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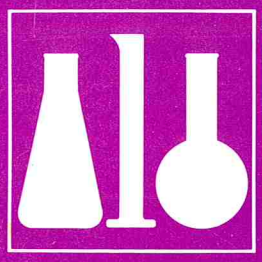

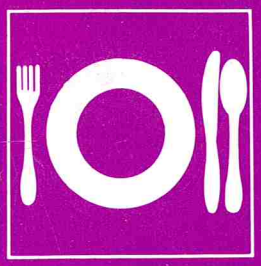

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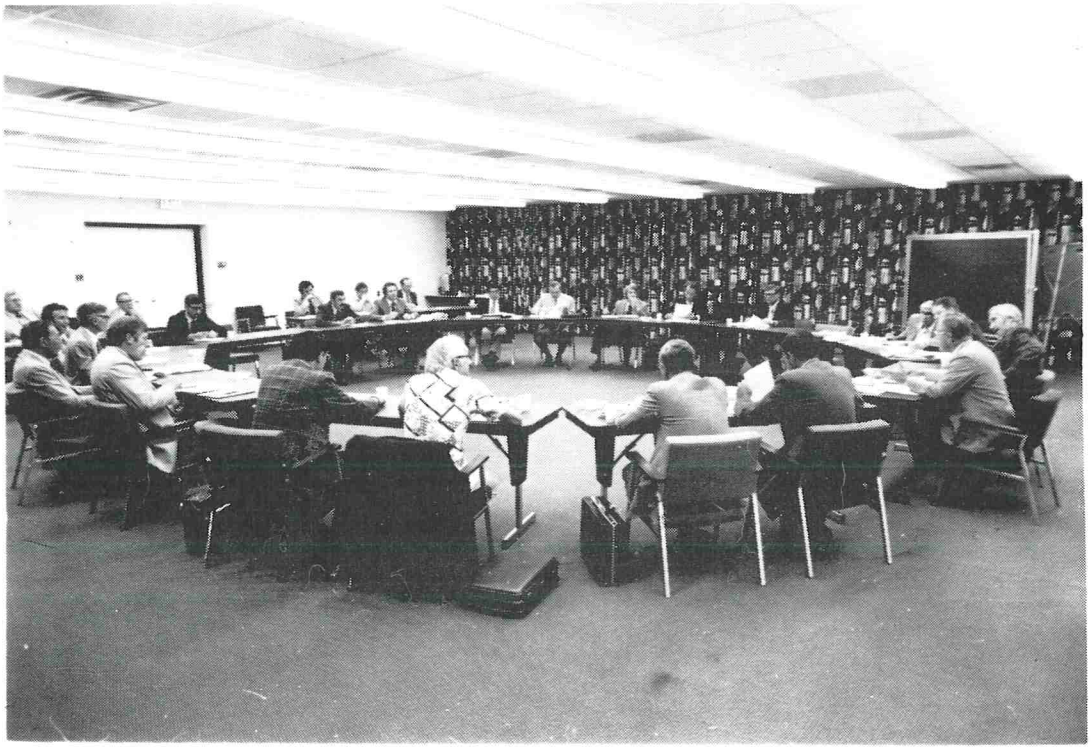

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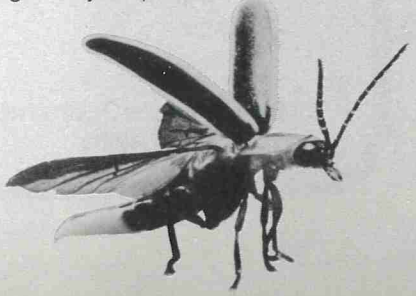

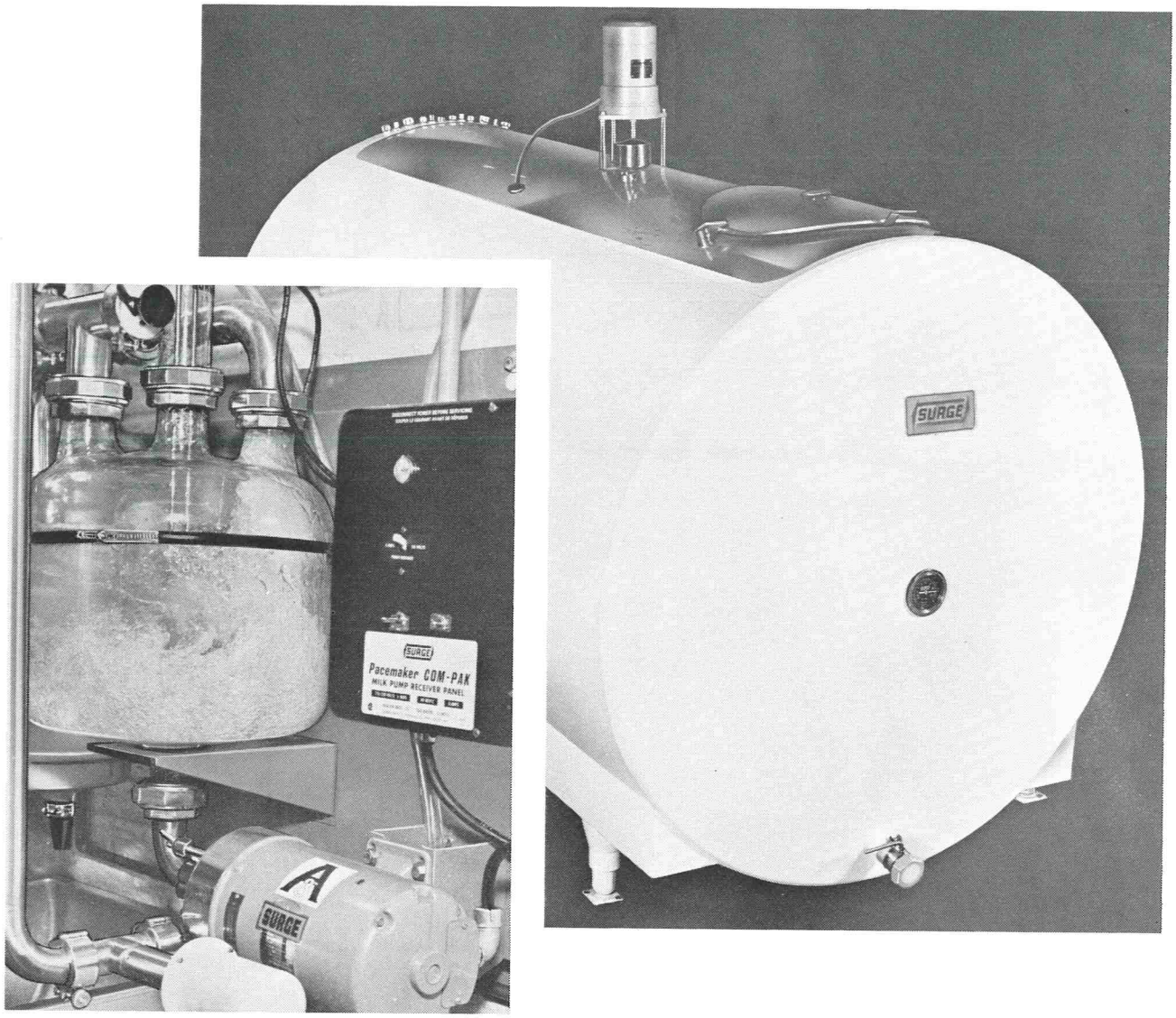

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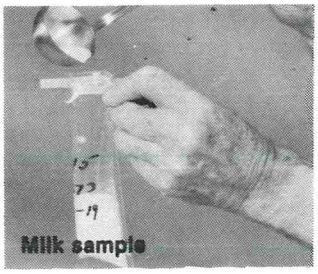
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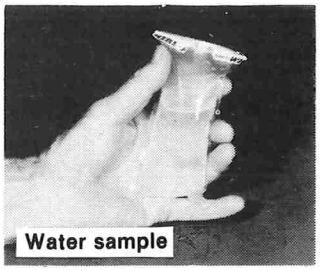
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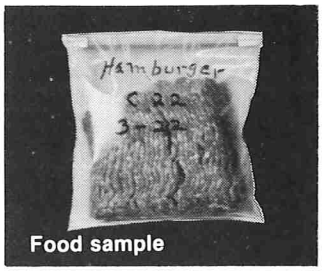
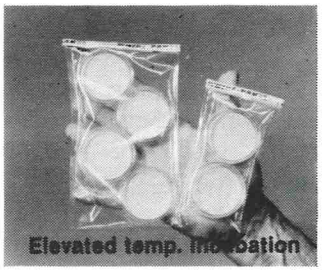


Food sample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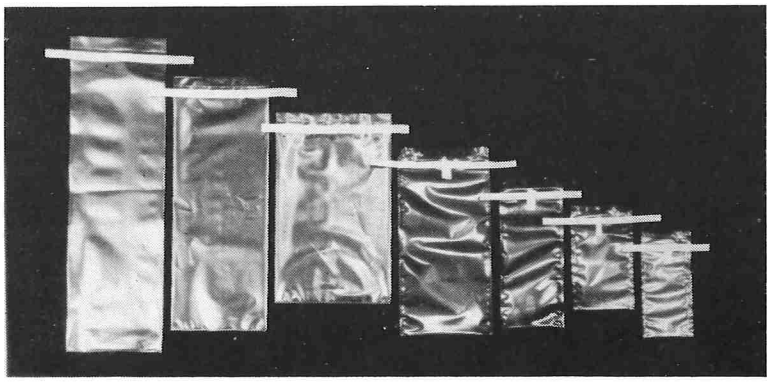

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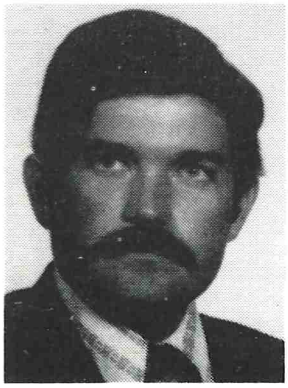

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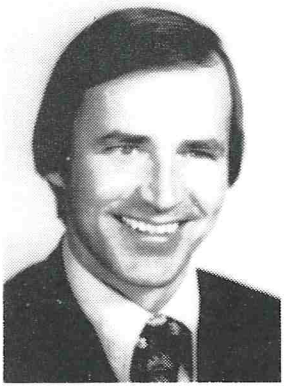

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General Interest Papers

**Asterisk designates person to whom inquiries regarding this paper should be addressed*

Journal of Food Protection Vol. 41, No. 7, Pages 510-513 (July, 1978)

Composition of Selected Commercial Salads from the Retail Market

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(Received for publication November 26, 1976)

ABSTRACT

Analyses were done to evaluate among- and within-brand variation in the composition of five types of commercial salads. Samples from five companies were collected at three different times and analyzed for fat, protein, fiber, ash, and moisture. Carbohydrate and caloric content were calculated. Wide variations in composition were evident both among brands and within brands, which indicated a need for ingredient specifications, ingredient listing, and application of basic control procedures in the operations examined.

Salads, traditionally prepared in the home, are becoming items of commerce in increasing amounts as homemakers seek more convenience foods. Salads are prepared, packaged, and offered for sale by several types of manufacturers. Closest correspondence to the home kitchen are small delicatessens which prepare salads in small batches and dispense them to their customers. Operations range in increasing size through supermarkets which prepare salads for sale on their premises or through other stores in their chain to manufacturers who specialize in salads.

Very little information on composition and variation in composition of these food materials is available in the literature. Bowes and Church (*3*) reported carbohydrate, fat, Ca, P, and calories for four salad types and Bradley (*4*) listed carbohydrate, protein, fat, Ca plus P, and calories for three types. No data on variation of any type were included in either report. The sales volume of these convenience foods is increasing (*1*) as they meet the desires of American consumers, but for them to be used intelligently in the diet, information on their composition will be necessary. The paucity of such information led to the work herein reported.

MATERIALS AND METHODS

Five salads were chosen to include main course types and meal adjuncts. These were cole slaw, pimento cheese, potato salad, chicken salad, and ham salad. Five companies were chosen to represent different classes of manufacturers; local delicatessens, grocery chains, and companies whose main product was prepared salads. To check within-company variations, samples were obtained at three different times at intervals of 1 month. Samples were collected at retail outlets.

Samples were prepared for analysis by blending 2 min at high speed in a Waring blender to obtain a homogeneous material. In most instances it was necessary to dilute the materials with distilled water to facilitate blending. The dilution was on a w/w basis and varied with salad type. Such dilution was incorporated into calculation of results. Portions of the blended samples were sealed in polyethylene bags and stored at −23 C until analyzed.

Analytical methods which were suitable for FDA nutrition labeling analyses were used. Protein was measured by the Kjeldahl method (*2*, #2.051), total ash by dry ashing for 4 h at 550 C (*2*, #14.006), fiber as crude fiber (*2*, #7,053 - 7.057), and moisture by drying to constant weight at 60 C under vacuum (*2*, #31.005). FDA Interim Methodology #2 (*6*) was used to determine fat, and carbohydrate was obtained by difference. Atwater factors were used to calculate calories (*5*). All analyses were done in triplicate.

Data were subjected to analysis of variance by a factorial arrangement of treatments in a randomized complete block design with three replications. Means from treatments indicated as significant at the 5% level of probability were compared by the Student-Newman-Keul's test (*7*).

RESULTS AND DISCUSSION

The five brands included in the study were from: A - a small delicatessen, B and C - salad operations in grocery chains, and D and E - companies whose primary business is the manufacture of salads. Objectives were to determine the composition of different types of salads and to assess variation among brands and over time within brands. Although caloric content is a result of, rather than a part of composition, calculated calories are presented with the compositional data for purposes of comparison.

With only one exception, ash in ham salad, replication effects were not significant at the 5% level of probability, and that exception was not significant at the 1% level. For most of the data the effect of time was significant, but time effects separated from brands were not pertinent, so effects of company - time interactions are tabulated to show variation within a company over time.

Variation among brands is shown in Table 1. The

[1]*Present address: Huttenbauer Foods, Inc., 2620 Broadway Street, Knoxville, Tennessee 37917.*

widest variation in overall nutrient content was among samples of cole slaw and pimento cheese which showed approximately 100% variation in caloric content. Much of this was in the content of fat which ranged more than three-fold among the five brands. It is noteworthy that salads from the small delicatessen, brand A, consistently had the highest nutrient content. Among the three "main dish" salads - pimento cheese, chicken, and ham salad - the fat content varied up to 200%. Two-fold differences in protein content were also evident in chicken and ham salad. Rather wide variations in other components were also evident.

One would expect the composition of a given product from one company to remain relatively constant from batch to batch, or over time. Examination of data in Table 2 shows that this assumption is not valid for commercial salads. Generally, variations in composition within brands were less than those among brands, but the magnitude of within-brand differences showed a notable lack of consistency. Within brands, the widest variation was observed in the fat content of cole slaw and potato salad.

The data indicate that commercial salads are far from being standardized products. This is not surprising since

TABLE 1. *Composition means of salads by brands*[1,2]

Salad and brand	Fat	Protein	Fiber	Ash	H_2O	CHO	Calories per 100 g
Cole slaw				(%)			
A	18.7a	1.3a	2.4a	0.9a	68.6a	8.0a	206a
B	16.7b	1.4a	1.7b	1.1b	69.9b	9.3a	193b
C	11.0c	1.1b	3.1c	1.4c	68.4a	15.0b	163c
D	6.9d	1.0b	1.1d	0.8a	77.8c	12.5c	116d
E	5.3e	1.1b	1.7b	1.2bc	78.9d	11.8c	99e
Pimento cheese							
A	47.8a	9.8a	0.8a	3.6a	28.1a	9.9a	509a
B	35.1b	11.3b	0.5b	3.0b	36.7b	13.4b	415b
C	21.8c	8.8c	0.5c	3.8c	50.9cd	14.2b	288c
D	17.6d	9.1d	0.4d	3.3d	50.0d	19.7c	273d
E	15.1e	8.7c	0.5e	3.9c	52.1c	19.6c	249e
Potato salad							
A	14.6a	1.8a	1.1a	0.8a	68.4ab	13.4a	192a
B	9.1b	1.9b	0.8b	1.1b	69.9b	17.3b	159b
C	6.6c	1.6c	0.8b	1.9c	67.8a	21.2c	151c
D	6.6c	1.2d	0.6c	1.4d	69.5b	20.7cd	147c
E	10.0d	1.7e	0.9d	1.3d	67.0a	19.2d	173d
Chicken salad							
A	31.9a	6.2a	0.9a	1.3a	49.0a	10.7ab	355a
B	29.1b	13.4b	0.8b	1.5a	45.6b	9.6a	354a
C	14.8c	11.2c	0.7c	2.0b	59.9c	11.5b	224b
D	13.2d	7.1d	0.9a	1.4a	63.8d	13.7c	201c
E	30.8e	6.6e	0.9a	1.7c	47.5e	12.4bc	354a
Ham salad							
A	26.7a	6.8a	0.5a	2.0ab	48.5a	15.5a	330a
B	27.0a	12.3b	3.4b	1.9a	50.1a	5.4b	314b
C	20.1b	8.8c	0.9c	2.2b	52.3b	15.7a	279c
D	14.4c	6.4d	0.7d	2.3b	60.4c	15.8a	218d
E	22.1d	7.2e	0.8d	2.0ab	52.8b	15.2a	288e

[1]Means within column groups of five followed by the same letter are not significantly different at the 5% level of probability.
[2]Means of nine observations, 3 replicates on 3 samples.

TABLE 2. *Composition means of salads: Company - time interactions.*

Salad and brand	Time	Means[1,2] Fat	Protein	Fiber	Ash	H_2O	CHO	Calories per 100 g
Cole slaw				(%)				
A	1	28.1a	1.3a	2.5a	0.8a	59.8a	7.5a	288a
	2	13.7b	1.3a	2.6a	1.0a	72.0b	9.4a	166b
	3	14.4b	1.4a	2.2b	0.9a	73.9c	7.2a	164b
B	1	14.5a	1.2a	1.8a	0.9a	71.2a	10.5a	177a
	2	19.1b	1.3a	1.8a	1.0a	73.0b	3.9b	192b
	3	16.6c	1.6b	1.5b	1.3a	65.4c	13.6c	210c
C	1	12.9a	1.2a	2.9a	1.3a	66.3a	15.3a	182a
	2	14.5a	1.3a	3.4b	1.6a	66.7a	12.5b	186a
	3	5.5b	0.8b	3.2c	1.2a	72.2b	17.2a	121b
D	1	9.0a	1.0a	0.9a	0.6a	75.8a	12.7a	135a
	2	4.6b	1.1a	1.2b	0.9a	79.8b	12.5a	96b
	3	7.1c	1.0a	1.2b	0.8a	77.7c	12.2a	117c
E	1	6.0a	1.2a	2.1a	1.3a	78.3a	11.2a	103a
	2	6.4a	1.2a	1.8b	1.4a	76.7b	12.6a	113a
	3	3.4b	1.0a	1.3c	1.1a	81.6c	11.6a	81b

TABLE 2. *continued*

Potato salad								
A	1	20.8a	2.3a	0.6a	0.6a	61.5a	14.3a	253a
	2	4.9b	1.5b	1.1b	0.7a	80.5b	11.3a	96b
	3	18.2c	1.6c	1.5c	1.1b	63.1a	14.5a	229c
B	1	5.0a	1.5a	1.0a	0.9a	71.0a	20.7a	133a
	2	12.4b	2.4b	0.8b	1.3b	67.9b	15.3b	182b
	3	10.0c	1.9c	0.7b	1.0a	70.7a	15.8b	161c
C	1	5.9a	1.6a	0.9a	1.9a	69.6a	20.1a	140a
	2	7.8b	1.6a	0.9a	2.0a	66.0b	21.7a	163b
	3	6.2a	1.6a	0.7b	1.9a	67.6ab	22.0a	150c
D	1	7.8a	1.3a	0.4a	1.6a	66.3a	22.5a	166a
	2	6.4b	1.0b	0.7b	1.4ab	71.4b	19.1b	138b
	3	5.4c	1.2c	0.8c	1.2b	70.9b	20.6ab	135b
E	1	10.6a	2.0a	1.1a	1.3a	70.0a	15.1a	164a
	2	8.8b	1.5b	0.8b	1.3a	69.5a	18.2b	158a
	3	10.6a	1.6b	0.8b	1.2a	61.5b	24.3c	199b
Pimento cheese								
A	1	45.2a	9.5a	0.8a	3.4a	31.7a	9.4a	482a
	2	48.9b	10.0b	0.8a	3.6a	26.5b	10.3a	521b
	3	49.3b	10.0b	0.7b	4.0b	26.0b	10.0a	524b
B	1	34.9ab	11.5a	0.6a	2.7a	36.9a	13.4a	414a
	2	37.3b	10.8b	0.5b	3.0b	34.3b	14.3a	434b
	3	33.4a	11.4a	0.5b	3.2b	39.0a	12.5a	396c
C	1	21.0a	8.9ab	0.4a	3.7a	51.1ab	15.0a	284a
	2	22.0a	9.2a	0.4a	3.9a	49.0a	15.6a	296a
	3	22.4a	8.4b	0.5b	3.9a	52.7b	12.1a	284a
D	1	15.8a	8.2a	0.5a	2.9a	49.9a	22.7a	266a
	2	17.6ab	9.0b	0.4b	3.5b	49.9a	19.7a	273a
	3	19.3b	10.0c	0.4b	3.3b	50.2a	16.8a	281a
E	1	15.5a	9.1a	0.5a	4.2a	50.3a	20.5a	258a
	2	14.0a	9.6a	0.5a	3.8b	53.0a	20.1a	241a
	3	15.8a	8.5b	0.5a	3.9b	53.1a	18.3a	249a
Chicken salad								
A	1	32.1ab	6.0a	1.1a	1.0a	48.9a	10.9ab	356a
	2	31.3a	6.1a	0.8b	1.5b	48.0a	12.4a	355a
	3	32.5b	6.4a	0.8b	1.5b	50.1a	8.8b	353a
B	1	30.4a	12.8a	0.9a	1.9a	45.1a	8.9a	361a
	2	28.4b	14.9b	0.7b	1.2b	46.0a	9.0a	351a
	3	28.5b	12.6a	0.8c	1.4b	45.8a	10.9a	351a
C	1	19.1a	13.6a	0.7a	2.1a	54.1a	10.5a	268a
	2	13.9b	9.3b	0.7a	1.9a	62.1b	12.1a	211b
	3	11.3c	10.8c	0.6a	1.9a	63.5b	11.9a	192c
D	1	12.6a	7.4a	1.0a	0.8a	63.0a	15.2a	203a
	2	15.0b	7.6a	0.8b	1.5b	63.3a	11.7b	212a
	3	11.9a	6.3b	0.9b	1.8b	65.1a	14.1ab	188b
E	1	32.5a	6.0a	0.8a	1.8a	47.4a	11.4a	363a
	2	29.1b	6.0a	0.8a	1.6a	47.9a	14.7b	345b
	3	30.9c	7.9b	1.1b	1.7a	47.3a	11.1a	354b
Ham salad								
A	1	34.9a	5.8a	0.5a	1.7a	40.3a	16.8a	405a
	2	22.2b	7.6b	0.5a	2.2a	53.3b	14.4a	287b
	3	23.1b	7.0c	0.5a	2.1a	51.9b	15.4a	299b
B	1	21.1a	13.5a	3.4a	2.3a	52.4a	7.3a	273a
	2	31.5b	12.7b	3.4a	1.7b	47.2b	3.5a	348b
	3	28.3c	10.7c	3.4a	1.6b	50.6a	5.3a	319c
C	1	19.7a	9.8a	1.1a	2.2a	47.9a	19.3a	294a
	2	21.2a	8.1b	0.7b	2.2a	54.6b	13.2a	276b
	3	19.4a	8.4b	0.7b	2.4a	54.4b	14.7a	267b
D	1	14.5ab	6.0a	0.7a	2.4a	59.9a	16.6a	220a
	2	15.3a	6.7b	0.8a	2.3a	59.6a	15.3a	226a
	3	13.3b	6.5b	0.6a	2.2a	61.9a	15.5a	208b
E	1	23.7a	7.5a	0.7a	2.0a	52.1a	14.0a	299a
	2	20.0b	6.5b	0.9a	2.2a	53.8a	16.6a	273b
	3	22.5a	7.5a	0.7a	1.9a	52.5a	14.9a	292a

[1]Means within column groups of three followed by the same letter are not significantly different at the 5% level of probability.

[2]Means of 3 observations.

each company uses its own scheme of formulation, developed by one means or another. The recipes used are based largely on experience and consumer acceptance of a company's product and change only rarely. Of course, each company uses different ingredients, in different amounts, and this would lead to variation among brands.

While composition was expected to be different among companies, the degree of variation among batches of

each company's product was rather disturbing. Differences in composition of ingredients could be one contributing factor. Due to the size of most of the operations, sources of ingredients change rarely. Only company E, the largest volume producer in the group, indicated that sources of ingredients changed in response to short term fluctuations in price. Even if sources were changed, most of the major ingredients, such as mayonnaise, salad dressing, processed cheese, ham and chicken meat, and potatoes do not vary enough in composition to account for the within-brand differences observed. In this study, the regular lack of consistency within brands, supported by observation of the operations, leads to the assumption that poor control of manufacturing procedures was responsible for much of the variation noted. These would include the basic operations of weighing, measuring, and mixing. One would expect larger operators, such as companies D and E, to perform better in this respect, but such was not the case. In all companies a need for operator training and supervisory control was indicated.

Overall means, ranges, and standard deviations are summarized in Table 3. The range data suggest that attempting to use mean values for nutrients and calories in meal planning with commercial salads would be an exercise in futility.

There is a demonstrated need for application of control procedures to the manufacture of commercial salads. Standardization and specifications for ingredients would contribute, as would ingredient listing. Perhaps more importantly, salad companies could benefit from training programs for workers and supervisors which detail the concept of consistent performance in simple unit processes such as weighing, measuring, and mixing. Cost savings through controlling amounts of ingredients used could be effected. If and when nutrition labeling is applied to such products, manufacturers will have to control composition to maintain compliance.

TABLE 3. *Overall means[1], standard deviations, and ranges of composition among five types of salads.*

Salad type	Composition						
	Fat	Protein	Fiber	Ash	Moisture	Carbohydrate	Calories
	(%)						(Per 100 g)
Cole salw							
Mean ± S.D.	11.7 ± 1.05	1.2 ± 0.12	2.0 ± 0.08	1.1 ± 0.19	72.7 ± 0.86	11.3 ± 1.47	156 ± 5.74
Range	3.2 – 28.6	0.8 – 1.7	0.9 – 3.5	0.6 – 1.9	59.6 – 82.0	2.6 – 17.7	79 – 292
Pimento cheese							
Mean ± S.D.	27.5 ± 1.53	9.5 ± 0.25	0.5 ± 0.03	3.5 ± 0.16	43.6 ± 1.43	15.4 ± 1.83	347 ± 10.47
Range	12.4 – 51.0	7.6 – 11.8	0.3 – 0.9	2.7 – 4.2	23.3 – 54.0	6.4 – 24.0	234 – 544
Potato salad							
Mean ± S.D.	9.4 ± 0.45	1.6 ± 0.06	0.9 ± 0.05	1.3 ± 0.18	68.5 ± 1.40	18.4 ± 1.61	164 ± 5.78
Range	4.3 – 21.6	1.0 – 2.4	0.4 – 1.5	0.5 – 2.2	60.0 – 81.9	9.7 – 25.4	90 – 258
Chicken salad							
Mean ± S.D.	24.0 ± 0.58	8.9 ± 0.28	0.8 ± 0.05	1.6 ± 0.22	53.2 ± 1.30	11.6 ± 1.52	298 ± 6.03
Range	10.8 – 33.1	5.5 – 15.0	0.6 – 1.2	0.7 – 2.2	42.6 – 65.7	7.7 – 17.2	184 – 365
Ham salad							
Mean ± S.D.	22.1 ± 0.96	8.3 ± 0.27	1.2 ± 0.08	2.1 ± 0.29	52.8 ± 1.83	13.5 ± 2.33	286 ± 7.38
Range	12.3 – 35.2	5.6 – 13.6	0.4 – 3.6	1.4 – 2.7	39.8 – 63.3	0.3 – 20.2	197 – 406

[1]Means of 45 observations; 5 brands, 3 times, 3 replications.

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A Survey of Tomatoes Home-Canned in Utah

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ABSTRACT

Sample jars of home-canned tomato products collected in Utah counties during the 1975 canning season were tested for pH, titratable acidity, vacuum, headspace and number of microorganisms. Each consumer was interviewed to determine the times and temperatures used to process each product. Three methods were used including waterbath, pressure canner and open kettle. Acidity was sufficient (< 4.6) in all samples to inhibit the growth of *Clostridium botulinum* with the exception of one sample (pH 6.8) containing excessive numbers of fungi. Four to five percent of the samples exhibited no vacuum after storage, contained microorganisms and were underprocessed.

Home-canned acid foods were implicated in 34 of the 722 incidence of *Clostridium botulinum* poisonings between 1899 and 1975. Tomato products were implicated in 17 of the 34 outbreaks (*8*).

Canned tomato products can support growth of *C. botulinum* and production of toxin if the pH of the product is greater than 4.6 (*11*). Other microorgansims which survive processing may cause the pH to rise. Tomatoes have been reported to be less acid than most other acid foods having a pH range between 4.0 and 4.5 (*9*). Farrow (*2*) reported that 7% of the 15,000 pH determinations done on canning tomatoes were above 4.5. Selection of varieties with a pH below 4.6 for canning should provide an ample margin to insure a safe process (*11*).

Investigations as to the reasons for the growth of *C. botulinum* in canned tomatoes have revealed that microorganisms other than *C. botulinum* were present in the tomatoes. These organisms apparently reduced the acidity due to their metabolic processes and/or stimulated growth of *C. botulinum* to the extent that toxin production was possible (*4,5,7,10*). It is reasonable to assume that the suspected jars of tomato product were inadequately heated and/or were contaminated by microorganisms after heating.

The purpose of this research was to establish what factors utilized by the consumer may affect the safety of home-canned tomatoes.

EXPERIMENTAL

Collection of samples

Fresh and home-processed tomatoes from 15 Utah counties, were collected during the 1975 canning season (Table 1). At the time the processed samples were obtained each consumer was interviewed to obtain information relative to this study. Variety of the fresh samples was obtained when information was available. Processed samples were inspected for adequate closure and seal, then incubated for 8 months at 55 F. This incubation temperature was selected since it represents the mean temperature of storage in all areas of Utah during the 8-month period following the tomato canning season.

TABLE 1. *Samples of tomato products collected during the 1975 canning season in Utah counties.*

Tomato product	Number of samples
Fresh tomatoes	249
Canned tomatoes	156
Canned juice	90
Mixed (low acid food added)	30
Total	525

Microbiological analyses

Numbers of aerobic organisms and fungi were obtained using the recommended methods published in the *Bacteriological Analytical Manual* (*3*)

Numbers of anaerobic organisms were obtained on Brewer Anaerobic Agar, under a GasPak hydrogen-carbon dioxide atmosphere, incubated at 30 C for 8 days.

Vacuum and headspace

Vacuum of each jar was measured using a Bourdon-type gauge calibrated to read vacuum from 0 to 30 inches. Readings were obtained after the surface of the closure and the gauge had been flame-sterilized with methanol. Headspace was measured using a depth gauge with horizontal bar and vertical rule calibrated in millimeters.

pH and titratable acidity

Contents of the jar were blended for 15 sec using a blade assembly with a screw-type lid to fit standard mason jars (Waring). pH readings were made after blending using a Coleman Model 38A meter standardized at pH 4. After centrifugation at 5000 rpm to effectively remove the lycopene pigment, aliquots of the blended product were titrated with standardized NaOH to a phenolphthalein endpoint (*1*). Titratable acidity was reported as ml of 0.1 N NaOH per 100 ml of product.

RESULTS AND DISCUSSION

Acidity of fresh tomatoes

During the early part of the 1975 growing season frosts were prevalent in Utah necessitating the replanting of many garden plots. This situation, along with the fact that most consumers did not keep a record of the variety, complicated collection of reliable data on tomato varieties and their associated acidity. Most of the fresh tomatoes which were tested for pH were not identified as to variety (Table 2).

There was greater variation between samples of the same variety than between different varieties. This variation was probably due to location and ripeness rather than genetic factors.

All of the fresh samples which were tested contained adequate acid to inhibit growth of *C. botulinum.* When the pH was as high as 4.4 and 4.5 selection of less mature fruit in preference to slightly over-ripe fruit would enhance the acidity of these raw tomatoes when processed by canning (*6*).

Inspection of canned tomato products

During collection of samples of processed tomato products from home-canners, each jar was inspected by removing the band (ring) and applying pressure to the lid to insure that some vacuum was present. When the jar was not sealed by vacuum, samples were discarded. The integrity of the seal has always been used by the consumer as a major criterion for safety. Loss of vacuum and release of a sealed lid has been used as positive indication of spoilage. When these conditions existed, the consumer has been advised to avoid consumption of the food since it would be considered a hazard. Inspection of the jars before incubation to insure that they were properly sealed provided one criteria for evaluating the effectiveness of the process. Gas production in a glass jar during incubation is a fair indicator of microbial growth. Some chemical reactions also produce gas; however, these are not as prevalent in glass containers as they are in steel cans. Most tomato products in Utah are stored from 8 to 12 months before consumption. Most storage areas were not completely dark so the incubated samples were stored in subdued light.

The sealing compound on the lid was inspected visually for variations in thickness. Bands were inspected for corrosion, rust and abnormal shape. In some instances lids other than those designed for home canning were used. The rim of the glass jar was also inspected for nicks and pits which may have contributed to sealing failures (Table 3).

TABLE 3. *Factors affecting the quality of closures from jars of home-canned tomato products.*

Factor	Number exhibiting factor	Percent exhibiting factor
Band removed from jar	62	22.4
Corroded or rusted band	38	13.7
Uneven distribution of sealing compound on lid	12	4.3
Other than standard lid and band used[a]	2	0.7
Rim of jar pitted or nicked	6	2.1
Total jars tested	276	

[a]One of the two jars contained a one-piece lid from a jar of commercially packed pickles and the other contained a one piece lid from a jar of mayonnaise.

Removal of the band after the jar has sealed followed by drying of the band before storage has been recommended by manufacturers as a procedure to avoid corrosion of the band and possible failure of the jars to seal. When the 38 corroded bands from this study were compared to 38 normal bands using new lids the rate of failure of jars to seal was significantly ($P < .01$) higher in the jars sealed with corroded rings following a water bath for 55 min (Table 4).

Uneven distribution or an inadequate amount of sealing compound on the lid can affect the frequency of sealing failure. In the opinion of the authors, manufacturers must assume responsiblity for the quality

TABLE 4. *Sealing failures in corroded and non-corroded bands.*[a]

Trial	Closure band	Number of jars not sealed[b]	Percent of jars not sealed
1	Non-corroded	1	2.6
1	Corroded	5	13.1
2	Non-corroded	0	0
2	Corroded	6	15.7

[a]Corroded and non-corroded bands with new lids were placed on quart jars containing water with ½-inch headspace. They were processed in a boiling water bath for 55 min.

[b]Jars containing no vacuum were judged as failures.

TABLE 2. *pH of fresh tomato samples collected in Utah counties during 1975.*

Tomato variety	No. of counties	No. of samples	Average pH	Range of pH
Unidentified	15	171	4.22	3.78 - 4.55
Beefsteak	3	4	4.01	3.90 - 4.10
Stone	4	11	4.18	3.80 - 4.33
Moscow	5	19	4.15	3.90 - 4.49
Fantastic	2	2	4.34	4.21 - 4.47
Early Girl	3	6	4.08	3.81 - 4.30
"Cherry"[a]	1	2	4.19	4.14 - 4.24
Del Monte 52 +24	2	6	4.16	3.90 - 4.30
Burpee Hybrid	3	6	4.18	4.02 - 4.30
Big Boy	4	14	4.20	3.94 - 4.55
DX 5212	3	3	4.12	4.00 - 4.30
"Yellow"[b]	5	5	4.17	4.03 - 4.35

[1] "Cherry" refers to tomatoes 2-3 cm in size and not a specific variety.
[2] "Yellow" refers to color and includes several varieties.

of the lid. During the 1975 canning season a greater amount of jar sealing failures could be attributed to poor quality lids than was evident in this study. However, jars which were not sealed at the time of collection in this study were discarded making a detailed study of sealing failure due to poor quality lids impractical.

Shortages of lids during the 1975 canning season prompted use of other types of lids. This practice has hopefully been discontinued now that an abundance of standard lids and jars are available to the home canner.

Microbial analysis

Numbers of microorganisms in acid foods, unless supplemented with more detailed isolation and identification techniques for specific organisms such as *C. botulinum*, do not necessarily provide evidence as to the safety of the food. However, the presence of fungi in canned acid foods is a good indication of inadequate processing techniques, survival of spores and/or lids that leak. The numbers of these organisms one might expect to find in home-canned tomatoes (Table 5 and 6) are interesting from the standpoint of distribution.

Numbers of anaerobic and aerobic bacteria per ml of juice were considered important only when the vacuum in the jar was very low or zero, suggesting that growth had occurred. Presence of fungi indicates gross underprocessing, contamination after heating and/or survival of heat resistant spores.

Vacuum and headspace

The average vacuum in all jars tested was 15 inches. The range was from 0 to 22 inches. Those with a vacuum of 0 inches were considered to be improperly processed (Table 7). The headspace recommended for tomato products by U.S.D.A. (*12*) is 1.27 cm, (1/2 inch). All of the samples measured in this study were adequate, including those exhibiting no vacuum.

pH and Titratable acidity

Variation of the pH of the processed tomato products was very similar to that of the fresh samples. The processed products averaged pH 4.13 with a range of 3.81 to 4.53. Much greater variation was observed in the titratable acidity which averaged 76 with a range of 13 to 231. The wide range in titratable acidity was due to the addition of acid to some samples by the consumer. Twelve consumers indicated that acid such as vinegar or lemon juice was added to the tomatoes. The amount of acid varied between one teaspoon and two tablespoons per quart.

Such a high number of samples with no vacuum must be accounted for by some processing techniques

TABLE 5. *Distribution of microorganisms in home-canned tomato products.*

Samples	Total number	Number containing microorganisms	Number containing fungi	Number containing aerobic bacteria	Number containing anaerobic bacteria
Tomatoes	155	41	3	22	29
Tomato juice	90	21	1	19	11
Mixed	30	7	0	5	3

TABLE 6. *Range of microorganisms per ml isolated from home-canned tomato products.*

Class	Number of samples	Range of numbers per ml isolated from tomato products					
		$1\text{-}10^2$	$10^2\text{-}10^3$	$10^3\text{-}10^4$	$10^4\text{-}10^5$	$10^5\text{-}10^6$	$>10^6$
Anaerobic bacteria	43	22	11	6	0	4	0
Aerobic bacteria	59	14	26	16	1	2	0
Fungi	4	3	0	0	0	0	1

TABLE 7. *Characteristics of tomato products exhibiting no vacuum after storage for 8 months.*

Sample	Processing method	pH	Titratable acidity	Head-space cm	Micro-organisms per ml	Kinds of microorganisms
Tomatoes	Water bath	4.36	58.1	4.3	500,000	Anaerobic
Tomatoes	Water bath	4.12	89.0	4.2	20	Anaerobic
					150	Aerobic
Tomatoes	Water bath	4.22	74.0	2.5	5,000	Anaerobic
					400	Aerobic
					10	Fungi
Tomatoes	Water bath	4.12	78.0	2.0	1,000,000	Aerobic
Tomatoes	Water bath	4.02	71.0	2.0	1,400	Aerobic
Tomatoes	Water bath	4.24	53.0	4.1	15	Fungi
Tomatoes	Water bath	6.88	13.0	5.1	2,500,000	Fungi
Tomatoes	Water bath	4.25	59.0	4.8	1,000	Anaerobic
Mixture	Water bath	4.29	55.0	2.7	50,000	Aerobic
Juice	Water bath	4.13	62.0	3.5	6,500	Anaerobic
					6,000	Aerobic
Juice	Open kettle	4.12	89.0	2.2	60,000	Aerobic

which are not adequate. The factors associated with the closures and jars previously discussed which contributed to seal failure could not account for 4 to 5% of the samples. However, they may contribute to the ultimate safety of the canned products. Lack of acidity in the raw tomatoes did not seem to be a contributing factor to the loss of vacuum in the canned products. Only one canned sample exhibited a pH higher than 4.6 after processing and storage. This was due to excessive fungal growth which was probably not present at the time of processing.

Processing times and temperatures were collected on all of the tomato products. lack of proper processing methods may be the major contributing factor to the high rate of samples exhibiting no vacuum. U.S.D.A. (*12*) recommended times and pressures for processing tomatoes in Utah (including adjustments for altitude) are given in Table 9.

Since the jars exhibiting no vacuum were processed by the water bath method evaluation of the processing times actually used was of major importance (Table 10). The single sample processed by the open kettle which exhibited no vacuum was heated to boiling for 2-3 min before being transferred to the jar.

Approximately 85% of the cold packed samples and 30% of the hot filled samples were underprocessed. The reliability of these data is based on advanced notice to the consumer that this information would be requested when the samples were obtained by requesting a source. Samples were collected during the canning season, a time when consumers would be most aware of this information.

Since all of the jars in the study had enough vacuum to prevent the removal of the lid at the start of the storage period, we assumed that at that time the jars were adequately processed. Loss of vacuum during storage may have been due to microbial growth since microorganisms were present. Leakage of the sealed lid was also considered as a reason for loss of vacuum; however, examination of the lids and jars did not indicate this to be a major factor. The processing time, although subject to error, strongly indicates that loss of vacuum may be related to processing time and/or temperature. Most of the jars exhibiting no vacuum were underprocessed. Further work needs to be done under controlled conditions to verify these results.

Re-emphasis as to the importance of time and temperature in home-canning could results in fewer jars of spoiled product, or product about which the safety is questionable. Education and the availability of reliable information are essential for both the novice and the experienced home-canner.

TABLE 9. *U.S.D.A. recommended processing times. temperatures and pressures for tomato products in Utah.*[a]

Processing method	Temperature	Time[b] (minutes)	Pressure (lbs.)
Water bath			
Cold pack	Boiling water	Qt. 55	
		Pt. 45	
Hot fill	Boiling water	Qt. 15	
		PT. 15	
Pressure cooker	Above 212 F	Qt. 10	7-10
		Pt. 10	7-10

[a]An average altitude of 5,000 feet was used.
[b]Time includes an altitude adjustment.

TABLE 10. *Processing times for home-canned tomato products using the water bath method.*

Minutes	Number of samples		Number of samples exhibiting no vacuum	
	Quarts	Pints	Quarts	Pints
COLD PACK				
<5	0	0	0	0
6 - 10	2	2	0	0
11 - 15	2	3	2	0
16 - 20	26	2	1	0
21 - 25	28	3	2	2
26 - 30	9	1	0	0
31 - 35	22	5	0	0
36 - 40	8	4	0	0
41 - 45	26	9	0	0
46 - 50	1	1	0	0
51 - 55	19	4	0	0
56 - 60	1	0	0	0
HOT FILL				
<5	0	0	0	0
6 - 10	8	2	0	1
11 - 15	4	7	0	1
16 - 20	9	6	0	0
21 - 25	6	1	0	0
26 - 30	4	3	0	1
31 - 35	5	2	0	0
36 - 40	1	0	0	0
41 - 45	2	0	0	0
46 - 50	0	1	0	0
51 - 55	5	1	0	0
56 - 60	0	0	0	0

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Incidence of Salmonellae in Raw Meat and Poultry Samples in Retail Stores

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ABSTRACT

An examination of 142 samples of raw beef, pork, and poultry from five retail stores in Athens, Georgia, showed an overall incidence of 14.8% contamination by salmonellae. Pork samples had the maximum contamination (21.5%). Samples were obtained in lots of about 25 over a 6-week period and the extent of contamination by salmonellae in the products purchased every week fluctuated over a narrow range (12.5 - 19.2%). Higher levels of contamination were observed in samples purchased from two national supermarkets than in samples purchased from a regional supermarket, a local grocery store and one national supermarket. *Salmonella typhimurium* and *Salmonella agona* were the serotypes isolated most frequently from the samples.

Livestock and poultry are the most important reservoirs of salmonellae implicated in human salmonellosis. The organisms are retained in the intestines, and occasionally in the tissues of apparently healthy livestock destined for human consumption (*4*). Present practices of poultry and meat animal production and distribution are conducive to the build-up of salmonellae (*3*). The likelihood of exposure to microbial contamination has increased significantly as a result of restricting movement of animals and of re-using the same physical facilities.

Only a limited number of surveys have been conducted during the past 30 years to assess the incidence of contamination by salmonellae of meats and poultry products sold in the retail market (*5,6,7,9,10,11*). A report to the Congress of the United States (*1*) from the General Accounting Office alleges that raw meats and poultry products are being sold to the public and that neither the Food and Drug Administration nor the Food Safety and Quality Service has current national data to show the extent of contamination level. This survey was conducted to assess the extent of contamination by salmonellae in retail samplings of meats and poultry purchased in the Athens, GA area.

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MATERIALS AND METHODS

Samples from retail outlets

Samples of raw meats and poultry were purchased from retail outlets in the Athens area once every week over a 6-week period. Five retail supermarkets were included in the survey. Three of the supermarkets were national chains. Meats and poultry purchased at these outlets had been packaged in a regional distribution facility located outside Athens. The fourth supermarket was a regional chain; however, retail cuts of meat and poultry were prepared at that store facility. At this supermarket meats were not packaged for display. They were displayed in stainless steel trays (approximately 100 cm × 35 cm) inside refrigerated display cabinets. When the customer requested a specified quantity of a certain meat, the employee removed the desired portion on wax paper, weighed it, and packaged the meat in brown kraft paper. Poultry samples were displayed in a pre-packaged condition in the same manner as for the national supermarkets. The fifth retail outlet was a small local grocery and meat store. The meat handling and selling practices at this store were similar to those in the fourth supermarket.

Samples of meat and poultry, in approximately 1-lb. quantities, were purchased from each of the retail outlets. The samples were transported to the laboratory in an insulated ice chest and processed in the laboratory within 2-h after delivery.

Microbiological examination of samples

Meat and poultry products were examined by the conventional cultural method as recommended by the Food and Drug Administration (*12*). Twenty-five g of each raw meat or chicken sample were weighed aseptically into each of two sterile pint size Mason Jars. Two hundred twenty five ml of selenite cystine broth were added to one jar and 225 ml of tetrathionate broth were added to the other jar. The samples were homogenized in the selective enrichment broth using an Osterizer blender (John Oster Mfg. Co., Milwaukee, Wisconsin) at low speed (speed #1) for 2 min. Those samples that could not be homogenized were cut into small pieces with sterile scissors and shaken thoroughly with the enrichment broth. A measured volume (2.2 ml) of sterile Tergitol 7 (Aloe Scientific Co., St. Louis, Missouri) was added to the samples to emulsify the fat. Samples were mixed thoroughly and incubated at 37 C for 24 h. One loopful of each selective enrichment culture was streaked on one plate each of Brilliant Green (BG) Agar and Hektoen Enteric (HE) Agar. Plates were incubated at 37 C for 24 h and examined for colonies showing characteristics typical of *Salmonella*. At least two colonies were picked from each suspect *Salmonella*- positive plate and transferred to Triple Sugar Iron Agar (TSI) and Lysine Iron Agar (LIA) slants. Those cultures showing reactions on TSI and LIA typical of salmonellae were inoculated into lactose broth, dulcitol broth, KCN broth, urea broth, and tryptone

broth (indole test) for biochemical identification. The TSI cultures, presumptively positive for salmonellae, were subjected to 'O' serology by the slide agglutination method using *Salmonella* polyvant 'O' antiserum (Difco). For the 'H' agglutination test, 1 loopful of the tryptone broth culture was transferred to 5 ml of trypticase soy-tryptose broth containing 0.3% yeast extract and incubated for 18 h at 37 C. An equal volume of formalinized saline (0.85% sodium chloride and 0.6% formalin) was added to each tube after incubation and the tubes were placed at room temperature for 1 h. H-agglutination tests were done on these formalinized cultures using *Salmonella* polyvalent 'H' antiserum (Baltimore Biological Lab., Baltimore, Maryland).

Serotyping of cultures

Cultures of salmonellae isolated from the meat and poultry samples were sent to the Veterinary Diagnostic Lab., Animal and Plant Health Inspection Service, Ames, Iowa for serotyping.

RESULTS AND DISCUSSION

An analysis of the meat and poultry samples and the incidence of contamination by salmonellae in each product type are shown in Table 1. The highest incidence of contamination by salmonellae was found in pork samples (21.5%) while the lowest incidence was observed in poultry (7.3%). An overall incidence of 14.8% was observed for all samples. Samples were collected in lots of about 25 during a 6-week period. Table 2 shows the details of analysis for the samples on a weekly basis. The levels of contamination on a weekly basis ranged from 12.5% to 19.2%. By comparing these levels to the overall incidence of 14.8%, it can be seen that the incidence of contamination was fairly constant throughout the 6-week period.

TABLE 1. *Incidence of salmonellae in meat and poultry samples.*

Product type	Number of samples	Number contaminated with salmonellae	Percent contamination
Beef	36	4	11.1
Poultry	41	3	7.3
Pork	65	14	21.5
Total	142	21	14.8

TABLE 2. *Incidence of salmonellae in retail samples purchased over a six week period.*

Week beginning	Number of samples	Number positive for *Salmonella*	Percent positive for *Salmonella*
April 5, 1977	22	3	13.6
April 12, 1977	21	3	14.2
April 20, 1977	26	5	19.2
May 9, 1977	24	3	12.5
May 16, 1977	25	4	16.0
May 23, 1977	24	3	12.5

A comparison of the extent of contamination by salmonellae of meats and poultry samples obtained from different retail outlets is shown in Table 3. The incidence of contamination was low in one of the national supermarkets and in the regional supermarket. The local grocery store had an incidence of contamination (16.7%) between the lowest (8.3%) and highest values (23.1%). Samplings from two national supermarkets indicated high levels of contamination.

TABLE 3. *Incidence of salmonellae in meat and poultry samples obtained from various retail outlets.*

Retail outlet[a]	Number of samples	Number contaminated with salmonellae	Percent contaminated samples
NAT 1	22	5	22.7
NAT 2	36	3	8.3
NAT 3	26	6	23.1
LOC	24	4	16.7
REG	34	3	8.8

[a]Legend: NAT 1,2 and 3 = National supermarket chains 1, 2 and 3, respectively.
REG =Regional supermarket chain.
LOC =Local grocery and meat store.

The national supermarkets displayed their meats and poultry samples in the prepackaged condition in refrigerated display cabinets. Meats for all the three national chain stores were packaged at centralized packaging facilities located outside the Athens area. Meats sold at the regional supermarket were not prepackaged. In view of the differences in meat handling procedures between the national supermarkets and the regional and local stores, it was initially anticipated that the incidence of contamination by salmonellae would be higher at the latter outlets. However, this view was not substantiated by the data in Table 3.

Since the maximum incidence of contamination by salmonellae occurred in pork samples, data on pork samples obtained from different sources were regrouped as shown in Table 4. Pork samples constituted 45 to 61% of all samples purchased from four of the retail outlets but only 20.6% of the samples purchased from the regional supermarket. The contamination level of pork samples ranged from 25 to 30% from all outlets except for one national supermarket. The level of incidence in pork samples purchased from that market (NAT 2) was only 5.8%, although 52.7% of the samples purchased from this outlet were pork.

Samples of pork sausage purchased from the various retail outlets could be divided into two categories. Most of the pork sausage purchased from the three national supermarkets had been procured in prepared and prepackaged form from meat packing companies. The national chain was acting solely as the retailer for these products. In contrast, all the pork sausage obtained from the regional and local stores was freshly ground and was packaged at the store. The prepackaged pork (21

TABLE 4. *Incidence of salmonellae in pork samples obtained from different outlets.*

Retail outlet[a]	Total samples	Pork samples	Percent pork samples	Number of contaminated pork samples	Percent contaminated pork samples
NAT 1	22	10	45.4	3	30.0
NAT 2	36	19	52.7	1	5.3
NAT 3	26	16	61.5	4	25.0
LOC	24	13	54.2	4	30.8
REG	34	7	20.6	2	28.6

[a]Legend: NAT 1,2 and 3 = National supermarket chains 1, 2 and 3, respectively.
REG =Regional supermarket chain.
LOC =Local grocery and meat store.

samples) showed a contamination level of only 19.0%, whereas the fresh pork sausage (13 samples) had an incidence of 38.5% for salmonellae.

The meat and poultry samples purchased from the retail outlets could also be classified on the basis of their relative costs. Meats such as pork sausage, ground hamburger, stew beef and pork shoulder blade steak were included in the 'expensive' category whereas samples such as pork neck bones, chicken gizzards, hearts, and wings were placed in the 'cheap meat' category. The total of 142 samples were thus divided into 101 'expensive' samples and 41 'cheap meat' samples. Contamination by salmonellae of expensive meats was 13.9% (14 of 101 samples) while that of cheap meats was 17.0%.

Difficulties were encountered in isolating salmonellae from Brilliant Green Agar plates, probably because of the overwhelming effect of lactose fermentors present in the food sample. Thomason and Dodd (*8*) encountered the same problem in isolating salmonellae from pork sausage and chicken livers.

Serotypes of salmonellae isolated from raw meats and poultry samples are shown in Table 5. *Salmonella*

TABLE 5. *Serotypes of salmonellae isolated from retail meat and poultry samples.*

Serotype	No. of isolations	Source
S. typhimurium	4	ground beef (1), chicken gizzards and hearts (1), chicken livers (1), pork sausage (1)
S. agona	4	pork sausage (2), pork neckbones(2)
S. anatum	3	pork sausage (1), ground beef (1), pork liver (1)
S. derby	3	pork sausage (2), ground beef (1)
S. blockley	1	chicken livers
S. infantis	1	pork sausage
S. newington	1	pork sausage
S. give	1	pork neckbones
S. litchfield	1	chicken wings
S. nienstedten	1	pork sausage
S. london	1	pork neckbones

typhimurium and *Salmonella agona* were isolated most frequently, followed by *Salmonella anatum* and *Salmonella derby*. All isolations of *S. agona* were made from pork meat. Every year since 1970 (*2*) the number of isolations of *S. agona* reported to the Center for Disease Control, Atlanta, GA, have increased. That serotype ranked fifth among the 10 most frequently isolated salmonellae from human sources in 1975. Results obtained in this investigation of the incidence of salmonellae in retail red meat and poultry samples are consistent with those of Wilson et al. (*10*) and Weissman and Carpenter (*9*). These workers observed no clear-cut differences between the meat and poultry samples purchased from national supermarkets and from regional and local stores.

A significant portion of meats and poultry products purchased from the retail market were contaminated with salmonellae. The problem appears to be serious in pork products. Results reported here underscore the need for proper handling and storage of meats brought into the home. Even if all precautions of proper storage and cooking of meats are taken, the likelihood of transmitting viable salmonellae from the contaminated meat to uncooked foods remains as a public health problem of considerable magnitude.

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Recovery of Salmonellae from Broiler Carcasses by Direct Enrichment

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ABSTRACT

Each of four serotypes of *Salmonella* (*S. anatum, S. montevideo, S. saint-paul, S. typhimurium*), inoculated at low levels on broiler carcasses (ca. 20 cells/carcass) was detected by direct enrichment of the whole carcass rinse fluid with either Selenite Cystine Broth (SC) or Selenite Brilliant Green Broth (SGB). Neither Selenite Brilliant Green Sulfa Broth (SBGS) nor TT Broth was effective in detecting the serotypes unless the entire broiler carcass with the rinse fluid was incubated with either of these enrichment broths. SBG and SC were effective as direct enrichment broths for recovering pure cultures of the four seroytpes subjected to sublethal heat treatment (53 C for 1 min) approximating that to which broiler carcasses are subjected during the commercial scalding process.

A number of different procedures have been used for detection of *Salmonellae* in processed poultry carcasses; but as yet, none has been accepted as standard for this food product. There is disagreement as to the sampling method, choice of enrichment media, selective plating media, confirmation methods and whether the initial step in the procedure should involve preenrichment of the sample in a nonselective broth or direct enrichment in a selective broth.

Edel and Kampelmacher (*4*) have recommended preenrichment for some foods because sublethal injury to salmonellae could occur during processing. Many investigators have used or recommended lactose broth as the preenrichment medium in the analysis of poultry carcasses for *Salmonella* (*2,3,9,11,12,23*). Other investigators working with raw food samples or poultry used the preenrichment technique but with nutrient broth (*13*), mannitol broth (*16*) or buffered peptone (*25*) instead of lactose. Still others have used or recommended direct enrichment techniques for this type product (*10,24*). The International Commission on Microbiological Specifications for Foods (*15*) recommends a modified procedure of Surkiewicz et al. (*23*) for isolating salmonellae from poultry. The modification involves shaking the carcass in a plastic bag with 300 ml of lactose broth, followed by the addition of 300 ml of double-strength liquid-enrichment broth.

Many different enrichment broths are available, so if the decision is made to use the direct enrichment method for detecting salmonellae on poultry carcasses, the question will arise as to which one(s) to use. It has been suggested that the optimum procedures and methodology for *Salmonella* should be determined for every different food product (*8,14*). The purpose of our study was to compare the efficiency of four commonly used enrichment media for direct enrichment of processed broiler carcasses.

MATERIALS AND METHODS

Experiment 1

Eviscerated broiler carcasses were obtained from a local processing plant. A strain of *Salmonella typhimurium*, resistant to 100 ppm of nalidixic acid, was used as the marker organism in this study. Cells of this culture were grown on Brain Heart Infusion (BHI) Agar (Difco) slants for 18 h and then washed from the slant with sterile physiological saline solution. The suspension was then diluted with sterile saline solution to an optical density (OD) of 0.2 at 540 nm, measured with a Bausch and Lomb Spectronic 20 spectrophotometer. Then 0.1 ml of the diluted suspension which contained 20 or fewer cells of the marker organism, was rubbed into skin on the breast, the thigh and under the wing of each of 40 carcasses with a sterile bent glass rod. The number of cells inoculated was determined from 0.1-ml aliquots spread on 10 plates of BHI agar. The inoculum for this experiment ranged from 6 to 20 cells per carcass.

After inoculation, each carcass was individually placed in a plastic bag, 300 ml of Selenite Brilliant Green (SGB) Broth (Difco) were added and the contents vigorously shaken for 1 min. The bird and the broth were then incubated together in the bag for 24 h at 35-37 C. After incubation, three loopsful of broth from each bag were streaked onto plates of MacConkey Agar (Difco) containing 100 μg of nalidixic acid per ml. After 24 h of incubation at 35-37 C, isolated colonies were picked from the plates and tested serologically to confirm the presence of the marker organism.

The above procedure was repeated with three other enrichment broths, Selenite Brilliant Green Sulfa (SGBS) Broth (Difco), Selenite Cystine (SC) Broth (Difco) and TT Broth (Difco).

Experiment 2

The procedure for this experiment was identical to that described for Experiment 1 with the following exceptions. Each inoculated carcass was placed in a polyethylene bag with 270 ml of sterile water and

vigorously shaken for 1 min. The carcass, after draining into the bag for 30 sec was removed and 30 ml of a concentrated solution of one of the four enrichment broths (SGB, SBGS, SC, TT) which yielded a single strength final concentration was added to the rinse fluid. The rinse sampling bag was placed in an 800-ml beaker and incubated for 24 h at 35-37 C.

Experiment 3

A low level inoculum (approximately 12 cells) of an 18-24 h culture of nalidixic-acid resistant *S. typhimurium* was pipetted directly into each of 10 milk dilution bottles, containing 100 ml of SBG, and incubated 24 h at 35-37 C. A loopful of broth from each bottle was then streaked onto plates of MacConkey Agar containing 100 ppm nalidixic acid. After a 24-h incubation of these plates at 35-37 C, colonies were tested serologically to confirm them as our marker organism. The experiment was repeated with SBGS, SC and TT as the enrichment broth.

Experiment 4

Each of 20 freshly processed raw broiler carcasses was dermally inoculated with a mixed suspension containing approximately 20 cells each of *S. anatum, S. montevideo* and *S. saint-paul*. The sites inoculated were breast, thigh and under the wing. The number of cells of each serotype in 0.1 ml of the suspension was determined by the procedure described for the inoculum used in Experiment 1. Each carcass was placed in a plastic bag, with 300 ml of SBG broth, shaken vigorously for 1 min and incubated in the bag with the broth for 24 h at 35-37 C. Next, three loopsful of the broth were streaked onto each of two BG Sulfa Agar (Difco) plates. After 24 h of incubation at 35-37 C, 10 typical colonies were picked (five from each plate) for each of the 20 samples, inoculated onto slants of Lysine Iron (LI) Agar (Difco), and incubated 24 h at 35-37 C. LI agar slants exhibiting a typical salmonellae reaction were subcultured onto BHI agar plates for determination of purity, then these cultures were serologically confirmed to be one of the three serotypes inoculated.

This experiment was repeated with another 20 carcasses and SC instead of SBG broth.

Experiment 5

Individual suspensions of *S. anatum, S. montevideo, S. saint-paul* and *S. typhimurium*, each containing about 20 cells, were inoculated into 10 tubes each containing 3 ml of sterile physiological saline tempered to 53 C. The tubes were put in a 53-C water bath and held for 1 min. Then, the contents of each tube were transferred to 97 ml of SBG broth. After 24 h of incubation at 35-37 C, three loopsfull of the broth were streaked onto each of two BG Sulfa Agar plates and the procedures previously described in Experiment 4 were used to recover the inoculated organism. Experiment 5 was replicated 10 times, five times each with SBG broth and SC broth.

RESULTS AND DISCUSSION

In experiments 1 and 2 (Table 1) direct enrichment into SBGS, SC and TT successfully recovered an inoculum of very few cells of *S. typhimurium* 100% (40/40) of the time when the carcasses were incubated in the bag with the enrichment broth. SBG yielded recovery in 75% (30/40) of the samples. However, when the carcass was not incubated with broth, results were significantly different. In this experiment SBG and SC performed at a 100% efficiency (40 out of 40 positive recoveries); however, the efficiencies of SBGS and TT in recovering the marker organism were drastically reduced. SBGS yielded only 10% positive recoveries of the inoculated organism, while TT was unable to produce a positive in any of the 40 samples. In Experiment 3 (Table 2), direct introduction of the marker organism into the various enrichment broths produced results nearly identical to those obtained in Experiment 2. The findings presented in Tables 1 and 2 suggest that SBGS and TT may exert a lethal or toxic effect on our marker organism. TT broth contains tetrathionate and thiosulfate and Palumbo and Alford (*18*) observed a lethal effect on bacteria by this tetrathionate-thiosulfate combination. The actual mechanism of this lethality is not known. However, since both tetrathionate and thiosulfate react with free sulfhydryl groups of enzymes and cause their inactivation (*19,20*), tetrathionate broth may interfere with the synthesis and/or activity of sulfur-containing enzymes or cell wall and membrane components. Palumbo and Alford (*18*) also reported that the tetrathionate-thiosulfate combination initially reduced the numbers of many organisms, but that regrowth followed. Since the inoculum level in our studies was so low, such an initial effect could have caused elimination of the marker organism. The toxic effect of TT broth was not observed in Experiment 1 (Table 1) when the carcass was incubated in the rinsing fluid. A possible explanation could be that in Experiment 1 some of the inoculated cells were protected against these toxic effects by the skin and tissues of the carcass. The TT broth plus the broiler carcass is a complex mixture of continuously changing compounds due to the variety of microorganisms present, so that protection in the very early stages of incubation could be critical.

TABLE 1. *Efficacy of four enrichment broths for recovery of* Salmonella[a] *artificially inoculated on broiler carcasses.*

Media	Carcass incubated with broth[b] No. of positives/No. of carcasses	Carcass rinse fluid[c] incubated with broth No. of positives/No. of carcasses
SBG	30/40	40/40
SBGS	40/40	4/40
SC	40/40	40/40
TT	40/40	0/40

[a]Strain of *S. typhimurium* resistant to nalidixic acid.
[b]Mean inoculum range was 6-20 cells per carcass.
[c]Mean inoculum range was 12-18 cells per carcass.

TABLE 2. *Recovery of* Salmonella typhimurium *inoculated directly into various enrichment broths.*[a]

Media	No. of positives/No. of samples
SBG	10/10
SBGS	4/10
SC	10/10
TT	0/10

[a]Mean inoculum was 12 cells per carcass.

The inhibitory properties of SBG and SBGS arise from the combined activities of selenite brilliant green and sodium taurocholate (*22*). While the addition of sulfapyridine (*17*) has been shown to increase the selectivity of SBGS and promote recovery of salmonellae amidst large numbers of extraneous organisms, it may be that the combined effects of sulfapyridine, selenite brilliant green and sodium taurocholate, are in certain instances, toxic (or at least cause an initial reduction in numbers) to certain salmonella serotypes. Such a possibility would explain the poor performance of SGBS

in our study (Table 1 and 2). Our results with this medium agree with those of Yamamoto et al. (*26*) who found SBGS to be too selective and less effective than SC or tetrathionate brilliant green for isolation of salmonlla from turkey tissues and fecal samples. In addition, Carlson and Snoeyenbos (*1*) found that SBGS produced major reduction of salmonellae between 24 and 48 h of incubation, especially at 43 C. They concluded that this medium was totally unsatisfactory for some salmonella strains. In contrast, Fagerberg (*5*) and Fagerberg and Avens (*6,7*) found SBGS to be superior to 11 other enrichment broths for recovering salmonellae from contaminated turkey carcasses.

Since recovery of the marker organism was best with SBG and SC in the first three experiments, in Experiment 4 we tested whether they would enable us to recover several salmonella serotypes amidst the extraneous organisms found on broiler carcasses. In every instance, both SBG and SC allowed us to recover low numbers of cells of *S. anatum, S. montevideo* and *S. saint-paul* (Table 3). According to Fagerberg and Avens (*8*) an ideal enrichment broth should not be too inhibitory to salmonellae yet should be selective enough to prevent overgrowth by competitive organisms. Our data suggest that SBG and SC met this requirement for isolating these three serotypes of *Salmonella* from broiler carcasses.

TABLE 3. *Comparison of SBG and SC for recovery of* S. anatum, S. montevideo, *and* S. saint-paul *from broiler carcasses.*

Medium	Serotype	No. of positives/No. of carcasses
SBG	*S. anatum*[a]	20/20
	S. montevideo[b]	20/20
	S. saint-paul[c]	20/20
SC	*S. anatum*	20/20
	S. montevideo	20/20
	S. saint-paul	20/20

[a]Mean inoculum for *S. anatum* was 15 cells per carcass.
[b]Mean inoculum for *S. montevideo* was 23 cells per carcass.
[c]Mean inoculum for *S. saint-paul* was 18 cells per carcass.

It has been suggested that sublethal injury can occur during processing of raw meats and poultry and that, therefore, the examination of such foods for salmonellae should include a preenrichment step (*4*). During processing, broilers are commonly scalded for 1 min in 53-C water and this process could conceivably result in injury to salmonellae. In Experiment 5 we found that pure cultures of *S. anatum, S. montevideo, S. saint-paul* or *S. typhimurium* at low levels (about 20 cells) subjected to these conditions could be detected by enrichment in either SBG and SC without a preenrichment step (Table 4). Bacteria on broiler carcasses during commercial scalding are probably exposed to even less heat than that in this experiment because of the insulating effects of feathers, skin and organic matter in the scald water; so sublethal injury to salmonellae present would not be likely. The results of this experiment indicate that salmonellae cells on carcasses receiving a 53-C scald during processing could be recovered by use of SBG or SC enrichment broths.

TABLE 4. *Recovery of salmonellae subjected to 53 C water for one min by direct enrichment.*

Serotype	Enrichment broth	
	SBG	SC
S. anatum	5/5[a]	5/5
S. montevideo	5/5	5/5
S. saint-paul	5/5	5/5
S. typhimurium	5/5	5/5

[a]No. of positives/No. of observations.

Many variables can influence the performance of an enrichment medium; they include temperature of incubation, time of incubation, extent of agitation, presence or absence of detergent emulsifiers, ratio of salmonella to competitors, material sampled, and the salmonella serotype(s) involved (*8*). It was beyond the scope of this study to compare all the possible variables or even to compare all the available enrichment broths for detecting salmonellae from processed broiler carcasses. In our study SBG and SC were superior to SBGS and TT for recovery of low numbers of various salmonella serotypes from carcass rinse fluid. These findings agree with those of Yamamoto et al. (*26*) and of Smyser et al. (*21*), who found SC to be excellent for detection of *S. typhimurium* from poultry feed and tissues.

SBGS or TT probably should not be used for the detection of salmonellae from broiler carcasses unless a preenrichment step is included or the broiler is in direct contact with the enrichment broth during incubation. Since additional time and labor are required for preenrichment, and since incubating the carcass would obviously be more expensive, direct enrichment of carcass rinsing fluid with SBG or SC would be the most practical way to detect salmonellae on non-frozen broiler carcasses.

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Stability of Sauerkraut Packaged in Plastic Bags[1]

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ABSTRACT

Commercially processed sauerkraut, packaged in plastic bags, was evaluated for product stability following storage at 2, 20, 32 C. When stored at 2 C in the presence of benzoic acid and potassium metabisulfite, the product was stable for more than 8 months, whereas at 20 and 32 C the shelf life was reduced to 20 and 13 weeks, respectively. The reductions in shelf life were due to development of excessive discoloration (browning) and objectionable flavor formation. These defects appear to be caused by chemical rather than microbiological actions. In addition to serving as effective antimicrobial agents, the chemical preservatives (presumably sulfur dioxide) showed protective effects in retarding losses of naturally-occurring ascorbic acid found in sauerkraut. Under similar temperatures of storage, fresh sauerkraut containing no chemical additives had shelf life values of 22, 1.5, and 0.75 weeks, respectively. When stored at 32 and 20 C, the major defects (swollen or broken bags) were attributed to the actions of yeasts. Storage at 2 C markedly arrested and reduced viable yeasts counts, thereby extending the shelf life of the bagged product.

Food products have been packaged in containers constructed from flexible plastic films for a number of years, and sauerkraut is no exception. Based upon production estimates, (Personal communication, W. R. Moore, National Kraut Packers Association, Inc., St. Charles, IL), more than 42 million lb. of "bagged kraut" were produced by the industry during the past year. Although sauerkraut packaged in plastic bags appears to enjoy a modest volume of sales, less than 14% of the kraut produced in the United States is distributed in flexible packages. This lower acceptance rate of the packaged product is undoubtedly due to the processing methods used to preserve the final product, since naturally fermented sauerkraut serves as the ingredient common to both the canned and bagged product.

The canned product attains commercial sterility by the "hot-fill" method (*2*), whereas preservation of the packaged kraut relies upon the chemical treatment afforded by the additives (benzoic or sorbic acid and potassium bisulfite). In using such contrasting processes for achieving preservation, it is assumed that the physical and chemical characteristics and the anticipated shelf life of the products will be dissimilar.

Although numerous factors may influence the shelf life of a highly acid food, recent studies by Wagner (*7*) show that sauerkraut packed in plain electrotin bodies was stable for 12 months, whereas fully enameled cans provided a minimum shelf life of 18 months. However, with packaged kraut the parameters determining product stability remain to be defined. Therefore, this report describes the effect of time and temperature of storage on the keeping quality of packaged sauerkraut.

MATERIALS AND METHODS

Sauerkraut, packaged in polyethylene-mylar pouches (2 lb. capacities), was prepared under commercial processing conditions. The commercial grade samples (201 bags) were comprised of fresh sauerkraut, brine, and preservatives (sodium benzoate and potassium bisulfite). The untreated product or fresh sauerkraut (201 bags) was of similar composition but devoid of chemical additives.

Upon arrival at the laboratory, the chemically treated and untreated samples were divided equally into three lots (67 bags per lot), placed in conventional shipping cartons, and stored in 2, 20, and 32 C constant temperature rooms.

Drained weight analysis showed that the average contents of the bag were comprised of 70% solids and 30% brine. Using this distribution ratio, well drained solids (140 g) and kraut brine (60 g) were removed aseptically from each bag and added to a blender containing 100 g of sterile distilled water. Following 3 min of homogenization, aliquots of the blendate were removed for chemical and microbiological analyses.

The lactic acid bacterial population was determined by the methods and medium described by Etchells (*1*), whereas the viable yeast counts were established by plating on Potato-Dextrose Agar, pH 3.5.

Total titratable acidity (expressed as lactic acid) and salt (expressed as sodium chloride) were determined by the methods previously described (*4*). Ascorbic acid and free sulfur dioxide were determined by iodiometric titrations (*3*).

During the first 6 weeks of storage, each bag was examined for physical intactness at weekly intervals and at bi-monthy and monthly periods thereafter. The latter schedules were adopted because of product stability and to conserve samples for prolonged testing purposes.

Microbiological and chemical analyses were done in triplicate at each designated test interval, i.e. bags were selected at random from each

[1]*Approved by the Director of the New York State Agricultural Experiment Station for publication as Journal Paper No. 3117.*

of the lots (treated and untreated) stored at each of the three selected temperatures.

The color, flavor, and textural characteristics were evaluated by persons knowledgeable with kraut grading procedures. The scoring methods and the maximum allowable score points (color:30, flavor: 30, and texture: 10) used for evaluating product quality were similar to those standards established for grading commercial kraut (*6*).

RESULTS AND DISCUSSION

Fresh sauerkraut, packaged without chemical preservatives (benzoate and bisulfite) and stored at 20 and 32 C, was extremely vulnerable to microbial spoilage. This spoilage was visible as evidenced by the pronounced distention of the bags and subsequent violent rupture of the flexible plastic film.

As shown in Fig. 1, more than half (34 of 67) of the bags incubated at 32 C ruptured within 18 h. The remaining samples underwent similar types of destruction at sporadic intervals throughout the 5-day storage period.

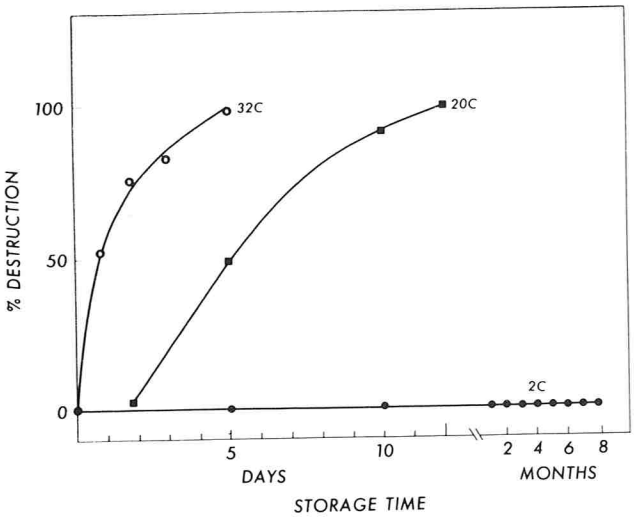

Figure 1. *Effect of temperature upon the burst rate of commercial sauerkraut packaged in plastic bags without chemical additives. Conditions: 67 bags were incubated at each of the respective temperatures. Initial temperature of product 12 C.*

As might be expected, bags stored at 20 C showed more reduced rates of spoilage than samples stored at 32 C. Under these conditions, simulating constant room temperature, the 50% destruction value was reached at the 5-day incubation interval, whereas an additional 7 days incubation were required to achieve complete destruction of all test samples.

A further reduction in storage temperature (2 C) decreased the incidence of spoilage most markedly; not a single bag showed a "Bloater" defect following 8 months of incubation.

These observations suggest that low temperature storage can serve as an alternative method for chemical additives; however, to achieve and maintain the control of reduced temperatures throughout the complete processing, distribution and consumer networks appears at the present time to be an idealistic rather than a practical solution to the preservation of bagged kraut.

It is well known that sauerkraut with its traditionally high salt and acid contents poses a hostile environment for many microorganisms; however, the fate and endurance of the naturally-occurring, acid-tolerant species in fresh kraut packaged in plastic bags and subsequently stored at refrigeration temperatures is less well defined. Therefore, the effects of such low-temperature storage (2 C) upon the viability of lactic acid bacteria and yeasts as well as the onset of undesirable sensory characteristics (color, texture, and flavor) during the 8-month storage term are shown in Fig. 2.

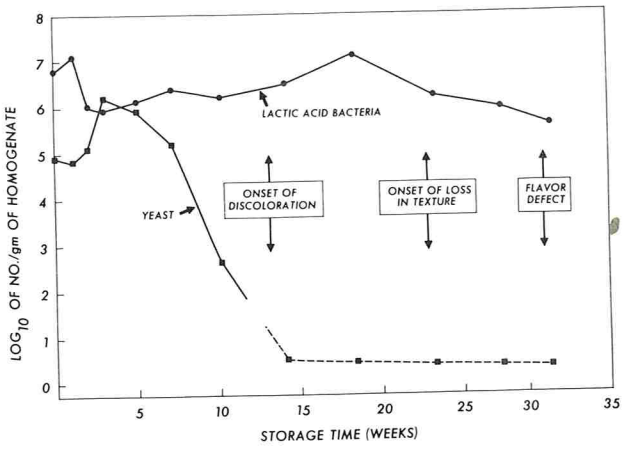

Figure 2. *Effects of low temperature storage (2 C) upon microbial viability and quality of sauerkraut packaged in plastic bags without chemical preservatives. ---- Estimated viable count.*

Based upon viable cell counts, the lactic acid bacterial population remained essentially unchanged (10^6 to 10^7 cells/g) throughout the 32-week incubation period. Since no attempts were made to differentiate the dominant species, the effects of low temperature storage upon the viabilities of heterofermentative and homofermentative lactic acid bacteria remain unknown. However, since the plastic bags showed no visible evidence of "swells" and the pH (3.40-3.62) and total titratable acid values (1.17-1.39%) of the product remained essentially unchanged throughout the course of this study, it appears that low temperature storage effectively arrested the major metabolic activities of the lactic acid bacteria. On the other hand, the yeast population displayed both growth and death characteristics during the above conditions of storage. For example, the viable yeast counts increased from 10^5 to 10^6 cells/g of product during the first 3 weeks of storage; however, following 14 weeks of incubation less than 10 cells/g of product were recovered.

Although the yeast population increased nearly 20-fold during the first 3 weeks of incubation, the bags showed no visual evidence of swelling. The failure to observe perceptible swelling of the plastic bags may be due to generation of CO_2 at a reduced rate and its subsequent diffusion through the flexible film. (The commercial grade polyethylene-mylar film used for packaging these sauerkraut samples does not provide a barrier totally

impermeable to gaseous diffusion).

Following 14 weeks of storage the yeast population declined to a level no longer detectable by the plate count method, i.e. 1.0-ml aliquots of sample yielded no viable cells when plated on PDA agar. Since methods for determining excessively low populations were not used, the maximal numbers of yeasts present remain unknown; however, the product was not devoid of viable yeasts following 20 weeks of storage at 2 C. This was demonstrated by removing four bags of product from low temperature storage and subsequently incubating them at 20 C. Following 21 days of incubation at the latter temperature each of the bags, previously showing no apparent abnormalities, developed perceptible "swell" characteristics and provided yeast counts in excess of 3×10^4 cells/ml.

Following 12 weeks of storage the untreated krauts began to develop a light pink color. Microscopic examination of the washed shreds indicated that this pink color was intimately associated with the shreds proper. Attempts to isolate pink yeasts or pigmented bacteria from the product resulted in consistent failure.

Following an additional 2 weeks of incubation, the discolored samples began to assume a buff or tan color characteristic which was accompanied by a loss in brilliance. This objectionable discoloration appears to be similar to that previously reported to occur in cabbage juice by Stamer et al. (5). Although color degradation was perceptible, the defect was minimal as the kraut scored 28 points (30 points, top quality) on the color scale used for grading canned sauerkraut.

The refrigerated product maintained excellent textural properties for more than 5 months. However, following 22 weeks of storage, the samples showed slight losses in crispness. Although the turgidity decreased throughout the remainder of the 32-week test period, the losses were not considered to be highly serious as the product received a score of 9 points (10 points, top quality) on the canned sauerkraut grading scale.

Flavor of krauts was judged to be of excellent quality following 7 months of storage. However, at the 8-month inspection interval, samples were judged to be highly unsatisfactory. The abrupt transition from an excellent to a sub-standard grade occurred without accompanying visible defects. The aroma of the product might best be described as "stale" with marked losses in pleasant bouquet, whereas the flavor was objectionably tart and accompanied by an extremely harsh, highly unpleasant, and lingering aftertaste.

The chemical additives, benzoic acid and sulfur dioxide or their salts, have been used for decades as preservatives of many foods and beverages. Although these compounds are frequently used for extending the shelf life of bagged kraut, their roles as antimycotic, antibacterial, and color-enhancing agents under various temperatures of storage are less well defined.

In examining the antimycotic properties of these chemicals, it was observed that yeasts were particularly vulnerable to these agents at each of the temperatures studied. For example, the initial viable yeast count, 9.4×10^4 cells/g, was reduced to less than 10 cells/g following 1 day of storage at 2, 20, and 32 C. Failure to recover viable yeasts throughout the course of the 8-month study period attests to the efficacy of these compounds as antimycotic agents for sauerkraut.

The effectiveness of these compounds as antibacterial agents appeared to be temperature dependent. For example, the population of lactic acid bacteria at the time of packaging was 4×10^6 cells/g; however, following 8 days of storage at 20 and 32 C, no viable cells were recovered. On the other hand, the samples stored at 2 C produced viable counts of 6.7×10^3 and 3.6×10^3 cells/g at the 8 and 14 day sampling periods respectively. However, no viable cells were recovered following 21 days and for the remainder of the study period.

Since the efficacies of the chemical additives upon microbial viability were measured as a function of two compounds (benzoate and SO_2), the effectiveness of each as a singular microcidal agent remains to be determined.

From a microbiological viewpoint, these data suggest that the currently recommended practice of immediatly placing the freshly packaged kraut under cooler conditions of storage or refrigeration has little merit or need. Packaging and storing the product at ambient temperatures (20 C or less) for several days provides a simple means for reducing microbial populations without adversely affecting product quality and significantly reducing shelf life.

This is not meant to imply that temperature and time of storage do not play vital roles in directing biochemical deteriorations, because kraut, as many foods treated with chemical additives, is vulnerable to non-microbial degradation. The inverse relationship between stability of color and temperature of storage was quite apparent, i.e. the first visual defect (browning) occurred with those samples stored for 14 weeks at 32 C. The intensity of this browning response became so pronounced following 16 weeks of storage that the samples were judged to be completely unacceptable to the consumer. Likewise, effects of reduced temperatures upon extending color quality were quite apparent in that the onset of similar degrees of discoloration required 29 weeks of incubation at 20 C, whereas no significant changes in color were detected with those samples stored at 2 C for 32 weeks.

The most objectionable sensory defect encountered in these shelf life studies was that of "off-flavor." Those samples stored for 16 weeks at 32 C developed highly aromatic, bitter, an medicinal flavor traits. These same undesirable flavor qualities were detected in those samples incubated at 20 C for 24 weeks; however, no flavor abnormalities were noted in those krauts held for 8 months at 2 C.

Of the sensory qualities evaluated, texture appeared to be the property least influenced by time-temperature relationships. Although undesirable flavor and color qualities dictated the time of sample rejection, all

samples held to their respective disposal dates possessed acceptable textural properties of firmness and crispness.

Since the sensory qualities (flavor, color, and texture) discussed above may singly or in concert establish final grade assignments to the product, the contributions of each factor and the effects of temperature upon the shelf life of sauerkraut are summarized in Table 1.

TABLE 1. *Anticipated shelf life of sauerkraut packaged in plastic bags.*[a]

Storage temperature (C)	Flavor		Color		Texture		Major defect		Maximum storage term	
	(–)	(+)	(–)	(+)	(–)	(+)	(–)	(+)	(–)	(+)
2	E/32	E/32	pink/10 tan/14	E/32	E/32	E/32	color	none	10	>32
20	—	E/19 P/22	—	E/22	—	E/22	gas	flavor	<1.5	20
32	—	E/13 VP/17	—	E/13 VP/17	—	E/22	gas	color & flavor	<0.75	13

[a]Polyethylene-mylar film; 2 lb. capacities. Experiment terminated following 32 weeks storage.
Numerical values expressed as weeks.
(–) No additives
(+) Additives (benzoic acid and sodium bisulfite)
E - Excellent; P - poor; VP - very poor qualities.

Since free SO_2 plays a vital role in producing and maintaining the desired color brightness in bagged kraut, the effect of temperature upon the fate of this highly reactive agent is shown in Fig. 3. As may be observed the SO_2 content was most rapidly depleted at 32 C, i.e. from 190 ppm (0.019%) to less than 10 ppm (0.001%) following 14 weeks of storage, whereas the SO_2 content of the samples stored at 2 C was never less than 140 ppm throughout the storage term. A comparison of the degradation rates of SO_2 in those samples stored at 32 and 20 C indicates that the former temperature produced a 50% loss in free SO_2 concentrations following 5 weeks of storage, whereas 10 weeks of incubation at 20 C were required to achieve a similar level of disappearance.

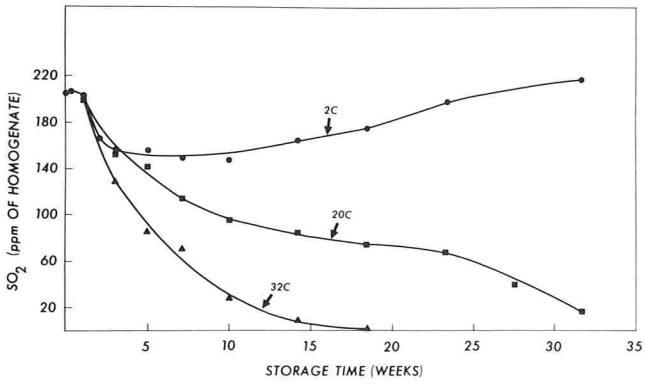

Figure 3. *Effects of time and temperature upon the levels of free SO_2 in sauerkraut packaged in plastic bags.*

Although the minimum levels of SO_2 required to maintain optimum color quality were not determined, it appears that discoloration was initiated at 32 C when the free SO_2 concentration was less than 20 ppm. (Estimated by comparing the time required for onset of discoloration, Table 1, to SO_2 levels obtained at a similar storage time, Fig. 3).

Since sauerkraut is a rich source of ascorbic acid, the effects of temperatures of storage and preservatives upon the lability of this naturally-occurring reductant were examined. As shown in Fig. 4, the ascorbic acid values (15 mg/100 ml brine) of those krauts stored at 2 C in the presence of benzoate and SO_2 remained essentially unchanged throughout the 32-week storage term. The effectiveness of refrigeration temperatures in extending the shelf life of vitamin C was very apparent in that storage at 20 and 32 C produced a 50% reduction in ascorbic acid content following storage periods of 12 and 3 weeks, respectively.

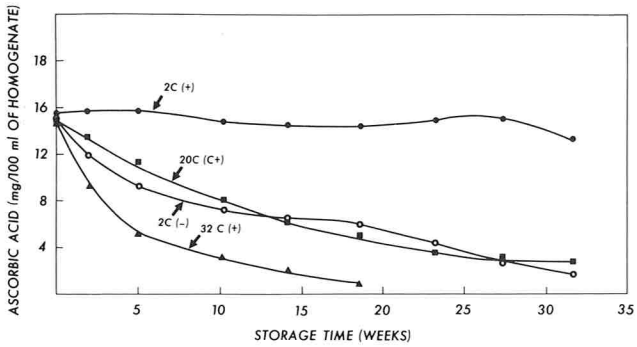

Figure 4. *Effects of preservatives and storage temperatures upon ascorbic acid content of sauerkraut packaged in plastic bags.* (+) *benzoate and bisulfite added,* (–) *no additives.*

The protective effects of the preservatives, benzoate and SO_2, on extending the shelf life of ascorbic acid were also noted. As shown in Fig. 4, the kraut samples stored at 2 C without preservatives suffered lossess of 7 mg/100 g of product within 10 weeks, whereas the treated samples showed no apparent losses following 32 weeks of storage. Although the specific roles of the individual additives were not examined in this study, it is assumed that SO_2 rather than benzoic acid functioned as the active protectant for ascorbic acid since the former is known to serve as an oxygen scavenger in many biological systems.

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Chemical Contaminants and Milk

Of all the foods on the marketplace, few are more carefully regulated and scrutinized for safety than milk. And few foods offer the outstanding nutrient profile; milk's nutrients are necessary for all age groups. But today's technology and increased use of various chemicals in the environment many occasionally lead to contamination of milk. Such accidental contamination is rare and potential danger to the public is minimal due to federal regulations. Nevertheless these dangers have been exaggerated through rumors and reports in the mass media.

PBB's: Polybrominated Biphenyls

PBB's are primarily used as a component of fire retardants. In 1973 there was a mix-up in Michigan and a batch of fire retardant was mistakenly added to animal food. The result was the condemnation and destruction of thousands of agricultural animals along with tons of milk, butter and cheese.

While no adverse effects could be detected in the general population, isolated cases of ill-effects were noted primarily by farmers who used the PBB contaminated animal food and chemical plant workers who were exposed to PBB's via non-dietary sources. Recent studies in Michigan have shown that PBB's are not readily taken up by plants or leached from the soil into water, thus contamination of animals and animal by-products via soil contamination appear unlikely.

Food and Drug Administration permits 0.3 parts per million (PPM), fat basis, PBB's in dairy foods, which provides a 100 fold margin of safety. While most consumers have heard of PBB's, only one percent of those surveyed in Michigan reported decreased milk purchases and consumption because of concern over milk contaminated with PBB's.

The PBB contamination in Michigan was the worst chemical accident in agricultural history. Although financial losses were great, there was no hazard to the consumer, thanks to strict regulation and observation.

PCB's: Polychlorinated Biphenyls

PCB's have a number of industrial applications as insulated agents in electrical equipment, in paints for silos and other metal structures, as lubricants in paper container manufacture, and as an ingredient in pesticides. Over the years, PCB's have been found as contaminants in various agricultural commodities, including dairy foods.

In 1970, the U.S. Department of Agriculture banned PCB's in pesticides. Voluntary action by industry has been effective in decreasing the use of PCB's even more. Such action has led to steadily declining levels of contamination in foods—far below FDA permitted levels. Ironically, since contamination has been proved avoidable, FDA has proposed lowering permitted levels of PCB contamination in dairy foods from 2.5 ppm to 1.5 ppm (fat basis). Today, game fish are the primary source of PCB's in the diet.

PCP's: Pentachlorophenol

PCP is an industrial chemical primarily used by the wood preserving industry and as an insecticide against termites in the south. During the production of PCP the final product is often contaminated with another class of chemical compounds-dioxins-which are a highly toxic class of chemicals. Present available data indicated that the quantities of the dioxins present in PCP production do not constitute a danger to man or the environment.

Contamination of milk with PCP can be oral (ingestion by dairy cows) or tactile (absorbtion through the skin of dairy cows). While exposure to PCP is minimal when good manufacturing practices are followed and manufacturers directions are followed when used in preserving wood, the American Wood Preserving Institute recommends that PCP's not be used in closed environments or on wood used for animal food storage or feeding.

Recently six Michigan dairy herds were quarantined due to suspected contamination of these animals with PCP or dioxins. Food and Drug Administration examination of milk from these herds proved negative. Clearly PCP's and its co-contaminant dioxins may have toxic effects. However, if used properly potential contamination of dairy herds and milk products are negligible.

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Modified Vogel and Johnson Agar for *Staphylococcus aureus*

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ABSTRACT

The formula for a modified Vogel and Johnson Agar is presented. In addition to the ingredients found in Vogel and Johnson Agar, the new medium contains, per liter: 5 g of beef extract, 2 g of deoxyribonucleic acid, 2 g of phosphatidyl choline and 780 units of catalase spread on the plates before inoculation. This new medium is as effective as Baird-Parker Agar in enumeration of stressed *Staphylococcus aureus*, and in enumeration of staphylococci from naturally contaminated processed food samples.

Staphylococcal selective media have been developed which inhibit the growth of other bacteria while allowing multiplication of *Staphylococcus aureus*. Optimum enumeration, however, can seldom be achieved using many of these selective media. Some of the selective media have used ingredients such as sodium chloride and mannitol (*7,8,9*), tellurite and lithium chloride (*18*), or tellurite, lithium chloride and glycine (*20,23,24*). Addition of egg yolk to Staphylococcus 110 agar (*6,13*) and to tellurite-containing agars (*3,4,10,16*) has also been suggested. Baird-Parker's Egg-yolk-Tellurite-Pyruvate Agar (BP) is currently recommended for use in routine enumeration of *S. aureus* from foods (*22*). Difficulties have been reported in its use, especially when used with milk products (*10,21*).

Several factors must be considered in the development of a selective medium for enumeration of staphylococci from a food product. Often a significant percentage of the organisms have suffered lesions during sublethal stress (heating, drying, freezing, freeze-drying) and are unable to develop into colonies using conventional media (*1,5,12,15*). Catalase has been shown to be useful as an additive (*5,11,19*) through hydrogen peroxide (H_2O_2) degradation. Increased sensitivity of *S. aureus* cells to H_2O_2 has been shown (*19*), as well as loss of media productivity, presumably as a result of H_2O_2 accumulation (*11,19*). This report presents the results of experiments designed to modify Vogel and Johnson Agar to develop a medium which would give enumeration equal to that of BP, and to provide additional information about the resulting colonies (nuclease test).

MATERIALS AND METHODS

Media

Baird-Parker Agar (BP), Vogel and Johnson Agar (VJ) and phosphatidyl choline-Vogel and Johnson Agar (PCVJ) were prepared using the ingredients listed in Table 1. Phosphatidyl choline and bovine catalase (EC 1.11.1.6) were obtained from Sigma Chemical Company (St. Louis, Missouri) and 1% Chapman-Tellurite, 50% egg yolk, beef extract and deoxyribonucleic acid were purchased from Difco (Detroit, Michigan). All of the appropriate components except the catalase, egg yolk and tellurite were suspended in 1 liter of water and autoclaved (15 min, 121 C, 15 psig). After tempering (48 C), tellurite and, where appropriate, egg yolk was added. Plates were then poured and stored overnight at room temperature. Approximately 1 h before use, 780 units of filter-sterilized catalase were spread on the top of each PCVJ plate.

TABLE 1. *Media composition.*

Component	BP (g/1) pH 7.0	PCVJ (g/l) pH 7.2	VJ (g/l) pH 7.2
Trypticase peptone	10	10	10
Yeast extract	1	5	5
Beef extract	5	5	—
LiCl	5	5	5
Glycine	12	10	10
Sodium pyruvate	10	—	—
Potassium phosphate dibasic	—	5	5
Mannitol	—	10	10
Phenol Red	—	0.025	0.025
Phosphatidyl choline	—	2	—
DNA	—	2	—
Agar	18	16	16
Tellurite (1%)	10 ml	10 ml	20 ml
Egg yolk (50%)	53 ml	—	—
Catalase	—	780 units per plate	—

Preparation of Staphylococcus aureus

Strains of *S. aureus* MF-31, 1889b, S-6, 181, 210, and 196E were grown in 200 ml of Tryptic Soy Broth (TSB, Difco) for 12 h as described by Iandolo and Ordal (*15*). Five frozen stocks of clinical isolates (#1,3,5, 7,8) obtained from anonymous sources, were grown in the same manner. One hundred milliliters of culture was then centrifuged for 10 min at 10,000 × g, the supernatant fluid discarded and the pellet suspended in 100 ml of 0.1 M phosphate buffer, pH 7.2. Samples to be heat-injured were diluted 1:10 in preheated phosphate buffer, pH 7.2, and stirred at 52 C for 20 min. Dilutions were made using 0.1%

peptone. Spread-plates were incubated at 35 C for 48 h before counting.

Naturally contaminated whey cream samples and low-temperature rendered ground beef samples were provided by anonymous sources. Low-temperature rendered ground beef had been heated to no more than 49 C to remove the fat. Whey cream samples were examined by thawing 5 g of the frozen cream, plating the first dilution directly, then diluting 1:10 (w/v) in 0.1% peptone for subsequent platings. Thawed low-temperature rendered ground beef samples (25 g each) were blended for 2 min in a Waring blender and diluted 1:10 (w/v) in 0.1% peptone. Appropriate dilutions were made before plating. Selected colonies isolated from each of the naturally contaminated food products were further confirmed as *S. aureus* by determining the presence of coagulase and heat-stable deoxyribonuclease activity (*22*).

RESULTS AND DISCUSSION

Baird-Parker Agar has been shown to be superior to VJ Agar for enumeration of stressed cells (*5*). Therefore, it was first necessary to determine what component of BP, other than sodium pyruvate, was responsible for the increased productivity of this medium. Egg yolk, which is composed of 48% water, 17% protein and 35% lipid (*17*), was found to be one necessary component (data not presented). Since lecithin (phosphatidyl choline) comprises approximately 25% of the total lipids, it was proposed that phosphatidyl choline could account for the increased productivity. Experiments determined that phosphatidyl choline, when added to VJ agar supplemented with DNA and beef extract and spread with catalase (phosphatidyl choline-Vogel and Johnson Agar-PCVJ agar) could increase enumeration of stressed cells. Use of levels similar to those of a whole egg yolk used in BP (2×10^{-3} g/ml medium) gave maximal enumeration of heat-injured organisms (data not presented).

Comparisons of VJ, PCVJ and BP agars for enumeration of heat-stressed *S. aureus* were made. Table 2 shows the results obtained using six laboratory strains. Heated cells of all strains plated on Tryptic Soy Agar with added 7% NaCl demonstrated increased sensitivity to this high-salt medium (data not presented). In five of the six strains, enumeration on PCVJ was statistically equal to that on BP. Strain 196E showed statistically higher counts on PCVJ than on BP. Colonies on both media were black and convex, with PCVJ being somewhat easier to count, because of the transparent nature of the medium and the yellow clearing zones around the colonies. Testing for deoxyribonuclease activity was done on PCVJ plates by adding several milliliters of 0.1 N HCl to the surface of the plate. Zones of clearing, which formed around the colonies usually in less than 2 min, provided confirmation of deoxyribonuclease activity.

Heat-stressed clinical isolates of *S. aureus* (Table 3) showed similar results. Four of the five isolates gave the same level of enumeration on both BP and PCVJ while one isolate gave higher counts on PCVJ. All five isolates were confirmed for deoxyribonclease activity, as described above. Two naturally contaminated food products were also used to compare the two media. They were low-temperature rendered ground beef (Table 4) and frozen whey cream samples (Table 5). The low-temperature rendered ground beef samples showed

TABLE 3. *Enumeration of heat-stressed*[a] Staphylococcus aureus *clinical isolates.*

Isolate number	CFU/ml[b] on BP	CFU/ml[b] on PCVJ	%BP[c]
1	4.0×10^9	5.1×10^9	128
3	1.6×10^9	1.4×10^9	88
5	2.2×10^9	2.4×10^9	109
7	2.3×10^9	2.9×10^9	126
8	1.7×10^6	4.7×10^6	275

[a]Cells were heated in 100 mM potassium phosphate buffer (pH 7.2) at 52 C for 20 min.
[b]Average of two separate trials, three plates per dilution per medium per trial.
[c]Percent calculated using the count on BP as 100.

TABLE 4. *Enumeration of* Staphylococcus aureus *from naturally contaminated low-temperature rendered ground beef.*

Sample number[a]	CFU/g on BP	CFU/g on PCVJ	%BP[b]
1	1.3×10^3	2.4×10^3	184
2	3.1×10^3	2.1×10^3	68
3	1.1×10^3	1.1×10^3	100
4	2.0×10^4	3.3×10^4	165
5	2.5×10^4	2.3×10^4	92
6	2.0×10^3	1.8×10^3	90
7	3.1×10^3	3.2×10^3	103
8	1.2×10^4	1.0×10^4	83

[a]Samples 1-5 from trial a, samples 6-8 from trial b.
[b]Percent calculated using the count on BP as 100.

TABLE 2. *Comparison of the enumeration of six heat-stressed*[a] *strains of* Staphylococcus aureus *on three test media with Baird-Parker Agar.*

S. aureus strain	Average CFU/ml on VJ	Average CFU/ml on VJ + C[b]	Sample size[c]	Average CFU/ml on BP (100%)	Average CFU/ml on PCVJ	Sample size[d]	$t_{calculated}$ ($t_{critical}$[e])
MF-31	2.86×10^9(60.5%)[f]	3.56×10^9(75.2%)[f]	4	4.73×10^9	4.80×10^9(101%)[f]	7	− 0.3809 (2.447)
210	1.01×10^9(28.1%)	9.84×10^8(27.4%)	4	3.59×10^9	2.42×10^9(67.4%)	7	1.5352 (2.447)
196E	3.75×10^7(21.3%)	1.60×10^8(91.0%)	4	1.76×10^8	3.20×10^8(182%)	6	− 2.7885 (2.571)[g]
S-6	1.23×10^8(33.8%)	1.43×10^9(39.4%)	3	3.64×10^8	3.96×10^8(109%)	6	− 1.0483 (2.571)
181	5.94×10^8(18.4%)	7.95×10^8(24.6%)	2	3.23×10^9	3.37×10^9(104%)	6	− 0.5122 (2.571)
1889b	1.01×10^8(2.4%)	6.30×10^7(1.5%)	1	4.20×10^9	4.25×10^9(102%)	4	− 0.3682 (3.182)

[a]Cells were heated in 100 mM potassium phosphate buffer (pH 7.2) at 52 C for 20 min.
[b]C-Catalase added at 780 units per plate.
[c]Number of separate trials, three plates per dilution per medium per trial, comparing VJ and VJ + C with BP.
[d]Number of separate trials, three plates per dilution per medium per trial, comparing PCVJ with BP.
[e]Paired *t*-test with n-1 degrees of freedom, comparing PCVJ with BP, calculated as described by Huntsberger and Billingsley (*14*).
[f]Percent calculated using the count on BP as 100.
[g]Statistically higher counts on PCVJ.

TABLE 5. *Enumeration of* Staphylococcus aureus *from naturally contaminated whey cream.*

Sample number	CFU/g on BP	CFU/g on PCVJ	%BP[a]
1	1.3×10^3	6.4×10^3	492
2	1.8×10^3	9.2×10^3	511
3	1.2×10^3	4.9×10^3	408
4	4.5×10^3	9.4×10^3	209
5	2.7×10^3	6.8×10^3	252
6	2.1×10^3	5.7×10^3	271
7	2.4×10^3	4.9×10^3	204
8	1.5×10^3	5.8×10^3	387
9	1.9×10^3	4.4×10^3	232

[a]Percent calculated using the count on BP as 100.

equal enumeration on the two media for one sample, higher counts on PCVJ for three samples, and better enumeration on BP for four samples. The average counts on PCVJ were 110% of those on BP for all eight samples. All nine whey cream samples exhibited better enumeration on PCVJ than on BP. With all naturally contaminated samples, PCVJ plates were easier to count than BP, especially when the inoculum was undiluted, as very few of the colonies cleared the egg yolk on the BP plates. The added differential character of deoxyribonuclease activity also made the plates easier to count after addition of HCl. All *S. aureus* colonies having deoxyribonuclease activity from these samples showed thermostable deoxyribonuclease activity.

This paper presents an alternative medium to BP for enumeration of *S. aureus.* Our results indicate that stressed organisms, as well as those found in naturally contaminated foods, can be enumerated with equal or improved efficiency on PCVJ agar. Tests with possible contaminating organisms which might be able to grow and give the same characteristics on PCVJ and are not *S. aureus* have not been done. Baird-Parker agar was recommended for *S. aureus* enumeration after a study of various previously used media (*2*). We have therefore limited these studies to comparisons of BP and PCVJ. It is possible that better enumeration of *S. aureus* could be achieved on a non-selective medium such as plate count agar. However, such procedures are not recommended for enumeration of *S. aureus* from foods. These results suggest that those interested in detection of *S. aureus* may want to undertake further development and testing of this medium.

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Thermal Injury and Recovery of *Bacillus cereus*

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ABSTRACT

A system to measure thermal injury to vegetative cells of *Bacillus cereus* B4ac was developed. After heating in 0.1 M potassium phosphate buffer, pH 6.0 for 5 min at 47 C, cells became both pH-and NaCl-sensitive. The cell population showed decreased viability at pH values less than 6.3 or greater than 7.3, and at concentrations of 2% NaCl or greater in plating media. Cells did not become sensitive to polymyxin B sulfate and/or phenol red at concentrations in which these substances are present in media routinely used to enumerate *B. cereus* in foods. Injury was normally detected as differential plating ability on Plate Count Agar (PCA) vs. PCA plus 2.5% NaCl. Injured cells could partially recover in 0.1% peptone, Brain Heart Infusion broth (pH 7.3), a mixture of 20 amino acids (10 or 50 μg/ml each), 1% glucose, and 0.1% peptone plus a protein synthesis inhibitor (chloramphenicol, 2.5 μg/ml) or 0.1% peptone plus a DNA replication inhibitor (nalidixic acid, 5 μg/ml). Cells recovered equally well at 20, 32 and 37 C. Recovery did not occur in 0.1% peptone plus an RNA synthesis inhibitor (rifampicin, 7.5 ng/ml) or in 0.1 M potassium phosphate buffer, pH 6.0 or 7.0.

There have been numerous studies on thermal injury (for a recent review see ref. *20*) and recovery (for a recent review see ref. *21*) of many microorganisms. Sublethal heating alters the cytoplasmic membrane (*1,9,11*) leading to leakage of various substances into the heating menstruum and increased sensitivity to various selective inhibitors such as NaCl (*9*), polymyxin (*8, 13*) and dyes such as methylene blue (*4*) to which the uninjured organism is resistant. There is also damage in some instances to ribosomal RNA (*2,13,18,19*), altered nutritional requirements (*2,3*), and damage to DNA (*6, 7*).

To date there have been no studies on thermal injury to *Bacillus cereus*, and few on the causes of thermal death in this organism (*5,16,17*). *B. cereus* has been implicated as a cause of foodborne disease (*5*). The usual procedure for isolation of the organism from suspect food involves use of a selective and differential agar medium [either KG agar (*10*) or MYP agar (*14*)] to enumerate *B. cereus* in the presence of other food microorganisms. Both of these media contain polymyxin B and phenol red. We began this study to determine if thermally injured *B. cereus* became sensitive to these components of the media normally used for their detection. In addition, we wished to determine conditions under which thermal injury and recovery would occur, and to obtain some information concerning the metabolic activities necessary for recovery.

MATERIALS AND METHODS

Growth and culture conditions

Stock cultures of *B. cereus* B4ac (from D. A. A. Mossel, Utrecht, The Netherlands), originally isolated from a food poisoning outbreak, were maintained on nutrient agar (NA) slants at room temperature. Overnight cultures incubated under static conditions at 32 C in 10 ml of Brain Heart Infusion broth (BHI) were started daily for use the next day. Three ml of the overnight culture were transferred to a 125-ml Erlenmeyer flask containing 27 ml of BHI (pretempered to 32 C) and incubated for 4 h on a New Brunswick reciprocal shaker (80 strokes/min) at 32 C. This procedure yielded a stationary phase culture with a low background of spores (usually less than 1 spore/10^6 vegetative cells).

Media and chemicals

Peptone, yeast extract, phenol red, Colbeck egg yolk enrichment, agar, Plate Count agar (PCA), BHI, Brain Heart Infusion agar (BHIA) and NA were all obtained from Difco, Detroit, Mich. KG agar (*10*) was prepared as follows. The basal medium was as described in (*10*), with the agar concentration reduced to 1.5%. To 900 ml of autoclaved basal medium, tempered to 50 C, were added 200 ml of Colbeck egg yolk enrichment (also at 50 C), and sterile polymyxin B sulfate to give a final concentration of 10 μg/ml. The normal pH of KG is 6.6-6.8. If another pH value was desired, the pH of the basal medium was adjusted before autoclaving. Plates were poured, allowed to harden and stored at 4 C until use (no longer than 1 week). The final pH was determined with a Markson 1207 contact electrode.

Polymyxin B sulfate was obtained from Pfizer (New York, NY) or Sigma (St. Louis, MO). Solutions of all of the above except rifampicin were prepared in glass-distilled H_2O and filtered through a 0.22-μ Millipore filter before use. Rifampicin was dissolved in absolute methanol, the solution diluted to 20% methanol with glass-distilled H_2O and filtered as described above. All rifampicin solutions were stored in foil-wrapped tubes to protect them from exposure to light. All of the above solutions were stored at 4 C and all but polymyxin B sulfate prepared freshly each week. Catalase (2500 units/mg) and sodium pyruvate were also obtained from Sigma.

Injury procedure

Cells grown as previously described were harvested by centrifugation in a Sorvall RC2B centrifuge at 9000 g for 10 min at 0-2 C. The supernatant fluid was discarded and the pellet resuspended in 30 ml of sterile 0.1 M potassium phosphate buffer (PB), pH 6.0 and recentrifuged. The supernatant fluid was discarded and this pellet was then resuspended in 10 ml of PB. Cell suspensions prepared in this manner contained 1-3 × 10^9 cells/ml.

Three ml of this suspension were then inoculated into 27 ml of prewarmed PB (47 C) in a 125-ml Erlenmeyer flask and incubated in a New Brunswick rotary waterbath shaker at 120 rpm. The cells were heated for the necessary time (usually 5-10 min) to give the desired level of injury.

Detection of injury

Samples were removed at intervals from the heated suspension, diluted in 0.1% peptone and 0.1-ml samples were spread on PCA and PCA plus 2.5% NaCl (PCAS). In some experiments 1% sodium pyruvate or a solution of catalase was added to the PCAS plates. In other experiments KG (pH∼6.8) and KG plates of higher or lower pH values were used. Uninjured cells grow equally well on all of the above media. Injured cells will grow only on PCA or KG (pH∼6.8). All plates were incubated overnight at 32 C.

Recovery conditions

One-ml aliquots of the heated suspension were diluted after the desired heating time into 9 ml of various unagitated media at different test temperatures to determine the conditions under which recovery would occur. Since the cells recover well in 0.1% peptone, this was used as the control medium in all recovery experiments. Recovery was assayed by diluting the inoculated recovery medium into 0.1% peptone before plating on PCA and PCAS, and following the increase in colony forming units on PCAS. In experiments involving metabolic inhibitors in the recovery medium, inhibitors were used at the minimum inhibitory dose for *B. cereus* B4ac as determined in this laboratory (unpublished data). All dilutions and platings were done as rapidly as possible to minimize any recovery that might occur in the peptone diluent. Since our primary interest was in determining whether or not recovery could occur, and not when it was complete, the injured cells were followed for 2 h for reasons of convenience. After 2 h approximately 10% of the cells had recovered, and the empirical presence or absence of recovery could be determined.

RESULTS

Injury and recovery

When vegetative cells of *B. cereus* B4ac were heated in PB, pH 6.0 at 47 C they became sensitive to increasing concentrations of NaCl added to PCA, (Fig. 1) while there was little change in the colony count on PCA alone (up to 7 min). The difference in counts between PCA and PCAS gives an estimate of the degree of thermal injury to the cell population. There was measurable injury by 3 min and by 10 min significant injury had occurred, as well as some degree of cell death.

If the injured cell population was diluted into 0.1% peptone and incubated for 2 h, the sensitivity to PCAS decreased as recovery occurred. Recovery occurred equally well at 20 C and 37 C (Fig. 2) as well as at 32 C (unpublished data). We would have expected a temperature effect, but the data reproducibly show none.

Recovery also occurred well in a mixture of 20 amino acids, (alanine, arginine, asparagine, aspartic acid, cysteine, glutamic acid, glutamine, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, trytophan, tyrosine, valine),

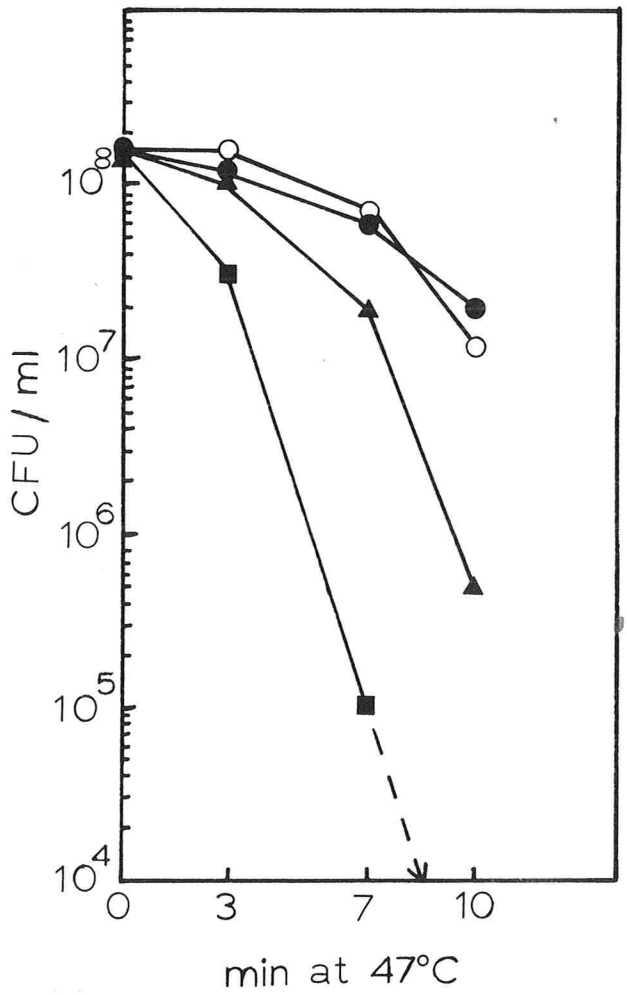

Figure 1. *Effect of heating* B. cereus *B4ac at 47 C in PB, pH 6.0, as measured by colony forming units (CFU)/ml detected by plating on PCA (0), PCA + 1% NaCl (●), PCA + 2% NaCl (▲), PCA + 3% NaCl (■).*

(10 or 50 μg/ml each), the amino acid mixture plus 1% glucose, amino acids plus purines and pyrimidines (adenine, thymine, guanine, cytosine and uracil at 10 μg/ml each), and 1% glucose alone. Recovery occurred most efficiently in 0.1% peptone or BHI. In the other media less recovery occurred than in BHI or 0.1% peptone, but there was no significant difference among these other media. The data for recovery in the amino acid mixture (10 μg/ml each) and in 1% glucose alone are shown in Fig. 3. No recovery occurred in PB, pH 6.0 or pH 7.0.

Recovery in the presence of metabolic inhibitors

After injury, cells were diluted into 0.1% peptone containing a protein synthesis inhibitor (chloramphenicol, 2.5 μg/ml), a DNA replication inhibitor (nalidixic acid, 5 μg/ml) or an RNA synthesis inhibitor (rifampicin, 7.5 ng/ml). Experiments were done at 20 and 37 C, with no significant differences in the results. Recovery occurred normally in the presence of either chloramphenicol or nalidixic acid, but not in the presence of rifampicin (Fig. 4). Recovery, therefore, requires RNA

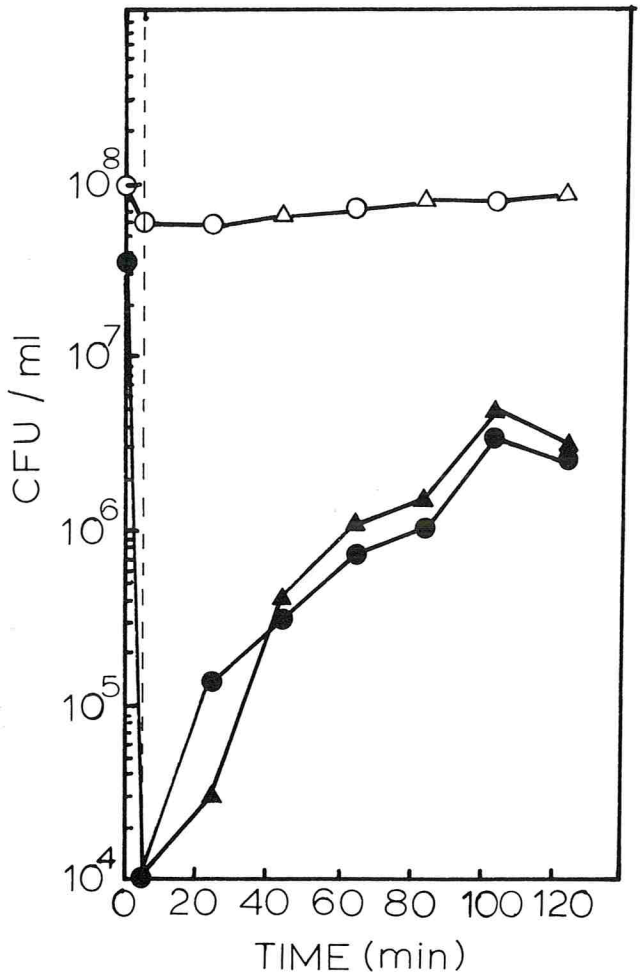

Figure 2. *Effect of incubation temperature on recovery of* B. cereus *B4ac on PCA* (0.1% *peptone at 20 C*) (0), *PCAS* (0.1% *peptone at 20 C*) (●), *PCA* (0.1% *peptone at 37 C*) (△), *PCAS* (0.1% *peptone at 37 C*) (▲). *The dotted line indicates the time of transfer from 47 C to recovery media.*

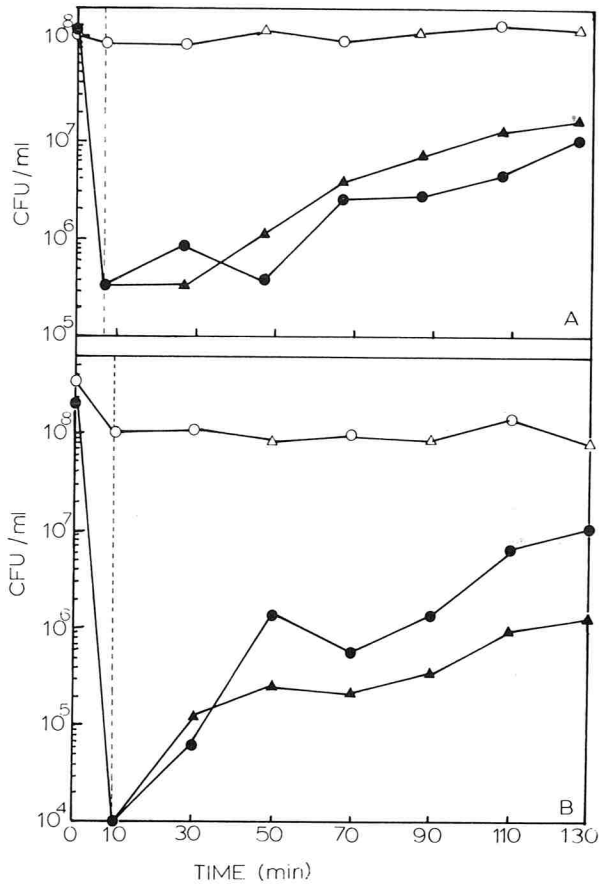

Figure 3. *Recovery of injured* B. cereus *B4ac as a function of medium composition at 20 C. The dotted line indicates the time of transfer from 47 C to recovery media. A. PCA* (0.1% *peptone*) (0), *PCAS* (0.1% *peptone*) (●), *PCA* (1% *glucose*) (△), *PCAS* (1% *glucose*) (▲). *B. PCA* (0.1% *peptone*) (0), *PCAS* (0.1% *peptone*) (●), *PCA* (*20 amino acids, 10 μg/ml each*) (△), *PCAS* (*20 amino acids, 10 μg/ml each*) (▲).

synthesis, but not protein synthesis or DNA replication. It has been reported for several other organisms (*20*) that thermally injured cells sustain ribosomal RNA damage, and that RNA synthesis is necessary for recovery from this injury.

Protection from injury

It has also been reported for some other organisms that thermally injured cells are protected from enhanced sensitivity to injurious substances such as NaCl by incubation in or plating on media containing 1% sodium pyruvate (*8*) or plating on media containing catalase (*12*). This is not true for *B. cereus* B4ac (Fig. 5). Neither incubation in or plating on media containing 1% sodium pyruvate nor use of plates spread with 0.1 or 0.2 ml of 0.02% (w/v) catalase had any protective effect for thermally injured cells on PCAS.

Injury on KG agar

We have also shown that thermally injured cells were extremely sensitive to pH changes in KG agar, but were readily able to grow on KG agar at its normal pH (6.8), and MYP agar (pH 7.0). Injured cells recovered and formed colonies on PCA (pH 7.0) and NA (pH 6.8), but not on BHIA (pH 7.4) (Fig. 6). The injured cells were sensitive to KG at pH values < 6.3 or > 7.3. At pH values within this range, however, thermally injured cells were not sensitive to the polymyxin B sulfate (10 μg/ml) or phenol red (0.0025%) in KG or MYP.

DISCUSSION

These experiments show that vegetative cells of *B. cereus* B4ac, known to cause foodborne illness, are sensitive to thermal injury. This manifests itself as sensitivity to increased levels of NaCl or to change in pH (< 6.3 or > 7.3) of the plating media used to enumerate the injured cells. The standard media used for enumeration of *B. cereus* from foods (KG and MYP), even though they contain both polymyxin B sulfate and phenol red, are not toxic to thermally injured cells. However, we wish to emphasize that whichever medium is to be used for enumeration of *B. cereus* that may have been thermally stressed, care must be taken to ensure that the pH is > 6.3 and < 7.3.

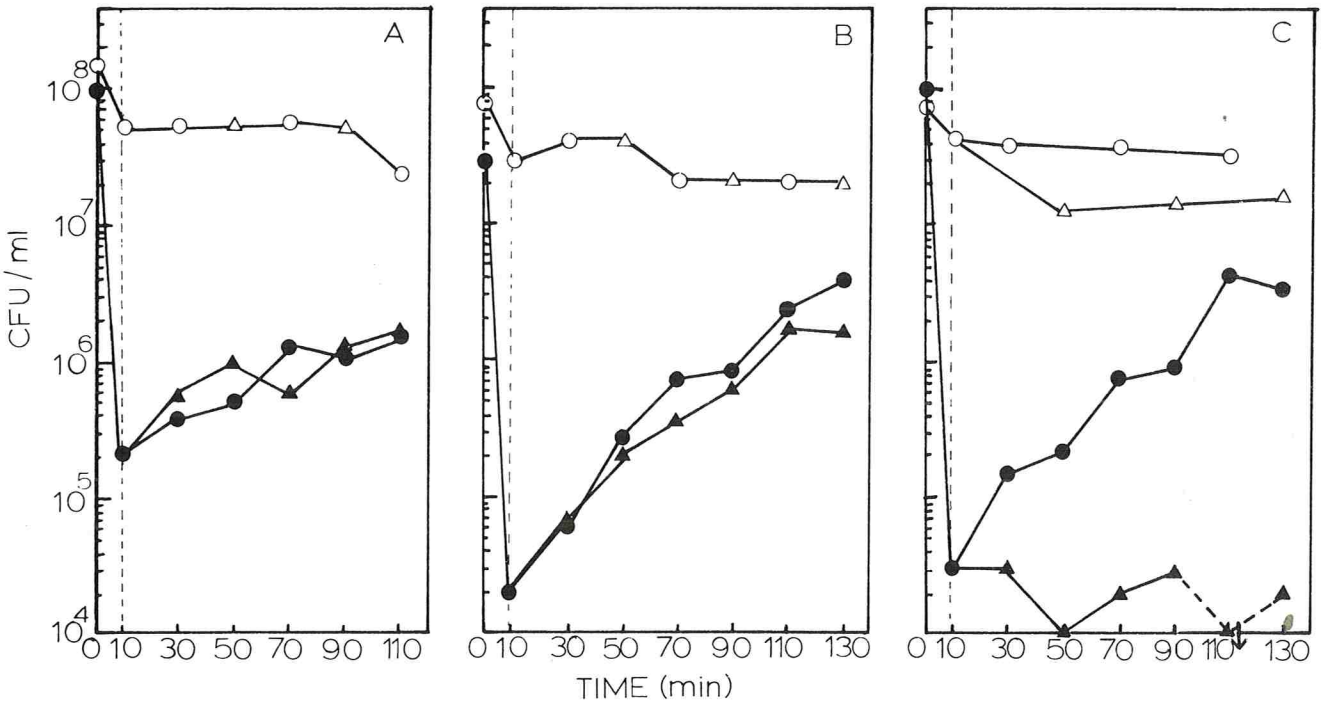

Figure 4. *Recovery of* B. cereus *B4ac in the presence of metabolic inhibitors in 0.1% peptone. The dotted line indicates time of transfer to recovery media. A. PCA (no inhibitor)* (0), *PCAS (no inhibitor)* (●), *PCA (chloramphenicol, 2.5μg/ml)* (△), *PCAS (chloramphenicol, 2.5μg/ml)* (▲), *all at 20 C. B. PCA (no inhibitor)* (0), *PCAS (no inhibitor)* (●), *PCA (nalidixic acid, 5μg/ml)* (△), *PCAS (nalidixic acid, 5μg/ml)* (▲), *all at 20 C. C. PCA (no inhibitor)* (0), *PCAS (no inhibitor)* (●), *PCA (rifampicin, 7.5 ng/ml)* (△), *PCAS (rifampicin, 7.5 ng/ml)* (▲), *all at 37 C.*

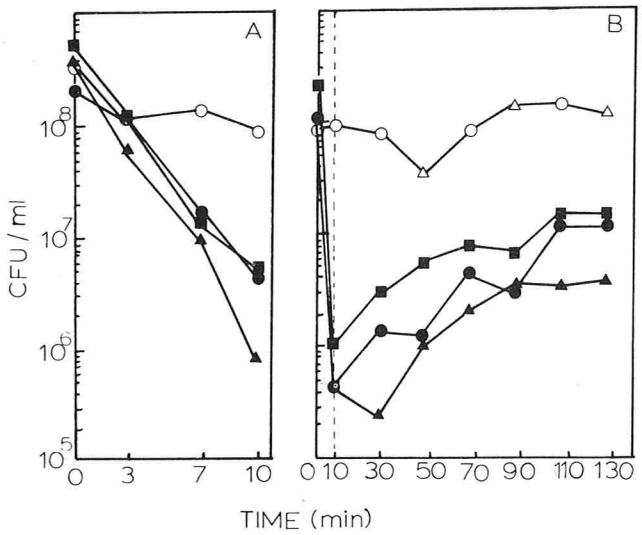

Figure 5. *Injury and recovery of* B. cereus *B4ac in the presence of catalase or pyruvate at 20 C. A. PCA* (0), *PCAS* (●), *PCAS + 1% pyruvate* (▲), *PCAS + 0.2 ml of 0.02% catalase* (■). *B. The dotted line indicates time of transfer to recovery media. PCA (0.1% peptone)* (0), *PCAS (0.2% peptone)* (●). *PCA (0.1% peptone plus 1% pyruvate)* (△), *PCAS (0.1% peptone plus 1% pyruvate)* (▲), *PCAS + 0.1 ml of 0.02% catalase (0.1% peptone)* (■).

Reparable injury in *B. cereus* seems to be RNA related, since rifampicin interfered with recovery. Neither DNA replication nor protein synthesis is required for recovery, since nalidixic acid and chloramphenicol did not interfere with this process. This result is in agreement with studies on other organisms (tabulated in ref. *20*). All of the thermally injured organisms tested (*Salmonella typhimurium, Staphylococcus aureus, Streptococcus faecalis, Pseudomonas fluorescens, Bacillus subtilis* and *Clostridium perfringens*) for a requirement for RNA synthesis to recover from injury did indeed require it. *S. typhimurium* and *C. perfringens* also require protein synthesis to recover from thermal injury. In addition *S. typhimurium* and *Escherichia coli* require DNA repair to recover from thermal injury. The other organisms listed were not tested for a requirement for DNA repair, and *E. coli* was not tested for RNA or protein synthesis requirements.

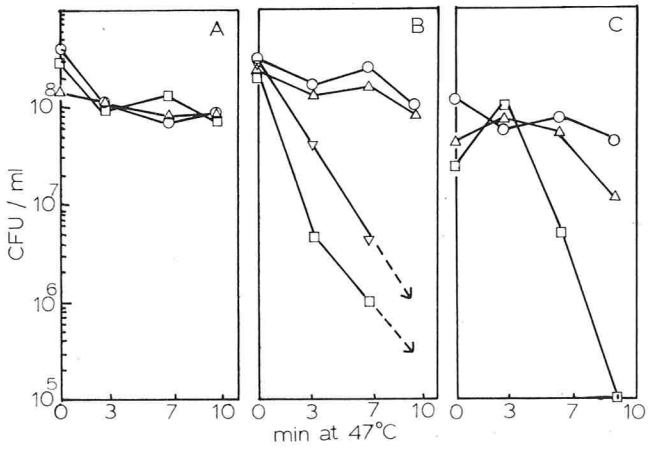

Figure 6. *Effect on plating media on the survival of thermally injured* B. cereus. *B4ac. A. PCA* (0), *NA* (△), *KG* (□). *B. KG (pH 6.8)* (0), *KG (pH 7.1)* (△), *KG (pH 7.7)* (□), *BHI* (▽). *C. KG (pH 6.8)* (0), *KG (pH 6.3)* (△), *KG (pH 5.6)* (□).

The phenomenon of "minimal medium recovery" has been observed for thermally injured cells (*6, 7, 22*). As the phenomenon was originally described (*6, 7*), cultures of *S. typhimurium* LT2 grown on a nutritionally poor medium were more sensitive to a nutritionally rich medium than to a nutritionally poor medium after thermal injury, while cells grown on a nutritionally rich medium were equally sensitive to both rich and poor media. Wilson and Davies (*22*) have done further experiments on *S. senftenberg* 4969, observing sensitivity to rich media of cells grown on either rich or poor media. We have done no experiments with *B. cereus* grown on poor media, but for thermally injured cells of *B. cereus* B4ac grown on BHI we saw equally good recovery in both a rich medium (BHI) and much poorer media (0.1% peptone, 1% glucose or a mixture of amino acids). "Minimal media recovery" therefore does not seem to be a factor in this system, at least for cells grown on a rich medium. Unexpectedly, temperature does not seem to be a major factor in recovery, since recovery occurred equally well at 20, 32 and 37 C. This is not true for *S. aureus* (*9*), for which increasing the temperature from 20 to 37 C increased the percent recovery from 34 to 80% in 2 h.

It has been reported for several organisms subjected to thermal stress (*S. aureus, S. typhimurium, P. fluorescens* and *E. coli*) that catalase (*12*) and pyruvate (*8, 12*) increase viable counts on media toxic for injured cells. Both of these substances are presumed to act by degrading H_2O_2 around or in the injured cells. This is not true for thermally injured cells of *B. cereus* B4ac. Neither catalase nor 1% sodium pyruvate in the selective medium (PCAS) increased viable counts on that medium. Recovery was not enhanced by 1% pyruvate in the recovery medium. Although this observation should be pursued further, we have concluded that H_2O_2 does not seem to be a major injurious factor to *B. cereus* B4ac under our experimental conditions.

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A Cost Analysis of Alternative Fluid Milk Packaging Systems[1]

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ABSTRACT

The fluid milk processing industry faces a problem arising from demands of consumers and environmentalists. Consumers want an inexpensive, high quality milk product in a convenient package, based on varying family needs. Environmental groups are concerned about the best container for packaging milk when the capacity of solid waste disposal systems, environmental quality, and natural resource utilization are considered. This study is intended to aid industry and government policy makers to evaluate the costs of five container systems: (*a*) paper, (*b*) returnable rigid plastic, (*c*) disposable blow-mold plastic, (*d*) plastic pouch, and (*e*) glass. The study is based on a non-random stratified sample of 28 firms in Washington, Oregon, and California. Costs were identified by departments or functions to reflect the various production or handling stages. The appropriate categories were combined for each type of fluid milk container to ascertain average unit costs. The average total dock cost for whole milk in all sizes of paper containers and gallon in-plant blow-mold plastic containers declines as the volume of fluid milk processed by a firm increases. Even with large variation in some cost categories by container type, there appears to be little difference in the average total dock cost among the returnable rigid plastic, paper, in-plant blow-mold plastic, and plastic pouch containers.

The fluid milk processing industry has undergone rapid technological change during the last 20 years with respect to fluid milk packaging. The once dominant glass bottle has been replaced by several alternative packages: paper containers, returnable rigid plastic containers, disposable blow-mold plastic containers, and the plastic pouch. These container systems were initially developed to fulfill several needs of the milk processing industry: consumer preference for convenience, product differentiation (a marketing tool often times used in a highly competitive market), and potentially lower unit costs of packaging and distribution.

Today, the milk processing industry is being confronted with new demands. The consumer is still demanding a relatively inexpensive, convenient product of high quality which meets state and federal health and safety regulations. At the same time, individuals and private and public institutions are voicing growing concern about solid waste management and environmental and natural resource utilization with respect to alternative raw materials used for packaging milk. However, before public policymakers, health officials, and industry representatives can adequately address these problems, basic data are required concerning the safety, toxicology, and/or contamination potential for all milk containers. In addition, the relative processing, packaging, and delivery cost structure of representative firms using various containers must be recognized. Given adequate and necessary data, a decision evaluating the comparative economic and environmental impacts of alternative packaging containers can be made.

The purpose of this report is to suggest a procedure to delineate, estimate, and compare typical costs of milk processors to process, package, and deliver milk to the point of the final sale through five containers: (*a*) refillable glass bottle, (*b*) returnable rigid plastic bottle, (*c*) paperboard carton, (*d*) disposable blow-mold plastic bottle, and (*e*) plastic pouch. This report illustrates procedures used in a study designed to identify and measure costs in the fluid milk industry in the Pacific Northwest. The research procedure involved identifying and surveying fluid milk processing plants utilizing these various packaging systems and then analyzing the data obtained to provide comparable summaries for each system, given its size, measured in volume of Class I fluid milk processed. Costs were estimated from a sample of milk processing plants in Washington, Oregon, and California.

Market Structure and the Relative Importance of Alternative Milk Packaging Containers

The market structure of processor-distribution fluid milk bottling plants in the three western states and the United States can best be characterized as changing to fewer but larger bottling plants. Recent reports show that the total number of fluid milk plants in the United States and in Oregon and California has decreased steadily since 1950 (*7,8*). This long-time trend has accelerated since 1965 with the number of fluid milk bottling plants

[1] *Technical paper 4744*

in the United States decreasing more than 58%. A USDA study indicates that, in general, the decline in the total number of processor-distributor fluid milk plants resulted from the closures of smaller plants. For a group of comparable federal order and state markets, the total number of plants decreased 36% between 1965 and 1970. The number of plants selling less than 4 million pounds of fluid milk per month decreased, while plants handling more than 4 million pounds per month increased in number (*7*).

Other changes in the structure of the fluid milk processing industry can be identifed. The most important of these are in the product, service, or container. The introduction and high acceptance of low-fat milk have changed the plants' product mix. Some integrated plants process and package only four or five fluid milk and cream products in a limited number and size of containers. Processing plants, in general have decreased the reliance on retail home delivery, moving increasingly to limited wholesale delivery to institutions and stores, and even to dock sales to independent sub-distributors. The result has been an expansion of a plant's market boundaries. Before 1950, few plants distributed milk more than 50 miles. Now, distribution of 100 to 200 miles is common, and in some areas of the West, milk moves up to 500 miles. The number of milk plants located in any given market area has declined sharply; the number of competitors in a market area has declined much less.

A consideration associated with changes in the structure of the fluid milk processing industry is the cost effect. Prior studies have shown that processing raw milk into a packaged fluid milk product is subject to economies of size (*4, 7*). There were large economies to be gained by processing at least 2 million lb. per month. Economies continued to occur beyond this volume, but at a rapidly diminishing rate.

The percentage of total fluid milk processed during one month of 1975 by size and type of container in Oregon and Washington is shown in Table 1. Paperboard is shown to be the predominant container, with 86.5% of the fluid milk being packaged in various sizes of that container. Two different plastic packages are summarized under the plastic category: milk packaged in returnable rigid plastic gallon and half-gallon containers which account for the majority of the 7.6% of sales, and milk packaged in gallon disposable plastic pouches. Institutional sales in plastic dispensing containers (bag-in-box) account for 3.4% of fluid milk sales. Glass, the least important container category, accounted for only 1.7% of fluid milk sold in November 1975.

The distribution of fluid milk container type and size for California plants is presented in Table 2. Several differences are apparent when the California data are compared with data for Oregon and Washington (Tables 1 and 2). Less fluid milk is packaged in paperboard containers in California than in Oregon and Washington. Secondly, a relatively larger proportion of fluid milk is sold in disposable blow-mold plastic gallon containers in California, amounting to 20% of the fluid milk sales. Milk plants in California package a larger percent of their fluid milk in half-gallon paper containers than plants in Oregon and Washington, 54.2 and 31.8%, respectively.

TABLE 1. *Percent of fluid milk by container type and size in Washington and Oregon, November 1975.*

	Type of container				
Container size	Paper	Plastic[a]	Plastic lined box	Glass	Total
			(Percent of fluid milk sales)		
Larger than gallons	—	—	3.4	—	3.4
Gallons	38.9	5.0	—	.2	44.1
Half-gallons	31.8	1.8	—	1.3	34.9
Quarts	6.4	.3	—	.1	6.8
Less than quarts	9.4	.5	—	.1	10.0
Total[b]	86.5	7.6	3.4	1.7	99.2

[a]This group is primarily composed of rigid returnable plastic containers, however, some disposable plastic pouch containers are included.
[b]0.8% of total fluid milk sales was in other containers (i.e., 10-gallon cans).
SOURCE: *Detail Summary of Size and Type of Containers and Method of Distribution,* Oregon-Washington, Inland Empire, and Puget Sound, Market Area, Federal Milk Market Administrator, Portland, Oregon, November 1975.

TABLE 2. *Percent of fluid milk by container type and size in California, October 1975.*

	Type of container				
Container size	Paper	Blow-mold plastic	Plastic lined box	Glass	Total
			(Percent of fluid milk sales)		
Larger than gallon	—	—	3.8	—	3.8
Gallons	0.4	20.0	—	*a*	20.4
Half-gallons	54.2	3.1	—	2.3	59.6
Quarts	5.8	0.1	—	0.4	6.3
Less than quarts	9.9	—	—	—	9.9
Total	70.3	23.2	3.8	2.7	100.0

[a/]Less than 0.05 percent.
SOURCE: *Dairy Information,* California Crop and Livestock Reporting Service, Sacramento, California, February 11, 1976, 11 pp.

Sample Size and Stratification

Given the structural characteristics of the fluid milk processing industry in the three western states, and the relative importance of each size and type of packaging container, a stratified sample based on the size of the processing plant and type of packaging container was used in this study. A non-random stratified sample of 28 firms was established, with some firms using two types of containers. For example, a processing plant may use the disposable blow-mold plastic container for gallon fluid milk, and paper containers for all other packaged milk.

The number of plants used in computing average unit costs for each relevant category in the sample, by size and type of container, is presented in Table 3. Firms were grouped into each category so relative cost comparisons could be made. Some plants included in Group II were not assigned to other size classes to avoid disclosure of confidentiality.

A DESCRIPTION OF COSTS

The data associated with the costs of processing, packaging, and delivering Class I milk were obtained by collecting accounting records from the sample firms for one quarter of the year. The time ranged from the first quarter of 1973 to the first quarter of 1976. Therefore, the data used for comparative analysis were standardized to permit comparisons among different accounting periods and different accounting procedures. The California State Department of Food and Agriculture's manual of auditing and costing procedures was used as a guide to standardize each plant's costs (*2*). In addition, this allowed use of relevant segments of a number of plant audits compiled by the California State Department of Food and Agriculture, which had been reviewed by each plant and published in the "Total Reasonable Necessary Unit Costs for Fluid Milk, Low Fat, and Non Fat" reports for various marketing areas in California.[1]

Raw Product, Processing, General Plant, and Delivery Expense

Raw product, processing, and packaging expenses are defined as all expenses incurred from the time the raw milk enters the processor's holding tank until the processed product is ready for sale at the processor's loading dock. These cost components, which will be presented and discussed separately for each type of container are: (*a*) raw product cost, (*b*) processing expense, (*c*) packaging labor expense, (*d*) container cost, and (*e*) general plant expense. The importance and the problems associated with estimating and using delivery expense for comparative analysis are discussed.

Raw product cost

Raw product cost for processing plants in the three states is computed on current raw product component costs, which take into consideration the amount of milkfat, solids-not-fat, and fluid required for each processed unit. The component costs vary according to the location of the plant and the minimum price established by the stabilization and marketing plan in effect for that particular market. In addition, the established price is for milk delivered to the plant with 1% allowance for plant loss for all components.

The cost of raw product used in this study is constant and representative of the price of Class I milk in the various marketing areas in the three western states, which was in effect during December 1975. This price is simply used for expository purposes. That is, the raw product cost of $10 per hundredweight (3.5% milkfat milk) is used for all the sample plants, even though the raw product cost for Class I milk in the plants surveyed ranged from $9.35 to $10.55 per hundredweight during December 1975. This procedure allows the study to focus on the relative costs associated with the various packaging systems, by size of plants rather than the specific locality of a plant.

Processing expense

The sample processor's records were analyzed and only those expenses that were applicable to the processing of fluid milk were included. The firm's payroll was analyzed, and with the assistance of supervisory personnel and some time studies, costs were allocated to various functions based on the amount of time required to perform or supervise each function. The functions, allocated on time basis, are: receiving and tanker washing, pasteurizing and standardizing, separating, case and carton handling, and cold room labor.

Building and equipment expenses associated with processing were allocated to various types and sizes of containers on a quart equivalency basis. These costs

[1] *Three reports presented at public hearings were used: (a) Southern Metropolitan Marketing Area — Zone 1, November 1974; (b) Central Coast Counties Marketing Area, August 1974; and (c) North Central Valley Marketing area—Zone 1, November 1973.*

TABLE 3. *Number of Class I milk processing plants in the non-random stratified samples, by size and type of container.*

Group	Monthly plant volume of Class I fluid milk (Million pounds)	Type of container: Returnable Paper	Disposable rigid plastic	Plastic blow-mold plastic	Gallon pouch	Glass
		(Number of plants)				
I	less than 2	2	—	—	—	—
II	2-4	3	4[a]	—	2[a]	2[a]
III	4-9	7	—	3	—	—
IV	more than 9	12	—	11	—	—
Total		24	4	14	2	2

[a] Plants range from 1 to 5 million pounds per month.

include depreciation of the building and equipment used in pasteurizing, standardizing, and separating of milk.

Two specific processing expense categories are isolated for analysis in this study: packaging labor expense and container cost. Packaging labor expense is the direct labor wages and fringe benefits associated with filling and casing fluid milk. For example, if an employee spends his entire time filling half-gallon paper containers his entire salary is allocated directly to the filling schedule for half-gallon paper. Sanitation and cold room labor is not included in this category. The labor expense used in this study was $8.19 per hour, which was representative of the wage rate and fringe benefits of plants in the three western states in 1975.

The second specific processing expense — container cost — was determined on the basis of unit costs obtained from the latest available price quotation of the principal supplier. It included the handle or cap in the case of plastic or glass. A 1% loss allowance was used for all types of containers. In addition, the royalties paid for the use of certain filling machines were included in the container cost. The container cost for returnable rigid plastic and glass containers includes the labor necessary for washing and visually inspecting each unit. It was estimated that, on the average, each rigid plastic container was reused 50 times, and each glass container was reused 30 times before being replaced.

For in-plant blow-molding, the container cost includes all costs associated with the blow-molding operation; resin, scrap loss, direct labor, utilities, label and cap, depreciation, property taxes, interest on investment, and maintenance. The average in-plant blow-mold gallon cost at plants where these costs were obtained was approximately eight cents per unit.

General plant expense

This expense category refers to all other expenses associated with processing Class I fluid milk in the plant. These costs include: occupancy expense, based on a square footage basis, maintenance; professional services; dues; real estate taxes; office expense, labor, and supplies; and general and administrative expenses incurred in the direction and administration of the company. All these expenses, with the exception of occupancy, are based on a quart equivalency.

Delivery expense

All expenses associated with the actual delivery process, once the product left the dock of the processing plant, were allocated to the delivery function for each plant in the sample. There was a large variation in the delivery expense between plants and in the ratio of delivery expense to the total cost of processing. This variation in delivery expense had a very low correlation with different types of containers.

Two previous studies of delivery costs helped identify the problem of analyzing delivery costs relative to size and type of container. Delivery expenses are affected by characteristics of the container. However, total delivery costs also vary substantially by: the quantity of milk delivered per customer stop, the size and density of the market area, the degree of vertical integration, and the size of truck used for delivery (*3,5*).

The delivery data for plants in this sample could not be analyzed to identify differences caused by the type and size of container delivered. The plants in this sample encompass the extremes of each of the variables listed above: a market size and density representative of Los Angeles and San Francisco to rather remote rural areas in Oregon and Washington, for example. In addition, the sample was too small to stratify to account for each combination of variables.

However, it should be noted that the California State Department of Food and Agriculture has developed two types of modifiers (indices) to allocate distribution costs in the audits of milk processing plants in the major metropolitan areas of California (*2*). The labor modifier is used to determine the unit cost of delivery labor, including supervision. A case modifier is used to determine the unit cost for all other delivery expenses, except labor, such as truck depreciation and gas and oil expense. These two modifiers show the relative costs of labor and other expenses in delivering different sizes and types of containers in the major marketing areas of Sacramento, San Francisco, and Los Angeles, California. The relative delivery costs of each size and type of container, as a percent of delivery cost of a paper quart container, are shown in Table 4. Half gallon paper containers have the lowest delivery expense. Glass and returnable plastic containers have a higher relative delivery expense than paper and disposable blow-mold plastic because of the additional cost of handling the container in the process of returning it to the processing plant.

TABLE 4. *Relative wholesale delivery costs as a percnt of the costs to deliver a paper quart container. California*

Container size	Type of container			
	Paper and blow-mold plastic		Glass and returnable plastic	
	Labor	Case	Labor	Case
	(Percent)			
Gallon	100.0	100.0	*a*	*a*
Half-gallon	87.5	89.0	150.0	155.0
Quart	100.0	100.0	150.0	135.0
Pints	170.0	114.0	170.0	140.0
1/2 pints	280.0	132.0	280.0	280.0

[a/] Gallon glass and returnable plastic containers were not included in the California study.

SOURCE: Calculated from the modifiers in *Manual of Auditing and Cost procedures for Processing and Distribution of Dairy Products.* California Department of Food and Agriculture, revised September 1974.

ANALYTICAL RESULTS

Estimates of Average Unit Costs

This analysis of costs utilizes a cost accounting technique which defines each cost category in the manner as described above. The volume of each product handled during the corresponding time the costs of each plant were obtained also was recorded. Using a "quart equivalent" as a standard unit or common denominator, the costs were allocated by departments or functions to reflect the relative importance of each product by size and type of container to total volume of product handled and cost of operation. Then the appropriate categories were combined for each packaged product to represent an average weighted unit cost at the final production stage (total dock costs).

Total Dock Expense

The unit costs, by container type and size, serve as the basis for analysis. However, the use of unit costs requires constant conversion between container sizes. This problem can be alleviated by converting the unit costs to a standard of 100 lb. of 3.5% milkfat milk. This procedure is utilized in the following analysis.

Three factors can be isolated to identify differences with respect to average total dock costs: plant size, type of container, and size of container. From Table 5, it is apparent that economies of plant size, with respect to average total dock expenses, are prevalent for both paper and in-plant blow-mold plastic containers. For example, for paper half-gallon containers, the average total dock costs for plants processing more than 9 million lb. of Class I fluid milk per month are 9% less than plants processing less than 2 million lb. per month. For firms with in-plant blow-mold containers, the average total dock costs decreased by 2% for plants processing more than 9 million lb. per month, relative to plants processing 4 to 9 million lb. per month.

The total dock expenses also vary by type of container. However, these costs can be analyzed only for identical containers within each given size of plant. For example, if the gallon paper container is used as a standard of comparison, the relative average costs of returnable rigid plastic and plastic pouch containers can be compared for plants processing 2 to 4 million lb. of Class I milk per month. The average total dock costs of rigid plastic containers are 1.9% less than paper, the same costs for the plastic pouch containers are practically identical to paper. The dock cost of 100lb. of milk in half-gallon glass containers is 6.4% more than the same quantity of milk in half-gallon paper.

The average total dock costs for in-plant blow-mold plastic containers also can be compared to the gallon paper container costs. For plants processing 4 to 9 million lb. per month, the average costs for in-plant blow-mold plastic containers are about 1% more than paper.

It is interesting to note that since the cost of the raw product is a constant in this study, and general plant expenses are a relatively small percent of total dock cost, the variation in total dock cost is primarily explained by the relative changes in average total processing and packaging expenses. Even though total processing expenses for rigid plastic and plastic pouch gallon containers are higher than for paper gallon containers, lower packaging expenses more than offset these cost disadvantages, and the gallon rigid plastic and plastic pouch containers have a slightly lower average total dock cost than paper. On the other hand, in-plant, blow-mold plastic and glass containers are higher than paper in all cost categories for a comparable container and plant size (*8*).

TABLE 5. *Average total dock expense by plant size and type and size of container, per 100 pounds[a] of 3.5 percent fluid milk[b]*

Type and size of container	Plant size			
	Group I	Group II	Group III	Group IV
	Less than 2 million lb./mo.	2-4 million lb./mo.	4-9 million lb./mo.	More than 9 million lb./mo.
	(Dollars)			
PAPER				
Gallon	13.621	13.034	12.401	—
Half gallon	13.512	12.893	12.316	12.230
Quart	14.414	13.484	12.656	12.502
Half pint	16.428	15.740	15.088	15.051
IN-PLANT BLOW-MOLD PLASTIC				
Gallon	—	—	12.521	12.242
RIGID PLASTIC				
Gallon	—	12.792[c]	—	—
PLASTIC POUCH				
Gallon	—	13.010[c]	—	—
GLASS				
Half gallon	—	13.723[c]	—	—

[a]One gallon of milk weighs 8.6 lb.
[b]A raw product cost of $10 per hundredweight (Class I milk) is used for all the sample plants.
[c]Plant size ranges from 1 to 5 million lb. per month.

The size of the container also affects the average total dock expenses. Paper is the only type of container with multiple sizes. The half-gallon paper container has the lowest average cost for all plant sizes. Using plants processing 2 to 4 million lb. per month as an example, the effect of container size can be shown. Total dock expenses for gallon containers are 1.1% more than half gallons, and quarts and half pints are 4.6 and 2.1% more than half gallons, respectively.

The results of this study are pertinent for fluid milk processors on the West Coast. The sampling and analytical procedures deem using averages, thus any given plant may have higher or lower costs than those presented here. In addition, cost comparisons are computed up to, and including, total dock costs. Implications of these cost comparisons should not be extrapolated to the store or consumer level. For example, what appears to be a cost advantage for rigid plastic or plastic pouch containers at the processor's dock may be reduced or even eliminated if total delivery and store handling expenses are considered. However, industry and policy makers can use this study as a benchmark to which they can adjust for specific situations and problems of interest.

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A Research Note

Group Comparative Study of VRB-2 Agar in the Recovery of Coliforms from Raw Milk, Ice Cream and Cottage Cheese

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ABSTRACT

The method of Hartman et al. for recovering stressed coliform bacteria was evaluated in a group study. The experimental medium, VRB-2, was more productive than VRB medium by 31% for raw milk, 70% for ice cream and 61% for cottage cheese. Differences in colony counts of coliforms caused by incubating for 48 vs. 24 h and by boiling vs. autoclaving were not statistically significant. Among 40 samples tested in two laboratories, counts obtained at 30 vs. 32 C were not significantly different.

It has been well documented that injured coliforms, which would otherwise not grow in selective media, will repair in nonselective media (*4*). Hartman et al. (*1*) developed a modified procedure for making coliform counts on solid media and reported that yields of coliforms on their Violet Red Bile-2 (VRB-2) medium were increased compared with standard Violet Red Bile (VRB) medium (*2*). Speck et al. (*5*) published a similar method at the same time.

In an effort to confirm the comparative performances of VRB and VRB-2 we undertook this group study. In addition to evaluating the medium, we tested incubation time and temperature and method of medium preparation.

MATERIALS AND METHODS

VRB and VRB-2 media (Difco) were hydrated according to Standard Methods (*2*) and aliquots were heat-treated by boiling for 2 min and by autoclaving at 121 C for 5 min. Dilutions of raw milk, ice cream and cottage cheese were prepared and plated according to Standard Methods (*2*) except as noted below. Some samples of ice cream and cottage cheese were inoculated with raw milk or with cultures of coliform bacteria. When this was done, cottage cheese was stored at 4 C and ice cream at − 20 C for 24 h before plating to allow organisms to be stressed. Ten locally-obtained samples per product were examined per laboratory, but not all products were examined in each laboratory, usually because samples with sufficient coliforms were not available (see Table 1).

Eight plates of each dilution were prepared per sample and duplicates were poured with VRB agar-boiled, VRB agar-autoclaved, VRB agar-2 boiled and VRB agar-2 autoclaved. VRB agar was poured in the usual way. The sample was mixed into a basal layer of 8 to 10 ml of Standard Methods agar in VRB-2 plates. On solidification, 8-10 ml of VRB-2 agar was poured onto the basal layer. Plates were counted after being incubated at 32 ± 1 C for 24 and 48 h. In two laboratories plates were also incubated at 30 ± 1 C. Data presented are for only 32 C except where otherwise noted. Average counts of duplicate plates were used in the statistical analyses.

Data (logarithms of counts) were analyzed by Analysis of Variance with main variables being laboratory, medium, treatment of medium and temperature of incubation. All possible interactions were tested. Means were differentiated by Least Significant Difference tests (*3*).

RESULTS AND DISCUSSION

The experiment was designed to determine whether medium, time of incubation or method of preparing the medium were significant variables in determining the coliform count of raw milk, ice cream and cottage cheese.

Geometric means of coliform counts by product, laboratory and medium appear in Table 1. The main effect of medium, averaged over other variables, was significant ($P < 0.05$) with each product. VRB-2 medium was more productive by 31% with raw milk, 70% with ice cream and 61% with cottage cheese. However, further breakdown of the data showed that within laboratory differences between the two media were only significant with raw milk in laboratory 3, with ice cream in laboratories 2 and 3, and with cottage cheese in laboratory 3. Laboratories in which significant differences were recorded were generally those in which counts were highest. This observation is not surprising since there is high variability among low counts such as are observed in counting coliform bacteria. Coefficients of variation were

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TABLE 1. *Geometric mean counts by product, laboratory and medium; percentages of difference and significance.*

Product	Lab[a]	Mean counts/ml VRB	VRB-2	Percentage difference[b]
Raw milk	1	10.4	12.0	15NS
	2	15.3	16.7	9NS
	3	14.9	35.4	138*
	4	29.4	41.5	41NS
	5	24.8	22.6	−9NS
	6	13.6	18.3	35NS
	Means	16.9	22.2	31*
Ice cream	2	6.4	10.9	70*
	3	8.9	18.8	111*
	4	7.3	10.0	37NS
	Means	7.5	12.7	70*
Cottage cheese[c]	1	9.1	12.2	34NS
	2	7.7	7.9	3NS
	3	9.4	41.2	338*
	7	28.0	31.3	12NS
	Means	11.7	18.8	61*

[a]n = 10 (samples per product per laboratory).
[b][(Count on VRB-2 - count on VRB ÷ count on VRB] × 100.
[c]Data in this section of table do not include counts made after 48-h incubation because of an accident in one laboratory.
NSNonsignificant.
* Significant at 5% level (individual percentages difference by LSD and means by ANOV).

.27, .47, and .46 for the milk, ice cream and cottage cheese analyses, respectively.

Counts (averaged over laboratories within products) made after 48 h of incubation were not significantly higher than those made after 24 h (Table 2). Even though differences did not prove statistically significant, counts among products were 11 to 19% higher after 48 h incubation. An insufficient number of colonies was confirmed as coliforms to determine whether the incidence of false-positive colonies differed with incubation time.

TABLE 2. *Geometric mean counts averaged over laboratories within products as affected by time of incubation and treatment of media.*

Product	n	Coliforms/ml Time of incubation 24 h	48 h	Treament of medium Boiled	Autoclaved
Raw milk	60	18.4	20.5	19.4	19.4
Cottage cheese	40	11.7	13.1	12.5	12.3
Ice cream	30	8.9	10.6	9.3	10.1
Average[a]		13.0	14.7	13.7	13.9

[a]Arithmetric averages of the means derived by logarithmic statistical analyses. Differences between individual pairs of geometric means were all insignificant ($P < 0.5$).

There was no significant main effect of boiling vs. autoclaving of the media (Table 2), and in no individual laboratory with any product was there a significant difference in response to heat treatment between the two media for the various products tested.

Only laboratories 1 and 4 determined counts at both 30 and 32 C; laboratory 1 for raw milk and cottage cheese, laboratory 2 for raw milk and ice cream. In none of the experiments was there a significant effect of temperature on coliform count. Mean counts at 30 vs. 32 C, respectively, averaged for milk 23.0 and 23.3, for ice cream 8.5 and 8.7, and for cottage cheese 9.5 and 10.3/ml.

CONCLUSIONS

The procedure in which VRB-2 medium was used was considerably more productive of coliform-type colonies than was the similar procedure in which VRB medium was used. Neither boiling nor autoclaving at 121 C for 5 min influenced productivity of either medium.

Extending the time of incubation from 24 to 48 h may be beneficial with ice cream. However, this was a minor variable, and there is little practical reason to consider increasing the incubation time. There is reason, however, to maintain a standard for time of incubation because of the tendency for counts to increase after 24-h of incubation. Decreasing the incubation temperature from 32 to 30 C should not change the value of the coliform count method when it is used with products examined in this study.

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Device for Microbiological Sampling of Meat Surfaces

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ABSTRACT

A coring device for sampling meat surfaces was developed. Aerobic plate counts of beef and pork carcasses were significantly higher when determined by the coring method than by the cotton or alginate swab technique.

Various methods have been developed for microbiological sampling of surfaces, and their advantages and disadvantages have been reviewed (*1,4,13*). The methods involve use of cotton or calcium alginate swabs (*4*), "swabpression" (*5*), various surface rinses (*2,3,14*), direct agar contact (*1*), tissue removal techniques (*6,7,8,15*), millipore filter (*11*), cellulose sponge (*10*), and vacuum (*1*).

Many of the above methods fail to give reliable results when applied to heterogeneous and uneven surfaces such as meat. Swab methods do not completely remove cells from meat surfaces (*14*). Direct agar contact methods are not adequate because the high levels of bacteria per unit area of meat surface completely obscure any quantitative reading (*1*). Tissue removal techniques followed by shaking or blending (*7,8,15*) give the highest estimates of the microbial flora on raw meat surfaces.

We developed a coring device for sampling meat surfaces; then we compared the efficiences of the core, cotton swab, and calcium alginate swab procedures for determination of microbial contamination on the surfaces of beef plates and pork bellies.

MATERIALS AND METHODS

Coring method

Core samples were removed with a tooled, steel, coring device (Fig. 1) attached to a 1/4-inch commercial roller-bearing drill. The coring device was inserted into the drill chuck and secured. When the drill was turned on, the screw tip of the corer penetrated the meat surface and directed the tissue into the path of the rotating blade. The blade then cut the tissue away from the sampling area to a depth set by the adjustable guideplate mounted on the outside of the coring device. The drill was turned on for about 2 sec so that the sample obtained consisted of one to three pieces; longer periods caused the corer to cut the sample into many pieces. The surface area sampled was 15 cm^2. An intial core sample was removed to a depth of 0.7 cm; directly beneath, a second core sample that penetrated 0.5 cm further was removed (1.2 cm total depth).

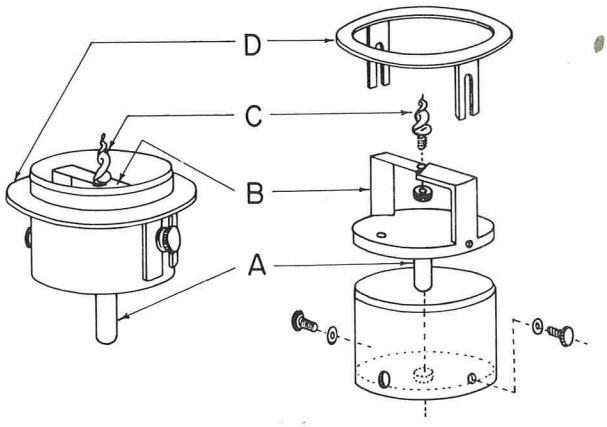

Figure 1. *Coring device for sampling meat surfaces. A = stem; B = blade; C = screw tip; D = guideplate.*

Each sample was removed from the corer with sterile forceps and blended with an Osterizer blender for 2 min in 100 ml of sterile Butterfield's phosphate diluent (*12*). Serial dilutions to 10^{-6} were prepared in 99-ml blanks of the diluent.

Between samples, the corer was removed from the drill, washed in hot soapy water, and rinsed in hot tap water. Next it was reattached to the drill and air dried with the drill turned on briefly. It was then submerged for 2 min in a heated ultrasonic water bath containing Basic H (Shaklee Corp., Emeryville, CA) cleaning solution, air dried as before, submerged for 2 min in a heated ultrasonic distilled water bath, redried, submerged in acetone, and redried.

Swabbing methods

A 15-cm^2 area of the meat surface, described by a circular paperboard template held by a sterile hemostat, was swabbed 30 sec with a cotton swab moistened with Butterfield's phosphate diluent. The cotton swab was then broken off into a tube with 10 ml of the same diluent and vigorously shaken on a Vortex mixer for 1 min. Serial dilutions to 10^{-6} were prepared in 99-ml blanks of the diluent. Immediately after the cotton swabbing, a core sample that penetrated 1.2 cm was taken from the swab area circumscribed by the paperboard template. Serial dilutions of the core sample were prepared as described above for the coring method.

Sampling with calcium alginate swabs was done as described above for cotton swabs, except that one-quarter strength Ringer's solution with

1% sodium hexametaphosphate was used instead of Butterfield's phosphate diluent. The 10-ml tube of diluent containing the alginate swab sample was mixed for about 5 min on the Vortex mixer until the swab dissolved. A core sample 1.2 cm deep was taken immediately after swabbing as described above.

Bacterial counts

Serial dilutions of the core and swab samples were pour plated in duplicate with Plate Count Agar (Difco), and the plates were incubated at 20 C for 72 h. Coliform bacteria were enumerated in duplicate on Violet Red Bile Agar (Difco) pour plates (with overlay) incubated at 35 C for 24 h.

Sampling plan

Each of the three sampling methods was done three times on the dorsal surfaces of each of three beef plates and three pork bellies (skin on) according to a Latin square design (*9*). Thus, there were a total of nine observations per sampling method for each species. Statistical analysis was based upon orthogonal polynomial contrasts of the ANOVA process. Variation due to differences between and within beef plates or pork bellies was statistically removed by blocking and the Latin square design.

RESULTS AND DISCUSSION

Coliform counts on both beef and pork were low and sporadic and could not be used as a basis for comparison of sampling methods. Table 1 gives the aerobic plate counts (APC's) for beef plates and pork bellies, as determined for each of the three sampling methods (alginate swab, cotton swab, and coring device). Also shown are the counts for the second samples (core), which were taken immediately after the first so that the number of bacteria remaining on the meat surface could be determined. The coring method gave significantly higher APC's than either the alginate or cotton swab method for both beef ($P < 0.05$) and pork ($P < 0.01$) surfaces. The first core failed to remove 0.5 to 2.0% of the total surface contamination, probably because the screw tip and the cutting edges of the corer carried some surface contaminants down into the sampling site directly below. Recoveries of microorganisms from beef and pork surfaces were greater for the cotton swab than alginate swab method but not significantly so.

The large differences in APC's between the coring and swabbing methods were primarily due to the inability of swabs to remove all of the bacteria from the irregular and hydrophobic surfaces of the beef and pork carcasses. Also, some of the microorganisms entrapped in the fibers of the cotton swabs may not have been released during vortexing. The alginate swabs were dissolved by sodium hexametaphosphate to free entraped microorganisms; however, there is evidence that alginate and sodium hexametaphosphate may be inhibitory to some microorganisms (*4,14*). Recoveries have been reported higher with alginate swabs than cotton swabs and vice versa (*4*).

The major disadvantage of the coring method is the time involved in cleaning and sterilizing the corer between samplings. If such a sampling device could be mass produced relatively inexpensively, a number could be maintained in the laboratory and sterilized by autoclaving before sampling.

The coring method used in this study gave a more accurate estimate of microbial contamination on chilled beef and pork carcasses than the widely used swab methods. Because the coring device does cause physical damage to the carcass, it may not be appropriate for routine quality control purposes in the meat industry. However, the corer is especially well adaptable to investigations in which accurate estimates of microbial surface contamination are essential.

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TABLE 1. *Comparison of three sampling methods for determination of aerobic plate counts (per cm²) on beef plate and pork belly surfaces.*[1]

Sampling method	First sample	Second sample (core)	Total	% Removal first sample/total
Beef				
Alginate swab	4.5×10^5 b	9.0×10^5 b	1.3×10^6 b	33.4
Cotton swab	5.1×10^5 b	5.7×10^5 b	1.1×10^6 b	47.2
Coring device	3.7×10^6 c	1.8×10^4 c	3.7×10^6 b	99.5
Pork				
Alginate swab	7.3×10^2 b	1.2×10^3 b	1.9×10^3 b	37.5
Cotton swab	5.5×10^2 b	6.1×10^2 b	1.2×10^3 b	47.3
Coring device	4.6×10^3 c	9.4×10^1 c	4.7×10^3 b	98.0

[1] Each value is the average count/cm² for 9 samples. Values for beef in the same column followed by the same letter are not significantly different ($P < 0.05$). Values for pork in the same column followed by the same letter are not significantly different ($P < 0.01$).

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Xanthine Oxidase

Considerable publicity has been given to an hypothesis that xanthine oxidase, an enzyme found in cow's milk, is linked to coronary heart disease. It has been maintained that when milk is homogenized, xanthine oxidase (XO) can be absorbed directly through the walls of the intestine, can enter the blood stream intact, and can then damage the walls of arteries, thus leading to arteriosclerosis and coronary heart disease.

Research experts stated that the XO concept was implausible since the large XO moledule would almost certainly be broken down during the digestive process and that if it were to enter the intestine intact, it could not be absorbed directly because of its vast size.

The Food and Drug Administration (FDA) published a statement that there was no cause for alarm, and that milk drinking should not be discouraged. After careful review of the XO hypothesis, the American Heart Association, National Heart, Lung and Blood Insitute, and American Medical Association similarly said that there was no need for consumer concern over the matter. Upon extensive scientific and medical examination of the XO hypothesis, the Life Sciences Research Office of the Federation of American Societies for Experimental Biology concluded that "the evidence supporting the hypothesis is inconclusive. Most consultants in this study expressed doubt that xanthine oxidase in bovine milk could be significant as a causal or risk factor in atherogenesis."

Recent research has shown that "xanthine oxidase absorption was minimal . . . consequently the data would not support the hypothesis of xanthine oxidase absorption." This work indicates in a definitive scientific manner that the xanthine oxidase molecule is largely broken down by the digestive process, and that what is not digested is not absorbed. As the XO hypothesis hinges on the concept that the XO molecule enters the bloodstream intact, this new work amply demonstrates that there is no substance to the XO hypothesis and that there is no danger in drinking homogenized milk.

It has been recently hypothesized that XO may be incorporated into fatty particles called liposomes and thus protected from the normal digestive processes. Experimental data, however, refute this latest hypothesis. By its nature, XO would be bound on the outside of the liposomes, and thus it would be accessible to the digestive process; tests show this to be ture, in that XO is readily metabolized. Moreover, there has been no demonstration that liposomes can serve as a carrier for any protein given orally, and that would include XO. Thus this newest hypothesis fails to stand up under scientific scrutiny.

The whole XO hypothesis remains amply refuted despite attempts to revive it.

Milk Allergy

There is great divergence of opinion in medicine regarding allergy in general, and milk allergy in particular. While it is true that a certain percentage of the infant population may react adversely to milk proteins, actual incidence remains controversial.

Symptoms of milk allergy may include asthma, nasal congestion, vomiting, and abdominal pain. Since these symptoms also occur with other allergies and with other diseases, the condition may be improperly diagnosed as milk allergy. Some physicians cite the incidence of milk allergy as being as low as 0.1 percent of infants, while others claim a figure as high as 30 percent. Most agree that the true range is between two and seven percent. Milk allergy is generally outgrown by age two.

Too often, a hasty diagnosis is made and mothers are urged by physicians to remove milk from an infant's diet. Frequently, the child never has milk replaced in the diet, and the individual may avoid dairy foods later in life. This may result in inadequate intake of nutrients in milk, notably calcium, riboflavin, and vitamins A and D.

Most medical authorities would support the following advice for physicians suspecting milk allergy in their infant patients. Remove milk from the diet for a two-week period of time. If symptoms remain, the physician must seek another cause and milk should be returned to the diet. If symptoms disappear, reintroduce milk to the diet. If no symptoms recur, they were possibly brought on by other than allergic reaction, and milk may remain in the diet.

Additional research must be performed to determine the actual incidence of milk allergy and to fully explore the area of iron deficiency anemia in infants.

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Aflatoxin is Degraded by Fragmented and Intact Mycelia of *Aspergillus parasiticus* Grown 5 to 18 Days with and without Agitation

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ABSTRACT

Mycelia of *Aspergillus parasiticus* NRRL 2999 degraded aflatoxins B_1 and G_1. Initially 9-day-old mycelia appeared to effectively degrade both aflatoxins; however, later experiments indicated that rates at which 9-day-old mycelia degraded aflatoxins B_1 and G_1 varied even though the mycelia were produced and evaluated under similar conditions. A comparison of the ratio of rates at which mycelia of different ages degraded aflatoxin B_1 and G_1 suggests that more than one mechanism may be involved in degradation of aflatoxin by the mold. Mycelia produced when incubation was quiescent or with agitation degraded aflatoxins B_1 and G_1. Fragmenting of mycelia greatly increased their ability to degrade aflatoxin, thus suggesting that the degrading factor(s) is/are intracellular constituent(s) of the mycelium.

Aflatoxin has caused the death of thousands of animals included ducklings, turkey poults, partridges, and pheasants (*2,5,7,11*). Associated with such deaths were biliary hyperplasia and extensive necrosis of the animals' liver (*2,7*). Other studies demonstrated that aflatoxin is a potent carcinogen and that it produced hepatic carcinomas in rats and rainbow trout after these animals received a diet containing the toxin (*18,28*). Since then, toxicity of aflatoxin has been demonstrated in a variety of laboratory and farm animals (*1,9,17*) and possibly in humans (*10,14,19*). Hence, the need to minimize hazards associated with aflatoxin by preventing it from entering our food supply or by removing and/or inactivating it once it has entered foods or feeds is evident.

It is not uncommon for commodities such as peanuts, wheat, cottonseed, rice, bran, corn, barley, and soybeans to become contaminated with aflatoxigenic molds which, if allowed to grow, will produce aflatoxin (*15*). Considering today's world food situation with millions of malnourished people, we cannot afford to sacrifice such aflatoxin-contaminated nuts and grains by plowing them under (*6*), nor can they be allowed to be consumed by an unsuspecting public who as a consequence may contract cancer years later. Consequently methods must be developed to treat aflatoxigenic foodstuffs so the toxin will be converted to a nontoxic form.

Numerous attempts have been made to detoxify aflatoxin in feedstuffs and still have a safe, nutritional, edible product (*17*). However, to date no single treatment has completely solved the problem. Although certain treatments may inactivate aflatoxin, such proposed treatments may lower the nutritional quality of the food, detectable amounts of aflatoxin may still remain, and/or toxic degradation products may be present. Therefore, further research on other possible methods to inactivate aflatoxin is needed.

In 1965, Ashworth et al. (*4*) observed that certain molds, including *Aspergillus niger* and *Aspergillus flavus*, when growing in an aflatoxin-containing liquid substrate or on toxin-containing peanuts, decreased the total amount of aflatoxin B_1 which was originally present. About a year later Ciegler et al. (*12*) observed the same phenomenon when aflatoxigenic strains of *A. flavus* and *Aspergillus parasiticus* were studied. They noted that these molds produced a maximum amount of aflatoxin when growing in a liquid culture in 20-liter fermentors and with futher incubation the total amount of aflatoxin decreased. Since then, other investigators have observed this same phenomenon (*3,13,21,22,24-26*). However, no one has yet fully elucidated conditions which are optimal for biodegradation of aflatoxin or the mechanism which controls it.

The objectives of our entire study were to delineate some of the conditions under which maximum amounts of aflatoxin are degraded by aspergilli and subsequently to suggest a possible mechanism for this phenomenon. Then, depending on the actual mechanism(s) for aflatoxin biodegradation, it might be possible to apply this information in devising ways to detoxify products that are naturally contaminated with aflatoxin. This paper reports observations on biodegradation of aflatoxin by mycelia of *A. parasiticus* of various ages, by mycelia from cultures that were aerated minimally and vigorously, and by mycelia that were intact or fragmented.

MATERIALS AND METHODS

Preparation of test mycelium

A slant of Mycological (Difco) agar was inoculated with *A. parasiticus* NRRL 2999 and incubated at 28 C for 7 days. Spores were then harvested by brushing the surface of the mycelium with a sterile wire loop and suspending the spores in sterile, deionized, distilled water. The final concentration of spores in the suspension was adjusted to 6 to 8×10^6 per ml. One milliliter of this spore suspension was used to inoculate 340 ml of glucose-salts medium in a 2-liter Erlenmeyer flask. The medium consisted of 50 g of glucose, 6.0 g of $(NH_4)_2SO_4$, 5.0 g of KH_2PO_4, 6.4 g of K_2HPO_4, 0.5 g of $MgCl_2 \cdot 6H_2O$, 2.0 g of glycine, 2.0 g of glutamic acid 10 mg of $FeSO_4 \cdot 7H_2O$, 5 mg of $ZnSO_4 \cdot 7H_2O$, and 1 mg of $MnSO_4$. Sufficient deionized, distilled water was added to make 1 liter. Glucose was sterilzed separately and aseptically added to the sterile glucose-free salts solution after the solution had cooled. Except for the experiment where the mold was grown with aeration, incubation was quiescent at 28 C for the desired number of days.

After incubation, the mycelial mat was aseptically removed from the medium, placed in a Buchner funnel containing four layers of cheesecloth, and washed with sterile, deionized, distilled water. The mycelial mat was aseptically damp-dried with sterile cheesecloth and weighed. Two milliliters of 0.067 M KH_2PO_4-NaOH buffer, pH 6.5, were added for each gram (wet weight) of mycelium and these were intermittently blended for 2 min at high speed in a Waring #7011 G blender (Waring Products, New Hartford, Conn.). To remove substances of small molecular weight which could serve as substrates for aflatoxin production and to remove as much aflatoxin as possible from this mycelial blendure, it was dialyzed overnight against several changes of 0.067 M KH_2PO_4-NaOH buffer, pH 6.5, at 2-4 C. After dialysis, 3.0 ml of this suspension were added to the aflatoxin-salts medium.

Preparation and use of aflatoxin-salts medium

A salts medium at pH 6.5 was prepared so it had the same composition as the aforementioned glucose-salts medium except that the carbon sources were not included. Fifty milliliters of this medium were added to 300-ml Erlenmeyer flasks which were stoppered with diSPo plastic plugs (Scientific Products, McGraw Park, IL) and sterilized. Aflatoxins B_1 and G_1, which were purified by column chromatography (*16*) followed by thin layer chromatography (*23,26*), were suspended in chloroform and added to each flask containing the salts medium. Fourteen such flasks were prepared for each experiment. Each flask was flushed with a stream of nitrogen until chloroform was removed. All flasks were covered with aluminum foil to avoid photochemical degradation of the aflatoxins.

After chloroform was removed, contents of two of the 14 flasks were immediately extracted with chloroform and the amount of aflatoxins present was determined. These two flasks served as controls; test results indicated the amount of toxin in each flask before the mycelium was added. Ten flasks were subsequently each inoculated with 3.0 ml of the previously described mycelial suspension. Contents of two of these flasks were immediately extracted with chloroform. The amount of aflatoxins present was the actual amount of each aflatoxin initially present for that experiment. Greater amounts of aflatoxin B_1 and G_1 were present in the inoculated than in control flasks because some aflatoxins were present in mycelia used as inocula. Of the remaining eight inoculated flasks, contents of two flasks were extracted with chloroform for aflatoxin determinations after 24, 48, 72 and 96 h. The uninoculated aflatoxin-salts mixture in the two control flasks was extracted with chloroform after 96 h of incubation. These data indicate the amount of nonspecific aflatoxin degradation which may have occurred during the 96-h incubation period. In no instance was there a notable difference between the amounts of aflatoxin B_1 and G_1 initially and after 96 h of incubation. All flasks were incubated at 28 C.

Extraction and analysis of aflatoxin from aflatoxin-salts reaction mixtures

A series of three extractions (100, 50, and 50 ml of chloroform) was used to remove aflatoxin from each 50 ml of reaction mixture. To remove water which may have been present, aflatoxin in the chloroform extract was filtered through anhydrous sodium sulfate. The chloroform extract was then concentrated to 100 ml and the amount of aflatoxin B_1 and G_1 present was determined.

Amounts of aflatoxin were determined by the method of Shih and Marth (*23*). This included alternately spotting 5-μl amounts of an aflatoxin standard, which contained 1.0 μg of B_1, 0.3 μg of B_2, 1.0 μg of G_1, and 0.3 μg of G_2 per ml between appropriate amounts of two unknown samples on thin-layer chromatography plates (Adsorbosil-1, 20 cm × 20 cm, 250-μm-thick layer, Applied Science Laboratories, State College, PA). Plates were developed in unlined tanks containing chloroform: methanol:water (98:1:1; vol/vol/vol). After development, plates were air-dried for 15 min and fluorescence of aflatoxins was measured with a fluorometer (Model 111, G. K. Turner Associates, Palo Alto, CA) equipped with a thin-layer plate scanner and a recorder (Model H, Leeds and Northrup Co., Philadelphia, PA). Calculation of aflatoxin concentrations was done according to the procedure of Pons et al. (*20*). Values for aflatoxin concentrations at each sampling interval, as given in figures, are averages of two determinations. Values reported as the change in aflatoxin concentration (%/24 h) were derived from the slope of amount of aflatoxin (μg) versus sampling period (h) plots. Slopes and lines of best fit for these plots were determined by linear regression analysis using methods of Steel and Torrie (*27*).

Determination of dry weight of mycelium

The amount of mycelium used as inoculum [in most experiments 3.0 ml of a mycelial blendure of 1.0 g of mycelium (wet weight): 2.0 ml of 0.067 M phosphate buffer, pH 6.5] was added to a predried weighing pan. Duplicate determinations were made for each sample. These samples were dried with vacuum at 740 mm of Hg for 4 h at 100 C in a vacuum oven (Precision Scientific Co., Model 29, Chicago, IL). After drying, samples were allowed to attain room temperature in a desiccator and were then weighed. They were dried for an additional 30 min at 100 C under vacuum at 740 mm of Hg. After this second drying, samples were again weighed. If these values agreed with (within ± 0.003 g) those obtained from the previous weighings, these values were used to calculate the dry weight of the mycelia which were used for tests. If values obtained after drying for the additional 30 min were not consistent with the first values, then mycelia were dried for an additional 30 min and weighed again.

RESULTS AND DISCUSSION

Effect of age of mycelia

Five- to 18-day-old mycelia of *A. parasiticus* NRRL 2999 were monitored for their ability to degrade aflatoxin. The age of the mycelium, as used in this paper, is the time in days between when a spore suspension was inoculated into glucose-salts broth and the mycelium produced from this spore suspension was harvested and used for these studies.

Figure 1 graphically illustrates changes in aflatoxin B_1 concentration caused by mycelia which were 5 to 11 days old. Similar information for 12- to 18-day-old mycelia is in Fig. 2. From slopes of curves it is apparent that aflatoxin B_1 was degraded at different rates depending on the age of mycelia. To more easily compare rates of aflatoxin degradation by mycelia of different ages, actual values for rates of change are given in Table 1. The percent change in aflatoxin concentration per 24-h period has been calculated from the slope of the line of best fit for each mycelium that was tested. Also in Table 1 are values for average dry weight of the 3.0-ml mycelial suspensions used at inoculum and the initial concentrations of aflatoxins. These values are important since, as will be shown in another paper, the amount of mycelium

used influences the rate of aflatoxin degradation. Similarly, the amount of aflatoxin initially present in the reaction mixture has a bearing on how much aflatoxin is degraded.

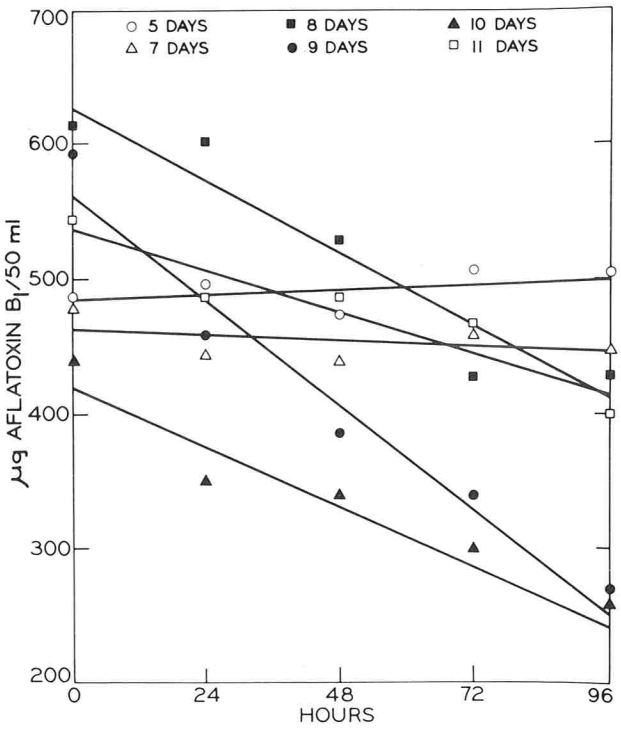

Figure 1. *Degradation of aflatoxin B_1 by blendures of 5, 7, 8, 9, 10, and 11-day old mycelia of* A. parasiticus *NRRL 2999 when incubated with aflatoxin in a salts medium, pH 6.5, at 28 C.*

On a dry weight basis, the amount of mycelium used for the different tests ranged from 0.09 to 0.13 g. Since these values could not be calculated until several hours after the aflatoxin-salts reaction medium had been inoculated, it was necessary to inoculate the medium on a wet-weight basis and compare values for dry weight after the fact. Furthermore, because it was not known how much aflatoxin B_1 and G_1 were present in the mycelium, the amount of aflatoxin initially present in each series of reaction mixtures varied depending on how much of each aflatoxin was added with the inoculum. Because of these variables it is difficult to say at what age the mycelium is optimal for degrading aflatoxin. However, more aflatoxin B_1 was degraded by mycelia which were

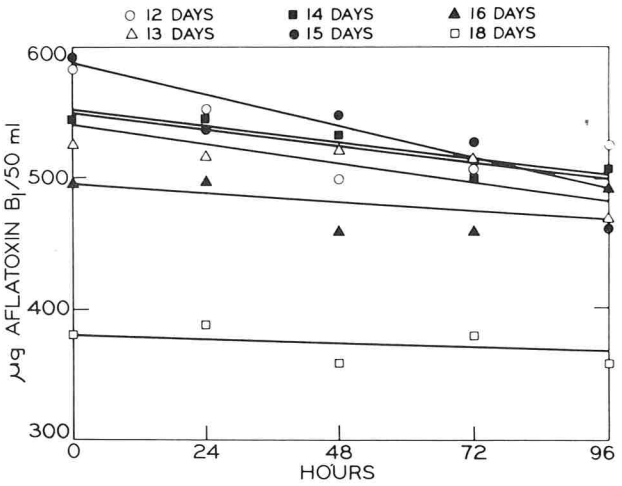

Figure 2. *Degradation of aflatoxin B_1 by blendures of 12, 13, 14, 15, 16, and 18-day-old mycelia of* A. parasiticus *NRRL 2999 when incubated with aflatoxin in a salts medium, pH 6.5, at 28 C.*

8, 9, 10, 11, and 15 days old than by mycelia of other ages, with the greatest amount of aflatoxin B_1 being degraded by 9-day-old mycelia.

Also in Table 1 are rates at which aflatoxin G_1 was degraded by mycelia of different ages. The most substantial rates of aflatoxin G_1 degradation were associated with 9, 10, 14, and 16-day-old mycelia; 9- and 10-day-old mycelia degraded aflatoxin G_1 most rapidly. Although initial amount of aflatoxin and dry weight of mycelia varied, a relative comparison of the rates at which aflatoxin B_1 was degraded to those at which aflatoxin G_1 was degraded by the same mycelia suggests that the aflatoxins were degraded by more than one mechanism. For example, the 8-day-old mycelium degraded aflatoxin B_1 approximately five times faster than aflatoxin G_1, whereas the 9-day-old mycelium degraded aflatoxin B_1 only about 1.6 times faster than aflatoxin G_1. However, degradation rates obtained with 12-day-old mycelia, which tests had starting aflatoxin concentrations similar to those of 8- and 9-day-old mycelia, were similar for both aflatoxins. Since the ratios between the rates at which aflatoxin B_1 and G_1 were degraded varied, more than one degrading mechanism might be suspected.

Since large amounts of aflatoxin B_1 and G_1 were degraded by 9-day-old mycelia, mycelia of this age were

TABLE 1. *Rates at which quiescently grown 5- to 18-day-old mycelia of* A. parasiticus *NRRL 2999 degraded B_1 and G_1.*

Age of mycelia (days)	Dry weight of inoculum (g)	Initial aflatoxin conc. (μg/50 ml)		Change in aflatoxin conc. (%/24 h)	
		B_1	G_1	B_1	G_1
5	0.11	487	197	+ 1.2	− 2.6
7	0.12	482	171	− 1.0	+ 4.6
8	0.13	615	235	−8.9	− 1.7
9	0.12	597	228	− 13.0	−8.2
10	0.12	439	248	− 9.4	− 10.3
11	0.13	543	219	−5.8	− 1.0
12	0.11	585	213	− 2.6	− 2.4
13	0.11	531	231	− 2.4	− 1.0
14	0.12	550	313	− 2.2	− 2.9
15	0.10	593	288	− 4.6	− 2.4
16	0.09	496	226	− 1.0	− 4.7
18	0.09	385	166	− 1.4	+ 1.7

used for later studies.

Variation in rates of aflatoxin degradation by mycelia which were the same age and grown under similar conditions

Mycelia of *A. parasiticus* NRRL 2999 were grown, quiescently on the glucose-salts medium at 28 C, harvested after 9 days of growth, and blended in the ratio of 1.0 g (wet weight):2.0 ml of 0.067 M phosphate buffer, pH 6.5. Such mycelia varied in their ability to degrade aflatoxins B_1 and G_1. For example, it was originally noted that these 9-day-old mycelia were extremely active and degraded 13.0% of the aflatoxin B_1 and 8.2% of the aflatoxin G_1 in 50 ml of reaction mixture per 24 h when 597 μg of aflatoxin B_1 and 228 μg of aflatoxin G_1 were present. However, other experiments with this same strain of mold, and mycelia of the same age and produced under similar conditions revealed that more or less aflatoxin B_1 and/or G_1 were/was degraded even though similar initial aflatoxin concentrations were used and the same amount (dry weight, 0.12 g) of mycelium was added. Data in Table 2 illustrate differences in rates of aflatoxin B_1 and G_1 degradation among eight different sets of mycelia which were grown under similar conditions. Rates at which aflatoxin B_1 was degraded ranged from 3.4%/24 h to 12.3%/24 h/50 ml of reaction mixture. It is evident that mycelia of the same strain of mold and grown under similar conditions vary in their ability to degrade aflatoxins. Similar variation has been noted in the ability of mycelia of the same strain of mold and grown under similar conditions to produce aflatoxins. Even though the same strain of mold was grown under identical conditions, mycelia grown in one flask produced either more or less aflatoxins than mycelia grown in an adjacent flask (*8*, Doyle and Marth, unpublished data). Hence, the biological variability which was observed in degrading of aflatoxins by mycelia is not surprising.

One can only postulate as to why this variation occurred. It may result because the mold did not always produce the same amount of substance(s) needed to degrade aflatoxin and this may be related to small differences in environmental conditions. It may also be related to small differences in the amount of degrading substance(s) released from mycelia, possibly because of variation in the extent to which mycelia were fragmented. Other hypotheses may be offered, but until the mechanism(s) for degradation of aflatoxin is (are) more firmly estabilised, such hypotheses are largely speculation.

Having observed that comparable mycelia vary in their ability to degrade aflatoxin, it is not feasible to definitively state that mycelia of a specific age will always degrade more or less aflatoxin than mycelia of another age. Until the reason(s) for this variation can be identified, mycelia of a specific age cannot be claimed as optimal for degradation of aflatoxin. Since different blendures of comparable mycelia varied greatly in their ability to degrade aflatoxin, the same mycelium or the same blendure of mycelia should be used to inoculate the entire series of reaction mixtures which are used to compare the effects different environmental parameters have on the mycelium's ability to degrade aflatoxins.

Degradation of aflatoxin by mycelia produced with aeration

Molds grown in submerged culture with aeration differ morphologically from those which are grown quiescently. Hence, an experiment was done to see if mycelia produced with aeration were also able to degrade aflatoxins B_1 and G_1. The same conditions as were previously described for quiescently growing cultures of *A. parasiticus* NRRL 2999 in a glucose-salts medium were used except that now cultures were incubated in a Controlled Environment Incubator Shaker (New Brunswick Scientific Co., Inc., New Brunswick, NJ) at 28 C and with agitation at 140 RPM. Constant agitation was employed from the moment of inoculation until the mycelial crop was harvested. Once harvested, mycelia were rinsed with sterile distilled water and blended [1.0 g (wet weight) of mycelium per 2.0 ml of 0.067 M phosphate buffer, pH 6.5]. Three milliliters of this blendure served as inoculum. Because of differences in growth characteristics of mycelia produced with and without agitation, the dry weight of 3.0 ml of inoculum from a mycelial blendure of the agitated cultures was only 0.05 g, compared to 0.11 to 0.13 g for 3.0 ml of inoculum from a mycelial blendure of quiescently grown cultures.

The rates at which aflatoxin B_1 was degraded by 5- to 12-day old mycelia cultured with agitation are illustrated in Fig. 3. Figure 4 gives similar information for aflatoxin G_1. Even though these cultures were morphologically different than those grown quiescently, it is apparent

TABLE 2. *Variation in rates at which comparable 9-day-old mycelia of* A. parasiticus *NRRL 2999 degraded aflatoxin B_1 and G_1.*

Experiment[a] number	Dry weight of inoculum (g)	Initial aflatoxin conc. (μg/50 ml)		Change aflatoxin conc. (%/24 h)	
		B_1	G_1	B_1	G_1
1	0.12	587	341	−3.4	−4.6
2	0.12	517	335	−4.6	−4.9
3	0.12	570	321	−4.6	−5.2
4	0.12	546	298	−5.0	−7.8
5	0.12	595	246	−7.7	−5.0
6	0.12	620	299	−8.0	−10.1
7	0.12	540	270	−8.0	−10.7
8	0.12	597	229	−13.0	−8.2

[a] A different blendure of mycelia was used for each experiment. Data are arranged from smallest to largest change in aflatoxin B_1.

that aflatoxin B_1 and G_1 were degraded by the mycelia produced with aeration. It is evident from data in Table 3 that large amounts of aflatoxin were degraded by mycelia that were 8, 9, 10, or 12 days old; however, because of variation in starting concentrations of aflatoxin, it cannot be definitively stated that mycelium of a specific age is substantially better for degrading

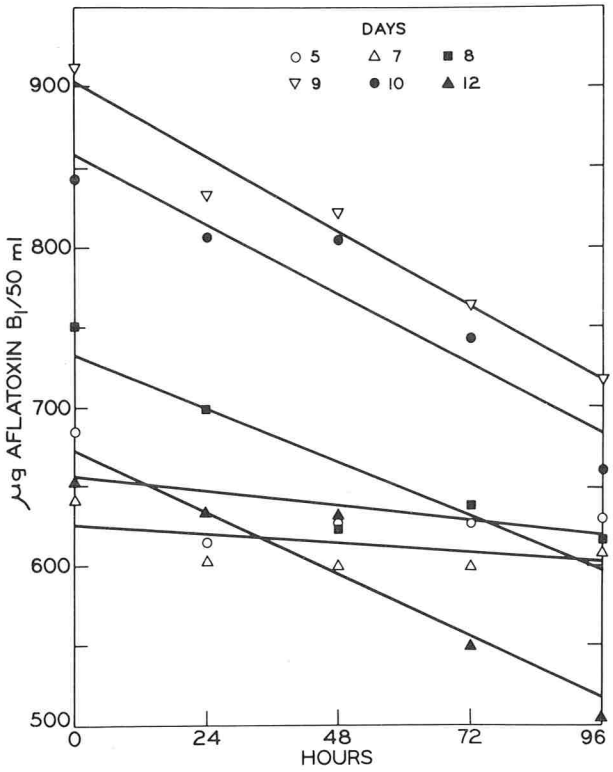

Figure 3. *Degradation of aflatoxin B_1 by blendures of 5, 7, 8, 9, 10, and 12-day-old mycelia of* A. parasiticus *NRRL 2999 which were originally grown with aeration and then incubated with aflatoxin in a salts medium, pH 6.5, at 28 C.*

aflatoxin than that of some other ages. In addition, if the variation in degradation of aflatoxin noted with quiescently grown mycelia also occurs for mycelia grown with aeration, it would still not be possible to absolutely identify mycelium of a certain age as being better for degrading aflatoxin than that of another age even if the starting aflatoxin concentrations were the same. Furthermore, even though the initial concentration of aflatoxin G_1 was 160 to 360 μg less than that of aflatoxin B_1 (Table 3), the rate at which aflatoxin G_1 was degraded was generally greater than the rate at which aflatoxin B_1 was degraded. Hence mycelia grown with aeration degraded aflatoxin G_1 more rapidly than B_1.

Effect fragmentation of mycelia had an aflatoxin degradation

Previous experiments involved using mycelia that were fragmented by blending in the presence of phosphate buffer. Ciegler et al. (*12*) observed that breakage or lysis of the mycelium was needed for aflatoxin degradation. To determine the necessity for fragmenting the mycelium, both fragmented (blended) and regular

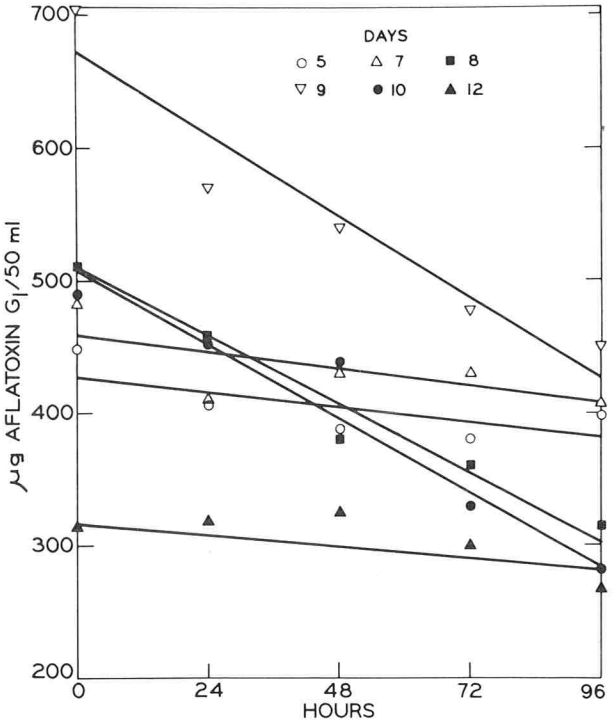

Figure 4. *Degradation of aflatoxin G_1 by blendures of 5, 7, 8, 9, 10, and 12-day-old mycelia of* A. parasiticus *NRRL 2999 which were originally grown with aeration and then incubated with aflatoxin in a salts medium, p 6.5, at 28 C.*

TABLE 3. *Rates at which aflatoxin B_1 and G_1 were degraded by 5- to 12-day-old mycelia of* A. parasiticus *NRRL 2999 when the mold was grown with aeration.*

Age of mycelia (days)	Initial aflatoxin conc. (μg/50 ml)		Change in aflatoxin conc. (%/24 h)	
	B_1	G_1	B_1	G_1
5	687	448	− 1.3	− 2.5
7	644	484	− 0.9	− 2.7
8	754	512	− 4.3	− 9.6
9	917	710	− 5.1	− 8.6
10	843	484	− 5.1	− 11.1
12	655	333	− 5.9	− 4.3

mycelia were compared for their ability to degrade aflatoxin. Quiescently grown and aerated cultures were used for this study.

Nine-day-old mycelia of *A. parasiticus* NRRL 2999, which were grown on the glucose-salts medium at 28 C, were used as inocula. Mycelia which were grown with aeration were small enough that they could easily be transferred with a wide bore pipette when 1.0 g of mycelium (wet weight) was suspended in 2.0 ml of 0.067 M phosphate buffer, pH 6.5. Hence, very few of the mycelial pellets formed during aerated growth were broken open or fragmented when used to inoculate flasks containing the aflatoxin-salts reaction medium. The quiescently grown mycelium grew as one large, continuous mass requiring that it be cut to obtain the desired 2.0 g (wet weight) used as inoculum. This procedure was not entirely desirable because edges of the mycelium which were cut were broken open, possibly resulting in release or exposure of "degrading factor(s)." To make sure aflatoxin was not absorbed by the mycelial

inoculum but was instead released into the medium for extraction, all reaction mixtures containing 2.0 g (wet weight) of non-fragmented quiescently grown mycelium were sonicated for 45 min before chloroform extraction.

Data in Fig. 5 indicate that little or no aflatoxins B_1, B_2, G_1, or G_2, were degraded by non-fragmented mycelia produced with aeration. In contrast, the non-fragmented quiescently grown mycelium degraded detectable amounts of aflatoxins B_1 and G_1 (Fig. 6). To more readily evaluate the aflatoxin-degrading ability of mycelia produced under different conditions, a quantitative comparison of the rates of aflatoxin degradation would be helpful. Furthermore, as will be discussed in another paper, the amount of inoculum and the starting concentration of aflatoxin must be considered when comparing rates of aflatoxin degradation. This information plus rates of aflatoxin degradation caused by non-fragmented and fragmented mycelia which were grown quiescently or with agitation are given in Table 4. Comparable experiments were also done using fragmented mycelia. To most accurately compare the ability of the non-fragmented mycelia to degrade aflatoxin to that of the fragmented mycelia, the 9-day-old mycelial crop obtained under each condition was devided in half. One-half of each mycelium was fragmented and the other half remained intact before tests were made.

The dry weight of fragmented and non-fragmented mycelia grown with aeration and used as inocula were similar as was the starting concentration of aflatoxins. Substantially more aflatoxins B_1, B_2, G_1, and G_2 were degraded by fragmented than non-fragmented mycelia.

Similarly, considerably less aflatoxins B_1, B_2, G_1, and G_2 were degraded by the non-fragmented, quiescently grown mycelium than by the same mycelium which was fragmented. An additional factor must be taken into account when comparing these rates for degradation of aflatoxin. The amount of inoculum used for the non-fragmented culture was 0.42 g on a dry weight basis, whereas that for the fragmented culture was 0.12 g (dry weight). As will be discussed in another paper, the amount of mycelial inoculum affects the rate at which aflatoxin is degraded. Larger amounts of inoculum result in increased rates of aflatoxin degration. Since the amount of non-fragmented, quiescently grown mycelial inoculum was 3.5 times greater than that of the fragmented, quiescently grown mycelial inoculum, substantially more of the aflatoxins should be degraded by the non-fragmented mycelium. However, the fragmented mycelial inoculum degraded 7.7% of the initial

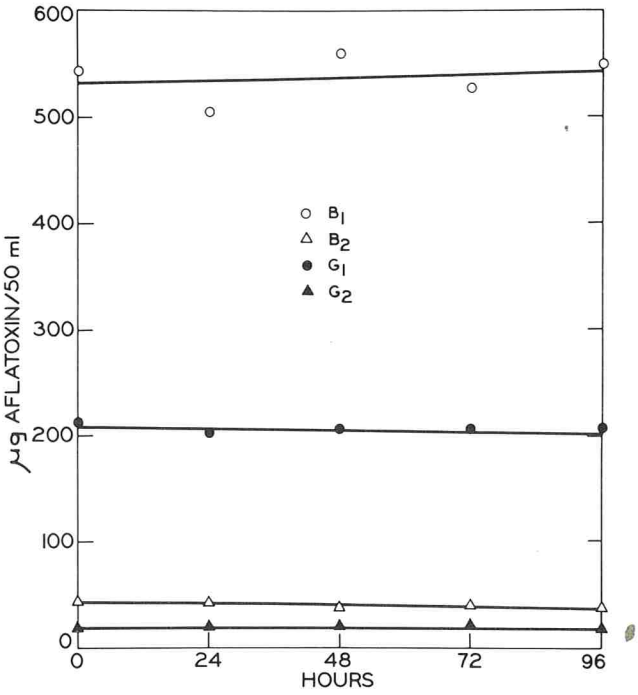

Figure 5. *Changes in the concentration of aflatoxin B_1, B_2, G_1, and G_2 caused by 9-day-old mycelia of* A. parasiticus *NRRL 2999 which were grown with aeration. Mycelia were not fragmented before they were inoculated into the aflatoxin-salts medium.*

aflatoxin B_1, 4.1% of aflatoxin B_2, 12.2% of aflatoxin G_1, and 7.5% of aflatoxin G_2 per 24-h interval, whereas the same non-fragmented mycelial inoculum degraded only 3.2% of aflatoxin B_1, 1.4% of aflatoxin B_2, 1.4% of aflatoxin G_1, and 1.8% of aflatoxin G_2 per 24-h interval.

It is possible that even less aflatoxin would have been degraded by the non-fragmented, quiescently grown mycelium had it not been cut to obtain the inoculum since some of the "degrading factor(s)" may have been exposed or released into the medium from the cut edges of mycelium. Some lysis of the non-fragmented mycelium may have also occurred thereby releasing "degrading factor(s)." It should be noted that starting aflatoxin concentrations were not equal when fragmented and non-fragmented, quiescently grown mycelia were tested. Initial concentrations for all four aflatoxins were greater for the non-fragmented than for the fragmented mycelia. Greater initial aflatoxin concentrations should favor greater rates of aflatoxin degradation (Doyle and Marth, unpublished data). Hence, this condition should favor increased degradation of aflatoxin by the non-fragmented, quiescently grown mycelium.

TABLE 4. *Degradation of aflatoxin B_1, B_2, G_1, and G_2 by fragmented and nonfragmented 9-day-old mycelia of* A. parasiticus *NRRL 2999 which were grown quiescently or with aeration.*

Mycelial inoculum	Dry weight of inoculum (g)	Initial aflatoxin conc. (µg/50 ml)				Change in aflatoxin conc. (%/24 h)			
		B_1	B_2	G_1	G_2	B_1	B_2	G_1	G_2
Non-fragmented, aerated	0.05	546	39	212	20	+0.7	−1.8	−0.2	+2.0
Non-fragmented, quiescent	0.42	645	50	287	17	−3.2	−1.4	−1.4	−1.8
Fragmented, aerated	0.05	580	40	238	19	−5.0	−3.5	−4.1	−0.5
Fragmented, quiescent	0.12	595	34	246	16	−7.7	−4.1	−12.2	−7.5

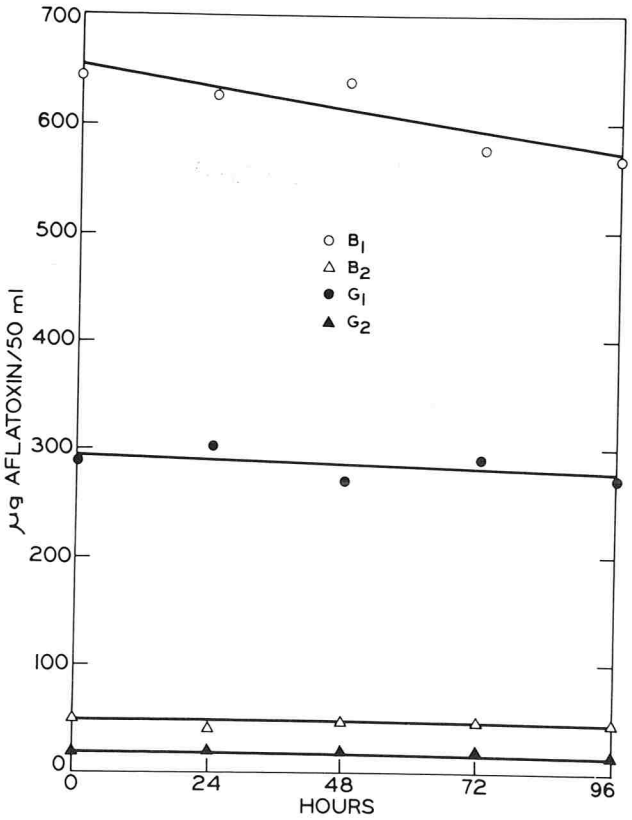

Figure 6. *Changes in concentration of aflatoxin B_1, B_2, G_1, and G_2 caused by 9-day-old mycelium of* A. parasiticus *NRRL 2999 which was quiescently grown. The mycelium was not fragmented before it was inoculated into the aflatoxin-salts medium.*

These data support the observations of Ciegler et al. (*12*), i.e. mycelial lysis is necessary for aflatoxin degradation to occur. In both instances, whether the inoculum was orginally grown quiescently or with aeration, fragmenting the mycelia resulted in substantially greater rates of aflatoxin degradation than those obtained from non-fragmented mycelia. This suggests that "degrading factor or factors" is/are present within the mycelium and by disrupting the integrity of the mycelium, this/these factor(s) is/are exposed or released into the medium and then can react with aflatoxin.

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The Minnesota Program for Prevention of Foodborne Illness: An Evaluation

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ABSTRACT

The Minnesota Quality Assurance Program for the Prevention of Foodborne Illness is a voluntarily attended, statewide education program to train foodservice owners, operators and managers in the methods of foodborne illness prevention. The education is conducted in 1-day seminars by trained sanitarians and foodservice personnel. It prepares the student to write a Quality Assurance (QA) program for his/her establishment to assure that there is no possibility of a foodborne disease outbreak. Certification is based on the approval of the Quality Assurance program. An evaluation after 9 months of operation indicates that most instructors performed adequately. Course content, including microbiological training, was well received. The QA written program requirement was supported by both instructors and students. Students strongly supported a recommendation that the QA document be mandatory for all foodservices and used as the basis for regulatory inspections.

Foodservice Manager education in the basic concepts of foodservice sanitation is a major goal of public health authorities (*1*). The expectation is that a properly educated restaurant foodservice manager/operator would be able to recognize and eliminate conditions capable of producing and transmitting foodborne illness. Implementation of this concept requires two components for its realization. The manager/operator must be able to (*a*) identify the problems involved in the operation and, having identified the problems, (*b*) take the necessary steps to eliminate them.

[1] *City of Golden Valley.*
[2] *University of Minnesota.*
[3] *These are taught either in 1-day (8-h) segments or as three 3-h segments spaced 1 week apart. Educational content is controlled by requiring all instructors to lecture from a common set of 43 overhead projection slides and to base their presentation on the contents of a special text developed by the Department of Food Science and Nutrition.*
[4] *Instructors to be qualified must have a BS in a food-related area, have experience as a sanitarian or foodservice manager and have completed a 1-day instructor training course taught by the program developer. They must also demonstrate competency in teaching.*

QUALITY ASSURANCE PROGRAM

The Minnesota Quality Assurance Program for the Prevention of Foodborne Illness represents a voluntary attempt to create the conditions by which foodservice manager/operators would learn how to identify the conditions capable of producing and transmitting foodborne illness and then be able to take action to eliminate problems. The program was developed and is managed by the University of Minnesota Agricultural Extension Service and Department of Food Science and Nutrition, and is sponsored by the Minnesota Restaurant Association and the Minnesota Department of Health.

The program consists of 8-h courses[3] on foodborne disease prevention in foodservice establishments. The courses are conducted every 4 months throughout the state by a group of 20 qualified sanitarians and foodservice personnel who are paid from the student course fee for teaching.[4] The program is self-supporting. It operates from a revolving fund at the University, and course fees pay all expenses of the program. The course prepares the manager/operator with a specific ability to conduct an operations hazard analysis, determine critical control points and specify quality assurance policies for the microbiological, physical, and chemical hazards in his or her operation. The quality assurance policies are specified by the student in the form of a Quality Assurance (QA) program. This is a policy document having sections on personnel QA duties, recipe QA procedures, training QA, cleaning and sanitizing QA and self inspection. It is tailored by the manager/operator to fit the individual physical facility, menu, organization and personnel characteristics of each foodservice establishment to assure that in each operation, there is no possibility of a foodborne illness outbreak (*2*). To complete the course the student must pass a test with a score of not less than 75% at the end of the 8-h course, and then return to his or her foodservice operation and write the QA program. This is then

submitted to the instructor and the Department of Food Science and Nutrition. When it is approved, the student is awarded the graduation certificate.

The training courses were first offered in October 1975. An evaluation was conducted in the summer of 1976 via a survey of 407 individuals completing the classroom portion of the course. The purpose was to determine, first, if the program was achieving its educational goals, and second, to obtain feedback for improvement of instruction. Responses were solicited from the 311 students from the commercial foodservice industry. Students in the not-for-profit sector were excluded to reduce the variability in the sample population.

COURSE EVALUATION

Course evaluation consisted primarily of a comparison of scores on the students pre-test and post-test, and an evaluation of student-prepared instructor-course rating sheets.[5] The evaluation also encompassed questioning of instructors on how they thought the course could be improved.

The students were also surveyed by mail to determine why they did or did not finish the course by completing a QA program. Other information was gathered in an attempt to learn the motivations that induce the student to take the course. They believed that the course should be mandatory for all commercial foodservice operators.

Those students who completed a QA program were asked about the adequacy of the program, whether or not they are complying with the program, and if not, what areas are a problem and for what reason. Food service establishments from the City of Golden Valley that had submitted a plan were interviewed to determine compliance with the U.S. Public Health Service 1962 Model Food Code and Ordinances (*3*), and their past history from Health Department records was also reviewed.

RESULTS AND DISCUSSION

The questionaire was returned by 69 (22%) of the students who did not complete the QA program and 16 (73%) of the students who did complete such a program. The overall improvement in knowledge was 40% as evaluated by the difference between the pre-test and post-test course scores.

The instructor rating sheets were useful in identifying problems and potential problems in teaching effectiveness of instructors. Two instructors, while receiving satisfactory numerical ratings, also received a few negative written comments on teaching methods.

Instructors generally indicated in their questionaire responses that they felt they had an adequate background and preparation for teaching the course. There was a feeling by instructors that the voluntary nature of the program precluded attendance by those who needed the instruction the most. Consequently, total QA program effectiveness would lag until a mandatory program is initiated. The instructors were enthusiastic about the program and apprarently actively promote attendance by foodservice operators whom they contact in the couse of their other duties as sanitarians.

As for the students, 85% felt the course was at least equal to if not better than, other courses previously attended. The 15% who indicated it was not at least equally as good centered their comments on the teaching techniques of the two instructors mentioned previously. Ninety-seven percent of the respondents indicated that the course provided useful information for prevention of foodborne illness in their restaurants operations. Of those who submitted QA programs, 66% indicated they had saved money and produced greater efficiency in their establishments because of the information presented in the course.

The respondents did not suggest changing the course content. The primary comment was "do not change anything." The course contains an intensive 2-h segment on microbiology of foodborne illness. The students felt that this information was very beneficial and interesting to them in establishing food handling procedures for prevention of microbiological growth. This was an important finding because there was some concern on the part of the course sponsors that the microbiology would be rejected as being too detailed.

Students were also asked how they felt about being required by the health department to have a Quality Assurance program. Eighty one percent (90% of those submitting QA programs) said they would like to be required to have a QA program and have the health department inspections based on that program. In addition, 92% of the students said they would recommend the course to their colleagues. This last statistic is supported by the number of additional individuals from the same company who attended subsequent courses.

Comments on the teaching facilities were also included in the survey. Instructors have the obligation to find free facilities wherever they present the course in the state. Some of these were apparently not very comfortable or conducive to teaching.

The student respondents who submitted QA programs differed in general characteristics from those who did not. They had more employer support and a higher educational level. It was noteworthy that 63% of those responding indicated that they had had post high school education.

Among those who did not submit a program, 60% indicated they were working on one, and 43% indicated it would be ready in 6 weeks. This seems to indicate good

[5] *The 10 question pre-test was given before beginning classroom instruction and the 40 question post-test given at the end of the 8-h course. Questions were intended to measure the student's knowledge of foodborne illness prevention. Increase in knowledge was measured by subtracting percent correct on the pre-test from percent correct on the post-test. The instructor rating sheet was completed before the student left the course.*

support for a program that is voluntary.

Reasons for not submitting QA programs were varied. Lack of any requirement to complete such a program allows completion to be put off until later. There were also some who indicated that they needed additional individualized assistance and encouragement. Palmer et al. (*4*) also found this in their sanitation training program. The need for individualized coaching and counseling was evident in three on-site evaluations. In these three foodservices, it was determined through interviews with management that QA policies as taught in the program were not fully implemented due to management not giving the program a high enough priority, and by management's failure to be able to fully apply what was taught. The 8-h course contains a great amount of information. There could be a greater retention of and ability to apply the course material if it were taught in a 15- or 24-h course, although 95% of the students indicated that they did not want a course any longer than 8 h. This learning effectiveness problem might be overcome if the local food sanitarian who inspects the restaurant would provide post-course assistance in resolving questions, and giving guidance in writing the QA program. Following this, the sanitarian must encourage and counsel the manager in the implementation of the written QA program.

SUMMARY

The actual course content appears to meet the needs of the students, as no changes were recommended by the respondents. The student's pre-test and post-test course examinations indicated that they gained quite a bit of new information in the course on the prevention of foodborne illness. The students had positive responses to questions asking if they received useful information, if the course was equal to if not better than others attended, and if they had saved money and increased efficiency because of application of knowledge gained in the course. A most important finding for future planning related to mandatory certification is that two out of three of the students supported the objective that Quality Assurance programs should be mandatory and that the programs should be used as a guide for the health department's inspection. One Minnesota municipality has begun a program to mandate such programs for all its foodservice establishments. A problem that remains is motivating all owner/operators to take time to submit QA programs and then to fully implement them. This motivation can be helped through an educational program of encouraging the consumer to look for the Quality Assurance Educational Certificate in foodservices, and fuller support by all foodservice inspectors in helping owner/operators with their individual sanitation programs.

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Foodborne Disease in Six Countries — A Comparison

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ABSTRACT

Foodborne disease data from six countries were compared. The number of outbreaks ranged from 48 for Australia to 6,109 for Japan, both over 5-year periods. *Salmonella* sp. , *Staphylococcus aureus* and *Clostridium perfringens* were the agents primarily responsible for illness in most of the countries. *Vibrio parahaemolyticus,* however, was the most significant agent in Japan. Meat and poultry were the foods most implicated in illness, but fish was also important in Japan and the United States. Foodservice establishments seem to have been the main places where food was mishandled, causing subsequent illness. less, however, is known of the contributory factors that led to such illness, although for the United States, the main one appears to have been improper holding temperatures. To permit a better and more complete comparison in the future, surveillance systems of different countries need to be developed on common criteria.

Foodborne disease is a world-wide problem and according to WHO is on the increase, at least in Europe (*13*). There are, however, relatively few data to assess the world situation. Foodborne disease surveillance systems have been organized in a limited number of countries, and few of these countries publish their data on a regular basis. In addition, some reports are not easy to locate, for they are produced for the local situation only. The present study compares foodborne disease statistics from six countries in four distinct geographic regions.

Food poisoning in England and Wales did not become a notifiable disease until 1938. The statistics of this disease were reported in the *Monthly Bulletin of the Ministry of Health and Public Health Laboratory Service* from 1949 until 1966 (*1*); since 1969 the data have been published in a scientific journal, but not on an annual basis. The latest statistics in print are for 1973-1975 (*12*). Japan has reported the number of persons ill from food poisoning since at least 1949, but detailed information has been available only since 1955 (*5*). The data for this comparison are for 1968-1972 (*14*), except those dealing with the food involved, which are for 1968-1970 (*5*). The United Stated has been collecting information on food-associated diseases since 1938, although the present annual Center for Disease Control publication began only in 1966. The years compared in this paper are 1973 (*9*), 1974 (*10*) and 1975 (*11*). Canada first reported detailed foodborne disease statistics in 1976, for 1973 data (*3*). Annual summaries for 1974 and 1975 are also available (*4,8*). Australia has not made any attempt to collect information on foodborne disease systematically; data for 5 years (1967-1971) were derived from a paper given at an international symposium (*7*). For the six countries data from two main time periods (1967-1972 and 1973-1975) are presented, which limits the accuracy of the comparisons, but is sufficient to show trends.

Facts on outbreaks, cases, etiological agents, foods implicated and places where the food was mishandled are discussed. Single (also called sporadic) cases were excluded from this study, as Canada, United States and England and Wales include them in their statistics while Japan and Australia do not; an exception was made for the comparison of foodborne disease mortality. Some microbiological agents, apparently implicated in food-borne incidents, have not yet been internationally accepted as causal agents. For the purposes of this presentation, they are called suspect, e.g., suspect *Pseudomonas aeruginosa.* The following definitions are to help interpret the data:

(*a*) *Outbreak.* An incident in which two or more persons experience a similar illness after ingestion of the same food and where epidemiological evidence implicates the food as the source of illness.

(*b*) *Single (sporadic) case.* One case, as far as can be ascertained, unrelated to other cases in respect to food consumed.

(*c*) *Microbiological.* Pertaining to viruses and to cells or products of cells of bacteria, yeasts, or microscopic fungi.

(*d*) *Parasitic.* Pertaining to round worms, tapeworms, protozoa or flukes which invade human tissue through the alimentary tract.

(*e*) *Plant.* Pertaining to whole or part of vegetable material, including flowers, trees, and mushrooms and to toxic substances produced by these.

(*f*) *Animal.* Pertaining to whole or part of an animal, including poisonous fish and shellfish where toxicity may be caused by bacterial or algal action.

(*g*) *Chemical.* Pertaining to substances produced by a chemical process, including metals, cleaning solutions, pesticides and rancid compounds.

Etiological agents were grouped into the categories shown in Table 1, even though this meant rearranging material from the national reports, e.g., those listed under "chemical" in the U.S. reports are put under "plant", "animal" and "chemical" headings according to the above definitions. "Natural poisons" which appear in reports from Japan are listed under the combined heading of "animal/plant."

RESULTS AND DISCUSSION

Outbreaks are shown in Table 1; Japan had the greatest number (6,109), Australia the least (48), both over 5-year periods. All countries recorded outbreaks of unknown etiology which ranged from 84.0% (Canada) to 0.6% (England and Wales). The large variation in the number of outbreaks where no agent was found probably reflects differences in efficacy of reporting rather than differences in analytical competence. Microbiological agents were primarily responsible for causing outbreaks of known etiology. Parasitic, plant, animal and chemical agents were less important, except for the natural poisons (plant/animal) of Japan which accounted for 11.2% of all outbreaks. For outbreaks where the etiology was known, the greatest number per 100,000 population was found in England and Wales (1.32) and Japan (0.59), although Canada had the greatest number of total outbreaks per 100,000 (2.18); the United States and Australia had relatively low figures (Table 2). The breakdown by etiology of cases and outbreaks is similar, except that proportionally more cases resulted from microorganisms than from the other agents (Table 3). This indicates that microbially-associated outbreaks tended to be larger than other outbreaks. On a population basis (Table 4), Japan had the highest number of cases per 100,000 for all agents (33.62). The United States and Australia had the lowest numbers.

The characteristics of specific microbiological agents are shown in Table 5. *Salmonella* was by far the dominant foodborne disease agent (86.6%) in England and Wales. In Canada and the United States *Staphylococcus aureus* was first, followed closely by *Salmonella.* Both these organisms were also significant in Australia (27.3%), but *Clostridium perfringens* was the most common foodborne pathogen there with responsibility for a third of the outbreaks. *C. perfringens* ranked third in the United States (12.7%) and Canada (11.1%). In Japan *Vibrio*

TABLE 1. *Foodborne outbreaks in six countries — number and percentage.*

Etiology	Canada 1973-75		U.S.A. 1973-75		England/Wales 1973-75		Japan 1968-72		Australia 1967-71	
	No.	%	No.	%	No.	%	No.	%	No.	%
Microbiological	199	13.8	316	26.4	1,946	99.4	3,217	52.7	33	68.8
Parasitic	8	0.5	45	3.8	—	—	—	—	—	—
Plant	2	0.1	11	0.9	—	—				
Animal	1	0.1	66	5.5	—	—	686[a]	11.2[a]	—	—
Chemical	21	1.5	20	1.7	—	—	60	1.0	—	—
Unknown	1,209	84.0	741	61.7	11	0.6	2,146	35.1	15	31.2
Total	1,440	100.0	1,199	100.0	1,957	100.0	6,109	100.0	48	100.0

[a]Plant/animal combined.
— = Not reported

TABLE 2. *Foodborne outbreaks in six countries — annual number per 100,000 population.*

Etiology	Canada 1973-75		U.S.A. 1973-75		England/Wales 1973-75		Japan 1968-72		Australia 1967-71	
	No. of out-breaks	No. per 100,000 population[a] per year	No. of out-breaks	No. per 100,000 population[a] per year	No. of out-breaks	No. per 100,000 population[a] per year	No. of out-breaks	No. per 100,000 population[a] per year	No. of out-breaks	No. per 100,000 population[a] per year
Microbiological biological	199	0.30	316	0.05	1,946	1.32	3,217	0.59	33	0.05
Other agents agents	32	0.04	142	0.02	—	—	746	0.14	—	—
Unknown	1,209	1.84	741	0.12	11	0.01	2,146	0.39	15	0.02
Total	1,440	2.18	1,199	0.19	1,957	1.33	6,109	1.12	48	0.07

[a]Based on 1973 estimates for each country (*6*): Canada 21,960,000; United States 209,650,000; England and Wales 49,045,000; Japan 108,810,000; Australia 13,090,000.
— = Not reported.

TABLE 3. *Cases of foodborne outbreaks in six countries — number and percentage.*

Etiology	Canada 1973-75		U.S.A. 1973-75		England/Wales 1973-75		Japan 1968-72		Australia 1967-71	
	No.	%	No.	%	No.	%	No.	%	%	No.
Microbiological	5,467	37.5	23,061	46.9	13,996	98.2	119,897	65.5	—	
Parasitic	116	0.8	314	0.6	—	—	—	—	—	—
Plant	4	0.0	52	0.1	—	—	3,029[a]	1.7[a]	—	—
Animal	43	0.3	588	1.2	—	—			—	—
Chemical	138	1.0	156	0.3	—	—	1,952	1.1	—	—
Unknown	8,805	60.4	25,043	50.9	250	1.8	58,022	31.7	—	—
Total	14,573	100.0	49,214	100.0	14,246	100.0	182,900	100.0	2,500	100.0

[a]Plant/animal combined.
— = Not reported.

parahaemolyticus was the most important agent (52.2%), followed by *S. aureus* (27.6%) and *Salmonella* (12.8%); no *C. perfringens* illnesses were reported. Reports of outbreaks from *Bacillus* sp. (mainly *B. cereus*) were restricted to the four western countries, which may reflect the relatively new status of *Bacillus* as a food-poisoning agent. In contrast, because of its seriousness, botulism is probably the most completely reported of all foodborne diseases; it was of some significance in Canada and the United States and, to a lesser extent, in Japan.

Escherichia coli has caused a few outbreaks in all the countries at one time or another, but no outbreaks were reported in the United States during the years of this study; the organism appears to have had its biggest impact in Japan. Apart from *Shigella* and hepatitis A in the United States, the other agents listed in Table 5 are of lesser significance. Fecal streptococci, *P. aeruginosa* and yeast and molds are not universally recognized as foodborne disease agents, although they were implicated in outbreaks in Canada, the United States and Australia. Japan listed but did not define "other bacteria".

Salmonella serotypes isolated from food and/or patients during outbreaks in Canada, the United States and England and Wales are shown in Table 6. The serotypes are listed in order of number of cases associated with the outbreaks (no information on outbreaks by serotype was available for England and Wales). Within the first 10 serotypes, four are common for Canada and the United States, five are common for United States and England and Wales, and three for Canada and England and Wales. *Salmonella infantis, Salmonella typhimurium,* and *Salmonella enteritidis* were the only serotypes found in the top 10 of all the countries compared. These rankings also compare favorably with those of serotypes from human and non-human isolations for each country; five or more serotypes in the first 10 were the same.

Foodborne shigellosis was reported only from the United States and Canada. When the data for the two countries are combined for 1973-1975, *Shigella sonnei* was the serotype both most frequently associated with foodborne illness (11 outbreaks) and with human isolations. Outbreaks also occurred due to *Shigella flexneri* types 2A and 3A, the next most frequently isolated serotypes from human sources in North America.

Non-microbiological agents that caused outbreaks are shown in Table 7. Australia, England and Wales did not

TABLE 4. *Cases of foodborne outbreaks in six countries — annual number per 100,000 population.*

Etiology	Canada 1973-75		U.S.A. 1973-75		England/Wales 1973-75		Japan 1968-72		Australia 1967-71	
	No. of cases	No. per 100,000 population[a] per year	No. of cases	No. per 100,000 population[a] per year	No. of cases	No. per 100,000 population[a] per year	No. of cases	No. per 100,000 population[a] per year	No. of cases	No. per 100,000 population[a] per year
Micro-biological	5,467	8.30	23,061	3.67	13,996	9.51	119,897	22.04	—	—
Other agents	301	0.45	1,110	0.18	—	—	4,981	0.92	—	—
Unknown	8,805	13.37	25,043	3.98	250	0.17	58,022	10.66	—	—
Total	14,573	22.12	49,214	7.83	14,246	9.68	182,900	33.62	2,500	3.82

[a]Based on 1973 estimates for each country (*6*): Canada 21,960,000: United States 209,650,000; England and Wales 49,045,000; Japan 108,810,000; Australia 13,090,000.
— = Not reported

TABLE 5. *Foodborne outbreaks in six countries caused by microbiological agents.*

Etiology	Canada 1973-75		U.S.A. 1973-75		England/Wales 1973-75		Japan 1968-72		Australia 1967-71	
	No.	%	No.	%	No.	%	No.	%	No.	%
Arizona hinshawii	—	—	1	0.3	—	—	—	—	—	—
Bacillus sp.	8	4.0	5	1.6	52	2.7	—	—	—	—
Brucella sp.	—	—	1	0.3	—	—	—	—	—	—
Clostridium botulinum	8	4.0	19	6.0	—	—	15	0.5	—	—
Clostridium perfringens	22	11.1	40	12.7	150	7.7	—	—	11	33.3
Escherichia coli	1	0.5	—	—	3	0.1	122	3.8	1	3.0
Salmonella sp.	58	29.2	106	33.5	1,685	86.6	412	12.8	9	27.3
Shigella sp.	2	1.0	14	4.4	—	—	—	—	—	—
Staphylococcus aureus	80	40.2	107	33.9	50	2.6	889	27.6	9	27.3
Vibrio cholerae	—	—	1	0.3	—	—	—	—	—	—
Vibrio parahaemolyticus	1	0.5	3	1.0	6	0.3	1,679	52.2	1	3.0
Yersinia enterocolitica	1	0.5	—	—	—	—	—	—	—	—
Group A *Streptococcus*	1	0.5	2	0.6	—	—	—	—	2[a]	6.1[a]
Other suspect streptococci	8	4.0	3	1.0	—	—	—	—		
Suspect *Pseudomonas aeruginosa*	1	0.5	—	—	—	—	—	—	—	—
Suspect mold and yeast	8	4.0	—	—	—	—	—	—	—	—
Other bacteria	—	—	—	—	—	—	100	3.1	—	—
Hepatitis A	—	—	14	4.4	—	—	—	—	—	—
Total microbiological	199	100.0	316	100.0	1,946	100.0	3,217	100.0	33	100.0

[a]All streptococci
— = Not reported

record illnesses caused by any of these agents. Trichinosis was the main parasitic disease in North America, but it was not reported in Japan. A large number of outbreaks, however, were caused by plants and animals in Japan, but the specific agents were not indicated. The United States had a significant number of fish-associated illnesses mainly caused by ciguatoxin and scombroid toxin; these poisonings tended to occur in tropical and sub-tropical areas, such as the southern coastal regions and island territories in the Pacific. Canada reported proportionally more chemical poisonings than the other countries; leaching of heavy metals from food containers was a prominant cause.

Case-to-outbreaks ratios for some etiological agents are shown in Table 8. Good correlation between Canada and the United States was obtained for *Bacillus* sp. and *C. perfringens*, and between all three countries for *C. botulinum*. For the other agents, however, there was more variation in the ratios. Ratios were generally higher for the United States than for Japan or Canada. *C. perfringens*, *Salmonella* sp., *Shigella* sp. and, to a lesser extent *S. aureus*, caused the larger outbreaks.

Deaths resulting from foodborne illnesses were reported for all countries except Australia (Table 9, which, however, excludes data for Japan because of limited information available). Data for single (sporadic) cases were included in this table, because deaths occurred in single cases, and because it was not possible to separate deaths from outbreaks and from sporadic cases for England and Wales. The ratio of deaths to cases expressed as a percentage ranged from 0.08% for the United States to 0.46% for England and Wales. *Salmonella* caused the death of more persons than any other agent in England and Wales (0.6%). In Canada and the United States, botulism had the highest mortality rate, though trichinosis and paralytic shellfish poisoning in Canada, and cholera, mushroom and chemical poisoning in the United States, also had serious consequences. Data available from Japan were limited to those for *V. parahaemolyticus* (17 deaths, 0.04%) and for

TABLE 6. *Comparison of* Salmonella *serotypes isolated during foodborne outbreaks in Canada, the United States and England and Wales, 1973-75.*

Canada					U. S. A.					England and Wales			
	Number of		Rank of serotypes[a] from			Number of		Rank of serotypes[b] from				Rank of serotypes from	
Serotype	Cases	Outbreaks	Human sources	Non-human sources	Serotype	Cases	Outbreaks	Human sources	Non-human sources	Serotype	Number[c] of Cases	Human sources	Non-human sources[d]
S. typhimurium	873	18	1	1	*S. newport*	3,583	8	2	4	*S. typhimurium*	8,396	1	3
S. enteritidis	323	9	2	5	*S. typhimurium*	2,035	23	1	1	*S. agona*	3,686	2	1
S. san-diego	244	3	>10	>10	*S. blockley*	708	3	>10	>10	*S. enteritidis*	2,602	3	7
S. saint-paul	184	5	3	3	*S. saint-paul*	647	4	6	7	*S. heidelberg*	1,178	4	>10
S. montevideo	140	2	6	6	*S. infantis*	504	8	4	9	*S. anatum*	1,009	5	6
S. eastbourne	107	2	>10	>10	*S. enteritidis*	451	11	3	>10	*S. indiana*	931	6	9
S. münchen	100	1	>10	>10	*S. reading*	403	3	>10	>10	*S. newport*	908	7	10
S. thompson	78	2	5	4	*S. oranienburg*	383	2	>10	10	*S. infantis*	698	8	2
S. paratyphi B	59	2	>10	>10	*S. agona*	296	4	7	>10	*S. hadar*	512	9	8
S. infantis	42	6	4	2	*S. dublin*	179	4	>10	>10	*S. bredeney*	494	10	10

[a]Data from Laboratory Centre for Disease Control, Health and Welfare Canada.
[b]Data from Salmonella Surveillance Report Nos. 121, 125, 126, Center for Disease Control, Atlanta, Georgia.
[c]Number of outbreaks not known.
[d]Ranking is within 20 serotypes only and is according to number of cases.

TABLE 7. *Foodborne outbreaks in Canada, the United States and Japan caused by parasitic, plant, animal and chemical agents.*

Etiology	Canada 1973-75		U. S. A. 1973-75		Japan 1968-72	
	Number	Percentage	Number	Percentage	Number	Percentage
PARASITIC						
Trichinella spiralis	8	25.0	44	31.0	—	—
Toxoplasma gondii	—	—	1	0.7	—	—
PLANT						
Mushroom poisons	1	3.1	8	5.6		
Other plant posions	1	3.1	3	2.1		
ANIMAL					686[a]	92.0[a]
Ciguatoxin	—	—	39	27.5		
Scombroid and related toxins	—	—	23	16.2		
Tetrodotoxin	—	—	1	0.7		
Paralytic shellfish poison and related toxins	1	3.1	3	2.1		
CHEMICAL						
Metals	7	21.9	7	4.9		
Monosodium glutamate	—	—	6	4.2	60[b]	8.0[b]
Other chemicals	14	43.8	7	4.9		
Total	32	100.0	142	99.9	746	100.0

[a]Plant and animal combined
[b]Chemical agent not specified
— Not reported

the total for all agents (228 deaths, 0.15%).

Foods associated with illness are shown in Table 10. A high percentage of outbreaks in England and Wales had no specific food associations (82.9%). Nevertheless, meat was the main vehicle involved in those countries (6.7%), in Canada (30.8%) and in the United States (22.3%). Beef was the most widely implicated product, with *C. perfringens* as important etiological agent. *Salmonella* was recovered from a variety of meat products, but *S. aureus* was most closely linked to ham. Poultry, too, was important, though less so in the United States (5% for the latter up to 22.9% for Australia). *Salmonella* and, to a

TABLE 8. *Foodborne outbreaks in three countries — case-to-outbreak ratio for selected agents.*

Etiology	Canada 1973-75			U. S. A. 1973-75			Japan 1968-72		
	Cases	Outbreaks	Ratio	Cases	Outbreaks	Ratio	Cases	Outbreaks	Ratio
Bacillus sp.	107	8	13	58	5	12	—	—	—
Clostridium botulinum	24	8	3	55	19	3	65	15	4
Clostridium perfringens	1,291	22	59	2,706	40	68	—	—	—
Salmonella sp.	2,248	58	39	7,178	106	68	23,142	412	56
Shigella sp.	81	2	41	2,013	14	144	—	—	—
Staphylococcus aureus	1,526	80	19	6,903	107	65	30,976	889	35
Vibrio parahaemolyticus	2	1	2	224	3	75	43,247	1,679	26
All microorganisms	5,467	199	28	23,061	316	73	119,897	3,217	37
Trichinella spiralis	116	8	15	314	45	7	—	—	—
Plant and animal	47	3	16	640	77	8	3,029	686	4
Chemical	138	21	7	156	20	8	1,952	60	33
Unknown	8,805	1,209	7	25,043	741	34	58,022	2,146	27
Total	14,573	1,440	10	49,214	1,199	41	182,900	6,109	30

— = Not reported

TABLE 9. *Deaths associated with foodborne incidents in Canada, the United States and England and Wales, 1973-75.*

Etiology	Canada			U. S. A.			England and Wales		
	Deaths[a]	Cases[b]	Percentage[c]	Deaths	Cases	Percentage	Deaths	Cases	Percentage
Clostridium botulinum	7	31	22.6	13	82	15.9	—	—	—
Clostridium perfringens	—	1,291	—	3	2,706	0.1	2	6,498	0.03
Salmonella sp.	1	2,253	0.04	10	9,534	0.1	131	21,428	0.6
Staphylococcus aureus	3	1,550	0.2	—	6,904	—	1	714	0.1
Vibrio cholerae	—	—	—	1	6	16.7	—	—	—
Vibrio parahaemolyticus	—	2	—	—	224	—	—	118	—
Hepatitis A	—	—	—	1	880	0.1	—	—	—
Trichinella spiralis	3	121	2.5	2	310	0.7	—	—	—
Mushroom poison	—	4	—	3	55	5.5	—	—	—
Paralytic shellfish poison	2	44	4.6	—	7	—	—	—	—
Chemicals	—	156	—	2	27	7.4	—	—	—
Others	—	407	—	—	3,067	—	—	350	—
Unknown	—	8,933	—	4	22,394	0.02	—	—	—
Total	16	14,792	0.11	39	46,196	0.08	134	29,108	0.46

[a]Deaths = number of deaths.
[b]Cases = number of cases, including single and sporadic cases.
[c]Percentage = ratio of deaths to cases expressed as a percentage.
— = Not reported.

TABLE 10. *Foods associated with foodborne outbreaks in six countries.*

Vehicle	Canada 1973-75			U. S. A. 1973-75			England and Wales 1973-75			Australia 1967-71			Japan 1968-72		
	Number	Percentage	Ranking	Number	Percentage	Ranking	Number	Percentage	Ranking	Number	Percentage	Ranking	Number	Percentage	Ranking
Meat	444	30.8	1	268	22.3	1	131	6.7	1	10	20.8	2	—	—	—
Poultry	137	9.5	2	60	5.0	4	103	5.3	2	11	22.9	1	—	—	—
Fruits and vegetables	111	7.7	3	39	3.3	6	—	—	—	—	—	—	—	—	—
Bakery foods	95	6.6	4	35	2.9	8	—	—	—	—	—	—	—	—	—
Fish and shellfish	84	5.8	5	112	9.3	2	10	0.5	5	6	12.5	3	1,270	35.4	1
Chinese foods	77	5.4	6	36	3.0	7	48	2.5	3	—	—	—	—	—	—
Salads	41	2.9	7	68	5.7	3	—	—	—	—	—	—	—	—	—
Dairy foods	36	2.5	8	42	3.5	5	32	1.6	4	—	—	—	—	—	—
Beverages	29	2.0	9	30	2.5	9	—	—	—	—	—	—	—	—	—
Eggs	3	0.2	10	—	—	—	—	—	—	—	—	—	—	—	—
Other foods	129	9.0	—	170	14.2	—	10	0.5	5	6	12.5	—	1,113	31.0	—
Unknown foods	254	17.6	—	339	28.3	—	1,623	82.9	—	15	31.3	—	1,203	33.6	—
Total	1,440	100.0		1,199	100.0		1,957	100.0		48	100.0		3,586	100.0	

— = Not reported

lesser extent, *S. aureus* were significant agents in poultry outbreaks. Fish and shellfish ranked high in Japan with 35.4% of the outbreaks, and in the United States with 9.3%; in Japan *V. parahaemolyticus* played an important role, whereas in the United States it was fish toxins that caused the greatest number of fish-related outbreaks. Dairy products accounted for less than 10% for all countries; and egg, excluding that used in products with multiple ingredients, was associated in only 0-0.2% of outbreaks.

Surprisingly, the factors that contribute to illness were not tabulated, except for the United States. In that country, where the factors were known (about 50%), the main one seemed to be improper holding temperatures (67%), followed by inadequate cooking (26%), poor personal hygiene (26%), contaminated equipment (19%) and food from unsafe source (13%), e.g., toxic plant or animal products.

Detailed information on places where food was mishandled was only available for Canada, the United States and England and Wales, although places of mishandling were not easily distinguishable from locations of outbreaks from the data given for England and Wales (Table 11). Foodservice establishments (particularly restaurants in the North American countries and hospitals in England and Wales) were the main places problems occurred (25.4-33.2%). Outbreaks also occurred because of problems in private homes in Canada and the United States (14.4-17.0%), but these are not specifically mentioned for England and Wales. Farms, however, were blamed for illness in these last-named countries; this was mainly as a result of consumption of unpasteurized milk contaminated with *Salmonella* sp. Even though food-processing establishments were responsible for relatively few outbreaks (0-5.3%), the impact of poor manufacturing practices in producing a widely-distributed product could be very great, e.g., the *Salmonella eastbourne* outbreak where infected chocolate candy caused several hundred cases of illness in both Canada and northeastern United States (2). Proper training of foodservice employees and education of those responsible for food in the home with due attention to factors that contribute to illness would help in reducing foodborne disease.

Amongst the data compared, the following similarities were found between countries: micoorganisms were the principal etiological agents involved in foodborne illness; some of the main *Salmonella* serotypes isolated during outbreaks occurred with similar frequencies in Canada, the United States and England and Wales; meat was the major food item associated with illness; and mishandling of food that led to disease was greatest at foodservice establishments in the countries where information was available. Differences were also noted: the restriction of botulism to North America and Japan and of trichinosis to North America; the high incidence of *Salmonella* in England and Wales, and of *V. parahaemolyticus* in Japan; and the greater significance of fish and shellfish in Japan and the United States than in other countries. Nevertheless, the data that are published are influenced by ways in which each country collects and reports them. For instance, the number of outbreaks due to unknown etiology ranging from 0.6% to 84.0%, and the lack of reporting of chemical poisonings for England, Wales and Australia, indicates different national approaches to surveillance of foodborne disease.

For the years compared, it is not possible to say whether some of these countries have less foodborne disease than others, or whether they have less efficient methods of data gathering. Therefore, although some conclusions can be drawn from the material presented, detailed comparisons will be difficult to make until more uniform systems of surveillance and reporting are followed.

TABLE 11. *Places where food in outbreaks was mishandled in Canada, the United States and England and Wales, 1973-75.*

Establishment	Canada		U. S. A.		England and Wales	
	No.	%	No.	%	No.*	%
Foodservice	425	29.5	398	33.2	497	25.4
Homes	245	17.0	173	14.4	—	—
Food processing	75	5.2	37	3.1	—	—
Retail food	52	3.6	—	—	29	1.5
Farms	2	0.2	—	—	48	2.4
Other[a]	13	0.9	591[b]	49.3[b]	1,383[b]	70.7[b]
Unknown	628	43.6				
Total	1,440	100.0	1,199	100.0	1,957	100.0

[a]Locations of outbreaks and places of mishandling are not clearly separated.
[b]Other and unknown combined.
— = Not reported.

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Food Additives and Safety

Probably owing to the increased national interest in foods and nutrition, some individuals have questioned the safety of food additives in our food supply in general and dairy foods in particular. Certainly a number of additives are used in certain dairy foods, such as colorings in cheese or preservatives in ice cream. There is, however, no need for consumer concern over the use of such additives.

Food additives are subjected to rigorous and extensive testing to prove their safety before the Food and Drug Administration approves their use.

In addition to safety, the following requirements must be met: 1.) additives must perform a useful function, 2.) additives must not be put into food to deceive the consumer, 3.) they must not cause a substantial reduction in the food's nutritional value, 4.) additives must not be used to obtain an effect that could be obtained by otherwise good manufacturing practices, and 5.) a method of analysis must exist to monitor additives' use in foods.

After a chemical substance meets these requirements, FDA defines a safe level—a small fraction of the highest percentage in the diet that can be fed to test animals without harm. The normal safe level is often based on a 100-fold safety factor of the "no adverse effect" level.

In 1976, FDA developed a new, far-reaching program to provide review of all food additives. Under this program, all food additives will undergo periodic scientific re-evaluation.

Before a proposed food additive is permitted for use, some significant direct or indirect benefit to the food consumer must be demonstrated and the degree of risk must be commensurate with the relative importance of the presumed benefit. For example, while prolonged intake of the high levels of vitamin D may be toxic, the addition of 400 International Units of vitamin D to each quart of milk is credited with the virtual elimination of rickets in this country.

On the other hand, there occasionally arises an erroneous statement as to additives in foods. For example, some have said that formaldehyde is added to milk. There is no basis for this claim. Formaldehyde is never added to market milk.

Similarly, confusion sometimes exists regarding chemical additives and their functions. A chemical called piperonal is a minor constituent of vanilla flavoring used in ice cream; it is present in minute amounts. But the same chemical structure is present in insecticides. Therefore people erroneously have said that insecticides are in ice cream. This is like saying cyanide, a deadly poison, is in lima beans or that arsenic is present in seafoods. Yes, they are naturally present, but in minute quantities and at the levels consumed they present no threat to health. We surely would not avoid these wholesome foods on this basis.

Chemical names and formulas are mysterious and perhaps even frightening-sounding to the untrained person. The fact remains, however, that everything in the world—food, medicine, and even water—has a chemical name and a chemical formula. Sodium chloride, for example, is the chemical name for ordinary table salt.

Today's food supplies in general, and dairy foods in particular, are nutritious and safe. Chemical additives are carefully regulated and should not be a concern for the consumer.

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Clostridium botulinum and Acid Foods[1,2]

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ABSTRACT

Outbreaks of botulism involving acid foods are rare. Of the 722 total botulism outbreaks reported from 1899 to 1975, only 34 (4.7%) involved acid foods. Home-canned acid foods were implicated in 34 of the 35 acid food outbreaks. *Clostridium botulinum* cannot grow at a pH of ⩽ 4.6; therefore, for a botulism hazard to exist in an acid food, a contamination with other microorganisms due to a process delivery failure and/or post-process contamination, (*c*) favorable composition of the food and storage conditions which are particularly conducive to *C. botulinum* growth and toxin production, and (*d*) metabiosis. The way each factor affects the botulism hazard in acid foods is discussed in this report. An acid food is safe from *C. botulinum* if the heat process kills all organisms capable of growth at a pH of ⩽ 4.6 and there is no post-process contamination.

Clostridium botulinum food intoxication (botulism) has been recognized as a public health problem since 1793 (*20*). Compared to outbreaks caused by other food poisoning microorganisms, outbreaks of botulism are rare but the fatality rate is high (*6*). In 1974 the fatality rate was 23%.

Seven types (A, B, C, D, E, F, and G) of *C. botulinum* are currently recognized on the basis of antigenically distinct toxins (*53*). Types A, B and E are the principal causes of the disease in man. Botulism caused by type A or B toxins is associated with canned vegetables, fruits and meats. Botulism caused by Type E toxin is associated with fish or various types of marine products.

Botulism is primarily considered a hazard of low-acid canned food; however, an often overlooked fact is that botulism occurs at a low rate in acid foods. Home-canned acid foods account for 97% of these acid food outbreaks. Home-canned acid foods were implicated in 34 (4.7%) of the 722 reported outbreaks of food-borne botulism from 1899 through 1975 (*6,9,10,35*). This number is too large to be neglected or written off to faulty diagnosis. Accordingly, *C. botulinum* must be regarded as a potential public health hazard in acid foods.

Outbreaks of botulism attributed to home-canned acid foods, especially tomato products, are a concern of consumers, governmental agencies and academic institutions (*23,42,45,46,50*).

This is a review, analysis and discussion of the problem of botulism in canned acid foods. The study leading to this report was carried out with the hope that if the conditions responsible for *C. botulinum* growth and toxin production in acid foods can be identified and put in perspective, then we will be better able to develop a positive control program.

OUTBREAKS OF BOTULISM INVOLVING ACID FOODS

In Table 1 are listed the number of botulism outbreaks that have been recorded in the United States from 1899 through 1975. Since 1899, 9% of the botulism outbreaks have been traced to commercially processed foods (Table 1). The products involved in these outbreaks have all been low-acid foods except for one outbreak involving tomato catsup in 1915 (*21,35*). This is a very good record for the canning industry considering that probably more than 775 billion cans of commercially canned foods have been consumed since 1930 (*33*).

Most botulism outbreaks have been traced to home-processed foods (Table 1). These foods were implicated in 72% of all outbreaks from 1899 through 1975. Home-processed low-acid foods accounted for 67.3% of all the outbreaks. During the past 76 years, 34 botulism outbreaks have been associated with consumption of home-canned acid foods. The 34 outbreaks attributed to acid foods are listed individually in Table 2. Some type of tomato product was the vehicle for botulism toxin in 17 (50%) of the 34 home-canned acid food outbreaks.

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[2]*Part of a thesis submitted by Theron E. Odlaug to the faculty of the Graduate School of the Univesity of Minnesota in partial fulfillment of the requirements for the degree of Doctor of Philosophy.*

TABLE 1. *Outbreaks of foodborne botulism attributed to commercially-processed or home-processed Foods, 1899-1975*[a]

Interval	Home-processed		Commercially-processed		
	Acid	Low-acid	Acid	Low-acid	Unknown
1899-1909	0	2	0	1	0
1910-1919	5	42	1	15	8
1920-1929	4	73	0	24	13
1930-1939	6	129	0	6	13
1940-1949	9	111	0	1	13
1950-1959	3	47	0	2	51
1960-1969	3	39	0	10	26
1970-1975	4	42	0	5	13
Total	34	485	1	64	137
Percentage of Total (%)	4.7	67.3	0.1	8.9	19.0
Total Number of Outbreaks 722					

[a]Data from 6, 9, 10, 35.

CONTAMINATION OF FOOD WITH CLOSTRIDIUM BOTULINUM AND OTHER MICROORGANISMS

C. botulinum spores are widely distributed in soil in the United States (*34,53*). Riemann (*53*) summarized the many reports on the prevalence of *C. botulinum* in soils. *C. botulinum* was found to be widespread in the many soil and sediment samples tested. These reports indicate that *C. botulinum* is ubiquitous in nature.

Fruits and vegetables, because of their intimate contact with soil, probably will be contaminated with spores of *C. botulinum*. There have been only a few studies on the incidence of botulinum spores on raw fruits and vegetables. Meyer and Dubovsky (*34*) found 6 to 33% of vegetable and fruit samples to be positive for *C. botulinum* spores. Hauschild et al. (*24*) found 15 *C. botulinum* spores per 100 g of unwashed mushrooms and 41 *C. botulinum* spores per 100 g of washed mushrooms. The National Canners Association (*40*) found 100 to 4000 bacterial spores per gram of harvested tomatoes. It can be assumed that *C. botulinum* spores were some fraction of this total.

Based on the studies just cited, it is probable that a large percentage of the raw fruits and vegetables in the home or at the processing plant will have *C. botulinum* spores associated with them. Undoubtedly some of these spores will still be present on the product when it is placed into containers.

When fruit and vegetable products are placed into containers, they will also be contaminated with other

TABLE 2. *Outbreaks of botulism attributed to acid foods, 1899-1975*[a]

Year	State	Product	Toxin type	cases	Deaths	pH	Other references[d]
1910	California	Pears	NA[b]	12	11		*49*
1915	California	Apricots	NA	5	5		*14*
1915	Illinois	Tomato catsup[c]	NA	2	0		*22*
1918	California	Pears	NA	1	0		*14*
1918	California	Apricots	A	8	6		*14*
1918	California	Apricots	NA	2	2		*14*
1922	California	Tomato relish	A	2	2		*11*
1924	Washington	Pickles	A	2	2		
1927	California	Pears	A	2	2	3.86	*36*
1929	Colorado	Tomatoes, green	NA	4	2		*22*
1931	Oregon	Applesauce	NA	2	1		
1933	Canada	Tomatoes	NA	3	1		
1935	California	Tomato juice	NA	2	0		
1936	California	Tomatoes	A	1	1		
1938	California	Tomatoes, green and peppers	NA	2	2		
1939	California	Okra, sourgrass and tomatoes	NA	5	2		
1940	California	Tomatoes	A	5	1		
1940	California	Apricots	A	3	0		
1940	Tennessee	Tomatoes	B	2	2	4.0	*58*
1941	Nebraska	Tomatoes	NA	3	2		
1942	California	Pickles, dill	A	4	3		
1943	California	Tomatoes	NA	2	1		
1947	New Mexico	Peaches	A	4	4		
1948	California	Tomatoes	NA	3	2		
1948	California	Pears	A	2	1		
1951	California	Tomatoes	NA	2	1		
1953	California	Huckleberry juice	A	1	0		
1954	California	Peaches	A	4	3		
1964	Kansas	Pickles	A	7	1		*2*
1965	Alabama	Tomato juice	NA	1	0		*3*
1969	Kentucky	Tomato juice	B	1	0		*4*
1973	Kentucky	Blackberries	B	2	1		*5*
1974	Alabama	Tomatoes	B	1	0		*7*
1974	Idaho	Tomato juice	A	1	1	4.2	*8*
1975	New Jersey	Applesauce	B	1	0		*9*

[a]Data from *6, 9, 10, 35.*
[b]NA, information not available.
[c]Commercially canned.
[d]Numbers refer to references cited.

microorganisms. These organisms are from several sources: normal microflora associated with the fruit or vegetable as it grows in the field, microorganisms that are deposited onto the product during food handling operations and microorganisms that are present on the container when the product is packaged (*16,30,56,57*).

EFFECT OF pH ON CLOSTRIDIUM BOTULINUM

A pH of 4.6 is generally considered to be the dividing line between low-acid and acid foods. Foods with pH values > 4.6 are considered low-acid and foods with a pH of ⩽ 4.6 are considered to be acid foods.

Data in the literature indicate that *C. botulinum* is not capable of growth and toxin production at a pH of ⩽ 4.7. Townsend et al. (*66*) conducted an extensive study on the effect of pH on growth and toxin production of *C. botulinum*. Tubes containing various foods at different pH levels were inoculated with $2.0\text{-}2.5 \times 10^6$ spores of a mixture of *C. botulinum* types A and B. The tubes were incubated at 30 C until growth was evident, or for as long as 1 year if no growth was evident. The lowest pH at which toxin was formed was 4.8. In tubes that contained a tomato substrate, 4.96 was the lowest pH at which growth and toxin production was observed. Recently, Huhtanen et al. (*25*) using an inoculum of 10^3 spores/ml, reported 5.24 as the lowest pH for toxin production in tomato juice for 11 strains of *C. botulinum* types A and B. Ito et al. (*27*) were able to demonstrate *C. botulinum* toxin production in cucumber puree with a spore concentration of 10^6/tube at pH 5.0, but not at pH 4.8.

Lechowich (*32*) presented data showing that the lowest pH at which *C. botulinum* could grow was greater than 4.6. Dozier (*15*), Ingram and Robinson (*26*), Bever and Halvorson (*1*), and Kadavy and Dack (*28*) have all shown in studies with *C. botulinum* that no growth or toxin production occurred at a pH of ⩽ 4.6.

Thompson and Tanner (*65*) inoculated cans of apricots, apples, blackberries, cherries, gooseberries, peaches, pears, plums, raspberries, strawberries, pickles, and sauerkraut with 2×10^6 to 2×10^8 *C. botulinum* spores. These cans of food were incubated at 37 C for 10 months; no botulinum toxin was detected.

Lagarde and Beerens (*31*) inoculated spores of a type A botulinum strain (grown from an isolate from canned peaches) into pear syrup; no toxin was produced when the pH was ⩽ 4.6. Odlaug (*43*) found that the spores grown from a *C. botulinum* type A strain, isolated from home-canned tomato juice in the outbreak of botulism in Idaho in 1974 (*8*), grew in tomato juice at pH 4.9 but not at pH 4.8. Odlaug (*43*) also found that spores grown from the *C. botulinum* type B strain, implicated in the outbreak of botulism in Alabama in 1974 (*7*) involving home-canned tomatoes, grew in tomato juice at pH 5.2 but not at pH 5.1. The inoculum in these experiments was 10^3/spore ml.

Results of these studies indicate that the minimum pH at which *C. botulinum* will grow and produce toxin is dependent on the initial pH, incubation temperature and time, and food substrate, as well as the concentration, type and strain of *C. botulinum* spores. However, in none of these studies was *C. botulinum* able to grow and produce toxin when the substrate pH was ⩽ 4.7. If the pH of all of the substrate remains below 4.6 then there will be no botulism hazard.

SURVIVAL OF CLOSTRIDIUM BOTULINUM SPORES IN ACID FOOD

The process that is used to preserve a food in a container is based on pH and other attributes of the product and container. In the heat preservation of low-acid canned foods, the sterilization process is designed to kill all the *C. botulinum* spores in the food product since surviving spores can grow and produce toxin. In the heat preservation of acid canned foods, the process is designed to kill those microorganisms that can grow and spoil the product, and not necessarily to kill sporeforming organisms such as *C. botulinum* because it cannot grow at pH ⩽ 4.6.

Organisms that are of concern in acid foods are: *Bacillus coagulans,* butyric anaerobes (e.g., *Clostridium pasteurianum*), mesophilic non-spore-forming bacteria (e.g., *Lactobacillus* sp.), yeasts, and molds. The spore-forming bacteria that are normally capable of growth in acid foods are characterized by D(100 C)-values of 0.1 to 0.5 min and the non-spore forming bacteria, yeasts and molds by D(65 C)-values of 0.5 to 1.0 min (*62*). Heat processes for acid foods are usually designed to kill these organisms.

There has been little work on the heat resistance of *C. botulinum* in acid foods because of its inability to grow at a pH ⩽ 4.6 (Ito, personal communication). Xezones and Hutchings (*68*) investigated extensively the effect of pH on the heat resistance of *C. botulinum* spores suspended in food. They found that there is a significant decrease in the spore D-value as the pH becomes more acid. The extrapolated D(100 C)-value for 62A spores in a tomato sauce-spaghetti-cheese product was approximately 6 min at pH 4.0 and 42 min at pH 7.0. The heat resistance of a type A strain, isolated from tomato juice in the outbreak of botulism in Idaho in 1974 (*8*) was shown by Odlaug and Pflug (*45,47*) to have an extrapolated D(100 C)-value in pH 7.0 buffer of 47 min and in pH 4.2 tomato juice of 18 min. These results indicate that *C. botulinum* spores have lower heat resistance in acid foods than in low-acid foods. However, even at the acid pH values, *C. botulinum* spores still have 10 to 200 times greater heat resistance then the spore-formers that normally grow in and spoil such foods.

C. botulinum spores, if present in an acid food, will remain viable for a considerable period of time. Odlaug and Pflug (*46*) have shown that *C. botulinum* spores stored in acid media (pH 4.2) survived up to 180 days with little or no decrease in numbers.

It will be normal for a fraction of the *C. botulinum* spore population present on foods to survive the heat processes given to acid foods and remain viable during

storage. Presence of viable *C. botulinum* spores in properly preserved acid foods does not constitute a public health hazard because of the inability of these spores to grow at a pH of $\leqslant 4.6$.

PROCESS FAILURES IN ACID FOODS

There are three possible areas of failure in the heat preservation of acid or acidified foods: (*a*) in process design or specification, (*b*) process delivery, and (*c*) post-process contamination.

A failure in process design occurs when the designed process is inadequate to preserve the specified product. Process design failures do not appear to have contributed to the botulinum hazard in acid foods. Acid food products such as tomatoes, tomato juice, applesauce, pickles, peaches and fruit juices, jams and jellies have been produced by commercial canners for years without a botulinum problem.

Delivery failures appear to be one cause of botulism in acid foods. There are two ways in which a process delivery failure can occur: (*a*) the product being processed is not the same as the product in the process design and (*b*) the scheduled (designed) process is not delivered to the specified product. Failures in process delivery may be due to human error or equipment failure.

Process delivery failures where the product was not the same as specified in the design have been a problem with some acidified foods. A product with a pH >4.6 that does not receive a minimum botulinum cook, and is acidified or inadequately acidified is a potential botulism problem. This was recently a problem with marinated mushrooms (*24*). In commercial acid food production a process failure is usually not equated with a botulism hazard. This is probably because the normal spoilage microorganisms of most acid foods tend to lower instead of increase the product pH (*41*).

Another possible process delivery problem, where the product was not as specified in the design, has been with low-acid tomatoes. There have been a number of studies dealing with the acidity of tomatoes that have been reviewed by Powers (*50*), who reported that depending on the variety, a high percentage of especially over-ripe tomatoes may have a pH above 4.6. However, the low-acid tomato problem does not account for all the botulism outbreaks involving acid foods especially when the epidiemiological evidence is considered. Of those botulism outbreaks listed in Table 2, in only three outbreaks were pH values reported and in none of the foods was the pH >4.2.

Acid food preservation using the hot-fill procedure at 85 to 90 C or heating to a center can temperature of 75 to 80 C, relies on a number of factors to effect preservation (heating time, pH, salt and/or sugar concentration, also type of acid added in acidified foods). Process failures can occur if the initial number of contaminating organisms is high, the heat process time and/or temperature is too low, or the pH is higher than in the design. A process delivery failure can occur in a hot-fill process allowing microorganisms present in the container or on the lid to survive if the container and product are cooled too rapidly after filling or the container is not inverted. Failure may also occur due to survival of encapsulated or occluded microorganisms that are not destroyed in the heat process.

The third type of process failure is post-process contamination. In a commercial operation poor quality containers, inadequate chlorination of cooling water and rough can handling can increase the probability of microorganisms leaking in and spoiling canned foods (*44,48,51*). The homemaker may also use poor quality containers, especially in terms of the closure. The risk from contaminated water entering the container due to a poor closure is low because the homemaker will probably air cool the prepared containers. Since an acid food product is by nature non-sterile, a container failure that allows oxygen leakage and permits aerobes to grow and spoil the product may also be considered post-process contamination.

CONDITIONS NECESSARY FOR CLOSTRIDIUM BOTULINUM GROWTH IN AN ACID FOOD WITH A PROCESS FAILURE

A process failure in acid foods either in delivery and/or post-process contamination will result in unwanted microorganisms being present in the product. If both *C. botulinum* and other microorganisms are present in a canned acid food because of a process failure, a botulism hazard may exist. Whether or not *C. botulinum* will grow and produce toxin will depend on several factors. These factors are the chemical composition of the food, physical state of the food, conditions of storage, and microorganisms interacting in the food.

Chemical composition of the substrate

The chemical composition of an acid food has an important effect on growth of *C. botulinum* and other microorganisms in the acid medium. The pH level, nutrients, redox-potential and antimicrobial constituents must be at levels in the food to permit germination of spores, outgrowth and toxin production. Nutrients in acid foods have been shown to be sufficient to allow growth of *C. botulinum* if the pH is changed to greater than 4.6 (*25,31*).

The redox potential in a food must also be at a level that allows growth. Spores of *C. botulinum* will not germinate when the redox-potential (E_h) is above a certain critical level. The redox-potential in a food is determined by the E_h poising capacity of the food itself, and the O_2 tension in the atmosphere and its access to the food (*38*). Smith (*59*) reported that the E_h of canned tomato sauce was -399.1 mv; tomato juice, -309.3 mv; peeled tomatoes, -295.7 mv; tomato sauce with bits, -271.1 m; and tomato bisque soup, -193.5 mv. Rapid growth of *C. botulinum* will take place in a suitable medium when the E_h is between -6 mv and -436 mv (*37*). Rowley and Anellis (*54*) indicate that an E_h of

greater than − 150 mv is needed to inhibit *C. botulinum* types A and B.

Physical state of the substrates

Water activity (a_w) is a critical physical factor in food. A_w values of $\leqslant 0.93$ are inhibitory to all types of *C. botulinum* (*53,60*). The water activity in canned acid food products such as tomatoes and tomato juice would not be a factor in preventing *C. botulinum* growth and toxin production. However, there are acid foods that have low a_w values (e.g. syrup-packed peaches, jams, jellies).

Conditions of storage

Storage temperature of a canned food and exposure of the contents to 0_2 are factors that will affect the growth of *C. botulinum* and other microorganisms.

The temperature will affect the growth of microorganisms. If storage is at < 10 F, growth of *C. botulinum* types A and B will not take place (*54*). The optimal growth temperature for *C. botulinum* is between 30-37 C (*53*).

Sugiyama and Yang (*63*) suggested that *C. botulinum* types A and B have an 0_2 tolerance that allows growth in the presence of 1 to 2% 0_2. Mossel and Ingram (*38*) described how molds and aerobic bacteria can develop in canned foods at very low oxygen levels. *Byssochlamys fulva* has been shown to grow on Potato Dextrose Agar at 0_2 levels as low as 0.27% (*29*).

Microbial interaction

The organisms present in a canned acid food because of a process failure will affect one another through differences in rates of development, antagonism or synergism (*38*). These are called implicit factors, a term suggested by Mossel and Westerdijk (*39*). In an acid food, if *C. botulinum* spores and other organisms are present, these factors will determine if growth and toxin production of *C. botulinum* will take place.

There have been three outbreaks in acid foods where organisms other than *C. botulinum* have been isolated. Table 3 lists these outbreaks and the organisms isolated. In all three cases the pH recorded for the food was below 4.6.

Meyer and Gunnison (*36*) did experiments with the organisms isolated from the canned pears in the 1927 outbreak (Table 3). They found toxin in tubes inoculated with the yeast, the *Lactobacillus* sp., and 5×10^8 spores of *C. botulinum*. They did not find toxin production when the *Lactobacillus* sp. was present along with *C. botulinum*. However, they did find toxin when the yeast was present along with the *C. botulinum* in the food. In tubes where no toxin was present, viable spores were still present. The pH values of the toxic syrups varied from 3.33 to 4.22. No other report on this project is known. Slocum et al. (*58*) inoculated organisms isolated from the canned tomatoes in the 1940 outbreak (Table 3) into 10 cans of commercially canned tomatoes and found no toxin after 32 days of incubation at room temperature.

Other investigators have studied the interaction of microorganisms with *C. botulinum* in acid media. Tanner et al. (*64*) detected botulinum toxin in low acid fruits inoculated with *C. botulinum* spores where a *Penicillium* sp. or *Mycoderma* sp. had grown in the food and shifted the pH from 3.25 to 4.9-5.4 in raspberries, and from 3.75 to 5.1-5.3 in cherries. Lagarde and Beerens (*31*) observed that a contaminant such as *Trichosporon* was able to raise the pH of pear syrup from 3.8 to 4.8 where *C. botulinum* produced toxin. Huhtanen et al. (*25*) were able to demonstrate toxin production in tomato juice (pH 4.2) where a *Penicillium* sp. or *Cladosporium* sp. was present to raise the pH at the surface above 4.6. Odlaug (*43*) was able to demonstrate *C. botulinum* toxin production in pH 4.2 tomato juice if *Aspergillus* sp. was present to raise the pH at the surface above 4.6.

Both Huhtanen et al. (*25*) and Odlaug (*43*) were able to demonstrate a pH gradient in tomato juice inoculated with molds when a heavy mold mat formed on the surface. The pH values near the mat were near neutrality, and the lower portions below the mat were more acid. Odlaug (*43*) showed that *C. botulinum* growth was greatest at the surface and that the counts decreased with distance from the mycelial mat. Toxin was present throughout the tomato juice. A non-hermetic experimental unit was used by Odlaug (*43*).

Huhtanen et al. (*25*) suggested that the reason that botulinum toxin could be found in a food with a pH of $\leqslant 4.6$ is that routine mixing of the product during analysis could destroy a pH gradient formed by acid-consuming microorganisms and show the food to have a pH in the high-acid range.

TABLE 3. Clostridium botulinum *outbreaks involving acid foods where other microbial species were isolated*

Date	Food product	Toxin type	*C. botulinum* isolated	Other microbes	pH of food	Reference
1927	Pears	A	Yes	*Lactobacillus* Yeast	3.86	*36*
1940	Tomatoes	B	Yes	*Bacillus* Diplococcus (*Streptococcus faecalis*?)	4.0	*58*
1974	Tomato juice	A	Yes	Diplococcus Yeast *Aerobacter agglomerans*	4.2	*8*

Odlaug (*43*) demonstrated that when a mold was present in tomato juice in a hermetic unit, the growth of the mold was restricted, probably due to lack of oxygen. The dry weight of the mycelial mat in the hermetic unit was approximately 10% of the dry weight of the mycelial mat in a non-hermetic unit. In the hermetic unit *C. botulinum* growth and toxin production could be demonstrated even though the pH (recorded with a combination electrode) on the mat, below the mat and at the surface of the tomato juice, was less than 4.6. Odlaug (*43*) could not demonstrate any filterable of dialyzable growth factors produced by the mold that would allow production by *C. botulinum*. It was concluded that *C. botulinum* and the mold had to occupy the same microenvironment for toxin production to be demonstrated. The toxin levels under these conditions were low (< 10 MLD_{50}/ml), and the increases in the *C. botulinum* population were small. Odlaug (*43*) suggested that growth of the mold (*Aspergillus*) at the surface of the tomato juice in a hermetic unit created microenvironments within the mycelial mat or directly below the mycelial mat where the pH was greater than 4.6 and where *C. botulinum* spores germinated, reproduced, and produced toxin.

It is interesting that Dickson (*14*), when reporting on the 1917 botulism outbreak involving apricots (Table 2), noted that the food was "moldy on the surface." The pH value of this food was not reported.

Recently, Fields et al. (*18*) isolated *Bacillus licheniformis, Bacillus subtilus*, and other organisms from home-canned tomatoes. Some of the cultures of *B. licheniformis* and *B. subtilis* inoculated into tomato serum were able to grow and raise the pH to a level above 4.6. *C. botulinum* was not tested for growth and toxin production in the presence of these organisms in an acid medium. Wentz et al. (*67*) have shown *B. licheniformis* to inhibit *C. botulinum* type A toxin production in brain heart infusion by production of an antibiotic, bacitracin. Stark et al. (*61*) showed that *Bacillus subtilis* could destroy preformed *C. botulinum* toxin.

Streptococcus lactis, Lactobacillus casei, Clostridium sporogenes, Clostridium bifermentans and *Clostridium perfringens* have all been shown to destroy botulinum preformed toxin (*12,61*). Saleh and Ordal (*55*) showed that *Lactobacillus bulgaricus* and *Streptococcus lactis* are capable of inhibiting *C. botulinum* growth and toxin production.

It is apparent that while microbes can destroy *C. botulinum* toxin or inhibit growth, they can also alter the environment to allow *C. botulinum* growth to take place where it normally would not. When one organism makes conditions favorable for growth of a second organism, the condition is known as metabiosis (*19*).

There appear to be two ways in which microorganisms in acid foods can create favorable conditions for *C. botulinum* growth and toxin production. One is where there is gross spoilage of the product that results in gross changes of pH throughout the product to a level where *C. botulinum* can grow. Since this type of spoilage would be readily apparent, the food would probably be discarded with no harm done. The second way of producing toxin is where growth of a microorganism does not produce gross but micro-scale changes in pH. In this condition, if a *C. botulinum* spore is present in the mold growth area, growth could take place. This type of spoilage may not be readily observable and therefore the food may not be discarded and be a hazard.

CONCLUSIONS

A review and analysis of the literature indicate that if the pH of a food is $\leqslant 4.6$ and no other viable microorganisms are present, then it should be impossible for any viable *C. botulinum* to grow and produce toxin. A canned food with a pH value of $\leqslant 4.6$ that receives a preservation treatment that inactivates all organisms capable of growing at pH $\leqslant 4.6$ and is free from post-process contamination, is safe frm *C. botulinum*.

For a botulinum hazard to exist in an acid food, pH $\leqslant 4.6$, there must be a number of contributing factors: (*a*) contamination of the product with large numbers of *C. botulinum* spores, (*b*) contamination of the food with other microorganisms due to a process failure in delivery and/or post-process contamination, (*c*) favorable composition of the food and storage conditions that are particularly conducive to *C. botulinum* growthand toxin production, and (*d*) metabiosis. The probability that all of the factors necessary for *C. botulinum* growth and toxin production will occur in a single container of an acid food is undoubtedly low as is the incidence of botulism in acid food.

There has not been a reported case of botulism attributed to the consumption of a commercially canned acid food in over 50 years. The problem of botulism in home-canned acid foods is rare but still occurs occasionally. There have been three deaths attributed to consumption of home-canned acid foods containing *C. botulinum* toxin in the last 20 years. The continuing problem of botulism in home-canned acid foods and its absence in commercially canned acid foods probably indicates that non-supervised people often fail to follow recommended heat preservation methods. The problem of botulism in home-canned foods can probably never be totally eliminated because of the laws of probability and human fallibility. In view of the relatively low risk of botulism from consumption of acid foods, would a control program be of benefit in reducing the risk to an even lower level? Since home-canning is an important means of preserving food for many families, a control program that would result in less home-canning of acid foods would not be beneficial to the population as a whole. It is probable that increased consumer education regarding the proper methods of home-canning will be beneficial in reducing spoilage losses. However, changes in the processing recommendations for acid foods may

do more harm than good unless that factor is specifically identified that is most important in contributing to a botulism hazard in each type of acid food. Any control program that is initiated should be carefully examined to determine if results of the program will be a lower incidence of botulism from the consumption of acid foods.

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Antibiotic Residues in Milk: Dye Marking Versus Direct Control

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ABSTRACT

Advantages and disadvantages of control for antibiotic residues in milk by compulsory dye marking of anti-mastitis preparations or by direct tests utilizing microbiological methods are reviewed. It appears feasible to construct a very strict direct control system utilizing highly sensitive microorganisms in laboratory methods or incorporated in industrially-made test kits. Useful methods whereby a dramatic decrease in both the incidence and amount of antibiotic residues can be attained are described. Compared to these systems, the compulsory addition of dye-markers to anti-mastitis preparations is unnecessary and even undesirable.

Antibiotic residues in milk are undesirable on the grounds of public health and good dairy practice. Therefore, in most countries an increasingly intensive control is being imposed on these residues. Control can be achieved in both a direct and an indirect manner. In direct control, use is made of microbiological test methods whereby the presence and, if necessary, concentration and nature of any antibiotics can be demonstrated. For indirect control, a marker dye is added to the antibiotic preparation such that the excretion rate of the dye correlates, as far as is possible, with that of the preparation administered. Most countries with intensive dairy farming use a direct control procedure, but indirect methods have been applied in the State of Victoria, Australia, since 1962 and later also in New Zealand, France and South Africa.

While in a growing number of countries the problem of control has been, or is being, reduced to acceptable proportions by detection based exclusively on microbiological methods, there are other countries in which an intention to introduce non-microbiological methods is still under discussion. It is therefore opportune to detail the various arguments in support of, or against, either system in the light of practical experience.

This paper will not deal with the already extensively described details of the various test methods, nor with equally well documented excretion patterns of many different antibiotics in milk; reference to two reviews will suffice (*6, 18*).

INDIRECT CONTROL [DYE-MARKING OF ANTIBIOTIC PREPARATIONS]

Locally administered antibiotics infused into the udder are responsible for the vast majority of incidences of residues in milk at concentrations which, even after high dilution on bulking, may remain detectable. In areas where use of a marker dye is compulsory, the immediately visible color warns against use of that milk and prevents its addition to bulk milk. This is an advantage over direct control methods as these are generally not applied until herd yields have been bulked. Added dyes, usually Brilliant Blue FCF ("Food Blue 3") and Green S ("Food Green 4"), can still be observed visually at a concentration of about 0.1 ppm. Concentrations as low as 0.005 ppm can be detected using ion exchange resin methods.

With such dye marking, even if there is no "unit-by-unit" excretion of dye and antibiotic (*2*), more than 95% of all antibiotics administered in combination with a dye can be excluded from milk intended for consumption. Compulsory dye marking has, however, some attendant serious disadvantages.

(*a*) Although most investigators (*3,10,14*) have noted a reasonable correlation between the excretion rates of dyes and antibiotics, marked deviations have also been reported. This is especially true for certain antibiotic substances (*9*) and in cases where vehicles having a slow excretion rate are included in the formulation (*4*).

(*b*) As residues in the milk of animals treated with antibiotics given other than by the intramammary route, e.g. by subcutaneous, intramuscular, or intra-uterine administration, cannot be detected by an indirect control, direct techniques cannot be dispensed with.

(*c*) Addition of the dye in itself constitutes a residue problem and can lead to discoloration of milk products should the dye enter fat globules and remain undetected (*1*). In a number of countries where milk is considered to be a basic food which should be kept as pure as possible, residues of this kind are in principle not allowed (*24*).

(*d*) Use of dye markers will have the effect of increasing the cost of antibiotic preparations, not only on account of the cost of providing and incorporating the marker, but also due to mandatory requirement to evaluate the excretion rate of each individual substance. Also, as there is no international harmonization of regulations governing detection of residues, costs are further increased by the resultant requirement to develop preparations to satisfy various standards. This inhibits large-scale batch production of each preparation.

(*e*) With certain antibiotics addition of a dye will decrease the stability of the product and thus shorten the shelf-life (*10,13*).

(*f*) As a result of contamination of clothing and hands, dairy farmers find it unpleasant to work with dye-marked preparations especially as high concentrations (up to 250 mg per instillation) are frequently needed.

(*g*) One disadvantage, however, may be even more important than those previously discussed. Where dye marking and indirect controls are made obligatory, direct control will be less intensive or even ignored. This would open the way for abuse of control procedures by use of non-dyed preparations obtained by official and unofficial channels of distribution. Also the imposition of dye marking requirements constitutes ethical pressure on veterinarians (*23*) which however is misplaced in countries where veterinarians have little or no influence on the distribution of antibiotics. It is true that in areas where compulsory dye-marking applies, about 95% of antibiotics administered locally as dye-marked preparations can be excluded from milk for consumption. However, milk from one quarter treated with a non-colored preparation is sufficient to vitiate the control procedure of a tanklot of milk.

DIRECT CONTROL PROCEDURES

Preparations containing penicillin still account for 80-90% of the locally applied anti-mastitis treatments. Therefore, it is not only the intensity of testing but also the sensitivity of the method as regards penicillin that determines the impact of control on the occurrence of antibiotic residues in milk. This explains why, in recent years, public health authorities of many countries have changed to test methods which are much more sensitive for penicillin. Levels of detection have decreased from 0.05 IU/ml (*Bacillus subtilis*) through 0.02 - 0.01 IU/ml (*Sarcina lutea* and *Streptococcus thermophilus*) to 0.003 IU/ml (*Bacillus stearothermophilus* var. *calidolactis*). This last test organism is utilized in the assay procedure described by Galesloot and Hassing (*7*) and a modification of this method is accepted as the official test by the International Dairy Federation.

In addition to these methods, which can only be pursued at well equipped laboratories, industries have developed ready-for-use test kits based on *S. thermophilus,* such as the "Interest" (*15*), or on *B. stearothermophilus* var. *calidolactis,* as in the "Delvotest-P" (*20*), "Enterotox" (*16*) and "Thermocult" (*22*). Several of these test meet many or all requirements; they are highly sensitive for penicillin, display satisfactory sensitivity for other antibiotics, are reliable and can be used practically in a rapid and economic fashion. *B. stearothermophilus* var. *calidolactis* has been accepted as the test microorganism of choice in most Western European countries and has recently been considered for acceptance in the USA (*17*).

The direct applicability of industrially-produced test kits makes it possible to check all incoming bulk milk. In addition individual supplier samples can be required and can be examined should the incoming bulk milk prove positive; this would enable suppliers of the positive portion of the bulk milk to be identified. Similarly, checks of farm milk are facilitated in that they can be carried out at more irregular intervals. Such test systems would permit appropriate measures to be taken more easily to prevent further incidents.

A major argument against direct control systems has always been that a risk exists that residues in bulk milk will be detected only after dilution with residue-free milk. However, experience has shown that initiation of direct control, based on a "sample-hold, bulktest, check-sample" procedure leads to a dramatic decrease in both the incidence and levels of antibiotic residues such that the total residue level of the bulked milk falls to levels generally far below acceptable or detectable levels. As a final check the finished dairy product can be tested.

DISCUSSION AND PRACTICAL EXPERIENCE

Compulsory dye-marking seemed a useful aid to control as long as no efficient direct test method existed. As indicated above, dye marking has a number of serious disadvantages. Thus with development of more sophisticated direct control systems, dye marking of antibiotic preparations seems to become obsolete.

Against direct control it has been argued, in particular by the dairy industry, that the necessary education of the pharmaceutical industry (which has to establish excretion rates) for this method, the veterinarians (who must instruct the farmer) and the farmer (who must carry out the instructions) would be too time-consuming, if not impossible. However, the major manufacturers of ethical veterinary preparations have done sufficient research on their products so that specific advice can be given to both veterinarians and farmers. Furthermore experience with some large cooperatives in various countries has shown that, using intensive checking with appropriate sanctions and combined with good instruction and technical support, the number of positive samples can be decreased markedly, even in a short period (*19*). Despite intensive control, the incidence of positive samples in, for example, The Netherlands and Denmark has stayed at around 0.2% for a number of years and it would seem that since such a level persists any further reduction is difficult to achieve. It is interesting to note that use of milking-cow preparations accounted for 63% of these

positive samples, dry-cow preparations for 9%, systemic therapy for 8% and penicillin teat-canal treatments for 5% of this incidence according to a regional study in the Netherlands (*18*). According to another report, 45% of positive samples appeared to result from a lack of communication between the farmer and the milker (*12*). Thus it seems that to reduce the incidence of positive samples the need to clearly identify treated cows should be stressed in the information provided.

In addition to provision of appropriate information, good service facilities provided by the dairy industry itself can stimulate appreciation of a control system. Examination, at the request of the farmer, of suspect milk samples can prevent economic sanctions and unnecessary waste of milk. In a collaborative study of this aspect, a dairy industry was found to obtain highly reliable results using an industrial test carried out by dairy technicians (*21*). In this way results of locally performed tests can be made available to the farmer before delivery is made. Legal sanctions against the supplier of contaminated milk are generally based on identification of penicillin residues. Where such regulations are closely restricted to residues of penicillin only, a shift in favor of the use of other antibiotics may occur. This accounts for the need for more simple methods by which the residues of other antibiotics can be identified.

As well as being highly sensitive for antibiotics some modern methods are sufficiently selective to be unaffected by other, naturally occurring, substances present in milk and thus avoid "false-positive" reactions. In one of the previously mentioned test methods (Delvotest-P) interference by such substances is rare, whether in bulk or farm milk and irrespective of preheating of samples (*20*). Even lysozyme concentrations which have an inhibitory effect in the disc assay utilizing *B. stearothermophilus* var. *calidolactis* do not interfere with this test method (*8*).

Finally, although further harmonization of test methods used in various countries is undoubtedly necessary, *B. stearothermophilus* var. *calidolactis* seems to be the test microorganism of choice.

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Delayed Chilling of Lamb — A Review

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ABSTRACT

Shortening of muscles by cold shock, enzymatic action, or both is a major cause of reduced meat tenderness. While chilling and enzymatic actions are highly correlated to tenderness, electrical stimulation and fatness are other factors which influence the tenderness. Emphasis on safety in foods has led to the belief that meat should be cooled as quickly as possible after death. However, evidence to be considered in this review shows that this practice of immediate chilling is in direct opposition to tenderness in lamb. Instead, delay in chilling improves tenderness by influencing factors such as enzyme activity.

Although refrigeration technology has been studied for many years, it is only recently that we have begun to understand the relationship between meat tenderness and chilling rates. These conditions require a considerable amount of knowledge in the area of refrigerant technology. Correspondingly, data suggest that CO_2 chilling and associated packaging systems can be used to facilitate shipment and storage for 11 days without significantly affecting freshness, consumer acceptance, odor and palatability (*16*). Also, a study on chilling of lamb carcasses in liquid N-cooled chambers indicated no adverse effects of liquid N chilling on quality (*1*).

An important aspect of the process, to which much research effort has been directed, is examination of pre-rigor muscle chilling and its relationship to tenderness. Locker (*8*) concluded that there was a relationship between postmortem shortening and tenderness. This relationship has been the subject of several investigations in the past decade (*11,19*). Postmortem shortening is dependent on temperature, but not all muscles show the same temperature-dependence (*13*). Other studies attributed a part of the increase in tenderness associated with the use of elevated chilling temperatures to increases in autolytic enzyme activity as well as to reduction of cold shortening (*12*).

Consumer reaction to frozen meat could be determined by its general appearance, but its lack of uniform tenderness seems to be of greatest significance.

CHILLING, COLD SHORTENING AND TENDERNESS

Rapid chilling reduces both weight loss and bacterial contamination, but if applied to pre-rigor meat "cold shortening" of muscle tissues may occur, leading to reduced tenderness. It has been shown that with lamb and muttom carcasses, "processing toughness" can be avoided if chilling is not below 10 C until rigor mortis is completed, which requires 16-24 h. However, this slow chilling introduces problems of microbial growth if the surface is too moist, and excessive weight loss unless the environment has a low drying effect. It has been found possible to achieve a satisfactory combination of conditions to avoid microbial growth or weight loss by careful control of air temperature, relative humidity (RH) and velocity (*5*).

Taylor et al. (*18*) worked with lamb carcasses, which were chilled rapidly and slowly (delayed) under controlled conditions. Sensory and objective measurements of tenderness (after cooling and conditioning at 0 C) showed that it was in all instances significantly improved by conditioning, but the rapidly chilled samples remained undesirably tough. The toughening of lamb meat induced by rapid chilling was prevented in carcasses given a pre-slaughter injection of papain solution at the normal commercial dose level of 18 mg/kg (*15*).

When boned-out cuts of meat are rapidly frozen while still in a pre-rigor state, massive contracture takes place with excessive loss of fluid of the meat subsequently thawed. Rapid chilling of strips of pre-rigor meat to below 10 C leads to unexpectedly rapid glycolysis and pH fall, contraction of up to 60% of the slack length (which is a "cold shortening" phenomenon) and extreme toughening (*9*).

Taylor et al. (*18*) reported that lamb is particularly vulnerable and because of its small carcass, it can undergo rapid temperature changes (Fig. 1). They showed (shear comparison and panel tests) that: (*a*) rapidly chilled meat had higher values of toughening

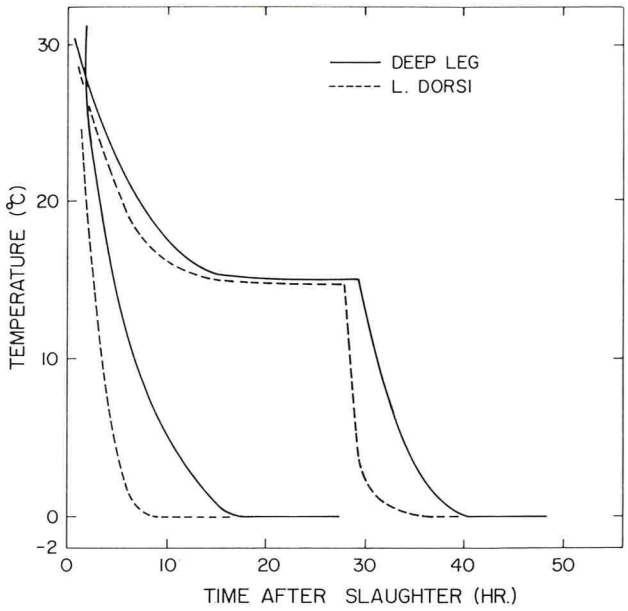

Figure 1 *Temperature changes in the longissimus dorsi and deep leg of lambs chilled by rapid and slow process to 0 C (by Taylor et al. 1972)*

than did the slowly chilled samples; (*b*) $MgSO_4$-treated rapidly chilled samples had significantly higher values than the control ones, whereas in the slowly chilled samples $MgSO_4$ had no significant effect; (*c*) panel scores for texture indication also showed a marked toughening for the rapid chilling, whereas conditioning resulted in an increase of tenderness in both $MgSO_4$-treated and control groups.

Results of Taylor and his colleagues clearly confirm the earlier observation of New Zealand workers that the toughening effect of cold-shortening in lamb muscle is commercially important when rapid freezing is employed. In comparison, all slowly cooled samples were tender initially and more tender under conditioning. Histological examinations showed that rapidly chilled shortened muscles contained a number of straight, highly contracted fibers while in the slowly chilled muscles the fibers were straight with long sarcomeres.

With advances in meat science it became possible to consider alternative methods of avoiding cold shortening. One method which is particularly interesting is high voltage carcass stimulation immediately after the death of the animal. This method had also been used to accelerate aging of beef by Harsham and Deatherage in 1951 (*3*) and to increase acceptance of frozen meat (*4*). The reasonable conclusion is that stimulation speeds glycolysis throughout the muscles of beef carcasses, as shown by the rapid pH fall, and that rigor is reached well before temperatures have fallen to levels inducing cold shortening (*4*).

In the meat packing industry, solidified CO_2 has been used for a number of years as a chilling medium. More recently, CO_2 snow and pellets have been used to facilitate chilling and to retard deteriorative changes during shipment of beef wholesale cuts and lamb carcasses. Lamb carcasses as well as beef wholesale cuts are being chilled with CO_2 pellets for shipment from packers to the retail market with claims of (*a*) reduced shrinkage, (*b*) improved appearances, (*c*) retarded bacterial growth, and (*d*) longer shelf life.

CHILLING, ENZYMES AND TENDERNESS

Proteolytic enzymes of plant origin have been used to tenderize meat since ancient times, but such products are now patented and under industry control. Kang and Rice (*6*) reported that the enzymatic action takes place in the meat only as the temperature rises during cooking and is halted as the enzyme is inactivated by further heating.

Rhodes and Dransfield (*15*), working with low and high dosage of enzyme (papain) preparation, used four groups, which were injected with substitute protein and then were slowly chilled. The slow chilling carcasses were delayed at ambient temperatures (appr. 15 C) for 6 h and then chilled at 0 C. For rapid chilling the carcasses were chilled at –2 C with 93% R.H. and transferred to 0 C approximately 6 h later when the temperature reached 5 C in the deepest part of the leg. Work of Rhodes and Dransfield indicates (Fig. 2): (*a*) rapid chilling about doubled the toughness values of the longissimus dorsi (LD) in the cuts roasted at 50 C and increased it by half when roasted to 65 or 85 C; (*b*) toughness values were reduced at all temperatures as the level of enzyme increased; (*c*) toughening produced by rapid chilling was more than eliminated by the tenderizing effect of the high level of enzyme at 65 and 85 C, while the two effects were about balanced at low level of enzyme at these temperatures; and (*d*) at 50 C the high level was only just sufficient to restore tenderness to that of the slowly chilled carcasses.

Smith et al. (*17*) proposed that delayed chilling improves tenderness by increasing enzymatic activity. This is supported by data showing that (*a*) higher muscle temperatures of the fatter lambs are conducive to greater amounts of autolytic enzyme degradation for longer periods; (*b*) connective tissues from fatter lambs were softer than those from thinner lambs (*14*). Although the evidence for more proteolysis in the tissues of the fatter lambs is not conclusive, the theory seems interesting, according to Pearson (*14*), and has some support. A study

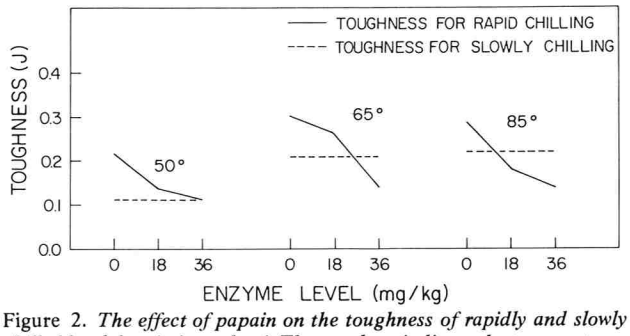

Figure 2. *The effect of papain on the toughness of rapidly and slowly chilled lamb longissimus dorsi. The numbers indicate the temperatures (c) to which the loin joints were roasted. Each number represents the mean of 10 observations on each of three lambs. (Rhodes and Dransfield 1973. J. Sci. Food Agri. 24:1582)*

by Smith et al. (*17*) proposed that the delay in chilling improves tenderness by increasing enzymatic activity.

CHILLING, FATNESS AND TENDERNESS

Newbold and Harris (*13*) indicated that some changes in the myofibrillar component of muscle during the period of the slaughter and the full development of rigor mortis could influence the tenderness of meat. The amount of muscle shortening decreases as the period between slaughter by pre-rigor chilling at 15-20 C lessens the myofibrillar toughness of lamb (*11*).

Marsh et al. (*10*) reported that cooling rate is influenced not only by ambient temperatures, humidity and air velocity, but also by the size of cooling body and the depth of tissue. It also pointed out that mature ewe carcasses sustained greater toughening from early freezing than did lamb carcasses (*20*). This is possibly because the blocky, thick and fat lamb carcasses cool more slowly than the angular, thin and lean mature ewe carcasses.

Smith et al. (*17*) showed that fatter animals usually produce meat that is more tender than that of leaner animals. These studies indicate that fatter animals tend to deposit greater quantities of marbling; but research during the last 50 years has not clearly demonstrated the relationship of marbling to tenderness, or the necessity of fat deposits for quality. Marbling might be related to tenderness by the insulatory effect of other fats (subcutaneous) in reducing the severity of cold shortening induced by low temperature chilling (*2*). Others at Texas A&M University suggested that carcass weight and fatness can affect tenderness by the way of cold shortening.

Smith et al. (*17*) studying the influence of fat thickness to tenderness, suggested that subcutaneous or intramuscular fat could affect the tenderness of muscles by insulating the muscle fibers during post-mortem chilling (changing the rate of temperature and decreasing the period of cold shortening).

The above study showed that fatter lamb carcasses: (*a*) chilled more slowly; (*b*) maintained enzyme degradation for longer periods of time post-mortem; (*c*) sustained less shortening of the muscle sarcomers; (*d*) had lower muscle pH values; (*e*) had softer or less perceptible connective tissue; and (*f*) were more tender than lamb carcasses with limited fat.

These observations support the hypothesis that deposits of increased quantities of subcutaneous or intramuscular fat increase tenderness via changes in post-mortem chilling rate. In other words, this supports the previous study and suggests that fatness improves tenderness by delaying carcass chilling, in contrast to other studies which have suggested the effect of fatness is solely to prevent muscle shortening.

CONCLUSION

This review has shown that the shortening of muscles by (*a*) cold shock, (*b*) enzymic action, (*c*) electrical stimulation, and (*d*) physical effect of fatness are some of the causes of reduced meat tenderness. Thus, extensive investigations will be required to prove whether or not the effect of fatness is strictly a physical one by preventing cold-shortening or also involves proteolysis and breakdown of the connective tissues due to a slower rate of cooling.

Since chilling and enzymatic actions are highly correlated to tenderness, meat packers using unsuitable processing methods can impair the tenderness of meat. Consideration therefore must be given not only to the requirements of the processors but also to the demand of the consumers for tender meat quality in relation to safety.

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News and Events

DFISA Elects Bjorgen, Nesbitt to Top Posts

Ralph L. Bjorgen, director of international sales, plastics and synthetics division, Norton Co., Akron, Ohio, was elected president of Dairy and Food Industries Supply Association at its 59th Annual Meeting at Palm Springs, April 17-19, 1978.

Arthur W. Nesbitt, President and chief executive officer, Nasco International, Fort Atkinson, Wis., was elected president-elect.

Bjorgen succeeds Duane Poulterer, chairman of the board, Germantown Manufacturing Co., Broomall, Pa., who served since 1976.

Bjorgen has been affiliated with the Norton Co. in marketing and sales since 1950. He was promoted to his present post in 1977 from director of sales.

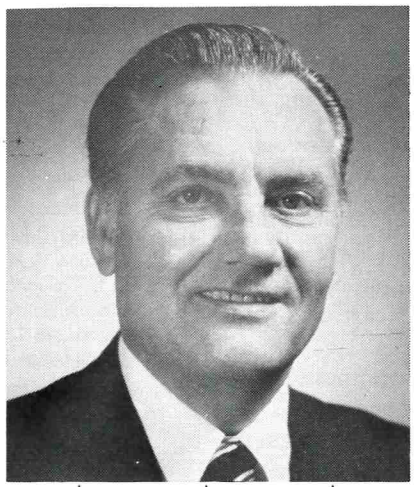
Norton Company is a diversified industrial manufacturer with 1977 sales of $848 million putting Norton number 272 on the "Fortune 500" list. It has more than 23,000 employees with 112 plant locations in 27 countries. Norton is the leading producer of abrasives and largest maker of diamond drilling and coring bits for the oil and gas exploration industries.

Norton's Plastics & Synthetics Division is one of the leading manufacturers of plastic materials. It is best known as the manufacturer of TYGON(R) Plastic Tubing, and markets to the food, dairy and beverage processing industries, the health care industry, industrial and clinical laboratories and general industry. Its TRANSFLOW(R) Tubing is also well known for use with milking machines, milking systems, and milk transport trucks.

Active in DFISA for 22 years, Bjorgen has held a seat on the board of directors since 1972. He served as chairman of the Expo committee and Expo credentials committee. He has been a member of the following committees: executive, employee relations, awards, membership development, marketing, customer relations, food industry liason and nominating. He has been a member of technical task committees for plastics, cleanability and sanitary fittings.

His industry memberships include the *International Association of Milk, Food and Environmental Sanitarians;* Sales and Marketing Executives International; Scientific Apparatus Makers Association, and National Association of Plastics Distributors. He has been active in all phases of Boy Scout work and the United Methodist Church of Ravenna, Ohio.

Bjorgen is a native of Rothsay, Minn. He attended Norwich University, the college of business administration, Kent State University, and completed training at the Graduate School of Sales and Marketing, Syracuse University. He lives at 418 Rosedale St., Ravenna, Ohio, with his wife LaVerne.



☆ ☆ ☆

Nesbitt joined Nasco International in 1959. The company manufactures plastic products for the dairy, food, medical and cosmetic industries and metal fabrications for the Defense Department. It distributes catalogs worldwide to agricultural and educational customers. Prior to joining Nasco, he was secretary-fieldman of the Pennsylvania Holstein Association.

Nesbitt was elected to the DFISA board of directors in 1974. He has served on the following association committees: annual meeting, awards, employee relations, Expo promotion, finance, membership development and nominating.

His affiliations include American Dairy Science Association, Dairy Shrine Club, International Association of Milk, Food and Environmental Sanitarians, Holstein Friesian Association of America, National Speakers Association, and World Dairy Expo. He is a director of the Bank of Fort Atkinson, deacon of the Congregational Church and past president of the Fort Atkinson Kiwanis Club.

He is a native of Enon Valley, Pa., and is a 1950 graduate of Pennsylvania State University in agricultural economics. Nesbitt and his wife Donna and daughter Sandra live at 711 Blackhawk Drive, Fort Atkinson, Wis.

DFISA is the national trade association of companies which manufacture equipment, products, and supplies for or render services to the food, beverage and dairy processing industries. DFISA membership consists of 425 companies located in the U.S. and abroad.

NSF News Release

Government, science and industry representatives are meeting under the auspices of the National Sanitation Foundation (NSF) to set new standards of performance, design and construction for home drinking water filters that claim health related benefits.

NSF, whose standards cover products and services that relate to the public health, has brought together representatives from:

EPA Office of Water Supply
EPA Office of Pesticide Programs
National Bureau of Standards
U.S. Army
Department of Housing and Urban Development
Veterans Administration
National Park Service
American Water Works Association
Conference of State Sanitary Engineers
American Society of Sanitary Engineering
University of Michigan
International Association of Plumbing and Mechanical Officials
Council of Local Environmental Health Administrators

Also participating in the deliberations are 16 manufacturers of drinking water treatment units.

In an April meeting at NSF headquarters in Ann Arbor, the Task Committees on Drinking Water Treatment Units are developing detailed new requirements which will specify how water filter units will be built and how they will perform in removing chemicals, bacteria and particles from drinking water.

When completed, the standards will establish performance test procedures that will be the basis for evaluating the claims made by the manufacturers of these units. Drinking water filter units that meet the standards may bear the NSF seal and may advertise the fact to the public.

The standards development and testing program is expected to be completed by mid-1979, and drinking water filter units will be qualified in the NSF laboratory shortly thereafter.

3A Adopts Standard For Culinary Steam

Criteria for the production of culinary steam, the technique of using steam in direct contact with the food product during processing, has been established by the 3-A Sanitary Standards Committees in the form of a new and separate 3-A Accepted Practice.

The action was taken at the 3-A spring meeting at Milwaukee May 2-4 as the result of the increasing importance of culinary steam in ultrapasteurization and aseptic processing. Originally considered years ago for foam and air-space heating in batch pasteurization of milk, provisions for producing the steam were initially included in the 1964 3-A batch pasteurizer standards. The new Accepted Practice recognizes the growing need for these unique criteria and their application to broad food processing in which the product is in contact with steam.

In other action, a series of updating amendments were adopted to the 3-A Practices for milk drying systems, for ice cream feezers (to provide for optional metal alloy); for fillers and sealers of singleservice containers (to provide for electroless coatings on filler parts); for fittings (to provide for electric automatic milk samplers), and for plastics (to add two new generic classes to the 3-A Plastics Standard).

Eight tentative standards, amendments or revisions were accepted and authorized for publication and eight were returned to the technical committee of Dairy and Food Industries Supply Association for revisions to be scheduled at the next meeting. Drafts were adopted for signing and publication will carry varying effective dates. All will be published in *Journal of Food Protection,* from which reprints will be available for distribution to the industry.

The 3-A program safeguards the public health through standards and practices for the cleanability of dairy processing equipment to protect the product against contamination from the equipment itself or foreign elements of dust, dirt or liquids. The program is conducted through the voluntary participation of dairy processors, equipment manufacturers, public health officials and sanitarians and their trade and professional associations. In general, 3-A standards and practices are accepted in most public health jurisdictions at the federal, state and local level. They are cited in the recommended Grade "A" Pasteurized Milk Ordinance of the U.S. Public Health Service.

Meat Curing Salts Won't Stop Aflatoxin

Nitrite and salt used in cured meat products do not prevent the possibility of aflatoxin food poisoning if the meat contains Aspergillus organisms and they're allowed to grow, according to University of Wisconsin-Madison food scientists.

K. R. Meier and E. H. Marth added aflatoxin producing mold organisms to the surface of beef sausages made with various concentrations of curing salt mixtures, then recorded mold growth and aflatoxin production after the sausages had been held at around 80 degrees for a week. In addition, they made the same kind of tests with laboratory culture media with various additions of salt and nitrite.

Aflatoxin production was lowest on sausages with 2 to 3 percent salt and no nitrite, and in general salt decreased aflatoxin production while nitrite increased it. Two percent salt appeared a bit better than higher or lower levels. Sugar is also normally added to sausage, but it seemed to have no effect in these tests.

Aflatoxin is a poison produced during the growth period of various species of Aspergillus molds. It can cause lack of appetite, reduced alertness and a staggering gait.

Aflatoxin can even kill. Exposure over long periods often promotes malignant liver tumors in animals and probably humans.

In view of the serious effect of aflatoxin, it's extremely important to keep Aspergillus molds out of foods and feeds, to destroy the organisms in contaminated materials quickly before they have time to grow, and to store critical foods in a way that does not permit mold growth.

Meier and Marth's research indicates the need to respect the dangers of aflatoxin production in sausages. The storage conditions they used would represent extreme mishandling of food. But the possibility exists that someone might hold sausage or other cured meats at a high temperature for some time, thinking that the curing salts used in processing would keep the product safe.

The research results clearly show that's not the case.

In other research on aflatoxin, Marth and G. G. Alderman found that oils from orange and lemon peels can suppress mold growth and toxin formation. They might be useful for reducing aflatoxin danger with some foods.

The researchers found that 2,000 to 3,000 parts per million of the citrus oils dramatically reduced aflatoxin formation in grapefruit juice to which they added Aspergillus mold spores and held at a high temperature for a week. The natural oils do a better job than the primary chemical component of the oils, but even very high concentrations don't completely prevent aflatoxin formation.

Other research suggests that a bland food such as milk could contain up to 1,000 parts per million of orange oil without adverse effects on the taste. More flavorful foods possibly would tolerate higher concentrations without affecting the taste.

This research was partially supported by the U.S. Public Health Service.

Diet and Hyperkinesis

During the past few years, controversy has emerged regarding a diet proposed to reduce hyperkinesis in children. The so-called Feingold or Kaiser-Permanente diet eliminates artifical food additives, colorings, and salicylates and all foods which may contain them. The diet is said to alleviate the symptoms of hyperkinesis, a childhood condition often marked by hyperactivity, short attention span, and reduced learning ability.

The originator of this diet, Dr. Benjamin Feingold, maintained that his patients improved significantly on this regimen. His observations, however, were not supported by carefully controlled scientific studies.

Several studies were subsequently initiated in an attempt to replicate Dr. Feingold's findings. These were done in well-designed, controlled conditions. No significant differences were seen between children placed on the additive-free diet and those whose diet was not altered in this way.

On the basis of the findings of these studies, the National Advisory Committee on Hyperkinesis and Food Additives concluded that artificial food additives in the diet do not substantially aggravate the behavior of children with learning disabilities and hyperkinesis. Thus, the Committee sees no reason to initiate major changes in food manufacture or labeling.

It is understandable that parents of children exhibiting symptoms of hyperkinesis would seek any possible help. Certainly additional research needs to be done in this area of medicine. At present there appears to be no justification for altering children's diets, or to deprive them of foods they both enjoy and need for their nutritional contributions, in hopes of alleviating the symptoms of hyperkinesis.

Cataracts and Dairy Foods

There is much confusion as to what causes cataracts, a condition of the eye resulting in blurred vision and often diagnosed by a grey or whitish spot over the pupil. In the midst of this confusion, erroneous statements have been made that yogurt and other dairy foods can cause cataracts. Basically, this confusion results from faulty logic.

While most cataracts are brought on by advancing age or physical or chemical injury, an inborn error of metabolism known as galactosemia can lead to the eye condition. Galactosemia is the inability to properly digest a common sugar known as galactose. Galactose, in turn, is found as a component of more complex sugars such as lactose (milk sugar) and raffinose (beet sugar). When these sugars are digested by enzymes in the gastric system, galactose is one of the simple sugars broken down for use by the body. People with galactosemia, however, cannot utilize galactose; and one possible result of the sugar's remaining unused in the body is cataracts.

Faulty logic, though, has led to the belief that high dietary intake of galactose can lead to cataracts in normal individuals. Galactosemia is an unfortunate, relatively rare condition. Symptoms of that disorder cannot be extrapolated to the general population.

Early confusion in this area arose when rats developed cataracts on a diet composed solely of yogurt. While no one eats a diet solely of yogurt, rats cannot digest large amounts of galactose. Except for the rare condition of galactosemia almost all humans have an abundance of the enzyme essential for complete galactose utilization.

All authoritative medical agencies would agree that there is no correlation between cataracts and consumption of dairy foods—or, for that matter, of sugar beets—for the general population.

Book Review

Fundamentals of Normal Nutrition 3rd Edition

By Corrine H. Robinson, R.D. (with assistance of Emma S. Weigley PhD., R.D.) New York: Macmillan Publishing Co., Inc., 1978. Pp 608 (approx) Hardbound. Price $12.50.

The author states that this text is written for students taking their first course in nutrition or for the lay person with some chemistry and biology background.

The 'brass tack' topics of digestion, absorption, metabolism, vitamins, minerals, and etc. are covered in the initial chapters of the book. Then Ms. Robinson procedes to discuss such current topics as: food misinformation, faddism, nutrition quackery, the role of fiber in the diet, and the national nutrition policy issue.

A strength of this text is that it is one of the author's goals to provide the reader with practical examples of the application of nutrition science. She accomplishes this goal by forcing the reader to view the information she offers not only from a personal standpoint but also from a public health and international standpoint. The reader is therefore encouraged to look at the influence of politics and legislation on nutrition issues . . . a topic which has been long neglected by many introductory texts.

In chapter 29, the author did an excellent job of familiarizing the reader with background information on some of the current trends in nutrition. From this chapter the student has a sound base to draw upon when faced with the future trends, related to: Medicare, maternal and child health and welfare programs, nutrition programs for the elderly, and food (and non-food) assistance programs. Basically this chapter tells where these programs are and in which direction they are or would like to be headed. A fringe benefit of the chapter is that these issues are presented in an especially easy-to-read and easy-to-understand fashion.

At the end of each chapter, the author furnishes problems and review questions for the students. In addition, a complete and current listing of references is provided. Throughout the book many helpful charts and tables are used to supplement the reading material. Pictures are in black and white.

A weakness of the book is that in her effort to keep step with the times the author may have provided information that would be confusing to students at the entry level. For instance, in her chapter entitled "Seeking New Answers and Direction," she outlines the "prudent diet" without discussing the *specifics* of why adapting the dietary suggestions included in the "prudent diet" might be beneficial for an individual.

In her chapter on lipids she states that "a moderate reduction of cholesterol intake, 400 to 500 mg daily, is probably desirable for all persons." This she says, "is readily achieved by restricting the intake of eggs to four per week.

Both of these highly controversial areas are of interest to student and lay persons. However, it seems that it would be easier for both the teacher and the reader if the less established material were set aside from the rest or outlined as "speculative."

Though the book may venture beyond its recommended level in places, it is thorough, complete, and current. Out of the classroom, it would be an excellent reference for both students and professionals. Instructors of nutrition for nursing students should take a close look at this text for it could be readily adopted for this use.

Gloria Potts
(Ahrendsen)
Senior-Student Dietitian
Iowa State University

Many Good Sites In Kansas City

Dear Conventioneers:

Welcome to our beautiful city! We can show you a real good time while you are here.

Kansas City has many sites for you to see and we will give the ladies a grand tour of the Harry S. Truman Presidential Library. Also, the historic town of Independence, Missouri and the home of Harry S. Truman, where Bess still resides. Then we will take you to the $64 million dollar Harry S. Truman Dual Stadium Complex, the only one of its kind in the country. You probably have seen the Kansas City Royals, and the Kansas City Chiefs play on television. Now you will see for yourself what the sportscasters call the Taj Mahal of sports. Then you will be served a lovely smogasbord in the dining area that looks out onto the ball field. The beautiful fountains will be on display.

Then, the buses will return you to the hotel about 3:45. This will give you time to get ready for the nite out on the farm, which is full of surprises, good food, music, and dancing.

On Tuesday morning another great day. The buses will leave the hotel around 10:30 for the Nelson Art Gallery and a tour of the famous Plaza Shopping Center of the world. Then on to the Cultural Square in Crown Center where you will be able to stay and have lunch at the elegant American Restaurant. Another beautiful smogasbord! You will see the worlds largest greeting card company, Hallmark Cards, and the scene of the $350 million dollar urban complex right out of the window where you will be dining. A view you will not forget!

On the final morning we will have a poolside luncheon at the Hilton Airport Plaza Inn, with the Lincoln Junior High School Drill Team.

Now, if you plan to stay the rest of the week in Kansas City, there are many more sites to see such as the Starlight Theatre at Swope Park, Worlds of Fun, Old Westport Area, Mission Hills residential area, the third wealthiest county in the United States where many celebrities and millionaires live in these magnificent estates.

Then, there are several dinner clubs, such as the Tiffany's Attic, and Waldo Astoria. Both feature a Las Vegas show with a delicious meal. If you are interested, reservations should be made as soon as possible. Both places are listed in the directory.

Sincerely,
Bernice G. Michaels
Ladies Hospitality

Association Affairs

Florida Plans for Next Years Annual Meeting

Dr. Ron Richter receives a watch and best wishes from R. L. Jolley on behalf of the Association.

The Florida Affiliate held its Annual Conference on March 14, 15, 16 in Orlando. The meeting was highlighted by a banquet at which Dr. Ron Richter was honored for his service to the Association and to the Florida Dairy Industry.

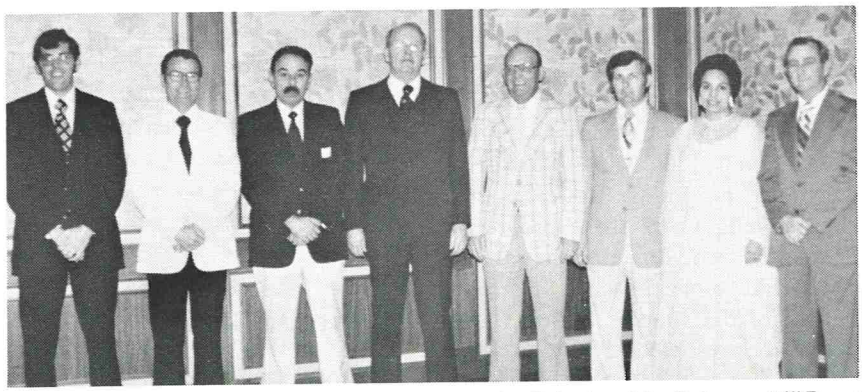
Conferees heard from an impressive group of speakers on such subjects as liquid sugar processing, antibiotic testing, orange juice lab procedures, sterile processing, goat milk, and aflatoxins, among others. We were especially pleased to have the State's Lt. Governor address the group on the topic of water quality.

At the meeting Joe Hayes of Tampa assumed the presidency of the Florida Association having been voted president-elect the previous year. Florida is proud to announce that Ms. Lupe Wiltsey of Borden Company, Miami, was chosen as president-elect at this year's meeting. We believe Lupe will become the first woman president of an I.A.M.F.E.S. affiliate.

New board members elected at the conference were: Tom Hart, past president and new secretary-treasurer, Fort Myers; Luther Lockaby, Plant City; W. A. Brown, Tallahassee; Dick Holtsclaw, Miami; John Koburger, Gainesville; and Ray McCown, Orlando.

The main topic of conversation in Orlando this year was next year's International Convention at the Sheraton Twin Towers which we will be hosting. We have been working on this now for nearly two years and Florida promises the best International meeting ever. Disney World, Sea World, and Circus World are just the beginning.

The 1977-78 Board voted to continue the Association's policy of awarding a free trip to the current year's International meeting as the door prize at our annual banquet. All members present are eligible. This year Dalton Dowdy of Jacksonville will be going to Kansas City as the guest of the Florida Affiliate.



New officers and directors are left to right: Tom Hart, Dick Holtsclaw, John Koburger, Bill Brown, Luther Lockaby, Joe Hayes, Lupe Wiltsey, and Ray McCown.

To: **ALL IAMFES MEMBERS**
From: **A SUSTAINING MEMBER, THE SINGLE SERVICE INSTITUTE, INC.**
Subject: **FREE MATERIALS OF INTEREST TO HEALTH PROFESSIONALS**

We would like to take this opportunity to bring to your attention various materials we have published on issues about which we feel we share a common concern.

"We" are the Single Service Institute -- the national trade association of manufacturers of single-use food service and packaging products, paper and plastic cups and plates among them. Our common concern is with public health and sanitation and the environmental problems that face our citizens. We feel single service products play a significant role in the health area, among consumers at home and especially in helping to ensure sanitation in public eating places. The sanitation value of paper and plastic single-use utensils has been endorsed in policy statements and resolutions passed by IAMFES and other leading professional societies in the public health and environmental fields.

In addition to health and sanitation, the Single Service Institute has long been concerned with environmental problems facing our country, such as litter and solid waste. We would like you to know our thoughts on these important matters, and, through you, to contribute to public discussion of them.

With this in mind, we feel that the following publications we have issued may be of interest to you as environmental health professionals.

- **Sanitation, Safety & Single Service** -- A compilation of studies and policy statements which relate single service to various public health and environmental issues.
- **What's Singular About Single Service?** -- A summary of the distinctive qualities and benefits of single-use food service utensils, along with a perspective on their environmental impact.
- **Single Service & Sanitation** -- A report on a research study comparing the microbiological quality of single service and permanent ware.
- **Single Service & Public Health** -- A report on the way public health and environmental professionals view the values of single service.
- **The Environmental Impact of "Disposables"** --An analysis of the environmental issues relating to single service items, and a review of the "trade-offs" involved in their use.
- **Keep Single Service Sanitary** -- A guide to food service operators in maintaining the high sanitation levels afforded by single service in public eating places.
- **Environment News Digest** -- A bi-monthly magazine devoted to public health and environmental problems and solutions. *END* is distributed free and you are invited to subscribe.

To receive a "sampler" containing these publications or to put your name on the *Environment News Digest* subscription list at no charge, write to:

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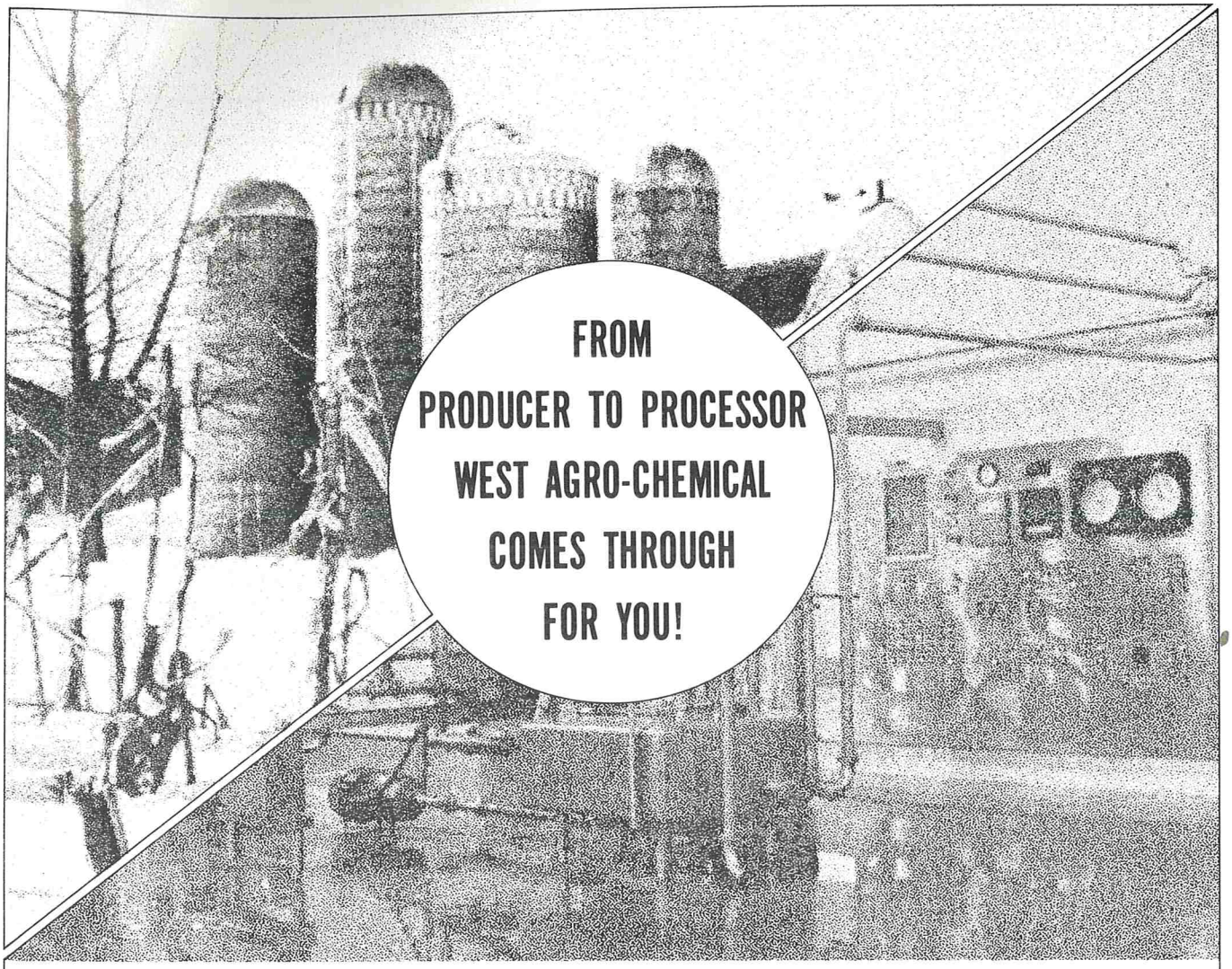

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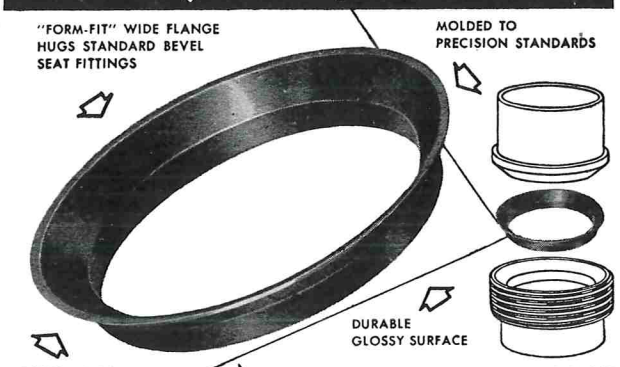

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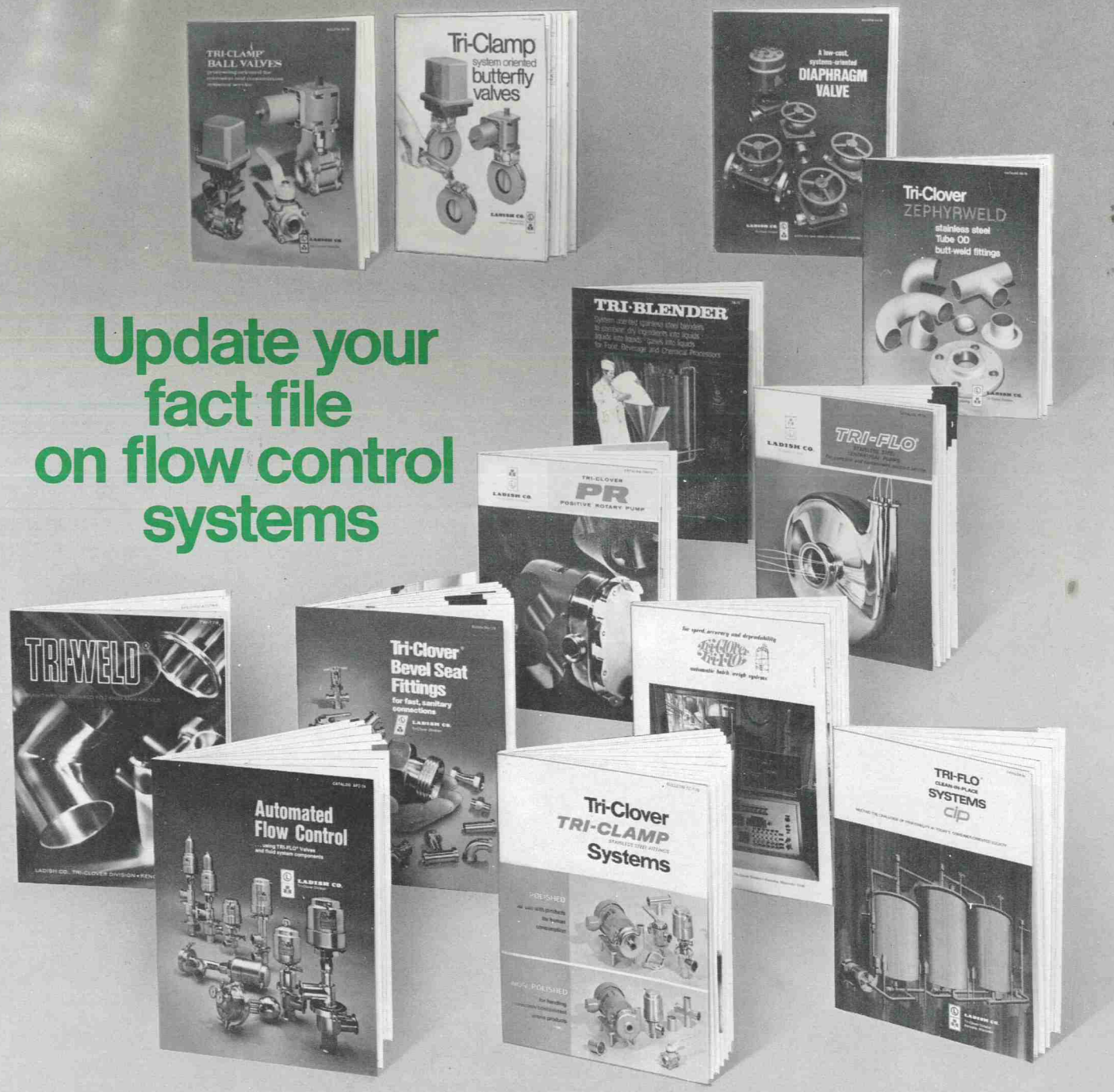

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Stimulation: The First Step Toward A Better Harvest

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It's milking time, and you are ready once more to harvest that milk crop you continue to work so hard for. You have provided the best nutrients to these dairy animals, and the best housing. You have raised them from calves and provided them with every benefit at your disposal to assure that they will be good producers. And now, they have just entered your milking parlor or are ready to be milked in the barn. Your next step, stimulation, will either make all your work worthwhile, or negate much of the effort you have put in.

Oxytocin Means Let Down

Stimulation is more than cleaning the udder before milking. Properly done, stimulation substitutes completely for the natural signal provided by the calf to tell the cow she is hungry. Oxytocin, a hormone released into the blood stream after stimulation, signals the milk making glands (alveoli) to release the milk they have produced. This squeezing out of tiny droplets of milk from each of the millions of alveoli is called "let-down." The let-down is directly related to the amount of oxytocin in the blood stream, and the amount of oxytocin present is directly related to the thoroughness of the stimulation.

Complementary Milk: Profits Left in the Udder

In tests conducted on a number of herds, we found that from three percent to twenty percent more milk was present in the udder than was being harvested, partly due to inadequate stimulation. The animals were first stimulated and milked in the normal way, by their regular milker, and production recorded just before complementary was obtained. Later, the cows were stimulated as usual and then, just before attaching the milking machine, they were injected with an adequate amount of oxytocin. The average cow gave in the area of ten percent more milk after receiving maximum stimulation with the additional oxytocin. This ten percent as complementary milk (instead of being part of the normal) represents profit lost for three reasons: First, this milk would not have been harvested during a normal milking. Second, the last of the milk is always richer in fat, and so the fat test would be lower. And last, with the complementary milk remaining in the alveoli, the cells become less active in producing milk. Over a normal lactation period, this can make a good cow produce far less than she is capable of. With proper stimulation, the amount of complementary can be reduced to about the same minimum as injecting oxytocin.

Thirty Seconds of Profitable Time

All results point to the fact that about thirty seconds is the amount of time necessary to achieve maximum stimulation and proper cleaning. This should be a vigorous massage—preferably with a disposable paper towel. Less time fails to provide the amount of needed oxytocin, and more than thirty seconds of stimulation does not increase the level. Time spent stimulating the animal will determine if she has received an adequate natural signal to allow maximum let-down. The thirty seconds you spend on each cow to assure proper stimulation may well be the most profitable time you use on the farm.