in the summer of 1947, he introduced the initial environmental Health programs to Northwestern South Dakota. He was instrumental in developing the first Milk and Food programs with the Northern Black Hills Health Unit and the Rapid City-Pennington County Health Department, along with other environmental programs in the entire Black Hills Region.

He began work with the Lincoln-Lancaster County, Nebraska Health Department in October, 1960, transferring to the Omaha-Douglas County Health Department in September, 1965 and started with the State Health Department in 1966. After a brief return to the Omaha Department, he returned to the State Health Department February 1, 1968, with the Division of Environmental Sanitation. In 1971 he was appointed Director of Environmental Sanitation, which later became the Division of Housing and Environmental Health.

A 1948 graduate of Oklahoma A&M, now Oklahoma State University, in Dairy Science, Fred participated actively in numerous local, state and national organizations, including the Nebraska and National Environmental Health Associations, IAMFES, Nebraska Dairy Technology Society, Nebraska Institute of Food Technologists. He was a Registered Sanitarian, serving three terms on the Board of Registration for Sanitarians.

### Sanitarians Needed for Foreign Service

The Cambodian Refugee Health Clearing House, (CRHCH), established in November, 1979, at the request of Surgeon General Richmond, is a referral service for voluntary agencies sending health workers to refugee camps in Thailand.

The Clearing House needs sanitarians who are willing to serve under primitive conditions, for six months or

### NSF Thermometers Guide Available

"Thermometers in Food Service — A Pocket Guide," is available from the National Sanitation Foundation.

The brochure includes information on the classification and characteristics of thermometers; calibration; information on selecting product thermometers; measuring product temperature; measuring frozen food temperature; and federal guidelines.

longer. Candidates should be in excellent health.

Particularly needed are persons with experience and expertise in water supply, wastewater and sewage management. For further information, contact the CRHCH at the following address: 2121 Virginia Avenue NW, Suite 302, Washington, DC 20037, 202-298-5901.

Single copies are available at no cost when a self-addressed stamped envelope is included with the request. Quantities of 50 are \$5.00. A camera-ready original for reproduction of the brochure is \$1.00.

For more information, contact: Education Service, National Sanitation Foundation, PO Box 1468, Ann Arbor, MI 48106, 313-769-8010.

**For Your Enjoyment  
Social Events at the 1980 IAMFES Annual Meeting**

Sunday, July 27  
6:00 - 9:00 P.M.  
Early Bird Reception  
Music and a light meal

Monday, July 28  
6:00 - 9:00 P.M.  
Gemütlichkeit Abendgesellschaft -  
Schottische, Polka, German Music for Listening. Beer, soft  
drinks, lamb, pork, chicken, and the fixings!

Tuesday, July 29  
6:30 - 8:00 A.M.  
Poolside Breakfast  
5:00 - 7:00 P.M.  
Wine and Cheese Party

Wednesday, July 30  
6:00 - 7:00 P.M.  
Reception (Open Bar)  
7:00 - 10:30 P.M.  
Awards Banquet  
Ethnic entertainment during and after banquet

Entertainment is sponsored by "Friends of IAMFES"  
Plan to arrive on Sunday and don't be disappointed — reserve  
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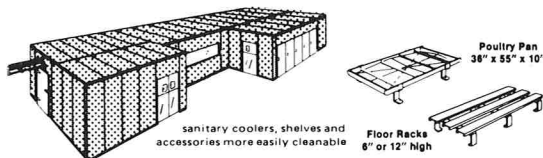
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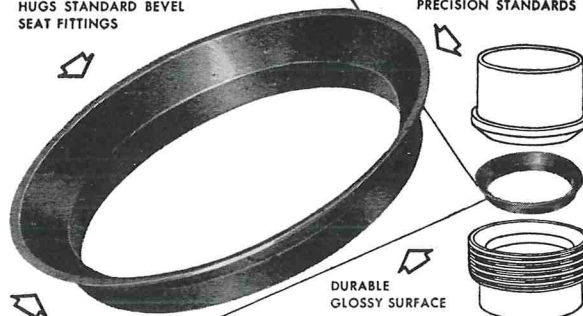
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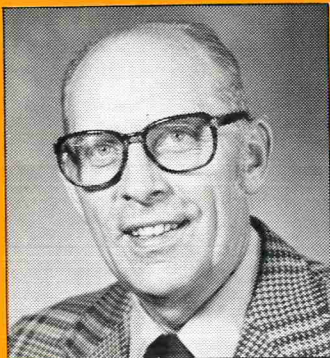
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# ***SOMEONE YOU SHOULD KNOW IN THE DAIRY INDUSTRY***



**Dr. Clarence C. Olson, Extension Dairyman**

*Professor Olson is a member of the Department of Dairy Science, University of Wisconsin, Madison. Born and raised on a Wisconsin dairy farm, and a graduate of the University of Minnesota, he began his extension career in North Dakota, taking responsibility in extension programs with both dairy producers and industry. Under his direction, the state saw a rebirth and expansion of Dairy Herd Improvement Association testing programs, the development of membership organizations to provide AI services, and a nearly-complete changeover from farm separated cream to bulk sale of high quality whole milk. He returned to his native state in 1961 to direct training programs for Dairy Herd Improvement Supervisors and Lab Technicians and, since 1973, has led membership promotion and educational programs aimed at helping dairymen and the industry make more profitable use of the Dairy Herd Improvement program.*

## **DHIA Is The Key To Progress**

"The role of the extension specialist in the scheme of American agriculture is to make things happen—to take an idea from the research lab and get it into daily use on the farm. That is called progress.

"For dairymen, we believe DHIA is the key to greater progress. The unique partnership with extension through this program can result in benefits to the individual dairyman and for the entire dairy industry. A team effort makes this possible.

"County agents work directly with dairymen through educational programs to help improve feeding, breeding and management practices. We provide special training for agents and help them in meetings and with development of teaching and reference materials; but more specifically, by analyzing herd data. Frequently, this involves a research idea that must survive the challenge of field testing in cooperating DHIA herds. For example, haylage and high moisture corn became staple items in diets of Wisconsin cows after DHIA records proved their greater nutritional value. The same process uncovers problems needing attention by researchers; then DHIA records provide measures of success in solving the problem. The benefits of this team-up of extension-research with cooperating dairymen through DHIA are very evident to the industry.

"Perhaps the best known benefit of DHIA has been the pooling of performance data to identify genetically superior sires and top cows who are chosen to become mothers of future sires. The use of better and better sires by our dairymen has resulted in improved productivity of dairy cattle to the extent that

the nation's milk supply is now provided with half as many cows. It would be interesting to speculate what the consumer would have to pay for milk and other dairy products if it were not for greater and more efficient milk production by the nation's dairy cows.

## **DHIA Is More Than You Think**

"It is easy for a program that has been around as long as DHIA to become stereotyped—not true, it is changing all the time. As new research suggests, we work with our state DHI committee to make changes in the DHIA program. These dairymen help evaluate these new options to determine how well they fit the needs of members.

"Through this evolutionary process, DHIA records are now more than just measures of cow performance. The current emphasis is on management guidelines for feeding, breeding, selection and for complete records of identification and ancestry.

"The newest option available to DHIA members is somatic cell testing to evaluate udder health. This effort at improving milk quality along with quantity is very appropriate. Not only is it a valuable tool for the herd owner and the veterinarian but it also monitors the cows environment in order to help prevent mastitis.

"Feed testing and ration balancing are other new options available to members. We do our best to encourage these practices so that the benefits of superior genetics and the investments made in raising a heifer calf to producing age may be realized in more net income via the bulk tank.

"In summary, if we have been effective in promoting 'progress' in our DHIA herds, then faster adoption of new research should result in greater production by DHIA cows. A look at the data shows this has happened because cows on DHIA are currently outproducing cows not on test by over 4000 lbs. of milk yearly. Therefore, the bottom line reads only one way—we believe that if you milk cows and you want to make your cows worth more, then DHIA will insure your future."



**SURGE**