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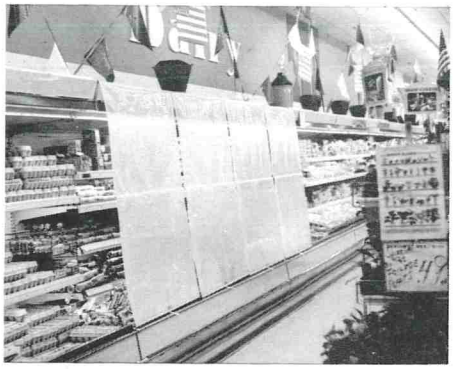

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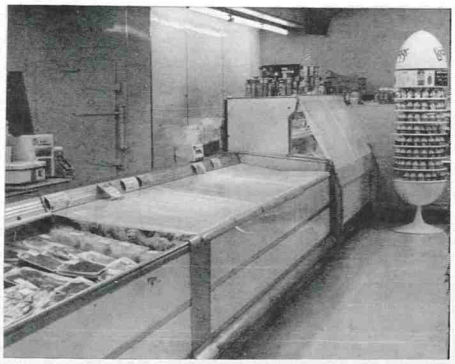

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J. Milk Food Technol. Vol. 39, No. 5, Pages 322-324 (May, 1976)

Validity of Members of the Total Coliform and Fecal Coliform Groups for Indicating the Presence of *Salmonella* in the Quahaug, *Mercenaria mercenaria*

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(Received for publication September 26, 1975)

ABSTRACT

To determine the relationship of most probable number (MPN) of the total and fecal coliform groups in shellfish and shellfish-growing waters to the presence of *Salmonella* in quahaugs (*Mercenaria mercenaria*), a microbiological survey of 214 samples of the quahaug, or hard-shell clam, was done over 24 months. For purposes of this study, waters were classified as safe for shellfish harvesting by one of two criteria: (*a*) a total coliform MPN of $\leq$ 70/100 ml of water or (*b*) a fecal coliform MPN value of $\leqslant$14/100 ml of water. None of the quahaug samples harvested from waters meeting these standards contained *Salmonella.* Additionally, *Salmonella* was not detected in any of the quahaug samples meeting the wholesale market quality standard of 230 fecal coliforms per 100 g of shellfish as specified by the National Shellfish Sanitation Program. An increase in the total coliform and fecal coliform MPN of the waters more closely paralleled an increase in the fecal coliform MPN, as compared to the total coliform MPN, of the quahaug meats. Five *Salmonella* serotypes, including *Salmonella parathyphi* B, were found singly and in combination in five (2.3%) of the quahaug samples in this survey.

During a recent 2-year interval, a microbiological survey was conducted of shellfish-growing waters and shellfish samples harvested from such waters. On the primary bases of economic importance and availability, this survey was restricted to the investigation of two types of shellfish: the Eastern oyster, *Crassostrea virginica,* and the quahaug, *Mercenaria mercenaria.* The project was concerned not only with updating the determination of the safety of the shellfish-growing waters and of the microbiological quality of shellfish harvested from these waters, but was also involved in investigating: (*a*) the relative reliability of the total coliform and fecal coliform groups in the growing waters to indicate the presence of *Salmonella* in the shellfish themselves, (*b*) the comparative efficiency of various analytical procedures for recovering *Salmonella* from shellfish, and (*c*) the relative occurrence of the various *Salmonella* serotypes in shellfish. The findings with the oyster samples have been reported previously (*2*); the data of the quahaug samples are given here.

MATERIALS AND METHODS

This survey, conducted during a consecutive 24-month interval, was initiated on July 1, 1972, and ended on June 30, 1974. The collection of quahaug samples and the water samples overlying these harvesting areas was done by the Northeast Technical Services Unit (NETSU), a field unit of the Food and Drug Administration's Shellfish Sanitation Branch, located at Davisville, Rhode Island. Sampling was dependent upon such factors as tidal movements, climatic conditions, and estuary size. From each of 8-10 sampling sites, enough shellstock was collected to yield a sample of approximately 500 g of shellfish meat and liquor. Additionally, water samples consisting of approximately 200 ml were collected above each quahaug sampling site. The shellfish and water samples were collected monthly from shellfish-producing estuaries in Rhode Island, Massachusetts, Connecticut, and New Jersey. Collection of samples was equally divided between areas approved for harvesting and adjacent conditionally approved, restricted, or prohibited areas. All areas were classified in accordance with the specifications of the *Manual of Operations* (*4*) of the National Shellfish Sanitation Program (NSSP).

During sampling operations, water depth over the actual sampling areas ranged from $\leq$ 1 to 30 ft. Salinities ranged from 5.4 to 32.6 parts per thousand, and water temperatures ranged from 2.6 to 8.7 C during November-April and from 10.9 to 27.9 C during May-October.

To effect a more even distribution of the analytical workload, all water samples and most of the quahaug samples from approved growing areas were examined by NETSU, whereas all shellfish samples from non-approved areas and the balance of the shellfish samples from the approved areas were sent for analysis by air express to the Division of Microbiology laboratories in Washington, D.C. Definition of samples, specification of sizes of analytical units, procedures for collection and shipment of water and quahaug samples, and techniques for recording hydrographic data have been detailed previously (*2*). The recommended procedures of the American Public Health Association were used in the determination of coliform numbers in waters and shellfish (*1*). To effect maximum recovery of *Salmonella* from the shellfish, both the lactose pre-enrichment and direct selective enrichment procedures of the *Bacteriological Analytical Manual* (*BAM*) (*5*) were used. Each of three 100-g portions of the shellfish sample was blended in a high-speed blender for 60-120 sec at 14,000 rev/min with 150 ml of 35 C-tempered lactose, selenite cystine (SC), or tetrathionate (TT) broth containing 10 mg of brilliant green dye per liter. The homogenates were poured into flasks containing the remainder (750 ml) of the 900 ml of the respective broths. After adjusting the pH of the broth mixtures to 6.8 $\pm$ 0.2, flasks were incubated in a walk-in incubator at 35 C. After 24 h of incubation, 100

ml of lactose pre-enrichment mixture was subcultured to flasks containing 900 ml of fresh, 35 C-tempered SC or TT broth. After 24 and 48 h of incubation, a portion of the contents of the flasks containing the selective enrichment broths was streaked with a 3-mm loop to plates of brilliant green, bismuth sulfite (BS) and *Salmonella-Shigella* agar (Difco). All plates were incubated and examined after a 24-h incubation period. The BS agar plates were examined after 24 h, but were incubated an additional 24 h at which time they were re-examined. When present, at least two colonies suspicious for *Salmonella* were picked per plate in tandem to triple sugar iron agar and lysine iron agar. Cultures giving reactions suspicious for *Salmonella* were submitted to biochemical screening and, if necessary, were definitively serotyped.

RESULTS AND DISCUSSION

The relationship of levels of total coliforms in the waters to the levels of total and fecal coliforms and the presence of *Salmonella* in the quahaugs harvested from these waters is shown in Table 1. Except for the first category of 0-70 coliforms/100 ml of water, the categories of water MPN were arbitrarily established. The upper limit of 70 coliforms/100 ml of water was chosen as the first category since the present standard for approved shellfish-growing waters specifies that the total coliform median MPN of the water does not exceed 70/100 ml, and that not more than 10% of the samples ordinarily exceed an MPN of 230/100 ml for a 5-tube decimal dilution test, or 330/100 ml for the 3-tube decimal dilution test, in those portions of the area most probably exposed to fecal contamination during the most unfavorable hydrographic and pollution conditions (*4*).

In addition to this growing-area standard, there is a quality standard established by the NSSP for shellfish at the wholesale market level. Shellfish having a fecal coliform MPN of $\leq$230/100 g and a total plate count not in excess of 500,000 organisms/g of shellfish are considered to be of acceptable quality at the wholesale market (*4*). The range of fecal coliforms in quahaugs harvested from waters with a total coliform MPN of $\leq$ 70 was from < 20 to 260. Only one of these samples had a fecal coliform MPN in excess of 230. Of the 40 quahaug samples examined in this category, 33 had indeterminant fecal coliform MPNs of < 20, reflecting a bacteriologically high-quality product.

Salmonella was not recovered in quahaug samples harvested from waters of the 0-70 and the 71-200 total coliform MPN categories. It was not until the total coliform MPN of the waters exceeded 200 that *Salmonella* was recovered from the quahaugs. The range of total coliform MPN of the waters at which *Salmonella* could be recovered was from 490 to 11,000.

Table 2 shows the relationship between level of fecal coliforms in the waters to the levels of total and fecal coliforms and the presence of *Salmonella* in quahaugs taken from these waters. Sixty-seven quahaug samples were harvested from waters having a fecal coliform MPN of $\leq$ 14. Fourteen was chosen as the upper limit of the first fecal coliform category since the recently recommended standard at the Eighth NSSP Workshop proposed that "the median fecal coliform MPN value for a sampling station shall not exceed 14/100 ml of sample and not more than 10% of the samples shall exceed 43 for a 5-tube, 3-dilution test or 49 for a 3-tube, 3-dilution test" (*3*). Forty-eight of the quahaug samples in the $\leq$ 14 category had indeterminant fecal coliform MPN of < 20 and none had MPN in excess of 230 fecal coliforms/100 g of quahaugs.

No *Salmonella* were recovered from any of the 67 samples in the $\leq$14 category. The range of fecal coliform MPN of waters at which *Salmonella* could be recovered in the quahaug meats was from 33 to 2300. Tables 1 and

TABLE 1. *Correlation of coliforms and* Salmonella *in quahaugs to the total coliform densities of overlying waters*

Total coliform MPN/100 ml of overlying waters	Quahaug samples examined		Total coliform MPN/100 g quahaug meats		Fecal coliform MPN/100 g quahaug meats		*Salmonella*-positive	
	No.	Percent[a]	Range	Median	Range	Median	No.	Percent
0-70	40	18.7	$<20-2.30 \times 10^3$	46	<20-260	<20	0	0
71-200	26	12.2	$<20-4.90 \times 10^3$	230	$<20-1.09 \times 10^3$	20	0	0
201-1,000	60	28.0	$<20-7.90 \times 10^3$	330	$<20-3.30 \times 10^3$	<20	2	3.3[b]
>1,000	88	41.1	$<20-1.72 \times 10^5$	130	$<20-3.30 \times 10^4$	49	3	3.4[b]
Total	214	100.0					5	
Overall average								2.3[a]

[a]Expressed as percentage of 214 samples.
[b]Expressed as percentage of samples in this category.

TABLE 2. *Correlation of coliforms and* Salmonella *in quahaugs to the fecal coliform densities of overlying waters*

Fecal coliform MPN/100 ml of overlying waters	Quahaug samples examined		Total coliform MPN/100 g quahaug meats		Fecal coliform MPN/100 g quahaug meats		*Salmonella*-positive	
	No.	Percent[a]	Range	Median	Range	Median	No.	Percent
0-14	67	31.3	$<20-5.42 \times 10^3$	70	<20-230	<20	0	0
15-70	51	23.8	$<20-7.00 \times 10^3$	220	$<20-3.30 \times 10^3$	<20	2	3.9[b]
71-200	29	13.6	$<20-7.90 \times 10^3$	330	$<20-1.10 \times 10^4$	<20	0	0
201-1,000	44	20.6	$<20-1.30 \times 10^5$	230	$<20-3.30 \times 10^4$	20	1	2.3[b]
>1,000	23	10.7	$<20-1.72 \times 10^5$	130	$<20-3.30 \times 10^4$	70	2	8.7[b]
Total	214	100.0					5	
Overall Average								2.3[a]

[a]Expressed as percentage of 214 samples.
[b]Expressed as percentage of samples in this category.

2 show that an increase in the total and fecal coliform MPN of the waters more closely paralleled an increase in the fecal coliform MPN, as compared to the total coliform MPN, of the quahaug meats. This finding would support the NSSP use of the fecal coliform indicator group, rather than the total coliform indicator group, in establishing the present wholesale market quality standard for quahaugs and other shellfish as well.

Of the total of 214 quahaug samples examined, 182 had fecal coliform MPN of ≤ 230 (Table 3). Even though some of these shellfish samples had been harvested from waters having a total coliform MPN exceeding 70 or a fecal coliform MPN exceeding 14, many of these samples were able to meet the NSSP wholesale market standard. The finding that *Salmonella* was not isolated from any of the 182 samples meeting the market quality standard substantially supports the validity and bacteriological safety of the NSSP standard. The range of total coliforms and fecal coliforms in the quahaug samples containing *Salmonella* was from 1,300 to 172,000 and from 490 to 33,000, respectively.

TABLE 3. *Correlation of* Salmonella *in quahaugs to the fecal coliform densities in quahaugs*

Fecal coliform MPN/100 g quahaug meats	Quahaug samples examined		*Salmonella*-positive	
	Number	Percentage[a]	Number	Percentage
0-230	182	85.0	0	0
>230	32	15.0	5	15.6[b]
Total	214	100.0	5	
Overall average				2.3[a]

[a]Expressed as percentage of 214 samples.
[b]Expressed as percentage of samples in this category.

The occurrence of such few numbers of *Salmonella*-positive samples led to an investigation of the sensitivity of the BAM method for recovering *Salmonella* from both oysters and quahaugs. As few as 8-10 *Salmonella* organisms/100 g of artificially contaminated shellfish could consistently be recovered, indicating an acceptable level of sensitivity. Additionally, the possibility of the methodology used being inadequate is further minimized when one considers that the methodology for recovering *Salmonella* from quahaugs was the same as that used in the analysis of oysters, where 11.1% of the oyster samples examined were positive for *Salmonella* (2). Of the five *Salmonella*-positive quahaug samplers, three were positive exclusively by direct enrichment in TT broth with added brilliant green dye, whereas the remaining two samples were positive by both the lactose pre-enrichment and direct selective enrichment procedures. *Salmonella typhimurium* and *Salmonella paratyphi B* were found individually in two of the samples, *Salmonella anatum* and *Salmonella thompson* were both found in each of two samples, and one sample contained *S. anatum, S. thompson,* and *Salmonella derby. Salmonella* was chosen as the representative of the potential pathogen load since the methodology for its detection is established, relatively standardized, and sensitive. In determining the overall significance of the data from this study, it must be kept in mind that other pathogenic bacteria, viruses, and parasites are capable of existing and proliferating in shellfish.

In this survey, *Salmonella* was not found in the quahaug when either the coliform or fecal coliform densities were within the recommended limits. Because of the relatively low number of *Salmonella*-positive quahaug samples, however, no conclusion can be made about the comparative efficacy of these two indicator groups to indicate the presence of *Salmonella* in quahaugs. It can be concluded, however, that both the present total coliform standard and the proposed fecal coliform growing-area standards were acceptable from the standpoint that the use of each resulted in the harvesting of a shellfish product capable of meeting or surpassing the present wholesale market quality standard and indicated the absence of *Salmonella* in this marketed product. The findings of this study support the conclusion that the bacteriological content of quahaugs is a reflection of the bacteriological conditions of the waters overlying the sites from which the shellfish were harvested.

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The authors gratefully acknowledge Captain James Verber, Chief, Northeast Technical Services Unit, and his staff for the collection of samples. Appreciation is expressed to Aida Romero and Paul Poelma, Division of Microbiology, for serotyping of *Salmonella* isolates. Special thanks are expressed to Daniel Hunt, Assistant Chief, Shellfish Sanitation Branch, for co-ordination of field activities.

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J. Milk Food Technol. Vol. 39, No. 5, Pages 325-327 (May, 1976)

Bacterial Growth and Vitamin Content of Milk[1]

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(Received for publication September 24, 1975)

ABSTRACT

Five brands of whole milk from retail stores were analyzed for quantitative vitamin changes when the samples had developed off flavor in storage at 10 C. Vitamins A, B_6, and niacin decreased by a small but significant amount. Average concentration of thiamine, riboflavin, and B_{12} were not changed during bacterial growth. Cultures for Dagano cheese and yogurt and two strains of *Streptococcus lactis* decreased the contents of Vitamin A, riboflavin, and B_{12} during growth. The averages for thiamine and B_6 content were not significantly changed. Niacin content was decreased by growth of each of the four cultures. Three strains of *Bacillus subtilis* and six other species of bacteria that caused off flavor in milk during growth produced no significant changes in contents of Vitamins A, B_6, B_{12}, and riboflavin. The mean contents of thiamine and niacin were decreased, but two species did not cause a decrease in niacin.

With the increasing emphasis on nutritional quality of foods, a greater knowledge of bacteriologically caused changes in nutrient content is important. Fresh pasteurized milk is regarded as one of the most nutritious foods, but it is also very perishable due to its ability to support growth of bacteria. Therefore, the dairy industry and consumers should be aware of any nutritional changes that can be caused by growth of bacteria in milk.

Available literature concerning quantitative changes in content of vitamins in milk caused by bacterial growth in general seems to be limited to dairy product cultures (*1, 4, 6, 7, 9, 11, 12*), for example vitamin requirements of the bacteria and also the changes in vitamin contents of cultured milks at the completion of acid development.

Peter (*10*) reported that *Escherichia coli* and *Aerobacter aerogenes* produced extracellular B_2, B_6, and niacin. Currently not enough is known about the quantitative effects of the growth of spoilage bacteria and culture organisms on vitamins in milk. Consumers should be informed if the growth of specific species or strains of bacteria is likely to alter the content of vitamins reported on the label in complying with Food and Drug Administration's nutritional labeling requirements.

The objectives of this investigation were to evaluate the quantitative changes of the more common vitamins in whole milk during heterogenous bacterial spoilage and to observe the influence of individual species during growth of milk. The vitamin content of cultured milks before and after bacterial growth was also studied.

[1]*Michigan Agricultural Experiment Station Journal Article No. 7414.*

EXPERIMENTAL

Duplicate samples of five commercial brands of pasteurized, homogenized, whole milk were purchased at local grocery stores for use in the heterogenous bacterial growth trials. One set of samples (control) was analyzed immediately. Others were held in a dark refrigerated room at 10 C until sensory evaluation indicated the development of no more than a definite off-flavor according to the ADSA milk scoring guide. This necessitated a variable holding period of 3 to 7 days. The pH varied from 6.6 to 4.7 and the standard plate count from 1.4×10^6/ml to 3.1×10^9/ml. Six trials were conducted during November to June. During the final trials, samples were allowed to warm to 21 C for brief periods to simulate conditions during use in some homes.

Dairy product cultures that were used included: (*a*) two different strains of *Streptococcus lactis;* (*b*) a yogurt culture of *Streptococcus thermophilus* and *Lactobacillus bulgaricus;* and (*c*) a Dagano cheese culture containing *Streptococcus lactis, Streptococcus diacetilactis, Streptococcus cremoris,* and *Leuconostoc cremoris.* Samples of milk were heated to 82 C for 30 min, cooled, and inoculated with 1% active culture. Controls were chilled to 4 C and analyzed. The other trial samples were incubated according to common commercial practices.

The following spoilage organisms were used in the study: *Enterobacter aerogenes, Pseudomonas fragi* (*5*), *Escherichia coli, Bacillus subtilis* (strains 705, A1, and Goldblith), *Bacillus cereus, Micrococcus flavus,* and *Alcaligenes viscolactis.* The organisms were maintained on slants of plate count agar and were prepared for the trials by growing them for 24 h in micro inoculum broth (Difco). They were then removed by centrifuging, resuspended in 0.9% saline solution, and 1 to 2 million added to a quart of pasteurized milk. The milk samples inoculated with *P. fragi* (*5*) were held at 10 C until an off-flavor developed. Samples with the other organisms listed above were incubated at 21 C.

Vitamin A was determined fluorometrically (*16*). Niacin was extracted by the A.O.A.C. method (*2*) and analyzed on a Technicon Auto Analyzer (*15*). Thiamine and riboflavin were extracted by methods described by Freed (*3*). The fluorescence of riboflavin before and after reduction was mesured by Auto Analyzer (*13*). Thiamine was determined by the thiochrome method (*14*). Vitamin B_6 was estimated by the cup-plate method (*17*) using *Saccharomyces carlsbergensis* as the test organism. For determination of cobalamin the samples were incubated with papain and sodium cyanide to liberate the bound vitamin as recommended by Gregory (*8*). Then the cobalamin content was estimated by using *Lactobacillus leichmannii* (*2*).

The results were analyzed for significance of difference at the .05 level. The concentrations of the six vitamins were studied in a split plot design. Mean values of experimental samples and controls were compared for each species or strain using a two-sided t test.

RESULTS AND DISCUSSION

Heterogeneous bacterial growth

Results of the vitamin analyses are summarized in Table 1. Samples from all five different companies

TABLE 1. *Vitamin contents of pasteurized market milk before and after bacterial growth*[a]

Sample brand	Vitamin A (IU/ml)		Thiamine (ug/ml)		Riboflavin (ug/ml)		Vitamin B_6 (mug/ml)		Vitamin B_{12} (mug/ml)		Niacin (ug/ml)	
	Control	Experimental sample	Control	Experimental sample	Control	Experimental sample	Control	Experimental sample	Control	Experimental sample	Control	Experimental sample
HA	1.40	1.35	.39	.41	1.70	1.81	.39	.33	1.98	1.87	1.25	1.07
IB	1.44	1.40	.39	.39	1.76	1.74	.41	.37	2.16	2.34	1.22	1.02
GC	1.38	1.31	.42	.39	1.83	1.80	.34	.30	2.53	2.63	1.13	1.00
OD	1.34	1.28	.40	.40	1.76	1.72	.45	.39	2.44	2.49	1.06	0.98
XE	1.36	1.31	.40	.39	1.77	1.76	.54	.49	2.29	2.16	1.23	1.05
Average	1.39	1.33[b]	.40	.40	1.76	1.77	.43	.38[b]	2.28	2.30	1.18	1.03[b]

[a]Mean of six trials.
[b]Significant difference between controls and experimental samples after bacterial growth.

TABLE 2. *Vitamin content of cultures after completion of growth*[a]

Organism	Vitamin A (IU/ml)		Thiamine (ug/ml)		Riboflavin (ug/ml)		Vitamin B_6 (mug/ml)		Vitamin B_{12} (mug/ml)		Niacin (ug/ml)	
	Control	Experimental sample	Control	Experimental sample	Control	Experimental sample	Control	Experimental sample	Control	Experimental sample	Control	Experimental sample
S. lactis A	.88	.73	.32	.32	1.86	1.72[b]	32	.35	1.00	.84[b]	1.37	1.43
S. lactis B	.93	.90	.32	.26	1.82	1.65[b]	22	.22	.94	.70[b]	1.14	1.10
Yogurt Culture	1.05	.98	.32	.18[b]	1.93	1.75[b]	30	.29	.82	.53[b]	1.01	.91
Dagano Culture	.73	.72	.33	.28	1.80	1.74	.39	.39	1.08	.50[b]	.91	.63[b]
Average	.90	.83	.32	.26[b]	1.85	1.72[b]	.31	.31	.96	.64[b]	1.11	1.02[b]

[a]Mean of three trials.
[b]Significant difference between controls and experimental samples.

showed slight average decreases of 4.3, 11.6, and 12.7% of Vitamins A, B_6, and niacin, respectively. Thiamine and riboflavin content increased 5.1 and 6.5%, respectively, for one brand of milk and decreased slightly or remained unchanged in the other four brands. The amount of Vitamin B_{12} increased slightly in three brands of milk and decreased slightly in two but these changes were not significant. Apparently the species of bacteria that grew during off-flavor development affected the content of these six vitamins. Although the data show variations in bacteria counts and acidity of the samples when analyzed for vitamin contents, the off flavors were approximately at the same degree of objection as determined by the expert panel.

Since Vitamin A is not considered necessary for growth of bacteria, it's relatively small average decrease of 4.3% may be attributed to chemical changes during the holding period.

Dairy culture growth

Since only three trials were conducted on each species (or strain) the lack of statistical significance does not necessarily indicate that no change of vitamin content was produced by the cultures during growth.

None of the four cultures (Table 2) had significantly less Vitamin A content than the milk when inoculated. Riboflavin and B_{12} averaged 7.0 and 33.3% decreases, respectively, after growth of each of the four cultures. Thiamine average content showed a significant decrease of 18.8% but no change in one strain of *S. lactis* culture. Gorner and Oravcova (*7*) observed a decrease in the thiamine and riboflavin content of yogurt. Emanuilov and Nachev (*6*) noted that Vitamin B_{12} content declined in yogurt during incubation.

The Dagano culture produced a significant decline in content of B_{12} and niacin. Strain A of *S. lactis* culture caused a slight increase in the amount of Vitamin B_6 and decreases in riboflavin and Vitamin B_{12} content. There was practically no change in thiamine content, and a small increase of 4.4% in niacin content. Strain B of *S. lactis* decreased riboflavin 9.4% and B_{12} 25.5%. Niven (*9*) reported that strains of *S. lactis* differ in their requirements for thiamine. This agrees with data in Table 2. Bambha et al. (*4*) observed a small decrease in B_{12} for 10 cultures of lactic acid bacteria.

Growth of spoilage bacteria

None of the nine species or strains of spoilage bacteria (Table 3) caused a significant quantitative effect on Vitamin A or Vitamin B_{12}.

Growth of *P. fragi* in milk caused a gain of 12.1% in thiamine. The other eight bacterial species or strains caused insignificant vitamin losses during growth. Riboflavin increased in five samples and decreased in four. Seven of the nine different species or strains of bacteria that increased Vitamin B_6 content during growth in the milk were *E. aerogenes, E. coli, B. cereus, A. viscolactis,* and each of the three strains of *B. subtilis.* Four species that increased the Vitamin B_{12} content during growth were *E. aerogenes, B. subtilis* Al, *B. cereus,* and *A. viscolactis.* During growth *M. flavus* significantly increased the niacin by 38.6%, but the slight increase during growth of *P. fragi* was not significant. Four other species of bacteria during growth insignificantly decreased niacin contents and two, *B. subtilis* Al and *Goldblith,* caused significant decreases of 29.4 and 37.8%, respectively. *A. viscolactis* growth caused slight increases in content of Vitamins B_6, B_{12}, niacin content and a significant increase of 9.1% in riboflavin. Results for *B. subtilis* Al indicated very slight increases in three vitamins: A, B_6, and B_{12}.

Above results suggest that after growth of heterogeneous species or single species of spoilage organisms in milk, generally only small, insignificant

TABLE 3. *Quantitative vitamin changes in milk during the growth of spoilage bacteria*[a]

Bacteria	Vitamin A (IU/ml)		Thiamine (ug/ml)		Riboflavin (ug/ml)		Vitamin B_6 (mug/ml)		Vitamin B_{12} (mug/ml)		Niacin (ug/ml)	
	Control	Experimental sample	Control	Experimental sample	Control	Experimental sample	Control	Experimental sample	Control	Experimental sample	Control	Experimental sample
E. aerogenes	1.17	1.12	.38	.37	1.77	1.83	.22	.31[b]	2.43	2.51	1.17	1.00
P. fragi	1.28	1.26	.33	.37	1.80	1.80	.34	.29	2.77	2.49	1.00	1.14
E. coli	1.27	1.24	.39	.32	1.81	1.78	.32	.36	2.47	2.36	1.01	.93
B. subtilis 705	1.08	1.00	.37	.33	1.79	1.75	.27	.29	2.78	2.73	1.43	1.22
B. subtilis Al	1.08	1.09	.37	.31	1.79	1.79	.27	.29	2.78	2.80	1.43	1.01[b]
B. subtilis Goldblith	1.08	1.08	.37	.33	1.79	1.80	.27	.28	2.78	2.70	1.43	.89[b]
B. cereus	1.08	1.01	.37	.33	1.79	1.70	.27	.30	2.78	2.88	1.43	1.24
M. flavus	1.18	1.19	.40	.35	1.75	1.98	.30	.28	2.71	2.71	.88	1.22[b]
A. viscolactis	1.18	1.17	.40	.35	1.75	1.91	.30	.32	2.71	2.80	.88	.99
Average	1.16	1.13	.38	.34	1.78	1.81	.28	.30	2.69	2.67	1.18	1.07[b]

[a]Mean of three trials.

[b]Denotes significant difference between controls and experimental samples.

increases or decreases in the content of six vitamins were observed. The few vitamins with significant decreases in content may be of concern to consumers of milk. A decrease re-emphasizes the desirability of preventing bacterial growth in the milk. Growth of two strains of *S. lactis* Cheddar cheese cultures, two species of bacteria in yogurt, and Dagano cheese culture with four species of bacteria also produce variable changes in contents of the six vitamins that were studied.

Numerous investigators have reported in literature on the adverse effects of chemical changes due to the light exposure and related factors such as type of package, storage time, and temperature. Bacterial growth as well as chemical reactions can have an effect on the nutritional quality.

CONCLUSION

Growth of spoilage or culture bacteria in milk may decrease, increase, or result in no change in Vitamins B_6, B_{12}, thiamine, riboflavin, niacin and Vitamin A depending on the specific species or strain. Generally, the changes in vitamin contents due to bacterial growth were minor when growth had progressed to the point that the milk was unacceptable. Consequently, the changes should be even less in market milks with an acceptable flavor.

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Survival of *Salmonella* in Cheddar Cheese

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ABSTRACT

Salmonella newport, S. new-brunswick, and *S. infantis* were singly added to Cheddar cheese milk to evaluate the behavior of *Salmonella* in Cheddar cheese made from the milk and to obtain survival times when cheese was stored at different temperatures. Of a total of 48 lots of cheese inoculated with *Salmonella,* detectable numbers of these pathogens appeared in 16 samples aged at 4.5 C for 9 months and from six samples stored at 10 C. The information obtained under the conditions of this study indicates that these enteropathogenic microorganisms when initially present in large numbers in milk can survive for extended storage periods in Cheddar cheese of high pH made from the milk.

The assumption that the "aging" process in Cheddar cheese manufacture tends to render the product safe for human consumption prompted an investigation to test its validity when enteric pathogens were present. Also, the length of this "aging" process necessary to effect complete disappearance of these organisms has not been clearly defined so as to satisfy the authors. Fabian (*7*) concluded that a 60-day holding time was too short since pathogens could survive the storage period, especially if the cheese were held at low temperatures, i.e., 4.5 C to 10 C. He added that a 90-day holding period would be preferable and 120 days still better. *Salmonella typhimurium* was found to remain viable in Colby cheese for 302 days when the cheese was held at 6.1 to 8.9 C (*17*). In an early study (*22*) viable *Eberthella* (*Salmonella*) *typhosa* organisms were detected in Cheddar cheese for 34 to 36 days when the cheese was held at 15.6 C. In a later study (*9*) storage of Cheddar cheese contaminated with *S. typhimurium* for 2.5-3 weeks at 13 C or 3.5-4 weeks at 7 C was required to achieve a 90% reduction in number of viable *Salmonella.* The initial inoculum added to pasteurized milk consisted of 25 to 100 cells of *S. typhimurium* per ml. These workers postulated that production of volatile fatty acids in the curd during curing could have been responsible for the decline in number of salmonellae. They stated further that salting of curd appeared to induce a reduction in multiplication rate of the bacteria.

Park et al. (*14*) reported that salmonellae survived during ripening of Cheddar cheese for up to 7 months at 13 C and 10 months at 7 C. They noted that growth of these pathogens during the early stages of ripening and subsequent extended survival of the salmonellae was probably due to, at least in part, high moisture (average 43.2%) and high pH (5.75 after overnight pressing) of the cheese. This high pH resulted from the use of a slow acid-producing starter culture.

The critical pH value for survival of *Salmonella* has been reported to be 4.5 (*6*) and 4.1 or lower (*16*) with a critical salt level of 8% NaCl. It was further noted (*8*) that the lowest pH for growth of *Salmonella* was 5.5, and that they grew best in low acid foods. It has been stated (*2, 11, 20, 21*) that the normal pH of Cheddar cheese is in the range of 5.0 to 5.5.

As a result of these reports, the pH was noted throughout the study and correlations made with it. Its importance in relation to survival patterns is considered to be of extreme importance as results indicated.

The time-temperature effect of pasteurization is sufficient to destroy *Salmonella* (*1, 3, 5, 8, 13, 15*). It is assumed that *Salmonella* recovered from pasteurized products results from recontamination or improperly operating equipment (*12*). This hypothesis was also tested in this study.

MATERIALS AND METHODS

Selection of serotypes

The three serotypes employed in this study were obtained from the Center for Disease Control in Atlanta, Georgia. *S. newport* and *S. infantis* were chosen since they are continually in the top 10 most commonly isolated serotypes of both human and non-human origin (*18*). *S. new-brunswick* was chosen because of its involvement in dried milk contamination (*10*).

Isolation procedure

The organisms were tested for purity by doing pertinent tests as described by the standard description chart of the Society of American Microbiologists and key characteristics of the genus *Salmonella* (*4, 19*). Also, a culture of each serotype was sent to the Public Health Service, Jackson, Mississippi for serotyping. This was done at the end of the study to ensure that the appropriate serotypes had in fact been used and were still present.

The salmonellae which were added to the cheese milk were initially grown in trypticase soy broth. A 24-h culture of these organisms was transferred to raw whole milk which had been steamed for 30 min on three successive days. The cultures were transferred twice in this milk medium to give the pathogens a period for adaptation.

The *Salmonella* cultures were plated immediately before addition to the cheese milk or utensils to determine the expected number of cells per milliliter of cheese milk.

The isolation procedure followed during the course of the study was a

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slight modification of the *Procedure for Examination of Dried and Liquid Milk Products for Salmonellae* (*19*). A Waring blender was used to thoroughly mix 100 g of cheese sample with 900 ml of phosphated, sterile water added as a diluent. The cheese had been aseptically extracted from 9.1+ kg blocks representing each vat of milk used. This procedure for mixing was the only major modification. A 1-10 dilution was prepared in all instances.

Following mixing of the sample, 20 ml of a .1% aqueous solution of brilliant green dye were added to inhibit any gram positive bacteria. Subsequent to incubation at 37 C for 24 h, the sample was enriched in Tetrathionate broth under the same incubation conditions. Then three selective plating media were routinely used throughout the study. Brilliant green (BG), brilliant green sulfa (BGS), and *Salmonella-Shigella* (SS) agars were selected due to the clarity of colony identification, sufficient selectivity, closeness of correlation between Standard Plate Count agar when compared in pure culture, and author's preference. Typical colonies were then picked and transferred to Triple Sugar Iron Agar slants (TSI slant). Isolates were biochemically and serologically identified by methods detailed by the U.S. Public Health Service (*19*).

Milk supply and cheese make procedure

All milk used in this study was obtained from a mixed dairy herd at the Mississippi State University Dairy Farm and was standardized to 3.8% butter fat in all instances. The milk was heated at 62.8 C for 30 min. Cheese was then made using the procedure outlined by Wilster (*23*). The quantity of milk used was 272.7 kg.

Addition of Salmonella cultures

Fifteen hundred milliliters of the *Salmonella* culture (containing on an average for each of the three serotypes of approximately 3.7×10^8 organisms per ml) were added to the 272.7 kg of milk so as to yield an average of approximately 3.7×10^5 salmonellae per ml of cheese milk for stages one and two. Under normal conditions, this is admittedly a very high inoculum; however, salmonellae do not gain entry into milk under normal conditions. If they were to be present in the milk accompanied by an increase in holding temperatures possibly due to faulty refrigeration equipment, such numbers could be present in the milk. The salmonellae were added at one of four stages of manufacture with each vat of milk representing one of the following four stages of addition: (*a*) to the raw milk; (*b*) immediately after pasteurization following cooling to 31.1 C (at the same time the lactic starter was added); (*c*) mixed with the lactic starter 1 h before its addition to the milk; and (*d*) via utensils, by pouring over cutting knives, rubber gloves, etc.

Storage of cheese

One block (9.1-10 kg) of cheese from each lot was placed in storage at 4.5 C and another block at 10 C. The cheese was cultured on the first day of storage to give a zero time (control). Samples from each vat and both temperatures were analyzed monthly thereafter for presence of salmonellae until two successive monthly tests yielded negative results, or for a total of 9 months. Initially the cheese was analyzed for fat, moisture, pH, and presence of absence of these pathogens. Also, the pH, and presence or absence of these pathogens. Also, the pH of the cheese was determined after the fourth and ninth months of storage for each vat whether positive or negative for *Salmonella.* The viable *Salmonella* present in the cheese after the fifth and ninth months were enumerated as described previously.

RESULTS AND DISCUSSION

To ensure that the experimental cheese did not differ compositionally from control cheese, a comparison of the average fat and moisture contents was made. The average fat content was 34.40% for all experimental cheese and 34.30% for control cheese. There was even less difference in moisture content with the averages being 35.62% and 35.70% for the experimental and control cheese, respectively.

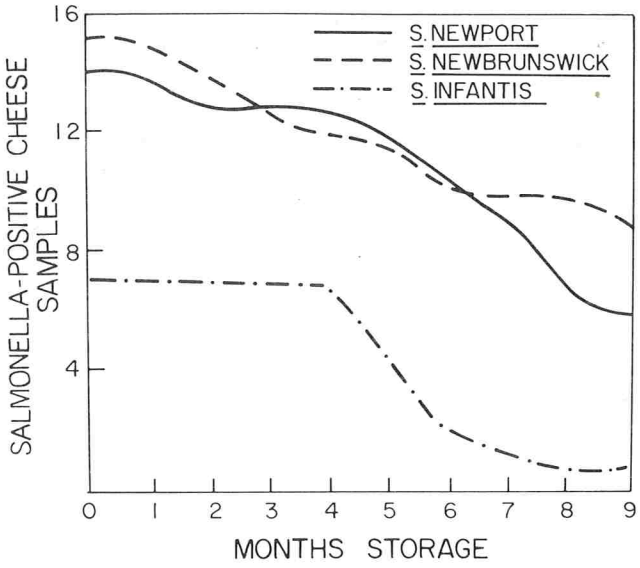

Figure 1. *Survival of* Salmonella newport, Salmonella new-brunswick, *and* Salmonella infantis *in Cheddar cheese stored at 4.5 C.*

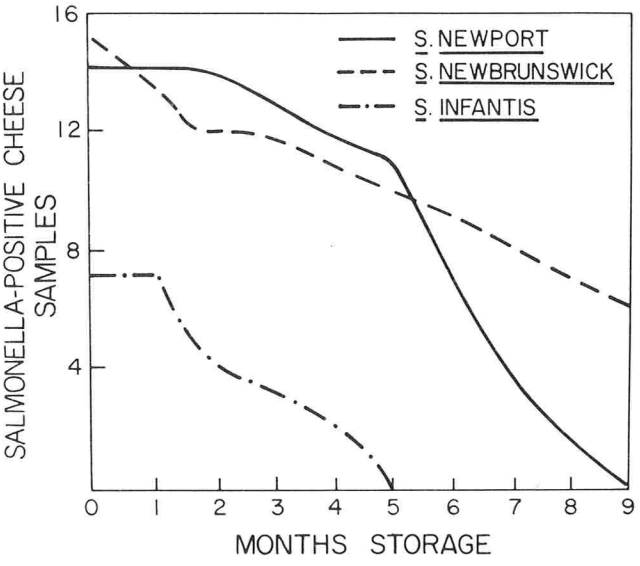

Figure 2. *Survival of* Salmonella newport, Salmonella new-brunswick, *and* Salmonella infantis *in Cheddar cheese stored at 10 C.*

Figures 1 and 2 show the survival of the three serotypes in cheese over a 9-month period at storage temperatures of 4.5 and 10 C, respectively. This includes all experimental cheese (all methods of contaminating cheese milk). These figures illustrate the variation in survival observed between the two storage temperatures. As expected, the pathogens survived longer when cheese was stored at 4.5 C rather than at 10 C. Also, data in these figures depict the variation in survival times among the serotypes. *S. new-brunswick* was the heartiest of the three with nine of the 16 contaminated cheese samples yielding positive results after the full 9 months of storage

at 4.5 C with six still positive when aging temperature was 10 C. On the other extreme there was only one *S infantis*-contaminated cheese sample still positive after 9 months of storage at 4.5 C and one when storage was at 10 C. This illustrates that *S. infantis* did survive well in cheese. There was some persistence of survival if these organisms were present at zero and particularly after 1 month's storage. A suitable explanation other than "serotype variation" was not found. *S. newport* was slightly less hearty than *S. new-brunswick* with six samples positive after 9 months at 4.5 C but none at 10 C.

These data indicate the importance of using more than one serotype or strain in research of this nature due to variations observed among the microorganisms. The recognition and acknowledgement of this fact is essential in the avoidance of drawing possibly erroneous conclusions for an entire group of bacteria.

Survival and pH

Chemical and physical factors related to commercial manufacturing procedures were researched with pH appearing to be the most critical factor. The lowest pH of a cheese positive for *Salmonella* after 9 months of storage at either 4.5 or 10 C was 5.70 with the highest being 6.2, an average of 5.85. The lowest pH value for negative samples was 5.4 with the highest being 5.8 (average of 5.55). Duplicate determinations were made on each sample of cheese tested. The pH measurements were made with a deionized water slurry of the cheese. Admittedly, this technique could have resulted in slightly higher readings than would normally have been expected; however, the same method was applied to all the cheese samples. The difference, therefore, between the pH of salmonellae-free cheese at 9 months of storage at either 4.5 or 10 C and the pH of cheese from which salmonellae were isolated appeared to be significant. Moreover, all cheese was compositionally normal and "legal." Also, the average milling acidity for all cheeses was 0.58% indicating normal acid development by the lactic starter organisms. Based on the milling acidity values obtained, many of which were 0.60% or higher, perhaps the pH of the cheese aged for 9 months should be lower than those recorded. At any rate, the higher pH values for the salmonellae-free cheese appear to be worthy of note, and while this difference of approximately 0.3 pH unit may not be significant, the difference does exist.

Thus, under conditions set forth in this study, a pH value below 5.70 would apparently contribute to the decline in the number of viable salmonellae. Conversely, the results observed indicate that a higher than average pH (thus, closer to neutrality) provide a greater chance for survival of these organisms in the cheese.

Correlation coefficients were calculated to determine the possible correlation between any and all of the following factors involved in making of the cheese: (*a*) starter acidity, (*b*) milling acidity, (*c*) number of turns in cheddaring process, (*d*) number of months positive for *Salmonella* at 4.5 C, and (*e*) number of months positive for *Salmonella* at 10 C. The only significant ($P < 0.05$) correlation (.791) was one between the two storage temperatures and survival as has been discussed previously. Also, the correlation (.477) between milling acidity and survival of 4.5 C approached significance at the 5% level of probability.

TABLE 1. *Comparison of the mean pH values for Cheddar cheese contaminated with* Salmonella *at 0.4, and 9 months of storage*[a]

Serotype	Number of lots of cheese inoculated with each serotype	Months storage		
		0	4	9
S. newport	16	5.56[b]	5.43	5.78
S. new-brunswick	16	5.41	5.42	5.79
S. infantis	16	5.34	5.30	5.53

[a]Cheese was stored at 4.5 and 10 C.
[b]Numbers represent pH values of cheese samples.

The data in Table 1 show the average pH values of the contaminated cheese stored at each temperature at 0 (tested on first day of storage), 4, and 9 months of storage. When these pH values were averaged over all storage times and temperatures, *S. newport*-, *S. new-brunswick*- and *S. infantis*-contaminated cheese were represented by pH values of 5.59, 5.54, and 5.39, respectively. Thus, *S. infantis*-inoculated cheese exhibited the lowest pH at both temperatures of aging and at any stage of the aging. *S. newport* and *S. new-brunswick* show very little difference in relation to pH. Since there was a lower survival rate for *S. infantis* in the cheese (Fig. 1 and 2), and since all manufacturing steps were the same as for the other serotypes, it is proposed that this lower pH had a significant relationship with regard to the reduced survival of this particular pathogen.

Survival and stages of addition

The data in Table 2 compare the actual survival of the pathogens with the four stages of addition of the *Salmonella* cultures. It was noted that addition at stages two and three resulted in greater survival than addition at stages one and four. This was expected because more

TABLE 2. *Comparison of the survival of* Salmonella *in Cheddar cheese with various methods of addition to the cheese milk-4.5 C.*

Months storage at 4.5 C	Stage of addition[a]			
	1	2	3	4
0	7[b]	11	10	9
1	7	11	10	9
2	4	11	10	9
3	3	11	10	9
4	3	11	10	8
5	2	10	9	6
6	0	8	9	5
7	0	8	9	4
8	0	6	7	4
9	0	6	7	3

[a]Stage 1 = Addition to raw milk.
Stage 2 = Immediately following pasteurization.
Stage 3 = Mixed with lactic starter before adding.
Stage 4 = Via utensils
[b]Number of samples positive for *Salmonella.*

viable organisms were placed in the cheese. The fact that there were some positive vats of cheese when the salmonellae were added before pasteurization (stage one) is alarming. This perhaps can be explained by the fact that pasteurization was not accomplished under the exact provisions of *Grade A Pasteurized Milk Ordinance* (1965) in that the milk was pasteurized in the cheese vat with non-mechanical agitation. This method was deemed necessary due to the inherent danger of running milk known to be contaminated with human pathogens through the regular pasteurization equipment of the MSU dairy plant. At any rate, a temperature of 62.8 C was achieved and maintained for 30 min. This does not rule out the possibility of contamination by psychrotrophic bacteria often associated with dairy products and equipment; however, every effort was made to preclude this possibility.

Needless to say, the stages of addition were actually incidental since the main objective of the study was to get the pathogens in the cheese and observe their behavioral patterns. However, this would possibly give the processor some guideline to follow in the analysis of his routine manufacturing procedures to ensure the avoidance of any possible practice which might render his cheese susceptible to contamination by *Salmonella.*

Salmonellae have been shown to be capable of survival in Cheddar cheese for extended storage periods when the pH of the cheese is abnormally high. Dairy products, while certainly not a major cause of salmonellosis, are subject to contamination by these organisms even though pasteurization should effectively destroy them. Since the "aging" process has been shown not to destroy these enteropathogenic organisms when the cheese milk was inoculated with an admittedly high level, extreme care should be taken to avoid any practice which would render this product susceptible to contamination by *Salmonella.*

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Effects of Fluorescent Light on Flavor and Ascorbic Acid Content in Refrigerated Orange Juice and Drinks[1]

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ABSTRACT

The effect of fluorescent light on flavor changes and ascorbic acid losses in orange juice and orange drink packaged in various containers was investigated. In general, flavor changes occurred more quickly and to a greater extent in glass and blow mold plastic packaged juices than in paperboard. Microbial growth in paperboard packaged orange juice may have caused flavor changes greater than those induced by light after 72 h of exposure. Ascorbic acid losses in light-exposed packages were much higher with reconstituted orange drinks, between 40 to 90%, than with orange juice, about 20%, following 144 h of exposure.

Although many investigations of light-induced changes in food, especially milk and milk products (*3*), have been reported, no studies on the effect of fluorescent light on orange juice products have appeared. These products are commonly marketed in containers and under light conditions known to have adverse effects on ascorbic acid and flavor in other foods.

Various studies (*2,8,9,12,14,16,19,20,22,*) have shown that many factors are involved in off-flavor development of orange juice and orange concentrate. These include storage temperature, oxygen levels, microbial development of diacetyl and acetyl methyl carbinol, volatile oils, lipid fraction present, and use of sorbates combined with processing temperatures. The need for control of the microflora in orange juice by pasteurization and temperature control has recently been demonstrated (*15*).

It appears that most of the literature on orange juice or orange drinks represents commercial citrus packers. While it is common practice among citrus packers to heat orange juice to inactive enzymes and to control microbial growth, similar methods are often not done in dairy plants. In a recent survey (*5*), only 1% of the dairies responding used a pasteurized hot-fill method. This represented only 1% of the juice processed. Forty percent of the dairies, representing 56% of the product, pasteurized the products and cold filled. However, 59% merely reconstituted or did no processing, and cold filled. This represented 43% of the volume.

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Many factors have been investigated relating to the stability of ascorbic acid in orange juice products. These include processing and storage temperatures (*6,10,13, 20*), type of containers (*7,13,17*), pH and certain sugars (*4*). It appears remarkable in view of the well known effect of light destruction of ascorbic acid, that this subject has received little or no attention.

Aerobic oxidation of ascorbic acid forms dehydroascorbic acid. While both of these compounds have antiscorbutic potency, dehydroascorbic acid is rapidly hydrolyzed to diketogulonic acid, which has no such activity. Possible flavor changes and ascorbic acid losses in orange juice under current retail marketing conditions should be of interest to the processor, the nutritionist and the consumer.

MATERIALS AND METHODS

One frozen orange juice concentrate and two orange concentrate drinks were obtained from a commercial packer. The frozen orange juice concentrate, packed in polyethylene-lined fiberboard boxes, was U.S. grade A fancy, with a stated Brix of 58° and a Brix-acid ratio of 15:1 to 18:1. One of the concentrated orange drinks consisted of 30% orange juice, citric acid, and orange oil fortified with an unstated level of ascorbic acid. The other concentrated orange drink was 10% orange juice, citric acid, and U.S. certified artificial color. Both of the orange drinks were in No. 10 cans and frozen by the packer.

Before reconstitution, these products were allowed to partially defrost by holding at 5 C for 12 h. Each product was reconstituted with tap water following label directions. The orange drink products also required sucrose. Mixing was done in a previously sanitized stainless steel vat. No heat was applied to any of the products.

Following reconstitution, the products were packed in various one-half gallon containers. The containers included blow mold plastic; white, black and orange colored polyethylene coated paperboard; and clear flint glass. Blow mold plastic and glass bottles were sanitized before filling, and the paperboard containers were preformed on a commercial filler. Paperboard containers were furnished by the International Paper Company, Philadelphia, Pennsylvania.

After filling, containers were placed in a commercial sliding door display case maintained at 6 ± 1 C. Adjustable shelves and containers were arranged to insure uniform lighting exposure. Illumination was by 40-watt cool white fluorescent lamps. Average illumination was 104 ft-c at the mid point of each container as determined by a Model 756 Weston meter. Average exposed surface of each container was 190-200 cm^2, varying slightly due to configuration of the package. Control samples, packed in glass, were held at 6 ± 1 C in an unlighted

refrigerator. Sufficient packages were filled to permit use of fresh samples at each examination period.

Included in observations after 24,48,72,120, and 144 h of exposure were organoleptic evaluations, ascorbic acid determinations, pH and acidity measurements, and limited microbiological examinations.

Organoleptic evaluations were by a panel of 10-12 women, all of whom had 2-3 years experience in food evaluation. The panel was also given preliminary training on orange juice and test procedures to be used. Organoleptic observations included a hedonic scale rating and multiple comparison procedure using methods as described by Larmond (*11*). The hedonic scale ranged from 1, dislike extremely, to 9, like extremely. Multiple comparison involved two tests. One involved submitting four samples; three from treatment packages plus an uncoded control. In the other multiple comparison, the remaining two treatment samples plus an uncoded control were submitted. In each instance an additional coded control was given as a reference. Comparison demands included better, equal, and inferior to the reference, as well as rating the differences between samples and control using a scale ranging from 1, extremely inferior, to 9, extremely better.

Ascorbic acid and acidity were determined at each observation time following the methods as described in A.O.A.C. (*1*). The pH was measured using a Corning Model 7 meter.

Separate batches of frozen concentrated orange juice were used in each study. During the second study on orange juice, abnormal flavors and obvious fermentation occurred leading to the discontinuance of this trial after 72 h. A third study on orange juice was conducted, and plate counts, coliform, and yeast and mold counts were determined at each observation time. Similar counts were made after 0, 72 and 144 h on each trial of the orange drinks.

The reconstituting, packaging, and flavor analyses were repeated to give two replications.

Statistical analyses involved a model describing each observation as the mean plus the effect of replication plus the effect of storage container plus a residual effect. The same model was used for each type of drink, for each variable (hedonic scale, multiple comparison score, and ascorbic acid content), and for each exposure time measured. The average of all taste panel members was used as the single observation. Likewise, the average of the two evaluations of the uncoded control in multiple comparison score was used as a single observation. The residual variances from the analyses of variance (*21*) for each exposure time were tested for homogeneity of variance by Bartlett's test (*21*) within type of drink and variable. The hypothesis of equal residual variances was never rejected ($P<0.05$). The values and degrees of freedom were pooled for a common estimate of residual variance with 25 degrees of freedom for ascorbic acid and 30 degrees of freedom for the hedonic and multiple comparison scores. A series of five single degree of freedom orthogonal contrasts were conducted. The first contrasted the effect of treatment containers (stored in light) versus control (stored in dark). The second contrasted plastic and glass versus all paperboard. The third contrasted plastic versus glass. The fourth contrasted white versus colored paperboard containers. The fifth contrasted orange versus black paperboard.

RESULTS AND DISCUSSION

Figure 1 shows the flavor quality as average hedonic scores for reconstituted orange juice after the indicated exposure times. F-values of container contrast are in Table 1. It is apparent that the paperboard-packaged product was preferred over that in glass or blow mold plastic after 24 h. As shown by Dimick (*3*) light transmission is much greater for glass or plastic than for paperboard. Figure 1 shows the control was preferred to

TABLE 1. *F-values of various container contrasts for reconstituted orange juice, 30% orange drink and 10% orange drink, using the mean hedonic scores of two trials for each*[a]

Type of contrast[b]	Exposure times (h) 0	24	48	72	120	144
	Orange juice					
A	0.12	2.27	12.67*	3.00	6.92*	14.32*
B	0.33	8.52*	19.59*	10.70*	12.22*	17.65*
C	0.06	1.87	0.00	3.95	0.38	0.14
D	0.42	0.62	0.18	0.00	0.02	0.33
E	0.76	1.25	2.22	0.25	6.17*	1.54
	30% Orange drink					
A	0.08	1.33	9.48*	0.92	29.56*	12.67*
B	3.17	15.56*	15.56*	14.81*	41.56*	29.04*
C	0.03	1.25	0.14	0.14	2.22	3.47
D	0.01	0.74	1.40	0.18	1.40	0.18
E	0.03	0.55	0.87	0.14	0.31	0.00
	10% Orange drink					
A	0.02	0.24	2.20	4.12	0.71	5.19*
B	0.00	0.98	2.20	0.12	0.63	2.51
C	0.92	2.35	0.15	0.92	1.32	0.15
D	0.01	0.01	0.11	1.48	0.20	3.14
E	0.33	0.04	0.33	1.80	0.00	0.00

[a],*Indicates significant difference ($P < 0.05$) within various container contrasts.

[b]A—Control vs trial containers
B—Plastic and glass vs paperboard
C—Plastic vs glass
D—White paperboard vs colored paperboard
E—Black vs orange paperboard

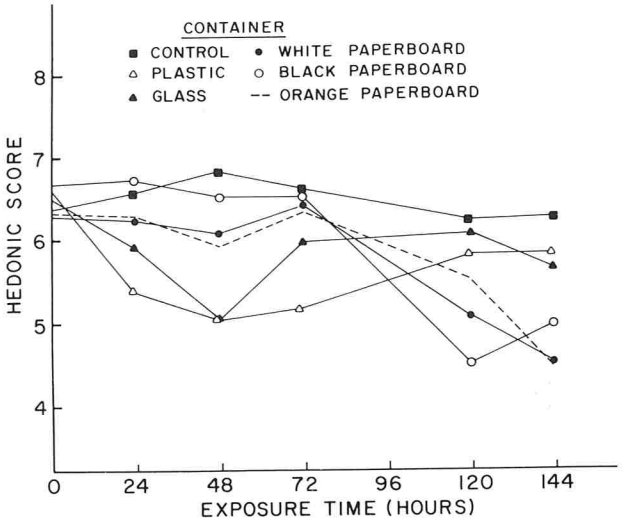

Figure 1. *Mean hedonic flavor scores from trained panel for reconstituted orange juice exposed to fluorescent light in various containers.*

TABLE 2. *Microbial counts on reconstituted orange juice exposed to fluorescent light in various containers (results of one trial)*

Exposure time (h)	Standard plate count per ml[a] Control	Plastic	Glass	Paperboard White	Black	Orange	Yeast and mold count per ml[a] Control	Plastic	Glass	Paperboard White	Black	Orange
24	2.556	2.653	2.279	2.623	2.477	2.892	1.954	2.041	2.000	2.079	2.041	2.114
48	2.591	2.491	2.342	2.322	2.477	2.663	2.079	2.041	2.079	2.000	2.301	2.230
72	2.672	2.653	2.431	2.949	2.544	2.556	2.301	2.477	1.954	2.505	1.813	2.362
120	2.362	5.544	4.898	6.322	6.505	6.580	1.653	5.301	4.643	5.929	6.301	6.146
144	2.924	5.813	5.580	5.716	6.204	5.863	1.939	5.623	5.279	6.079	5.886	5.851

[a]Logarithms of actual counts.

the light-exposed product as exposure time progressed. While organge juice exposed in glass or plastic appeared to suffer flavor deterioration more rapidly than in paperboard, after 48 h the juice in paperboard changed rapidly in flavor. As shown in Table 2, neither standard plate nor yeast and mold counts increased in the glass-packaged control. However, all counts increased in orange juice in the various trail containers, including glass, as storage time progressed. The only difference between the two glass-packaged samples, control versus experimental, was light exposure. It is possible that light-induced free radical formation yielded compounds stimulating microbiological activity. The same phenomenon may have occurred with the plastic packaged orange juice. Another possibility may have been oxygen availability, especially with the paperboard-packaged juice. Glass and plastic bottles were completely filled, whereas the paperboard bottles, as usual commercially, had considerable free headspace. The question of oxygen permeability of the paperboard also cannot be ignored.

The freshly reconstituted orange juice averaged approximately 53 mg of ascorbic acid/100 ml. Ascorbic acid losses (Figure 2) for reconstituted orange juice in the

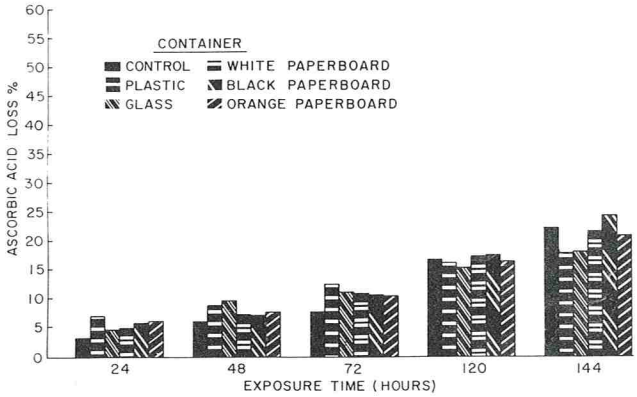

Figure 2. *Ascorbic acid loss for reconstituted orange juice exposed to fluorescent light in various containers.*

control and treatment packages varied slightly at examination times, averaging between 20 and 25% after 144 h. Package effect with this product was generally negligible. F-values for container contrasts of ascorbic acid are shown in Table 3.

The acidity of the reconstituted orange juice, as citric acid, was 0.70±.02 mg% and the pH 3.9-4.0. Little change was noted in either during the time of observation in any of the samples.

Data on the orange drink made from 30% orange juice, citric acid, and orange oil are shown in Fig. 3. Container contrast of flavor score data are listed as part of Table 1. With this product packaged in glass or plastic, light-induced changes appeared more rapidly and to a greater extent than in paperboard, and these continued through the 144 h of observation.

Original ascorbic acid levels in the 30% orange juice averaged approximately 22 mg/100 ml. Losses were much higher, in excess of 40%, and progressed more

TABLE 3. *F-values of various container contrasts for reconstituted orange juice using the mean ascorbic acid content of two trials*[a]

Type of contrast[b]	Exposure time (h)				
	24	48	72	120	144
	Orange juice				
A	1.48	1.06	2.86	0.00	0.74
B	0.00	1.03	0.50	0.89	6.30*
C	1.25	0.09	0.20	0.09	0.00
D	0.23	0.00	0.02	0.00	0.22
E	0.00	0.03	0.00	0.14	1.56
	30% Orange drink				
A	5.41*	33.36*	19.83*	126.96*	211.31*
B	5.16*	0.90	0.52	77.23*	210.10*
C	0.49	0.06	6.09*	0.78	7.05*
D	2.45	2.16	0.89	0.00	1.21
E	2.34	0.02	1.21	0.12	0.12
	10% Orange drink				
A	17.34*	28.17*	99.23*	99.23*	60.80*
B	0.73	2.04	31.74*	41.61*	8.64*
C	0.02	0.02	0.10	1.60	0.40
D	0.67	0.41	0.30	0.41	0.07
E	0.62	4.22	0.10	0.02	0.02

[a],*Indicates significant difference ($P < 0.05$) within various container contrasts.

[b]A—Control vs trial containers
B—Plastic and glass vs paperboard
C—Plastic vs glass
D—White paperboard vs colored paperboard
E—Black vs orange paperboard

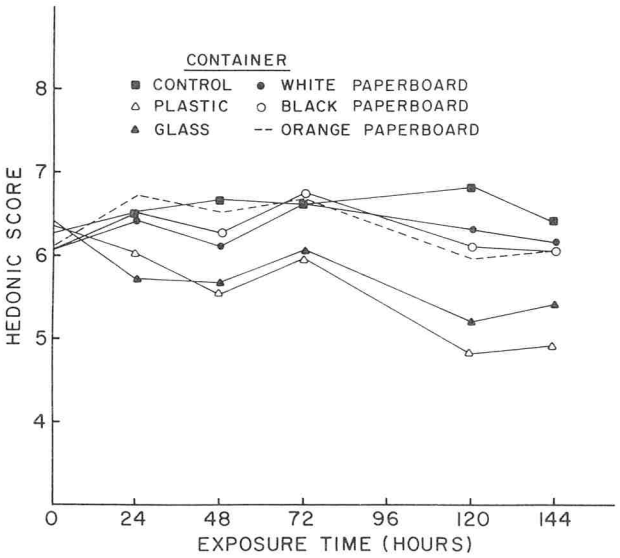

Figure 3. *Mean hedonic flavor scores from trained panel for reconstituted orange drink (30%) exposed to fluorescent light in various containers.*

rapidly percentage-wise with the 30% orange product (Fig. 4, Table 3) than was found with reconstituted orange juice. While intial losses of ascorbic acid were higher in plastic and glass that in paperboard, after 120 h this was reversed, and subsequent losses of this vitamin were much higher in paperboard.

No evidence of any microflora was found in the 30% orange drink at 0, 72, and 144 h observations. The acidity as citric acid was 0.53±0.2 mg%, and the pH approximately 3.3. Neither varied appreciably throughout the observation period. Lack of microbial activity may have been due to, at least in part, the low pH.

Flavor data from the second orange drink, containing 10% juce, citric acid and U.S. certified color are shown in Fig. 5 and as part of Table 1. Container influence (Fig.6, Table 3) was less evident with this product than either of the other two. On the other hand, loss of ascorbic acid, only 3.5 mg/100 ml originally, was rapid and pronounced.

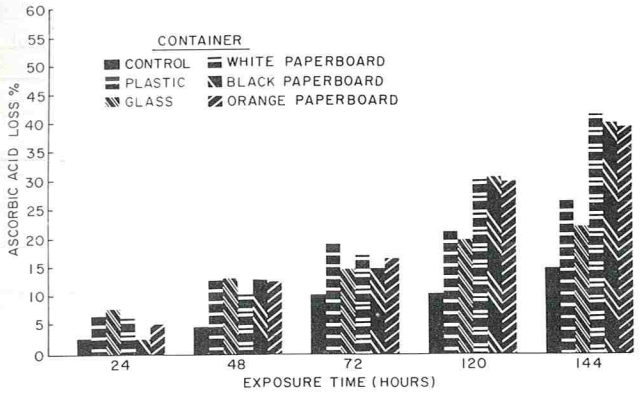

Figure 4. *Ascorbic acid loss for reconstituted orange drink (30%) exposed to fluorescent light in various containers.*

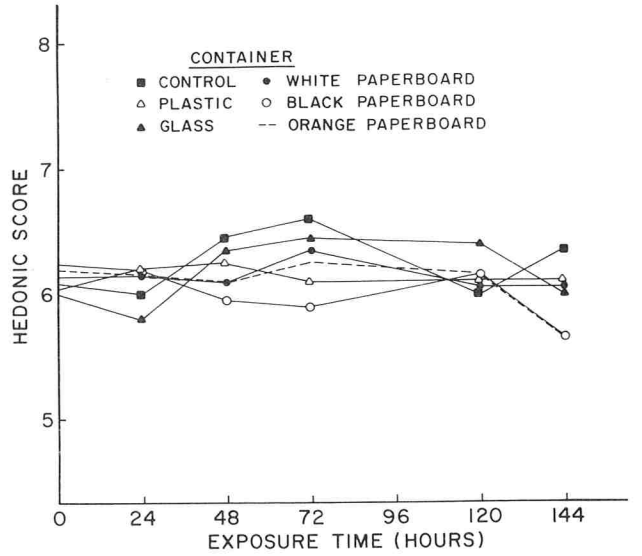

Figure 5. *Mean hedonic flavor scores from trained panel for reconstituted orange drink (10%) exposed to fluorescent light in various containers.*

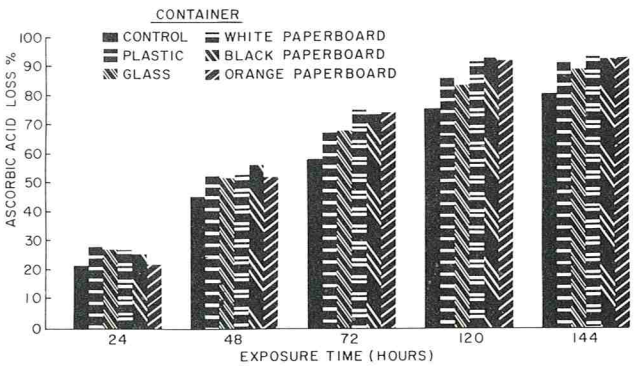

Figure 6. *Ascorbic acid loss for reconstituted orange drink (10%) exposed to fluorescent light in various containers.*

No microbial growth was evident in the 10% orange drink at 0, 72, or 144 h. The acidity, as citric, was 0.32-0.34 mg% and the pH approximately 3.0 and both remained constant throughout the study period.

Perhaps percentage losses of ascorbic acid in these products is less meaningful than the quantity available. As shown in Table 4, even after 144 h orange juice retained approximately 42 mg/100 ml, or about 80%, of the original ascorbic acid. With the 30% reconstituted drink, containing about 22 mg/100 ml originally, 13 to 17 mg/100 ml were present after 144 h. Of the 3.5 mg of ascorbic acid/100 ml in the 10% reconstituted drink, only about 0.3 mg/100 ml was left after 144 h. Orange juice, and to a lesser extent, orange drinks, are often relied on as a source of this vitamin. These data point out the need for processing and/or packaging to prevent losses.

TABLE 4. *Concentration of ascorbic acid in mg/100 ml for reconstituted orange juice and orange drink, using the mean of two trials*

Type of container	Exposure time (h)					
	0	24	48	72	120	144
	Orange juice					
Control	53.56	51.84	50.29	49.34	44.62	41.66
Plastic	—	49.86	48.84	46.81	44.87	44.00
Glass	—	51.11	48.41	47.44	45.29	43.90
White paperboard	—	50.93	49.59	47.66	44.28	42.06
Black paperboard	—	50.39	40.65	47.75	43.94	40.62
Orange paperboard	—	50.31	40.30	47.88	44.46	42.37
	30% Orange drink					
Control	21.89	21.30	20.87	19.63	19.41	18.71
Plastic	—	20.43	19.11	17.78	17.26	16.11
Glass	—	20.17	19.02	18.70	17.59	17.10
White paperboard	—	20.51	19.61	18.21	15.32	12.88
Black paperboard	—	21.30	19.11	18.72	15.24	13.17
Orange paperboard	—	20.73	19.16	18.31	15.37	13.30
	10% Orange drink					
Control	3.51	2.75	1.90	1.47	0.85	0.66
Plastic	—	2.52	1.67	1.13	0.49	0.33
Glass	—	2.53	1.68	1.11	0.56	0.37
White paperboard	—	2.53	1.64	0.87	0.28	0.22
Black paperboard	—	2.60	1.54	0.91	0.24	0.24
Orange paperboard	—	2.55	1.67	0.89	0.25	0.23

Multiple comparison data on these three products reinforce the hedonic data and are omitted for brevity.

SUMMARY

Studies were done on three reconstituted orange concentrate products. The three products included orange juice concentrate and two orange concentrate drinks containing different amounts of orange juice. The main purpose of the study was the investigation of the

protection offered by various containers on flavor and ascorbic acid stability when orange juice products are exposed to fluorescent light.

With reconstituted orange juice, microbial growth was found. Flavor changes and ascorbic acid losses with this juice were probably due to a combination of microbial growth and light effects. The development of off-flavors with reconstituted 30% orange concentrate drink appear to be related to the light protection offered by some containers. Flavor data on the 10% orange drink suggest it was less affected by light than the other two products, and the panel was less able to identify flavor differences attributable to light-induced changes in the various packages. This may have been due to lower concentrations of off flavor precursors, and higher sugar content.

In general, paperboard containers gave more protection against flourescent light-induced flavors than plastic or glass. This was confirmed by statistical analysis of variance ($P<0.05$). Also, a significant difference was found between the unexposed and exposed samples which confirms the occurrence of the photochemical reactions. No significant differences were found among the three paperboard containers. The results indicate a significant difference between plastic and glass in favor of the latter after exposure of 144 h.

The stability of ascorbic acid varied among the three commercial products studied. These losses varied from about 20% in orange juice up to greater than 90% in the 10% orange drink. The variation in loss might have been due to the concentration of ascorbic acid, pH, oxygen levels, and microbial activity, as well as light exposure.

It would appear desirable for dairies processing orange juice or orange drinks to use pasteurization or to hot pack, where feasible. Especially with reconstituted orange juice this could reasonably be expected to extend acceptable flavor characteristics and possibly prevent some loss of ascorbic acid.

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Commensalism and Competition in Mixed Cultures of *Lactobacillus bulgaricus* and *Streptococcus thermophilus* [1]

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ABSTRACT

Cultures of *Streptococcus thermophilis* and *Lactobacillus bulgaricus* produced more acid in mixed than in single strain culture. Growth of *S. thermophilus* in mixed culture was enhanced during the exponential phase and reached higher numbers in the stationary phase than when grown alone. *L. bulgaricus* was inhibited in the exponential and stationary phases of growth in mixed culture. *L. bulgaricus* liberated Seitz-filterable compounds during its growth that stimulated growth and acid production of *S. thermophilus*. These compounds are believed to be responsible for the commensal response observed in mixed cultures. Because of its rapid growth, *S. thermophilus* was a better competitor than *L. bulgaricus* for limiting nutrients in the medium. This resulted in inhibition of the growth of *L. bulgaricus*. The competitive and commensal response was optimal at 37 C and at a ratio of numbers of lactobacilli to streptococci of 2:1 at inoculation.

Manufacture of cultured dairy products depends at some point on controlled production of lactic acid. Generally, selected strains of organisms are added to milk at some stage of manufacture to bring about these changes. Single species seldom are sufficient, so mixtures of cultures possessing desirable capabilities are most often used. These organisms usually have complex nutritional requirements for growth and activity. When several species are combined for propagation in a medium with finite growth nutrients, many different growth patterns or associations result. Thus, the controlled fermentation of milk using selected starters presents an unusual opportunity to study the interactions of the organisms.

Effects of such associations have been identified in some dairy products. For example, the inhibitory action of nisin and diplococcin produced by some lactic streptococci was discovered in studies of delayed acid production in starters for the manufacture of Cheddar cheese (*13*). Strain dominance in mixed cultures of *Streptococcus lactis* and *Streptococcus cremoris* became evident in similar investigations (*6,16*). Little information is available, however, on the associative growth of *Lactobacillus bulgaricus* and *Streptococcus thermophilus*, the organisms used to manufacture yogurt and Italian and Swiss cheeses. Most reports of their associative growth stress a symbiotic relationship (*1,4,8,14,15*) in which growth and acid production of both organisms are stimulated. *L. bulgaricus* is believed to produce large quantities of amino acids that stimulate growth and acid production of *S. thermophilus* (*1, 4, 14, 14*). *S. thermophilus* produced formic acid, which similarly stimulated *L. bulgaricus* (*8*). These reports emphasized the stimulation of acid production by mixed cultures which should result in shorter manufacturing times for products and economic savings.

The purpose of this investigation was to describe the interactions of a single-strain pair of *S. thermophilus* and *L. bulgaricus* that resulted in stimulation of acid production because of commensalistic and competitive growth.

MATERIALS AND METHODS

Cultures

Single strains designated *L. bulgaricus* 1 and *S. thermophilus* A were studied. These cultures were obtained from the culture collection of the Department of Food Technology, Iowa State University. This pair was selected from a survey of pairs exhibiting stimulation of acid production as assessed by a modified activity test of mixed cultures (N. J. Moon, M. S. Thesis, Iowa State University, 1974). Cultures were maintained in 10 ml of sterile, reconstituted skimmilk. All trials were conducted in 800 ml of skimmilk unless stated otherwise.

Enumeration of viable organisms and plating technique

Lee's agar (*9*) was used for enumeration of *S. thermophilus* in mixed and pure culture, but was modified to increase recovery efficiency by addition of 0.3% carboxylmethyl cellulose, 0.1% Tween 80, and 0.05 *M* phosphate buffer. *S. thermophilus* colonies were counted after a 30-h incubation at 32 C. At this time and temperature, *L. bulgaricus* will not form colonies on this medium.

Viable numbers of *L. bulgaricus* in mixed and pure culture were determined by colony formation on LBS agar (BBL, Division of Bioquest, Cockeysville, MD). *S. thermophilus* did not form colonies on this agar. Pour plates were used for enumeration according to standard procedures (*2*).

Developed acidity

Milk samples withdrawn from the culture flask were titrated to the phenolphthalein end point with 0.10 *N* NaOH, and the percentage of developed acidity calculated as lactic acid.

Stimulation or inhibition of developed acidity

The acidity produced by mixed cultures was expressed as the percentage difference between the mixed-culture-developed acidity

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(MCDA) and the sum of the pure-culture-developed acidities (PCDA) from the equation,

$$\% \text{ difference} = [(\text{MCDA} - \Sigma \text{ PCDA})/\Sigma \text{ PCDA}] \times 100$$

If the percentage difference is negative, inhibition has occurred. A positive percentage difference would indicate stimulation.

This equation is reliable only if the total developed acidity is not greater than about 0.9%. At this acidity the streptococci are inhibited (7). *L. bulgaricus* is somewhat more aciduric.

Optimum ratio of organisms to produce stimulation of acid production in mixed cultures

The *S. thermophilus* inoculation level was held constant at 0.5% (v/v), while the inoculation level of *L. bulgaricus* was varied from 0.02% to 1.0%, producing numerical ratios of lactobacilli to streptococci of 0.3:1 to 20:1 at inoculation. Pure culture controls were always included. Two-tenths percent of *L. bulgaricus* corresponded to about 2.5×10^6 organisms/ml and 0.5% addition of *S. thermophilus* to 1.2×10^6 organisms/ml. The incubation temperature was 37 C and the percentage of stimulation or inhibition of developed acidity and viable numbers were determined as noted.

Temperature optimum for stimulation of developed acidity

A linear temperature gradient for incubating cultures was produced by placing heating bars on one end of an aluminum block and circulating refrigerated water through the opposite end. The 1.3-m long block had 18 parallel series of holes (22 × 110 mm) for placement of culture tubes (18 × 125 mm). The block was insulated on five sides by 10 cm of expanded mica packing. The entire assembly was placed in a lucite box. Temperature fluctuations were minimized (± 0.5 C) by fitting the lucite box with a hinged lid that was kept closed during experiments. To facilitate rapid warm up of sample tubes 1 ml of water was placed in each hole of the block. With this arrangement temperature equilibration time was about 5 min for a 10 C difference between sample tube and the block.

For temperature optima studies, sterile milk tempered to 30 C was inoculated with 0.5% (v/v) *S. thermophilus* and 0.2% (v/v) *L. bulgaricus* and 15 ml was dispensed into sterile culture tubes. This procedure was followed to avoid errors in inoculating small volumes of milk with reproducible inoculum sizes. The tubes were then placed in the prewarmed aluminum block to obtain the desired temperatures 47, 42, 37, 32, and 21 C.

Cell-free filtrate preparation

Pure cultures were incubated for 4 h at 37 C. At this time the milk had not coagulated. The cells were removed by centrifugation at 12,000 × *g* for 15 min at 4 C. The supernatant fluid was adjusted to pH 4.5 with sterile 1.0 *N* HCl to precipitate the casein. The precipitate formed after 30 min was removed by centrifugation at 12,000 × *g* for 15 min at 4 C. This precipitate also contained cells not removed in the first centrifugation. The supernatant fluid was adjusted to pH 6.8 with 1.0 *N* NaOH. The slight precipitate that formed at this stage was removed by centrifugation at 12,000 × *g* for 15 min at 4 C. This precipitate obtained at pH 6.8 contained few cells, < 100/g. The supernatant fluid was sterilized by passage through a prewashed Seitz filter. For heat stability tests, 15 ml of Seitz filtrate was transferred aseptically to a sterile screwcap culture tube and held in water baths at 100 C for 10 min or 80 C for 10 min, or was autoclaved at 121 C for 15 min.

The presence of active compounds was assessed only in the final Seitz filtrate and pH 6.8 precipitate. Other fractions were not tested because they contained high numbers of cells. Filtrates were tested by addition of 10% (v/v) to freshly inoculated pure cultures of the test organism. A 10% addition of 0.75% saline to a second culture served as a control. The pH 6.8 precipitate was tested for activity by addition to sterile growth media at 1% (w/v). The control contained no additions. The assay cultures were incubated for 10 h, and tested every 2 h for an increase in cell numbers and developed acidity.

RESULTS AND DISCUSSION

Growth and stimulation of acid production.

Preliminary studies with these selected strains of *L. bulgaricus* and *S. thermophilus* indicated that stimulation of acid production occurred in mixed culture. With 0.5% *S. thermophilus* and 0.2% *L. bulgaricus* inocula, stimulation of the mixed culture was observed for the first 10 h of incubation at 37 C (Fig. 1).

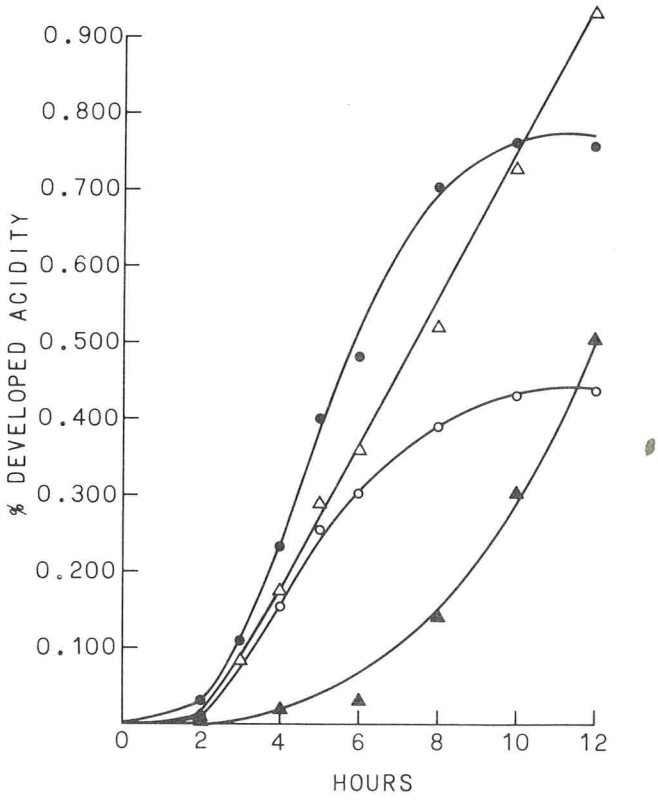

Figure 1. *Developed acidity of mixed and pure cultures of* L. bulgaricus *and* S. thermophilus. *Closed circle: Mixed culture. Open triangle: Expected value (sum of developed acidity of both cultures). Open circle:* S. thermophilus. *Closed triangle:* L. bulgaricus.

Previous investigators have shown that stimulation of acid production in mixed culture is due to enhanced growth of streptococci. Our results further emphasize this effect (Fig. 2). Growth of *S. thermophilus* at about 4 h was increased by about 50%. This growth response of the streptococci can be termed commensalism. Later (4 to 8 h), viable numbers of *S. thermophilus* decreased in the mixture, and to a lesser extent in the control. The early sharp decline of *S. thermophilus* in the mixture is unexplained, but probably is not due to ph (4-h pH was 5.8). The sharp decline in *S. thermophilus* after 15 h may be due to pH (20-h pH was 3.8).

Growth of *L. bulgaricus* in mixed culture was less vigorous than in the control. In mixed culture, the exponential period of growth was not maintained, and the stationary phase occurred sooner and with fewer numbers of cells present than in the pure culture. Growth of *L. bulgaricus* in the mixture was retarded throughout most of the growth cycle.

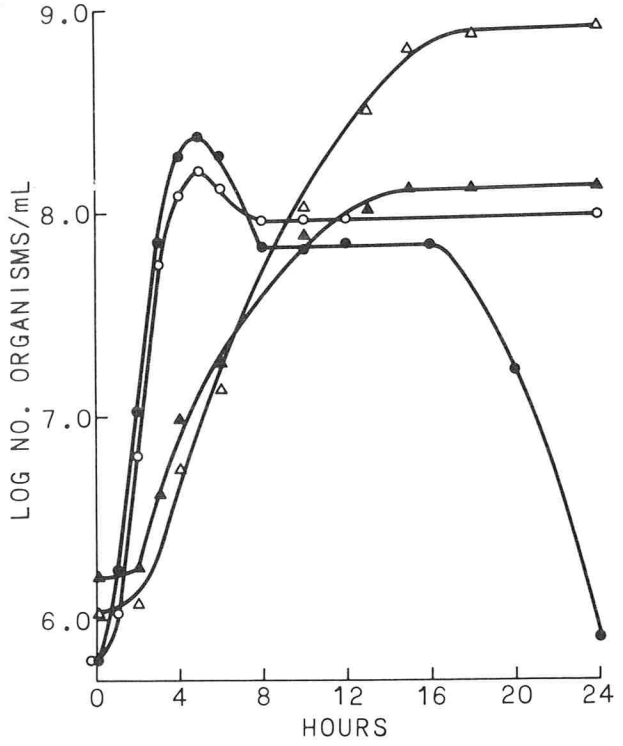

Figure 2. *The growth of mixed and pure cultures of* L. bulgaricus *and* S. thermophilus *at 37 C. Open circle:* S. thermophilus *control. Closed circle:* S. thermophilus *in mixed culture. Open triangle:* L. bulgaricus *control. Closed triangle:* L. bulgaricus *in mixed culture.*

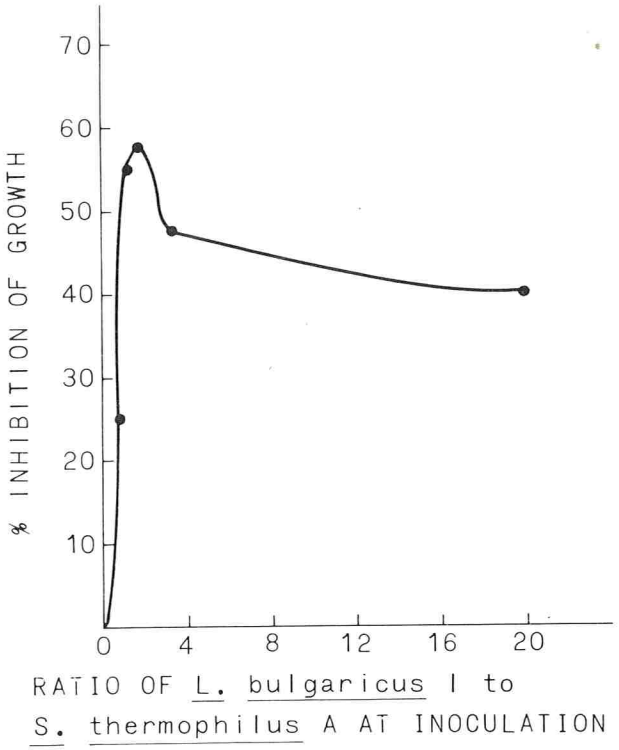

Figure 3. *Percent inhibition of the growth of lactobacilli in the exponential phase as a function of the ratio of* L. bulgaricus *to* S. thermophilus *at inoculation.*

Competition and L. bulgaricus

Inhibition of *L. bulgaricus* throughout its incubation in mixed culture has not been previously reported. Inhibition of an organism in mixed culture may theoretically be due to crowding, suboptimal pH, competition for nutrients, or the presence of an inhibitory compound (*10*). Crowding and pH differences were unlikely causes for inhibition of *L. bulgaricus* in mixed culture because the control *L. bulgaricus* culture grew to greater cell densities and lower pH.

No inhibitory compounds produced against *L. bulgaricus* by *S. thermophilus* seemed to be present because cell-free filtrates and pH 6.8 precipitates from *S. thermophilus* cultures did not inhibit growth or acid production of *L. bulgaricus.* It is possible that such a factor could be removed by coprecipitation or attachment to the Seitz filter. To explore this possibility, we altered the ratio of cell numbers at inoculation. We reasoned that if *S. thermophilus* produced an inhibitory factor, inhibition of *L. bulgaricus* would be maximal when *S. thermophilus* predominated in the inoculum. Conversely, when *L. bulgaricus* predominated in the inoculum, minimum inhibition would be expected. Figure 3 shows that maximum inhibition occurred at a ratio of *L. bulgaricus* to *S. thermophilus* of 2:1 rather than when *S. thermophilus* predominated (such as at ratios of 1:15). Minimum inhibition occurred either at very high or very low ratios of *L. bulgaricus* to *S. thermophilus.* Thus, the presence of an inhibitory factor against *L. bulgaricus* produced by *S. thermophilus* was very unlikely.

As a final alternative, we considered the possibility of inhibition of *L. bulgaricus* by competition with *S. thermophilus* for essential nutrients. Often, when an inhibitory factor cannot be demonstrated, competition for essential nutrients restricts the growth of one species in mixed culture (*12*). In competitive environments where nutrient sources are limited, the faster-growing organism often has a greater advantage (*3*). At optimal conditions for stimulation of acid production, *S. thermophilus* grew much more rapidly than *L. bulgaricus* (Fig. 2). It may be that inhibition of *L. bulgaricus* was due to the competitive and rapid utilization of some essential nutrient by the faster growing *S. thermophilus.* A competitive growth advantage for *S. thermophilus* also is suggested by the temperature optimum for stimulation, 37 C (Fig. 4). This temperature is closer to the growth optimum for *S. thermophilus* (40-45 C) than for *L. bulgaricus* (45-50 C) (*5*). The lack of stimulation at higher temperatures may be due to faster growth and better competitive ability of *L. bulgaricus* at these temperatures. No attempt was made to discover the identity of the proposed limiting nutrient. Because both of these organisms are known to be fastidious, any one of a large number of compounds could be responsible.

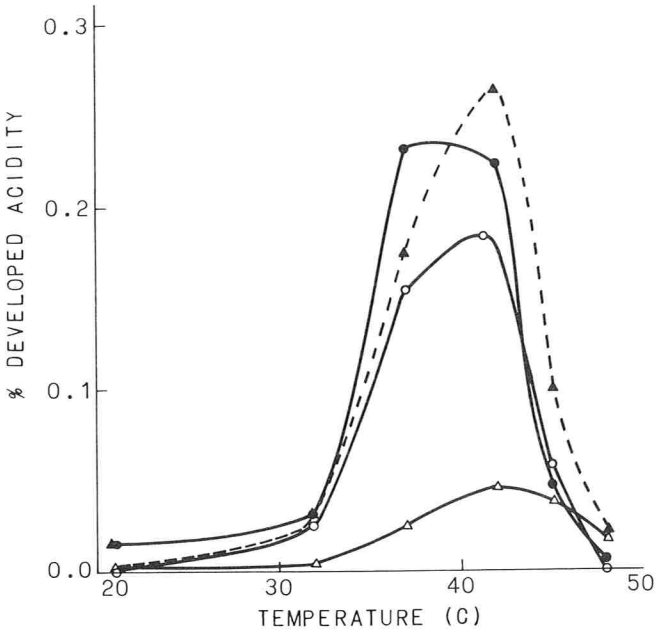

Figure 4. *Developed acidity of mixed and pure cultures of* L. bulgaricus *and* S. thermophilus *after 4-h incubation as a function of temperature. Closed circle: Mixed culture. Open circle:* S. thermophilus. *Open triangle:* L. bulgaricus. *Closed triangle: Expected value (sum of developed acidity of* L. bulgaricus *and* S. thermophilus).

Commensalism and S. thermophilus

Other workers have indicated that *L. bulgaricus* produced growth factors for *S. thermophilus.* Our results further substantiate this claim. A 10% addition of cell-free Seitz-filtrates of *L. bulgaricus* culture stimulated the acid production of *S. thermophilus* by 26% (Table 1). This is somewhat less than the 38% observed in mixed culture. This difference may be due to some dilution effect in the filtrate preparation. Additions of more than 10% were not as stimulatory to *S. thermophilus,* probably because of depletion of nutrients essential for *S. thermophilus* by *L. bulgaricus* in the original pure culture.

The stimulatory agents in the cell-free filtrate were only partly heat stable, suggesting the involvement of more than one factor (Table 1). The identity of compounds was not determined, but they could be any of several water-soluble, filterable, partly heat labile compounds known to stimulate *S. thermophilus* and other lactic acid organisms (*11*).

TABLE 1. *Stimulation*[a] *of developed acidity of* S. thermophilus *by the addition*[b] *of heat-treated Seitz filtrates of 4-h* L. bulgaricus *culture*

Addition	% Stimulation developed acidity
Seitz-filtrates	26.5
Autoclaved Seitz-filtrates	15.0
Seitz-filtrates heated 80 C 10 min	18.2
Seitz-filtrates heated 100 C 10 min	15.4

[a]Developed acidity determined after 4-h incubation and % stimulation determined by comparing with the control containing no addition of filtrate.
[b]Added at 10% (v/v).

Stimulation of acid production in mixed cultures was temperature-dependent, having an optimum of 37 C (Fig. 4). This temperature would presumably favor *S. thermophilus* as previously discussed. The optimum ratio of cell numbers at inoculum for stimulation of the growth of *S. thermophilus* was 2:1 (*L. bulgaricus* to *S. thermophilus*) (Fig. 5). *S. thermophilus* was maximally stimulated in the exponential phase of growth at this ratio. Presumably, the stimulating factors are produced in optimal amounts at this ratio and temperature.

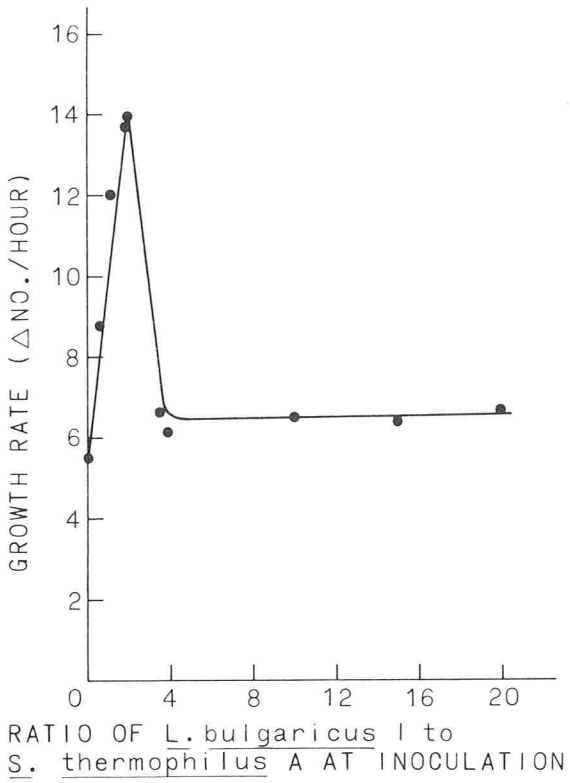

Figure 5. *Stimulation of the growth of* S. thermophilus *in the exponential phase as a function of the ratio of* L. bulgaricus *to* S. thermophilus *at inoculation.*

Commensalism and competition are two terms that have been applied to growth of these organisms in mixed culture. *L. bulgaricus* produces compounds that stimulate growth and acid production of *S. thermophilus.* The rapid growth appears to deplete the medium of some unknown compound essential for *L. bulgaricus.* The exact identity of the compounds involved in this instance is unknown but may be any of several known growth factors for *S. thermophilus* and *L. bulgaricus.* Inhibition of *L. bulgaricus* by *S. thermophilus* may be significant when mixed cultures of these organisms are used for product manufacture. Reduced growth of *L. bulgaricus* could result in defective body characteristics in cheeses, and flavor problems in yogurt. These results illustrate the complexities of growth interactions of mixed cultures. It is likely, however, that

similar interaction complexities exist and play a significant role in the population dynamics of natural environments and fermented foods.

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Flavor Acceptability of Unfermented and Lactic-Fermented Soy Milks

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ABSTRACT

Soy milks prepared from whole Harosoy soybeans by a hot grind process and milks prepared from soybean flour defatted by a Cornell University patented process were evaluated for flavor acceptability by a 27 member taste panel at the New York State Agricultural Experiment Station using fresh homogenized cow's milk as standard. Hot grind soy milk was rated significantly different and distinctly inferior to cow's milk in flavor. Soy milk prepared from defatted flour (U.S. Patent No. 3,721,569) by aqueous extraction and addition of 2.5% refined soy oil and 2% sucrose was rated slightly inferior to fresh cow's milk in flavor. Soy milk from the defatted flour was also lactic fermented to prepare a yogurt-like product whose flavor acceptability was compared with fermented cow's milk. The fermented soy milks had a satisfactory gelatinous curd and an acceptable flavor; however, the taste panel preferred the higher acidity of the fermented cow's milk.

Soy milk with a protein content similar to that of cow's milk is a beverage which could contribute to infant and child nutrition in areas of the world where cow's milk is insufficient in supply or too costly for the general population. Soy milk is a commercial success in Hong Kong where approximately 90,000,000 bottles are sold each year (*7*). Relatively large quantities are also produced and sold in Taiwan, Bangkok, and Singapore. A soy milk satisfactory to 6th grade Filipino school children was developed by Steinkraus et al.(*9*). Soy milks which are successful among Oriental populations accustomed to soybean products are generally quite sweet and sold as soft drinks. However, in the Western world, soy milks to date have become commercially successful only in the medical area where infants allergic to human or cow's milk can generally tolerate soy milks. The Western consumer seems to be quite critical of soybean flavor. Thus, much effort has been directed toward elimination of "beany" flavors in processing soy milks. One approach has been to heat the soybeans either before or during initial processing to destroy lipoxidase to minimize development of undesirable flavors due to degradation or oxidation of lipids (*6, 11*). A second approach has been complete extraction of lipids to remove the substrate which can lead to development of off- and beany flavors (*8*). A possible third approach is the use of fermentation to modify and improve flavor. This approach has been quite successful with fungal fermentations such as those used in production of soy sauce, miso, and Indonesian tempeh. Fermentation has been less successful in production of fermented soy milks and soy cheeses. Hang and Jackson (*3, 4*) prepared cheese-like products from soy milk, skim milk powder, rennet extract, and lactic starter cultures. They reported residual beany flavor. Wang et al. (*10*) reported that the beany flavor was masked by fermentation of soy milk with *Lactobacillus acidophilus.*

In most studies which have been done on soy milks in the Western World flavor acceptability has been cursory or lacking. Generally, soy milks have been compared with other soy milks to determine if a flavor improvement has been achieved. This study was made to compare the flavor acceptability of soy milks produced by the best technology we are aware of with fresh, homogenized cow's milk, a well-known, highly acceptable flavor standard. Then the best-flavored soy milk was fermented with lactic organisms and compared with fermented cow's milk.

MATERIALS AND METHODS

Fresh, whole homogenized cow's milk used as standard was purchased as required.

Preparation of soy milk 1 (hot grind)

Dry, whole soybeans (variety Harosoy) were thoroughly washed and soaked in water at 60 C until the absorbed water was about 1 ml/g dry weight. Soak water was decanted and beans were washed. Beans were ground in a Waring Blendor for 5 min (3 min at low, 1 min at medium, and 1 min at high speed) with boiling water. The ratio of beans to water was 1:9 wt/vol and the temperature of water during grinding between 85-95 C. Use of boiling water inactivated the enzyme, lipoxygenase, during grinding (*11*). The resulting suspension was filtered under reduced pressure using a Buchner funnel equipped with a 7-inch standard Agway milk filter with one layer of coarse pad on top of a fine pad. The resultant soy milk was dispensed in 160-ml screw cap bottles, autoclaved for 15 min at 121 C, and held at 5 C until used.

Preparation of soy milk 2 (defatted beans)

The process developed by Steinkraus (*8*) was used for defatting the soybeans. Soy flour (40 mesh) prepared from dehulled soybeans was extracted with 95% ethanol (1:2 soy flour to solvent wt/vol) followed by ethanol (95%)-chloroform (1:1 vol/vol) mixture until a clear filtrate appeared. After each extraction the solvent was removed by filtration under reduced pressure using a Buchner funnel equipped with 6.5-inch Agway premium milk filters. The residue from the last extraction was dried at 40 C under vacuum to completely remove the solvent and powdered to uniform size in a Braun mixer (Type MX3, Max Bruan, Frankfurt, Germany).

One hundred grams of defatted soy flour was comminuted with 1 liter of distilled water in a Waring Blendor for 10 min (6 min low, 2 min

medium, and 2 min high speed). The resulting slurry was centrifuged at 4080 × *g* for 20 min and the supernatant fluid (extract 1) was collected by filtration under reduced pressure through a Buchner funnel equipped with 6.5-inch Agway premium milk filters. The residue was again comminuted with 1 liter of distilled water, centrifuged, filtered, and the supernatant fluid collected (extract II). Extracts I and II were combined, warmed to 70 C and 2.0% (wt/vol) sucrose and 2.5% (vol/vol) refined soybean oil (Proctor and Gamble, Cincinnati, Ohio) were added. The mixture was blended for 1 min and homogenized three times in a Model 15M homogenizer (Manton-Gaulin, Everett, Mass.) at 8000 psi to obtain uniform distribution of fat. The soy milk thus prepared was dispensed in 160-ml screw cap bottles, autoclaved for 15 min at 121 C, and held at 5 C until used.

Cultures and conditions for production of fermented milks.

Streptococcus thermophilus (Marschall) and *L. acidophilus* (ATCC 4356) were kindly supplied by Dr. H. B. Naylor, Laboratory of Bacteriology, Cornell University, Ithaca, New York. *Lactobacillus plantarum* (B-246) was given to us by Dr. J. R. Stamer, N.Y.S. Agricultural Experiment Station, Geneva, New York. The cultures were maintained by bi-weekly transfers in sterile litmus milk and held at 5 C between transfers. An incubation temperature of 30 C was used for *L. plantarum* and 37 C for the other organisms. All cultures were transferred daily for 3 days in soy milk prepared from defatted beans before they were used as inocula to manufacture fermented milks. *S. thermophilus* was carried similarly in fresh, homogenized cow's milk for the control. All milks were fermented for 16 h before evaluation for flavor acceptability.

Analytical procedures

Protein was determined by a slightly modified semi-micro Kjeldahl method (*1*) replacing mercuric oxide and potassium sulfate with a Kjeldahl tablet containing sodium sulfate and selenium as the catalyst (British Drug Houses Ltd., Poole, England).

Titratable acidity was determined using 10-g samples and titrating with 0.1 N NaOH using phenolphthalein as indicator. Changes in pH were measured with a Beckman Zeromatic pH meter.

Fat, ash, and moisture values were determined using standard A.O.A.C. (*1*) procedures.

Analytical data on the unfermented soy milks 1 and 2 and cow's milk are given in Table 1.

TABLE 1. *Analyses of unfermented soy milks and cow's milk*

Component	Cow's milk (%)	Soy milk 1 (Hot grind) (%)	Soy milk 2 (Defatted beans) (%)
Protein (N × 5.71)	3.44	3.31	3.04
Fat	3.70	2.20	2.41
Ash	0.70	0.49	0.33
Moisture	87.1	91.77	92.18
Other matter (by difference)	5.06	2.23	2.04
pH	6.4	6.4	6.6
Titratable acid (as lactic)	0.16	0.17	0.11

Flavor evaluation

A multicomparison difference test described by Larmond (*5*) was used to evaluate flavor acceptability of fermented and unfermented soy milks. The taste panel consisted of staff members at the N.Y.S. Agricultural Experiment Station. Fresh, whole homogenized cow's milk purchased commercially was used as the flavor standard for evaluating the flavor of the unfermented soy milks. Cow's milk fermented with *S. thermophilus* was the flavor used for judging the flavor of the fermented soy milks. Samples were 45 ml in volume, served at a temperature between 45 and 50 F (7-10 C). Water was available for panel members who wished to rinse between samples.

Samples were assigned numerical values 1 to 9 with "no difference between the sample and the flavor standard" equaling 5, "extremely better than flavor standard" equaling 1, and "extremely inferior to flavor standard" equaling 9.

RESULTS AND DISCUSSION

Hot grind soy milk (soy milk 1) rated significantly different (1% level) and extremely inferior to the fresh cow's milk control, Table 2. It was also significantly different (1% level) from and inferior to soy milk 2. Thus, soy milk 1, although considered to be a much superior product than traditional cold water extracted soy milk (*2*), would require much further improvement before it would be as acceptable as cow's milk to American consumers. Soy milk 2 prepared as an aqueous extract of defatted soybeans with the addition of 2% sucrose (wt/vol) and 2.5% (vol/vol) refined soybean oil was rated as only slightly different (no significant difference statistically) and only slightly inferior in flavor to fresh cow's milk. It was much superior to soy milk 1 which possessed beany, objectionable flavor.

TABLE 2. *Taste panel evaluation of unfermented and fermented soy milks*

Type of soy milk	Average[a] flavor score[b] (27 member panel)
Unfermented soy milk 1 (hot grind)	8.75
Unfermented soy milk 2 (defatted beans)	5.90
Soy milk 2 (defatted beans)-fermented with *S. thermophilus*	6.60
Soy milk 2 (defatted beans)-fermented with *L. acidophilus*	6.85
Soy milk 2 (defatted beans)-fermented with *L. plantarum*	7.26

[a]The scores represent the acceptability rating of the samples compared with the flavor standards. Fresh homogenized cow's milk purchased commercially was used as the flavor standard for unfermented soy milks. The flavor standard for fermented soy milks was fresh cow's milk fermented with *S. thermophilus*.

[b]The ratings were assigned numerical values 1 to 9 with "no difference between the sample and the flavor standard" equaling 5, "extremely better than flavor standard" equaling 1, and "extremely inferior to flavor standard" equaling 9.

Soy milk 1 was not used in later fermentation experiments as the soy milk itself was considered too beany and objectionable to be compared further with either unfermented or fermented cow's milk.

It was found that fermented soy milks with a yogurt-like gelatinous curd could be produced from soy milk 2 using *S. thermophilus, L. acidophilus,* or *L. plantarum. S. thermophilus* produced the most acid of the three (0.51%) (Table 3) and rated closest to cow's milk fermented with the same organism. The soy milks fermented with *L. plantarum* and *L. acidophilus* were rated inferior to fermented cow's milk principally

TABLE 3. *Acid production by lactic acid bacteria in cow's milk and in soy milk 2*

Organism	Cow's milk (Control) Time	pH	% TA	Soy milk 2 Time	pH	% TA
Streptococcus thermophilus	0 h	6.40	0.17	0 h	6.65	0.11
	16 h[a]	4.46	0.64	16 h[a]	4.35	0.51
Lactobacillus plantarum				16 h[b]	4.80	0.36
Lactobacillus acidophilus				16 h[a]	4.50	0.43

[a]37 C
[b]30 C

because of their lower acidity. The fermented soy milks had a smooth texture and were virtually devoid of objectionable flavor as only one panelist out of 27 recorded a slightly beany taste. Three panelists described the mouth-feel as chalky and six recorded persistent aftertaste. No panelist judged the fermented soy milks as unacceptable in flavor.

This study suggests that aqueous extracts of dehulled, pulverized soybeans defatted by a Cornell University patented process (*8*) and fortified with 2% (wt/vol) added sucrose and 2.5% (vol/vol) added fat can be used to formulate soy milks nearly as acceptable in flavor as fresh, homogenized cow's milk. Addition of cow's milk flavor would be expected to further improve the flavor acceptability of the soy milks.

The study also indicates that fermented soy milks with an acceptable flavor and yogurt-like texture can be produced. Flavor acceptability will likely be further improved compared with fermented cow's milk if the acidity of the fermented soy milks is increased.

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Effect of Freezing, Additives, and Packaging Techniques on the Quality of Processed Blue Crab Meat[1,2]

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ABSTRACT

Experiments were conducted to study the effect of freezing technique on quality of Blue crab meat. Freshly processed crabmeat was used as a control. The treatments were evaluated by a trained sensory panel and analyses for trimethyl amine (TMA), total volatile base (TVB), and pH. The first experiment involved comparisons of crabmeat pre-treated with malic acid vs. phosphate, can vs. vacuum packaging in plastic pouches and storage at −18 and −30 C for 8 months. The results indicated that freezing and storing of crabmeat in a conventional air-circulating freezer (−18 C) significantly reduced the quality (aroma, flavor, texture, appearance) when compared to fresh crabmeat. Even when stored at −30 C, the quality was substantially lower than that of fresh crabmeat after 1½ months of storage. The two packaging methods and application of malic acid or phosphates did not prevent quality deterioration. A second experiment included a similar design except that the effect of freezing crabmeat with dichlorodifluoromethane ("Freon" Food Freezant) was compared with conventional freezing. The same packaging method was used but the additives were omitted. Freezing with "Freon" for 1½ month caused no significant difference in meat qualtiy when compared with fresh crabmeat (stored 0 days) but quality of the frozen crabmeat decreased significantly after 1½ months. Storage at −18 C was unsatisfactory for both freezing methods. TMA, TVB, and pH analyses were not of major value in assessing the quality of the fresh or frozen crabmeat.

Since Blue crab (*Callinectus sapidus*) meat is a highly perishable product which is harvested seasonally, it is usually sold within a limited marketing area. A satisfactory means of extending the shelf life of crab-meat would enable marketing to be done in a wider geographic area, thereby expanding consumer demand and reducing seasonal fluctuation.

Processing usually involves cooking of whole crabs by steam for approximately 10 min at 121 C, air cooling, and hand picking to remove the meat. Currently processed Blue crab meat is marketed as fresh, refrigerated, sterilized, pasteurized, or in some instances, as a frozen product (*14, 20, 22*). There are limitations to all of these methods of preservation. The major problem with refrigerated, fresh crabmeat is the limited shelf life of approximately 10 days. The other methods usually result in a decrease in one or more quality attributes (*5, 11, 12, 23*).

According to Dassow *et al.* (*8*), frozen crabmeat very rapidly becomes spongy and fibrous in texture and loses the delicate characteristic flavor of the fresh product. Gagal and Magar (*12*) reported that slowly frozen crabmeat appeared drier, more fibrous, and tougher than quickly frozen crabmeat after 5 months of storage at -18 C. Winter *et al.* (*24*) reported that fluctuations in storage temperature have an adverse effect upon the chemical and physical properties of crabmeat.

Several methods of freezing crabmeat have been proposed. The most widely used is air blast freezing at approximately -20 to -30 C. Cryogenic freezing has been accomplished with liquid nitrogen (boiling point -196 C), carbon dioxide (sublimation point - 79 C) or fluorocarbons (i.e. dichlorodifluromethane, boiling point -30 C). With the development of equipment for application and acceptance of dichlorodifluoromethane ("Freon" Food Frezant) for food products, cryogenic freezing with liquid "Freon" has been proposed as a method of freezing crabmeat (*2, 3, 6*). It has been observed that "Freon" freezing can be faster at -30 C than liquid nitrogen at -196 C (*1*). Shrimp and tuna have been successfully frozen using "Freon" (*8, 15*).

Strasser (*19*) reported that it is essential to have packages with a low water vapor permeability, a low oxygen transmission rate and resistance to absorption of oils and water to adequately preserve crabmeat. A minimum of air space or the injection of gas to remove oxygen is also desirable (*21*). Thus, a moisture and vapor impermeable vacuum package should be advantageous.

Polyphosphates have been used to increased the water holding capacity of crabmeat (*11*). Farragut (*10*) and Spinelli *et al.* (*19*) have proposed combining sodium tripolyphosphate and sodium chloride as a dipping solution. However, sodium chloride promotes oxidation rancidity in frozen meats and its use in a dipping solution may be of questionable value due to the development of off-flavors (*17*). Gardner, in 1966 (*13*) reported on the use of various food acidulants for improvement of product keeping quality.

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The objective of this investigation was to determine the effect of combining selected freezing methods, food additives, and packaging techniques on the quality of Blue crab meat during fresh and frozen storage.

MATERIALS AND METHODS

Experimental procedures

Two experiments were done in this investigation. The Blue crab meat used in these experiments was obtained from commercial firms who processed Blue crabs by cooking for 10 min at 121 C with steam, air cooling the crabs, then removing the meat by hand picking and chilling (1-3 C) for subsequent use in the experiments. Freshly picked meat from the body of the crab, designated as special crabmeat, was combined and thoroughly mixed by hand in a sanitized steel vat. In Experiment I, the crabmeat was randomly divided into treatment lots as follows: (*a*) untreated control, (*b*) treated with .5% phosphate (Freeze Guard Phosphate Blend No. 31777, Calgon Co.), and (*c*) treated with a solution containing 28.5% malic acid, 5% sucrose, and 1% starch. The additives were applied by dipping the crabmeat into the desired solution, holding for 1 min, removing and draining free of excess fluid before weighing and packaging. Care was taken to assure that the solutions thoroughly covered all particles of the crabmeat. The particle size of special crabmeat was small and thorough coverage was obtained. The product was packed tightly in 1-lb. cans that were then sealed and stored at 1, −18 and −30 C. Conventional freezing consisted of placing the individual containers on racks at the indicated frozen storage temperature. Evaluations were made after 0, 7, and 14 days for product at 1 C and after 1½, 3 and 8 months for product held at −18 and −30 C. In addition, a portion of the untreated fresh crabmeat was divided into 1-lb. units, packaged in nylon/ saran/polethylene pouches (No. P85OS Pouch, Cryovac Div., W. R. Grace Co.) having thickness of 1 mil/0.1 mil/2.5 mil, respectively. Pouches were vacuumized, heat sealed, placed in storage at 1, −18, and 30 C and evaluated at the same time intervals as the canned crabmeat. All treatments were replicated six times.

For Experiment II, the crabmeat was prepared as described for Experiment I and randomly divided into treatment lots of: (*a*) fresh, (*b*) conventionally frozen, and (*c*) frozen with "Freon." The crabmeat for each of these three lots was packaged in both cans and vacuum pouches as described for Experiment I. Freezing was accomplished by conveying the crabmeat into liquid "Freon" Food Freezant. The dwell time of the crabmeat in the liquid "Freon" was a fraction of a second, accomplished by conveying it under a spray of "Freon" and then equilibrating the crabmeat on a conveyor in vapors of "Freon" using a Mini Mark IV Freezer (E. I. duPont DeNemours and Company, Wilmington, Del.). The dwell time of the crabmeat in the liquid "Freon" was a fraction of a second with maximum freezant contact time being less than 30 sec and the equilibration time being approximately 2 min. Inlet temperature of the crabmeat was approximately 4 C with outlet temperature approximately −20 C. The "Freon" was partially recovered by circulating "Freon" through a series of heat transfer elements in contact with the "Freon" vapors. The "Freon" was kept cooled to approximately −40 C by additons of dry ice (carbon dioxide). After freezing, the crabmeat was immediately packaged in either cans by packing firmly and sealing or in the plastic pouches followed by vacuumizing and sealing. Both were immediately transferred to the storage freezer. "Freon" frozen crabmeat packaged in the can had considerable air space, whereas the air was removed from the pouches by vacuumization. The packaged, fresh crabmeat was evaluated after 0, 7 and 14 days of storage at 1 C. The conventional and "Freon" frozen samples were each sub-divided, stored at −18 and −30 C, and evaluated after 1½, 3, and 8 months. Each treatment lot was replicated 6 times.

Analytical procedures

Crabmeat samples were blended with an equal weight of distilled water and the pH was determined.

Total Volatile Base was determined by the method of Shewan and Ehrenberg (18) using Conway dishes. Proximate analyses for ash, protein and moisture were determined by standard procedures (*4*) and total lipids by the acetone-water extraction method of Dambergs (*7*). Trimethyl amine (TMA) was analyzed by the method of Dyer (*9*) as modified by Murray and Gibson (*16*).

Fluid loss during storage periods was determined by removing the sample from storage, wiping the package surface free of frost and water, opening the container, and weighing the crabmeat immediately. The sample was placed on a mesh grid over a pan, enclosed in a plastic bag, sealed and stored at 3 C for 36 h. The sample was removed from the plastic bag, inverted with the mesh grid onto a sheet of waxed paper and the mesh grid removed. The sample was immediately weighed and fluid loss determined by the difference in weights.

Cooking loss was determined by removing 50 g of thawed and drained meat, placing it in a tared beaker, heating the beaker in boiling water for 10 min, and immediately draining for 5 min. The crabmeat was reweighed and cooking loss calculated by the difference in weights of heated and unheated product.

Crabmeat was heated as described for the cooking loss evaluation and organoleptically evaluated for aroma, texture, flavor, and appearance by a five-member panel trained to identify changes in intensity of the specific quality characteristics. The panel members were screened and tested for threshold sensitivity to sweet, sour, bitter, and salt before the beginning of the experiment.

The statistical treatment of the data was accomplished by using crosstreatment averages to dilineate the specific treatments within each designated treatment lot. This is an acceptable statistical approach which indicates the degree of significance which is caused by a designated treatment regardless of other imposed treatments.

RESULTS AND DISCUSSION

Data for Experiment I on the effects of packaging method (can vs. vacuum pouch) and food additives (phosphate vs. malic acid) under fresh frozen storage conditions on chemical, physical and sensory evaluations of crabmeat are presented in Table 1. The method of packaging did not profoundly affect the quality of either the fresh or frozen crabmeat. Even where significant differences were observed, as was found for the fluid losses during storage and cooking, there was not a consistent trend for all sub-treatments. Thus, vacuum packaging did not produce the improvement in storage stability of the crabmeat that was anticipated from the report of Stansby et al. (*20*), wherein a minimum of air space in the package was reported to aid in maintaining the quality of crabmeat.

As would be expected the pH of the crabmeat receiving the malic acid treatment was significantly lower than the control and that treated with phosphate. The protein content of treated crabmeat, with malic acid, was significanlty lower which suggested removal of protein during the dipping process. Also, the TMA content of treated fresh crabmeat with malic acid was significantly lower with a similar trend for the frozen crabmeat indicating its potential in preventing the development of TMA. However, fresh crabmeat treated with malic acid was rated significantly lower by sensory evaluation than the control samples. The same was found for frozen crabmeat, except that malic acid assisted in maintaining a satisfactory appearance. The use of malic acid was expected to prevent discoloration and adverse textural changes but we could not determine from this study that it was sufficiently beneficial in maintaining overall quality to warrant its use.

TABLE 1. *The effects of packaging method, storage condition and additives on various chemical, physical and sensory properties of crabmeat*[1,2]

	Can						Vacuum pouch	
	Fresh (n = 6)			Frozen (n = 12)			Fresh (n = 6)	Frozen (n = 12)
Analyses	Control	Phosphate	Malic acid	Control	Phosphate	Malic acid	Control	Control
Chemical analyses								
Moisture %	78.23	78.30	76.97	78.05	77.90	77.21	77.91	77.57
Protein %* *	18.96a	18.05b	15.97c	17.25d	17.32d	15.13e	18.84a	17.94b
Fat %* *	2.12a	2.05a	2.28a	1.80b	1.91b	1.86b	2.12a	2.12a
Ash %	2.18	2.38	1.90	2.29	2.49	2.01	1.88	2.14
pH* *	7.87a	7.86a	6.46b	7.96a	7.95a	6.61b	7.86a	7.93a
TMA mgN/100/g* *	8.32a	7.42a	5.93b	2.13c	2.13c	1.87c	14.92d	2.12c
TVB, mg base/100 g* *	4.03a	4.33a	3.73a	3.32b	2.27b	2.18b	4.73a	2.18b
Physical changes								
Fluid loss %* *	0.71a	0.57a	0.69a	3.20c	2.43b	2.59b	1.28a	2.68b
Cooking loss %* *	4.37a	3.99a	5.70b	3.10c	2.83c	3.50c	3.42c	3.53c
Sensory rating[3]								
Aroma* *	4.55a	4.03b	3.87b	3.98b	3.87b	3.73b	4.48a	3.73b
Texture* *	4.48a	4.34a	3.84b	4.00b	3.94b	3.96b	4.60a	4.16b
Appearance* *	4.32a	4.37a	4.33a	3.21b	3.19b	3.43c	4.27a	3.18b
Flavor* *	4.50a	4.44a	3.86b	3.71b	3.68b	3.58b	4.68a	3.86b

[1]This table represents the means of all storage times and temperatures within each designated treatment lot.
[2]Values followed by the same letter (a, b, c, d, e) in any given row indicate no significant differences.
[3]Rated on 5 point scale with: 5 = highest, 1 = lowest quality.
* Significant at .05 level, * * Significant at .01 level.

The phosphate mixture used in this study did not result in a substantial improvement in either fresh or frozen crabmeat quality. The results indicated a reduction in fluid loss upon thawing of the frozen crabmeat which confirms previous findings (*10, 19*).

The most significant differences were found between the fresh and frozen treatments. TVB, TMA, and cooking loss levels were significantly lower in the frozen than fresh samples, regardless of the treatment. However, the sensory evaluations for aroma, flavor, texture and appearance indicated that the quality of frozen crabmeat was significantly lower than that of fresh crabmeat regardless of additive or packaging treatments. Thus, it was concluded that the sensory methods were more critical for evaluating the quality of crabmeat than the chemical methods.

TABLE 2. *The effects of storage temperatures on various chemical, physical and sensory properties of crabmeat*[1,2]

	Fresh	Frozen	
	1 C	—18 C	— 30 C
Chemical analyses			
TMA mgN/100 g	9.15	2.12	2.00
TVB mg base/100 g	4.21	2.20	2.78
pH	7.51	7.63	7.59
Physical changes			
Fluid loss %* *	0.81a	3.38b	2.06c
Cooking loss %	4.32	3.22	3.26
Sensory rating[3]			
Aroma*	4.23a	2.63b	4.02a
Texture*	4.32a	3.85b	4.18a
Appearance*	4.33a	3.11c	3.40b
Flavor* *	4.37a	3.51c	3.90b

[1]This table represents the means of all storage times, additives and packaging materials within each designated treatment lot (n = 24).
[2]Values followed by the same letter (a, b, c) in any given row indicate no significant differences.
[3]Rated on 5 point scale with: 5 = highest, 1 = lowest quality.
* Significant at .05 level.
* * Significant at .01 level.

The means for the effect of various fresh and frozen storage temperatures are shown in Table 2. The chemical evaluations (TMA, TVB, and pH) did not indicate differences due to storage temperatures. Fluid loss was significantly lower for fresh crabmeat than that stored at -18 and 30 C, with the -30 C samples being lower than those stored at -18 C. The cooking loss was appreciably higher for the crabmeat stored at 1 C. Thus, there was an inverse relationship between fluid and cooking losses. The crabmeat stored at -30 C was rated significantly higher than that stored at —18 C for all sensory characteristics (aroma, texture, appearance, and flavor) but the crabmeat stored at —30 C was rated significantly lower in flavor and appearance than fresh crabmeat with no significant differences for aroma and texture. It is evident from these results that extremely low storage temperatures (-30 C) are essential in maintaining the quality of crabmeat.

The means for the effect of storage time for all treatments are shown in Table 3. Length of storage was of primary importance in causing a decrease in the quality of both fresh and frozen crabmeat. The increased in TMA and TVB were substantial with extended storage time of fresh crabmeat but they remained relatively low in frozen crabmeat, being approximately equal to the initial values for fresh crabmeat. The aroma, flavor, texture, and appearance rating decreased significantly with increased storage time for both the fresh and frozen samples. The sensory ratings for fresh crabmeat did not show a substantial decrease in quality until storage day 14; being unacceptable by then. In no instance were the frozen samples rated as high in quality as the initial ratings for fresh crabmeat. When the ratings for frozen and fresh crabmeat are compared, the aroma and texture values were comparable after 1 1/2 mo frozen storage and 7 days fresh storage. However, appearance and

TABLE 3. *The effects of storage time on various chemical, physical and sensory properties of crabmeat*[1,2]

Analyses	Fresh storage, days			Frozen storage, months		
	0	8	14	1.5	3.0	8.0
Chemical analyses						
pH	7.93	7.51	7.49	7.61	7.66	7.58
TMA mgN/100 g* *	1.93a	9.26b	16.25c	1.82a	1.92a	2.45a
TVB, mg base/100 g* *	2.40a	4.00b	6.23c	3.10a	2.33	2.04a
Physical changes						
Fluid loss, %* *	.99a	0.64a	0.80a	3.80b	1.47c	2.91d
Cooking loss, %* *	5.17a	4.61a	3.40b	3.56b	4.06b	2.09c
Sensory rating[3]						
Aroma* *	4.60a	4.53a	3.58b	4.28a	3.93b	3.80b
Texture* *	4.70a	4.15a	3.88b	4.46c	3.97b	3.61b
Appearance* *	4.39a	4.58a	4.01b	3.56c	2.93d	3.28c
Flavor* *	4.51a	4.61a	3.60b	4.01b	3.88b	3.71b

[1]This table represents the means of all storage temperatures, additives and packaging methods within each designated treatment lot (n = 8) for storage, days; n = 16 for storage, mos)
[2]Values followed by the same letter (a, b, c, d) in any given row indicate no significant differences.
[3]Rated on 5 point scale with: 5 = highest, 1 = lowest quality.
* Significant at .05 level.
* * Significant at .01 level.

TABLE 4. *Effect of freezing method and storage time on various chemical, physical and sensory properties of crabmeat*[1,2]

Storage		Moisture	Protein	Fat	Ash			Fluid loss	Cooking loss	Sensory rating[5]			
Temp.	Time	(%)	(%)	(%)	(%)	TMA**	TVB*	(%)	(%)	Aroma**	Texture	Appearance**	Flavor**
Fresh	0 Day	77.90	19.07	1.97	2.21	1.60a	2.20a	0.98a	5.05	4.60a	4.65	4.40a	4.55a
	7 Day	78.50	18.56	2.18	2.15	9.20b	4.70b	0.48a	4.95	4.90a	4.55	4.60a	4.80a
	14 Day	78.29	19.26	2.22	2.18	14.15c	5.20b	0.67a	3.10	4.15b	4.00	3.95a	3.80b
Conv.	1½mo	78.05	17.25	1.80	2.20	1.85a	5.35b	4.94d	3.58	4.38b	4.45	3.38b	4.08b
frozen[3]	3 mo					1.98a	2.50a	1.75b	3.58	3.65b	3.75	3.10b	3.35b
	8 mo					2.58a	2.10a	2.92c	2.15	3.90b	3.80	3.15b	3.70b
Freon	1½mo	78.35	18.38	1.81	2.14	1.45a	3.20a	1.18a	2.85	4.73a	4.63	4.45a	4.58a
frozen	3 mo					2.15a	1.90a	0.91a	3.95	3.75b	4.45	3.83a	3.78b
	8 mo					2.30a	2.15a	1.84b	1.73	4.05b	4.60	4.25a	4.08b
Fresh	0 Day	77.69	17.94	2.02	2.18	2.30a	2.60a	1.51a	4.35	4.80a	4.90	4.40a	4.80a
	7 Day	77.79	18.97	2.11	2.06	9.30b	3.80b	0.86a	3.10	4.70a	4.45	4.60a	4.90a
	14 Day	78.26	19.60	2.25	2.11	33.15d	7.90c	1.46a	2.80	3.95b	4.30	3.80a	4.00b
Conv.	1½mo	77.57	17.94	1.88	2.14	1.83a	2.30a	3.33c	4.38	4.23a	4.58	3.68a	4.20a
frozen[4]	3 mo					2.00a	2.10a	2.06b	4.18	3.20b	4.15	2.63b	3.48b
	8 mo					2.53a	2.15a	2.65c	2.05	3.75b	3.75	3.25b	3.90b
"Freon"	1½mo	78.20	17.60	1.75	2.18	1.48a	2.55a	4.23d	2.90	4.80a	4.23	4.45a	4.65a
frozen	3 mo					2.05a	2.35a	3.05c	4.00	3.30b	4.45	3.78a	3.58b
	8 mo					2.35a	2.30a	1.38a	2.08	3.80b	4.30	4.00a	4.20a

[1]This table represents the means of all storage temperatures within each designated treatment lot.
[2]Values followed by the same letter (a, b, c) in any given row indicate no significant differences.
[3]Conventional freezing consisted of placing the individual containers on racks at the designated freezer storage temperature.
[4]Frozen with "Freon," The E. I. duPont DeNemours & Co.
[5]Rated on 5 point sacle with: 5 = highest, 1 = lowest quality.
* Significant at .05 level; * * Significant at .01 level.

flavor values were lower for the frozen samples after 1 1/2 month than the fresh samples after 7 days of storage.

The effect of the "Freon" freezing method was compared with fresh and conventionally frozen crabmeat in Experiment II, by the same experimental design for storage times and temperatures and packaging methods as for Experiment I.

The effects of freezing method and storage time on the quality of crabmeat for Experiment II are indicated by the data presented in Table 4. There was no significant difference between the two packaging methods as found in Experiment I. Also none of the treatments significantly affected the proximate composition of the crabmeat. Significant increases were observed in TVB and TMA values with increased storage time for fresh crabmeat. These values did not increase during frozen storage for either method of freezing. In general, the chemical analyses were of little benefit for evaluating frozen samples. "Freon" treated samples had lower fluid losses than conventionally frozen samples, with all frozen treatments being higher than fresh.

The organoleptic ratings for "Freon" frozen samples were higher than those for conventionally frozen crabmeat. After 1 1/2 month, the "Freon" frozen crabmeat was rated comparable to fresh crabmeat stored for 7 days.

Data on the effect of storage temperature are presented in Table 5. At the same storage temperature, TMA and TVB values were lower for "Freon" than conventionally frozen crabmeat. Samples stored at -30 C were rated higher in all quality attributes by sensory evaluation than the samples stored at -18 C. In fact, the crabmeat frozen with "Freon" and stored at -30 C was

TABLE 5. *The effect of freezing method and storage temperature on various chemical, physical and sensory properties of crabmeat*[1,2]

	Storage temperature				
	1 C	— 18 C		30 C	
Analyses	Fresh	Conv.[3]	Freon[4]	Conv.[3]	Freon[4]
Chemical					
TMA, mgN/100 g* *	11.62a	2.16b	1.95b	2.09b	1.98b
TVB, mg base/100 g* *	4.38a	3.55b	2.56c	3.30b	2.25c
pH	7.86	7.98	7.93	7.91	7.89
Physical					
Fluid loss %* *	0.99a	3.92b	2.14b	1.96b	2.04b
Cooking loss %	3.89	3.23	3.23	3.40	2.61
Sensory rating[5]					
Aroma* *	4.52a	3.65b	3.96b	4.05b	4.17b
Texture*	4.54a	3.83b	4.40b	4.33a	4.83a
Appearance* *	4.29a	3.03b	4.12a	3.37b	4.13a
Flavor* *	4.59a	3.56b	3.99b	4.01a	4.29a

[1]This table represents the means of all storage times and packaging methods within each designated treatment lot (n = 12).
[2]Values followed by the same letter (a, b, c) in any given row indicate no significant differences.
[3]Conventional freezing consisted of placing the individual containers on racks at the designated freezer storage temperature.
[4]Frozen with "Freon," the E. I. duPont DeNemours & Co.
[5]Rated on 5 point scale with: 5 = highest, 1 = lowest quality.
* Significant at .05 level, * * Significant at .01 level.

rated comparable to fresh crabmeat (0 days storage). However, when the data in Table 4 are examined, it it evident from the decrease in quality with storage time that the major advantages for "Freon" freezing and low temperature storage (-30 C) were during the first 3 months. Thus, the combination of "Freon" freezing and a -30 C storage temperature was determined to be of value for a relatively short term of storage.

In conclusion, freezing of crabmeat with "Freon" was found to be superior to conventional freezing but temperature and time of storage were the major factors in maintaining quality regardless of freezing technique. When stored for up to 1 1/2 month at -30 C, the "Freon" frozen crabmeat was not significantly different in quality from that of fresh crabmeat (stored for 0 days). It can be concluded that crabmeat may be frozen by the "Freon" method and held for periods up to 1 1/2 month at -30 C without a substanial deterioration in quality from that of fresh picked crabmeat. On the basis of these results, the conditions for preserving crabmeat by freezing must be closely controlled. It is doubtful if current practices in the industry can achieve this control. This conclusion is based upon the current storage and distribution problems wherein freezer storage temperatures are not routinely held as low as -30 C. Also, due to the requirements of transfer and transportation systems a uniform storage temperature is not routinely maintained. However, with further technological applications by the industry, IQF techniques in conjunction with better control of storage temperatures may be within the realm of practicality for handling frozen crabmeat. Based upon previous work (21, 22) and the results of this investigation, the application of presently available additives was not found to be advantageous. However, with the advantages shown for rapid freezing, such as "Freon," and low temperature storage, the possibility of additive applications should be studied further in an attempt to overcome the difficulties with freezing and storage constraints.

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A Research Note

Importance of Brands of Dehydrated Culture Medium

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ABSTRACT

Selenite Brilliant Green Sulfa broths, one prepared from basic ingredients according to formula and brands A and B available in dehydrated form, were compared for effectiveness of recovering salmonellae. Distinctly different percent recoveries of salmonellae were achieved among the three types of media.

Brands of dehydrated bacterial culture media may affect corroboration of analytical results among different laboratories. Reliable results and continuity of laboratory analyses may depend greatly on brand of dehydrated medium employed. Different brands of the same type of medium may yield quite different results. An example of such a problem is reported herein. Perhaps researchers and laboratory technicians should consider the importance of using the same brand of medium throughout analyses if they wish to reproduce or corroborate results, so as not to fall victim to erratic results because of brand differences. A comparison of brands of media before locking into routine analyses or initiating experimental research would be advisable.

MATERIALS AND METHODS

Two different brands, designated as A and B, of Selenite Brilliant Green Sulfa (SBGS) broth, used as an enrichment for *Salmonella* recovery, and a laboratory-prepared SBGS broth made from basic ingredients according to formula were simultaneously compared during 75 analyses for salmonellae. All broths were incubated at 37 C for 24 h. Communication with manufacturers about the particular lots of brand A and B used in these experiments confirmed the medium should be effective for its purpose during the period of the experiments. The analyses were conducted on excised skin samples of artificially contaminated raw turkey tails. Five replicate tails were contaminated, by immersion in 0.1% aqueous peptone inoculum fluid, with either *Salmonella typhimurium, enteritidis, anatum, panama, cubana, worthington, javiana, meleagridis, cerro, poona, newington, tennessee, manhattan,* or *heidelberg* at very low contamination levels [between 1-50 *Salmonella* organisms per excised sample by Most Probable Number determinations (*1*)]. Incubated broths were streaked to Brilliant Green Sulfa agar, from which after 24-h incubation, typical pink *Salmonella* colonies were picked and inoculated into Triple Sugar Iron agar. Further confirmation included urea, tryptone, dulcitol, malonate and lysine decraboxylase biochemicals and serogroup somatic antigen antisera.

RESULTS AND DISCUSSION

Results shown in Table 1 illustrate a distinct difference in effectiveness between brands A and B and show the poor quality of the laboratory-made SBGS. Brand A was much more effective in enriching for salmonellae (91.4% recovery) than brand B (64.3% recovery). There was no apparent relationship between serotype and recoverability from a given brand, rather brand A was consistently better than the other two broths. However, with *S. anatum, panama* and *tennessee,* brand B was slightly better than brand A.

Differences among the broths were apparently due to brand and, therefore, probably due to how manufacturers prepare the media and the specific type and/or brand of ingredients they use. Users should determine the brand which gives them the most positive results for their type of work and then consistently use

TABLE 1. *Comparison of three types of Selenite Brilliant Green Sulfa (SBGS) broth based on percent positive of 14 salmonella serotypes from skin samples excised from artificially contaminated raw turkey carcass tails*

	Percent positive *Salmonella* recoveries[a]														
Type of SBGS	typhimurium	enteritidis	anatum	panama	cubana	worthington	javiana	meleagridis	cerro	poona	newington	tennessee	manhattan	heidelberg	Average
Brand A	100	100	80	80	100	60	100	100	100	100	100	80	80	100	91.4
Brand B	100	60	100	100	40	0	40	20	100	60	60	100	80	40	64.3
Laboratory-made	60	40	80	100	60	20	20	20	0	60	0	0	20	0	34.3

[a]Percent refers to the number of tails from which the specific serotype was recovered and identified out of five contaminated tails per brand/serotype treatment

this brand to avoid variation in their work due to this factor. Results indicate specifically that brand of SBGS is a very important factor to consider when analyzing for salmonellae from poultry meat.

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Biologically Active Amines in Food: A Review

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ABSTRACT

Biologically active amines are normal constituents of many foods and have been found in cheese; sauerkraut; wine; and putrid, aged, or fermented meats. These low molecular weight organic bases do not represent any hazard to individuals unless large quantities are ingested or natural mechanisms for their catabolism are inhibited or genetically deficient. Tyramine, histamine, and phenethylamine, which can arise from enzymatic decarboxylation of the corresponding amino acids, are strongly vasoactive. Histamine, a capillary dilator, produces hypotensive effects while tyramine and phenethylamine cause a rise in blood pressure. Phenethylamine has been implicated in the onset of migraine headache attacks. The occurrence, mechanism of formation, and catabolism of these compounds is reviewed.

INTRODUCTION

Biologically active amines (biogenic amines) have been defined as aliphatic, alicyclic, or heterocyclic organic bases of low molecular weight which arise as a consequence of metabolic processes in animals, plants, and microorganisms (*28*). Biogenic amines are generally either psychoactive or vasoactive. Psychoactive amines act on the neural transmitters in the central nervous system, while vasoactive amines act, either directly or indirectly, on the vascular system (*43*). Pressor amines are vasoactive amines that cause a rise in blood pressure. In 1901, Barger and Walpole (*5*) first identified tyramine, phenethylamine, and isoamylamine as the pressor principles of putrid meat. Tyramine was the most active of the pressor amines. It is now well known that phenethylamine derivatives including tyramine, dopamine, and norepinephrine cause a marked increase in blood pressure when injected intravenously into mammals. Serotonin and histamine are also strongly vasoactive (*30*). Histamine, in contrast to tyramine, is a strong capillary dilator and can produce hypotensive effects.

Biologically active amines are normal constituents of many foods, but they usually do not represent any hazard to individuals unless large amounts are ingested or the natural mechanism for the catabolism of one or more of the amines is inhibited or genetically deficient. Monoamine oxidases (MAO) and diamine oxidases (DAO) oxidatively deaminate the amines and play a major role in their degradation. Monoamine oxidase inhibitors (MAOI) are drugs that block the oxidative deamination of certain primary amines. These drugs have been primarily used for the treatment of depression in psychiatric patients. Hypertensive attacks during MAOI treatment were first reported in 1955 (*49*). Blackwell (*7*) first observed an association between hypertension crises of patients on MAOI (tranylcypromine) and possible dietary precipitants (cheese). Asatoor et al. (*3*) implicated tyramine in cheese as the primary causative agent. During the early 1960's many reports of hypertension crises of patients on MAOI were reported in the literature. The principle foods implicated in these first attacks included cheese and yeast extract. In addition to the reports of hypertension crises in the literature, there are reports connecting tyramine, serotonin, and phenethyamine to migraine headaches (*1, 2*). The role of amines in migraines is presently being investigated.

Since Asatoor et al. (*3*) associated tyramine in cheese to pressor attacks in MAOI patients, considerable research has been completed dealing with the appearance of biologically active amines in foods. The objective of this review is to present information on the physiological effects of various biologically active amines, to summarize the quantitative data that are available in the literature, and to discuss factors that lead to amine formation.

TYRAMINE AND HYPERTENSIVE CRISES

Hypertension attacks during treatment with iproniazid, a monoamine oxidase inhibitor (MAOI), were first reported by Ogilvie in 1955 (*49*). Initially, MAOI were used to treat patients with pulmonary tuberculosis and later for general treatment of depression in psychiatric patients. With increased use of the drugs, more pressor attacks were reported. Davies (*19*) reported hypertension attacks in patients on nialamide and Clark (*17*) and Blackwell (*6*) observed cases of hypertension crises in patients on tranylcypromine. Up until this time no connection betwen the attacks and dietary factors had been observed. Blackwell in 1963 (*7*) first reported that cheese may be the dietary precipitant in the hypertensive crises.

Although Van Slyke and Hart (*61*) originally isolated tyramine from cheese in 1903, and Barger and Walpole (*5*) reported in 1901 that tyramine was the major pressor principle in putrid meat, new investigations into tyramine contents of foods were not initiated until Asatoor et al. in 1963 (*3*) associated tyramine in food to the hypertensive attacks noted in MAOI patients.

Asatoor et al. (*3*) examined several cheeses for tyramine and found concentrations up to 2 mg of tyramine/g of cheese and 25.4 μg/g of wine. Controlled studies of a hospital patient given tyramine and cheese containing tyramine showed that there was only a minor response to the cheese or tyramine in the absence of MAOI. This individual, after MAO inhibition with paraglyline, showed a marked increase in blood pressure, accompanied by headache with ingestion of both cheese and tyramine.

Tyramine acts pharmacologically by releasing norepinephrine from tissue stores which in turn causes a rise in blood pressure (*28*). Tyramine has 1/20-1/50 of the ability of epinephrine to increase blood pressure (*3*). MAO inhibitors increase the tissue stores of norepinephrine and thus potentiate the action of tyramine. Symptoms of hypertensive crisis include high blood pressure, headache, fever, and sometimes perspiration and vomiting (*11, 30*). MAO inhibitors still being used as antidepressants include isocarboxazid, nialamide, phenelzine sulfate, and tranylcypromine sulfate (*50*).

Numerous other reports of hypertensive crises occur in the literature. Foods that have been implicated include chocolate (*39*), yeast extract (*10*), beef liver (*12*), chicken livers (*32*), broad beans (*34*), and pickled herring (*48*). It appears that tyramine is the major offender in precipitating hypertensive crises. In man, 20-80 mg of tyramine injected intraveneously or subcutaneously cause a marked elevation of blood pressure (*3*). In individuals on MAOI, as little as 6 mg taken orally can cause a rise in blood pressure. The tyramine contents of fruits, vegetables, cheese, and other foods are given in Tables 1, 2, and 3. It appears unlikely that tyramine in fruits and vegetables could precipitate hypertension attacks unless large quantities are consumed. Cheeses and some sausages contain much higher concentrations of tyramine than do fruits and vegetables and are considerably more dangerous to the tyramine-susceptible individual. Of the other foods, yeast extract, salted dried fish, pickled herring and the meat samples indicated in Table 3 could possibly be dangerous.

TABLE 2. *Histamine and tyramine contents of cheeses*[a]

Cheese	Histamine (μg/g)[b]	Tyramine (μg/g)	References
Cheddar	0-1,300	0-1, 500	*3, 8, 23, 35, 55, 62*
Camembert	0-480	20-2,000	*3, 35, 55, 62*
Emmenthaler	—	225-1,000	*3, 35*
Brie	0	0-260	*35, 55, 62*
Stilton blue	0	460-2,170	*35, 55, 62*
Processed	0	0-50	*35, 55, 62*
Gruyere	—	516	*35*
Gouda	0-850	20-670	*23, 55, 62*
Brick, natural	—	524	*55*
Mozzarella	0	0-410	*55, 57, 62*
Blue or Roquefort	0-2, 300	27-1,100	*55, 62*
Boursault	0	110-1,116	*55, 62*
Parmesan	0-58	4-290	*55, 57, 62*
Romano	0-161	80-238	*55, 57, 62*
Provolone	10-525	38-150	*55, 57*
Swiss	0	0-1,800	*62*
Colby	0-500	100-560	*62*
Edam	0	300-320	*62*
Cottage	0	0	*62*
Others	0-2,600	0-660	*62*

[a]Table adapted from Lovenberg (*43*).

[b]A dash means the cheese was not tested for this amine and 0 indicates that the level of the amine was below the detection threshold. Values represent ranges or quantities present in the selected samples and should not be interpreted as averages.

HISTAMINE

Histamine has been implicated in several outbreaks of food poisoning. Ferencik et al. (*26*) showed that several samples of tuna fish, which gave intoxication symptoms in people after consumption, contained considerable quantities of histamine (204-464 mg/100 g). Doeglas et al. (*22*) indicated histamine to be the cause of

TABLE 1. *Vasoactive amines in plant foods*[a]

Plant substance	Amine in μg/g or μg/ml[b]					
	Serotonin	Tryptamine	Tyramine	Dopamine	Norepinephrine	Reference
Apple	—	0	—	—	—	*55*
Avocado	10	0	23	4-5	0	*59*
Banana (peel)	50-150	0	65	700	122	*59*
Banana (pulp)	28	0	7	8	2	*59*
Egg plant	0	0	—	—	—	*59*
Grape	0	0	0	0	0	*59*
Grapefruit juice	—	—	0	—	—	*55*
Orange	0	0.1	10	0	+	*59*
Passion fruit	1-4	—	—	—	—	*27*
Pawpaw	1-2	—	—	—	—	*27*
Pineapple juice	25-35	—	0.36	—	—	*13, 55*
Plantain	45	—	—	—	—	*59*
Potato	0	0	1	0	0.1-0.2	*59*
Blue plum	0	5	—	—	—	*59*
Red plum	10	0-2	6	0	+	*59*
Red blue plum	8	2	—	—	—	*59*
Raspberry	—	—	13-93	—	—	*4*
Spinach	0	0	1	0	0	*59*
Tomato	12	4	4	0	0	*59*

[a]From Lovenberg (*43*)

[b]A dash means the food was not tested for this amine, 0 means that the level of the amine was below the detection threshold, and + indicates the material contained a trace of the amine.

TABLE 3. *Histamine and tyramine contents of foods other than cheese*[a]

Food substance	Histamine (μg/g)[b]	Tyramine (μg/g)	References
Beer and ale	—	1.8-11.2	*35, 55*
Wines	0-22	0-25	*35, 45, 51, 55, 58*
Yeast extracts	210-2,830	0-2,256	*9, 35, 55*
Fish			
Tuna	2,040-5,000	—	*26, 36, 37*
Salted dried fish	—	0-470	*55*
Pickled herring	—	3,000	*48*
Meat			
Meat extracts	—	95-304	*55*
Beef liver	—	274	*12*
Chicken liver	—	100	*32*
Sausage	0.74-410	0-1237	*14, 21, 52*
Miscellaneous			
Soya sauce	—	1.76	*55*
Sauerkraut	7-200	20-95	*44*

[a]Table adapted from Lovenberg (*43*).

[b]A dash means the food was not tested for this amine, and 0 indicates that the level of the amine was below the detection threshold. Values represent quantities present in selected samples and should not be interpreted as averages.

intoxication from the consumption of a 2-year-old Gouda cheese. A recent outbreak of scombroid poisoning occurred in February, 1973, and involved 254 cases associated with the consumption of canned tuna fish (*15, 16*). Histamine is reported to be one of the principle compounds leading to scombroid poisoning (*37*). Although the toxicity of histamine is controversial, ingestion of 70-1000 mg will usually cause clinical symptoms of intoxication (*33*). Blackwell et al (*9*) reported that less than 225 mg of histamine taken orally usually does not produce symptoms, although susceptible subjects (allergy, asthma, or peptic ulcers) might be adversely affected by smaller quantities. Following intraveneous injection of 0.1 mg of histamine phosphate, facial flushing, pulse quickening, a fall in the blood pressure, and a rise in the cerebrospinal fluid pressure occurs within 20 sec. The onset of histamine headache occurs 1 min after injection (*30*). Unlike tyramine, histamine acts as a powerful capillary dilator (*28*). The symptoms of scombroid poisoning are similar to the physiological effects induced by histamine injection and include nausea, vomiting, facial flushing, intense headache, epigastric pain, burning sensation in the throat, dysphagia, thirst, swelling of the lips, and urticaria (*16*).

Although tyramine is the major precipitator of hypertension attacks in MAOI patients, Blackwell et al. (*10*) found the presence of histamine, along with tyramine, in yeast extracts to alter the nature of the hypertensive attack. These workers concluded that MAOI facilitated the absorption of both tyramine and histamine from the intestine and potentiated their action. The presence of histamine could explain the occurrence of the persistent headache in hypertension crises and the fall in blood pressure these workers observed after its initial rise. Lovenberg (*43*) warns that use of aminoguanidine, a potent inhibitor of diamine oxidase, may potentiate effects of histamine-containing foods on the patients involved.

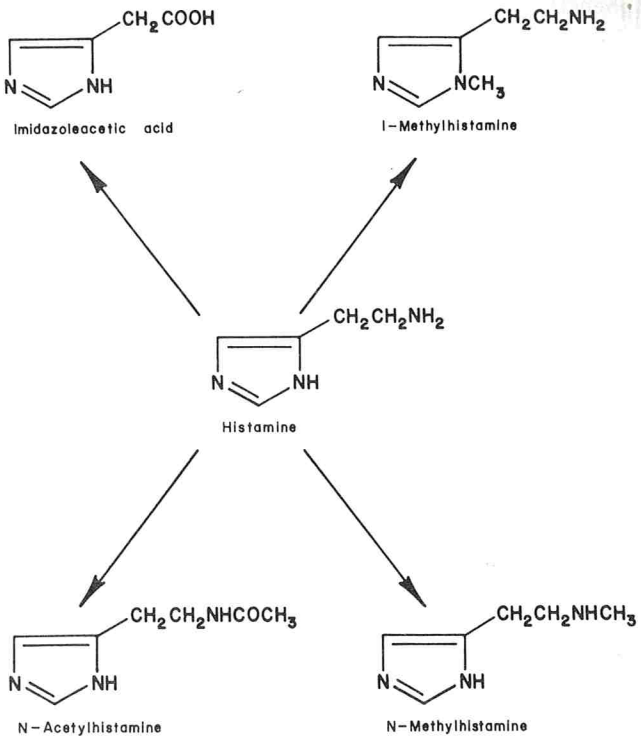

Figure 1. *Histamine catabolic routes.*

Histamine can be catabolized by several routes (Fig. 1). It can be oxidatively deaminated by diamine oxidase, methylated to form 1-methylhistamine, or its side chain can be methylated or acetylated (*28*).

Histamine is commonly found in foods that have been aged or fermented. The histamine content of various cheees (Table 2) varies from 0 to 2,600 μg/g. It seems possible that cheeses, which contain high concentrations of histamine could lead to symptoms of histamine intoxication. Doeglas et al. (*22*) reported the instance of one individual showing intoxication symptoms from ingestion of a Gouda cheese containing 850 μg histamine/g. The histamine contents of other foods are listed in Table 3. Histamine is present in high amounts in yeast extracts and samples of tuna fish known to have caused scombroid poisoning. Tuna fish have been reported to contain up to 5000 μg histamine/g (*37*).

OTHER AMINES

Phenethylamine, like tyramine, causes an increase in blood pressure by liberating norepinephrine from tissue stores (*28*). Phenethylamine has a potency of only 1/200-1/500 of that of epinephrine (*3*) and is not as important as tyramine in precipitating hypertensive attacks. Chocolate is one food that has been incriminated in a hypertensive crisis (*39*). It was suggested that vanillin, a catechol derivative, was the precipitant, but chocolate is also known to contain substantial amounts

of phenethylamine (*2*). Sandler et al. (*54*) reported that a 2-oz. bar of chocolate contains at least 3 mg of phenethylamine but no tyramine. In this instance, phenethylamine may have been the dietary precipitant.

Chocolate has also been implicated as a dietary cause of migraine headaches (*2*). Sandler et al. (*54*) tested this hypothesis by giving phenethylamine (3 mg) and lactose, the control, to individuals who suffered from migraine headaches precipitated by chocolate. In this test, 18 out of 36 individuals reported a headache after taking phenethylamine and only 6 out of 36 reported a headache after lactose. The phenomenon had also been observed with tyramine (*31*). Sandler et al. (*54*) also tested the human platelet MAO activity in migrainous subjects. These subjects had a significantly ($p < 0.001$) lower activity than the control patients when either phenethylamine or tyramine was used as substrate.

The presence of tryptamine has been reported in tomatoes (*64*) and other fruits and vegetables (Table 1), and cheese (*56, 62*). The levels of tryptamine reported in cheese (0-1, 100 μg/g) are generally lower than the levels reported for histamine or tyramine. Although tryptamine has a pharmacological action similar to tryamine, there are no reports of tryptamine intoxication or of hypertensive crises due to tryptamine.

Serotonin has been widely discussed as a possible cause of migraine headache (*1*), but the evidence is not clear that serotonin is directly related to this disease. Serotonin is present in fairly large amounts in bananas (*63*) and is present in other fruits and vegetables (Table 1). Hodge et al. (*34*) reported a case of hypertensive crisis due to 3, 4-dihydroxyphenylalanine in broad beans. Although there are no definite reports of intoxication due to serotonin or norepinephrine, elevated levels of the metabolites of these substances are biochemical signals of the diseases pheochromocytoma and malignant carcinoid (*43*). Therefore, consumption of bananas and/or pineapple juice may give rise to false positive tests for these diseases (*18*). Doeglas and Nater (*23*) reported that histamine in foods may give rise to false positive scratch tests for food allergies.

FORMATION OF TYRAMINE AND HISTAMINE IN FOODS

Although foods normally contain small amounts of tyramine and histamine, formation of large amounts of these amines has been reported only in aged, fermented products or products such as tuna fish that has undergone spoilage. The factors that govern formation of amines include: (*a*) availability of free amino acids, (*b*) presence of microorganisms that can decarboxylate the amino acids, and (*c*) favorable conditions for the growth of the microorganism and for production of decarboxylase enzymes. It is well known that, in the ripening of cheese, liberation of amino acids occurs, Dierick et al. (*21*) observed a general increase in free amino acids with little change in the concentration of free tyrosine during sausage ripening. However, these workers reported an increase in the presence of tyramine during sausage ripening, indicating that although free tyrosine is produced, it is decarboxylated to form tyramine. Microorganisms commonly found in sausage fermentations include *Pediococcus, Lactobacillus, Streptococcus,* and *Micrococcus* (*20*).

Rodwell (*53*) tested 34 strains of *Lactobacillus* for their ability to decarboxylate amino acids. Most of them showed no activity toward any amino acid with only *Lactobacillus pentoaceticus* Rudensis showing slight tyrosine decarboxylase activity. Four *Lactobacillus* spp., including *Lactobacillus* 30a, had histidine decarboxylase activity. Lagerborg and Clapper (*41*) tested 33 strains of *Lactobacillus* and found that three strains could produce CO_2 from tyrosine. One of the strains could produce CO_2 from histidine. It is well known that the group D streptococci produce tyrosine decarboxylase. Gale (*29*) studied production of tyramine by *Streptococcus faecalis* and found that six out of seven strains produced tyrosine decarboxylase. The optimal pH of the tyrosine decarboxylase in washed suspensions of the cells was pH 5.0, and cultures grown at 27 C had the same activity as cultures grown at 37 C. The activity of the washed suspension varied considerably with the pH of the media. Non-carbohydrate media at pH 5 yielded 30-40 times more activity than did a non-carbohydrate medium at pH 7. Media containing carbohydrate showed high activity due to the production of lactic acid which lowered the pH. Other bacteria known to produce tyrosine decarboxylase include *Betabacterium* spp., *Clostridium aerofoetidum, Clostridium sporogenes, Escherichia coli, Proteus mirabilis,* and *Pseudomonas reptilivora* (*47*).

Yeast extract has been reported to contain large amounts of histamine and tyramine. This product is made by a process which involves plasmolysis and autolysis (*9*). Conditions are carefully controlled to permit maximum enzyme activity, and most of the protein is reduced to a soluble form within 24 h. It is possible that tyramine and histamine are formed by the yeast enzymes or by contaminating bacteria present in this ideal medium.

In the production of Cheddar cheese, the only microorganisms present known to have tyrosine decarboxylase activity are the coliforms and *Streptococcus* group D (*S. faecalis, S. faecium,* and *S. durans*) (*8*). Raw milk is ofen pasteurized before acidification or fermentation. This step should kill the coliforms. However, it is possible that tryosine or histidine decarboxylase may remain active following the heat treatment. It is also possible that further contamination may result from faulty sanitation. The production of acid by dairy starter cultures (*Streptococcus* group N) should also help to control coliforms. However, coliform counts of 1 million/g of cheese may develop in cheese made from raw milk exposed to poor hygiene or to delayed acidification (*8*). The more heat and acid resistant group D streptococci may compete with the starter culture and produce tyramine.

Tyramine and histamine are also formed during the fermentation of cabbage in the production of sauerkraut (*45, 46*). Mayer et al. (*45*) reported that sauerkraut with a low histamine and tyramine content could be produced by inhibiting growth of pediococci by early interruption of the fermentation just below pH 4. This produces a mild, low-acid sauerkraut which can be pasteurized to achieve stability. Mayer et al. (*46*) observed that the histamine contents of sauerkraut increased simultaneously with the appearance of *Pediococcus cerevisiae.*

Mossel (*47*) compiled a list of microorganisms that can produce histamine from histidine. These microorganisms include *Betabacterium* spp., *Clostridium perfringens, Enterobacter aerogenes, E. coli, Proteus morganii, P. reptilivora,* and *Ristella, Salmonella,* and *Shigella* spp. He reported that all of these microorganisms except *Ristella* are of frequent and numerous occurrence in foods and thus could be important in the production of histamine. Ferencik (*25*) implicated *Hafnia* strains, *P. morganii,* and hemolytic *E. coli* in the formation of histamine in toxic samples of tuna fish. In addition to *P. morganii,* Ienistea (*36*) has mention that *Achromobacter histaminum* and *Escherichia freundii* have been isolated from fish containing large amounts of histamine.

Histamine is thought to be one of the main toxicants in scombroid poisoning. Scombroid poisoning is so named because the fish implicated in cases of poisoning, tuna and skipjack, belong to the suborder *Scombroidei.* These fish have a higher concentration of basic amino acids and imidiazole derivatives than that found in the normal musculature of slaughter animals and other fish (*25*). This may be an important factor in the formation of higher levels of histamine in their flesh. Since histidine decarboxylase is an inducible enzyme, the higher levels of histidine will favor its induction. Also, increased levels of free histidine will favor rapid formation of histamine (*24, 25*). A limiting factor in the formation of histamine in fish muscle is the release of histidine from muscle proteins. Ferencik (*25*) stated that autolytic proteases are much more important in this respect than the proteolytic enzymes of the contaminant microflora.

CATABOLISM OF TYRAMINE AND HISTAMINE IN FOOD

Destruction of histamine or tyramine by bacteria affects the amount of these amines in food (*36, 37*). Compared to data on formation of histamine and tyramine, little information is available on the catabolism of amines by bacteria. In man, tyramine may undergo one of several different catabolic reactions (Fig. 2). Oxidation by monoamine oxidase is one of the more important pathways. Histamine also undergoes several different catabolic reactions of which deamination by diamine oxidase is a significant pathway (Fig. 1). Many bacteria have amine oxidase activity (*38*). Yamada et al. (*65*) and Kumagai et al. (*40*) crystallized and described the properties of a tyramine oxidase from *Sarcina lutea.*

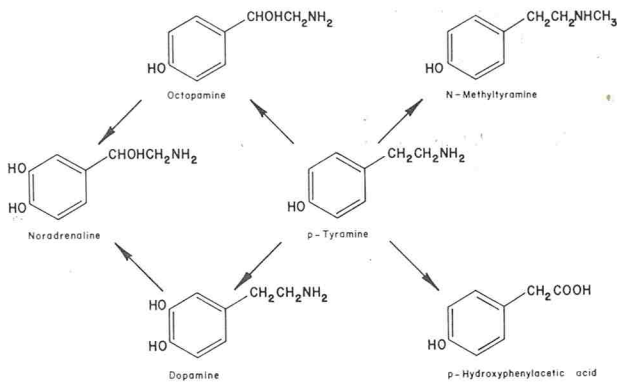

Figure 2. *Tyramine catabolic routes.*

This enzyme oxidatively deaminates tyramine in the presence of oxygen and form p-hydroxyphenyl-acetaldehyde, ammonia, and hydrogen peroxide. *Aspergillus niger* and *Trichosporon* sp. possess amine oxidases that oxidize a wide range of primary amines (*42*). Numerous bacteria possess diamine oxidase and are able to degrade histamine. Some of these bacteria include *Pseudomonas aeruginosa, E. coli, Proteus vulgaris, Serratia flava,* and *Clostridium feseri* (*36*). In the intestines of man, bacteria, in particular *E. coli* and *E. aerogenes,* are capable of acetylating histamine, thus rendering it inactive (*36, 60*). It is possible that these bacteria, when present in foods through contamination, or in the normal microflora, may carry out this reaction. Since several types of bacteria have the capability of degrading tyramine and histamine, it appears likely that the catabolism of amines by bacteria may play an important role in the final concentration of these amines in food.

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Detection of Salmonellae in Foods—Past, Present, and Future: Activities and Attitudes of the Food Industry[1]

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ABSTRACT

The increased emphasis to reduce salmonellae contamination of foods since 1967 has had tremendous impact upon the food industry. While there has been improvement made in the incidence of food product contamination, it is debatable whether this has led to a reduction in the incidence of human foodborne salmonellosis. Industrial microbiologists recognize and support the need for controlling salmonellae in foods. Certain changes in educational materials are needed to provide the positive attitude that salmonellae can be controlled.

An information leaflet (*6*) released in 1972 by the U.S. Department of Health, Education and Welfare states in the first paragraph: "Contamination of food by *Salmonella* poses a significant public health problem. There are more than 1,400 known serotypes of this organism, all capable of causing infection in man. These bacteria are found throughout the environment. Outbreaks of salmonellosis—or *Salmonella* infection—have been reported in increasing numbers in recent years." This perpetuates the philosophy promoted for the past 10 years by several federal and state agencies and some individuals in industry and is overdue for a change. This concept has been a hindrance to correcting the problem with which we are all concerned.

The fact that there are over 1,400 known serotypes of salmonella is of academic but not practical interest. During the 5 years from 1968 through 1972, 10 serotypes accounted for 66.1 to 72.2% of the salmonellae isolated from humans. The annual total number of different serotypes isolated from humans ranged from 154 to 189 during that time. From a practical standpoint, the problem of human salmonellosis is limited to a mere handful of serotypes out of the more than 1,400 serotypes and variants now known to exist.

ARE SALMONELLAE REALLY UBIQUITOUS?

The second point in the paragraph is that "these bacteria are found throughout the environment." This same impression was conveyed in an article in the first issue of *FDA Papers* under the title: "Salmonella—the ubiquitous bug" (*5*). The FDA Task Force Report (*3*) released last year also states that, "salmonellae exist in the environment of man in profuse numbers and may be isolated with relative ease from water, food, air, sewerage, milk and other environmental sources." To read through the lists of food product recalls during the past 10 years one might conclude that the salmonellae are indeed ubiquitous. The truth, as I have seen it develop through food plant investigations, is that this is a false impression. Others closely associated with industry have had similar experiences. A more accurate assessment of the problem of salmonellae contamination in most food plants is that one, and possibly several, serotypes can become part of the indigenous microflora of the food processing environment. The bacterium gains entrance to the food plant environment by some means, establishes itself, and multiplies as part of the normal flora. Once these sites are detected and corrections are made, it is possible to eliminate the organism. In some instances this might more correctly be stated as bringing the population of salmonellae under control to the point where it is not detectable. It is possible for the same serotype(s) to recur to detectable levels some months or a year later as a result of relaxed sanitation or some change in processing. The point to be made is that salmonellae need not be ubiquitous in a food processing environment. There is a reason for the presence of the salmonellae and the bacteria can usually be brought under control.

To continually reinforce the impression that (*a*) there are more than 1,100, 1,400, 1,700 or whatever the number will be tomorrow, and (*b*) salmonellae are ubiquitous has caused confusion and created a sense of hopelessness in the minds of food industry management. Many individuals in the food industry are of the belief that salmonellae are indeed everywhere: in the air, dust, soil, water, on shoes, in birds, people, etc; and, therefore, the task is hopeless. In some instances this attitude has led to needless closing of plants and lost jobs. This false impression must be corrected if we are to succeed in making the improvements we all seek. A positive attitude by food industry management is needed, and it is essential that educational materials begin to take this into consideration.

ARE THERE MORE OUTBREAKS OF SALMONELLOSIS?

The third point in the paragraph of the leaflet is that

[1]*Presented at the 74th Annual Meeting of the American Society for Microbiology, Chicago, Illinois, May 12-17, 1974.*

outbreaks of salmonellosis have been reported in increasing numbers in recent years. In April 1962 the national *Salmonella* surveillance reporting program was instituted. Figure 1 shows data on the reported incidence of human salmonellosis in the U.S. for the years 1942-1964 (*1*). The graph for salmonellosis other than typhoid fever is alarming. Although it was frankly admitted that this increase in non-typhoid salmonellosis might have been due to increased reporting, the impression conveyed 10 years ago to industry and others was that the problem was becoming worse and had to be brought under control. A major effort was undertaken by the FDA and some state agencies to detect and eliminate salmonellae contaminated foods. This was a traumatic experience for all: industry, government and the consumer.

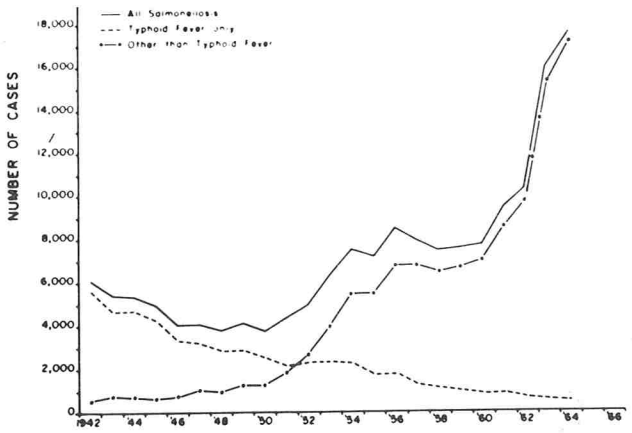

Figure 1. *Reported human isolations of salmonellae-United States, 1942-1964.* (———) *All Salmonellosis;* (----) *Typhoid fever only;* (·—·) *Other than typhoid fever.*

The 1972 annual Salmonella Surveillance Report summarized the salmonellae isolations for the years 1963-1972 as shown in Fig. 2 (*2*). This covered the period since the inception of the national reporting program. Using a logarithmic scale, the figure conveys the impression that the number of human isolations has generally leveled off. The total number of human isolations ranged from 18,649 to 26,110 from 1963 through 1972. Has it really leveled off? If the number of isolations shown in Fig. 2 were plotted on the graph used for Figure 1, it is obvious that the number of isolations did not level off (Fig. 3).

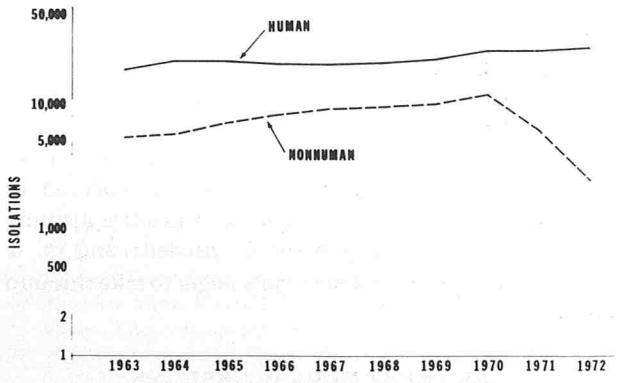

Figure 2. *Reported human and nonhuman isolations of salmonellae-United States, 1963-1972.*

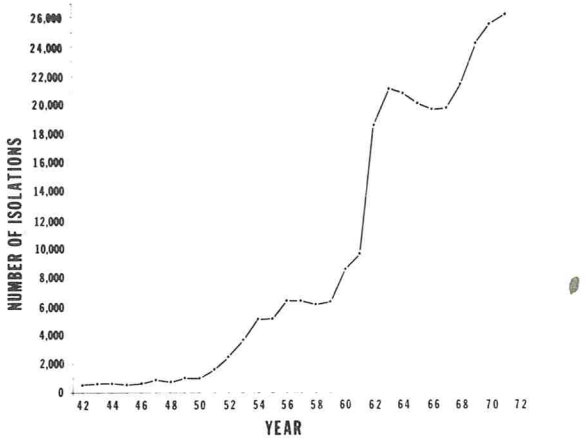

Figure 3. *Reported incidence of human salmonellosis-United States, 1942-1972.*

The problem of salmonellae in foods was reassessed by the USDA and FDA in separate task force reports issued on August 14, 1973 (*3, 4*). The FDA Task Force reported several significant findings. Among them were:

(*a*) "FDA efforts during the past 5 years have demonstrated an effectiveness in controlling the overall incidence of food-borne salmonellosis caused by foods other than meat." This statement was based upon the data in Table 1. The Task Force Report further stated,

TABLE 1. *Incidence of outbreaks of human salmonellosis attributable to meat and meat products as opposed to other foods*[a]

Product	1963-66		1967-71	
	Number	Percent	Number	Percent
Meat and meat products	27	63	53	75
Other foods	16	37	18	25
Total	43		71	

[a]Source: FDA *Salmonella* Task Force Report (3).

"Since the inception of intensified FDA efforts in 1967, the incidence of foodborne salmonellosis attributable to foods other than meat has dropped to 25% of the reported outbreaks, while the estimated total number of human isolations has remained relatively constant." With certain assumptions "this trend suggests a 25-35% reduction in the incidence of salmonellosis caused by foods except meat since the base years." Is such a conclusion valid? Another interpretation of the data in Table 1 is that the number of reported outbreaks involving meat and poultry products fluctuated upward during the 1967-1971 period. The number of outbreaks

involving meat and poultry products ranged from 3-19 per year during this period. In the meantime, the number of outbreaks attributed to other foods remained steady and certainly did not decline.

(*b*) "Concerted FDA efforts to eliminate known *Salmonella* problems in particular food commodities have been successful." In FY 1967, FDA concentrated almost half of its sample analysis efforts on five product categories (i.e. eggs, dry milk, candy, drug substances of animal origin including gelatin, and miscellaneous foods such as starches and sugars). "In FY 1967, 8.6% of these samples were positive, but in FY 1972 only 0.8% of the samples in the same category were positive" (Table 2).

TABLE 2. *Results of samples of food and feed products analyzed by FDA between the years FY 1967 and FY 1972*[a]

Categories	FY 1967		FY 1972	
	Number Samples Examined	No. (%) Positive	Number Samples Examined	No. (%) Positive
Top 5 product categories	2849	244 (8.6%)	368	3 (0.8%)
All other categories except animal by-products	3239	254 (4.0%)	1337	70 (5.2%)

[a]Source: FDA *Salmonella* Task Force Report (*3*).

These five categories that received intensive review by FDA "no longer constitute a significant health threat to the consumer." Relative to the data in Table 3, the report

TABLE 3. *Summary results of* Salmonella *examinations conducted by FDA*[a]

FY	No. samples, human food	No. positive (%)
1967	5360	303 (5.7%)
1968	4406	244 (5.5%)
1969	3702	196 (5.3%)
1970	3664	220 (6.0%)
1971	3282	225 (6.9%)
1972	1499	59 (3.9%)
1973, 1st Qtr.	299	19 (6.4%)

[a]Source: FDA *Salmonella* Task Force Report (*3*).

states the "low incidence of contamination appears constant for other processed human foods which, in some part, is due to continued surveillance by FDA but probably is due more so to heightened industry awareness an care resulting from FDA actions. This low level of continuous contamination of miscellaneous processed foods appears not to threaten the public health."

IMPACT ON INDUSTRY

The crash program approach initiated by FDA in the middle 1960's to eliminate salmonellae from the nation's food supply has had tremendous impact upon industry. Industry will not easily forget the experience. Although the data in Table 1 showing the improvement claimed in the FDA Task Force Report are not convincing, the incidence of salmonellae contaminated foods undoubtedly has been reduced. However, the data which are available at this time fail to show that the pattern and extent of human food-borne salmonellosis has changed. It does appear that FDA is viewing and interpreting surveillance data through different eyes or with a different attitude.

The chief executives of several corporations have established corporate policies that their products will not be released for sale if contaminated with salmonellae. Such policies have had a strong effect on the actions of microbiologists working for these corporations. Also, there has been increased research and a strengthening of quality assurance programs.

Food industry microbiologists do recognize the public health significance of salmonellae contaminated food. They support the need to monitor their products and conduct research as it applies to their products. It is important to them that they be satisfied that their products are safe and of high quality. Their livelihood depends upon product acceptance in the market and they know more than most the effect which a product recall would have upon their companies and their jobs. It is for this reason that industrial microbiologists are concerned with the direction of future regulatory policies. Programs should be developed and sustained at the corporate, university, and government levels which will continually and progressively lead toward improved salmonellae control and, hopefully, a reduced incidence of foodborne salmonellae outbreaks. Meanwhile, it is urged that the effort be conducted in a less tumultuous atmosphere. Let not industry, government, and the public be subjected to the trauma of another crash program in the future.

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Procedures for Surveillance of Bulk Milk Sampling

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ABSTRACT

Surveillance of methods for collection of raw milk samples and their transmittal to laboratories is essential to the interpretation of laboratory results for official grading purposes. At least 15,000 farm bulk tank truck drivers collect milk samples each day. The accuracy of thermometers and sanitization and/or sterilization of sample transfer instruments, agitators, and containers is important. Agitation of milk, collection of temperature controls and samples, and storage in insulated sample cases, containing a suitable refrigerant during transmittal to the laboratory, are necessary critical operations in collection of representative samples. Accurate records indicating time, date, and temperature at collection must accompany each series of samples. Samples must arrive at the laboratory between 32-40 F (0-4.4 C) and examinations should begin within 36 h after collection. Surveillance of sample collectors is done at least biennially by Food and Drug Administration (FDA) approved State milk sampling evaluation officers who have met FDA criteria for certification.

Sample collection is a fundamental step in the process of evaluating the milk producer's product to determine its compliance with legal bacteriological and chemical standards and for butterfat content, as a basis for payment. The importance of proper procedures used in collecting, handling, and transporting samples from farm to laboratory cannot be overemphasized. It is imperative that each sample be representative of the volume from which it is collected, and that it arrives at the laboratory in the same condition it left the bulk tank, with no change in its bacteriological, chemical, or physical condition.

Following World War II, the dairy industry underwent a major change in the method of producing, handling, and transporting raw milk from farms to milk plants. Development of farm bulk milk cooling/holding tanks and the tank-truck pickup system brought about an entirely new approach in administration and operation of milk sanitation/quality control programs not only for regulatory agencies but also for industry.

To serve the needs for public health and consumer acceptance and yet meet demands of present day economics is a challenge faced today by every regulatory agency engaged in milk control. Consideration must, therefore, be given to the most efficient and economical means available for collecting raw milk samples. Because raw milk is produced and transported into the community from ever-expanding production areas, collection of milk samples from the farm can be the most expensive, time-consuming activity in milk control work. Before the advent of farm bulk milk cooling/holding tanks, the sanitarian or technician from the laboratory collected samples at the milk plant or receiving station. Today, the bulk-tank milk hauler has become the key figure in the producer-processor-milk regulatory agency relationship. His is a three-fold, overlapping job. The milk hauler not only represents the producer and the processor, but also the milk regulatory agency.

As the receiving agent, weigher, and sampler, the hauler becomes the official, and often the only judge of the quality and volume of milk received. When his obligations include collection and delivery of samples to the laboratory for analysis, he becomes a vital part of the regulatory and quality control programs. The hauler's operating habits directly affect the quality and safety of the milk committed to his care, and any deviation committed by him may result in the producer being debited on official inspections or sanitation ratings. Contaminated samples with high bacteria counts may result in the producer losing his market. A producer may suffer the loss of payment if his milk sample tests show abnormal milkfat content which is caused by a deviation in sample collection practices.

Recognizing tentative problems resulting from this transition in methods of producing and transporting raw milk, a few State and municipal milk sanitation agencies took definite steps to assure that raw milk samples were properly collected, especially when bulk tank truck operators were given the responsibility of collecting such samples. Driver training programs were established in which the truck operators' responsibilities for truck maintenance (cleaning and sanitizing), grading of the product, weights and measures, as well as the technique of sample collection, were discussed. Successful completion of training programs and subsequent examinations determined whether licenses or permits would be granted to the individual drivers.

In 1963, the National Conference on Interstate Milk Shipments (NCIMS) recognizing that many states had neglected to include the inspection and supervision of sample collection procedures used by bulk tank truck

operators in their milk sanitation programs, wrote into the NCIMS agreements that State Laboratory Survey Officers would include evaluations of sampling procedures in laboratory surveys. In 1971, to provide a more thorough approach in this area of milk sanitation, agreements were modified to include the State Milk Sanitation Rating Officer in the sampling evaluation program (*6*).

The Laboratory Development Section (LDS), Food and Drug Administration, was given the responsibility for supervising State sampling surveillance programs. A nationwide program has now been established whereby individuals who have been designated by the State as the Sampling Surveillance Officer(s) are standardized and certified in the procedures of sample collection by personnel from the LDS. This program is being carried out in accordance with the procedures that govern the voluntary Cooperative State-Public Health Service Program for the Certification of Interstate Milk Shippers (*6*). Accordingly, sampling procedures must conform to the specifications outlined in Chapter 3, "Sampling Dairy Products," of the 13th Edition of *Standard Methods for the Examination of Dairy Products* (SMEDP) (*2*) and the requirements of the *Grade A Pasteurized Milk Ordinance—1965 Recommendations of the U.S. Public Health Service* (*4*).

Sampling evaluations conducted by the FDA support the concern of NCIMS that sample collection techniques in many instances are faulty and that many deviations in sampling practices are committed. There are over 250,000 Grade-A raw milk producers involved in the Interstate Milk Shipper (IMS) Program who are graded on the basis of bacteriological counts or receive payment on the basis of laboratory milkfat tests. The dollar value of milk rejected on the basis of microbiological examinations or loss in milkfat payment due to improperly collected samples for both the producer and processor can only be a matter for speculation.

On the Grade A side of the industry there are at least 15,000 unskilled or semi-skilled bulk tank truck operators who collect milk samples from the dairy farms for official bacteriological, or chemical analysis, or milkfat determinations in the laboratory. It is therefore essential that sanitary and microbiological principles are applied in collection of milk samples that will eliminate any methods or conditions that might contaminate the samples.

RECOMMENDED PROGRAM FOR BULK MILK HAULERS

The unique position of the farm bulk milk hauler makes him a critical factor in the enforcement-industry relationships. Because of this fact, it is strongly recommended that the following criteria be considered by the enforcement agencies when implementing a farm bulk milk haulers program. These criteria should be a prerequisite in the acceptance of industry-collected samples by official agencies and should include at least the following.

Training

The bulk milk hauler must be trained to understand the importance of bulk milk collection and the techniques of sampling. The new milk hauler as well as relief drivers, must be told why and instructed how in the proper procedures of picking up milk and collection of samples. This initial training is industry's responsibility and can be accomplished under the supervision of the dairy plant fieldman, route supervisors, or a milk hauler whose training and experience in techniques and practices qualify him for this responsibility. Training also includes classroom sessions in which the sanitarian describes pickup practices and demonstrates sampling procedures and care of samples. Basic considerations of sanitation and personal cleanliness, which are important to the protection of milk quality, are discussed. Officials administering weights and measures frequently participate in these programs and provide instruction in the measuring of milk and the keeping of required records. Extension Dairy Specialists provide expertise in the grading of milk by holding organoleptic and taste panels. An examination is usually administered at the conclusion of this program; prospective bulk milk haulers who fail the test are denied permits until deficiences are corrected.

Regularly scheduled (12-18 months) refresher short courses for farm bulk milk haulers conducted by sanitarians and officials administering weights and measures assist in increasing and maintaining the efficiency of the hauler.

Qualifications

The bulk milk hauler's qualifications should include experience, which may constitute a required period of observation apprenticeship in which the prospective hauler accompanies a permittee in the performance of his duties.

Application for licenses or permits should be supported by suitable references testifying to the character and integrity of the candidate, plus the satisfactory completion of the training sessions that may include a written examination.

Routine inspection of haulers and operations

Routine inspections are required. This provides the regulatory agency with an opportunity to check both the condition of the hauler's equipment and the degree to which he is observing required pickup and sampling practices.

The sanitarian's inspection will provide useful information on the overall attitude of the farm bulk milk hauler to his work. The sanitarian should see the inherent danger of carelessness on the job and should require its correction. Frequent visits by the sanitarian are recommended in such instances. Periodic evaluations of sampling procedures shall be conducted by the State Milk Sanitation Rating Agency or State Laboratory

Survey Agency. Specific items included in judging hauler performance are:

(*a*) *Personal appearance.* The hauler should be neat, should wear clean outer garments and head covering, and should have clean hands.

(*b*) *Care of equipment.* Milk hose, outlet valves, etc., should be protected from contamination until used. Milk hoses should remain capped except when connected to bulk cooling/holding tanks, and should be rinsed free of accumulation of milk when the delivery hose is attached. Routine cleaning and sanitizing of the bulk tank outlet is the responsibility of the producer; however, when there is leakage of milk, the hauler should clean and sanitize the bulk tank outlet valve before attaching the delivery hose.

(*c*) *Protection of the milk.* Tank lids should be kept closed during the emptying time. Ports should be used for sampling and measuring, and for checking the odor and appearance of the milk. The delivery hose should be disconnected and capped before rinsing the bulk cooling/holding tank after it is emptied. The manhole cover of the bulk transport tank should not be opened at any time during pickup or transport of milk to the milk plant, receiving station, or transfer station.

(*d*) *Protection of the samples.* Sampling and the care of samples shall be in substantial compliance with *Standard Methods for the Examination of Dairy Products,* the details of which are discussed below.

Universal sampling system

It is recommended that when bulk milk haulers collect raw milk samples, the "universal sampling system" should be employed whereby milk samples are collected every time milk is picked up at the farm. This system permits the enforcement agency laboratory, at its discretion, at any given time, and without notification to industry, to select and analyze samples collected by the hauler. The use of the "universal sample" enables the regulatory agencies to have more faith in the validity of samples collected by industry personnel.

PROCEDURES FOR BULK MILK SAMPLE COLLECTION

The implications of poor sampling techniques are obvious. Laboratory results for bacterial counts, milkfat tests, or chemical determinations will not be valid if sample collection procedures are faulty. As we have already indicated, inaccurate bacterial counts could cause milk producers or processors to have their products eliminated from the market. Improperly collected samples for milkfat tests could cause the loss of monetary payment to the producer. From the public health standpoint, faulty sampling techniques will prevent the laboratory from determining the sanitary quality of the milk product tested.

Regardless of the type of sample collected and the type of laboratory analysis made, certain basic items of equipment and procedures are required.

Equipment

A test thermometer should be carried by every sample collector; it may be mercury-filled or dial type, with graduation intervals not exceeding 2 F. The accuracy of the thermometer is to be checked every 2 years with a thermometer certified by the National Bureau of Standards, or one of equivalent accuracy. When samples are collected from farm bulk tanks, storage or balance tanks, and cans or vats, it is suggested that a metal thermometer be used to avoid possible breakage. The accuracy of dial type thermometers should be checked every 3 months, or more often, if there is reason to question their accuracy.

Sample transfer instruments may be stainless steel dippers with long handles and a capacity of at least 10 ml or more, single-service plastic or paper sampling tubes, or other equivalent means. All sampling instruments must be protected from contamination before use. Dippers may be carried in sanitizing solution tubes and held in place and protected with rubber stoppers. Sterile sampling tubes and other sampling devices must be carried in cases with tight covers. Sampling cocks on large storage tanks may be used, provided they have been thoroughly cleaned and properly sanitized before the sample is drawn.

Sample containers may be either multi-use or single-service. Multi-use containers are sterile and should be large enough to hold a minimum of 15 ml with adequate space to permit thorough mixing. Sterile, evacuated sampling tubes may also be used to collect samples. Single-service containers may be presterilized polyethylene bags, or other suitable nontoxic, nonsterile plastic containers of adequate size. All nonsterile plastic containers and closures must comply with the same bacteriological standards as single-service containers for pasteurized products (*3, 5, 6*). There should be enough space on the container to allow for proper identification of sample contents.

Sample cases in which to transport samples to the laboratory should be rigid, either metal or plastic. The cover, sides, and bottom of the cases must be completely insulated, with adequate space for coolant (ice) and necessary racks or compartments to protect samples from possible contamination due to ice water.

Cases used to ship samples to a laboratory via common carrier must be provided with a locking or sealing device. Warning labels such as "This Side Up" must be attached to the top of the case.

Cleaning, sterilizing, and sanitizing of equipment

All multi-use sampling instruments must be thoroughly cleaned and rinsed before sterilizing or sanitizing. Immediately after use, all multi-use instruments must be rinsed with tap water and resanitized. Multi-use containers must be cleaned and sterilized before reuse. Methods of sterilization and sanitization are not discussed here but may be found in SMEDP (2).

Bulk milk sampling

To promote fast and efficient milk sample collection practices for farm bulk milk, the following step-by-step procedures are discussed in the sequence that they most frequently occur at the dairy farm. Our observations indicate that when a milk hauler follows these procedures in their prescribed order, he does a much better job sampling with less time spent at each pick-up:

(*a*) Upon arrival at the dairy farm, unload the milk transfer hose and electrical pump cord from the truck, slip the hose through the hose port, and connect it to the tank outlet valve. Rinse the outlet valve if there is any indication of milk leakage.

(*b*) After the hose is connected, wash and dry hands before measuring and sampling the milk. Use the hand washing facilities provided in the milkhouse; do not use the equipment wash vats.

(*c*) Examine the milk carefully for odor and appearance by lifting the sample port cover and smelling the milk for off-flavors. Next, lift the tank lid briefly to observe the surface of the milk for acceptability. Look for frozen, lumpy, curdled, or churned milk or any foreign matter. Close the tank lid and keep it closed throughout measuring, sampling, and emptying of the milk. Record all abnormalities and report them to the farmer, the plant manager, and the fieldman.

(*d*) Next, measure the milk. If the measuring stick is stored in the tank, first dry it with a single-service sanitary towel to insure an accurate reading. If the State requires heating the stick in hot water, use care to prevent contamination during handling and drying. If measuring sticks are stored outside of the bulk tank, clean, sanitize, and dry them as prescribed before insertion into the milk. If, upon arrival in the milkhouse, the agitator is running, continue the operation for the required agitation time (at least 5 min), then take the sample as described below. After collecting the sample, turn off the agitator to allow the milk to become quiescent, and then measure the milk.

(*e*) Usually, however, when the hauler arrives at the farm, milk will be quiescent and may be measured before sampling. Agitate the milk at least 5 min or according to the tank manufacturer's specification. Larger tanks (1,500 gal or more) will require at least 10-min agitation. If necessary, determine agitation time for individual tanks according to the criteria found in SMEDP (*1*). Insufficient agitation can be the major single cause of variations in tests conducted by a laboratory.

(*f*) During the agitation period (at least 5 min), determine the temperature of the milk. Sanitize the test thermometer, insert it into the milk, and then compare the temperature of the test thermometer with the tank thermometer. It is important that both the farmer and milk hauler know that the milk tank thermometer is accurate. Many tank thermometers do not work or are not accurate. When this fact is known, take the milk temperature with the test thermometer each time milk is picked up. Use the time while the milk is being agitated to fill out the weight slips, identify the sample container, and record the required information, i.e., time of collection, date, milk temperature, and any unusual conditions observed. All data collected must accompany the sample to the laboratory.

(*g*) Also, during the agitation period bring the sampling equipment into the milkhouse. Use care before and during sampling to protect the sampling instruments from unnecessary exposure and possible contamination. Transfer or carry dippers or sampling tubes into the milkhouse in their solution tubes or instrument cases.

(*h*) The sample containers must be handled aseptically. Avoid dropping the containers, laying them down on wet or soiled surfaces, or otherwise contaminating them. Do not carry presterilized plastic bags or other types of containers in clothing pockets.

(*i*) If multi-use, thick-walled glass containers are used, precool them before taking the sample. These containers retain atmospheric heat which will raise the temperature of the sample (generally only 2-4 oz) well above the required 32-40 F (0-4.4 C) storage temperature.

(*j*) If a dipper is used, remove the dipper from the sanitizing solution, drain out the sanitizer, and rinse the dipper at least two times in the milk to remove any residual sanitizer before transferring the sample. Keep the sampling dipper in sanitizing solution (100 ppm of chlorine) or other approved bactericidal solution until next use.

(*k*) To prevent any drippage of milk back into the tank, transfer the sample into the container over the sink or floor drain, but not over the tank.

(*l*) To permit proper mixing of the sample at the laboratory, fill the container no more than ¾ full. If a plastic bag is used, twirl it closed without expelling the air to allow sufficient space to mix the sample.

(*m*) Sample the producer's total milk supply. If there are two or more tanks at a stop, sample each tank.

(*n*) Collect two samples at the first sampling location; one sample is for temperature control. Identify the temperature control by "T.C.," "control," or some similar manner. Laboratories have been instructed to use the first sample collected as the temperature control when no control has been collected.

(*o*) As soon as the sample has been taken, place it in the sample case and maintain the temperature between 32-40 F (0-4.4 C). Make sure the cooling medium (ice, ice and water, gel ice) is maintained at the proper levels. Ice should not cover the tops of the containers. Do not bury the containers in the ice. Ice water should not be higher than the milk in the sample containers. Use racks or compartments to maintain the sample containers in a vertical position to assure maximum protection.

(*p*) After sample is taken and placed in the sample case, rinse the dipper in tap water and replace it in the sanitizing solution.

(*q*) Deliver samples promptly to the laboratory for testing within 36 h after collection. If the samples are to

be shipped to the laboratory via common carrier, use a tamper-proof shipping case.

LABORATORIES' RESPONSIBILITIES

As soon as the samples are submitted to the laboratory for analysis, determine the temperature of the samples. Use a precooled test thermometer. If a temperature control has not been submitted, use the first official sample collected as the temperature control. Make the required entries on the sample identification form, showing temperature, time, and date of receipt. Store samples at 32-40 F (0-4.4 C) until tested. When samples are tested, record the time and date of analysis as well as the temperature of the temperature control. This same information should also be recorded at each interim sample transfer point (plant or transfer station) from the time of sample collection until receipt at the testing laboratory.

CONCLUSION

The sample collector, the sanitarian, and/or the bulk tank truck hauler are all totally responsible for the sample collected from the moment the milk sample is taken until it is in the hands of the analyst at the laboratory. The sample collector must handle the sample so as not to alter it and to insure that there will be no change in the product except to maintain the sample at the desired storage temperature. The test of any milk sample is only as accurate as the sample delivered to the laboratory. This fact makes the collection, handling, and transportation of samples the most important step in the milk sanitation and laboratory control program. Following the foregoing standard sampling procedures can eliminate contaminated samples and can provide the milk producer or processor and enforcement agencies with valid laboratory results.

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The National Sanitation Training Program: Canadian Restaurant Association's Answer to Safe and Sanitary Foodservice

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ABSTRACT

There are an estimated 120,000 cases of bacterial food poisoning occuring in the Canadian foodservice industry each year. The Canadian Restaurant Association in co-operation with all Provincial departments of health and Health and Welfare Canada, developed a sanitation code to help the foodservice operator understand every essential sanitation requirement. In conjunction with the code, a national sanitation training program was developed to assist in the total education in foodservice sanitation of all personnel in the Canadian foodservice industry. To date, 3,200 personnel have successfully completed one of the national sanitation training programs and gained a more responsible level of foodservice sanitation awareness. The program as well as being accepted by all Provincial departments of health and Health and Welfare Canada, has officially been endorsed by the Canadian Public Health Association and the Consumers Association of Canada.

Today, the Canadian foodservice industry is enjoying unprecedented growth. There are more dining-out facilities in every town and city throughout Canada. Due to public acceptance, the fast food section of our industry is enjoying a phenomenal increase in its share of the away from home, food dollar. Canadian restaurant receipts for public sit-down restaurants in 1973 totalled $1.5 billion, an increase of 12.6% over 1972. Sales in 1974 exceeded $1.75 billion. Total receipts for the foodservice section of the hospitality industry were $3.1 billion and estimates for 1975 indicate total receipts will be $4 billion.

The hospitality industry currently employs more than 350,000 persons and this represents 4% of the total number of people employed in Canada. A total of 25-30,000 new employees are required in the foodservice industry each year. Canadians now eat one out of every three meals away from home, by 1980 this figure is expected to rise to one in every two.

As a businessman, you may say that the Canadian foodservice industry is in a very health position. Generally speaking it is, but we do have a serious problem in the industry.

A PROBLEM OF SANITATION

The Health Protection Branch of Health and Welfare Canada advises us that an estimated 120,000 cases of bacterial food poisoning occur in commercial foodservice operations annually. This represents 30% of the estimated 400,000 cases occuring annually. For your information 50% of all cases occur in the home, 10% in non-commercial operations, and 10% have unknown origins. When we apply the total number of estimated food poisoning cases against the Canadian population, we realize that 1 person in 55 will be an unfortunate recipient of food poisoning.

During the past year, the Consumers Association of Canada has analyzed fast food outlets products. Hamburgers and milk shakes were sampled and their bacteriological content scrutinized. Unfortunately the results haven't been very encouraging. Both total and fecal coliforms were found in some of the samples.

Last year, Medical Officers of Health, particularly in Ontario were regularly informing the news media of poor sanitation practices in restaurants. The subsequent press coverage culminated in journalistic sensationalism with headlines such as "filthy restaurants face crackdown," "standard of cleanliness in local restaurants shocking."

Not all foodservice operations in Canada are kept in unsanitary conditions. On the contrary, most operations or retail outlets where food is sold, are conscious of their role in providing safe, sanitary foodservice to the dining-out public. Members of the Canadian Restaurant Association must abide by a Code of Ethics that maintains the concept that, it is the operators *duty* to maintain a high standard of sanitation and service in the selection and serving of food to guests.

However, in the foodservice industry today there seems to be a void between the operators cognizance of sanitation and the actual grass roots level of application. This void is primarily due to the lack of education and understanding of what actually constitutes safe and sanitary foodservice.

Although we as professional Public Health people realize that foodservice sanitation is a pretty straightforward matter, most industry people consider the subject to be a complete mystery. Ask any waitress why milk turns sour and her reply may be "because it is old" or it "wasn't kept in the refrigerator." If only someone could tie together the two variables of time and temperature with the elusive germ, our waitress would understand the first fundamental principle of foodservice sanitation.

SANITATION CODE

To overcome this problem, a little more than 10 years

ago, the Canadian Restaurant Association decided that an effective way to achieve higher sanitary standards in the industry was through introduction of a Sanitation Code which should: (*a*) have outline and complete explanation of all foodservice requirements, (*b*) be written in easily understood language with a simple reference, (*c*) be in compliance with existing Public Health Regulations, and (*d*) be available to anyone in the Canadian Foodservice Industry and Public Health Departments.

In essence, such a Code would eliminate the mystery of foodservice sanitation for the concerned operator. For almost a decade, work proceeded quietly on a proposed Sanitation Code. For the most part, it was a behind-the-scenes project by the Canadian Restaurant Association, which at times appeared to be "an impossible task." The original Code was found to be highly technical. The second draft was less so but tended to be voluminous.

After drafts 3 and 4 were reviewed by Public Health Officials in each province and the Federal Government, the final draft became a reality and was presented to the foodservice industry on April 28, 1974. To date, 40,000 copies of this Code are in circulation throughout Canada and other countries.

The prime objective in formulation of the Sanitation Code was to focus attention on the need for training within the Canadian Foodservice Industry. Since the Canadian Restaurant Association acts as one of the voices of the industry, it was felt that the Association should undertake a leading role in the introduction of the Code and implementation of a Training Program.

TRAINING PROGRAM

An Advisory Council of Senior Public Health Officials from all the provinces and the Federal Government, together with a Training Program Committee comprising Industry and Government people, worked diligently towards a National Sanitation Training Program. It was decided from the onset that the program would be: (*a*) national in scope, thereby ensuring a uniform standard of foodservice sanitation knowledge to the entire Canadian Foodservice Industry; (*b*) provincial in its working environs so that the grass root level of application could be achieved; (*c*) taught only by Public Health Inspectors who we felt were the best qualified instructors; and (*d*) highly visual in the sense that modern 16 mm color films, cassette projectors, and slides would be used intermittently throughout the program.

After evaluating all the different types of foodservice sanitation programs offered in Canada and the United States, and taking into consideration the contents of the Sanitation Code, it was agreed that the program should be divided into several courses. In this way the management level would be separated from and require more detailed instruction than the other foodservice personnel.

Course No. 1—was designed for owners, managers and supervisors. It covers the entire Sanitation Code and lasts 12 h or 2 course days.

Course No. 2—is for the front of the house people such as waiters, waitresses, bartenders, and service personnel. This course takes 6 h or 1 course day to complete.

Course No. 3—is for chefs, cooks, dishwashers, and preparation staff. Like Course No. 2, the duration of this course is 6 h.

Course No. 4—Fundamentals of foodservice sanitation is a quick introduction to the topic of safe and sanitary foodservice.

If we may for a moment, let us look in detail at the content of the first lesson of the four that comprise Course No. 1. Participants are first introduced to a film, "Sanitation, Why All The Fuss." This film gives an overview of the subject matter. Interpretation of the terms familiar to the foodservice industry are next explained. We want all participants to have the same understanding of these words. As an example, the Code refers in several places to food contact surfaces. Each of us may think of this as meaning some particular surface such as a butcher's block or a sandwich counter. However, the term means any and every surface, a knife blade, the inside of a pan, the top of a work table, a plate or any surface where food may make contact or touch at some stage of its journey from the receiving area to the customers plate.

Next, the principles of microbiology are explained. The primary objective is to let the participants know what bacteria are, how they grow, and where they come from. The transmission of infectious diseases relates microbiology to foodservice employees and to their hygiene and state of health. Here it is advantageous to carry out the black light experiment, using a fluorescent dye on the participants' pencils. In this way, without embarrassing anyone, participants can readily see that their hands touch many parts of their bodies, when the dye is illuminated by the black light.

A film cassette is then viewed and this discusses the microorganisms *Staphylococcus, Clostridium perfringens, Salmonella, Streptococcus* and their associated transmission. Petri plates with organisms are also viewed. Personal hygiene and food handling practices are dealt with next. After another film cassette on this topic, an examination of 50 questions pertaining to subjects taught is given. This first lesson takes approximately 3 h to teach including general discussion. Lessons 2, 3, and 4 follow the same format of showing audio-visual aids in conjunction with lecture material and culminating with examinations.

These courses are being carried out with the active co-operation of the Provincial Departments of Health, their Public Health Inspectors acting as instructors. In certain instances, industrial representatives and senior Canadian Restaurant Association members are invited to speak at the programs. All of the instructors use an *Instructor's Manual* prepared by the Association. It includes a text of each course, the audio-visual aids, and

questionnaires. This manual helps the instructors immeasurably but more than that, it ensures continuity of material from coast to coast.

At the Canadian Restaurant Association (CRA) Region level, Public Health Committees have been formed to act in liaison with the Public Health Department. Together they schedule the times and places where one or all of these four courses will take place.

To bring you up to date, 3,510 participants have registered for the program. The courses have taken place in British Columbia, Alberta, Saskatchewan, New Brunswick, Nova Scotia and Newfoundland. Manitoba, Ontario, and Prince Edward Island will commence in September, October, and November (1975), respectively. Numerous private CRA member companies such as Canadian National, Cara Operations, Canadian Institute for the Blind, and General Foods have also inquired about obtaining one of the courses for in-house training for their management and staff.

Attendance at these courses vary from 218 owners, managers and supervisors in Calgary to 11 in Estevan, Saskatchewan. We have a good turnout from the ethnic groups in our industry. As an example, 38 Chinese operators attended the St. John's, Newfoundland program along with 118 other owners, managers, and supervisors from that city. All types of foodservice operations are represented including church groups, hospitals, jails, fast food, and restaurants.

Why do we have such a successful attendance rate of over 60 persons per session? Why is it that the Mama and Papa operations attend alongside the multi-unit foodservice outlets? Primarily, the success of the National Sanitation Training Program rests in the co-operative spirit with which a trade association works with government. This combination cannot be beaten. An example of this cooperative effort is the advertising of the programs.

Three weeks before a program, the Regional CRA people issue a press release outlining the merits of the National Sanitation Training Program. Two weeks before the program, CRA's Regional President sends a letter asking for participation from all the foodservice operations in the selected area. A brochure, "Information On The National Sanitation Training Program," is enclosed. One week before the program, the Provinces Public Health Department issues a letter from the Minister of Health asking that the foodservice operator attend. This letter is hand delivered to the operator by the local Public Health Inspector. Another press release is issued by the Canadian Restaurant Association 2 days before the program. At this time the use of the other forms of media, such as television and radio are encouraged. As you can well imagine, by using this format we gain a good attendance.

PROBLEMS

I have described the tremendous success of this National Sanitation Training Program. However, with success there are *problems*. The first is the popularity of the program. Everyone seems to want the program at the same time. To compound the problem, Public Health Inspectors can only allot so much of their working days to education. During the summer months it is impossible for them to conduct these courses due to sewage disposal installations and building lot inspections. Likewise the months of July, August, and December are out, for they are the busiest months for the industry.

The second major problem is one of finances. Last April, our Association launched a fund raising campaign to raise $200,000 for the next 3 years, so that the program could be carried out successfully. Since government was supplying the manpower for these programs and foodservice operators were paying their management and staff to attend, it was felt that a leading role in financing could be expected from the suppliers to our industry. While they wholeheartedly support the objectives of the National Sanitation Training Program, they feel their financial support is either very limited or not warranted. Their premise is that those who receive the program should pay for it. Fortunately CRA's Advisory Council and National Executive Committee are looking at alternate ways of financing this program.

THE FUTURE

Turning now to future objectives. Given that the aforementioned problems can be resolved, the Canadian Restaurant Association hopes to graduate 30,000 owners, managers, and supervisors from the program in the next 3 years. With active government participation, this can be achieved. In conjunction with this, it is an Association policy to work towards some form of certification in the industry. By certification we mean that a person entering our industry must have a solid background in subjects such as foodservice sanitation, accounting, personnel procedures, and merchandising. These courses would have to be successfully completed so that a person would be qualified to obtain a license to either operate, or supervise a foodservice operation.

CONCLUSION

In conclusion, the Canadian dining-out public has a right to expect safe and sanitary foodservice. It is the responsibility of both government and industry to provide the mechanisms whereby people are educated to a satisfactory level of competence. The Canadian Restaurant Association believes that through the total education of every foodservice person in the industry, the 120,000 cases of bacterial foodpoisoning in commercial operations will be limited, if not entirely eradicated.

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Organization of Environmental Programs at the State Level

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ABSTRACT

There are no standard "models" to be followed, but there are some basic organizational principles to be considered when organizing environmental agencies at the state or local level. These include (*a*) organizational visibility, (*b*) programming on a multiple goal basis, (*c*) freedom of interagency communication and coordination, (*d*) operating with a mission of public service and consumer protection, (*e*) responsiveness to public sentiment, (*f*) ease of regulatory actions, (*g*) comprehensive programming, (*h*) legislation designed for rapid, equitable results instead of procedural delays, (*i*) line item budgets for the environmental agency, (*j*) programmed for environmental protection rather than environmental utilization and development, and (*k*) regulations and standards promulgated by a board or commission representing balanced public interests. The foregoing principles may be attained in a variety of organizational arrangements ranging from an appropriate environmental agency within a health department to a separate, free-standing environmental agency or department. However, adherence to the foregoing principles is necessary if there is to be an effective environmental protection effort.

It is understandable that everyone has a different opinion about organization of environmental programs in this decade of the environment. The issue of organization of environmental programs is viewed from significantly differing perspectives depending on whether one is an employee involved, one who is regulated by these programs, one who is a citizen activist, or whether one is a political leader attempting to balance the interests of the various parties involved. Because of these differing perceptions, a student of the organization of environmental programs quickly comes to the conclusion that there is definitely no standard model to be followed and it is indeed doubtful if any two state organizations resemble each other very closely. Many of us usually remember the "good old days" when this was not true and it was generally assumed that most environmental health programs were organized within the framework of the traditional state health departments. But with emphasis on consumer protection, comprehensive environmental management, organizational visibility of environmental programs, importance of citizen imput and participation, and effective regulatory actions, the organizational picture has changed radically within the past 5 to 10 years.

Additionally, the organizational arrangements and interrelationships are further confused by the differing definitions and vocabulary used concerning environmental programs. Some states have chosen to reorganize and place emphasis on something called "pollution control" which terminology can, by definition, be used to cover almost any environmental program desired. However, such states have usually confined such "pollution control" programs to those involved in the air-water-wastes syndrome. Others have retained the terminology "environmental sanitation" or "environmental health" and typically have consigned such programs to their state health departments. Still other states have used terminology such as "environmental protection," "environmental improvement," or even "environmental quality." The latter terminology generally being utilized in the most comprehensive sense to cover problems such as air, water, solid wastes, environmental chemicals, environmental injuries, noise, shelter, food, and radiation. And still other states have gone considerably further by combining not only environmental protection functions but also attempting to intertwine programs of environmental protection with programs of environmental utilization or development. The latter, while seeming to be the most comprehensive, are inherently dangerous in that the obvious mixture of goals poses one of the most dangerous conflict-of-interest situations to be found in government today.

My background has primarily been in management of environmental health and environmental protection programs. For this reason, it may be desirable to briefly set the stage by describing the nature of three different types of organizations with which I have been privileged to be associated.

FIRST EXAMPLE

The City of Albuquerque, New Mexico had a reasonably traditional approach to "public health" until the mid 50's. Locally, all environmental and personal health activities were under the jurisdiction of a medical health officer to the end that there was a fairly traditional textbook approach. Some of us began questioning this concept and organization and over a period of time prevailed upon the various local governing bodies and the state legislature to form a city Department of Environmental Health (it later became a city-county Department of Environmental Health) completely separate from the local public health functions that were

assigned to a Department of Preventive Medicine and Personal Health. In 1975, this concept is reasonably commonplace and certainly well accepted. However, in the mid 50's, implementation of this type of organization literally took place over the prostrate bodies of several medical health officers, the State Health director, and some other leaders in the medical community. At the time of this separation and the creation of the two distinct departments, the environmental health activities were constrained to rather perfunctory activities in the field of milk and food sanitation and meat inspection and involved a total of about 17 personnel. The environmental health programs had been stuck with the then time-honored formula of being allowed something like ⅓ the number of "sanitarians" as there were public health nureses.

Following a complete organization and budgetary separation with enhanced visibility, public information, planning, and programming, the Department of Environmental Health took on added functions involving water supply, water pollution control, air polution, radiation protection, occupational safety and health, insect and rodent control, pure food control, and housing conservation and rehabilitation. Later, the Department spawned the city programs of Model Cities, low-rent public housing, and Urban Renewal, and was also given the quite questionable "privilege" of administering the Refuse Division and Animal Control Division. Within a few years, the Department had grown from 17 to something like 460 personnel and a multi-million dollar budget.

SECOND EXAMPLE

In 1967, the Governor of New Mexico, by Executive Order, merged the New Mexico Department of Public Health and the New Mexico Department of Welfare into an umbrella Department termed the Health and Social Services Department. While this merger seemingly focused increased emphasis on problems of personal health and welfare, it also provided the opportunity to gain increased visibility, scope, and effectiveness for environmental health activities. I was privileged to be appointed director of the newly formed Environmental Services Division of the Health and Social Services Department. Top management of the Department was so engrossed in the overwhelming problems of welfare and environment. Following the election, we proposed that the environmental protection agency include all programs previously assigned to the Environmental Services Division, that there be a budget sufficient to include all personnel in the Environmental Services Division plus all environmental health personnel previously known as county or district sanitarians, that it be based on the pursuit of goals including but much broader than mere "health," and that new and necessary programs be authorized. During the legislative process, the terminology for the proposed agency was changed from Environmental Protection Agency to the New Mexico Environmental Improvement Agency and it was organized within and as an integral component of the New Mexico Health and Social Services Department. From an authorized strength of something like 37 personnel assigned to the Environmental Services Division in 1967, the agency expanded and prospered to a strength of 260 in 1973 and included such programs as food protection, air quality, water quality, water supply, radiation protection, occupational safety and health, noise control, solid wastes management, environmental chemicals, insect and rodent control, swimming pool safety and sanitation, subdivision control, etc.

THIRD EXAMPLE

In 1973, we requested legislative authorization and funding to organize a comprehensive consolidated state laboratory system to serve all state and many federal agencies desiring laboratory support services. This was the first step in organizing the Scientific Laboratory System to which I was appointed as director in August 1973. I had long been concerned about fragmentation and duplication of laboratory services, the lack of a clear and explicit service-oriented mission for the laboratory, and the need for a modern laboratory facility. The Scientific Laboratory System is now well-established and is routinely serving such agencies as the Environmental Improvement Agency, the State Health Agency, the State Welfare Agency, the New Mexico Traffic Safety Commission, the Game and Fish Department, Department of Corrections, Department of Hospitals and Institutions, New Mexico Racing Commission, Indian Health Service, Forest Service, Veterans Administration, local law enforcement agencies, and others on a fee-for-service basis.

pinpointed and noticeable during this "age of the environment" which began in the late 60's and will no doubt continue far into the future. There is no longer any doubt that the environment must be managed and will be managed. The only remaining questions relate to "how" and "by whom." Traditionally trained and experienced "environmental healthers" have frequently not exhibited the management knowledge and capability to cope with or show leadership regarding the new found public and political pressures, organizational trends, expanded program methodology, legislative demands and mandates, broadened program scope, and evolving program goals. All too frequently our environmental health leaders have been viewed as negative obstructionists rather than constructive leaders and have exhibited territorial defense mechanisms in lieu of creating, promoting, and justifying effective program and organizational concepts to meet the public clamor for a quality environment. "There go my people and I am their leader" has become a truism.

Environmental and personal health

First of all, and particularly for persons usually interested in environmental health programs, it may be necessary to indicate the difference between environmental health programs and personal health programs. Environmental health programs are simply organized methods of solving environmental problems which have a significant health component through means of manipulating or managing the environment. Contrarywise, personal health programs should be addressing those health issues which can best be handled by manipulating the individual.

Goals

Basic to the problem of management inadequacies has been the lack of an understandable, stated goal for environmental health programs and agencies. A goal may be simply defined as an "ultimate desired condition". Even though a goal may be stated in somewhat nebulous terminology, such a statement is still necessary as a means of maintaining consistent program direction. A suggested goal of environmental programs might be "insuring an environment that will confer optimal health, safety, comfort, and well-being on this and future generations." You will notice that I use the terminology "health, safety, comfort and well-being" which signifies my belief that few, if any, environmental problems can be successfully solved on a "health" basis only.

Mission

Another important and basic factor in many environmental agencies and programs is the statement of a mission. Simply stated, a mission is a statement indicating an agency's constituency or clientele. For example, an environmental agency should have a mission of consumer protection and public service. A laboratory should have a mission of providing service to other agencies and departments. Certain types of agencies such as an agriculture department have a mission of promoting and protecting a given industry. Conflicts of interest occur when such missions are mixed with the resultant "fox in the henhouse" syndrome. It is patently impossible to have a mission of consumer protection coupled with a mission of protecting and promoting a given industry or other special interest group. These situations do exist and continuously result in the public being defrauded instead of being protected.

Since many environmental agencies have not fully developed the concept of a mission, these agencies have been ready prey for those businesses and industries which they are empowered to regulate. This has frequently resulted in the regulating agencies actually protecting or even promoting the interests of those they are charged with regulating.

Equally as onerous is the situation wherein an agency having a clear legal mandate of public service and consumer protection is saddled with a board of commission loaded with special interest groups such as representatives of polluting industries. This poses another conflict of interest which defrauds and effectively disenfranchises the citizenry.

Even laws and regulations must be viewed with skepticism to determine if they are really designed to provide for rapid and equitable resolution of alleged violations or if they are so couched in hazy definitions and procedural delays as to serve the purpose of protecting the polluter.

Program scope

Another management concept worth understanding is that of program scope and program-problem relationships. A "program" may be defined as a rational grouping of methods or activities designed to solve one or more problems. An environmental "problem" may be defined as "a reasonably discrete environmental factor having an impact on man's health, safety, comfort, or well-being".

Program scope is usually defined by a governmental body such as the Congress, a legislature, a board, council, or commission. However, to understand the value of and need for having major environmental health and environmental protection regulatory programs managed within a single agency, it is imperative to understand program-problem definitions and interrelationships. Much of the recent environmental program fragmentation at federal, state and local levels might have been prevented if environmental program managers, citizens, and political leaders had a working concept of these relationships.

A few examples of environmental "problems" with a biased indication of their relative importance or level of priority might be in order, as follows: (*a*) Level I: population numbers and density; (*b*) Level II: energy, transportaition, and land-use; and (*c*) Level III: air pollution, solid wastes, water pollution, food,

environmental injuries, environmental chemicals, noise pollution, radiation, and water supply.

Society, through its legislative processes, has generally decreed a degree of curative environmental management through formal environmental programs for the type of problems listed in Priority Level III. However, formal programming to effectively address the more basic and preventive issues in Levels I and II has not been allowed or decreed. Those listed in Level II are now being widely discussed but thus far most efforts have been restrained to "skirting and flirting." It will undoubtedly be many decades before formal programming is seriously considered to deal with the most basic and highest priority issues—those of (*a*) population numbers and density, and (*b*) population life styles and resource consumption of the human animal. Environmental health and environmental protection agencies, therefore, are usually only dealing with the by-products of the basic problems. Programs designed to solve the Level III problems are, therefore, actually curative rather than preventive. The basic issues are not yet subject to programming. However, such basic problems are still environmental and solutions must have input from environmental agencies and personnel

TABLE 1. *Program-problem relationships*[a]

	Environmental problems													
Program examples	Population nos. & dist.	Energy needs	Land-use	Transportation	Air pollution	Water pollution	Solid wastes	Env. injuries	Biol. insults	Env. chemicals	Food safety	Radiation	Noise pollution	shelter
Air quality	X	X	X	X	X	X	X	X		X				
Food quality					X	X	X	X	X	X	X	X	X	X
Radiation protection		X						X				X		
Solid waste mgmt		X	X		X	X	X	X	X	X				
OSHA					X	X	X	X	X	X	X	X	X	X
Insect & rodent control							X		X		X			
Water supply						X	X		X	X		X		
Noise control			X										X	
Env. cont. of rec. areas					X	X	X	X	X					
Institutional env. control					X	X	X	X	X	X	X	X	X	X
Housing cons. & rehab.					X	X	X	X	X	X	X		X	X
Hazardous substances & product safety								X	X	X				
Subdivision control	X	X	X	X	X	X	X							
Water quality	X		X			X	X	X	X	X	X	X		

[a]X = Aids in solving problems

Table 1 may be helpful to more fully understand program-problem relationships, and the need to have major environmental regulatory efforts centralized rather than fragmented.

When studying program-problem relationships in Table 1, it can be seen that it is inefficient, uneconomical, and administratively inappropriate to separate certain environmental programs since several appropriately designed programs may aid in solving any given environmental problems. Table 1 also indicates the improper and inefficient design of many environmental programs, e.g., Food Quality. A properly designed food quality program, for example, should not be aimed at solving only the food problem but should have an impact on other problems as indicated.

Institutional setting

The question of organizational or institutional settings for environmental health programs is another management concept that has completely dumbfounded many of the old-style "public healthers." Everyone manages the environment to some degree. Dozens of agencies at all levels of government have a share of the action in terms of regulation, education, research, demonstration, and consultation. For reasons of operational economy and program effectiveness, it is important and valid to recommend that major environmental regulatory functions at each level of government be managed within a single agency. I have previously indicated that this can be explained and supported in terms of environmental and administrative program-problem interdigitation.

The type and organizational location of this environmental agency is another matter. Historically, relatively narrow, single-purpose (i.e., health) environmental health programs were almost solely the province of health departments and the health profession at all levels of government. Public and political clamor and concern over the rapidly deteriorating environment in the late 1960's caused a widespread re-evaluation of environmental problems, program goals, program scope, program effectiveness, program support, environmental legislation, as well as program organization and institutional settings. Programs were shifted to new and/or different agencies for a variety of reasons—some valid and some questionable. Eager citizen environmentalists and citizen action groups sometimes confused change with progress. Public and environmental officials generally exhibited a high degree of territorial defense and a relatively low titer of organizational and program management knowledge. Powerful polluter lobbyists delighted in the opportunity to retard and confuse environmental management through repeated reorganizations and by placing environmental personnel and agencies in positions of greater "political responsiveness". The federal Environmental Protection Agency has been touted as a model for state environmental agencies and this, in turn, has led to further undesirable program fragmentation in many states imbued with the desire to follow the federal "model."

The federal government must also share or accept responsibility for imposition on states of narrowly oriented, single-purpose codes conceived through tunnel vision. The federal codes and regulations relating to such problems as food, milk, occupational health, air pollution, water pollution, etc. are all beautiful examples of the administrative problem of single-problem-oriented

codes which truly result in a disservice and extra expense to our taxpayers.

Another issue basic to an environmental organization is the determination of the complete spectrum of problem-solving methods which can or should be utilized to solve the previously listed scope of environmental problems. And next comes the highly important process of developing "programs" which, as previously defined, are "rational groupings of activities designed to solve one or more environmental problems."

It is in this process that we truly need some organizational and management creativeness and innovation if we are to deliver environmental service efficiently and effectively. Again, this relates to the previously mentioned issue of single problem codes. Table 1 indicated the basic interrelationship between a number of environmental problems which should best be handled within one organizational framework. No doubt, many of our programs should properly be repackaged and renamed. Even industry has learned that products must be repackaged, retitled, and repromoted occasionally to provide the best sales possible. Having properly designed programs which address various important environmental problems might also be a step toward discouraging the practice of continuing to apply undue effort toward a problem which has been basically solved. Program personnel may not desire to completely solve the problem if it means that they would have to go out of business. Therefore, they frequently invent new aspects of the problem or, still worse, apply new and unnecessary requirements to the solution of the problem. For example, if 10 ft^2 of floor area is good, 20 is better; if one wash vat is good, two are better; if 100 ppm, 200 is better; or if a count of 30,000 is good, 15,000 is better. I am sure we must all admit to having seen examples of this type of program nonsense.

CONCLUSIONS

Very simply, the environment can be defined as "that which surrounds." We should all understand the value of approaching the environment on a comprehensive basis with comprehensive programming and we should also understand the ecological and administrative interrelationships of programs. Environmental health programs are essential services of state government, an unquestionably good investment, and they are ususally expected and demanded by our taxpayers.

Other items in common for most environmental health programs are those necessary program resources. Many of the basic environmental programs require a common type of manpower, equipment, facilities, legislation, and laboratory support services.

There are no standard "models" to be followed, but perhaps there are some basic organizational principles to be considered when organizing environmental agencies at the state or local level. These include (*a*) organizational visibility, (*b*) programming on a multiple goal basis, (*c*) freedom of interagency communication and coordination, (*d*) operating with a mission of public service and consumer protection, (*e*) responsiveness to public sentiment, (*f*) ese of regulatory actions, (*g*) comprehensive programming, (*h*) legislation designed for rapid, equitable results instead of procedural delays, (*i*) line item budgets for the environmental agency, (*j*) programmed for environmental protection rather than environmental utilization and development, and (*k*) regulations and standards promulgated by a board or commission representing balanced public interests.

The foregoing principles may be attained in a variety of organizational arrangements ranging from an appropriate environmental agency within a health department to a separate, free-standing environmental agency or department. In any event, however, adherence to the foregoing principles is necessary if there is to be an effective environmental protection effort.

I recall reading a provocative *Journal of Milk and Food Technology* editorial written by one of our esteemed leaders some 20 years ago. It was entitled "The Changes Have Already Taken Place" and was written by our friend and former president of IAMFES, Dick Adams. Truly, many of the organizational changes have already taken place while many of us have had our heads buried in the sand bemoaning the changes but failing to provide organizational and program leadership. Obviously, the environment will be managed and the only qestions really involve how and whom.

And since programs and organizations require manpower, a few words about manpower. When one grasps the magnitude and scope of environmental problems, understands their vital importance to this and future generations, scans the maze of organizational arrangments for delivering programs, and views the variety of useful program methods, it becomes obvious that the scope of environmental manpower required is as broad as the environment. Such manpower necessitates educational achievements through a spectrum from the lowest assistant or inspector through the various types of doctoral-level environmentalists. Truly, the environmental programs demand an alliance of physical scientists, life scientists, social scientists, engineers, planners, technicians, laboratory scientists, veterinarians, lawyers, physicians—the list is endless and all types are necessary.

Traditionally, environmental programs were erroneously thought to be (and perhaps were) the province of engineers, with other professions such as "sanitarians" playing an ancillary and subordinate role. That manpower concept is now kown to be inappropriate and archaic. The mantle of environmental program leadership now falls to those who earn it, be they "doctors, lawyers, or Indian chiefs."

A final thoughts about the environment and the economy. It isn't a case of "versus" or "either/or." The environment and the economy are not contradictory expectations or values and, in fact, are mutually interdependent. We can't have an economy without an

environment. And two basic ecological considerations should be kept foremost in mind when considering the environment and economy: (*a*) everything is connected to everything else, and (*b*) we should strive for the greatest good for the largest number over the longest period.

I am advised that "ecology" and "economy" are both derivatives of the Greek work "ecos" (oikos) which means house. An economist was a keeper of the house and an ecologist is a keeper of the big house we all live in - - - or our environment, the place in which we are all going to spend the rest of our lives.

ACKNOWLEDGMENT

Presented at the 62nd Annual Meeting of the International Association of Milk, Food, and Environmental Sanitarians, Toronto, Ontario, Canada, August 10-13, 1975.

Kultures and Kurds Klinic Awards

Mr. Clarence Davis (left) and Mrs. Debby Miller receive the prestigious Neil C. Angevine Superior Quality Award on behalf of T. G. Lee Foods, Orlando, Florida, from Mr. Angevine, Assistant Secretary of the American Cultured Dairy Products Institute.

The 1976 Kultures and Kurds Klinic, sponsored by the American Cultured Dairy Products Institute, was held March 29-31 in Arlington, Virginia. The Klinic drew 175 delegates from thirty-one states, Mexico, Canada, Denmark and Japan.

This year's Neil C. Angevine Superior Quality Award, given to the dairy plant with the highest cumulative score for all cultured products evaluated at the Annual ACDPI Training Schools, was presented to T. G. Lee Foods, Orlando, Florida. Pine State Creamery, Raleigh, North Carolina, placed second in the over-all products category, with third place going to A & P National Dairy Division, Ft. Washington, Pennsylvania.

First place certificates of merit for quality products were awarded to the following organizations: Cass-Clay Creamery, Fargo, North Dakota (buttermilk); T. G. Lee Foods and Pine State Creamery, (sour cream); A & P National Dairy Division, Ft. Washington, Pennsylvania (plain yogurt and all categories yogurt); Anderson Erickson, Des Moines, Iowa (lemon yogurt); Land O'Lakes 19-19, Clinton, Iowa (strawberry yogurt); and Purity Dairies, Nashville, Tennessee; Land O'Lakes, Minneapolis, Minnesota; Ludwig Dairy Corporation, Dixon, Illinois and Pine State Creamery (cottage cheese).

PROGRAM

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

In cooperation with
Associated Illinois Milk, Food and
Environmental Sanitarians, Inc.
August 8-12, 1976

Arlington Park Hilton **Arlington Heights, Illinois**

Harold E. Thompson, Jr.
President
I.A.M.F.E.S., INC.

REGISTRATION

Sunday, August 8—1:00 p.m.-5:00 p.m.
Monday, August 9—8:00 a.m.-5:00 p.m.
Tuesday, August 10—8:00 a.m.-5:00 p.m.
Wednesday, August 11—8:00 a.m.-12:00 Noon
Thursday, August 12—8:00 a.m.

REGISTRATION FEE—$15.00
Banquet—$15.00
Ladies Registration—$9.00
Students—No Charge for Registration
National Mastitis Council Registration—$1.00

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JOURNAL OF MILK AND FOOD TECHNOLOGY

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SUNDAY, AUGUST 8, 1976

1:00- 5:00 p.m.—Registration-
1:30- 5:30 p.m.—Executive Board-
6:00- 7:00 p.m.—Early Bird Reception
8:00-11:00 p.m.—Executive Board

MONDAY, AUGUST 9, 1976

8:00 a.m.-5:00 p.m.—Registration

Special Meetings

9:00 a.m.-12:00 noon—Executive Board
1. Report on Local Arrangements
2. Report of Executive Secretary
3. Report of Sanitarians Joint Council

1:00 p.m.- 3:00 p.m.—Affiliate Council
1. International Program
2. Discussion of Association Awards
3. Affiliate Input to Journal of Milk and Food Technology
4. Discussion of possible IAMFES and NEHA unification
5. Future Meeting Location Recommendations
6. Election of Affiliate Council Officers
7. Other Pertinent Topics

1:30 p.m.- 5:00 p.m.—Executive Board
1. Regular Agenda
2. Report of Journal Management Committee
3. Committee Chairmen
4. Report of Affiliate Council
5. Meet with Past Presidents

1:30-5:00 p.m.—Individual Committee Meetings
3:00-5:00 p.m.—Farm Methods Committee
6:00-7:00 p.m.—Reception

(Individual Committee Meetings are open to all registrants)

TUESDAY, AUGUST 10, 1976

8:00 a.m.-5:00 p.m.—Registration
8:00 a.m.-9:00 a.m.—Executive Board

Morning - General Session

Henry V. Atherton, Presiding

9:30 a.m.—A Salute to America's 200th Anniversary: "Spirit of America" (film)
9:35 a.m.—INVOCATION-C. Bronson Lane
9:40 a.m.—ADDRESS OF WELCOME
Dr. Joyce Lashof
Director of Public Health
Illinois Department of Public Health
9:55 a.m.—PRESIDENTIAL ADDRESS-Harold E. Thompson, Jr.
10:25 a.m.—KEYNOTE ADDRESS-to be announced
11:10 a.m.—Take the Bushel Off the Light-Clarence K. Luchterhand, Chief-Milk Certification, Wisconsin Division of Health
11:40 a.m.—ANNOUNCEMENTS
Program Highlights
Local Arrangements
New Nominating Committee
Other Announcements

Afternoon - Milk Sanitation Session
Howard Hutchings, Presiding

1:30 p.m. DOOR PRIZE DRAWING

1:45 p.m. Statistical Summary of Public Health Evaluations of Milk Quality on Louisiana Dairy Farms. Richard M. Garrett and J. H. Gholson, Louisiana State University, Baton Rouge.

2:00 p.m. The Interstate Milk Shippers Conference-Present Status of the I.M.S. Program. Hubert H. Vaux, Indiana State Board of Health, Indianapolis.

2:30 p.m. Dating of Food Products-Politics or Public Health? C. Bronson Lane, Dairy and Food Nutrition Council of Florida, Orlando.

3:00 p.m. Sporeformers in Raw Milk and Their Outgrowth in Heated Milk at 7°C. E. M. Mikolajcik* and N. T. Simon, Ohio Agricultural Research and Development Center, Columbus.

3:15 p.m. Milk Break

3:30 p.m. Microorganisms Isolated from Bread Doughs of Bakeries in Shiraz, Iran. Reza A. Tadayon, Pahlavi University, Shiraz, Iran.

3:45 p.m. Antibiotics in Milk-Current and Future Methodology. James W. Messer, Food and Drug Administration, Cincinnati.

4:20 p.m. A Collaborative Study to Determine the Feasibility of Using 0.40, 0.20, 0.14, and 0.10-Inch Diameter Discs to Measure Sediment in Fluid Milk. Committee Report, Earl O. Wright, Chairman.

Afternoon - Food Sanitation Section
David Fry, Presiding

1:30 p.m. DOOR PRIZE DRAWING

1:45 p.m. Thermal Inactivation of Clostridium botulinum, Type A Toxin in Acetate Buffer. J. G. Bradshaw*, J. T. Peeler, and R. M. Twedt. Food and Drug Administration, Cincinnati.

2:00 p.m. Food Service Certification Programs-Panel Discussion

2:00 Sanitation Training For Food Service Managers-A Must! A. Sidney Davis, Food and Drug Administration, Washington, D.C.

2:20 Foodservice Certification-The NIFI Program. Chester G. Hall, National Institute for the Foodservice Industry, Chicago.

2:40 Foodservice Certification-The Florida Program. A. W. Morrison, Florida Department of Health and Rehabilitation Services, Jacksonville.

3:00 Discussion

3:20 p.m. MILK BREAK

3:35 p.m. Microbiological Studies on Aging of Intact and Excised Beef Muscle. C. R. Rey, A. A. Kraft* , and F. C. Parrish, Jr. Iowa State University, Ames.

3:50 p.m. Toxicological Considerations in the Selection of Flexible Packaging for Foodstuffs-Fred B. Shaw, Continental Can Co., Inc. Chicago.

4:25 p.m. Bacteriology of Ground Beef and Soy-extended Ground Beef. I. Wilfred Obioha* and A. A. Kraft, Iowa State University, Ames.

TUESDAY, EVENING, AUGUST 10, 1976

7:00-9:00 p.m. EVENING DISCUSSION GROUPS

7:00 p.m. FOOD SANITATION

7:00 p.m. MILK SANITATION

WEDNESDAY, AUGUST 11, 1976

8:00 a.m.-5:00 p.m. REGISTRATION

Harold E. Thompson, Jr., Presiding

8:30 a.m. DOOR PRIZE DRAWING

8:45 a.m. ADDRESS

9:15 a.m. IN DEFENSE OF TECHNOLOGY-Fergus M. Clydesdale, University of Massachusetts, Amherst.

9:45 a.m. VALUE OF NATIONAL ASSOCIATION OF DAIRY FIELDMEN AFFILIATING WITH IAMFES. Sidney H. Beale, Michigan Milk Producers Association, Detroit.

10:10 a.m. MILK BREAK

10:20 a.m. DOOR PRIZE DRAWING

10:30 a.m. ANNUAL BUSINESS MEETING

1. Report of Executive Secretary
2. Report of Secretary-Treasurer
3. Committee Reports
4. 3-A Symbol Council Reports
5. Report of Resolutions Committee
6. Report of Affiliate Council
7. Old Business
8. New Business
9. Election of Officers
   Ivan Parkin-Parlimentarian

Afternoon - Milk Sanitation Section
James Meany, Presiding
Chicago Board of Health

1:30 p.m. DOOR PRIZE DRAWING

1:45 p.m. Toxinogenic Potential of Molds Isolated From Moldy Cheese Trimmings. Lloyd B. Bullerman, University of Nebraska, Lincoln.

2:05 p.m. A Look At the U.S. Whey Processing Industry. Warren S. Clark, Whey Products Institute, Chicago.

2:40 p.m. Assessing Post-Pasteurization Contamination-Predicting Shelf Life. Carlton E. Parmelee, Purdue University, Lafayette, Indiana.

3:15 p.m. MILK BREAK

3:30 p.m. Enumeration and Fate of Enteropathogenic and Nonpathogenic Escherichia coli During the Manufacture of Camembert Cheese. J. F. Frank* , E. H. Marth, and N. F. Olson. University of Wisconsin, Madison.

3:50 p.m. Somatic Cells in Milk-Significance and Relationship to Milk Composition. Loris H. Schultz, University of Wisconsin, Madison.

4:30 p.m. Characterization of Psychrotrophic Bacteria from Aged Beef. M. Valland and A. A. Kraft*. Iowa State University, Ames.

Afternoon - Milk Production Section
James M. Hellnich, Presiding
Kraft Foods

1:30 p.m. DOOR PRIZE DRAWING
1:45 p.m. History and Role of the IAMFES Farm Methods Committee. Melvin W. Jefferson, Virginia Bureau of Dairy Services, Richmond.
2:20 p.m. Testing for Antibiotics in Milk-Field Observations. Peter R. Schoech, Yankee Milk, Inc., Newington, Conn.
2:55 p.m. Freezing Points and Milk Adulteration. Sidney E. Barnard, Pennsylvania State University, University Park.
3:30 p.m. MILK BREAK
3:45 p.m. Present Status and Trends in Tuberculosis and Brucellosis in Cattle. E. A. Schilf, United States Department of Agriculture, Washington, D.C.
4:20 p.m. National Association of Dairy Fieldmen. Annual Business Meeting.

Afternoon - Food Sanitation Section
William Embry, Presiding
Evanston Health Dept.

1:30 p.m. DOOR PRIZE DRAWING
1:45 p.m. Biodegradation of Aflatoxin by Toxigenic Aspergilli. M. P. Doyle* and E. H. Marth, University of Wisconsin, Madison.
2:05 p.m. The Significance of Mycotoxins to Food Safety and Human Health. Lloyd B. Bullerman, University of Nebraska, Lincoln.
2:35 p.m. Developments in Food Virology. Dean O. Cliver, University of Wisconsin, Madison.
3:05 p.m. The Evaluation of a Fast Food Management Training Program-One Year Later. Bruce Jackson* , Jack Hatlen, and Bradley Palmer. University of Washington, Seattle.
3:20 p.m. MILK BREAK
3:35 p.m. Handling Perishable Foods. Sidney E. Barnard* , Morris G. Mast, and Gerald R. Kuhn, Pennsylvania State University, University Park.
3:50 p.m. Textured Vegetable Protein, New and Growing Food Category. Thomas L. Welsh, Miles Laboratories, Inc., Chicago.
4:25 p.m. Present Status and Trends in the Aseptic Packaging of Milk Products and Juices. Harold Wainess, Harold Wainess and Associates, Northfield, Ill.

Indicates person presenting paper.

WEDNESDAY EVENING, AUGUST 11, 1976

6:00-7:00 p.m. RECEPTION
7:00 p.m. ANNUAL AWARDS BANQUET
Harold E. Thompson, President, Presiding
INVOCATION-Ivan E. Parkin
INTRODUCTIONS
PRESENTATION OF AWARDS
Earl O. Wright, Chairman

1. Past Presidents Award
2. Citation Award
3. Honorary Life Membership
4. C. B. Shogren Memorial Award
5. Sanitarians Award
   Sponsored by:
   Klenzade Products, Inc.
   Division Economics Laboratories
   Pennwalt Chemical, Inc.
   Diversey Corporation, Inc.
6. Industry and/or Education Award
   Sponsored by:
   Milking Machine
   Manufacturers Council of the Farm and Industrial Equipment Institute
7. Samuel J. Crumbine Consumer Protection Award
   Sponsored by:
   Single Service Institute, Inc.

INSTALLATION OF OFFICERS
9:00 p.m. ENTERTAINMENT
10:00 p.m.-12:00 a.m. DANCE-CASH BAR

THURSDAY, AUGUST 12, 1976

8:00 a.m. Registration-NATIONAL MASTITIS COUNCIL
Phillip J. Hermsen, AMPI, Chicago
7:30 a.m. EXECUTIVE BOARD-BREAKFAST MEETING

NATIONAL MASTITIS COUNCIL
1976 Summer Meeting

PROGRAM

8:00 a.m. REGISTRATION-Phillip J. Hermsen, AMPI, Chicago
Ewing H. Row, Presiding
Hoard's Dairyman, Fort Atkinson, Wis.
8:30 a.m. GREETINGS-Richard Mochrie, President National Mastitis Council,
North Carolina State University, Raleigh
THEME-A Quality Glass of Milk
8:45 a.m. DELVOTEST P ANTIBIOTIC TEST
Phillip B. Nelson, Enzyme Development Corp., N.Y.
9:15 a.m. REASONS FOR ANTIBIOTIC CONTAMINATION, Donald P. Wesen, North Carolina State University, Raleigh
9:45 MILK BREAK
10:00 a.m. WHAT IS HIGH QUALITY MILK?

Edward W. Custer, Mississippi State University, Mississippi State.

10:30 a.m. COOLING PROBLEMS AND MILK QUALITY, Karl Girton, Girton Manufacturing Company, Millville, Pa.

11:00 a.m. CLEANING AND SANITIZING PROBLEMS AND MILK QUALITY, James R. Welch, Klenzade Products Division, Economics Laboratory, Inc., St. Paul, Minn.

11:30 a.m. DISCUSSION AND QUESTIONS

12:00 Noon LUNCH

1:30 p.m. USING SOMATIC CELL COUNTS IN AN EFFECTIVE MASTITIS CONTROL PROGRAM, Allan N. Bringe, University of Wisconsin, Madison.

2:00 p.m. LABORATORY TESTS AND HOW THE RESULTS CAN HELP THE DAIRYMAN Charles Morrow, Dairymen, Inc., Spartanburg, S.C.

2:30 p.m. DISCUSSION AND QUESTIONS

2:45 p.m. MILK BREAK

3:00 p.m. DI's QUALITY PROGRAM FROM THE COW TO THE CONSUMER, William Arledge, Dairymen, Inc., Louisville, Ky.

3:45 p.m. R. L. MATHIS CERTIFIED DAIRY's PROGRAM FOR RAW CERTIFIED MILK, Jack Mathis, R. L. Mathis Certified Dairy, Decatur, Ga.

4:30 p.m. DISCUSSION AND QUESTIONS

ENTERTAINMENT
MEN AND WOMEN

SUNDAY, AUGUST 8, 1976

6:00 p.m.-7:00 p.m. RECEPTION

MONDAY, AUGUST 9, 1976

6:00 p.m.-7:00 p.m. RECEPTION

TUESDAY, AUGUST 10, 1976

9:15 p.m.-10:30 p.m. WINE AND CHEESE PARTY

WEDNESDAY, AUGUST 11, 1976

6:00 p.m.-7:00 p.m. RECEPTION

7:00 p.m. BANQUET & ENTERTAINMENT

ENTERTAINMENT FOR THE LADIES

(Ladies are invited to attend any of the meeting sessions)

MONDAY, AUGUST 9, 1976

TOUR Woodfield Mall, Schaumburg, Ill.

TUESDAY, AUGUST 10, 1976

TOUR 1 Kitchens of Sara Lee, Deerfield, Ill.

TOUR 2 Art Museum, Sears Tower, etc., Chicago, Ill.

WEDNESDAY, AUGUST 11, 1976

TOUR Hagar Pottery, Dundee, Ill., and Long Grove Village, Long Grove, Ill.

THURSDAY, AUGUST 12, 1976

TOUR Woodfield Mall, Schaumburg, Ill.

News and Events

National Mastitis Council Meeting

The 1976 National Mastitis Council Annual Meeting was held in Louisville, Kentucky on February 17 and 18. Program Chairman Dr. R. D. Mochrie is to be congratulated for the informative and well-rounded program presented. Three hundred members and guests were in attendance for all or part of the two-day program which included a research report from one international authority on mastitis control. Credit for an efficiently run meeting goes to local members William L. Arledge and Dr. John H. Nicolai, Jr.

Officers elected were: Dr. R. D. Mochrie, President, and Mr. J. R. Welch, Vice President. Dr. J. C. Flake was re-elected Secretary-Treasurer.

The following reports and actions at the Board of Directors' meetings held prior to and at the conclusion of the NMC Annual Meeting are of special interest:

Drug Labeling and Cow Identification: This new committee authorized by the Board is to be chaired by John B. Adams, and will include representatives of the veterinary profession, dairy industry, and animal drug industry. Objectives of the committee include: development of a petition to FDA on mandatory labeling of drugs and/or package inserts for milk withdrawal times and slaughter times, and the development of a uniform system for identification of treated cows.

Research Committee: Chairman Dr. James W. Smith reviewed plans for expanded activities for the committee and sub-committees in 1976, which will involve teat dips, coliform mastitis, and drug residues. An important task for the committee is to update the Current Concepts of Bovine Mastitis and its Supplement, a major treatise availabe in the NMC educational program.

Teat Dip Committee: This committee expects to continue to work with FDA and review any proposal FDA makes on regulating teat dip products. The committee has worked out identification symbols for teat dips, udder washes and sanitizers. Many NMC member teat dip manufacturers have agreed to the principle of these symbols.

As customary, the NMC 1976 Summer Meeting will be held in conjunction with the I.A.M.F.E.S. meeting. See this issue for the program of the NMC Meeting on August 12.

Wisconsin Food Service Convention

The Wisconsin School Food Service Association is planning a special convention for members and friends. Their 19th Annual Convention will be held June 14, 15 and 16, 1976, at the Cartwright Center, University of Wisconsin at LaCrosse and the Holiday Inn, LaCrosse, Wisconsin. The theme of the convention is "Trails to Excellence" and it will feature workshops, seminars and general sessions designed to reflect training needs for all personnel.

For further information, contact Laureal Huibregtse, Publicity Chairperson, 5301 Monona Drive, Monona, WI 53716.

Pennsylvania Dairy Fieldmen's Conference

The 1976 Pennsylvania Dairy Fieldmen's Conference will be held June 8 and 9 at the J. O. Keller Conference Center on the campus of The Pennsylvania State University, University Park, PA. An evening program is also planned for June 7. Topics to be included this year include a review of Pennsylvania Department of Agriculture programs and regulations relating to milk production and dairy products, an update on the new USPH Pasteurized Milk Ordinance, and pesticide regulations and case histories. Other subjects will include milking machine design and related economic factors, energy conservation on the farm, and Department of Environmental Resources dairy farm guidelines and regulations. A discussion will be given relative to the possible inclusion of somatic cell counting, using the Foss Somatic cell counter, as part of the DHIA program.

For further information contact: Agricultural Conference Coordinator, 410 J. O. Keller Conference Center, University Park, PA 16802.

Ag Engineers Summer Meeting

The American Society of Agricultural Engineers sponsors two national meetings each year. This year the ASAE Summer Meeting will be held June 27-30, 1976 at Lincoln, Nebraska.

The meeting will include six half-day periods during which general and concurrent technical sessions will be held for a total of 74 sessions during the course of the meeting. The Food Engineering Division of ASAE is sponsoring several sessions on topics of interest to food engineers and allied technologists. These will include a general theme session on meat packing, a session on sugar beet processing and one on storage and handling of sugar beets. A session relating to physical properties of food materials as well as one on general food engineering topics will also be held. Other divisions of ASAE are sponsoring sessions which may be of interest to the food industry, including the mechanization of vegetable harvesting and handling.

Further information, along with a copy of the complete program and registration material may be obtained by writing directly to ASAE, 2950 Niles Road, St. Joseph, Michigan 49085.

Book Review

Achieving Sanitation with Cost-Savings

Food Store Sanitation, A Profit Protection Program, by Don C. Rishoi. Published by Chain Store Publishing Corporation, New York, N.Y. 1976. 302 pp. $15.95.

As the author stresses, sanitation and economics are partners, and the emphasis of this book is to show the retail food store operator how to practice sanitation toward profitable ends. The book covers many areas, such as microbiology, hygiene, cleaning and sanitizing, equipment construction and maintenance, rodent and insect control, problems of consumer education, sanitation in the various food store departments, and the sanitation program. In addition, consideration is given to legal aspects, with a section on the AFDOUS retail food market code. With such broad coverage as this, it is inevitable that some areas may be better treated than others. For example, a paragraph on food poisoning bacteria indicates that these organisms are likely to be found when the numbers of other, non-harmful, types of bacteria are low. This in itself could be misleading the reader into thinking that high bacterial counts are conducive to "germ-free" products (a strange anomaly which the author does explain). Without this review becoming unduly cumbersome, it might be said that the author might better have stayed with the simple concepts more in keeping with the theme of the book. This is only a minor criticism of the book. In general, principles and explanations of actions needed are given in a clear manner designed for the retail store worker who should find it very valuable in achieving sanitation with cost-savings. The book also may be considered as a troubleshooter's manual to some extent, since individual potential problems are effectively dealt with. In some cases, perhaps too much detail is noted, but it is better to have such detail than errors of omission.

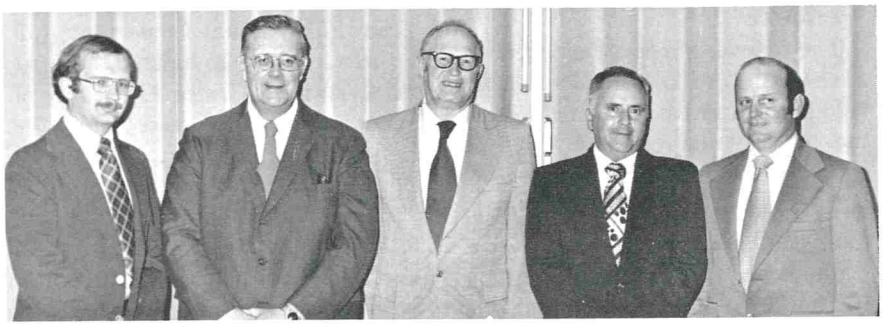
Of particular value to the retail store operator are the guidelines given under Departmental Sanitation. This chapter spells out very clearly and specifically rules of good sanitary practices for each of the individual store departments. The author gives practical reasons for the recommendations and rules, and no operator should have any trouble in understanding the material.

The book is a "must" for all food store operators, managers, and executives. It should serve well for use in training programs for retail food store personnel, and that is one of the greatest values in food quality assurance and consumer protection. Food sanitarians and inspectors should, therefore, find the book to be very helpul in their operations.

A. A. KRAFT
Department of Food Technology
Iowa State University
Ames, Iowa 50011

Association Affairs

Virginia Dairy and Food Industry Workshop



Officers installed at the Virginia Association's annual meeting (from left to right): A. N. Smith, International Representative; W. H. Gill, Sec'y.-Treasurer; L. C. Morgan, Second Vice-President; D. E. Henderson, First Vice-President and L. T. Lester, President.

The Virginia Association of Sanitarians and Dairy Fieldmen sponsored a two and one-half day "Dairy and Food Industry Workshop" on the campus of Virginia Polytechnic Institute and State University at Blacksburg, Virginia on March 8, 9 and 10, 1976. This meeting was held under the Continuing Education Program with units of credit available to those who participated.

Seventy-five members and guests attended the workshop. The program was designed to cover topics of interest in the latest developments in both the dairy and food industries.

Some highlights of the program included a report by Dr. C. W. Heald on the Fossomatic electronic cell counting machine in use at the VPI DHIA Lab; the need for rapid tests for antibiotic residues in milk by Dr. Ralston Read, Deputy Chief, Division of Microbiology, FDA, Washington, D.C.; factors encountered in marketing milk and dairy products by Dr. Roger H. Wilkowske, Program Leader, Dairy Marketing and Food Technology, USDA, Washington, D.C.; and a report on federal water control standards by John Adams of the National Milk Producers Federation. Panel discussions were presented on problems caused by coliforms in milk and food products and on consumer expectations regarding milk quality and milk pricing by Prof. Georgia Crews, VPI, Mrs. Betty Gross and Dr. W. T. Boehrn, VPI.

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Iowa—March 22, 1976, Ramada Inn, Ames.
Kansas—October 1-3, 1975, Holiday Inn, Manhattan.
Kentucky—February 22-23, 1977, Stouffer's Inn, Louisville.
Michigan—March 1976.
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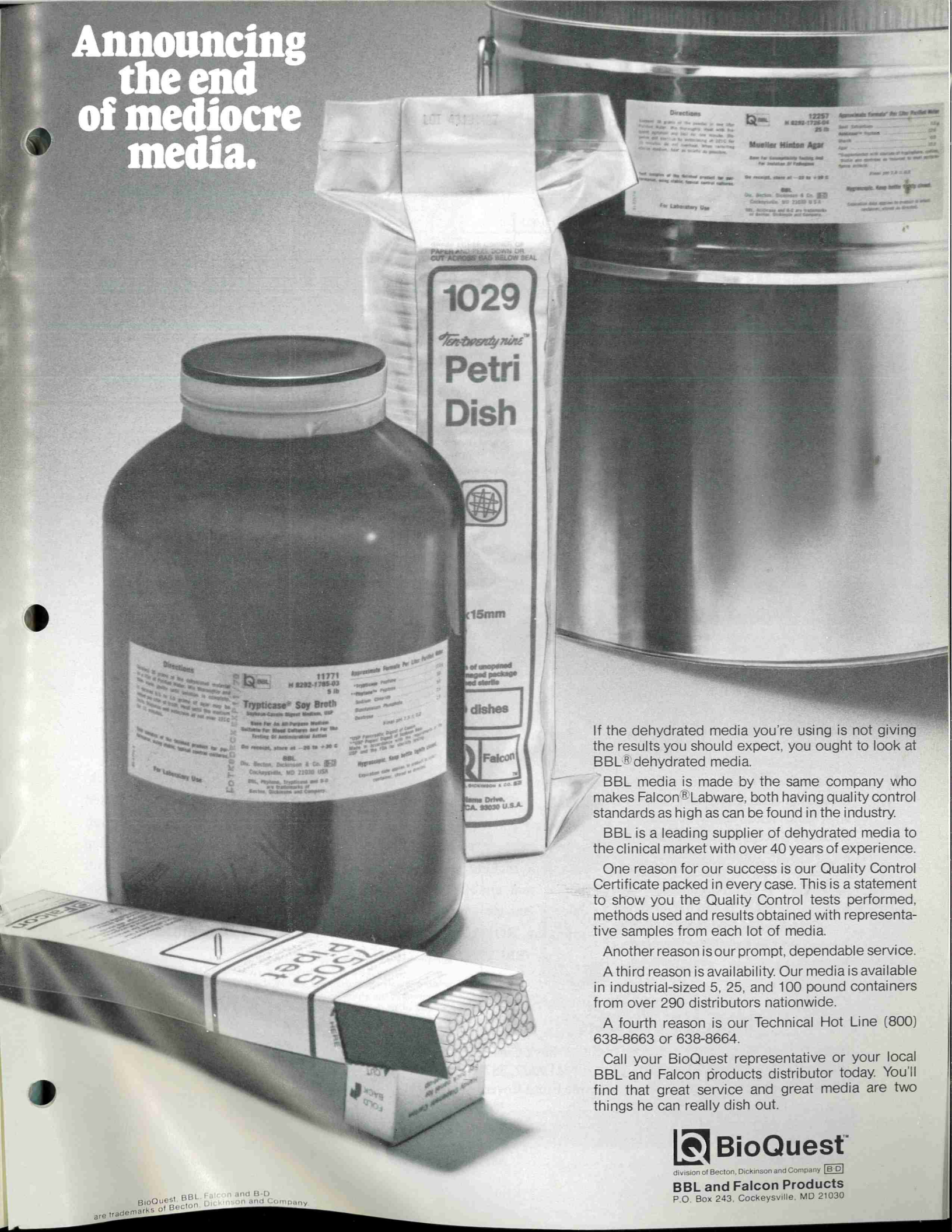

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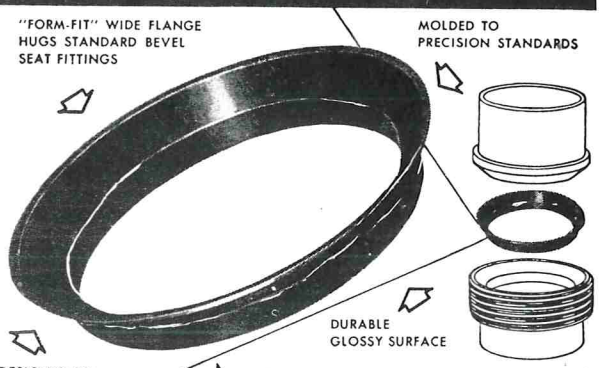

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Dairy authorities speak out on better cow milking

Stephen B. Spencer
Extension Dairy Specialist,
Pennsylvania State University

What's your score on vacuum?

An inadequate vacuum pump can affect the operation of the milking units drastically. Excessive "drop-off," slower milking, incomplete milking and an increase in the incidence of mastitis are likely results.

As a dairyman, your net dollar income depends on the vacuum pump and it probably is used more hours each year than most farm tractors.

Few people would hitch six plows behind a four plow tractor and head for the field to do a day's work. Yet many of these same people milk cows with a short vacuum supply and never question whether the pump is adequate.

Vacuum pumps used to be rated according to the number of units that could be operated. Today we measure the pump capacity in terms of Cubic Feet of air per Minute (CFM). Just as "horsepower" is more reliable than "plow rating" as an indication of tractor size, "CFM" is more reliable than "unit rating" when sizing a vacuum pump.

CFM output depends upon vacuum level. As vacuum level increases, the CFM output decreases. It's important to operate the system at the vacuum level specified by the manufacturer or the output of the pump will be altered.

The important consideration of any vacuum pump is the CFM output at the milking vacuum level. The pump must have adequate CFM output to meet the vacuum requirements of the system and provide sufficient reserve to maintain a constant vacuum level.

Vacuum Requirement
The milking unit is the most important of the machine components which admit air into the system. The air consumption of milking units varies depending upon shell and inflation size, pulsation rate and length and size of pulsated air tubes. Typically, the air requirement of a milking unit while it is not milking is three to four CFM. The pulsator consumes 50 to 70% of this volume. Considerably larger air pumping capacity (a reserve) must be provided to make a milking system operational. Other components which consume air are such things as vacuum operated door openers, milk metering devices, and the vacuum regulator. The requirements of each component must be added together to determine the system requirements.

Vacuum Reserve
The vacuum reserve is the air pumping capacity which remains after the vacuum requirement of all components has been satisfied. That's the problem. We've thought in terms of the vacuum reserve as the amount of CFM capacity that's *left over.* We really should be thinking in terms of a *base reserve* for the operator(s) before we begin to compute the system requirements.

The reserve is all-important in order to maintain vacuum stability. The reserve is necessary in order to make allowances for operator usage and possible leaks in the system or other contingencies.

The most important reason for an adequate vacuum reserve is to provide for the amount of air that the operator will use. The operator is the largest user of the vacuum reserve. Some operators are very wasteful of the available reserve. This occurs as units are being attached and removed. Improper unit adjustment is also a significant factor. When teat cups start to leak and "squeal" during milking, the vacuum reserve is depleted rapidly.

Some operators may deplete vacuum reserve as much as 30 or more CFM for short periods of time. The careful operator will use but half that amount during the milking process. The real test of any milking system is when a milking unit falls off. It takes huge reserves of air just to keep the remaining units on the cows. Reserve tanks aid a little during these occurrences but basically the vacuum pump must be relied upon to maintain vacuum level. It all adds up to the fact that an adequate pump is a *must* for every dairyman.

Research in Ireland, Wisconsin, Pennsylvania and California indicates that inadequate vacuum reserve is associated with higher leucocyte counts. In plain language it means that mastitis can result if your vacuum pump isn't large enough.

What's your Vacuum Score?
Don't make a mistake and just assume that your pump is putting out enough air. Have it checked with an air flow meter once a year. Many dealers are equipped to do this for you.

And how will you know for sure that they're not just trying to sell you a pump? Frankly, I've found most dealers to be very reliable in this respect.

There have been many different recommendations about pump sizes. It's hard to give one that's exactly right for each system. Here's a guide for you to check your vacuum needs. It's based upon the New Zealand Standard. The American Standard would give values equal to one-half of the New Zealand Standard.

For bucket users:
Allow 4 CFM per unit + 20 CFM base reserve.

For pipeline users:
Allow 5 CFM per unit + 40 CFM base reserve for the first operator and 20 CFM for each additional operator.

The resulting CFM values would give you the minimum size vacuum pump capacity. If your system has more than this, fine. If you have less vacuum capacity than this you should carefully investigate your vacuum needs.

This method of determining vacuum capacity is different from what you may have seen before. A 50 percent reserve is commonly used. While a 50 percent reserve may be satisfactory on a system of six or more units, our field studies indicate that using a 50 percent reserve is not adequate for the smaller system.

Remember this: There is no substitute for an adequate vacuum system. Make sure you know your score on your vacuum needs.