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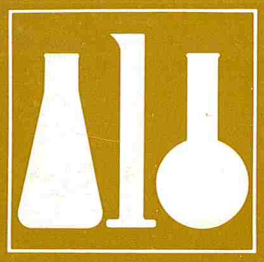

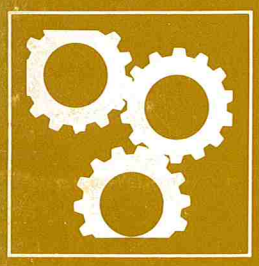

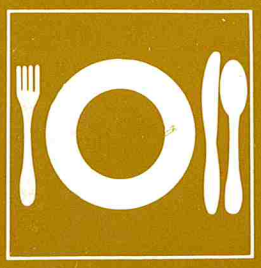

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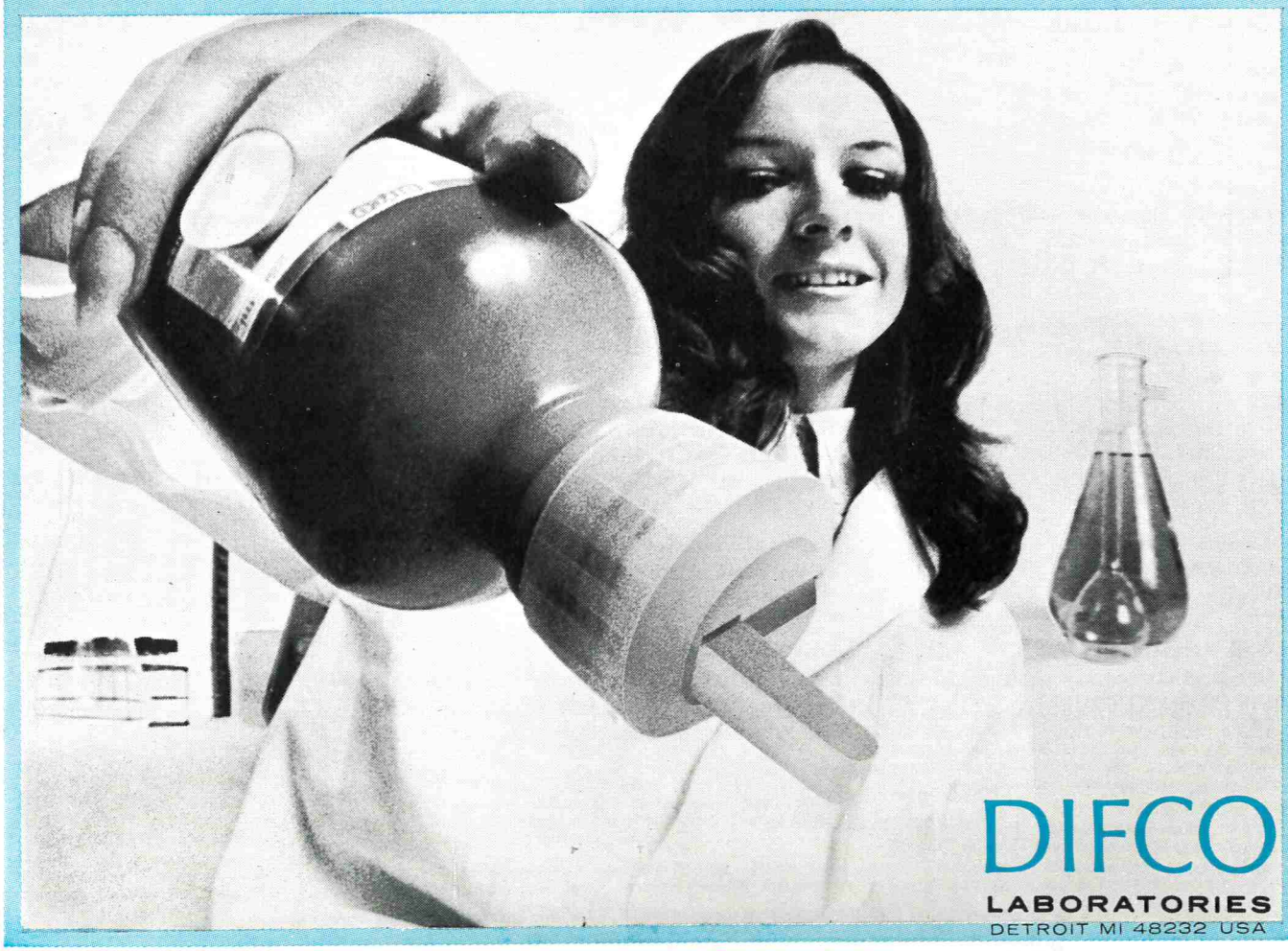

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Effect of Temperature on Growth and Alpha Toxin Production by *Clostridium perfringens*[1]

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(Received for publication June 21, 1978)

ABSTRACT

Growth and alpha toxin production by a strain of *Clostridium perfringens* was determined in Thioglycollate medium, beef broth with ground beef, and beef broth with ground beef and soy protein. Incubation temperatures ranged from 15 to 50 C. In Thioglycollate medium, maximum alpha toxin production occurred at 35 C and was 40 times greater than that observed at 45 C. However, generation time and maximum population were approximately the same at 35 and 45 C. At 15 C, a two log cycle reduction in viable counts occurred within 6 h. Irrespective of incubation temperature, alpha toxin levels in Thioglycollate medium declined as the incubation period was extended beyond the stationary growth phase. In the beef broth with ground beef system which was studied at 35 C only, the organism grew slower and produced less toxin than in Thioglycollate medium. The amount of alpha toxin detected was influenced to a greater extent by the incubation time and temperature, the holding time beyond the stationary growth phase, and the growth medium than by the population level of *C. perfringens*.

It has been suggested that quantification of alpha toxin produced by *Clostridium perfringens* can be used as an index of growth of the organism (*8*). Results obtained by the alpha toxin procedure may be influenced by several factors. For example, a wide variation has been demonstrated between strains of *C. perfringens* with respect to alpha toxin production (*6,11,15*). Temperature and substrate are known to influence biological activity of all microorganisms and would also likely affect the growth and alpha toxin production of *C. perfringens*.

This paper reports on the effects of incubation time and temperature as well as on effect of the nature of the substrate on estimation of numbers of *C. perfringens* by alpha toxin assay.

MATERIALS AND METHODS

Test organism

The strain (OSU 1) of *C. perfringens* used in the experiment was obtained from the Department of Animal Science, The Ohio State University, Columbus, Ohio. The organism was propagated in Thioglycollate medium.

Test media

The test media included Brewer's Thioglycollate medium (Difco) and beef broth (RJR Foods, Inc., Winston-Salem, North Carolina) with added ground beef or a mixture of ground beef and Promine-D (Central Soya, Chicago, Illinois). The beef broth was blended with 20% ground beef, or 14% ground beef and 6% Promine-D and sterilized (121 C for 15 min) in the blender jars. These media were blended well before use and distributed into previously sterilized 50-ml screw-capped Erlenmeyer flasks. No attempt was made to control final E_h of the beef/soy protein systems.

Measurement of growth and alpha toxin production

One tenth milliliter of an overnight broth culture of *C. perfringens* was inoculated into 40 ml of the test medium contained in an Erlenmeyer flask. For the Thioglycollate medium, incubation was carried out at 15, 25, 35, 45, and 50 C in a temperature-controlled water bath. However, when the beef broth with ground meat and/or soy protein were used as the test media, optimum incubation temperature (35 C) was used. Samples were taken at selected time intervals in sterile test tubes (12 × 100 mm) for the measurement of viable counts, production and pH. The viable cell population was estimated by pour plates prepared with SFP agar (*17*) without addition of Polymyxin B sulfate and Kanamycin sulfate. Alpha toxin production was measured by the Hemolysin Indicator (HI) plate test of Duncan and Harmon (*5*) with modifications suggested by Park and Mikolajcik (*12*). The pH was measured using a PHM 62 Standard pH Meter (Radiometer, Copenhagen).

Growth curves were constructed by plotting the logarithm of the colony-forming units (CFU) versus incubation time. The generation time was calculated by the formula: $Gt = t/n = t/(3.3 \log_{10} b/a)$ where Gt (generation time) is equal to the time, t = the elapsed time between measurement of a, the initial population, and b, the final population, divided by the number of generations, n (number of generations being equal to $3.3 \log_{10} b/a$.

RESULTS AND DISCUSSION

Growth and alpha toxin production in Thioglycollate medium.

Results for cell growth, toxin production and pH changes at 25, 35, 45 and 50 C are shown in Fig. 1-4. Data at 15 C are not presented because the organisms failed to grow and exhibited a two-log reduction in counts within 6 h of incubation.

In general, increases in alpha toxin activity closely paralleled population increases and a population of at least 400,000/ml was required before alpha toxin activity could be detected. These data are in agreement with those found by Harmon and Kautter (*7*) who showed that there is a relationship between *C. perfringens* population in a food sample and alpha toxin activity. It was observed that the incubation temperature strongly influenced the rate of alpha toxin production and maximum yield of alpha toxin. The optimum temperature for alpha toxin production was 35 C (Fig. 2). The rate of alpha toxin production was closely related to the growth rate,

[1]*Approved as Journal Series Article 101-78, Ohio Agricultural Research and Development Center, Wooster.*

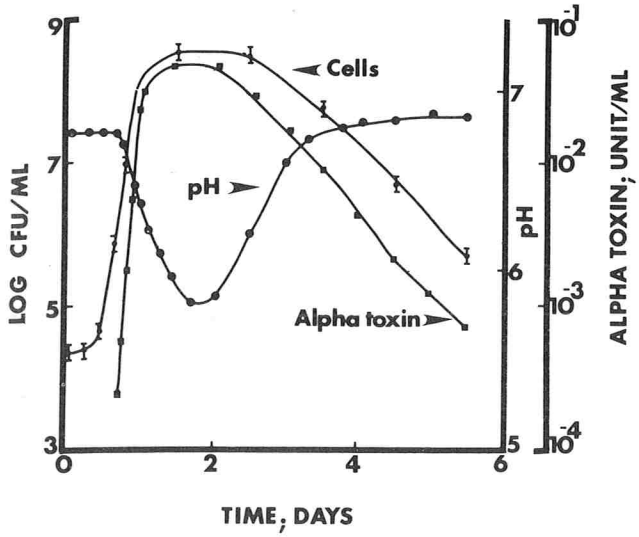

Figure 1. *Growth, pH, and alpha toxin production of* C. perfringens *in Thioglycollate medium at 25 C. Data are based on 3 trials.*

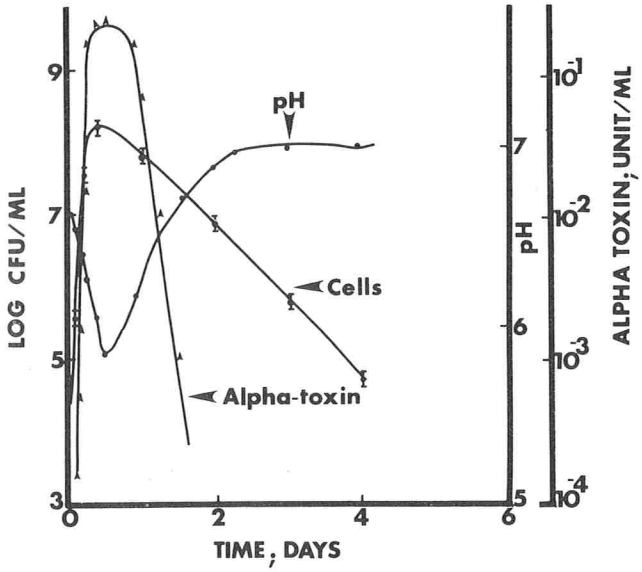

Figure 2. *Growth, pH, and alpha toxin production of* C. perfringens *in Thioglycollate medium at 35 C. Data are based on 3 trials.*

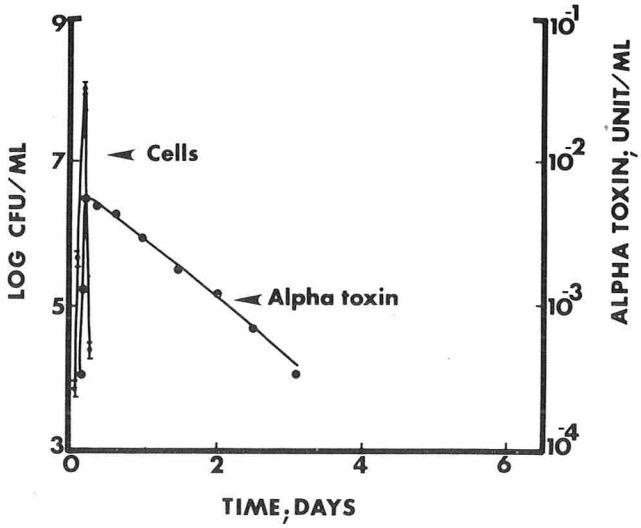

Figure 3. *Growth and alpha toxin production of* C. perfringens *in Thioglycollate medium at 45 C. pH was not determined. Data are based on 3 trials.*

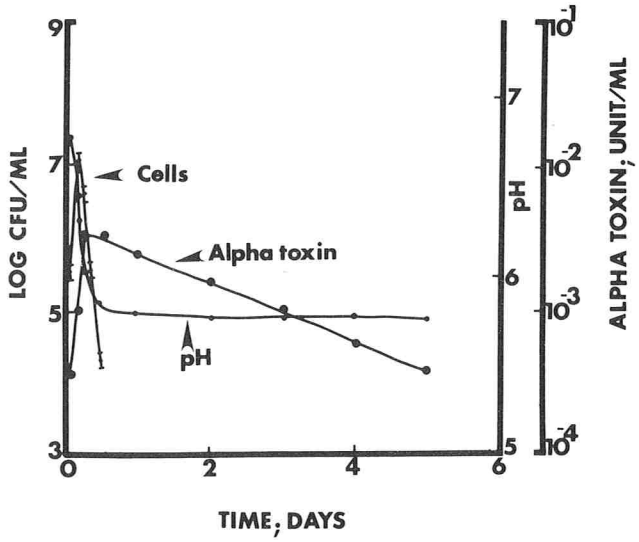

Figure 4. *Growth, pH, and alpha toxin production of* C. perfringens *in Thioglycollate medium at 50 C. Data are based on 3 trials.*

irrespective of the incubation temperature. However, the maximum yield of alpha toxin did not correlate well with the growth rate. For example, the maximum yield of alpha toxin at 35 C was 40 times greater than at 45 C, although the growth rate at 35 C was practically the same as that at 45 C.

The relationship between the viable count of *C. perfringens* on SFP base agar and the amount of alpha toxin produced at various incubation temperatures is presented in Fig. 5. At a population level of 10^8, *C. perfringens* produced approximately 1.0×10^{-1} unit of alpha toxin/ml at 35 C and 6.5×10^{-3} unit/ml at 45 C.

The optimum temperature for *C. perfringens* on the basis of minimum generation time was found to be 35 to 45 C. Rey et al. (*14*) observed the optimum growth temperature for this organisms as being between 30 and 40 C; Breed et al. (*1*), 35 and 37 C; Collee et al. (*4*) and Smith and Holdeman (*20*), 43 and 45 C. Although our strain had a generation time of 19 min., others have reported generation times of 9.5-12.5 min (*16*), 8.5 min (*2*) or 24-32 min (*19*). Apparently, generation time at optimum temperature is subject to such variables as strain, growth media, age, size of inoculum, pH and redox potential among others. At 45 C (Fig. 3), the growth was almost as fast as at 35 C, i.e. the difference in generation times between 35 C and 45 C was almost negligible (Table 1). Even at 50 C (Fig. 4), the growth was fairly rapid. At 25 C (Fig. 1), the growth was considerably slower than at 35 C. The time required to reach the stationary phase ranged from 4 to 5 h at 35, 45 and 50 C, in contrast to 22 h at 25 C.

At different incubation temperatures, the maximum viable population reached at the end of logarithmic growth phase also varied. It was approximately 2.2×10^8

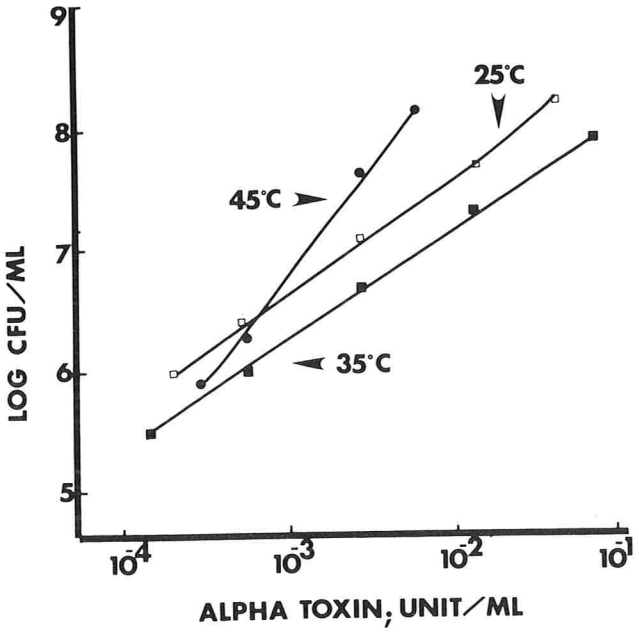

Figure 5. *Relationship between viable counts of* C. perfringens *and alpha toxin production in Thioglycollate medium at different incubation temperatures.*

TABLE 1. *Generation time, Gt; maximum yield of alpha toxin, Y; and time required for 90% reduction of alpha toxin, Dt, in Thioglycollate medium at different incubation temperatures.*

Incubation temperature	Gt	Y	Dt
(c)	(min)	(unit/ml)	(h)
25	61.5	4.47×10^{-2}	43.1
35	19.3	2.24×10^{-1}	6.3
45	19.7	5.63×10^{-3}	60.3
50	22.6	3.55×10^{-3}	121.2

organisms per ml at 25, 35 and 45 C and 1.3×10^7 organisms per ml at 50 C. In general, after the stationary phase the higher the incubation temperature, the more rapid the decline phase. The number of organisms developing in a food system is important because it is generally accepted that 100,000-1,000,000 *C. perfringens*/ml are required for an infective dose. Others (*9,10*) suggest the total dose to be 100 million organisms.

Stability of the alpha toxin was apparently dependent on the temperature and time of incubation. Generally, the destruction rate of alpha toxin closely followed first order kinetics. At the incubation temperatures examined, alpha toxin was most unstable at 35 C. At all incubation temperatures, the decline of alpha toxin activity occurred at the end of the exponential growth phase and the longer the incubation time after the stationary phase of growth had been attained, the more alpha toxin was destroyed. Shemanova et al. (*18*) demonstrated that the rapid decline of phospholipase C activity in the culture supernatant fluid coincided with the appearance of maximum proteolytic activity. However, Nord et al. (*11*) detected little proteolytic activity and doubted that proteolytic degradation was responsible for the rapid loss of enzyme activity. This discrepancy still needs to be resolved.

In general, pH of the medium dropped when maximum growth and alpha toxin production were attained (Fig. 1-4). The optimum pH for alpha toxin activity and production was reported to be approximately 7.0-7.2 by Pivnick et al. (*13*).

Growth and alpha toxin production in beef broth with ground beef and Promine-D

Before proceeding to the study of alpha toxin production by *C. perfringens* in beef broth with ground beef and Promine-D, recovery of known amounts of alpha toxin suspended in beef broth was determined. The recovery was essentially 100%, i.e. quantification of alpha toxin using beef broth as diluent yielded practically the equivalent hemolytic zone diameter as that observed when Thioglycollate medium was the diluent.

When *C. perfringens* was inoculated into beef broth with 20% ground beef and incubated at 35 C (Fig. 6), the organism grew slower (generation time, 156 min) and produced less alpha toxin (.008 unit/ml) per the same number of organisms than when it was grown in Thioglycollate medium (Gt, 19.3 min; .224 unit/ml). However, the final population level was slightly higher in the beef broth/ground beef system than in the Thioglycollate medium.

The increased use of soy protein extenders for meat products prompted the study of the effect of soy protein isolates on growth and alpha toxin production of *C. perfringens*. When 6% Promine-D was blended with 14% ground beef in beef broth under conditions where anaerobiosis was not controlled, viable counts of *C. perfringens* decreased two log cycles within 24 h (Fig. 6). No alpha toxin was detected. Busta and Schroder (*3*), using Trypticase (BBL) in Thioglycollate medium as a protein control, demonstrated that some proteins had stimulative effects on growth of *C. perfringens*, whereas

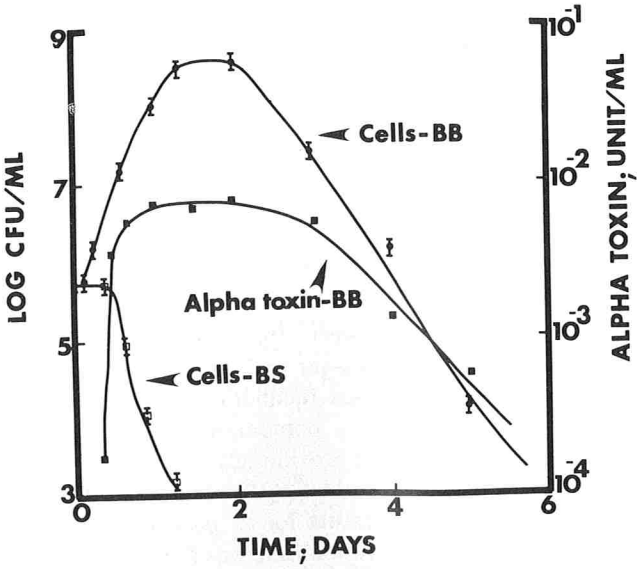

Figure 6. *Cell numbers and toxin production of* C. perfringens *at 35 C in beef broth plus 20% ground beef (BB) and cell numbers in beef broth plus 14% ground beef and 6% soy protein (BS). Data are based on 3 trials.*

others were inhibitory. They postulated that a modification in protein availability through manufacturing processes of soy protein and the presence or absence of some inhibitory factors might play a part in its effect on growth. Schroder and Busta (*16*) also have reported that under actual meat loaf conditions, addition of soy protein to beef did not affect the growth of *C. perfringens*.

In Fig. 7, the relationship between substrate, population levels and production of alpha toxin is shown. At a population level of 10^7, *C. perfringens* produced approximately 7.1×10^{-3} unit of alpha toxin/ml in Thioglycollate medium and 4.0×10^{-4} unit/ml in beef broth blended with 20% ground beef.

Thus the amount of alpha toxin produced in any food sample may be influenced by the nature of the substrate as well as the time and temperature at which it is being held. However, Harmon and Kautter (*8*) have maintained that the type of food associated with the different *C. perfringens* food poisoning outbreaks appeared to have little effect on population estimates based on the quantification of alpha toxin.

Overall, the results have demonstrated that if population levels of *C. perfringens* are to be estimated from the amount of alpha toxin detected in a food system, prior knowledge of incubation time and temperature, storage time and composition of the food is essential.

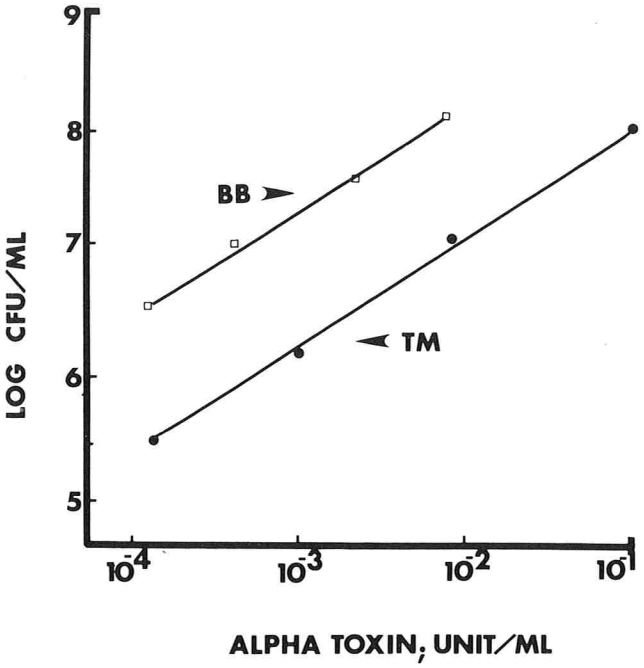

Figure 7. *Relationship between viable counts of* C. perfringens *and alpha toxin production in Thioglycollate medium (TM) and beef broth with 20% ground beef (BB).*

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Comparative Study of Procedures for Quantification of *Vibrio parahaemolyticus* in Seafoods

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(Received for publication January 8, 1979)

ABSTRACT

A collaborative study was made comparing the efficacy of combinations of two broths and two agar media for recovery and quantification of *Vibrio parahaemolyticus* and *Vibrio alginolyticus*. Broths used in the study were Glucose Salt Teepol broth (GSTB) and Horie broth (HB); agars were Thiosulfate-Citrate-Bile Salts-Sucrose agar (TCBS) and water blue-alizarin-yellow agar (WB). Frozen shrimp samples seeded with high, medium and low concentrations of *V. parahaemolyticus* and medium concentrations of *V. alginolyticus* were used in this study. Results showed that in general all media using HB gave MPN values 10 times greater than combinations employing GSTB. The HB-WB combination gave *V. parahaemolyticus* recoveries 10-fold higher than the other media combinations tested.

Since the initial isolation of *Vibrio parahaemolyticus* in 1950 from Japanese victims of seafood poisoning (*8*), the world wide distribution of these organisms in sea water and sea fish has been accepted (*2*). Because many formulae are purported to be effective for isolating and identifying *V. parahaemolyticus* (*12*), laboratories must select an appropriate procedure. The Food and Drug Administration (FDA) *Bacteriological Analytical Manual* (BAM) (*7*) specifies, as pre-enrichment, Glucose Salt Teepol Broth (GSTB) to be subsequently streaked on Thiosulfate-Citrate-Bile Salts-Sucrose agar (TCBS) in a protocol designed to isolate the organism from contaminated seafoods. Both GSTB and TCBS have been used extensively since first described in 1963 (*1,11*). Another teepol-containing medium, described in 1963 by Sakazaki (*14*), is water blue-alizarin-yellow agar (WB). Horie et al. (*10*) devised a broth used successfully for isolation of *V. parahaemolyticus* from plankton and fish. Studies by FDA (Division of Microbiology, unpublished) and Beuchat (*3,4*) indicate that Horie broth (HB) and WB may be superior to GSTB and TCBS, particularly in recovery of cold-stressed organisms. The main difference in the composition of these media is the inhibitors employed. HB is a non-selective broth and GSTB contains teepol. Teepol is also used in WB agar. The selectivity of TCBS agar depends on oxgall and sodium cholate.

This report describes a study constrasting the efficacy of GSTB with HB and TCBS with WB. Nineteen analysts in three university, three food industry, and eleven FDA laboratories participated. Shrimp were inoculated with mixtures of *V. parahaemolyticus* and *Vibrio alginolyticus*. These organisms are often isolated from common environments and food samples; however, their close association, similar habitat, morphology and growth requirements can be confusing to the analyst.

MATERIALS AND METHODS

The organisms used in this study, supplied by Robert M. Twedt, FDA, Cincinnati, OH, were *V. alginolyticus* No. Z106 and *V. parahaemolyticus* No. 553, K-type 4, Kanagawa positive. Cultures for sample seeding were grown in Trypticase Soy Broth (BBL) with 3% (w/v) NaCl (TSBS) for 24 h at 35 C. Viable counts were determined by the plate count technique, using Trypticase Soy agar (BBL) with 3% (w/v) NaCl (TSAS). The final viable count in the sample was made after frozen storage for 5 days. The initial inoculum levels were adjusted to provide a loss of one log due to freezing and storage.

Frozen, raw, peeled shrimp, demonstrated to be free of indigenous *Vibrio* bacteria, were chopped into small pieces and placed in sterile Oster blenders. The chopped shrimp were seeded with *V. alginolyticus* at a level that would provide a final viable count of 4000/g. The shrimp were then divided into three groups and further seeded with *V. parahaemolyticus* at levels that would provide final viable counts of approximately 70,000, 7000, and 700/g, respectively. The samples were diluted 1:10 with 3% saline-10% glycerol solution, blended to a homogeneous slurry, weighed into sample jars and frozen at −30 C. Each microbiologist received six samples that were shipped frozen in dry ice; two from each of the 70,000, 7000, and 700/g *V. parahaemolyticus* inoculum levels.

Each medium, ingredient and chemical employed was from a single manufacturer's batch, lot or control number. All participating analysts were provided with the same materials and formulae.

A common analytical procedural outline was followed by each microbiologist. The scheme is shown in Fig. 1. GSTB and HB were inoculated in parallel with the serially diluted sample, into three tubes at each of six dilutions, to determine the most probable number (MPN). One-gram portions were examined by inoculating double-strength GSTB and HB with 10 ml of the sample as received. After broths were incubated for 20 h at 35 C, all tubes were streaked on TCBS and WB plates with a 3.0-mm loop. After incubating the plates for 20 h at 30 C, one characteristic *V. parahaemolyticus* colony/plate was selected for the biochemical confirmation tests shown in Table 1.

RESULTS AND DISCUSSION

After frozen storage, expected counts for the three sample sets designed to contain high, medium and low *V. parahaemolyticus* concentrations were 70,000, 7000 and 700/g, respectively. Geometric means of analysts' data and estimates of replicate error are given in Table 2. For purposes of statistical analysis, all MPN values reported as $>$ 110,000/g were given the value of 240,000/g. More than half of the high concentration sample values were reported as $>$ 110,000/g when media combinations of HB-TCBS and HB-WB were used. In general, all media

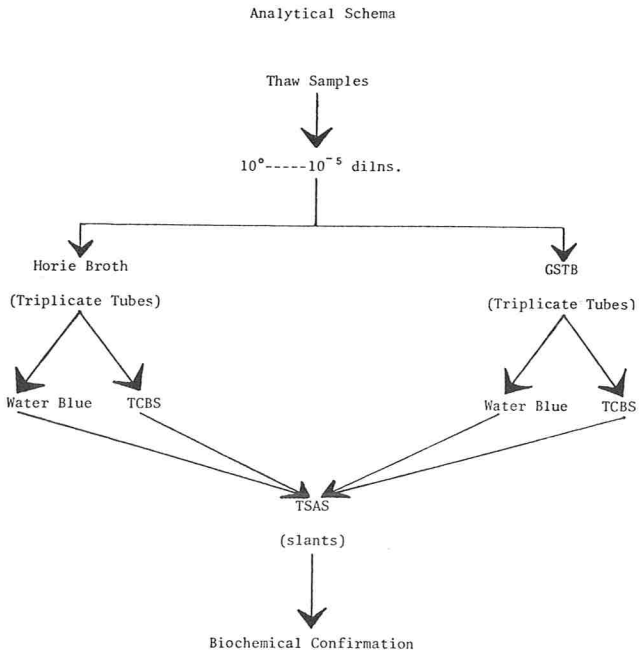

Figure 1. *Analytical schema for* V. parahaemolyticus.

TABLE 1. *Confirmation tests for* V. parahaemolyticus.

Test	Reaction
Cytochrome oxidase	+
Glucose aerobic	+
Glucose anaerobic	+
Sucrose fermentation	–
Lysine decarboxylation	+
Growth at 42 C	+
Halophilism, 8% NaCl	+
Halophilism, 10% NaCl	±
Acetoin (V.P.)	–
Triple Sugar Iron Agar Slants	alkaline slant, acid butt, no H_2S
Morphology (gram stain)	rods (negative)

using HB gave MPN values 10 times greater than those combinations employing GSTB. A non-parametric (*6*) test of significance (Kruskal-Wallis H test) was performed. Data were ranked for each concentration to determine if one condition had a significantly different average rank. All three tests (H = 17.7, 125.2, 10.9) were significant at the $a = 0.05$ level. The HB combinations seemed to be the best choice.

Components of variance (*15*) were computed for the medium and low concentrations in the HB-WB media combination. The high concentration contained too many > 110,000 values to be included in the computation. Estimates of experimental error (σ^2A) and interactions between analysts and samples (σ^2AB) were 0.11302, 0.36833 and 0.10039, respectively. The sum of these components is 0.76272. Variance values of the magnitude this study yielded are unacceptable for a regulatory procedure. However, the HB-WB combination gave *V.parahaemolyticus* recoveries 10-fold higher than other media in the study.

A previous study (*5*) demonstrated that HB was superior to GSTB in the recovery of *V. parahaemolyticus* from chilled and frozen crab meat. The work of Ray et al. (*13*) demonstrates the need to consider providing a non-selective medium for injury-repair before more selective media and may be the clue to the superiority of HB in this study. Additionally, the study contrasted WB agar with TCBS agar. Most participants in this study found it difficult to differentiate *V. alginolyticus* from *V. parahaemolyticus* colonies on WB agar. Although not indicated in the procedure, it was found that identifiable colonies would develop on WB agar with an additional 20 h of aging at room temperature. Our data and all recent publications (*5,9,13*) support the necessity for continued research in the methodology of *V. parahaemolyticus* detection.

ACKNOWLEDGMENTS

We are grateful to the following analysts who participated in the study: D. Aird, Food and Drug Administration (FDA), Dallas; L. R. Beuchat, University of Georgia; D. F. Brown, FDA, Division of Microbiology; J. Codor, Minneapolis Center for Microbiological Investigations, FDA; R. R. Colwell, University of Maryland; E. Gutter, FDA, Cincinnati; J. Kaper, University of Maryland; D. Kennedy, Thomas J. Lipton Company; J. Liston, University of Washington; S. Ludwig, FDA, Denver; R. McDonald, FDA, Los Angeles; P. McLaughlin, FDA, New York; K. O'Brien, FDA, Baltimore; G. K. Peterson, General Mills, Inc.; E. Prohaska, FDA, Boston; D. Pusch, The Pillsbury Company; E. Singleton, FDA, Atlanta; F. Stanley, FDA, Seattle; J. T. Peeler for the statistical evaluation; J. Lovett and R. M. Twedt for their editorial assistance.

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TABLE 2. *Geometric Means and Replicate Variances for Four Sets of* V. parahaemolyticus *Broth and Solid Media.*

		Broth			
		GSTB		HORIE	
		Solid media			
Concentration		TCBS	WB	TCBS	WB
High	Geometric Mean	23,000 (18)[a]	21,000 (17)	130,000	130,000
	S^2	0.37477	0.47746	b	b
Medium	Geometric Mean	2,500 (17)	2,700 (16)	25,000 (18)	35,000
	S^2	0.40040	0.14888	0.17810	0.15917
Low	Geometric Mean	250 (17)	240 (16)	1,300 (16)	1,800 (11)
	S^2	0.13533	0.16818	0.43372	0.06419

[a]Degrees of Freedom.
[b]More than 10% of values > 110,000 (set equal to 240,000).

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Harmon, *con't. from p. 917*

biology. He is a systematic, conscientious teacher, one who is attentive to students' perception of subject matter. His research has continued throughout his academic career, and the list of his publications is impressive. At the 1966 ADSA Annual Meeting, he received the Pfizer Award. Dr. Harmon has been a longtime member of the Editorial Board of the *Journal of Food Protection.*

The Award of Honor is presented in recognition of unusually outstanding contributions to the welfare of the ADSA. Dr. Harmon served the ADSA in 1966 as its representative at the World Dairy Congress in Munich, Germany. He has accepted numerous committee assignments, was a member of the Board of Directors from 1968 to 1971, was Vice-President in 1974-75 and President in 1975-1976. He then served in the positions of treasurer and Chairman of the General Arrangements Committee, which organized the combined meeting of the American Dairy Science Association and the American Society of Animal Science. at Michigan State University.

Friend, *con't from p. 917*

After receiving her M.S. degree, Friend worked as a research technician in the Biochemistry Department of the University of Nebraska Medical School, Omaha, for two years. She then moved with her husband, who is in the U.S. Air Force, to Texas and then Illinois. In June 1978, after her husband was sent to Korea, she returned to the University of Nebraska to continue work toward the Ph.D. degree.

She has been involved in a number of research areas, but has especially excelled in enzyme biochemistry, particularly the understanding of lactase immobilization and its significance to the dairy industry. Her Ph.D. work has further improved the process by which lactase is made more stable and immobilized. She also developed ways by which lactase can be regenerated once inactivated.

Economical enzymatic hydrolysis of lactose in dairy products can be a significant development for the dairy industry for several reasons: production of low lactose dairy products suitable for consumption by lactose intolerant individuals is made possible; approximately one-half billion pounds of lactose per year which is currently being wasted can be made usable; cost savings for waste treatment of cheese whey can be realized; the fermentation in producing industrial alcohol from whey for the manufacture of gasohol can be accelerated.

Sandine, *con't from p. 917*

tured products. His research has considered the importance of bacterial physiology and genetics in understanding control of fermentative processes.

Dr. Sandine holds membership in Phi Kappa Phi, Sigma Xi, Gamma Sigma Delta, American Society for Microbiology, IAMFES, and ADSA. He has served on award selection committees for ADSA and on the editorial boards of the *Journal of Dairy Science* and *Journal of Food Protection.* He has appeared as an invited speaker at numerous conferences and seminars.

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Extension of Shelf-life of Fresh, Whole Broilers, Using a Potassium Sorbate Dip

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(Received for publication January 15, 1979)

ABSTRACT

The shelf life of fresh, whole broilers was extended by dipping freshly chilled carcasses into a 5% (w/v) solution of potassium sorbate for 30 sec. One hundred broilers were removed from a processing line immediately following the final chill tank. Fifty carcasses were dipped in water for 30 sec, and 50 were dipped in the sorbate solution for 30 sec. The birds were allowed to drain and then individually bagged and stored at 3 C until spoilage odors were noted. The control birds were stored for 10 days when spoilage was evident, and the sorbate-treated birds were stored for 19 days, at which time spoilage was evident.

The relatively short shelf-life of fresh, unfrozen poultry has been an industry problem for some time. The initial microbial count greatly influences broiler shelf-life (*1*). Fresh broilers in retail outlets normally have an initial contamination level of 10^4 to 10^5 microorganisms per cm^2 (*4,14,15*) and normally can only be stored for 1 or 2 days at 3-5 C and still maintain their freshness (*10*). The major concern of processors is control of spoilage organisms (*Pseudomonas* spp.), which produce off-odors and are the cause of consumer rejection (*9*).

Reviews by Barnes (*3*), Brune and Cunningham (*4*), Dawson et al. (*5*), Mountney (*10*) and Walker and Ayres (*14*) have stated the importance of *Pseudomonas* spp. in causing spoilage of poultry stored at refrigerator temperatures. These organisms cause an off-odor when they are present in high numbers (10^7 cells/cm^2). Extension of shelf-life of fresh poultry, therefore, depends mainly on the control of pseudomonas (*3*). The most important method to delay spoilage of fresh poultry is to practice good sanitation in the processing plant, and to hold carcasses at or near 32 F (*6,10*).

Antibiotics (chlortetracycline and oxytetracyline) were used as preservatives for fresh poultry in the early 1960s. Use of 30 ppm of chlortetracyline extended the shelf-life of whole carcasses from 7 to 12 days at 4.4 C (*2*) in a laboratory test. Vaughn and Stewart (*13*) reported that it was difficult to ascertain the effect of antibiotics under commercial conditions, that resistant strains of bacteria developed and that the antibiotics were only effective against some bacteria and ineffective against yeasts and molds. Due to the threat of forming antibiotic-resistant strains of bacteria and variable results, use of antibiotics to preserve fresh poultry is no longer allowed. Dawson and Stadelman (*6*) reviewed the effect of chlorine on fresh poultry. They found that when carcasses were immersed in chill water containing 20 ppm of chlorine, bacterial counts were lower than the control groups for the first few days of storage, but shelf life was not extended.

Perry et al. (*11*) reported on extension of poultry shelf-life by processing with sorbic acid. Putrid odors were noted in the control breasts after 5 days of storage at 45 F. A treatment with 7.5% sorbic acid spray resulted in a 4-day shelf-life beyond that of the control poultry parts. The authors also noted that after 12 days of storage at 45 F the poultry parts did not exhibit any putrid odor even though bacterial counts were relatively high. Barnes (*3*) stated that when the chief spoilage organisms are inhibited, organisms producing less offensive odors may be present in much higher numbers without affecting acceptability of the product. Spoilage cannot be measured solely by total numbers; one must identify types of organisms present as well. Kaloyereas et al. (*8*) reported that ice containing glycol diformate and sorbic acid extended the shelf-life of poultry. Robach and Ivey (*12*) reported that a 5% potassium sorbate dip significantly reduced the total plate count of chicken breasts when compared to counts from untreated breasts. The same authors also observed that a 5% potassium sorbate dip markedly reduced growth rate of salmonellae inoculated onto the surface of the chicken breasts. Perry et al. (*11*) were unable to produce a poultry spoilage microflora capable of growing in the presence of 0.10% sorbic acid. The authors also demonstrated migration of sorbic acid into the poultry muscle.

The purpose of this study was to determine the shelf-life of sorbate-treated broilers and compare it to the shelf-life of conventionally-treated broilers when both groups were held at refrigerator temperatures.

EXPERIMENTAL

Sample preparation

The broilers involved in the shelf-life study were obtained from the Milford, Delaware processing plant of Shorgood Poultry Division, Bayshore Foods, Incorporated.

One hundred freshly slaughtered and chilled broiler carcasses were removed from the processing line immediately following the final chill tank. Fifty birds were dipped in water and 50 birds were dipped in a 5% (w/v) potassium sorbate solution for 30 sec. The birds were allowed to drain, and then were individually packaged in oxygen-permeable polyethylene bags. They were then placed in storage boxes and covered with ice. Eighty carcasses (40 from each group) were air-freighted to the Monsanto Company Research Laboratory in St. Louis, MO and 20 (10 from each group) were left at the processing plant for observation there.

All carcasses were stored at 3 C for the duration of the test. Sorbate analysis was done as described by Robach and Ivey (*12*). Sensory evaluations (odor and touch) were made daily.

Total plate count

Total plate counts (TPC) were determined at appropriate intervals by swab sampling 5 birds from each group. Internal counts were made by swabbing a 1-cm² area inside the body cavity, and external counts were made by swabbing a 1-cm² area of the upper breast. Swabs were made in the same area for each bird. Serial dilutions were made using 0.005 M phosphate buffer (pH 7.2) as the diluent. TPCs were determined in duplicate using Plate Count Agar (Difco). The plates were then incubated at 20 C for 72 h before counting.

Pseudomonas count

Pseudomonas counts were determined by plating the swab samples on *Pseudomonas* P agar (Difco) and incubating plates at 20 C for 72 h. Different colony types were picked and tested for oxidase activity. All isolates were tested using the API-20E differentiation system (Analytab Products, Plainview, NY).

RESULTS AND DISCUSSION

A 30-sec dip in 5% potassium sorbate solution left an average residue of 0.12% sorbate (as sorbic acid), based on the weight of the bird. The log phase of growth, as determined by the total plate count was longer for the sorbate-treated broilers than that of the control broilers (Fig 1). Subsequent growth of the microorganisms was not as rapid on the sorbate-treated broilers as was the growth on the control birds. In their review, Elliott and Michener (*7*) reported that off-odors appeared from poultry when the log number of bacteria reached from 6.5 to 8.0 per square centimeter. They also reported slime formation when the microbial population reached a log number of 7.5 to 9.0 per square centimeter.

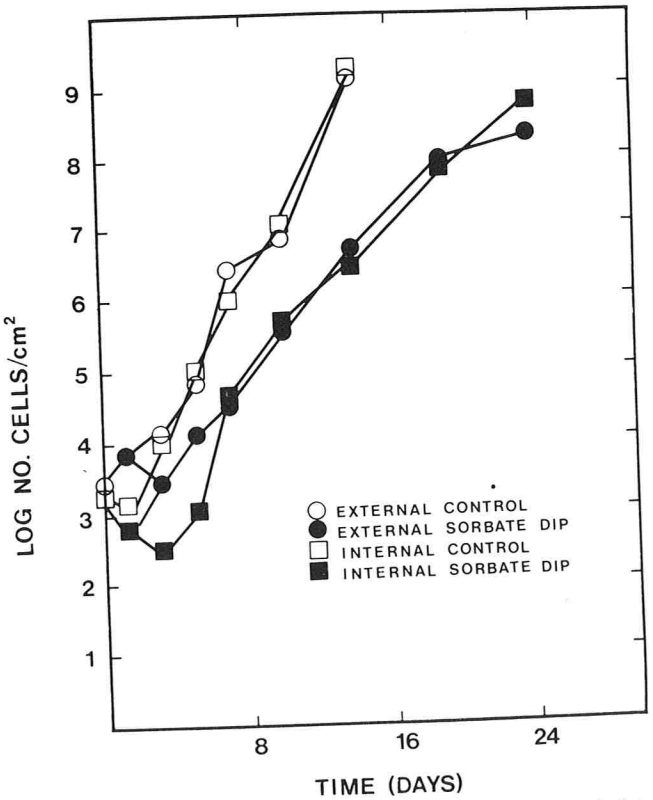

Figure 1. *The effect of a 5% potassium sorbate dip on the total plate count of whole broilers stored at 3 C.*

Spoilage odors appeared from the control birds on the tenth day of storage at 3 C (Fig. 1). The average log number of bacteria per square centimeter was 6.97 in the body cavity and 6.76 on the breast. Both these log numbers fall into the 6.5 to 8.0 range. The sorbate-treated birds did not exhibit off-odors until the 19th day of storage at 3 C (Fig. 1). The average log number of bacteria per square centimeter was 7.92 in the body cavity and 7.97 on the breast, both numbers being in the 6.5-8.0 range. Figure 2 shows that members of the genus *Pseudomonas* were the predominant bacteria present at the time of spoilage in both the sorbate-treated and control broilers.

Based on both microbiological and organoleptic evaluations, sorbate-treated whole broilers were stored for 19 days at 3 C until considered spoiled, while the control broilers could be stored only 10 days at 3 C before spoiling.

Results obtained in this study are in agreement with reports from Perry et al. (*11*) and Robach and Ivey (*12*).

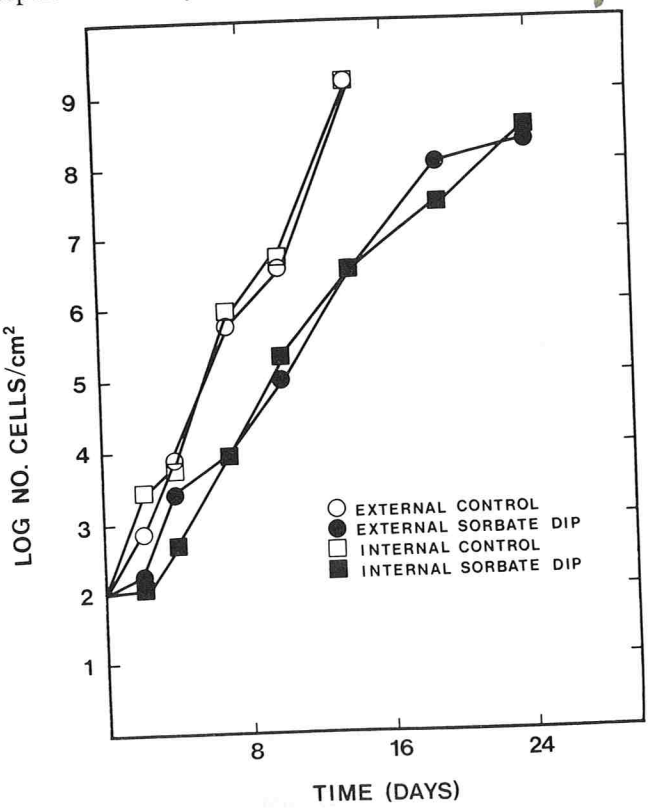

Figure 2. *The effect of a 5% potassium sorbate dip on the* Pseudomonas *count of whole broilers stored at 3 C.*

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The author extends his appreciation to George E. McCabe and Pat Sigler of Shorgood Poultry for their cooperation.

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Harper, *con't from p. 917*

The winner of the teaching award this year grew up in a midwestern university town where his father was a teacher. He graduated from his home-town university in 1946 in Dairy Manufacturing and like so many other outstanding people in the dairy industry, earned his M.S. and Ph.D. at University of Wisconsin. He joined the faculty of still another outstanding university, Ohio State, in 1949 and remains there today. Dr. Harper has authored or coauthored over 160 publications, including chapters in books used as texts in dairy manufacturing courses. He has prepared a variety of teaching materials for students, including mimeographs, slide sets, and video tapes. He now teaches 3 undergraduate and 3 graduate courses.

Professor Harper is also active in less formal aspects of teaching. He serves as an advisor to undergraduate and graduate students, is a member of his department's teaching and curriculum committee, and has been a member of a similar college-wide committee. He has contributed to the development of a system for formal evaluation of courses and instructors, of aids to instructors for effective use of new teaching mechanisms, and of systems for student evaluation of courses and instructors.

Perhaps his most unique characteristic as a teacher is an ability to encourage and develop independent thinking, along with correlation of knowledge gained in other courses to solve problems. His students are presented relevant practical problems which require that they utilize prior training, current literature, and logical thought to suggest solutions. Former students express appreciation at having learned this approach to problem-solving.

Gilliland, *con't from p. 917*

the yield of cells, and of decreasing the damage by freezing and storage are highly important to the cultured products industry. Research conducted by Dr. Gilliland and his associates on the inhibition of psychrotrophic bacteria by lactobacilli and pediococci adds to the basic knowledge and practical applications in the field of food science. Likewise, the inhibition of foodborne pathogens by *Lactobacillus acidophilus* is of practical significance. He and his associates have developed several new methods for growing, enumerating, and identifying Lactobacilli.

Dr. Gilliland won the Outstanding Teacher Award in the College of Agriculture after only 3 semesters of teaching at Oklahoma State University. He also serves on the Editorial Board of the *Journal of Food Protection.*

Call for Papers for Energy Conservation Conference

Papers are being solicited for the Second Annual Conference on Industrial Energy Conservation Technology and Exhibition, co-sponsored by the Texas Industrial Commission and the U.S. Dept. of Energy. The Conference will be held in Houston, April 13-16 at the Hyatt Regency Hotel.

Papers are sought which will focus on several areas as they directly relate to energy efficiency improvement in industrial operations.

Authors will be invited to present papers of 20-25 minutes duration. A short discussion period will be allowed following each paper. Each prospective author should submit a 300-350 word abstract of his or her proposed presentation by December 15, 1979, to:

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Inquiries concerning the papers or the conference should be addressed to:

Mr. M. A. Williams, P.E.
Technical Program Director
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Austin, Texas 78731
(512)345-8052

Journal of Food Protection Vol. 42, No. 11, Pages 858-861 (November, 1979)

Inhibition of *Clostridium botulinum* Types A and B by Phenolic Antioxidants

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(Received for publication February 28, 1979)

ABSTRACT

Butylated hydroxyanisole (BHA) was inhibitory to growth of three proteolytic strains of *Clostridium botulinum* in prereduced thiotone yeast-extract glucose medium (TYG) (pH 7.0) at 37 C. There was outgrowth and toxin production by strain 10755A spores when 25 ppm of BHA was present in the medium, whereas growth was inhibited by 50 ppm of BHA. Strains 62A and 213B were inhibited by 25 ppm of BHA throughout an incubation period of 7 days. Butylated hydroxytoluene (BHT) and propyl gallate (PG) were less effective in inhibiting outgrowth of *C. botulinum* spores than was BHA. Spore outgrowth and toxin production were inhibited in the presence of 200 ppm of BHT in the TYG. PG exhibited the least inhibitory activity of the antioxidants tested. None of the levels (25 to 200 ppm) of PG tested delayed outgrowth or toxin formation for more than 24 h.

The antimicrobial effects of the phenolic antioxidants have not been extensively studied. Ward and Ward (*9*) reported that 10,000 ppm of butylated hydroxytoluene (BHT) was only slightly inhibitory to *Salmonella senftenberg* 775W. Chang and Branen (*1*) found that 150 ppm of butylated hydroxyanisole (BHA) in nutrient broth inactivated *Staphylococcus aureus* and 400 ppm of BHA was lethal to *Salmonella typhimurium*. Robach et al. (*4*) reported that *Vibrio parahaemolyticus* was inhibited by 50 ppm of BHA in Trypticase Soy Broth containing 2.5% NaCl, whereas 400 ppm of BHA was required to inhibit growth in a crab meat homogenate.

The objective of this study was to determine what effect phenolic antioxidants may have on outgrowth and toxin formation in a laboratory medium by spores of three strains of *Clostridium botulinum*.

MATERIALS AND METHODS

Identification and maintenance of the test organisms

A lyophilized culture of *C. botulinum* 10755A (NCTC 2021) was obtained from the VPI&SU Anaerobe Laboratory. Lyophilized cultures of *C. botulinum* 62A and *C. botulinum* 213B were obtained from the Swift Research Center. The cultures were identified using the results of biochemical tests, gas chromatographic analysis of the volatile fatty acid fermentation products and toxicity tests as described by Holdeman et al. (*2*).

The lyophilized cultures were inoculated into a prereduced chopped meat glucose medium and incubated at 37 C for 24 h. Spore suspensions prepared according to the method of Rowley and Feeherry (*5*), were diluted to 5.0×10^8 spores/ml, and stored at 4 C in glass-distilled water.

Antioxidants

Butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) were obtained from the Eastman Chemical Company (Kingsport, TN). Propyl gallate (PG) was obtained from Naarden International (Owings Mill, MD). The BHA, BHT and PG were dissolved in 95% ethanol to a final concentration of 2% (w/v), filter sterilized (Millipore 0.22μ) and stored in sterile glass-stoppered flasks at room temperature.

Growth media

The basal growth medium (TYG) consisted of 1% thiotone (BBL), 1% yeast extract (Difco), 0.1% glucose, 0.05% cysteine hydrochloride and 0.0001% resazurin. The dehydrated media used in all experiments were taken from the same control lot to insure constancy of the media.

TYG used in the culture tube experiments was anaerobically prepared and dispensed according to the methods of Holdeman et al. (*2*). The prereduced TYG was adjusted to pH 7.0 ± 0.1 with 8 N sodium hydroxide and 5 N hydrochloride acid, dispensed into 18 × 150 mm anaerobic culture tubes (Bellco Glass, Inc., Vineland, NJ), stoppered under oxygen-free nitrogen and autoclaved at 121 C for 15 min in a tube press (Bellco Glass, Inc., Vineland, NJ). The tubes were allowed to cool at room temperature before addition of the various antioxidants. BHA, BHT and PG were aseptically added to the desired concentrations while sparging the tubes with anaerobe-grade carbon dioxide using the VPI Anaerobic Culture System (Bellco Glass, Inc., Vineland, NJ).

Growth studies

Culture tubes containing 10 ml of the prereduced TYG broth and appropriate amounts of antioxidants were inoculated using the methods described by Holdeman et al. (*2*). The spore inoculum was prepared by diluting the refrigerated spore crop in prereduced 0.1% peptone, 0.05% cysteine hydrochloride, 0.001% resazurin (pH 7.0) to a concentration of approximately 5×10^4 spores/ml. The spore suspension was heat-shocked for 10 min at 80 C, 0.1 ml of this spore suspension was anaerobically inoculated into each tube and tubes were incubated at 37 C.

The initial inoculum level was approximately 3×10^2 spores/ml. Counts were made in prereduced PYA according to the procedure of Pierson et al. (*3*) and Shoemaker and Pierson (*6*). Growth of the three test organisms in culture tubes containing TYG was monitored by determining the absorbance of the cultures at 600 nm in a Bausch and Lomb Spectronic 20 Spectrophotometer. Absorbance readings were made at 24-h intervals and duplicate tubes were used at each level of antioxidant.

Detection of toxin

Two-ml samples were withdrawn from each culture when turbidity was observed. The culture fluid was centrifuged at $20,000 \times g$ for 10 min (10 C) to remove cells and debris. The resulting supernatant fluid was tested for toxicity by injecting intraperitoneally 0.5 ml into each of two white ICR mice (18-22 g). When samples showed the presumptive presence of toxin, two more mice were inoculated with the toxic culture fluid, and two mice were inoculated with a toxin-antitoxin (antitoxin obtained from the Center for Disease Control, Atlanta, GA) mixture that was diluted 1:10 in phosphate (0.05 M)-gelatin (0.1%) buffer (pH 6.2) (*2*). All of the chemical additives were tested for toxicity to mice at the highest level employed in this study by inoculating 0.5 ml of TYG broth containing the additives intraperitoneally into two mice. In all cases the mice were observed for 3 days.

[1]*Present Address: Monsanto Industrial Chemicals, Co., 800 N. Lindbergh Boulevard, St. Louis, Missouri 63166*

RESULTS AND DISCUSSION

Butylated hydroxyanisole

The data obtained for the effect of BHA on spores of *C. botulinum* 10755A, 62A, and 213B in prereduced TYG broth (pH 7.0) are presented in Table 1. Overall, BHA appeared to have a profound effect on outgrowth and toxin formation by *C. botulinum* spores. Strains 10755A and 213B had outgrowth and toxin in the medium within 1 day, whereas at 2 days significant growth and toxicity were observed with strain 62A. Addition of 50 ppm of BHA to the TYG medium with strain 10755A was inhibitory to the spores through 21 days. Addition of 25 ppm of BHA was completely inhibitory to both strains 62A and 213B throughout an incubation period of 7 days.

The antimicrobial effects of BHA are relatively unknown; only recently has research been directed to this area (*1,4*). These data indicate that BHA may hold some promise as an antimicrobial agent in foods in addition to its well known role as an antioxidant (*8*). The similarity of structure between BHA and the esters of parahydroxybenzoic acid has been alluded to in earlier reports (*1,4*). With such a similarity in structure, the mechanism of action could also be closely related. The phenolic ring of BHA could interfere with the cell membrane or compete with coenzyme for protein, as suggested by Wyss (*10*). Another possible mode of action could involve the ability of BHA to be a hydrogen donor, thus acting as a reducing agent. Many clostridia lower the oxidation-reduction potential of their environment by production of large amounts of electrons. Smith (*7*) studied the effects of several reducing agents on growth of spores of *C. botulinum* type E and reported that excessive amounts of a reducing agent were inhibitory to outgrowth of the spores. It is possible that the antioxidants inhibit or compete with the electron donor systems within the cells and thereby inhibit growth.

Butylated hydroxytoluene

Table 2 outlines the results obtained on the effect of BHT on outgrowth and toxin formation by *C. botulinum* spores in prereduced TYG broth. When the medium contained 25 ppm of BHT, outgrowth of 10755A spores was delayed 1 day, whereas addition of 50 and 100 ppm of BHT inhibited outgrowth and toxin production for 3 days. BHT at 200 ppm inhibited outgrowth of the spores for the entire incubation period. BHT was generally more effective against the 62A and 213B spores, as also was observed with BHA. Addition of 25 ppm of BHT delayed outgrowth and toxin formation of the 62A spores until the third day of incubation, whereas 100 ppm of BHT inhibited the spores for the entire incubation period. When 25 ppm of BHT was added to the TYG, outgrowth and toxin formation of the 213B spores were first observed after 3 days of incubation. Addition of 50 ppm of BHT gave a similar result, whereas outgrowth and toxin formation in the presence of 100 ppm of BHT were not observed until the seventh day of incubation. When 200 ppm of BHT was incorporated into the growth medium, outgrowth and toxin formation were not observed during the incubation period.

While not as effective as BHA, BHT still exhibited an inhibitory effect on *C. botulinum* spores. BHT is generally not as effective an antioxidant as is BHA (*8*). If the mode of action is related to the antioxidant properties of the compound, it could be surmised that the more effective the compound is as an antioxidant, the more effective it will be as an antimicrobial agent.

Propyl gallate

Table 3 presents the results obtained on the effect of PG on outgrowth and toxin formation by spores of *C. botulinum* type A and B in prereduced TYG broth. Propyl gallate was not as effective as BHA or BHT in inhibiting growth and toxin production by the three strains of *C. botulinum*. Addition of 25, 50 and 100 ppm

TABLE 1. *The effect of butylated hydroxyanisole on the growth and toxin production of* C. botulinum *type A and B spores incubated at 37 C in prereduced TYG broth.*

Strain	Concentration (ppm)	Absorbance[a] after days of incubation							
		0	1	2	3	4	5	7	21
10755A									
	0	0	0.78[b]	0.67	0.54	0.37	0.35	0.23	0.10
	25	0	0	0	0	0	0	0.19[b]	0.09
	50	0	0	0	0	0	0	0	0[c]
	100	0	0	0	0	0	0	0	0[c]
	200	0	0	0	0	0	0	0	0[c]
62A									
	0	0	0.02	0.84[b]	0.67	0.51	0.50	0.44	NT[d]
	25	0	0	0	0	0	0	0[c]	NT
	50	0	0	0	0	0	0	0[c]	NT
213B									
	0	0	0.71[b]	0.51	0.40	0.34	0.32	0.22	NT
	25	0	0	0	0	0	0	0[c]	NT
	50	0	0	0	0	0	0	0[c]	NT

[a]Absorbance at 600 nm, average of duplicate tubes.
[b]Day toxin first observed.
[c]Non-toxic at the end of incubation period.
[d]NT - not tested.

of PG delayed toxin formation by 10755A for 1 day, whereas 200 ppm delayed growth until the second day of incubation, and toxin formation until the third day. When 62A spores were subjected to 25, 50 and 100 ppm of PG in the growth medium, outgrowth was slightly delayed, but toxin was present on the second day of incubation, just as in the control. When 200 ppm of PG was present in the TYG, growth and toxin production were delayed by 24 h. Addition of 25 and 50 ppm of PG to the growth medium had very little effect on outgrowth and toxin formation by 213B spores, whereas addition of 100 and 200 ppm of PG delayed toxin formation by 24 h. PG showed the least promise of the antioxidants tested as possible inhibitors of *C. botulinum* spores.

Toxin challenge studies

Death of the test mice and survival of the mice inoculated with the toxin-antitoxin mixture in all instances indicated neutralization of the toxin by the antitoxin. The results also showed that death was due to the toxin and not to other components present in the supernatant fluid. All mice inoculated with TYG broth containing the various antioxidants survived, indicating that at the levels employed in this study, none was toxic to mice.

ACKNOWLEDGMENT

This study was supported by the VPI&SU Agricultural Experiment Station.

TABLE 2. *The effect of butylated hydroxytoluene on the growth and toxin production of* C. botulinum *type A and B spores incubated at 37 C in prereduced TYG broth.*

Strain	Concentration (ppm)	Absorbance[a] after days of incubation							
		0	1	2	3	4	5	7	21
10755A	0	0	0.78[b]	0.67	0.54	0.37	0.35	0.23	0.10
	25	0	0	0.90[b]	0.82	0.58	0.31	0.11	0.04
	50	0	0	0	0	0.54[b]	0.59	0.11	0.02
	100	0	0	0	0	0.22[b]	0.20	0.18	0.01
	200	0	0	0	0	0	0	0	0[c]
62A	0	0	0.02	0.84[b]	0.67	0.51	0.50	0.44	NT[d]
	25	0	0	0	0.85[b]	0.50	0.40	0.31	NT
	50	0	0	0	0.75[b]	0.45	0.40	0.31	NT
	100	0	0	0	0	0	0	0[c]	NT
	200	0	0	0	0	0	0	0[c]	NT
213B	0	0	0.71[b]	0.51	0.40	0.34	0.32	0.22	NT
	25	0	0	0	0.16[b]	0.41	0.34	0.32	NT
	50	0	0	0	0.13[b]	0.51	0.35	0.33	NT
	100	0	0	0	0	0	0	0.17[b]	NT
	200	0	0	0	0	0	0	0[c]	NT

[a]Absorbance at 600 nm, average of duplicate tubes.
[b]Day toxin first detected.
[c]Non-toxic at end of incubation period.
[d]NT - not tested.

TABLE 3. *The effect of propyl gallate on the outgrowth and toxin production of* C. botulinum *type A and B spores incubated at 37 C in prereduced TYG broth.*

Strain	Concentration (ppm)	Absorbance[a] after days of incubation							
		0	1	2	3	4	5	7	21
10755	0	0	0.78[b]	0.67	0.54	0.37	0.35	0.23	0.10
	25	0	0.03	0.26[b]	0.29	0.30	0.25	0.17	0.05
	50	0	0.02	0.28[b]	0.29	0.26	0.19	0.16	0.04
	100	0	0.01	0.35[b]	0.26	0.24	0.18	0.12	0.14
	200	0	0	0.35	0.40[b]	0.36	0.19	0.13	0.05
62A	0	0	0.02	0.84[b]	0.67	0.51	0.50	0.44	NT[c]
	25	0	0	0.59[b]	0.43	0.30	0.19	0.17	NT
	50	0	0	0.58[b]	0.45	0.28	0.18	0.15	NT
	100	0	0	0.60[b]	0.34	0.29	0.15	0.09	NT
	200	0	0	0	0.47[b]	0.42	0.39	0.20	NT
213B	0	0	0.71[b]	0.51	0.40	0.34	0.32	0.22	NT
	25	0	0.69[b]	0.43	0.25	0.16	0.15	0.06	NT
	50	0	0.55[b]	0.54	0.25	0.13	0.12	0.05	NT
	100	0	0.11	0.60[b]	0.31	0.15	0.10	0.04	NT
	200	0	0	0.37[b]	0.42	0.10	0.07	0.05	NT

[a]Absorbance at 600 nm, average of duplicate tubes.
[b]Day toxin first detected.
[c]NT - not tested.

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Edmondson, *con't. from p. 913*

chairman of the dairy department from 1962-67, and chairman of the department of food science and nutrition from 1967-69. He's served on a number of university committees, including the university Interdisciplinary Nutrition Committee, Committee on Human Subjects in Research, Agriculture Honors Committee, Graduate Faculty Senate, and the Missouri Council for Agriculture. As an advisor, Dr. Edmondson works with 30 to 40 undergraduate students each year and carries a heavy advisement load in the sanitary sciences graduate program.

The achievements of this year's Educator Award recipient include affiliate activities, work in other organizations, as well as community activities. Dr. Edmondson has been a member of the Missouri affiliate of IAMFES for over 25 years, serving as secretary and, since 1957, program chairman. In 1977-78 he worked on a committee to rewrite the constitution and bylaws of the new state organization, the Missouri Milk, Food and Environmental Health Association. He was also the first president of that group, which merged the local affiliates of IAMFES and the National Environmental Health Association. Dr. Edmondson served IAMFES as program coordinator for the International's 1978 Annual Meeting, held in Kansas City, MO.

Dr. Edmondson has also served other sanitation-related organizations, including the Interstate Milk Shipper's Conference, Missouri Public Health Association, Institute of Food Technologists, and American Dairy Science Association. His community and service establishments include serving as president of his district water board from 1968-74, president of the county board of education, 1971-77, and chairman of the board of trustees of the Missouri United Methodist Church, 1969-74. Dr. Edmondson is also frequently a source of counsel for persons in the community needing public health and food sanitation advice.

The National Future Farmers of America (FFA) selected Dr. Edmondson as superintendent of their milk quality and dairy foods contest. He annually organizes and supervises the contest which involves over 100 high school students nationwide. Among previous awards he's won are the American Dairy Association's Cow Bell Award, its service award, Honorary American Farmer, awarded by the FFA, and recognition for outstanding work with the Food Technology Conference in 1975 of the St. Louis Institute of Food Technologists.

Thompson, *con't from p. 914*

Thompson then transferred to the Kansas City regional office where he remained until he accepted the position of Chief, Milk Sanitation Program of the United States Health Service.

In 1970 the U.S. Public Health Service presented its Commendation Medal to Thompson for continued high quality work, noteworthy technical and professional contributions to the science and administration of public health.

Thompson has actively promoted milk and food sanitation as a member of IAMFES as well as through serving on the Board of Directors of the National Mastitis Council, the Executive Committee of the National Conference on Interstate Milk Shipments, the Steering Committee of the 3A Sanitary Standards Committees, and the Joint FAO/WHO Committee of Governmental Experts on the Code of Principles Concerning Milk and Milk Products.

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A Research Note

Patulin in Apple Juice from Roadside Stands in Wisconsin

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ABSTRACT

High-performance liquid chromatography was used to determine the amount of patulin in 40 samples of apple juice sold at roadside stands in Wisconsin. Samples were taken during juice-producing seasons in 1976 and 1977. Nine of 11 samples obtained in 1976 contained from a trace (approximately 10 μg) to 350 μg of patulin per liter. Fourteen of 29 samples obtained in 1977 contained from a trace to 204 μg of patulin per liter. The average concentration of patulin in all samples was 51 μg/l; most samples contained less than 50 μg/l.

Patulin [4-hydroxy-4H-furo (3,2c) pyran-2(6H)-one] is a toxic secondary metabolite produced by some molds in several genera of fungi. It is acutely toxic to mice (*2*) and is mutagenic to yeast cells (*8*). Patulin also acts as a teratogen (*3*) and produces tumors in rats (*4*).

Apples are sometimes rotted by the patulin-producing *Penicillium expansum* and hence occasionally contain patulin (*1,6,10*). Thus it is not unusual that investigators also found patulin in apple juice (*9*). Some researchers have done limited surveys on the frequency of occurrence and amount of patulin in apple juice. Ware et al. (*10*) noted that 8 of 13 commercial apple juices contained from 44 to 309 μg of patulin per liter. Patulin at concentrations of 6 to 16,400 μg/l was also found in 8 of 20 samples of home-made apple juice (*6*).

Work described in this report was done to determine if patulin occurred in apple juice as sold at roadside stands in Wisconsin.

MATERIALS AND METHODS

Samples of apple juice were obtained from roadside stands or directly from producers. All juices were unpasteurized, unclarified and none contained additives.

Apple juice was tested for patulin using high-performance liquid chromatography, as described by Ware et al. (*10*). All samples were kept refrigerated for no more than 2 days after collection and before analysis.

Chromatography was accomplished using a Waters pump (Model M6000A, Waters Assoc., Milford, MA), a Schoeffel detector (Model SF 770, Schoeffel Inst. Corp., Westwood, NJ) and a Whatman Partisil-10 column (Whatman Inc., Clifton, NJ). All solvents used in the analysis were obtained from Burdick and Jackson Laboratories (Muskegon, MI) and were of spectrophotometric grade. Solvents (Aldrich Chemical Co., Milwaukee, WI) used for extractions and column chromatography were analytical reagent grade.

RESULTS

Results obtained from our tests are given in Table 1. Samples from 1976 contained an average of 115 μg of patulin per liter. This compares to 26 μg/l in samples from 1977. The amounts of patulin found in 1976 were more uniformly distributed over the entire range of concentrations than were the amounts found in samples from 1977. Most samples from 1977 contained less than 50 μg of patulin per liter. The difference in results from the 2 years is also illustrated by the smaller percentage of samples containing patulin in 1977 (48%) as compared to 1976 (82%). The ranges of values for samples containing patulin were approximately 10 to 350 μg/l in 1976 and 10 to 204 μg/l in 1977.

DISCUSSION

Fifty eight percent of the samples tested in this study contained patulin. This was similar to the value of 61% obtained by Ware et al. (*10*) when they examined 13 samples. However, our results differed in that most of our samples had less than 50 μg/l, whereas the positive samples of Ware et al. had a minimum concentration of 44 μg of patulin per liter.

TABLE 1. *Amounts of patulin in apple cider sold at roadside stands in Wisconsin (1976-77).*

Concentration (μg/l)	1976		1977		Combined	
	No.	%	No.	%	No.	%
< 10	2	18	15	52	17	43
10	2	18	2	7	4	10
11-50	0	0	8	28	8	20
51-100	2	18	2	7	4	10
101-200	2	18	1	3	3	8
201-300	2	18	1	3	3	8
> 300	1	9	0	0	1	3
Ave. (μg/l)	115.2		26.0		50.7	
Std. dev.	121.5		45.3		83.2	

Differences noted for samples from 1976 and 1977 could have resulted from one or several factors. First, there may have been variability in sampling of juice. In 1977, a larger number of samples were obtained from more sources throughout the state than in 1976; samples in 1976 were obtained primarily from southern Wisconsin. Second, differences in environmental conditions (samples from northern and southern versus samples from only southern Wisconsin) to which apples and/or juice were exposed could account for some of the variation in results. Third, there may have been differences in quality control among producers and between years. It is possible that such factors as market prices or crop yields could have encouraged use of apples of lower quality in 1976 than in 1977. Fourth, there may have been differences in elapsed time between pressing of juice and taking of samples.

A question which has not been answered is that of health hazards resulting from patulin in apple juice. If one assumes that humans have the same oral LD_{50} as do mice, 35 mg per kg of body weight, (*2*), 70-kg persons would each have to consume about 2.45 g of patulin to cause death of 50% of the persons in a given group. To get this amount of patulin it would be necessary to consume about 150 liters of juice which contains 16,400 mg of patulin (*6*) per liter. Thus the possibility of fatality from drinking apple juice contaminated with patulin is virtually nonexistant for healthly humans. However, one should keep in mind the possibility of effects other than death being caused by sublethal doses of patulin.

Although observations on humans are lacking for this aspect of patulin's toxicity, the fact that patulin is a possible carcinogen or teratogen should be enough reason to limit its content in foods.

There is evidence of cumulative toxic action when 25 mg of patulin per kg of body weight are given orally to mice daily for 14 days. This toxic action includes pulmonary edema in surviving mice (*2*). Other sublethal effects which have been noted in animals are accumulation of liquid in the peritoneal cavity and extensive hemorrhages (*7*).

Our results show that apple juice from roadside stands in Wisconsin contained patulin about as often as did commercial apple juice in the Washington D.C. area. Results also show that there was a wide range in amount of patulin in samples but most contained relatively small amounts of the mycotoxin.

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Ascorbic Acid and Ascorbate Cause Disappearance of Patulin from Buffer Solutions and Apple Juice

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ABSTRACT

High-performance liquid chromatography was used to measure patulin in studies on the effect of ascorbic acid or ascorbate on stability of the mycotoxin. First, patulin was added to give a final concentration of 5000 μg per liter of buffer at pH 7.5 and 25 C to which 2% (w/v) of sodium ascorbate was added at Day 0 or Day 6. The rate of disappearance of patulin increased immediately after addition of ascorbate. Second, patulin was added to give a final concentration of 5000 μg per liter in a buffer at pH 3.5 and 25 C to which 3, 1, 0.5, 0.15 or 0% (w/v) of an ascorbic acid: ascorbic mixture (Vit C) was added. The amount of patulin decreased at a faster rate in samples which contained the Vit C than in the control samples without Vit C. Rates of disappearance of the mycotoxin increased with an increasing concentration of Vit C. Third, patulin was added to give a final concentration of 300 μg per liter in samples of apple juice to which 5 and 0% Vit C was added. Samples were held at 10 C throughout the experiment. Patulin disappeared from apple juice when Vit C was present, but the mycotoxin was quite stable in its absence.

Patulin [4-hydroxy-4H-furo (3,2c) pyran-2(6H)-one] is a toxic secondary metabolite produced by some molds in several genera of fungi. It is acutely toxic to mice (*4*) and is mutagenic to yeast cells (*12*). Patulin also acts as a teratogen (*5*) and produces tumors in rats (*7*).

Apples are sometimes rotted by the patulin-producing *Penicillium expansum* and hence occasionally contain patulin (*3,8,15*). Patulin can also be present in apple juice. Ware et al. (*14*) found that 8 of 13 commercial apple juice samples contained from 44 to 309 μg of patulin per liter, whereas Brackett and Marth (*2*) noted that 23 of 40 samples of apple juice from roadside stands in Wisconsin contained from 10 to 350 μg of patulin per liter. The maximum concentration of patulin found in apple juice thus far is 16,400 μg per liter (*9*).

Little work dealing with removal of patulin from foods has been reported. Lovett et al. (*10*) found that trimming decayed tissue from rotted apples decreased patulin levels in juice made from those apples by 93 to 99%. Filtration through activated charcoal completely removed patulin from apple juice which contained 30 μg of the toxin per ml (*13*).

We investigated the effects of ascorbic acid and sodium ascorbate on patulin. The purpose of this research was to see if ascorbate could be used to inactivate patulin in apple juice.

MATERIALS AND METHODS

Preparation of test materials

Experiments were done using buffers at pH 7.5 or 3.5 and apple juice as the reaction liquids. The buffer at pH 7.5 was prepared by adding 0.1 N NaOH (Mallinkrodt Inc., St. Louis, MO) and 0.1 N Na_2HPO_4 (Mallinkrodt Inc., St. Louis, MO). Enough patulin (Calbiochem, La Jolla, CA) was then added to the solution to give a final concentration of 5000 μg of patulin per liter of solution. Two-hundred and fifty ml of this solution were then dispensed into each of three 450-ml screw-capped bottles. One bottle served as a control to which no sodium ascorbate (Calbiochem, La Jolla, CA) was added. The second and third bottles received enough ascorbate to give 2% (w/v). The ascorbate was added to the second and third bottles on Day 0 and Day 6, respectively. The pH value of the solutions containing ascorbate was maintained by adding 1 ml of 0.1 N NaOH per bottle. Samples were analyzed for amount of patulin after 0, 2, 4, 6, 9 and 14 days at 25 C.

The solution at pH 3.5 was Sorensen's glycine buffer (*6*). A combination of ascorbic acid and ascorbate (this combination will be referred to as Vit C) was used in place of the individual acid or salt to minimize the problem of maintaining pH by the buffer. Concentrations of Vit C used were 0, 3, 1, 0.5 and 0.15% (w/v). A 350-ml quantity of each concentration was dispensed into a 450-ml screw-capped bottle and enough patulin was added on Day 0 to give a final concentration of 5000 μg/l. Experiments using this buffer also were done at 25 C.

Samples of apple juice were prepared by pasteurizing them in a steamer for 15 min followed by cooling to 4 C. Enough patulin was added to give a final concentration of 300 μg/l. No patulin was added to the control. Samples were analyzed for patulin on days 0, 3, 7, 11, 14 and 21.

Quantification of patulin

Amounts of patulin were determined by use of high-performance liquid chromatography (HPLC). Samples of apple juice were analyzed using the method described by Ware et al. (*14*). Buffer samples were analyzed in a similar manner but clean-up with column chromatography was eliminated.

HPLC was done using a Waters pump (model M6000A, Waters Assoc., Milford, MA), a Schoeffel detector (model SF 770, Schoeffel, Inst. Corp., Westwood, NJ) and a Whatman Partisil-10 column (Whatman Inc., Clifton, NJ). All solvents used in the analysis were from Burdick and Jackson (Burdick and Jackson Laboratories, Muskegon, MI) and were of spectrophotometric grade. Solvents (Aldrich Chemical Co., Milwaukee, WI) used for extractions and column chromatography were analytical reagent grade. All results are expressed as averages of duplicate samples.

RESULTS

Effect of ascorbate on patulin at pH 7.5

Figure 1 illustrates the effect of ascorbate, added initially and on Day 6, on concentration of patulin. Patulin disappeared rapidly when sodium ascorbate was added at Day 0 or Day 6. However, patulin disappeared at the same rate as in the control before sodium ascorbate was added to the sample at Day 6.

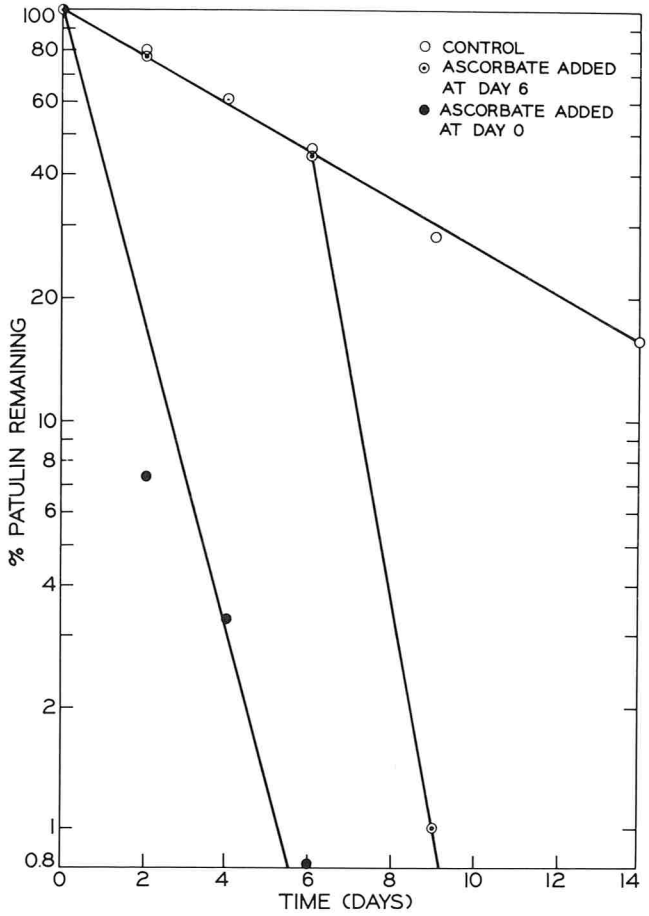

Figure 1. *Decrease of patulin content in a buffer at pH 7.5 to which 2% ascorbic acid was added on Day 0 and Day 6.*

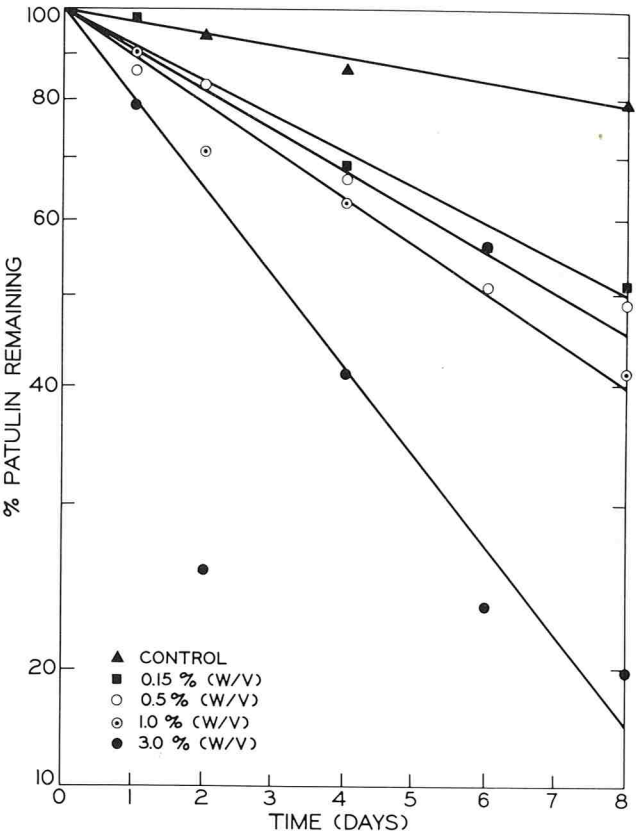

Figure 2. *Decrease of patulin content in a buffer at pH 3.5 to which 3.0, 1.0, 0.5, 0.15 and 0% Vit C was added.*

Effect of Vit C on patulin at pH 3.5

Figure 2 shows the changes in concentration of patulin caused by different amounts of Vit C at pH 3.5. The data show that the most rapid disappearance of patulin occurred when the highest concentration of Vit C was tested and that the rate of disappearance decreased as the concentration of Vit C decreased.

Effect of Vit C on patulin in apple juice

Figure 3 gives data to show that addition of Vit C caused a more rapid disappearance of patulin than occurred in the control sample where patulin was quite stable. However, the decrease in amount of patulin was not as rapid as in the previous experiments.

DISCUSSION

Addition of ascorbate or a mixture of ascorbate and ascorbic acid always increased the rate at which patulin disappeared from solution and also the amount that disappeared when compared with controls. Increasing the concentration of ascorbate or Vit C also accelerated the rate of disappearance of patulin. The amount of patulin in the apple juice decreased slower, with and without Vit C added, than it did when buffers were used. Presumably this is a result of the lower temperature at which the experiment was done and the greater stability of patulin in apple juice than in buffer solutions.

The mechanism by which patulin disappeared is not known. However, it is possible that a metal-catalyzed oxidation of ascorbate or ascorbic acid occurred. This reaction yields singlet oxygen or free radical forms of a metal-ascorbate complex (*11*). Aurand et al. (*1*) describe this mechanism in their discussion of singlet oxygen in lipid peroxidation. The singlet oxygen generated by a series of reactions could attack the conjugated double bonds of patulin. This would change the structure of patulin and account for the decrease in detectable patulin (absorbance at 254 nm).

Disappearance of patulin probably reflects inactivation since its structure is likely to have been changed. If ascorbate does, in fact, cause inactivation of patulin, it might be of value in treating apple juice that contains the mycotoxin. This would be of most value in juices which are to be stored for several weeks or longer before consumption so enough time would elapse to allow for degrading a substantial amount of patulin. Addition of ascorbate or ascorbic acid would also be of most value in juices which contain a minimum of patulin. This would increase the possibility that patulin in the juice would be decreased to less than a detectable level.

It might also be possible to treat apple juice containing larger amounts of patulin. This could be done by adding larger concentrations of ascorbate and subsequently diluting this treated juice with untreated, patulin-free

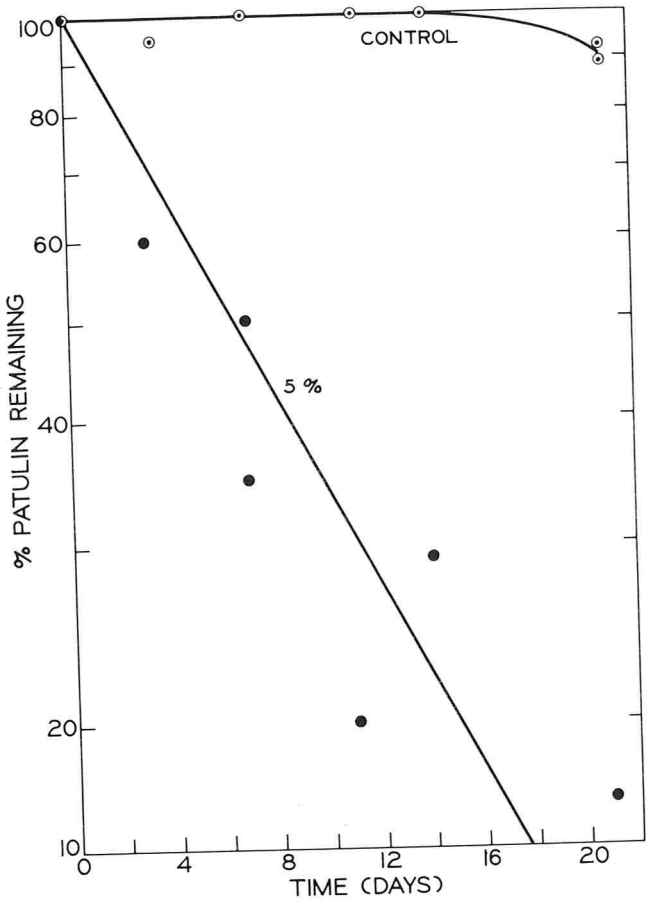

Figure 3. *Decrease of patulin in apple juice to which 5.0% Vit C was added.*

juice. This would serve to reduce the concentration of ascorbate to an acceptable level.

One needs to be concerned with the possibility that toxic products might result from the reactions just discussed. More work must be done to be sure the products of the reaction are nontoxic.

Another possibility which cannot be overlooked is that the reaction is reversible. One would not expect this to happen if patulin were inactivated by singlet oxygen. However, the possibility of the reaction being reversible and patulin being "regenerated" at a later date would have to be investigated before ascorbate could be used to treat apple juice.

ACKNOWLEDGMENTS

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Superoxide Dismutase Activity in Bovine Milk Serum

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ABSTRACT

Superoxide dismutase activity was shown to be present in bovine milk serum and was quantified by measuring the capacity of retentate from dialyzed milk serum to inhibit reduction of cytochrome c by xanthine-xanthine oxidase-generated superoxide anion. One unit of enzyme was defined as the quantity of superoxide dismutase which inhibits cytochrome c reduction by 20%. By this definition 19,500 units of enzyme were present per liter of retentate from dialyzed milk serum. This amount is equivalent to about 2.4 mg of purified bovine erythrocyte superoxide dismutase per liter. Polyacrylamide gel electrophoresis of a partially-purified superoxide dismutase from acid whey, followed by staining for enzymic activity, confirmed the presence of the enzyme in milk serum which was identical in electrophoretic properties to those of bovine erythrocyte copper-zinc superoxide dismutase. Pasteurization at 63 C for 30 min did not decrease superoxide dismutase activity in milk serum. Heating of purified bovine erythrocyte-superoxide dismutase at 100 C for 1 min resulted in almost complete loss of enzymic activity, whereas the partially-purified superoxide dismutase from acid whey still retained 40% of the original activity under these conditions. Bovine milk superoxide dismutase may be an important naturally-occurring antioxidant for increasing oxidative stability of milk and other dairy products.

The enzymic activity of a blue-green metalloprotein present in bovine erythrocytes, known also as erythrocuprein, was discovered by McCord and Fridovich in 1969 (*22,23*). The protein was subsequently renamed superoxide dismutase (SOD) because it catalyzes the dismutation of superoxide anion (O_2^-) radical to hydrogen peroxide and triplet oxygen:

$$O_2^- + O_2^- + 2H^+ \xrightarrow{SOD} H_2O_2 + O_2$$

Since that time, SOD has been observed widely in nature. It has been isolated and characterized from many animal, plant and microbial sources (*7,19,28*). At the time this work was completed (*9*), Hill (*10*) and later Asada (*1*) reported the occurrence of SOD in bovine milk. More recently, Holbrook and Hicks (*12*) reported on the variation of SOD in bovine milk and measured concentrations of enzyme similar to those found by Hill (*10*).

The production of superoxide anion in many biological processes is well documented (*4,7*). It is generally thought (*7,20*) that generation of this radical might lead to the formation of other oxidative species such as hydrogen peroxide, singlet oxygen and hydroxyl radical, which have been implicated in lipid peroxidation and destruction of biological membranes (*8,15,17,20,30*), as well as degradation of macromolecules (*21*). The SOD which catalytically destroys superoxide anion is thought to protect living cells from oxidative damage (*7,15,17*). Presumably, any biological system in which oxygen is present and superoxide anion is normally produced is likely to contain SOD as a defense against oxidative reactions initiated by oxygen species (*24*). Bovine milk has several systems present which are able to generate superoxide anion. Among these are enzymes such as xanthine oxidase (*7,14*) and lactoperoxidase (*11,14*). The spontaneous oxidation of tetrahydropteridines (*27*), hemoglobin (*26*), reduced flavins and quinones (*25*) results in formation of superoxide anion, and these can be in milk. Therefore, the possibility that SOD exists in the secretory cell, and secondarily in milk, was investigated.

In this study we confirm the occurrence of SOD activity in serum of bovine milk, as previously reported (*9*); however, the quantity of SOD present in milk from our source was approximately 10-fold greater than that reported by other authors (*1,10,12*). A simple procedure for isolation of partially-purified SOD from bovine milk has been elaborated and electrophoretic mobility and thermostability of the enzyme obtained from this procedure have been examined. The importance of SOD for the oxidative stability of milk is discussed.

MATERIALS AND METHODS

Enzymes

Enzymes were purchased from the following sources: milk xanthine oxidase from ICN Pharmaceuticals, Inc., Cleveland, OH; beef liver catalase from Boehringer Mannheim Biochemicals, Indianapolis, IN; purified bovine erythrocyte superoxide dismutase from Truett Laboratories, Dallas, TX. A partially-purified bovine milk superoxide dismutase was prepared from acid whey by fractionation with ammonium sulfate and ion exchange chromatography (Fig. 1).

Reagents

Xanthine monosodium salt was purchased from ICN Pharmaceuticals, Inc. Horse heart muscle ferricytochrome c and ethylenediaminetetraacetic acid disodium salt (EDTA) were from Aldrich Chemical Co., Milwaukee, WI. Bovine serum albumin (fraction V), L-methionine and nitro blue tetrazolium chloride (NBT) were from Sigma Chemical Co., St. Louis, MO. The reagents for polyacrylamide gel electrophoresis, carried out as described by Davis (5), were obtained from Eastman Organic Chemicals, Rochester, NY. All other chemicals were reagent grade. Deionized water was used in all assays.

[1]*University of Wisconsin.*
[2]*University of Kentucky.*

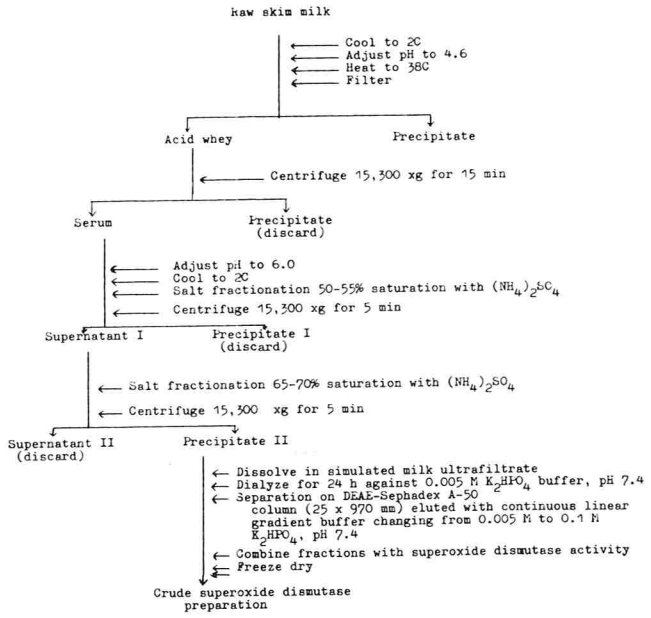


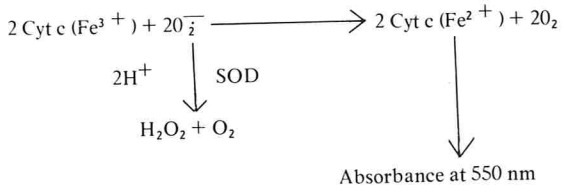
Figure 1. *Isolation of superoxide dismutase (SOD) from bovine milk. Simulated milk ultrafiltrate was prepared as described by Jenness and Koops (13).*

Milk serum preparation

Samples of raw bovine milk were obtained from the University of Wisconsin dairy herd and contained milk pooled from several breeds of cows. The milk was cooled to 4 C and used the same day for preparation of milk serum as follows. The skim milk fraction obtained by centrifugation at 1000 × *g* (10 min, 25 C) was further centrifuged at 100,000 × *g* (60 min, 4 C). The 100,000 × *g* supernatant fluid containing the milk serum fraction was then dialyzed for 6 h against 25 volumes of 0.05 M potassium phosphate buffer, pH 7.8 at 4 C. Retentate from dialyzed milk serum was used for assay of SOD activity.

Assay of SOD activity.

The assay was based on the ability of SOD to dismutate superoxide anion and thereby to specifically and efficiently compete with electron acceptors for superoxide radical. SOD activity in milk serum was measured spectrophotometrically from the amount of inhibition of the reduction of cytochrome c by the superoxide anion produced enzymatically in the xanthine-xanthine oxidase reaction:



The conditions for the assay were similar to those described by Weser and Voelker (32). The reaction was carried out at 25 C in a cuvette (1-cm light path) in a final volume of 1 ml (air-equilibrated) containing 50 mM potassium phosphate buffer, pH 7.8, 0.33 mM xanthine, 0.027 mM ferricytochrome c, 0.1 mM EDTA, 16 nM catalase and 210 nM xanthine oxidase. A similar solution without xanthine oxidase served as the blank. Cytochrome c reduction was measured at 550 nm in a Beckman Acta III double-beam spectrophotometer as a function of time. The cytochrome c reduction reached a plateau after 20 min. SOD decreased the amount of cytochrome c reduction at the plateau level, and the percent inhibition of cytochrome c reduction was directly proportional to bovine erythrocyte SOD concentration from 0 ng SOD/ml to 250 ng SOD/ml. This latter concentration of SOD yielded about 40% inhibition (Fig. 2). One unit of SOD activity was defined as that amount of SOD required to cause a 20% inhibition of cytochrome c reduction using the reaction conditions described above.

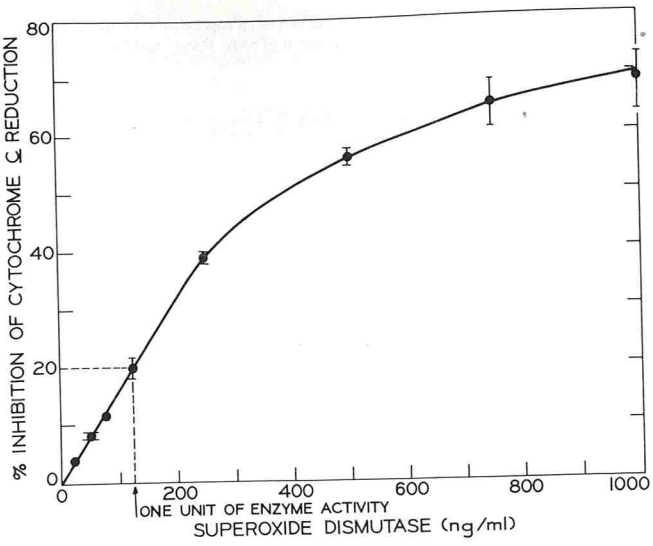


Figure 2. *Inhibition of cytochrome c reduction by purified bovine erythrocyte superoxide dismutase (SOD). Superoxide anion was generated by the xanthine-xanthine oxidase reaction. Each point on the curve represents an average of two measurements, each in duplicate. Bars represent upper and lower limits of values.*

Only that part of the curve where the percent inhibition of cytochrome c reduction was directly proportional to SOD concentration was used to calculate the amount of SOD present in milk.

Assay of SOD activity on polyacrylamide gels

Samples of purified bovine erythrocyte SOD or the crude preparation of bovine milk SOD were dialyzed against Tris-glycine buffer, pH 8.3 for 24 h at 4 C. The retentate obtained after dialysis was diluted with the same buffer to the desired enzyme concentration, dissolved in a sucrose solution (40% w/v) and applied onto the polyacrylamide gels. The gels were prepared and the samples subjected to disc electrophoresis according to the procedure of Davis (5). Gels were stained for SOD activity with NBT in the presence of photogenerated superoxide anion (2). The photogenerated superoxide anion reduced the dye to insoluble blue diformazan except in those areas where SOD destroyed the radical, resulting in formation of achromatic bands.

Protein assay

Protein concentrations were assayed in milk serum or partially-purified SOD preparations by the method of Lowry et al. (18) using bovine serum albumin or pure SOD protein as standards, respectively.

RESULTS AND DISCUSSION

The assay for enzyme activity was based on the capacity of SOD to inhibit reduction of ferricytochrome c by superoxide anion. Since ascorbic acid present in milk serum might interfere with the assay, it was removed by dialysis (MW cutoff ca. 14,000) against 0.05 M potassium phosphate buffer (pH 7.8 at 4 C) before the analyses were started.

As indicated in Fig. 3, retentate from dialyzed milk serum possessed the same qualitative inhibitory activity as purified bovine erythrocyte SOD in the assay of cytochrome c reduction by superoxide anion generated in the xanthine-xanthine oxidase system. Equivalent amounts of boiled, heat-inactivated serum retentate did not decrease cytochrome c reduction, thus eliminating the possibility of a nonspecific inhibition of cytochrome c reduction by the serum. As shown in Fig. 4, the decrease in cytochrome c reduction was directly related to the

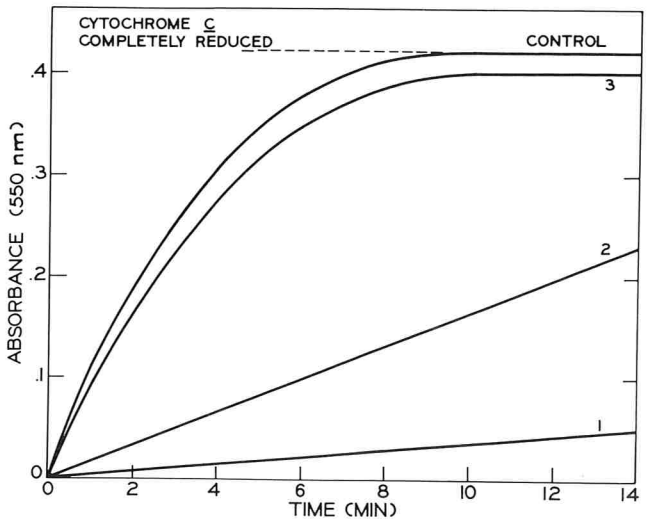


Figure 3. *Inhibition of cytochrome c reduction by: curve 1: 2.0 μg/ml of bovine erythrocyte superoxide dismutase (SOD); curve 2: 25 μl of dialyzed milk serum retentate; curve 3:25 μl of dialyzed milk serum retentate boiled for 10 min. Cytochrome c was completely reduced using the xanthine-xanthine oxidase system (32).*

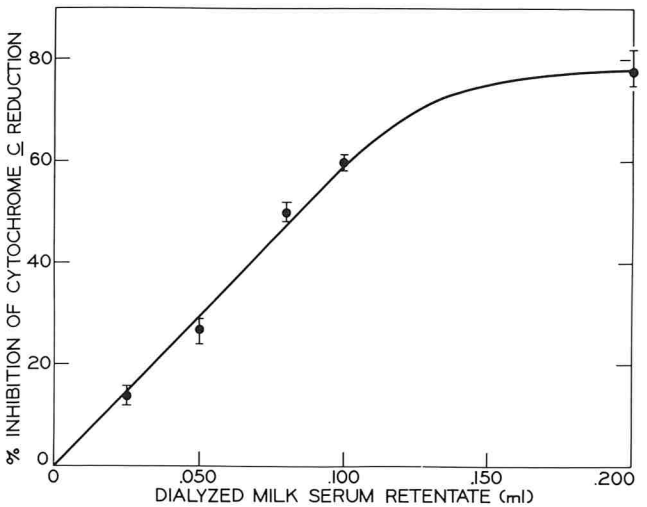


Figure 4. *Inhibition of cytochrome c reduction by dialyzed milk serum retentate. Superoxide anion was generated by xanthine-xanthine oxidase reaction. Each point on the curve represents an average of two measurements, each in duplicate. Bars represent upper and lower limits of values.*

quantities (up to 0.1 ml) of retentate added, and, subsequently, this range was used for assaying of SOD activity in serum.

Concentration of SOD in bovine milk serum was calculated from the standard curve (Fig. 2) prepared for purified bovine erythrocyte SOD. As indicated in Table 1, an average of 19,500 units of SOD per liter was measured in retentate from dialyzed milk serum. This was equivalent to 2.4 mg of purified bovine erythrocyte SOD per liter. The concentration of SOD in bovine milk reported by other authors was lower than that presented in Table 1; Hill (*10*) estimated about 0.15 mg/liter, and Asada (*1*) found about 0.32 mg/liter. Differences in milk origin, activity of standard enzyme, as well as in methods of assaying and calculating enzyme activity, might account for these variations. The acid whey fractionation of bovine milk followed by a two-step purification yielded an increase in enzyme specific activity of about 260-fold, indicating the enzyme is not associated with the casein fraction.

SOD isolated from bovine milk according to procedures in Fig. 1, and commercial bovine erythrocyte SOD were simultaneously subjected to polyacrylamide gel electrophoresis. After staining for SOD activity, two closely migrating achromatic bands located at the same distance from the cathode were detected for the enzymes from both sources (Fig. 5). The observed electrophoretic patterns of the enzymes were similar to those for the

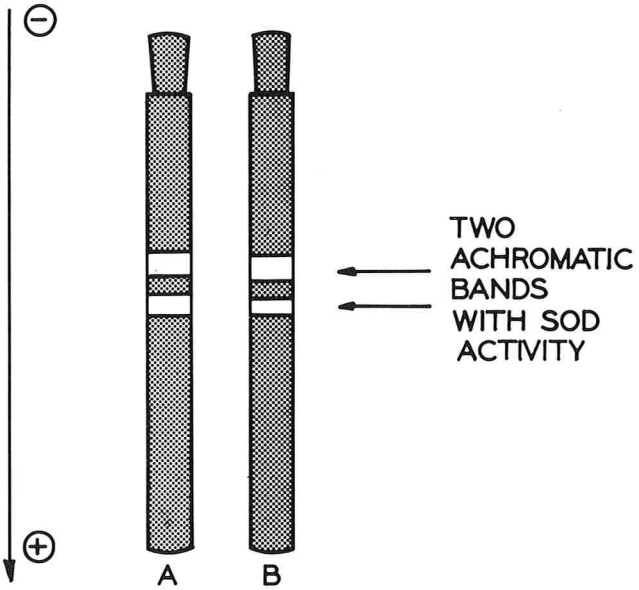


Figure 5. *Disc gel electrophoresis of superoxide dismutase, stained for enzyme activity with nitro blue tetrazolium in the presence of photogenerated superoxide anion. Gel A, 2 μg purified bovine erythrocyte superoxide dismutase, Gel B, 150 μg of protein from crude enzyme preparation isolated from bovine milk as shown in Fig. 1.*

TABLE 1. *Superoxide dismutase activity in bovine milk serum assayed by inhibition of cytochrome c reduction using the xanthine-xanthine oxidase system.*

Sample	Protein[a] (mg/ml)	Superoxide dismutase μg/ml	Superoxide dismutase units[b]/ml	Superoxide dismutase units[b]/mg protein
Dialyzed serum retentate[c]	8.07 ± 0.18	2.44 ± 1.20	19.5 ± 9.6	2.4 ± 1.2
Dialyzed serum retentate pasteurized at 63 C for 30 min[d]	7.89 ± 0.09	2.31 ± 0.60	18.5 ± 4.8	2.3 ± 0.5
Crude preparation of bovine milk superoxide dismutase[d]	0.48 ± 0.01	36.75 ± 4.36	294.0 ± 32.0	612.5 ± 67.4

[a]Protein concentration was assayed by method of Lowry et al. (*18*).
[b]Enzyme units were calculated according to the definition given in the Materials and Methods.
[c]Mean and SD calculated from eight separate samples.
[d]Mean and SD calculated from four separate samples.

copper-zinc SOD from bovine erythrocytes reported by other authors (*2,3,19*). Therefore, polyacrylamide gel electrophoresis further confirmed the presence of SOD in milk, and suggested that milk SOD is probably identical to the copper-zinc enzyme present in bovine erythrocytes. There is a possibility that SOD derived from bacterial or leukocytic cells is also present in milk. However, those cells have manganese- and/or iron-containing SODs which migrate more toward the cathode during gel electrophoresis (*7,28*). No bands representing those enzymes were detected on the polyacrylamide gels.

Purified SOD was less stable to heat than SOD in crude preparations (Table 2). When the same amount of enzyme in either the pure or the crude form was heated at 100 C for 1 min, almost all activity of the highly purified enzyme was abolished, whereas about 40% of the initial activity was still present in the crude preparations. Heating at 100 C for 5 min, however, also destroyed enzymic activity in the crude preparation. Low-temperature pasteurization of milk serum (63 C, 30 min) did not decrease SOD activity to any great extent (Table 1). About 96% of the original SOD activity remained after pasteurization. SOD is considered to be one of the most stable enzymes with regard to temperature, pH changes, storage conditions and resistance to many denaturating reagents (*6,31*). The greater resistance of the SOD enzyme to thermal inactivation in the presence of whey proteins should be beneficial for preserving SOD antioxidative activity in thermally processed dairy products.

TABLE 2. *Heat-inactivation of superoxide dismutase (SOD) purified from bovine erythrocytes or crude superoxide dismutase fractionated from acid whey of bovine milk.*[a]

Heating time (min)	% Of initial activity[b]	
	Erythrocyte SOD	Milk SOD
0	100.0	100.0
1	4.3 ± 0.2	40.4 ± 1.0
5	0	1.8 ± 0.2
10	0	0

[a]Samples containing 2.5 μg/ml of erythrocyte or milk enzyme in 0.05 M potassium phosphate buffer, pH 7.8, were incubated at 100 C. Aliquots were removed at the indicated time intervals, cooled in an ice bath, and assayed for SOD activity in terms of their inhibition of the reduction of cytochrome c in the xanthine-xanthine oxidase system.
[b]Mean and SD calculated from four separate samples.

Milk SOD might be an important naturally-occurring antioxidant component of milk. In the presence of suitable substrates, the release of superoxide anion in milk by milk enzymes such as xanthine oxidase or lactoperoxidase, is expected. Other biochemical compounds could also generate superoxide anion during spontaneous oxidation. If the amount of SOD endogenously present in milk is sufficient to keep superoxide anion at a very low steady-state level, it will inhibit oxidative reactions initiated by the superoxide anion and by other active oxygen species derived from it. However, the amount of superoxide anion generated in milk by various sources under various conditions in relation to the SOD activity present in milk remains uncertain at the present time. The spontaneous development of oxidized flavor observed in certain fluid milks (*29*) might be related to an insufficient amount of endogenous SOD in milk. Studying the correlation between the amount of endogenous SOD in milk and the susceptibility of milk to spontaneous oxidation might add valuable information for explanation and prevention of these problems. Recently, Holbrook and Hicks (*12*) concluded that the concentration of SOD in individual cow's milk did not account for the large variation in oxidative resistance in raw milk that had not been exposed to light.

Thus the endogenous level of SOD may be insufficient to act as an antioxidant under certain conditions. Apparently milk does not have sufficient SOD to prevent oxidation and degradation of milk constituents by photogenerated superoxide anion upon exposure of milk to light (*16*); however, the SOD may be adequate to scavenge any superoxide anion formed during storage in the dark. Therefore, it is important to determine the conditions which lead to formation of superoxide anion and other active oxygen species.

Additional research may indicate that oxidation of milk and other food products could be slowed or prevented by addition of enzymes such as SOD to destroy superoxide anion and catalase to destroy hydrogen peroxide, thus providing a new class of food antioxidants.

ACKNOWLEDGMENTS

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institutions. This research also led to the drafting of US legislation to protect the right of pre-trial and post-trial detainees in jails and prisons.

Among the developments which have taken place in the District of Columbia food sanitation program while under Dr. Walker's direction are amendments of general food regulations, including open dating of products, health examinations of employees after illness, weighted inspection forms, four rather than two inspections per restaurant per year, display of inspection results in sub-standard establishments, and identification of fat content of ground meat. A certificate of merit program was established to motivate and reward the food industry and to inform the public as to which establishments consistently maintain a superior level of cleanliness.

Dr. Walker's education began in Springfield, TN, where he was born and grew up. In 1954 he received a B.S. in biology from Kentucky State University. Following four years' service with the Air Force Medical Service Corps, he worked as a research associate with the Bureau of Environmental Health in Washington. Dr. Walker completed his master's degree in environmental health at the University of Michigan and worked as a public health sanitarian, first in Philadelphia, then Detroit and surrounding areas. Next he taught at the College of Medicine, Howard University, while beginning studies in environmental hygiene at Johns Hopkins University. He completed additional studies in water supply and pollution control at the University of Kansas while working parttime as a research associate in the Environmental Health Research Laboratory. In 1966 he became a research fellow and Director of Environmental Research Laboratories for the School of Public Health at the University of Minnesota where he completed his Ph.D. Following public health service in Ohio and New Jersey, he assumed his present position.

Throughout other commitments, Dr. Walker has served as a consultant to a number of public health agencies, continued teaching and lecturing at Howard University, George Washington University, and Meharry Medical College of Nashville. He has contributed numerous articles to professional journals and served as a consultant to a number of public and professional organizations. The recipient of many awards, Dr. Walker has been honored with the Robert W. Browning prize for disease prevention, outstanding service award of the American Civil Liberties Union, Special Achievement Award of the American Correctional Food Service Association, Outstanding Service Commendation of the U.S. Environmental Protection Agency, Editor's Award for outstanding contribution to the *Journal of Environmental Health*, Special Commendation of the U.S. Department of Justice, Most Distinguished Alumni Award of Kentucky State University, and Distinguished Environmental Health Scientist Award of the National Environmental Health Association.

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Fate of Selected Pathogens Inoculated into Foods Prepared in Slow Cookers[1,2]

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ABSTRACT

Staphylococcus aureus, Clostridium perfringens, Salmonella cholerae-suis, and *Salmonella typhimurium* were inoculated (10^8 cells or spores) into two slow cookers containing green bean casserole, baked navy beans, chicken cacciatore, barbecued ribs or pork pot roast, and their fate determined after cooking. Heating patterns also were determined at three positions inside the two cookers. None of the foods cooked in either of the slow cookers contained detectable levels of *S. aureus* or salmonellae. The similarity between *C. perfringens* vegetative and spore counts indicate that only spores were present in the cooked foods. Except for the green bean casserole cooked using a low temperature setting, cooking resulted in a 0.44-1.67 and 0.36-1.54 log count reduction, respectively, of vegetative cells and spores of *C. perfringens*. Counts of vegetative cells and spores after cooking the green bean casserole were approximately .18 and .30 log counts higher than the uncooked counts. The mean times for the coldest areas in Cooker A to reach 50 C were 2.57 and 0.97 h, respectively, for the low (80 watts) and high (160 watts) temperature settings. The mean times for the coldest areas in Cooker B (removable liner) to reach 50 C were 2.35 and 0.52 h for the low (130 watts) and high (260 watts) temperature settings, respectively. Results suggest that when the recommended quantities of ingredients are used and the proper cooking procedure followed, foods prepared in the slow cookers studied do not present a health hazard.

Some researchers and consumers have questioned the microbiological safety of electric slow cookers, because of the low temperatures and long times used to cook most foods. A study using 13 continuous-heat cookers found that foods reached 60 C in a "sufficiently short time," but that problems may arise when the "keep warm" setting is used with thermostatic models (*1*). Sundberg and Carlin (*8*) reported that the temperature in the center of roasts cooked in a Rival Crock Pot remained within the growth range of *Clostridium perfringens* for approximately 2 h. The heating patterns in slow cookers were studied by Brackett and Marth (*4*). They found that the foods studied remained in a temperature range suitable for the growth of *C. perfringens* and *Staphylococcus aureus* for an average of 1.75 h and 1.50 h, respectively. However, growth sufficient to cause a health hazard should not occur if the slow cookers are used according to the manufacturer's directions.

This study was undertaken to determine the effect of cooking five foods in two types of slow cookers on *C. perfringens, S. aureus, Salmonella typhimurium* and *Salmonella cholerae-suis*. In addition, heating profiles were determined during cooking of the foods in the two cookers.

MATERIALS AND METHODS

Slow cookers

The two 4-quart slow cookers with glass lids (Cooker A and Cooker B) used in this study were purchased from a department store in Lexington, KY. Cooker A (Sears Slow Cooker, model 400.646200) had a manual temperature control; Cooker B (Sears Crock Watcher, model 400.646400) had an auto-shift temperature control which allowed the cooker to be started on the high temperature setting, and after 1-3/4 h it automatically shifted to the low temperature setting. Cooker B had a removable liner.

Recipes

The recipes used were for pork roast, barbecued ribs, chicken cacciatore, baked navy beans and green bean casserole. All recipes were obtained from the instruction booklets packaged with the slow cookers. Ingredients and amounts used for preparation of each food are listed in Table 1. The minimum recommended cooking times were used.

Cultures

S. aureus UK 36, *C. perfringens* UK 92, *S. typhimurium* UK 29, and *S. cholerae-suis* UK 28 used to inoculated the uncooked foods were obtained from the Department of Animal Sciences, University of Kentucky. The two *Salmonella* species were grown separately and combined before inoculation, while the other two species were used alone.

Trypticase soy broth and nutrient broth were used for growth and maintenance of *S. aureus* and the salmonellae. These cultures were transferred daily for 3 days before each uncooked food was inoculated with approximately 10^8 cells of a 15- to 18-h actively growing broth culture. *C. perfringens* was grown anaerobically for 18 h at 37 C in thioglycollate broth, heated for 10 min at 80 C, cooled and 1 ml was used to inoculate sporulating agar slopes. The slopes were incubated anaerobically (BBL Gas Pak) at 37 C for 48 h and then placed at 2 C for 5 days. Growth was harvested using sterile physiological saline solution, and the washings were stored at 2 C until used. Trypticase soy broth was inoculated with a portion of the harvested cells, heated for 10 min at 80 C, cooled and each uncooked food was inoculated with approximately 10^8 spores.

Enumeration

Twenty-five grams of food sample were weighed directly into 18 × 30-cm sterile polyethylene bags and 225 ml of diluent were added. The mixture was homogenized for 20 sec using a Stomacher 400 (Cooke Laboratory Products, Alexandria, VA). Phosphate-buffered diluent was used for enumeration of staphylococci and salmonellae, while 0.1% peptone water was used for enumeration of *C. perfringens*.

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[1]*Published with the approval of the Director of the Kentucky Agricultural Experiment Station as journal article no. 78-5-214.*

[2]*Mention of a trademark or proprietary product anywhere in this text does not constitute a guarantee or warranty of the product, and does not imply its approval to the exclusion of other products that may also be suitable.*

TABLE 1. *Ingredients in foods tested in slow cookers.*[a]

Recipe	Ingredient	Amount
Green bean casserole	green beans	1 lb can
	French-fried onion rings	3 oz can
	grated Cheddar cheese	1 cup
	condensed cream of chicken soup	1 13 oz can
	water	1/4 cup
Baked navy beans[b]	dried navy beans	3 cups
	water	7 cups
	chopped onion	1/2 cup
	salt pork	1/2 lb
	molasses	1/4 cup
	ketchup	1 cup
	brown sugar	1/4 cup
Chicken cacciatore	frying chicken pieces	3 lb
	shortening	3 tablespoons
	onions	2 medium
	canned tomatoes	1 lb can
	tomato sauce	8 oz can
	green pepper	1/3 cup
	water	1/4 cup
Barbecued ribs	pork spareribs	3 lb
	onion	1 large
	barbecue sauce	2 cups
Pork pot roast	pork roast	4 lb
	onions	2 medium
	water	1 cup

[a]Spices (salt, pepper, curry powder, dry mustard, oregano, basil, celery flakes, bay leaf, sage) were added according to manufacturer's recommendations.
[b]Beans were soaked overnight before cooking.

The number of *S. aureus* in uncooked foods was determined using surface spread plates of Baird-Parker agar. The 3-tube most probable number technique using trypticase soy broth plus 10% NaCl and Baird-Parker agar was used for enumeration and identification of staphylococci in the cooked foods. Isolates were tested for catalase and coagulase activity (*3*). Pour-plates of SPS agar incubated anaerobically (BBL Gas Pak) were used to determine the vegetative count of *C. perfringens* in both uncooked and cooked foods. Black colonies were confirmed as *C. perfringens* (*7*). To detect the number of spores that survived cooking, 10 ml of cooked sample were heated for 10 min at 80 C, cooled and plated with SPS. Results of this test were recorded as the spore count for *C. perfringens*. Salmonellae in the uncooked foods were determined using surface spread-plates of brilliant green agar (BGA) and xylose lysine desoxycholate agar (XLD). *Salmonella*-suspicious colonies were picked to triple sugar iron agar (TSI), and slants showing reactions typical of salmonellae after 24 h at 37 C were considered to be the two *Salmonellae* species (*2*). Salmonellae in the cooked foods were determined by pre-enrichment of 25 g in 225 ml of nutrient broth for 18 h at 37 C, followed by enrichment in selenite cystine broth for 24 h at 37 C. Plates of BGA and XLD were streaked with the enrichment broth and then were incubated at 37 C for up to 48 h. *Salmonella*-suspicious colonies were picked to TSI agar slants for presumptive identification as salmonellae.

Heating profiles

Copper-constantan thermocouples (Omega Engineering, Inc., Stanford, CT) and a Honeywell Electronik 112 recorder (Honeywell, Ft. Washington, PA) were used to determine and record temperatures during cooking of the foods in the two cookers. The thermocouples were positioned at three points in the cookers: top center, bottom side and mid- or bottom-center. None of the thermocouples touched the sides or bottom of the cookers. The side and center thermocouples were inserted into the meat pieces for the ribs, roasts and chicken recipes. Tape held the thermocouples in place and was used to seal the gaps between the cooker and lid caused by the thermocouple leads.

RESULTS AND DISCUSSION

The uncooked foods were inoculated with an average of 4.0×10^8 *S. aureus*, 3.4×10^7 *C. perfringens*, and 2.8×10^8 *S. typhimurium* and 3.3×10^8 *S. cholerae-suis*. Results obtained before and after cooking each food containing the test species are given in Tables 2, 3 and 4. Variations in the counts obtained for the uncooked foods may have been due to differences in inoculum size, a failure to uniformly mix the inoculum with the food, differences in particle size of ingredients or differences in total contents of the recipes.

None of the foods cooked in either of the slow cookers contained detectable levels of *S. aureus* (per g) (Table 2) or *Salmonella* (per 25 g) (Table 3).

Colonies on Baird-Parker agar from cooked food samples did not have the same characteristics as colonies from the staphylococcus inoculum or from the inoculated uncooked foods. All isolates obtained from the inoculum and from uncooked foods were coagulase-positive, while

TABLE 3. *Number of* Salmonella *in inoculated foods before and after cooking in slow cookers.*

Recipe	Temperature setting	Uncooked food (CFU × 10^3/g) Cooker A	Uncooked food (CFU × 10^3/g) Cooker B	Cooked food
Roast	low	195.0	315.0	ND[a]
Ribs	low	4.0	NT[b]	ND
Ribs	high	NT	25.0	ND
Navy beans	low	0.2	0.1	ND
Chicken cacciatore	low	0.2	NT	ND
Chicken cacciatore	high	NT	29.3	ND
Green bean casserole	low	0.2	NT	ND
Green bean casserole	high	NT	0.1	ND
Green bean casserole	auto-shift	NT	0.2	ND

[a]ND = None detected per 25 g.
[b]NT = Not tested.

TABLE 2. *Numbers of* Staphylococcus aureus *in inoculated foods before and after cooking in slow cookers.*

Recipe	Temperature setting	Uncooked food (CFU × 10^5/g) Cooker A	Uncooked food (CFU × 10^5/g) Cooker B	Cooked food (MPN/g) Cooker A	Cooked food (MPN/g) Cooker B
Roast	low	7.7	5.8 7.6[a]	< 3	< 3
Ribs	low	NT[b]	0.6	NT	< 3
Ribs	high	0.8	8.1	< 3	< 3
Navy beans	low	2.6	1.6	< 3	< 3
Chicken cacciatore	low	12.8	9.2	< 3	< 3
Chicken cacciatore	high	0.7	5.5	< 3	< 3
Green bean casserole	low	27.7	8.9	< 3	< 3
Green bean casserole	high	3.1	NT	< 3	< 3
Green bean casserole	auto-shift	NT	1.4	NT	< 3

[a]Count of inoculated uncooked food after storage at 4 C for 12 h.
[b]NT = Not tested.

the isolates from the cooked foods were coagulase-negative. The presence of, or level of, enterotoxin in the cooked foods was not determined. While *S. aureus* was not detected in the cooked foods, it is not known if conditions were suitable for enterotoxin production during the time the foods were cooking. Generally, 4 to 6 h are required to produce toxin levels sufficient to cause illness. Staphylococcus populations of 10^6-10^7 per gram are required before these toxin levels are produced (5,6). The foods used in this study were not held at temperatures suitable for growth of or enterotoxin production by *S. aureus*. While the level of *S. aureus* in the uncooked foods was much higher than normally found in properly prepared and handled raw foods, no *S. aureus* was detected in cooked foods. The results indicate that conditions necessary to cause staphylococcal illness will not be obtained during the preparation of foods in slow cookers.

No salmonellae were detected per 25 g of cooked food (Table 3). The two slow cookers were effective in reducing the level of salmonellae from 10^4 to 10^5/g to less than 1 per 25 g.

The similarity between the *C. perfringens* vegetative and spore counts indicate that only spores were present in the cooked foods (Table 4). Except for the green bean casserole which was cooked using a low temperature setting, cooking resulted in a 0.44-1.67 and 0.36-1.54 log count reduction, respectively, of vegetative cells and spores of *C. perfringens*. These counts, after cooking the green bean casserole, were approximately 0.18 and 0.30 log counts higher than counts of the uncooked food. Sundberg and Carlin (*8*) reported a decrease of 3.96 and 0.98 log counts for *C. perfringens* vegetative cells and spores, respectively, in roasts after cooking in a Rival Crock Pot. They cautioned against holding cooked foods at room temperature for an extended time, because the temperature during the last half of cooking could stimulate germination and outgrowth of *C. perfringens* spores. In addition, the cooking process expels oxygen and kills competing organisms which permits rapid growth of *C. perfringens* in cooked foods held at room temperature. The spore count increased 0.3 log and the vegetative count 0.1 log when ribs cooked on low in Cooker A were kept covered and held at room temperature for 6.25 h after the cooker was unplugged.

Heating patterns obtained for the five products cooked in each cooker using the low temperature setting are shown in Fig. 1 to 3. Heating rates using the high temperature setting were much more rapid than those obtained using the low setting and are not shown in the figures. Erratic air temperature patterns were observed after the temperature began to level off for the ribs cooked in Cooker B using the high setting, and for the green bean casserole cooked using the high and low setting in both cookers. The erratic temperature patterns were assumed to be due to condensate from the lid dripping onto the thermocouple and causing a fluctuation in the recorded temperature. The temperature variation ranged between 3 and 23 degrees. The maximum temperature recorded was used for comparisons with the other heating patterns.

The times for various locations in each cooker to reach 46 and 50 C, respectively, are shown in Table 5. The mean times for the coldest areas in Cooker A to reach

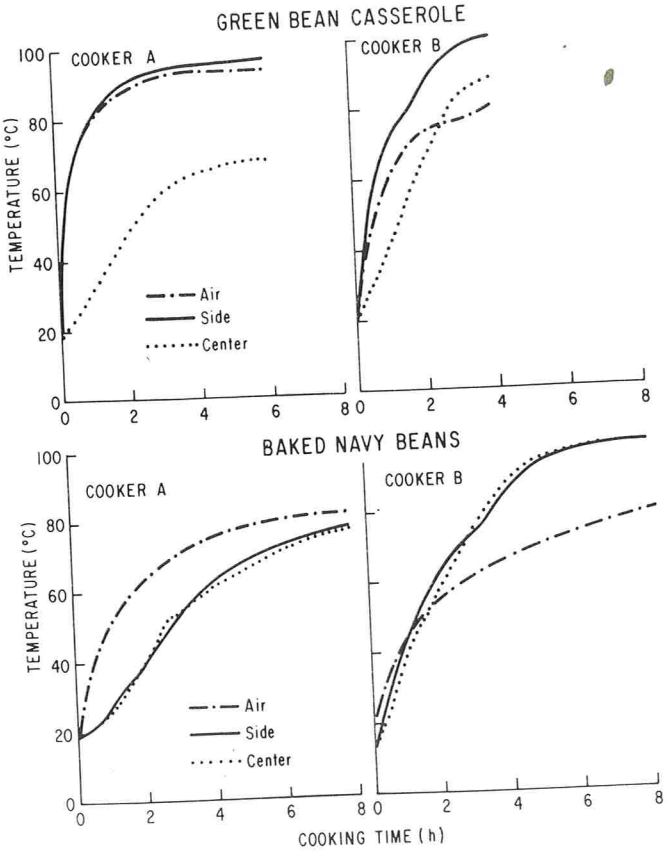

Figure 1 *Changes in temperature of green bean casserole and baked navy beans during cooking in two slow cookers.*

TABLE 4. *Number of* Clostridium perfringens *in inoculated foods before and after cooking in slow cookers.*

Recipe	Temperature setting	Uncooked food (CFU × 10^4/g)		Cooked food (CFU × 10^4/g)			
				Cooker A		Cooker B	
		Cooker A	Cooker B	Vegetative	Spore	Vegetative	Spore
Roast	low	NT[a]	18.0	NT	NT	1.9	1.2
Ribs	high	1.2	NT	.44	.53	NT	NT
Navy beans	low	6.2	6.1	.26	.17	.14	.18
Chicken cacciatore	low	5.6	5.0	.59	.56	.86	1.1
Chicken cassiatore	high	5.8	1.1	1.3	.68	.04	.10
Green bean casserole	low	5.6	4.1	7.9	10.8	6.4	8.8
Green bean casserole	high	4.2	NT	.09	.12	NT	NT
Green bean casserole	auto-shift	NT	2.1	NT	NT	.08	.06

[a]NT = Not tested.

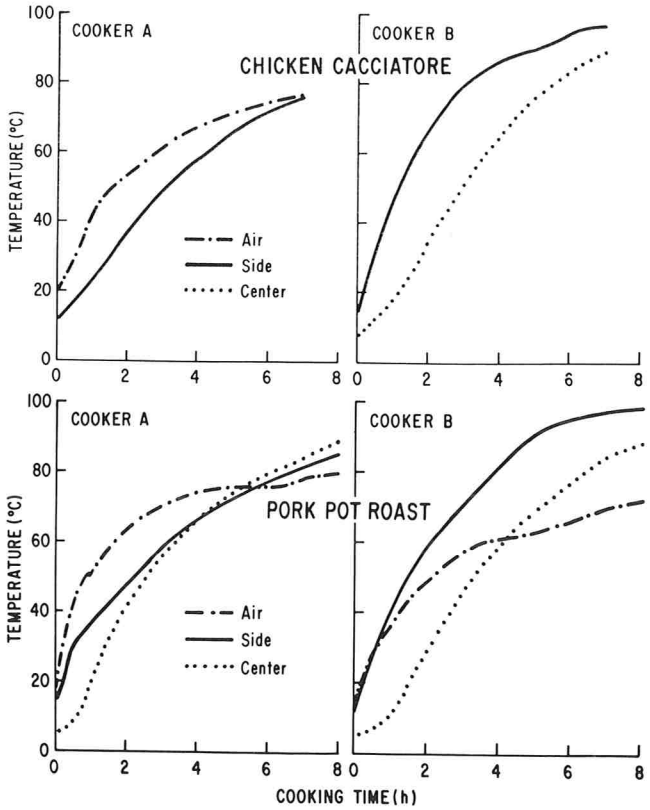

Figure 2. *Changes in temperature of chicken cacciatore and pork pot roast during cooking in two slow cookers.*

50 C were 2.57 and 0.97 h, respectively, for the low and high temperature settings. The mean times for the coldest areas in Cooker B to reach 50 C were 2.35 and 0.52 h for the low and high temperature settings, respectively. The upper limits for growth of salmonellae, *S. aureus* and *C. perfringens* are 45.6, 46 and 50 C,

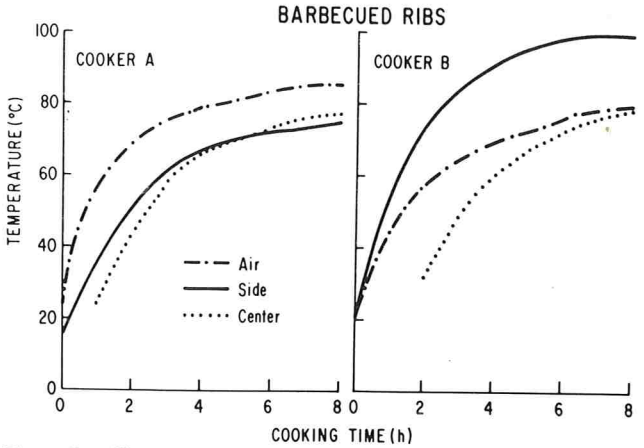

Figure 3. *Changes in temperature of barbecued ribs during cooking in two slow cookers.*

respectively (*3,5,7*). The results from this study indicate that lethal temperatures were obtained in sufficient time to prevent growth and/or toxin production by the test pathogens. Brackett and Marth (*4*) reported that the mean times for the coldest area to reach 50 C in beef stew, baked beans, and meat loaf cooked on low setting were 2.0 h for a Rival Crock Pot and 1.8 h for a West Bend Bean Pot.

The heating patterns obtained were influenced by several factors. Consistency of food and the distance from the heating elements affected heat transfer. Heating patterns also were affected by wattage of the cooker. The air temperature in Cooker A consistently reached 46 and 50 C more rapidly than in Cooker B; however, the temperature of the food reached higher levels in Cooker B than in Cooker A. Cooker B used 130 watts on low setting and 260 watts on high setting compared to 80 and 160 watts for Cooker A.

TABLE 5. *Times for different foods prepared in slow cookers to reach 46 C and 50 C.*

Recipe	Cooker	Cooking temperature	Hours to reach 46 C/hours to reach 50 C		
			Air	Side	Center
Green bean casserole	A	low	0.15/0.21	0.22/0.28	1.94/2.14
	B	low	0.57/0.67	0.43/0.52	1.20/1.35
	A	high	0.16/0.17	0.46/0.51	1.43/1.53
	B	high	0.27/0.30	0.51/0.57	0.69/0.72
	B	auto-shift	0.28/0.34	0.39/0.48	0.92/1.02
Baked navy beans	A	low	0.83/1.00	2.45/2.72	2.38/2.62
	B	low	1.09/1.42	1.15/1.30	1.30/1.50
Chicken cacciatore	A	low	1.29/1.70	2.69/3.04	NT[a]
	B	low	NT	0.97/1.12	2.65/2.90
	A	high[b]	0.11/0.12	0.57/0.72	0.71/0.81
	B	high	0.39/0.44	0.37/0.42	0.44/0.51
Barbecued ribs	A	low[c]	0.44/0.58	1.54/1.84	2.16/2.40
	B	low[c]	1.04/1.31	0.71/0.85	2.79/3.00
	A	high[d]	0.11/0.16	0.62/0.74	0.00/0.57
	B	high[d]	0.21/0.27	0.00/0.11	0.27/0.33
Pork pot roast	A	low[c]	0.65/0.83	1.83/2.12	2.26/2.51
	B	low[c]	1.73/2.08	1.28/1.48	2.99/3.23
	A	low[d]	0.37/0.50	2.24/2.61	NT
	B	low[d]	0.55/0.78	0.80/0.99	1.86/2.12

[a]NT = Not tested.
[b]The chicken cooked on the high setting was browned before slow cooking.
[c]Not broiled before slow cooking.
[d]Broiled before slow cooking.

The amount and type of food also influenced the heating patterns. The coldest area in the roast and ribs cooked in Cooker B and the chicken cooked in Cooker A took at least 3 h to reach 50 C. These pieces of meat were not broiled or browned before cooking and in each instance more meat was used than was recommended. The extra meat used was 0.18 kg of ribs, 0.34 kg of roasts and 0.36 of chicken. An increase in the amount of meat caused an increase in the time the foods were in the growth range of the test bacteria. Brackett and Marth (*4*) reported similar increases in the time to reach 46 and 50 C when their cookers were overloaded with up to three times the recommended amount of ingredients.

The results reported in this study are based on a limited number of cultures. Hence the heat sensitivity observed in this study pertains only to these cultures. It is possible that different results would have been obtained if more strains had been studied or if strains isolated from foods rather than laboratory strains had been used to inoculate the foods.

The results of this study indicate that when the recommended quantities of ingredients are used and the cooking procedures given in the recipes are followed, foods prepared in the two slow cookers studied do not present a health hazard.

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International Commission on Microbiological Specifications for Foods (ICMSF), Conference on the Sanitary Quality and Microbiological Safety of Fishery Products, U.S.-Japan Joint Panel on Toxic Microorganisms, International Standards Organization, and Conference on Global Impacts of Applied Microbiology, among others.

Co-author of *Dairy Microbiology*, a college textbook, Dr. Olson has also written more than 100 journal papers, technical bulletins, and pamphlets. He helped write, co-author, and edit several ICMSF books. Professional organizations of which Dr. Olson is a member, in addition to IAMFES, include the Institute of Food Technologists, American Dairy Science Association, American Society for Microbiology, and the American Academy of Microbiology.

Originally from Roseburt, OR, Dr. Olson received his B.S., M.S., and Ph.D. degrees from the University of Minnesota. His Ph.D. research concentrated on the heat resistance of coliform bacteria in milk. From 1939-67, Dr. Olson advanced at the University of Minnesota from instructor in the department of dairy husbandry to professor in food science and industries and professor in microbiology. His teaching responsibilities included introductory courses in general microbiology, dairy and food microbiology, and a course in milk regulatory control for senior veterinary students. His research emphasized microbiological aspects of production, processing, distribution, and public health safety of milk and dairy products.

Among other awards Dr. Olson has received are the IAMFES Citation Award and the Achievement Award of the Minnesota Sanitarians Association. He is also a member of Gamma Sigma Delta, Society of the Sigma Xi, and a charter member of the American Academy for Microbiology.

Committee Report Sanitarians Joint Council

The Sanitarians Joint Council (SJC) is composed of two representatives from the Environmental Health Section, American Public Health Association; International Association of Milk, Food and Environmental Sanitarians, Inc.; National Environmental Health Association; and the National Society of Professional Sanitarians.

Considerable time was spent discussing "sunset laws" and their effect on the various Sanitarian Registration Acts; revision of the SJC Model Registration Act; and credentialing and licensure. Those present felt that the Model Act should be revised, with legal input, to meet a need until credentialing is widely accepted.

The Sanitarians Joint Council is going to redefine the objectives and purposes of the organization. The revised document "Organization of the Sanitarians Joint Council" will be submitted to the parent organizations for reaffirmation of their support of the SJC. This action is based on the fact that the SJC has accomplished many of the previous objectives and other objectives are no longer applicable as a result of changing trends, concepts and philosophy.

Respectfully submitted,
Harry Haverland
Secretary-Treasurer
Sanitarians Joint Council

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Presence and Distribution of *Salmonella* Species in some Local Foods from Baghdad City, Iraq

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ABSTRACT

A total of 353 local food samples were cultured for *Salmonella* species using mannitol broth and two selective enrichment media, tetrathionate brilliant green bile broth and selenite cystine broth. Fifteen *Salmonella* species and serotypes were isolated: *Salmonella paratyphi* B, *Salmonella typhimurium*, *Salmonella* 4,5,12:-:-, *Salmonella muenchen, Salmonella senftenberg, Salmonella lille, Salmonella alachua, Salmonella* 6,7:-:-, *Salmonella anatum, Salmonella enteritidis, Salmonella havana, Salmonella eppendorf, Salmonella emek, Salmonella california,* and *Salmonella* 1,3,19:-:-. *Salmonella* was recovered from 27% of food samples examined. The occurrence of some species was as high as 11% of the samples. *S. lille* and *S. alachua* were isolated and are reported for the first time in Iraq. Seventy food samples (19.8%) harbored one type of *Salmonella*, 11 (5.9%) harbored two types and four (1.1%) harbored three types. Isolation of *Salmonella* from foods is affected by types of enrichment media. Tetrathionate brilliant green bile broth was superior to selenite cystine for *Salmonella* recovery from most foods. Some species prefer certain enrichment media for growth and multiplication. Use of more than one type of selective medium increases the chance of isolation of more *Salmonella* species and serotypes.

After isolation of *Salmonella* by Gaffky in 1884 (*4*), the number of species and serotypes increased gradually, but it increased rapidly in the last 20 years to reach 1744 (*31*) by 1974. Thereafter, serotypes were added every year, based on classification of the Kauffman-White Scheme. Since Budd in 1856, on the basis of epidemiological evidence, suggested the infectious nature and transmission of typhoid fever, the concern about *Salmonella* is also increased because it affects health, food and economy of humans. The classical chain of *Salmonella* infection is feedstuffs-animal-foods-man.

The following criteria are used for tentative identification of *Salmonella* (*3,4,6,7,8,16*): gram negative rods; motile (except *Salmonella pullorum* and *Salmonella gallinarum*) by peritrichous flagella; fermentative to glucose, mannitol, maltose, and sorbose, except *Salmonella typhimurium, Salmonella paratyphi* C, *Salmonella enteritidis, Salmonella typhi* and *S. gallinarum* which do not produce gas; do not ferment lactose, sucrose, or salicin except *Salmonella arizona* (subgroup III) and *Salmonella houtenae* (subgroup IV); unable to liquefy gelatin except *Salmonella daressalam* (subgroup II), *S. arizona,* and *S. houtenae;* do not produce indol except *Salmonella panama, Salmonella eastbourne*, and some strains of *S. enteritidis*; do not produce urease; reduce trimethylamine oxide to trimethylamine; aerobic but some are facultatively anaerobic (*S. enteritidis, S. typhimurium* and *S. paratyphi* C); aerogenic except *S. typhi*; reduce nitrate to nitrite; utilize citrate as carbon source except *S. typhi* and *S. paratyphi* A; decarboxylate lysine except *S. paratyphi* A; do not grow in KCN medium except *S. houtenae*; positive in the MR and catalase tests; and negative in the VP and oxidase tests. The ultimate criteria in classification of the species are serological tests based on somatic and flagellar antigens, phase 1 and 2 (*4,17*).

Because *Salmonella* occurs in small number in food, compared to competing microorganisms, it is necessary to employ enrichment media for its isolation. Tetrathionate and selenite broth that are suitable for isolation of salmonellae from clinical materials have a definite short coming when used for isolation of the bacteria from food (*25*). Among the problems involved in isolation are: (*a*) Impairment of the selectivity of the media by the sample (*26*). (*b*) The physiological dormancy of *Salmonella* in food products; thus to increase its recovery from food products, it needs to be preenriched in non-inhibitory media, like mannitol broth, before employment of selective broth (*28,29*). In addition, the time of incubation in preenrichment medium is critical (*19,21, 24*). Examples of the continuing outbreaks of *Salmonella* infection from food are those of 1965 and 1966 caused by *S. newbrunswick* (*20,22*). After the reports on new isolation methods (*15,30*), a comparison study between four methods for detection of *Salmonella* in food appeared. Included were the conventional fluorescent antibody, rapid direct fluorescent antibody technique, microcolony fluorescent antibody and enrichment serology. The latter is simpler to perform, with fewer false-positive results and is more economical than other methods (*1,18,27*).

Although official statistics on salmonellosis in Iraq are not available to us, enteric fevers and food poisoning outbreaks due to *Salmonella* are not uncommon. In a research project we are conducting on *Salmonella*, the bacterium has been frequently isolated from a wide variety of sources and from clinical cases for which tests were done in private laboratory work. Moreover, the frequency of isolation of *Salmonella* from poultry farms in Iraq is becoming alarming. This investigation was aimed at finding the occurrence and distribution of *Salmonella* species in local foods in the City of Baghdad,

and to find the most appropriate culture media for isolation of the bacteria from such sources.

MATERIALS AND METHODS

Samples

Three hundred and fifty three local food samples were collected in Baghdad, Iraq; they comprised raw chicken meat 47, raw vegetables (tomatoes, celery, lettuce, salad green) 43, baked cakes covered with cream 36, frozen kubba that was stuffed with meat and green raisins 34, raw milk 34, raw meat (beef and lamb) 33, home-made sweet cheese 26, non-carbonated soft drinks (orange and sherbert) 24, commercial olives exposed to house flies, other insects and dust 19, raw eggs 15, local hand-made sweet "Datly" exposed to air 14, home-processed raw cream 14, yogurt and house butter 8, fresh grapes and dates 6.

Media

All media used were dehydrated and prepared by Difco. For preenrichment from different food samples, mannitol broth was used (*11,14*). For selective enrichment media, tetrathionate brilliant green bile broth and selenite cystine broth were employed. For milk samples, neutral red lysine iron cystine broth (*15*) was employed. The differential media were brilliant green sulfa agar and xylose lysine desoxycholate agar.

Physiological tests

The following were employed: T.S.I. (Eiken Chemical Co. Ltd.); urea agar (Difco); lysine decarboxylase medium; gelatin liquefaction medium (Difco); mannitol motility medium (Difco); fermentation of glucose, lactose, salicin, sucrose and dulcitol; peptone water (Difco), MR-VP medium (Difco); Simmon citrate agar (Difco); malonate broth (Difco); potassium cyanide broth (Difco). The serological diagnostic media were tryptic soy agar and tryptic soy semisolid (Difco) using the Graigie tube method. Antisera were imported from Hoechst (W. Germany).

Isolation of Salmonella *from foods*

The following technique was employed to isolate *Salmonella* except when dairy products were tested. Ten g of food sample were added to 10 ml of mannitol broth and homogenized with a blender at 4000 rpm under aseptic condition. To the homogenate was added 90 ml of mannitol broth and the samples were incubated at 37 C for 18 h. One ml was transferred to 9 ml of tetrathionate brilliant green bile broth and then incubated at 37 C for 24 h. Another one ml was transferred to 9 ml of selenite cystine broth, incubated at 43 C for 24 h. One loopful from each of the above selective media was streaked on two differential media, brilliant green sulfa agar and Xylose lysine desoxycholate agar, and plates were incubated at 37 C for 24 h. Five *Salmonella*-suspect colonies were picked randomly from each plate for biochemical and serological tests. For milk and milk products, 10 ml or 10 g were placed in 100 ml of neutral red lysine iron cystine broth (*15*) which was incubated at 39 C for 24 h, and then processed as mentioned above.

Cold drinks were concentrated by centrifugation of 40 ml at 1000 *g* for 30 min in a refrigerated centrifuge, and then were reconstituted to 4 ml with physiological saline solution. One ml of the latter was transferred to 9 ml of tetrathionate brilliant green bile broth and another 1 ml to 9 ml of selenite cystine broth; they were incubated as above.

After tentative identification of the isolates, serologic groups and specific serotypes were determined. All isolates were confirmed at the National Salmonella Institute of Iraq. Further confirmation was obtained from Professor Le Minor at the Pasteur Institute in Paris.

RESULTS AND DISCUSSION

Of 353 food samples, 95 samples (27%) harbored *Salmonella*. These were distributed in the following types of local food: Kubba and cakes 6.2%, meat 4.8%, raw milk 3.1%, chicken and sweets 1.7% of each, olives 1.4% (Table 1). The occurrence was extremely high in certain types of food, mainly kubba, cakes and meat. Due to the known difficulties encountered in isolation of *Salmonella* from food and the relative suitability of the preenrichment and enrichment media, these positive values might be lower than reality, and thus the negative samples may not mean the absence of this bacterium. Fifteen *Salmonella* serotypes were identified, of which *S. paratyphi* B was the most frequently encountered in food samples (11%). This was followed by the occurrence of *S. typhimurium*, *Salmonella* 4,5,12:-:- and *S. enteritidis* (Table 2). In other investigations (*2,9*), the most predominant *Salmonella* species and serotypes reported in foods were *S. typhimurium*, *Salmonella heidelberg*, *Salmonella thompson*, *Salmonella tennessee*, *Salmonella montevideo*, *Salmonella newport*, and *Salmonella oranienburg*. Among the species, *Salmonella lille* and *Salmonella alachua* were isolated for the first time in Iraq. The serological formulae for the isolated species are also shown in Table 2. *S. paratyphi* B was isolated from a wide variety of local foods and in a high percentage as shown in Fig. 1. *S. enteritidis* was found in raw milk and could be of high incidence, while *Salmonella* 4,5,12:-:- and *S. typhimurium* were common contaminants in other foodstuffs. This type of distribution emphasizes the

TABLE 1. *Presence of* Salmonella *in local foods in Baghdad.*

Local foods	No. of samples	Positive samples		
		No.	Specific food (%)	Out of total (%)
1. Kubba	34	22	64.7	6.2
2. Cakes	36	22	61.1	6.2
3. Meats	33	17	51.5	4.8
4. Raw milk	34	11	32.3	3.1
5. Sweets	14	6	42.9	1.7
6. Chicken	47	6	12.8	1.7
7. Olives	19	5	26.3	1.4
8. Vegetables	43	3	7.0	0.85
9. Cheese	26	2	7.7	0.57
10. Cream	14	1	7.1	0.28
11. Eggs	15	0	0	0
12. Yogurt & butter	8	0	0	0
13. Fruits	6	0	0	0
14. Cold drinks	24	0	0	0
Total	353	95		27

TABLE 2. Salmonella *species isolated from local foods*[a].

No. Salmonella	No. of positive samples	% Of total	Serological formula
1. *S. paratyphi* B[b]	39	11	1,4,5,12:b:1,2
2. *S. typhimurium*	16	4.5	1,4,5,12:i:1,2
3. *Salmonella* 4,5,12:-:-	16	4.5	4,5,12:-:-
4. *S. enteritidis*	13	3.7	1,9,12:gm:1,7
5. *S. muenchen*	9	2.5	6,8:d:1,2
6. *S. senftenberg*	7	2.0	1,3,19:gst:-
7. *S. lille*[c]	5	1.4	$6,7:Z_{38}$:-
8. *S. anatum*	2	0.57	3,10:eh:1,6
9. *S. alachua*[c]	2	0.57	$35:Z_4Z_{23}$:-
10. *S. emek*	2	0.57	8,20:gms:-
11. *Salmonella* 1,3,19:-:-	1	0.28	1,3,19:-:-
12. *S. california*	1	0.28	4,12:gmt:-
13. *S. havana*	1	0.28	1,3,23:FG (S):-
14. *S. eppendorf*	1	0.28	1,4,12,27:d:1,5
15. *Salmonella* 6,7:-:-	1	0.28	6,7:-:-

[a]Total number of foods examined was 353,
[b]D-tartrate negative,
[c]Were isolated for the first time in Iraq.

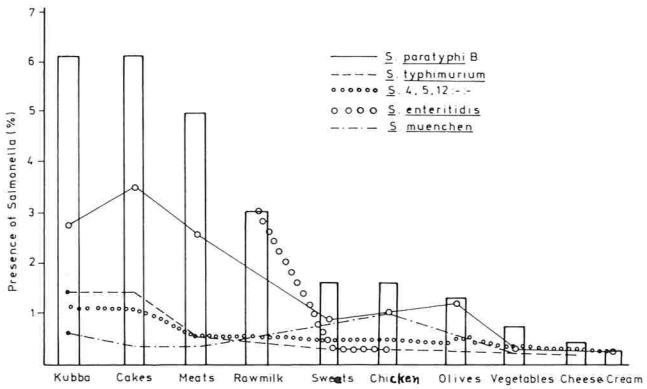

Figure 1. *The presence of* Salmonella *in some local foods.*

importance of the investigation of the source of contamination.

Eleven *Salmonella* serotypes were isolated from meats; they were mainly *S. paratyphi* B and the other 10 serotypes were *S. typhimurium, S. senftenberg, S. lille, Salmonella* 4,5,12:-:- *S. emek, S. alachua, S. california, S. anatum, S. muenchen* and *Salmonella* 6,7:-:-.

Since the classical chain of infection reported is feedstuff-animals-foods-man (*13*), strict bacteriological inspection and control is of vital importance. Eight different *Salmonella* serotypes were isolated from the commonly used food in Iraq, kubba; they were *S. paratyphi* B, *S. typhimurium, Salmonella* 4,5,12:-:-, *S. muenchen, S. senftenberg, S. lille, S. alachua* and *Salmonella* 1,3,19:-:-. The frequent isolation of *Salmonella* from kubba samples could be attributed to food handlers, contaminated water, kitchen utensils, beside the meat and other stuffings used. The last factor is confirmed by the isolation of the same serotypes from meat and kubba samples; furthermore, the species isolated were only found in meat, as in the presence of *S. senftenberg* and *S alachua.*

Cake samples contained six *Salmonella* serotypes; *S. paratyphi* B, *S. typhimurium, Salmonella* 4,5,12:-:-, *S. muenchen, S. havana* and *S. eppendorf.* A high contamination rate of cake samples (Table 1) may be due to food handlers or one of the constituents like cream and egg. The role of egg in the contamination of cake mixes is illustrated by some human outbreaks (*5*).

One *Salmonella* serotype was isolated from 70 (19.8%) food samples, two serotypes from 21 (5.9%) and three serotypes from 4 (1.1%), as shown in Table 3. Reports on frequency of *Salmonella* multiple infections are increasing (*23*).

TABLE 3. *Number and percent of local foods*[a] *harboring one or more* Salmonella *serotype.*

Salmonella	Number	%
One serotype	70	19.8
Two serotypes	21	5.9
Three serotypes	4	1.1

[a]Total food samples = 353.

The isolation of *Salmonella* from foods was found to be affected by types of selective enrichment medium used. As seen in Table 3, *S. paratyphi* B was isolated from 32 samples by using tetrathionate brilliant green bile broth but from only 20 samples using selenite cystine broth. However, the incubation temperature for the former was 37 C while for the latter it was 43 C. The high temperature used for selenite cystine broth has less inhibitory effect on *Salmonella* and the Arizona group than on other competing gram-negative rods (*12*). A *Salmonella* isolate recovered with one selective enrichment medium may not be recovered with another (*10,12*). When the identification of 20 colonies from each food sample (10 colonies from each of the two selective media) was evaluated, tetrathionate brilliant green bile broth revealed the highest number of *Salmonella* colonies (55.3%) in comparison to selenite cystine broth (44.7%), Table 5. Needless to say, there is an overlap in isolation of serotypes by selective media that were used. It seems that some *Salmonella* serotypes don't grow or at least are not enhanced in tetrathionate brilliant green bile broth, as noticed with *Salmonella* 6,7:-:-, *S. havana* and *S. eppendorf.* The reverse seemed true with *S. california* and *Salmonella* 1,3,19:-:-. It is evident from Tables 4 and

TABLE 4. *Effect of selective enrichment media on the isolation of* Salmonella *serotypes from local foods.*

Salmonella serotype	No. of samples	Positive samples (1)[a] No.	(1)[a] %	(2)[b] No.	(2)[b] %
S. paratyphi B	39	32	82.0	20	51.3
S. typhimurium	16	10	62.5	13	81.3
Salmonella 4,5,12:-:-	16	9	56.3	9	56.3
S. muenchen	9	8	88.9	5	55.6
S. senftenberg	7	3	42.9	4	57.1
S. lille	5	2	40	3	60
S. anatum	2	2	100	1.	50
S. emek	2	1	50	1	50
S. alachua	2	1	50	1	50
S. enteritidis[c]	2	2	100	1	50
S. california	1	1	100	0	0
S. havana	1	0	0	1	100
S. eppendorf	1	0	0	1	100
Salmonella 1,3,19:-:-	1	1	100	0	0
Salmonella 6,7:-:-	1	1	0	1	100

[a](1) Tetrathionate brilliant green bile broth at 37 C.
[b](2) Selenite cystine broth, at 43 C.
[c]Eleven isolates of *S. enteritidis* were picked up in neutral red lysine iron cystine broth.

TABLE 5. *Enhancement of two selective enrichment media on the growth of* Salmonella *serotypes.*

Serotypes	Isolation of *Salmonella* colonies from: (1)[a]	%	(2)[b]	%	Total isolates
S. paratyphi B	124	66.0	64	34	188
S. typhimurium	42	51.2	40	48.8	82
Salmonella 4,5,12:-:-	32	50.8	31	49.2	63
S. muenchen	27	60.0	18	40.0	45
S. senftenberg	11	64.7	6	35.3	17
S. lille	3	21.4	11	78.6	14
S. alachua	1	9.1	10	90.9	11
Salmonella 6,7:-:-	0	0.0	10	100.0	10
S. anatum	5	71.4	2	28.6	7
S. enteritidis	3	50.0	3	50.0	6
S. havana	0	0.0	5	100.0	5
S. eppendorf	0	0.0	5	100.0	5
S. emek	3	75.0	1	25.0	4
S. california	3	100.0	0	0	3
Salmonella 1,3,19:-:-	1	100.0	0	0	1
Total	255	55.3	206	44.7	461

[a](1) Tetrathionate brilliant green bile broth, 37 C.
[b](2) Selenite cystine broth, 43 C.

5 that the multiplication rate of specific types differs in both media, and generally tetrathionate enrichment was superior to selenite cystine for *Salmonella* recovery from most foods. This coincided with Mohr's results (*18*), but the use of more than one type of selective medium increases the chance of isolation of more species.

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Sixty-Seventh Annual Meeting of IAMFES

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Journal of Food Protection Vol. 42, No. 11, Pages 881-882,887 (November, 1979)

Adaptability of *Streptococcus thermophilus* to Lactose, Glucose and Galactose

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(Received for publication February 12, 1979)

ABSTRACT

Significant differences in growth response to lactose, glucose and galactose were found among different strains of *Streptococcus thermophilus*. Some strains fermented all three carbohydrates (group A), whereas other strains utilized lactose and glucose only (group B) and one strain grew on lactose alone (group C). Characteristically, most lactose-grown strains in either group A or group B showed slow adaptation to either glucose or galactose after transfer to a medium containing either of these carbohydrates. Growth on glucose did not influence growth patterns following the transfer of either group A or group B strains to lactose broth. Lactose-grown group A and group B strains were restricted in growth following addition of galactose to the medium, whereas glucose-grown strains were not. The results suggested differences in carbohydrate transport mechanisms and utilization.

Lactic streptococci play a major role in the preparation of many dairy foods. Much information has accumulated on the metabolism of carbohydrates by this group of microorganisms (*5,7,13*). The evidence suggests that metabolism of lactose in group N streptococci (*Streptococcus cremoris, Streptococcus lactis,* and *S. lactis* subsp. *diacetylactis*) involves an energy-dependent group translocation mechanism catalyzed by a phosphoenolpyruvate (PEP)-mediated phosphotransferase system (PTS), and several membrane-bound, stereospecific catalytic proteins (*6*). Similar transport mechanisms had been described in several other genera of microorganisms (*11*). Group translocation involves the chemical modification of a carbohydrate during transmembrane transport, resulting in the conversion of lactose to glycosyl-β(1,4)-galactoside-6-phosphate (lactose-P), and the subsequent cleavage of the latter by β-D-phosphogalactoside galactohydrolase (β-Pgal) to glucose and galactose-6-phosphate (*9*).

Information is limited on carbohydrate transport systems and metabolism in *Streptococcus thermophilus*, a closely related thermotolerant microorganism that plays an essential role in the preparation of yogurt and certain cheese varieties. In a preliminary report, Reddy et al. (*10*) presented data on lactose transport in galactose- and glucose-grown cells of *S. thermophilus* and concluded that only galactose-adapted cells were induced to transport lactose, the transport of which was apparently dependent on a PEP-PTS system.

In our laboratory, adaptation tests with different strains of *S. thermophilus* showed that free galactose was not utilized by all strains, indicating possible differences in carbohydrate transport systems and(or) sugar metabolism. This paper reports on the growth characteristics of different strains of *S. thermophilus* in broth media, as influenced by preadaptation to lactose, glucose and galactose.

[1]*Agricultural Research, Science and Education Administration, U.S. Department of Agriculture.*

MATERIALS AND METHODS

Cultures

Thirty one strains of *S. thermophilus* were obtained from colleagues and commercial sources (Table 1). The cultures were maintained in sterile 10% reconstituted nonfat dry milk and Hogg and Jago basal medium (*4*), containing 3% tryptone (Difco), 1% yeast extract, 0.2% beef extract and 0.5% KH_2PO_4, and having a final pH of 6.5 before sterilization. The basal medium was supplemented with lactose, glucose or galactose, as needed, at 0.5% concentration. The cultures were incubated at 37 C for 17-24 h, and stored at 4 C. Weekly transfers of all cultures were made.

The identity of the cultures was tested by several techniques. All strains grew at 45 C and yielded a negative test for arginine decarboxylase activity, according to the method of Mikolajcik (*8*). All strains gave negative results when tested for the presence of Lancefield's group N streptococcus antigen against Bacto Streptococcus Antiserum (Difco), using the capillary tube assay of Fung and Wagner (*3*). Antigen was prepared by the autoclave method (*2*). Maltose and mannitol fermentation tests, and the test for the ability to grow in the presence of 2% NaCl, were all negative, except for strains ST/AH and Is. These two strains were considered atypical.

Adaptation to carbohydrates and growth measurements

Carbohydrate adaptation experiments were carried out in lactose, glucose and galactose media, in which final carbohydrate concentration was 50 mM. Cultures initially grown in lactose medium were transferred (1%, v/v) to glucose and galactose media and incubated at 37 C for 24 h. Cultures showing growth after 24 h were subcultured daily for 5 days.

The growth characteristics of cultures were studied turbidometrically at 660 nm with Zeiss PMQ II spectrophotometer. The inoculum was prepared from a 14-h-old culture by centrifuging at 10,000 × *g* for 10 min and resuspending the pellet in 50 mM of K_2HPO_4-KH_2PO_4 buffer, pH 7.0. Appropriate dilutions of the cell suspensions were made so that a 0.1-0.2 ml aliquot added to 50 ml of fresh medium gave an initial absorbance of 0.01 at 660 nm. The carbohydrate concentration in these experiments was 10 mM, unless otherwise indicated. During incubation at 37 C, samples were withdrawn at convenient intervals for absorbance readings. Growth experiments were repeated three times.

RESULTS AND DISCUSSION

The results of a survey of different strains of *S. thermophilus* showed that growth responses to lactose, glucose and galactose were variable. Twelve strains fermented all three carbohydrates (group A), 18 strains fermented lactose and glucose but not galactose (group B) and one strain fermented lactose only (group C) under

the experimental conditions employed. These observations suggested that not all strains of *S. thermophilus* had the potential to transport galactose or glucose to the interior of the cell. Failure to grow on galactose also may have resulted in some strains (group B) from lack of the D-tagatose-6-phosphate or the Leloir pathway, which are present in lactic streptococci (*1*).

The cultures were studied in detail to evaluate the effect of preadaptation on the initial growth response to fermentable carbohydrates. Group A lactose-grown strains appeared to be transiently cryptic with respect to glucose and/or galactose utilization and showed a prolonged adaptation phase, with the exception of strains Is, P-137 and 19258, following transfer to either glucose or galactose broth (Fig. 1). The adaptation phase, which was characterized by very slow growth, lasted up to 7 h or, in some instances longer, depending on the strain. However, following prolonged incubation (24-48 h) and repeated transfers in glucose and galactose media, the growth response to the hexoses became more rapid and, with glucose, became as immediate as that observed in the lactose medium. A similar phenomenon in *Lactobacillus bulgaricus* was observed by Snell et al. (*12*) who studied carbohydrate utilization in this microorganism.

In several cultures (strains 4, 7, L-225, 4074, 7132, and 9353), the effect of increased monosaccharide concentration on growth was also checked. Supplementation of the glucose with galactose (10 mM), or increasing the glucose concentration to 20 mM and 100 mM had only a minimal effect on the initial growth response of five strains. The exception was strain 7, which grew nearly as well in 20 mM glucose as in 10 mM lactose medium.

The initial growth response of group A strains to galactose, with the exception of the atypical strain Is, was also slow when glucose grown cells were transferred to galactose broth. Several galactose-grown group A strains

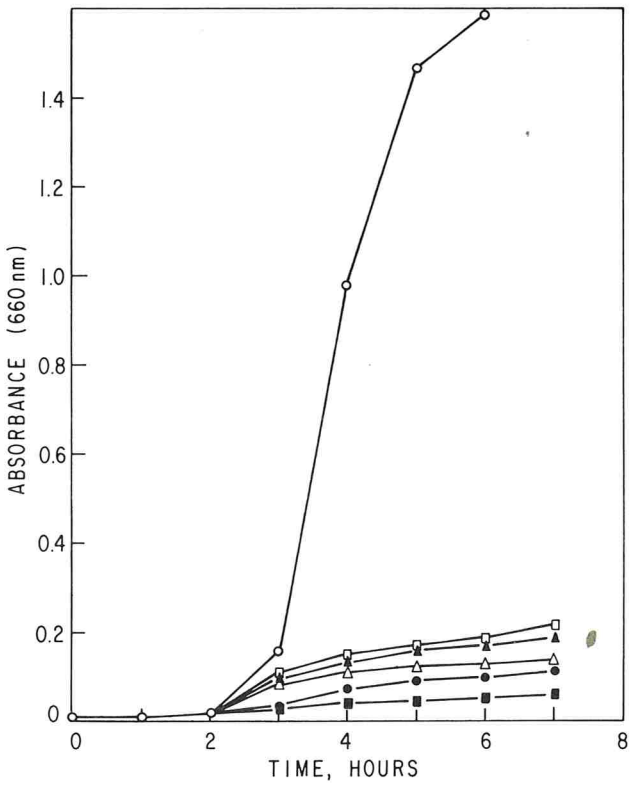

Figure 1. *Effect of preadaptation on the growth of* Streptococcus thermophilus *strain 9353 in lactose (L), glucose (G), and galactose (Ga) media.* ○, *L to L (10mM);* ●, *L to G (10mM);* △, *L to G (20mM);* ▲, *L to G (100mM);* □, *L to G (10mM) + Ga (10mM);* ■, *L to Ga (10mM).*

(4, L-225, P-371, 4074, and 9353) showed slow initial growth when transferred to 10 mM glucose broth, while others remained unaffected or grew even more rapidly (strains Ds, Is, 7, 404, 7132, and 19258). On the other hand, following transfer to lactose broth, all glucose-grown group A strains of *S. thermophilus* showed growth patterns that were indistinguishable from those observed in cases of lactose-to-lactose transfer.

TABLE 1. *Adaptability of* Streptococcus thermophilus *to carbohydrates.*

Group A (L, G, GA)[a]		Group B (L, G)		Group C (L)	
Strain	Origin[b]	Strain	Origin	Strain	Origin
		4109	Hansen	406	INRA
4074	Hansen	6069	"		
7132	"	7024	"		
9353	"	6097	"		
7	Microlife	9	Microlife		
4	Miles	8	"		
19258	ATCC	YB/ST	"		
371	NIZO	3	"		
L225	"	33	"		
Is	"	ST/AH	"		
Ds	"	19987	ATCC		
MC	UN	14485	"		
404	INRA	3641	NRRL		
		ST_S	NIZO		
		EB-8	UN		
		SFi-1	Nestle		
		SFi-3	"		
		391	INRA		

[a]L: lactose, G: glucose, GA: galactose.

[b]Hansen, Chr. Hansen's Laboratory, Inc., courtesy of R. L. Sellars; Microlife, Microlife Technics, courtesy of E. R. Vedamuthu; Miles, Miles Laboratories, Inc.; ATCC, American Type Culture Collection; NIZO, Netherlands Institute for Dairy Research, Ede, The Netherlands; UN, University of Nebraska, courtesy of K. M. Shahani; INRA, National Agricultural Research Institute, Jouy-Eu-Josas, France, courtesy of J. Auclair; NRRL, Northern Regional Research Center, U.S. Department of Agriculture; Nestle, Nestle Products Ltd., Lausanne, Switzerland, courtesy of T. Sozzi.

Strains of *S. thermophilus* that could not ferment free galactose (group B) also appeared to be cryptic with respect to glucose metabolism, and showed slow initial growth, with the exception of strains 14485 and 19987, when lactose-grown cells were transferred to glucose broth. Strains in this group showed normal growth patterns when glucose-grown cells were transferred to lactose broth, similar to the phenomenon observed with group A strains.

The results showed that most group A strains had difficulty metabolizing glucose and galactose, and most group B strains were slow to initiate growth on glucose, when a lactose-grown culture was inoculated into a medium with a monosaccharide as the carbon source. Since streptococci generally rely on glycolysis to ferment sugars (5), it was unlikely that group A and group B lactose-grown strains of *S. thermophilus* lacked the enzymic potential to metabolize glucose and/or galactose. It was more likely that in the transiently cryptic cells, one or more stereospecific components of monosaccharide transport systems were not induced when cells were growing in a lactose medium. On the other hand, glucose- and most galactose-grown group A strains, as well as all glucose-grown group B strains, initiated growth in lactose broth without showing a long adaptation phase. This suggested that transmembrane transport of lactose was, at least in part, constitutively expressed in most group A and group B strains of *S. thermophilus*, regardless of previous growth on glucose or galactose.

Lactose-grown strains of all three groups (A, B and C), except the atypical strains Is in group A and ST/AH in group B, grew more slowly than the control, when galactose (25 mM or 50 mM) was added to lactose broth at the start of incubation (Fig. 2). Glucose and the nonmetabolizable sugar alcohol mannitol failed to induce a similar change in growth pattern. The growth patterns of glucose-grown strains of either group A or group B, however, were not influenced when glucose medium was supplemented with galactose.

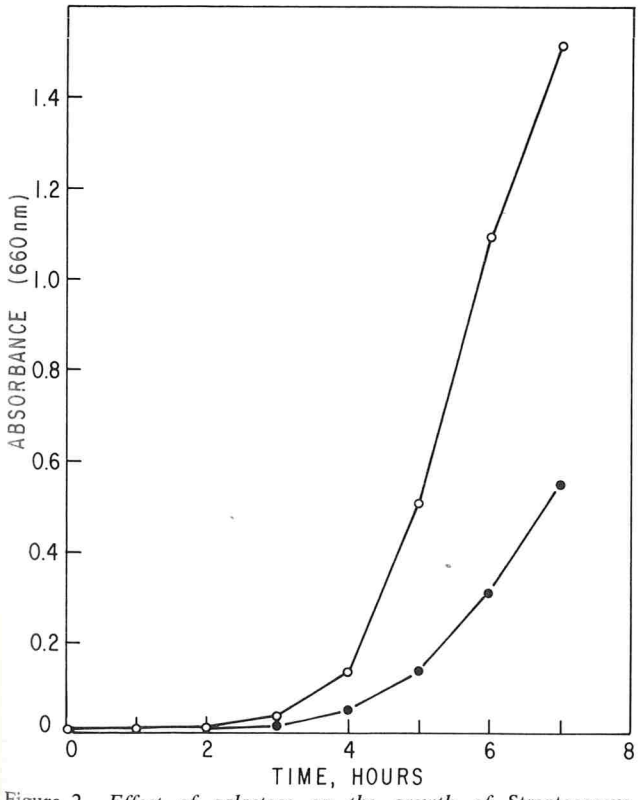

Figure 2. *Effect of galactose on the growth of* Streptococcus thermophilus *strain Ds in lactose broth.* ○, *L (10mM);* ●, *L (10mM) + Ga (25mM).*

The data suggested that in broth culture galactose interfered with lactose but not glucose metabolism in *S. thermophilus*. Group A strains, which fermented galactose, were not as susceptible as group B strains. The growth-suppressive effect of galactose is possibly the result of the inihibition of one or more catalytic proteins involved in the transmembrane transport of lactose.

Most strains in both groups A and B apparently have inducible transport systems specific for glucose or galactose, or both, whereas the transport of lactose is constitutively expressed. The inducible nature of hexose transport systems was supported by the finding that most lactose-grown strains required 6-7 h before initiation of rapid growth following transfer to media containing glucose or galactose.

Carbohydrate uptake studies with strains adapted to lactose, glucose and galactose are in progress.

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Journal of Food Protection Vol. 42, No. 11, Pages 888-889 (November, 1979)

A Research Note

Purchased Cell Cultures for Detecting Foodborne Viruses

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ABSTRACT

Cell cultures purchased from three different manufacturers generally showed adequate and comparable sensitivity to poliovirus which had been inoculated experimentally into extracts of ground beef.

Methods to recover foodborne viruses have evolved recently to the point that any food microbiology laboratory can carry out the extraction procedures (*4,6*). Nevertheless, virus will only be detected if it produces a perceptible infection in a laboratory host (*2*). The laboratory host of choice is ordinarily tissue culture, and unfamiliarity with tissue culture procedures seems to be a major deterrent to virologic investigations in many food microbiology laboratories.

Present methods produce food extracts which could be submitted to a clinical virology laboratory for testing. If this is not feasible, one might well wonder whether the tissue cultures offered for sale in the catalogs of several companies would serve adequately for this purpose. The present study was designed to compare tissue cultures obtained in 1976 from three different manufacturers and those prepared in our own laboratory, from the standpoints of cost and of sensitivity to virus in a model food extract. Vendors were selected solely on the basis of prices quoted. They represent the low, middle, and high ranges of prices charged for apparently comparable cultures at the time of these experiments; they are named here only for accuracy and completeness of reporting.

MATERIALS AND METHODS

Primary African green monkey (*Cercopithecus aethiops*) kidney tissue cultures were purchased from Industrial Biological Laboratories (IBL, Rockville, Md.), Grand Island Biological Company (GIBCO, Grand Island, N.Y.), and Flow Laboratories (FLOW, Rockville, Md.). Established African green monkey kidney tissue cultures (Vero, originally obtained from Flow Laboratories) were prepared in our laboratories by methods previously described (*5*). Poliovirus type 1, strain CHAT, was obtained from the American Type Culture Collection (Rockville, Md.) and was passed and assayed by the plaque technique in our laboratory.

Table 1 shows cost per culture, as well as other pertinent information. There was very little difference between manufacturers; most cultures were received in good condition, despite the severe winter weather, with two exceptions. First, of the three lots from IBL, one was received dead, and another was only 50% confluent when received and had to be grown to confluency. Second, the GIBCO cultures began to deteriorate after 8 days of incubation at 37 C, while all others were viable for at least 10 days, in the absence of virus.

Replicate ground beef extracts prepared by polyelectrolyte filtration methods (*3*) were inoculated with 11, 1.1 and 0.11 plaque-forming units (PFU) of poliovirus type 1 per 100 ml of unconcentrated extract. Each 100 ml was divided among three culture flasks (ca. 33 ml/flask) as previously described (*4*). The flasks were incubated at 37 C, with a change to maintenance medium at 24 h, and were examined daily for cytopathic effects (CPE) for 10 days. Experimental trial 1 was performed on January 6, trial 2 on February 2, and trial 3 on February 12.

RESULTS AND DISCUSSION

Results were recorded on the basis of whether virus effects were seen in each culture (Table 2). These were interpreted on the basis of the three-tube MPN table in *Standard Methods for the Examination of Water and Wastewater* (*1*); because the volume of inoculum had been 33 ml, rather than 10 ml per culture, numbers derived from the table were divided by 3.3. Of nine cultures of each kind inoculated with virus-containing beef extract in each trial, the number showing virus effects ranged from four to six, except for the Flow cultures in the second trial. This lot evidently was completely insensitive to PO1. Despite a wide range of

TABLE 1. *Comparative specifications of African green monkey kidney cell cultures.*

Producer	Culture type	Unit price 1976	Unit price 1978	Condition when received	Survival on experiment
IBL[a]	Primary	$2.75[b]	—	1 lot good 1 lot dead 1 lot 50% confluent	> 10 days
Flow	Primary	$5.00[b]	$2.80[b]	2 lots good	> 10 days
GIBCO	Primary	$6.50[c]	$7.10[c]	2 lots good[d]	8 days
FRI[e]	Established (Vero)	$0.50[b]	$0.64[b]	Not applicable	> 10 days

[a]Some problems experienced with billings; primary cultures no longer produced.
[b]Per 25-cm² styrene flask.
[c]Per 30-cm² glass bottle, though 48-cm² glass bottles were shipped on one occasion.
[d]One lot was received a week later than promised.
[e]Produced at the Food Research Institute; this strain subsequently died out.

TABLE 2. *Detection of poliovirus in ground beef extracts, using cell cultures from various producers.*

Producer	Experimental trial	Virus present (PFU/100 ml)				MPN	95% confidence limits	
		11	1.1	0.11	0		lower	upper
IBL	1	3[a]	1	0	0	13[b]	2	64
	3	3	1	1	0	23	4	70
Flow	1	3	1	1	0	23	4	70
	2	0	0	0	0	< 1	—	—
GIBCO	1	3	2	1	0	45	9	133
	3	3	1	1	0	23	4	70
FRI	1	2	2	0	0	6	1	14
	2	3	1	0	0	13	2	64
	3	3	1	0	0	13	2	64

[a]Number of cultures showing virus effect, of three inoculated.
[b]In PFU/100 ml of sample, at highest tested virus concentration.

point estimates for the other cultures, all of the 95% confidence intervals included the true value of 11 PFU/100 ml; this indicates that the sensitivities of these cultures, as a function of producer and of lot, was within the experimental error inherent in sampling for virus.

We compared purchased cultures on the basis of price because their unit cost was much greater than that for cultures we produce. The relatively high unit cost might well not be a serious deterrent to those who will use only small numbers of cultures and would rather not equip their laboratories for production. Neither the quality of the cultures nor of the accompanying account services are clearly functions of the producer's price. The incidence of such problems as the lot of cultures which was insensitive to polioviruses shows that the consumer who delegates cell culture production must still do some careful quality control; this would include diligent record-keeping, close observation of uninoculated control cell cultures, and frozen storage of control cell culture materials for possible later verification of results. The changes in costs since these experiments were performed in 1976, as shown by the 1978 prices in Table 1, do not appear to us to affect the validity of these conclusions.

ACKNOWLEDGMENTS

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Crumbine Award, *con't from p. 918*

including food protection, to more than 1,300,000 residents of Suffolk County. It covers an area of 922 square miles of eastern Long Island bounded by the waters of the Atlantic Ocean and Long Island Sound.

According to Charles W. Felix, Director, Environment, Health and Public Affairs of the Single Service Institute, "More than any other entry, the Suffolk program proved itself to exceed the norm in the four principal areas of measurement: program improvement, innovative and effective use of evaluation, effectiveness of planning and management, and information and education activities."

The Crumbine Award jury, Felix said, placed special emphasis on the Suffolk County organization's unique research project on sanitation and health effects in the shellfish industry of the Great South Bay of Long Island. This area is a leading shellfish producer, yielding between 50 and 80 percent of the hard clams marketed in the United States.

The Award jury also praised the Division for "the rare epidemiological accent given to traditional food sanitation practices" and for "remarkably effective utilization of limited manpower." The agency's program was further cited for its encouragement of no-smoking sections in restaurants and communications efforts in professional journals.

The Crumbine Award consists of a bronze medal and an engraved plate mounted on a walnut plaque. Bronze medallions are also presented to individual public health officials who are directly responsible for the winning agency's program.

Established by the Single Service Institute in 1954, the Crumbine Award takes its name from the Kansas State Health Officer and public health pioneer who in 1909 first banned common drinking cups from public facilities. The Institute is the national trade association of manufacturers of single-use food service and packaging products.

Journal of Food Protection Vol. 42, No. 11, Pages 890-894 (November, 1979)

Heat Treatment of Cultured Dairy Products

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ABSTRACT

By heat-treating cultured dairy products, lactic acid bacteria as well as contaminants are eliminated so that a considerable prolongation of shelf-life can be achieved. In general, a temperature of 70 C and a holding time of 30-60 sec are sufficient to eliminate lactic acid bacteria as well as contaminants, primarily yeasts and molds. The heat resistance of the thermophilic lactic acid bacteria proved to be 10-15 C higher than that of the mesophilic lactic acid bacteria. The different steps of the technology are reviewed. The manufacture of dessert products based on whey proteins is also proposed. In the conclusion, it is pointed out that this technology is recommended only if recontamination of the product during the filling process is avoided.

The problem of heat-treating cultured milk products to prolong their shelf life is not new. A U.S. patent published in 1914 describes the manufacturing process of canned cream-cheese which had been heated at 100 C for an extended period. For several decades a "hot-pack-cream-cheese", a kind of double-cream cheese heat-treated at 69-70 C and subsequently hot-filled in bags has been known in the U.S.; the shelf-life of this product supposedly being more than 60 days (*5*).

Pasteurization of yogurt was first patented by Klebs in 1928 "as a process for manufacturing from milk a creamy, vitamin containing yogurt beverage with a long shelf life characterized in that the milk is fermented in a closed package at approx. 45 C, sealed, quickly heated to 60 C and held at this temperature, then followed by fast cooling" (*14*).

STORAGE LIFE

The increasing popularity of cultured milk products, which is partly due to their combination with fruit, also required development of new technologies to obtain a prolonged shelf-life which is imperative for optimal distribution and sale. By heat-treatment of the finished product and subsequent aseptic filling this aim can be reached.

The shelf-life of cultured milk products depends on the microflora therein. Though lactic acid bacteria are eliminated during heat-treatment of cultured milk, the problem of contamination during the filling process still exists and can only be solved by aseptic filling (Fig. 1). To prolong the shelf-life, the following requirements regarding the technological process must be met: (*a*) avoidance of post-acidification (after-acidification) bitter taste, (*b*) elimination of contaminants such as coliforms,

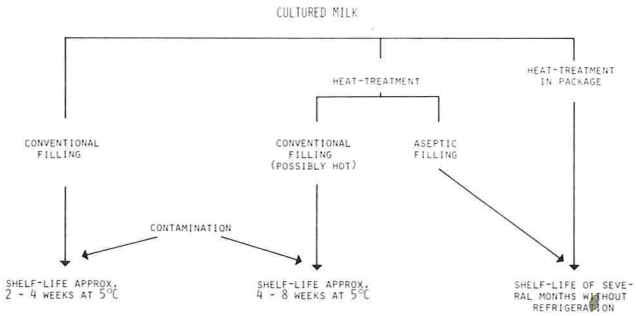


Figure 1. *Dependence of shelf-life of cultured milk on the technological process.*

yeasts and molds and (*c*) avoidance of unnecessary loss of valuable nutrients so that the economic consequences will result in easier distribution, access to more distant markets and easier shopping and storing for the consumer.

MICROBIOLOGICAL ASPECTS OF HEAT-TREATMENT

By heat-treating cultured milk products it is intended to more or less eliminate the lactic acid bacteria as well as contaminants such as yeasts and molds so that during the long period of storage there will be no metabolism of microorganisms such as post-acidification and proteolysis. Beside the microbiological aspects of this technology, consistency and structure of the product should remain unchanged.

First, the microbiological aspects of heat-treatment of cultured milk, including quark, will be discussed. Flüeler and Puhan (*6*) investigated the influence of temperature and H-ion concentration on elimination of lactic acid bacteria and other contaminants during heat-treatment of yogurt and quark. With decreasing pH in the product, heat-treatment results in a progressive reduction of the thermophilic and mesophilic lactic acid bacteria. The thermophilic lactic acid bacteria, however, resisted higher temperatures than did the mesophilic lactics. In yogurt with pH 4.55, 97.6% of the lactic acid bacteria survived after heat-treatment at 65 C, 22 sec, whereas at pH 3.82, 99.99% of the lactic acid bacteria were eliminated at the mentioned temperature/time relation. In quark, however, only 0.01% of the mesophilic lactic acid bacteria survived heat treatment at 60 C, 60 sec and pH 4.5.

At the holding time of 22 sec for yogurt and 60 sec for quark, and the same pH in both products, it became evident that for eliminating 99.98 - 99.99% of the lactic

TABLE 1. *Elimination of thermophilic lactic acid bacteria*[a] *during heat treatment of yogurt (6).*

Holding time, 22 sec at	Survival rate (%) at pH			
	4.55	4.28	4.05	3.82
55 C	100	92.1	88.4	79.5
60 C	100	79.4	76.6	61.2
65 C	97.6	77.4	6.16	0.002
70 C	0.015	0.001	0.001	0.001
75 C	0.001	0.001	0.001	0.001

[a]Before heat-treatment: 6×10^9 microorganisms/ml.

TABLE 2. *Elimination of mesophilic lactic acid bacteria*[a] *(homo- and heterofermentative) during heat-treatment of quark (6).*

Holding time, 60 sec at	Survival rate (%) at pH		
	4.50	4.25	4.05
50 C	3.56	1.33	0.438
55 C	0.174	0.026	0.019
60 C	0.005	0.008	0.014
65 C	0.003	0.004	0.006
70 C	0.001	0.001	0.001

[a]Before heat-treatment: 3×10^7 microorganisms/ml.

acid bacteria, the temperature for the thermophilics had to be approximately 15 C higher than that for the mesophilics. The remaining 0.01 to 0.02% of lactic acid bacteria practically showed no activity so that after storage for 2 months at 20 C, post-acidification could be measured neither in yogurt nor quark.

Flüeler (5) also investigated elimination of *Escherichia coli, Enterobacter aerogenes, Pseudomonas fluorescens, Kluyveromyces fragilis, Geotrichum candidum, Bacillus cereus* and *Clostridium sporogenes* during heat-treatment of cultured milk. In yogurt with pH 4.07, heat treatment at 55 C was sufficient to inactivate *E. coli* completely. However, 40% of *E. coli* survived heat treatment at this temperature at a slightly higher pH of 4.55. In quark, *E. coli* was inactivated completely at 55 C.

In yogurt, 60 C can be considered as the minimum temperature to inactivate *K. fragilis* and *G. candidum* completely, whereas in quark this minimum is slightly higher, i.e. between 60 and 65 C. The reason for this greater heat resistance of these two microorganisms in quark is probably due to the higher solids content and consequently lower lactic acid concentration.

TEMPERATURE OF HEAT-TREATMENT

A survey on development of heat-treatment technology for cultured dairy products from the basic investigation by Schulz (*14*), followed by Klupsch (*8*), Siegenthaler (*16*), Flüeler and Puhan (*6*), Robinson and Tamime (*13*), Egli and Egli (*4*), Kohl (*10*), Puhan (*11*) and Id (*7*) leads to the conclusion that the temperature range lies between 60 and more than 75 C, whereby no distinction is made between mesophilic and thermophilic lactic acid bacteria. However, the observations made by Flüeler and Puhan (*6*) revealed that, apart from differences in heat resistance of lactic acid bacteria, the minimum temperature for inactivating contaminants and lactic acid bacteria, depending on the pH of the product, is also different. These results have been summarized in Fig. 2.

TABLE 3. *Elimination of* Escherichia coli[a] *during heat-treatment of yogurt and quark (6).*

Temperature	Survival rate (%) at pH			
Yogurt - holding time 22 sec.				
	4.55	4.28	4.07	3.83
55 C	40.0	0.02	0	0
60 C	0.76	0	0	0
65 C	0	0	0	0
Quark - holding time 60 sec.				
	4.50	4.25	4.05	
50 C	5.57	0.94	0.23	
55 C	0.08	0.001	0	
60 C	0	0	0	

[a]Added: 5×10^5 microorganisms/ml or g.

TABLE 4. *Elimination of* Kluyveromyces fragilis[a] *during heat-treatment of yogurt and quark (6).*

Temperature	Survival rate (%) at pH			
Yogurt - holding time 22 sec				
	4.55	4.28	4.07	3.83
55 C	62.96	4.67	7.77	0.15
60 C	0.06	0	0	0
65 C	0	0	0	0
Quark - holding time 60 sec				
	4.50	4.25	4.05	
50 C	100	64.05	69.07	
55 C	36.38	23.19	12.64	
60 C	0.06	1.35	0	
65 C	0	0	0	

[a]Added: 1×10^5 microorganisms/ml or g.

TABLE 5. *Elimination of* Geotrichum candidum[a] *during heat-treatment of yogurt and quark (6).*

Temperature	Survival rate (%) at pH			
Yogurt - holding time 22 sec				
	4.50	4.28	4.07	3.83
55 C	8.25	18.64	0.10	0
60 C	0	0	0	0
Quark - holding time 60 sec				
	4.50	4.25	4.07	
50 C	53.75	40.10	33.62	
55 C	32.61	5.09	8.35	
60 C	2.40	0	1.48	
65 C	0	0	0	

[a]Added: 6×10^4 microorganisms/ml or g.

Contrary to Siegenthaler's opinion (*16*), according to which the pasteurization temperature of the finished product "should be chosen as high as possible" and "that the upper limit is reached where a negative influence on the cultured milk product by cook-flavor or changes in consistency and structure occur", we are of the opinion that the temperature should be kept as low as possible.

Without discussing the problem of whether the original name can still be used for heat-treated cultured milk products, two technologies of more recent date should be mentioned where the product is indeed undergoing heat treatment with the lactic acid bacteria remaining alive.

For manufacture of yogurt Battistotti et al. (*3*) and Bottazzi (*2*) use special thermoresistant *Lactobacillus bulgaricus* and *Streptococcus thermophilus* strains which

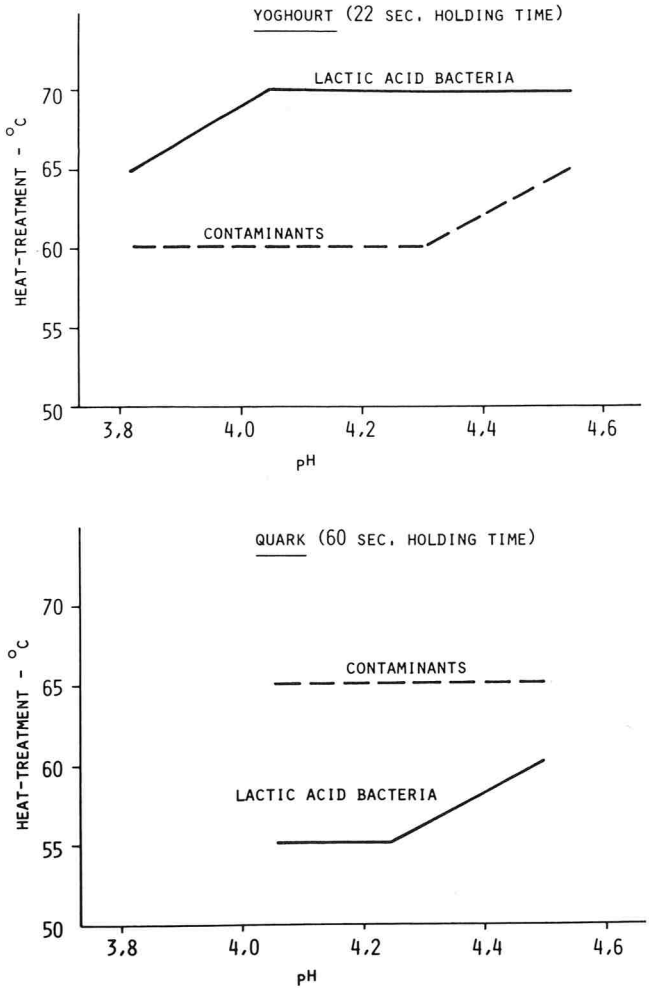
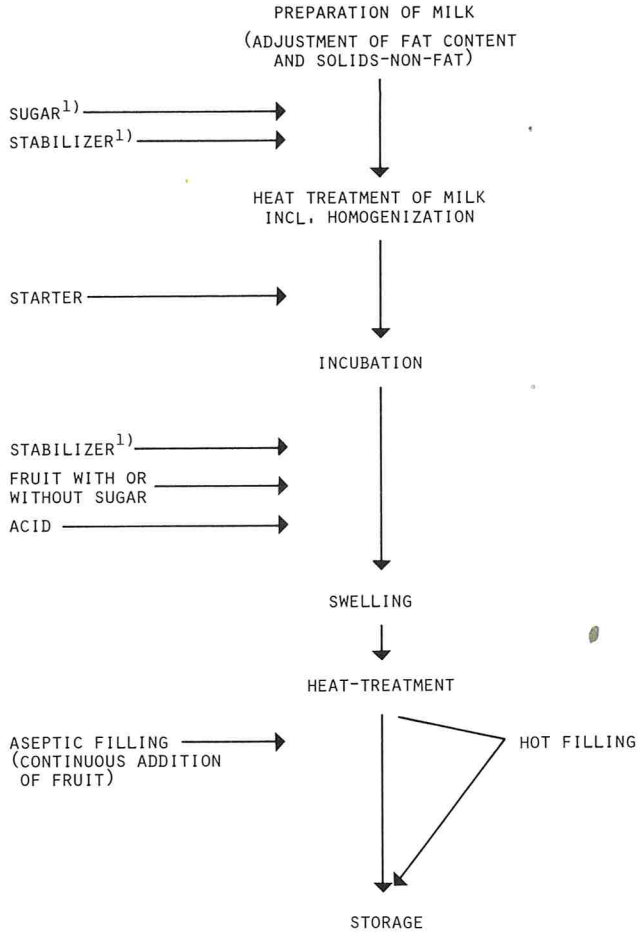


Figure 2. *Temperature limits for eliminations of lactic acid bacteria and contaminants from yogurt and quark (6).*



[1)] ALTERNATIVES

Figure 3. *Manufacturing procedure of heat-treated cultured milk products.*

survive heat-treatment at 65 C. According to a HF/UHF multiple frequency method for heat-treating cultured milk products described by Bach (*1*), it is possible to inactivate yeasts and molds at a temperature below 60 C without damaging the added starter culture. Heat-treated cultured milk products in which the lactic acid bacteria remained active following heat-treatment must be stored at low temperatures, whereas the storage temperature for products with inactivated lactic acid bacteria can be higher.

TECHNOLOGICAL ASPECTS OF HEAT-TREATMENT

The purpose of heat-treating cultured milk products should be to prolong their shelf-life without, however, having any negative influence on their quality. But these two factors, heat and acidity, favor syneresis of the casein followed by a separation of precipitated casein and whey. It is thus the technologist's task to take measures to avoid such adverse effects on the quality of the product. Figure 3 shows the technological process for heat-treatment of cultured milk.

Contraction of the acid-precipitated casein is influenced by the following factors (*14,16*): pH of the product; fat-, protein-, and sugar-content; heating temperature and -time of the milk before fermentation; proteolysis; type of hydrocolloid (stabilizer) and heating temperature of finished product.

Practical experience showed that with increasing heat-treatment of the milk for production of cultured milk products, contraction of the gel and thus its tendency towards syneresis diminishes considerably. In general, it is true that extensive denaturation of whey proteins has the effect of a stabilizer, increasing at the same time the viscosity of the product. The investigations of Schulz (*15*) show that the more intensively milk is heat-treated before fermentation, the higher the pH can be at which the cultured milk product is pasteurized (Fig. 4).

Higher fat and sugar contents are also beneficial for stability of the product, whereas the opposite applies to the casein content. As regards acidity, the product can be heated more easily when the pH is lower. If there should not be enough lactic acid, it is recommended to adjust the pH, depending on the product, with an accuracy of ± 0.05 by adding a mixture of citric and tartaric acid. In general, heat-treatment is carried out within the range of pH 4.40-4.10. At a pH below 4.0, a cultured milk product can generally be pasteurized without stabilizer (*14*).

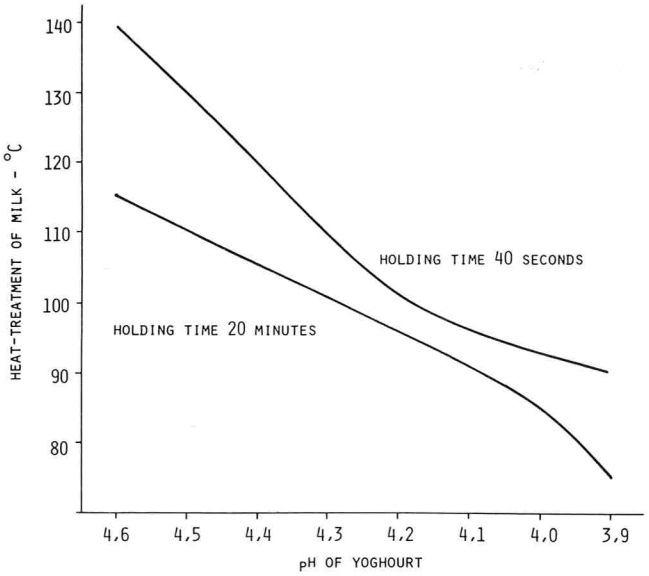


Figure 4. *Influence of heat-treatment of milk on the upper pH-limit for pasteurizing yogurt (15).*

Hydrocolloids are practically indispensible in heat-treating of cultured milk products as they stabilize the proteins, form gels and increase the viscosity (*9,14*). Hydrocolloids such as starch, gelatin or pectin may be added during the preparation of milk before fermentation or after fermentation but before heat-treatment if guar gum, locust bean gum, etc. are used. When selecting type and quantity of hydrocolloids, it must be kept in mind that these are merely additives necessary for heat-treatment and therefore should not change the properties of the finished product.

Heat-treatment of the product is followed by the filling process whereby it is of utmost importance that any contamination with yeasts and molds as well as lactic acid bacteria must be avoided. This is possible by either aseptic filling or hot filling. When filling the product aseptically it is cooled before filling. If the second method is applied, the product should be filled at the temperature it was heat-treated. To eliminate probable molds and yeasts, the temperature should not fall below 65 C (Fig. 2). Cooling of the packed product should be fast to avoid possible changes. Impeccable packing material, high hygienic standard, elimination of unsealed cups and thorough cleaning and disinfection of the machines following use as well as disinfection before filling are imperative for successful hot-filling of heat-treated cultured milk products (*8*).

In connection with heating and cooling, thought should also be given to the mechanical damage of the coagulum, especially that of low-fat products. Unsuitable pumps as well as too long and narrow pipes may have a negative influence on the consistency. By using stabilizers, the consistency of the product can be improved. However, the greater the mechanical damage was, the less effective the stabilizers will be. For this reason, the viscosity of aseptically-filled products is always less than that of hot-filled products.

Heat-treatment is mainly applied to cultured milk products with fruit and flavor added. Some flavor components are heat-labile. Furthermore, certain stabilizers such as locust bean gum and guar gum decrease the intensity of the flavor. For these reasons the heat-treated cultured milk product needs an overdose of flavoring material (*12*).

WHEY-PROTEIN DESSERTS

Finally, I would like to mention the heat-treatment of milk-type desserts. The technology of these products, which are based on sweet or cultured milk, is relatively simple and the problems of shelf-life are similar to the afore-mentioned products.

Due to the low pH value of the fruit component, production of desserts with added fruit is possible only by using cultured milk. New possibilities appear by using whey proteins obtained by ultrafiltration. These can easily be mixed with fruit and gel is formed quickly at temperatures between 75 and 90 C. By a suitable combination of whey-protein concentrate and stabilizers it is possible to produce acid milk-based products without lactic acid fermentation (*17*).

CONCLUSION

Even with the technical possibilities and the technological know-how of today for considerably prolonging the shelf-life of cultured milk products, I am of the opinion that these possibilities should not lead to a conversion of fresh products into canned food with a shelf-life of several months without refrigeration. Heat treatment of cultured milk products should not be considered as a possibility to correct lack of hygiene during production but rather as technological progress which is sensible only if recontamination of the product during the filling process will be avoided.

TABLE 6. *Influence of heat-treatment of UF-retentate on gelling of whey-protein (17).*

Protein (%)	Heating temperature (C)					
	65	70	75	80	85	90
14	90′	25′	2′15″[b]	50″	30″	25″
12	Non gelling	Non gelling	2′30″	60″	35″	30″
10	"	"	5′15″	1′10″	40″	30″
8	"	"	7′	1′30″	45″	40″
6	"	"	Non gelling	Non gelling	Non gelling	Non gelling
4	"	"	"	"	"	"
2	"	"	"	"	"	"

[a] 90 min.
[b] 2 min, 15 sec.

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Interim Report of the Applied Laboratory Methods Committee 1979

The activities of the three subcommittees of the Applied Laboratory Methods Committee have been limited during the past year due to resignations of two sub-committee Chairpersons and increased job responsibilities of the third Chairperson. New Chairpersons are Berry Gay, Jr. - Subcommittee on Laboratory Methods for the Examination of Water and Other Environmental Samples and Clair Gothard - Subcommittee on Laboratory Methods for the Examination of Milk and Milk Products.

Members of the Milk Methods Committee have served in various capacities in the preparation of new editions of APHA "Standard Methods for the Examination of Dairy Products." The 14th Edition has been published and Chapter Chairpersons will soon be selected for the 15th edition. Two members of this committee have been appointed to serve in control capacities and we anticipate additional requests for committee participation of members.

The new Chairpersons will be revitalizing their subcommittees, making changes or additions where applicable. All Chairpersons will be prioritizing subcommittee projects for development during calendar years 1979-1980.

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Fermentation of Soy Milk by Lactic Acid Bacteria. A Review

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ABSTRACT

Fermentation of soy milk with lactic acid bacteria offers a means of preserving soy milk and the possibility of modifying the characteristic flavor and texture to make it more acceptable to Western taste. It is possible to make soy milk yogurt-like products with acceptable texture and clean acid flavor. The choice of fermenting organisms is limited to those that can ferment the sugars typical of soy milk, i.e. stachyose, raffinose or sucrose, unless sugars fermented by the desired culture(s) are added to the soy milk. Utilization of stachyose and raffinose in soy milk should decrease its tendency to produce flatulence in the intestinal tract and therefore improve the digestibility and acceptability. Further research is needed regarding activity of the lactic organisms in soy milks.

Soy milk is a milk-like product obtained by aqueous extraction of soybeans. Since the protein content of soy milk is similar to that of cow's milk, it can contribute to infant and child nutrition in areas of the world where cow's milk is in short supply or too expensive for the general population. Soy milk is widely used in parts of East Asia, including Thailand, Taiwan, Hong Kong, and Singapore. A soy milk satisfactory to 6th grade Filippino school children was developed by Steinkraus et al. (*47*). However, in other parts of the world soy milk to date has been used primarily in feeding infants allergic to human or cow's milk.

Development of a beany flavor during manufacture of soy milk has limited its use in populations other than those accustomed to soybean foods. Thus much effort has been directed toward elimination of beany flavor in processing of soy milk. One approach has been to heat the soybeans either before or during initial processing to inactivate lipoxygenase to minimize development of undesirable flavors due to degradation or oxidation of lipids (*31,32,54*). A second approach has been complete extraction of lipids to remove the substrate which leads to development of off-flavors (*43*). A possible third approach is the use of fermentation to modify and improve flavor. This approach has been quite successful with fungal fermentations. Fungi such as *Rhizopus oligosporus* (*23,44,45*) *Neurospora sitophila* (*46,48*), and *Aspergillus oryzae* (*51*) and bacteria such as *Bacillus natto* (*17*) have been successfully used to prepare fermented soybean foods. Their preparation and the action of these organisms on soybean constituents has been studied in detail (*5,6,9,10,33,40,50,52*). Recently Gray (*14*) reviewed the use of these organisms in food fermentations. Use of lactic acid bacteria in preparation of fermented soybean foods has received increased attention recently. The information on the behavior of these organisms in soy milk is in scattered form. This review summarizes the pertinent literature on the growth and activity of lactic organisms in soy milk.

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FERMENTED PRODUCTS

Kellogg (*19*) was the first to prepare a fermented product by using a lactic culture in soy milk. A butter-like product was obtained by using *Lactobacillus acidophilus*. Later on Gehrke and Weiser (*11,12*) found that soy milk is a satisfactory medium for growth of lactic acid bacteria. However, they also found that lactic cultures produced less acid in soy milk than in cow's milk. *Streptococcus citrovorus* (*Leuconostoc cremoris*) *Streptococcus paracitrovorus* (*Leuconostoc dextranicum*), and *Streptococcus lactis* produced about half as much acid in soy milk as in cow's milk. In contrast to this, no significant differences in the volatile acidity measurements were observed in either of the milks.

Ariyama (*7*) developed a process for manufacture of a synthetic yogurt from soybeans with protein (9.8%) and mineral contents higher than those of cow's milk yogurt. The soy milk was supplemented with 15% sucrose and fermented with *Lactobacillus bulgaricus* or cow's milk yogurt cultures to obtain a desired product. Studies conducted on fermentation of carbohydrates by the Subcommittee on Taxonomy of Lactobacilli (*49*) and other workers (*27,38*) have clearly established that *L. bulgaricus* does not use sucrose. The fermentation reported by Ariyama (*7*) must have been obtained by using cow's milk yogurt as it contains both *Streptococcus thermophilus* and *L. bulgaricus*. *S. thermophilus* ferments sucrose (*27*) and hydrolysis products of sucrose are utilized by *L. bulgaricus*. It appears that acid production in the product may, therefore, have been due to the activity of cow's milk yogurt organisms rather than of *L. bulgaricus* alone as stipulated by Ariyama (*7*). Moreover, in view of the very high solids content, this product would be much different in appearance and consistency from that of dairy yogurt.

Streptococcus faecalis has been used to prepare a cheese-like product from soybeans (*20*). Soy milk was

supplemented with casein, glucose, milkfat and vegetable oil. The mixture was treated with *S. faecalis*, rennet extract and calcium chloride. The resulting curd was processed according to the conventional method of cheesemaking.

Use of *S. thermophilus* in preparation of a cheese-like product from soy milk has been investigated by Hang and Jackson (*15,16*). They concluded that satisfactory cheese could be prepared by lactic fermentation of soy milk. The cheese thus obtained was superior in body and texture and contained less moisture than the product obtained using acetic acid or salt precipitation. Incorporation of skim milk and rennet extract along with lactic culture improved the flavor of the product. Skim milk stimulated growth of the organisms because of supplementation of soy milk carbohydrates with lactose. The soy milk constituents did not show any change except for the carbohydrates which were presumably used for acid production.

Obara (*34*) suggested that no satisfactory product can be made from soy milk by the conventional cheese-making process. He, therefore, treated the curd obtained by salt precipitation with proteolytic enzymes before inoculating with *Streptococcus cremoris* and *S. lactis*. Among the single enzymes tested, papain gave the best results with respect to flavor and texture. Trypsin and molisin were found unsatisfactory because of poor digestibility and inferior flavor of the product. A combination of papain, bioprase and pronase exhibited a higher rate of ripening as well as more desirable flavor and texture than other enzyme combinations or single enzymes tested.

A combination of *S. thermophilus* and *L. bulgaricus* has been used by Yamanaka et al. (*55*) in preparation of a sour milk beverage from soybean protein, cow's milk and sucrose. They also added certain amino acid mixtures to mask the characteristic flavor of soybean protein. An amino acid mixture containing alanine, arginine, aspartic acid, sodium glutamate, lysine, methionine and glycine appreciably masked soy flavor. Replacement of this mixture with proline or a mixture of proline and alanine gave equally good results.

ACID PRODUCTION IN SOY MILK

S. thermophilis produces remarkably greater amounts of acid in soy milk than other lactic cultures. Matsuoka et al. (*24*) found that *S. thermophilus* produced a greater amount of acid in soy milk than *S. lactis* and *L. bulgaricus*. However, the cheese-like product made from soy milk using *S. thermophilus* darkened significantly during ripening. Kim and Shin (*21*) also described greater acid production by *S. thermophilus* than *S. cremoris* and *L. bulgaricus*. However, contrary to eariler findings (*15*), they observed that the amount of acid produced by *S. lactis* subsp. *diacetylactis* was comparable with that of *S. thermophilus*. They also prepared a cheese-like product using *S. thermophilus* and smeared the surfaces with *Penicillium caseolyticum* and sodium chloride. The proteolysis observed during ripening was due to action of the mold on soybean protein.

Acid production by a number of lactic cultures in soy and soy-skim milk combinations as compared to skim milk alone has been studied by Yamanaka and Purukawa (*56*). Acid production by *S. thermophilus, S. faecalis, L. acidophilus, L. bulgaricus* and *Lactobacillus casei* was higher in soy-skim milk combinations containing up to 70% soy milk than in skim milk alone. They also observed that supplementation of soy-skim milk combinations with glucose enhanced acid production by all cultures whereas addition of sucrose increased acid production by *L. acidophilus* only. They concluded that curd texture became harder with increasing proportions of soy milk.

Angeles and Marth (*1*) reported that acid production in soy milk was not always directly related to the growth rates of the organisms. They found that *S. thermophilus, Lactobacillus delbrueckii, Lactobacillus plantarum* and *L. dextranicum* produced greater amounts of acid in soy milk than did other species of lactic acid bacteria tested because of their ability to utilize the sugars. They did not study use of individual sugars by the organisms. Increased acid production by *S. lactis, S. cremoris, S. lactis* subsp. *diacetylactis, L. casei* and *Lactobacillus helveticus* in soy milk on supplementation with glucose, whey powder, or lactose showed that either the carbohydrates present in soy milk are a limiting factor or the lactic cultures tested do not fully utilize them.

Acid production in the medium depends upon growth of the organisms and their ability to ferment the available carbohydrates. The fermentable carbohydrates in soybean and soybean products are water soluble, low molecular weight oligosaccharides such as sucrose (5.0%), raffinose (1.1%) and stachyose (3.8% (*18*). Mital et al. (*27*) tested a number of lactic acid bacteria for their ability to utilize soybean oligosaccharides for acid production. They found that organisms such as *S. thermophilus, L. acidophilus, Lactobacillus cellobiosis* and *L. plantarum,* which utilized sucrose, the major fermentable sugar in soybeans, exhibited significant growth and produced substantial amounts of acid in soy milk. Although *Lactobacillus buchneri* ferments sucrose, it exhibited a rather slow rate of growth and produced less acid in soy milk than those organisms. The poor growth and acid production in soy milk by *L. bulgaricus* was explained by the authors on the basis of inability of this organism to ferment sucrose and other soybean carbohydrates.

Lipolytic and proteolytic activities of lactic cultures in soy milk have also been studied (*3,4*). Most of the cultures tested did not hydrolyze soy lipids. However, *L. casei, L. delbrueckii* and *S. thermophilus* liberated some free fatty acids in soy milk and in MRS broth fortified with 2% soy lipids, *L. delbrueckii* and *S. thermophilus* also exhibited proteolytic activity in soy milk. Both were, however only weakly proteolytic as compared to *Bacillus cereus* and *Micrococcus conglomeratus*.

Attempts have been made to improve the flavor and texture of soy-cheese by incorporating skim milk and by mold ripening (*39*). The addition of skim milk was insufficient to mask the dominating beany flavor or appreciably change the texture of the finished cheese because of the effect imparted by the fibrous soybean matter. Mold ripening brought about desirable changes in the texture of the product but the improvements were off-set by the development of bitter flavors, presumably resulting from proteolysis.

PROCESSING SOY MILK

Various processing conditions and methods of preparation of soy milk affect acid production by lactic cultures in soy milk. During soaking of soybeans, carbohydrates leach into the soak water which is discarded. This practice reduces the carbohydrate concentration in the resulting soy milk medium and thus decreases acid production by lactic cultures. Lo et al. (*22*) found that as the soaking time for soybeans increased, larger quantities of water-soluble solids leached out in the soak water and were lost during the manufacturing process. Analysis of dry solids in soak water showed that mainly carbohydrates were lost. Badenhop and Hackler (*8*) found that soaking soybeans in 0.05 N sodium hydroxide yielded milk with a pH of 7.37. Such an alkaline pH will not favor the growth of lactic cultures. Mital et al. (*27*) observed that all lactic cultures exhibited higher growth and acid production in soy milk prepared by a hot grind method than in soy milk prepared from dehulled defatted soybeans. They concluded that loss of some of the growth factors during solvent extraction of the defatted beans explains the difference in the behavior of the organisms in the two milks.

The effect of heating on acid production in soy milk has been studied by Angeles and Marth (*2*). They found that heat treatment of soy milk (60 C for 15 min) enhanced acid production, whereas heating of soy milk to 80 C for 5-60 min greatly reduced acid production as a result of marked increases in the concentrations of inhibitory substances such as sulfhydryls and toxic volatile sulfides. More severe heating at 100 or 120 C progressively improved the quality of soy milk as a substrate. The beneficial effect of severe heating on acid production was attributed by the authors to expulsion of toxic compounds and a decrease in the oxidation-reduction potential of the medium.

Soybean oligosaccharides such as raffinose and stachyose contain $a(1 \rightarrow 6)$ galactoside linkages. Since the human gastrointestinal tract does not possess an *a*-galactosidase (*13*), the metabolic fate of these oligo-saccharides is uncertain. Some investigators (*35,36, 37,41,42*) have suggested that raffinose and stachyose are responsible for flatulence often experienced following consumption of soy products. With a view to removing these oligosaccharides by lactic fermentation, Mital et al. (*26*) tested a number of lactic cultures for *a*-galactosidase activity. They found that the enzyme is constitutive in *L. buchneri, L. brevis, L. cellobiosis, Lactobacillus fermentum* and *Lactobacillus salivarius* subsp. *salivarius* and present in the soluble fraction of the cell. However, it could also be induced in *L. plantarum* (*25*). Fermentation of soy milk with lactic cultures possessing *a*-galactosidase activity reduces raffinose and stachyose contents (*28*). However, rapid utilization of sucrose resulted in a pH low enough to inhibit further use of higher saccharides and thus proved to be a limiting factor in their complete removal.

The changes in pH of soy milk fermented with *L. acidophilus* and *L. bulgaricus* have been compared by Wang et al. (*53*). *L. acidophilus* significantly lowered the pH of soy milk, indicating its ability to use soy milk carbohydrates for acid production. In conformity with the findings of Mital et al. (*27*), they also found that *L. bulgaricus* did not change the pH of soy milk, reflecting the inability of this organism to ferment sucrose, the major soy milk carbohydrate. They also observed that beany flavor was masked by fermentation of soy milk with *L. acidophilus*.

FLAVOR OF SOY MILK

Generally, the flavor of soy milks is unacceptable in the Western World. Usually soy milks have compared to other soy milks to determine if a flavor improvement has been achieved. Mital and Steinkraus (*29*) compared the flavor acceptability of soy milks prepared by a hot-grind process and an extraction process utilizing defatted soybean flour to that of fresh homogenized cow's milk, a well known, highly acceptable flavor standard. Hot-grind soy milk was significantly different and distinctly inferior to cow's milk in flavor. Soy milk prepared from defatted soybean flour 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 prepared from 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 milk had a satisfactory gelatinous curd and an acceptable flavor. Reduced acid production in the fermented soy milks was an impediment to higher acceptability. Later Mital et al. (*30*) found that acid production in fermented soy milks could be enhanced by enrichment of soy milk with sucrose, glucose or lactose and further increased by using selected lactic cultures.

CONCLUSION

Use of lactic cultures in improving the acceptability of soy products holds great promise. However, more information is needed regarding activity of these organisms in soy milk. Use of soybean oligosaccharides essential for acid production needs further investigation if lactic cultures are to be used in preparation of fermented soy products. Use of oligosaccharides in soy products for acid production will also make the products less flatulent. Also, information on proteolytic and lipolytic activities of lactic cultures needed for prepara-

tion of products such as cheese is still insufficient. Investigations along these lines will be of great help in formulating acceptable soy foods for human consumption in the future.

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Report of The Journal Management Committee

The Journal Management Committee met on August 12, 1979 and discussed the actions taken on previous recommendations as well as the current status of the Journal. As in the past, the Committee decided to reaffirm a few previous recommendations that had not been acted upon by the Executive Board and to make new recommendations for consideration by the Board as follows:

1. The Committee recommends that a feasibility study be conducted of splitting the *Journal* into two monthly publications with contents as follows:
   (a) New Journal
      (1) Articles and speeches of interest to the practicing sanitarian and fieldman
      (2) News and Events
      (3) Association Affairs
      (4) Program-Annual Meeting
      (5) 3A Standards
      (6) E-3A Standards
      (7) Letters to the Editor
      (8) Abstracts of papers published in the *Journal of Food Protection*
      (9) Advertisements
      (10) Coming Events
      (11) Sustaining Membership
   (b) *Journal of Food Protection*
      (1) Scientific Papers
      (2) Advertisements
      (3) Sustaining Membership
2. The Committee recommends that the Assistant Managing Editor of the *Journal* review other publications to identify and obtain material of direct interest to the practicing sanitarian for reprinting in the *Journal*. (Repeat from 1978)
3. The Committee recommends that the Instructions to Authors material be expanded by adding a section specifically for the writing of articles for the practicing sanitarian. (Repeat from 1978)
4. The Committee commends the printing of information from *Morbidity and Mortality Reports* of CDC and recommends that this kind of coverage be extended to the *Federal Register*.
5. The Committee recommends that the source of the material printed as filler in the main section of the *Journal* and in the News and Events section be identified at the end of each article. (Repeat from 1978)
6. The Committee recommends that a Directory of Members of IAMFES be published.
7. The Committee recommends that a Committee be appointed to seek sustaining members from the food industry.
8. The Committee recommends that the *Journal* name, volume and data be published on each page of the *Journal* that is used for scientific papers.
9. The Committee recommends that the page charge for scientific articles be increased to $30.00. It further recommends that a $35.00 page charge be placed into effect if the added revenue is needed for initial support of the increased costs of publishing two journals.
10. The Committee recommends that the IFT and Am. Dietetic Assoc. meetings be attended and a suitable display be prepared to call attention to the IAMFES and the *Journal*.
11. The Committee recommends that the Asst. Managing Editor appoint a committee of knowledgeable sanitarians and a committee of dairy fieldmen whose responsibilities would be to develop lists of subjects of current concern that should be developed and published in the *Journal* as well as to identify people who could prepare these articles. (Repeat from 1978)
12. The committee recommends that the size of type used for abstracts, titles of figures and tables, and column headings in tables be increased.

R. B. Read, Jr., Ph.D.
Chairman

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Agriculture's Challenge in Florida

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We in the Florida Department of Agriculture and Consumer Services share a concern with you that has become even more critical with the recent trend to higher food prices. We, like you, are concerned that the public have access to an adequate food supply that is wholesome, nutritious and palatable. Unfortunately there are a few who would take shortcuts in this area to help offset higher prices which result in consumer resistance at the supermarket. We cannot allow this to happen because substandard foods at any price are not bargains. And I think the consumer would agree with us on that point.

INSPECTION

We have inspectors in the field who constantly monitor the quality of foodstuffs offered for sale in Florida. On-site inspections in slaughter-houses, packing plants and in supermarkets help insure that consumers have access to the quality food items that they have come to expect. We also certify scales and other weighing devices so that the purchasing public is sure to receive the correct quantities that they are paying for.

In addition to our on-site inspections, we have road-guard checkpoints which have been strategically placed on the major highway routes coming in and going out of the state. Inspectors at these stations serve many important functions and high on the list of these duties is to make sure that agricultural shipments to and from Florida are certified disease-free.

Our dairy inspection station located on I-75 rejected a total of 40 tanker loads of milk destined for consumption here in Florida during 1978. With nearly 2900 tankers coming through the station that year, you might feel that the 40 tankers that were rejected represent an insignificant number, roughly 1.4%. However, had our inspectors not been there to turn those trucks around, approximately 220,000 gallons of substandard milk would have been offered for sale on the grocery shelves in Florida. To me that figure is not insignificant. Hopefully the day will come when we are not forced to reject any milk shipments coming into the state. Technological advances coupled with industry awareness that Florida will not accept shipments of substandard dairy products may some day bring us closer to that goal.

As should be obvious from the remarks I just made, Florida is forced to rely on domestic milk imports to meet the consuming needs of its citizenry. I believe Florida's dairy industry has responded to the challenge of meeting this demand by maximizing in-state production and using milk from other states to see that the total dairy needs of the state are met. We currently import about 6% of our total consumption, but this figure is expected to rise, primarily due to a rapidly-expanding population. With cooperation of everyone involved, from producer to consumer, I remain confident that Floridians will continue to have adequate supplies of milk and other dairy products at prices that are fair.

AGRICULTURE IN THE NATIONAL ECONOMY

Despite some of the problems that all of us in the agricultural sector have faced recently, I still believe that American agriculture can be a major force in improving and then stabilizing the economy of our nation. In the 7 months from October, 1978 through April, 1979 our agricultural trade balance showed a surplus of $9.1 billion. At the same time, our non-agricultural trade balance showed a deficit of $22.4 billion. This comes to a total trade deficit of $13.3 billion. Hence American agriculture is doing more than its fair share for this country in the international trading arena. I firmly believe that the major challenge facing both American and Florida agriculture is to do more business on the export market. We have the production capabilities and the market for our farm products is there. We must not let any past political or ideological differences stop us from fostering these markets.

A few months ago I hosted a group of 21 high-level representatives of the People's Republic of China who were here for a 6-day agricultural tour of our state. They had a real interest in our technology but no less an interest in our agricultural products. Now the People's Republic of China is by no means a wealthy country, but I suspect that they have something much more valuable than currency with which to barter. I think they have oil. As a matter of fact, I think they have lots of oil. What they lack is the technology to explore for and discover the oil. We have the technology to find that oil, and we also have the meat, corn, citrus, phosphate and a host of other products that we can trade them for that oil. It's a possibility that I feel is worth pursuing, especially when we are now enjoying such amiable relations with China. If we don't at least explore those possibilities, it may be something we will severely regret 10 years from now. Let's face it; until we more fully develop alternative

forms of energy, we're going to be highly dependent on foreign oil. If we can replace the money that goes to purchase it with agricultural products, we may some day soon see our balance of payments climb out of the red. In the context, I think food for crude is a reasonable alternative.

ENERGY PROBLEMS

Let me briefly bring you up to date on transportation and related energy problems that have troubled us here in Florida and have significantly contributed to rising food prices. Although I will concentrate on the Florida situation, it should become obvious to you that it is reflective of a more extensive national problem.

Transportation tie-ups in May and June clearly illustrate the problem facing the food industry and consumers in Florida and throughout the United States. Without adequate, reliable transportation, both the Florida farmer and the national consuming public suffer.

The transportation problems affecting Florida agricultural have national implications since most of our fresh fruits and vegetables go to market outside our state. Because of this, what happens in other States and on a national level will have profound effects.

The strike by regulated, organized truckers early this spring, followed by the shutdown of independent haulers of exempt commodities later, presented problems that could not be resolved solely within our State. National remedies must be forthcoming if such actions to halt the flow of food from producing areas to consumers are to be avoided in the future.

One of the complaints of independent haulers during their shutdown was that other States have weight and length restrictions on trucks that haul exempt produce. Other complaints were the sudden rise in costs of diesel fuel, restrictions on backhauls and inflexibility of State regulation on rates.

To seek a solution to the conflict of interstate regulation, I have called on those States with significantly different weight and trailer length regulations to offer legislative relief. Alternatively, I have sought to establish interstate highway corridors on which State regulations would not apply. Federal interstate highways in other States should have the same cargo-carrying capacity as Florida interstates.

Deadhead returns of truck combinations add to the rising cost of transporting Florida crops. Where such regulations no longer serve any useful or rational purpose, they should be reduced or eliminated to facilitate backhauls and spread the cost of the round trip.

Expansion of the agricultural exemptions on cargo to include farm production input items such as fertilizer, feed, seed and implements would give exempt haulers more flexibility in scheduling routes over which they must travel on the return trip to producing areas. This would ease the backhaul problem tremendously.

Each dollar a consumer spends in a supermarket for food includes about 60 cents for marketing; the farmer's share is now about 40 cents. Transportation is a major and increasing component of the marketing cost of food, and present and future fuel shortages and rising costs will make the transportation component even more important in the ultimate price of food.

If retail food prices rise from 12 to 15% this year, it is not likely that the farmer's share of the consumer food dollar will increase, since the cost rise will be attributable to the increased cost of hauling, handling and associated inputs. Included in the mix of price increases affecting the retail price of food at the supermarket are higher prices for tractor-trailer combinations, higher insurance rates to cover the more expensive equipment, wage increases for drivers and such hidden costs as product losses through transportation failures due to misallocations of diesel fuel.

The Florida food industry's dependence upon truck transportation is evidenced by the losses incurred in the late June shutdown by watermelon growers in North Florida. Conservative estimates of farmer losses ran to about $1 million. Losses to farmers and shortages in the supermarket would have been much greater if the truck shutdown had occurred during the vegetable harvest season in South Florida.

Florida shipments to major markets throughout the Eastern U.S. are almost totally dependent today upon trucks, while as recently as 1968, about 25% of the fresh commodities were shipped by rail. The railroads have given up the fresh produce traffic for a number of reasons, including the former abundance and relative low cost of diesel fuel and the increased convenience of trucks to shippers.

However, the truck strike and refusal of exempt haulers to move cargo clearly illustrates the dangers to our food industry of an unbalanced transportation system. In my view, high priority must be given to a national effort to encourage railroads to put modern refrigerated equipment into use and regain its position as a major transporter of perishables. The recent deregulation of railroad shipment of fresh commodities is a step in the right direction, although it must be admitted that rates for rail shipments rose markedly during the truck shutdown.

Shortages of rail cars are a constant problem for agriculture. The current shortfall in rail cars is estimated at 235,000, especially for equipment to haul bulk farm commodities. It may be necessary and desirable to provide some kind of government incentives to the railroads and to the shippers to add new rail equipment.

Without modern equipment, railroads cannot compete. In 1930, the nation's railroads carried 74% of intercity freight ton-miles; by 1977, that share was reduced to 36%. The rail's share of total freight revenues dropped even more precipitously — from 70% in 1930 to 21% in 1977. This does not encourage investment in modern equipment or improved trackage, although in 1978 the railroads spent an estimated $10 billion on new

locomotives, cars and track improvements.

With ample justification, railroads point out that Federal subsidies to highway construction and waterway carriers makes the competition unfair. Government takeover and operation of the rails is not the solution. Since investor-owned railroads offer the most efficient means of moving agricultural products, it is in the national interest to provide incentives to railroads and shippers on a basis which is at least comparable to those of highway and waterway users.

Greater public understanding of the problems involved in food transportation and marketing, and a unified national approach to solving those problems, will be necessary to minimize the impact of the energy crisis on our food industry.

IN CONCLUSION

As I conclude let me say that Florida will continue to take a leadership role in areas of significant national concern. We will continue to encourage the expansion of our agricultural export markets through our sponsorship and participation in export seminars, trade shows and related efforts.

Our Department and Industry people will continue to take an active role in national organizations such as your own, which seek to improve the quality of life here and abroad. I'm proud that Jay Boosinger, our Dairy Division Director, is currently the Chairman of the National Conference on Interstate Milk Shipments in addition to being Vice-President of NASDA's Dairy Division. Red Larson, one of our prominent Florida dairymen, is now serving as President of the National Milk Producers Association. I could go on but I won't.

Staphylococcal Food Poisoning at Wedding Reception

A number of guests at a wedding reception in Sussex County, Delaware, earlier this year contracted acute gastrointestinal disease. Among the 107 guests present, 64 had one or more of the following symptoms: vomiting, nausea, abdominal cramps, or diarrhea. Thirty-eight required emergency room attention but none were hospitalized.

Among the 103 guests from whom food histories could be obtained, chicken salad was the food implicated in the illness. The attack rate among those who consumed the salad was 76% as compared to 9% of those who did not eat it. Coagulase-positive *Staphylococcus aureus* was later isolated from the chicken salad and the food grinder used for preparation.

Most of the food for the reception was prepared in private homes. Chicken used for the salad was cooked, deboned, and stored in a refrigerator in a large, plastic washtub on March 8.

The following day the salad was prepared in a grinder and returned to the same tub. On the day of the reception, March 10, the salad was not refrigerated for a period of about 7 hours as it was transported to and used at the reception. Persons serving the salad noted that salad from the central part of the container felt warmer than that from the top, indicating uneven and inadequate refrigeration.

Excerpted from Morbidity and Mortality Weekly Report, Vol. 28, No. 37, Sept. 21, 1979.

Journal of Food Protection Vol. 42, No. 11, Pages 903-905 (November, 1979)

Presidential Address

HOWARD HUTCHINGS

South Dakota State Health Department, Pierre, South Dakota 57501

(Received for publication August 15, 1979)

It is with a great deal of honor that I stand before you this afternoon and welcome each of you to the 66th annual meeting of the International Association of Milk, Food and Environmental Sanitarians (IAMFES). Our organization has a long and proud history. We can be thankful for those who organized and have carried on the professional tradition of International. It is with a great deal of pride that I have been able to serve as president during this past year. It is a very humbling experience, first of all to be nominated and elected, and then actually to serve on the Executive Board, climaxing this year as president. I look back hardly believing that it is over, and yet when I look forward there are many things the organization should be doing.

In many organizations, the president's address comes at the beginning of his term--in IAMFES it is traditional that it comes at the end. It is time to look back and reflect on all the great things that have been accomplished. I personally feel that looking back should only be done long enough to take aim on where you want to go forward. In these few paragraphs I would like to present some ideas and possibly challenges, not so much about what has been done, but challenges for the future: namely, *professionalism* and *communications.*

A REVIEW

In looking back, there are several things of which we need to be cognizant. At the moment, our revenue is down somewhat; in fact, it is at a deficit. I am sure Mr. Wright will comment on this during his report at our business meeting. At the same time our membership is up a little, which is very encouraging. The *Journal* has been the largest ever and reflects the continued professional format for which our organization is known. Inflation has not side-stepped our group. The cost of everything, as you well know, is up. Rent more than doubled, the cost of printing the *Journal* is up considerably. These have all contributed to our temporary financial deficit.

As you know, our dues were raised effective July 1, 1979. This has not yet produced any significant change, but will counter-balance some of the current inflation problem. The sustaining membership program, which was organized a few years ago, has begun to shape up and presently there are over 20 sustaining members. This should continue to be an active, growing part of putting our organization on a continual sound financial basis.

Another great plus is completion of the *Procedures to Investigate Waterborne Disease Outbreaks.* A few copies are available today (August 15, 1979) from an early press run. This will be a very welcome companion to the booklet on investigation of foodborne disease outbreaks.

A two-color brochure about membership is about ready to be finalized and should be an added plus in communicating to people the world over what professional sanitarians and IAMFES are all about.

In a brief moment of looking back, I feel one of the greatest achievements that has been made this year is the final determination of the merger or jointure activity between IAMFES and the National Environmental Health Association (NEHA). As I am sure all of you know, for at least 15 years there has been continuing dialog between our group and NEHA concerning a possible merger. There have been ad hoc committees, numerous groups of individuals and the executive boards of both organizations dealing with ways and possibilities of merger. It seems the word *merger* and the later term *jointure* had almost become a fetish. Each president and executive board had to deal with the concept every year. It would seem a new twist or new variety or new suggestion came up and became a dominant topic of conservation that far outweighed the importance of what really should have been occurring.

Last August, just 1 year ago, the ad hoc committee made up of members of both of our great organizations met in Kansas City and recommended its own termination. It is a tribute to this committee, named by both our organizations for the sole purpose of jointure, to realize at this time that merger was not the answer. The committee recognized the almost forced nature of what had developed was out of place with the professional nature and mood of the organizations. It was their suggestion to the respective boards that a permanent joint committee with staggered terms for continuity be established. This committee is not aiming at jointure or merger but instead at exploring ways of mutual cooperation and of working together whenever possible. Several areas were suggested for exploration.

In an Executive Board meeting in October, NEHA named four members with one, two, three or four-year terms to serve on the joint committee. At the November, 1978 meeting of our Executive Board, we named four

people to the committee. They are Clarence Luchterhand, Henry Atherton, O. M. Russell and Harry Haverland. There have been one or two meetings, I believe, without a quorum each time, but contact has been made between these two committees. Some areas that this joint committee is charged with include: (*a*) identification of employment and professional advancement opportunities, (*b*) cooperation and leadership in the registration and licensure process of sanitarians, which is also called credentialing, (*c*) joint publication of manuals and guidelines, (*d*) planning of joint national meetings, (*e*) cooperation and sharing of information among technical committee and (*f*) production of guidelines on a membership program. These suggestions have been used to identify the scope of the joint committee.

I feel this is probably the greatest accomplishment that our organization has made this past year. I believe we have once and for all placed the emphasis where it should be, on cooperation and mutually working together to achieve a professional attitude for sanitarians all across the country. The idea of a forced merger is not now dominant in our thinking. If, at some later time a merger comes, it will be because of a natural process of growing and sharing ideas together. Any sort of common denominator must come from working together--not because a committee deemed it was the thing to do on a given schedule or on a certain date.

THE FUTURE

The IAMFES is well known throughout the world, representing the ultimate in the total food protection picture. This, as it relates to public health, production, storage and processing of food products, points to us as specialists in food protection. We, as the IAMFES, are not trying to solve all the environmental problems of the world. We are a multidisciplinary organization of gathered expertise dedicated to improving all aspects of food protection.

I believe the future looks bright, if we approach it as professionals. As any strong and vibrant professional society or organization, we have much talent and expertise among researchers, field sanitarians, educators and leaders of industry and of enforcement or regulatory organizations. Our organization has a tendency to move slightly forward regardless of yearly elections, inflation, depression, etc. I believe it is time we stand together as professionals and attack in an aggressive manner a major problem that confronts all organizations; that is, image.

Let me cite an example or two of what industry does when faced with an image crisis. If I mention a certain food product normally marketed as a hamburger but one that is hot and juicy, all of you know the trade name to which I am referring. Do you realize that this past year the Wendy's Hamburger chain has increased its sales by 45% through a vigorous advertising campaign. Its advertising budget for this year will equal 4% of its entire budget (from 31 million to somewhat over 40 million dollars for advertising alone.) Today there are 31 foreign food companies owning restaurants and/or chains of restaurants in the United States.

Seven years ago McDonald's, the fast food leader in total dollar volume of sales, had a system-wide sales total of 784 million dollars. In 1979, its overseas sales alone will top that total. That is the result of aggressive selling of an image. Decide the thing you do best and let the public know what it is. We have a good product in professionalism, we have the ultimate organization for total food protection in the world today. I would like to challenge our group, not just the incoming president, but each of us to start a communication program that will sell IAMFES as an entity of specialization that is second to none. To do this we must show, through leadership and aggressive professional action, that we are the best.

I certainly don't have the answer on how this should be done. I am sure no individual has all the answers. I believe that as an organization we do have the knowledge to put together an approach that will lead to improvement; not just in membership, not just in dollars, but in image and stature for providing service and leadership throughout the world in food protection. I feel very strongly about the need for increased communication of what we are, what we have and what we can do.

I am sure every president has been concerned about how better to communicate to members. I feel there needs to be an improved method or organizational scheme to involve the Affiliate Council and the local membership in a stronger manner. Again, I have no idea how this can best be accomplished. Others have shown concern and yet we continue to waste opportunities because Affiliates have not been made aware of problems that could be addressed. There must be a method to increase the flow of information from the Affiliate Council, from the affiliate members, the state groups or city groups to the individual sanitarian and back to the Executive Board.

I would like to see the Chairman of the Affiliate Council be made a member of the Executive Board. If this is not possible in any way other than as a non-voting member, this would certainly be a suitable start. It would seem that the Chairman, if he could attend all the Executive Board meetings and be involved at the decision-making level, might provide a start for increasing the informational flow from the individual members to the Executive Board.

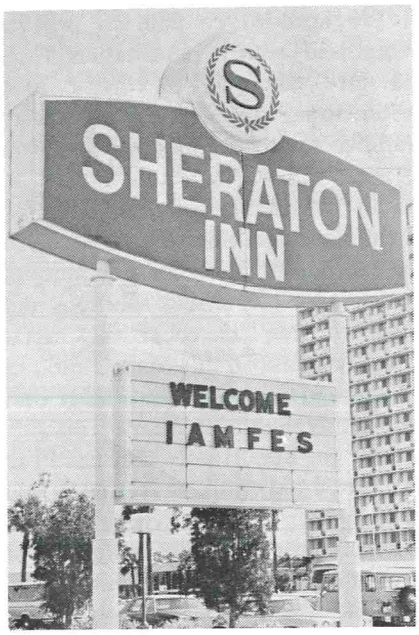
As I indicated before, I don't have the answers. Maybe our new president has some thoughts, but I hope, collectively, as professionals we can contribute toward the end of marketing what we know best; that is, professional food protection. At present the food industry, and I am using the term to include milk, food and all aspects of food, is one of the fastest growing industries in the world. The percentage of the gross national product that is spent for food increases yearly at an escalating rate. The number of meals eaten out and the number of food products that are created each year is

overwhelming to those of us whose memory goes back just a few years. It behooves us as professionals in an organization that has been in existence for over 66 years, to provide strong and vibrant leadership in this growing market place of the world.

I would like to see the IAMFES reach out and become more active in the world as a professional organization. We have a long history of working with Canadian sanitarians who have been counted among our members. I would like to see other countries become just as active. I feel we need to promote ourselves with membership in other countries and tap the world-wide pool of food protection experts. I am not speaking of membership dollars, I am speaking of membership expertise in the professional realm of food protection. Food protection has indeed become a world-wide phenomenon and certainly cannot be bound by continental lines or national borders. We have subscriptions in about 70 countries and are receiving articles for the *Journal* from scientists in many countries. We need to be able to provide professional leadership and solicit information from those countries that are emerging as major food processors. We have something to offer--I feel we need to promote ourselves and become a prominent professional organization.

I would like to close with this challenge, again not to the new president or the Executive Board, but to each of us who proudly considers himself or herself a member of the IAMFES. We need to promote, to seek solutions and to share these in a way that we can collectively better serve mankind. We need to establish this organization as a prominent pool of total food protection knowledge that is available to everyone at any time.

I thank each of you for the opportunity that has been afforded me this past year. I only hope that each of you will feel as much as I do that it is a privilege to be a member — an active member — in this fine group of dedicated workers in the field of food protection.



Above, left, the Sheraton Twin Towers rolls out the red carpet for IAMFES. Above, right, Executive Board stops work for a photo. Members are, front row, left to right, Earl Wright, Howard Hutchings, Henry Atherton, Dave Fry, Bob Marshall. Back row, left to right, Dick Brazis, Jan Richards, Bill Arledge, Harry Haverland, Elmer Marth, Bill Kempa, and Leon Townsend. Below, leaders of the Florida Association of Milk, Food and Environmental Sanitarians, hosts for this year's Annual Meeting, were Dick Jolley, left, Chairman of the Local Arrangements Committee, and Lupe Wiltsey, President.

1979 IAMFES Annual Meeting

Once again, an excellent educational program highlighted the IAMFES Annual Meeting. And while the educational aspects provided enough reason to attend the 66th Annual Meeting, the lush palms and orange groves of Florida, and the color and excitement of Disney World helped make this a very memorable event.

True, a few showers caught those IAMFES members who spent their free Tuesday afternoon at Disney World, but it wasn't enough to dampen spirits or spoil a good time.

The meeting, held at the Sheraton Twin Towers in Orlando, Florida, August 12-16, attracted 300 persons from around the country, as well as some foreign members and visitors.

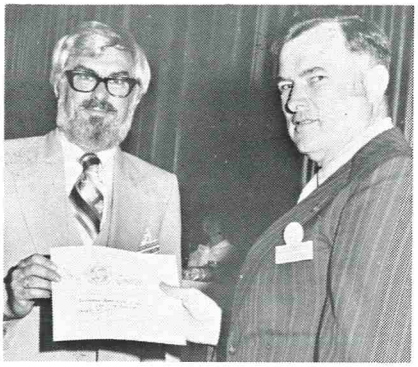
Hard-working committee members arrived to begin meetings on Saturday and Sunday and were entertained at the end of the weekend with an "early bird reception." Most conference attendees arrived to start the 1979 Meeting in full force Monday afternoon with the opening general session.

Throughout the conference, one frequently heard comments concerning the high quality of the program. As a result, many persons desired but were unable to attend several sessions being held at the same time.

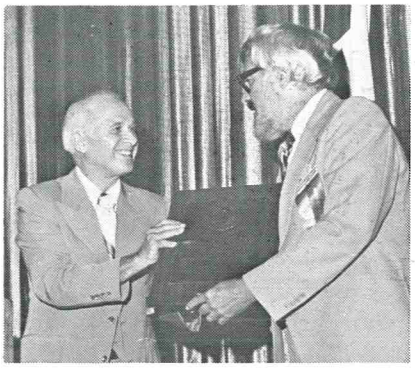
A good crowd attended the Annual Awards Banquet, a highlight of the meeting, at which IAMFES honored Dr. Bailus Walker, Jr., Dr. Joseph Edmondson, Dr. Joseph Olson, and Harold Thompson for their achievements and contributions to the Association and the field of milk, food and environmental sanitation. The New York State Association of Milk and Food Sanitarians was named the outstanding affiliate of the Association. The Samuel Crumbine Consumer Protection Award, offered by the Single Service Institute each year, was presented this year at the IAMFES meeting. It was awarded to the Suffolk County, New York Health Department.

The Executive Board welcomed its two new members at the 66th Annual Meeting. They are Dr. Robert Marshall of the University of Missouri, Columbia, MO, and Dr. A. Richard Brazis of Fairmont Foods, Omaha, NE.

Much was accomplished by the Executive Board and Committees at this Annual Meeting. A change in



Howard Hutchings presents a charter to Ken Whaley, representing IAMFES' newest affiliate, the Tennessee Association of Milk, Water and Food Protection.



William Kempa presents the Past President's plaque to outgoing IAMFES President, Howard Hutchings, at the Annual Awards Banquet.

William Kempa, 1979-80 President, accepts the official gavel from Charles Felix of the Single Service Institute.

the composition of the Executive Board to include the Affiliate Council Chairman as an ex-officio member, discussion of proposed major changes in the *Journal of Food Protection,* the completion of "Procedures to Investigate Waterborne Disease Outbreaks," and planning for next year's annual meeting, to be held concurrently with NEHA's Annual Meeting, were major items. Much other work and planning took place, however, as witnessed here.

EXECUTIVE BOARD MEETINGS

The following reports were given and business conducted during the meetings of the Executive Board:

Auditor's Report. Earl Wright presented the Auditor's report. Association income for the year totaled $68,695, offset by $60,584 in expenses, for income of $8,111. *Journal of Food Protection* income amounted to $76,101, as compared to *Journal* expenses of $94,440, for a loss of $18,339. The net loss of the Association and *Journal* was $10,228. Much of the increased pricetag of the *Journal* was due to the costs of producing a larger volume, as well as the increases in postage and printing.

Budget Report. Harry Haverland presented the 1979-80 proposed budget. Projected net income for the Association for 1979-80 is $11,250, while projected net loss for the *Journal of Food Protection* is $1600, for a net income for both of $9,650. The Board discussed ways the Association might increase total income. The Board voted to increase direct subscriptions from $32 to $50 per year and agency subscriptions from $25 to $40 per year. Discussion also favored increasing the manuscript service charge of articles printed in the *Journal*, with a recommendation that the *Journal* Management Committee first study the effect various increases might have on the volume of manuscripts submitted to the *Journal.*

Jan Richards reported on new publications produced by the Association, including the new membership brochure and "Procedures to Investigate Waterborne Disease Outbreaks." Work has also started on a career information pamphlet, to be available later this year or early in 1980.

Jan also reported on membership increases in the Association. The Ontario affiliate demonstrated the largest increase with 50 new members.

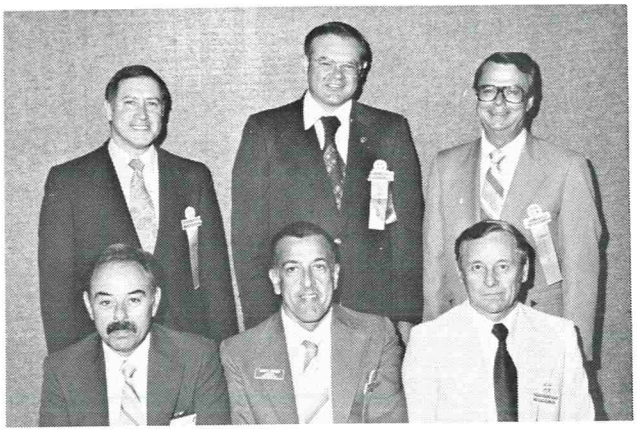
Executive Secretary's Report. Earl Wright presented his report, including highlights of changes in the Association in the five years he's served in the office. He emphasized a need for work on membership increases and direct attention to the Association's goals.

Planning for new affiliate groups was discussed by the Board. Earl Wright noted that Tennessee developed as a solid affiliate and was chartered this year after several years of initial groundwork. He also indicated that the most promising areas for new affiliate groups appear to be Georgia and Ohio. Discussion also concentrated on the need for follow-up and revival of some affiliate groups which continue to exist, but have dwindled in membership and involvement in recent years.

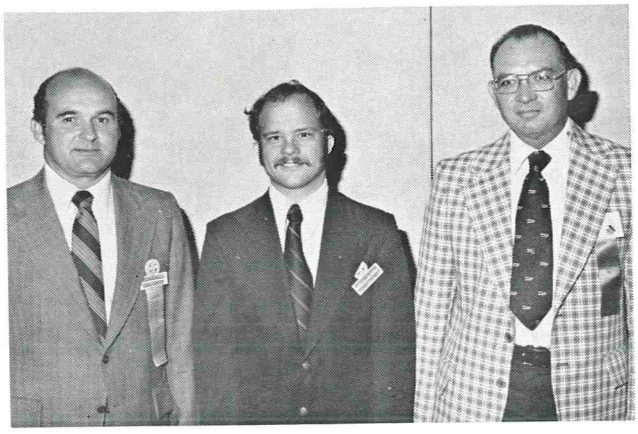
Awards Committee. Henry Atherton reported results of the Awards Committee's selections. (See section on award winners). Discussion concerning the Shogren Award followed. Several board members noted that a cash award, rather than a savings bond, might be easier for affiliate groups to use.

Bridge Committee. Harry Haverland reported that the Bridge Committee has had some difficulty finding a common meeting time this year. Howard Hutchings reported on the NEHA meeting in June in Charleston, SC. He noted that improved communications with NEHA is a key priority for successful concurrent meetings next year, as well as successful joint projects between the Associations. Earl Wright highlighted reasons for the decision to change the IAMFES meeting headquarters in Milwaukee from the Pfister Hotel to the Red Carpet Inn. Rate increases of $10 and more in prices quoted this year by the Pfister made it necessary to change hotels to keep room rates in the $35-45 range. While this means IAMFES and NEHA meeting sites are now several miles apart, busing for common events would be a way to accomodate the distance.

Next the Board discussed new *Journal* advertising rates and the possibilities of exhibiting at several



Milk Sanitation speakers, front, left to right, included: J. A. Koburger, S. E. Barnard, and M. P. Dean. Standing, left to right, D. K. Bandler, F. W. Bodyfelt, and R. Mykleby.



General session speakers included, left to right; J. Byrnes, C. S. Hickey, and K. L. Smith.

large industry meetings. It was noted that booths or exhibits at some of these meetings could help increase exposure, awareness, and membership in the Association. Meetings suggested included the Dairy and Food Expo, Institute of Food Technologists' meeting, and the American Dietetic Association meeting.

Billing for renewals of affiliate memberships could be streamlined in the future, the Board discussed. As the *Journal* mailing list and affiliate membership list is computerized, Earl Wright noted that it would be possible and perhaps easier for affiliates as well as the main office if affiliate members were billed directly.

3A Symbol Report. Carl Kroppman reported for Richard Whitehead. One Standard was completed this year, with several others updated and prepared for action at future meetings of the Committee.

Journal Management Committee. Dr. R. B. Read presented the report, the highlight of which was a proposal to develop and publish two journals, one oriented to sanitarians and fieldmen and the other a scientific publication. The practical journal would contain Association news and events, articles and coverage of speeches of interest to practicing sanitarians and fieldmen, 3A and E3A Sanitary Standards, abstracts of papers published in the *Journal of Food Protection*, as well as other general interest news and items. Other recommendations of the Committee included increasing the page charge to $30 or $35, as needed; attending expositions and meetings of other associations to increase awareness of the *Journal of Food Protection* and IAMFES; and developing committees to recruit sustaining members in the food industry, suggest subjects of current interest to practicing sanitarians and fieldmen, for use in the *Journal.*

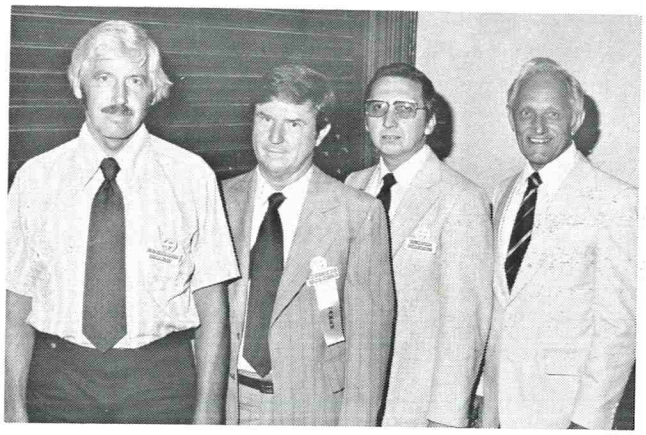
The Committee also suggested that financial analysis begin to determine costs of producing two new proposed publications.

Henry Atherton reported that Bob Bradley of the American Dairy Science Association had contacted him regarding the possibility of a joint meeting at some time between ADSA and IAMFES. ADSA has chosen meeting sites through 1983, while IAMFES has committed sites through 1982.

Applied Laboratory Methods Committee. Dick Brazis presented this report. Activities of the Committee were limited somewhat this year due to resignations of two Subcommittee chairpersons and increased job responsibilities of the third. Members of the Milk Methods Subcommittee assisted in the preparation of the new edition of the APHA "Standard Methods for the Examination of Dairy Products." Dick submitted his resignation as Chairperson of the Committee in order to attend to new duties as Secretary-Treasurer of the Executive Board.

Milwaukee Local Arrangements. Paul Pace, Chairman of the Local Arrangements Committee for next year's Annual Meeting, to be held concurrently with the NEHA Annual Meeting, presented details of current plans for that meeting. Discussion followed concerning policies of cross attendance at technical sessions of the two meetings, preparation of programs, and registration fee policies. The following recommendations resulted: that each association print its own program, at the time and in the form it sees fit, and include the other association's program; that each organization have its own registration fee; that IAMFES open its technical sessions to members of both organizations; that each group arrange for transportation of its own members to joint events; that social events be financed on a per capita basis.

Sustaining Membership Program. Dale Termunde reported that letters were sent to many potential sustaining members, the result being a growth in number of memberships from 17 to 22. Dale indicated the growth was not as great as he'd hoped, but that it indicated the Committee was proceeding along the right path. The Committee has a goal of 50 Sustaining Memberships



Above left, Food Sanitation section speakers, left to right, included: R. Blankenship, J. Lovett, D. A. Schiemann, and O. W. Kaufmann. Above right, Milk Sanitation speakers, seated, left to right, were: E. Todd, M. Rhodes, E. Mikolajcik. Standing, left to right: L. Smoot, S. E. Barnard, E. H. Marth, and S. M. El-Gendy. Right, Food Sanitation speakers, seated, left to right, included: J. Wyatt, C. S. Hickey, and G. C. Holland. Standing, left to right; M. C. Robach, R. J. Alvarez, K. J. Baker, and D. Greenaway.

Sessions solid, well-attended

for 1980, and a later goal of "80 in 1980" was mentioned.

The Board turned its attention to future Annual Meetings as bids were made by several cities for the 1982 meeting. George Spaulding of the Louisville, KY Convention Bureau, presented a slide-talk about the city's convention facilities. John Schilling extended an invitation to St. Louis for the 1982 convention, and William Kempa noted that Edmonton, Alberta, is interested in hosting the IAMFES Meeting. The Board voted, after discussion, to hold the 1982 meeting in Louisville, KY.

Sanitarians' Joint Council. Harry Haverland presented the report of the Council. Much time was spent by the Council discussing "sunset laws" and the effect they will have on various sanitarian registration acts. They also worked with revision of the SJC Model Registration Act, credentialing and licensure. Those present at the SJC meeting felt the Model Act should be revised, with legal input, to meet a need until credentialing is widely accepted. Harry noted that the SJC will be redefining its goals and objectives as it has accomplished many of its previous objectives, and others which were set are no longer applicable due to changing trends, concepts, and philosophy.

AFFILIATE COUNCIL

The Affiliate Council met in two sessions, one at the beginning of the Annual Meeting, with a second at the close of the meeting.

Among reports presented at the Affiliate Council meeting were the following: Earl Wright reported on the status of IAMFES and NEHA joint projects. Two committees have been formed, one by the Executive Board of each organization, to become a "Bridge Committee." The committee aims to work together on projects of common interest to the two Associations.

The *Journal of Food Protection,* again, can use assistance of affiliate members for story leads and papers of interest to practicing sanitarians and fieldmen, for use in the magazine. Jan Richards will help with writing and rewriting, if necessary, of such articles if essential information and materials are provided.

Henry Atherton reported for the Awards Committee. He noted that more affiliate groups should apply for the C.B. Shogren Award, which recognizes the top affiliate group of IAMFES each year. Discussion followed concerning development of a certificate of merit which could be awarded to persons who have helped the Association meet its goals, but who might not otherwise be recognized for their efforts.

Gail Holland of the Ontario affiliate detailed the work of that group in recruiting new members and reviving membership, in general, this

year. The Ontario group was recognized as having the largest increase in membership, with 50 new members. Among changes there were a new name, the Ontario Food Protection Association, a new logo, updated newsletters, and stronger programs.

Ken Whaley reported on progress in Tennessee, site of the newest affiliate of IAMFES. That group was chartered this year and held its first annual meeting in March.

Howard Hutchings reported on work of the Bridge Committee, whose members are studying jointure between IAMFES and NEHA. IAMFES members are Clarence Luchterhand, Harry Haverland, Oak Russell, and Henry Atherton. Howard noted that the Committee will work on common projects, with less pressure at this time to merge the two organizations. If jointure of the two associations is to come about, he noted, it will be as a result of cooperative solutions to problems common to the two groups. Howard also addressed the need for stronger communications between affiliate members, the Affiliate Council and the Executive Board.

Howard also discussed the availaability of central billing service through the IAMFES central office. As mailing lists for the *Journal* are already computerized, renewal notices could come from the office in Ames, just as they do for direct members and subscribers.

The Affiliate Council discussed central billing and, on a related issue, voted to recommend to the Executive Board that quarterly printouts of direct and affiliate members from each affiliate area be sent to officers of the group.

Sid Barnard, of the Pennsylvania affiliate, discussed several items affecting the Pennsylvania group which might be of concern to other affiliates. Pennsylvania's group is made up solely of persons associated with the dairy industry and there is a need there to offer affiliation with the Association to persons whose interests are food-related. The current Pennsylvania affiliate is not interested in diversification, he noted. Sid emphasized a need for greater communications between affiliates, as well as with the Executive Board and central office. An information or newletter exchange is needed, he said. Earl Wright offered that if affiliate secretaries send 25 copies of their programs and newsletters, the International office can distribute them.

Dave Fry conducted the election of officers for the Council and Leon Townsend was elected Chairman, with Archie C. Holliday elected as Secretary.

The Council voted unanimously to recommend to the Executive Board that the Chairman of the Executive Board be included as a member of that body. The Council also discussed the possibility of meeting twice at each Annual Meeting, and it was suggested that the Chairman recommend this change to the program chairman for next year's annual meeting.

At the second meeting of the affiliate council, Chairman Leon Townsend made an appeal to affiliates to provide more news and articles of general interest for the *Journal of Food Protection.*

Leon indicated that he will work with the Executive Board to develop an Award of Merit, as recommended at the earlier Council meeting. He noted, that on the item of central billing of annual dues, he will distribute a questionnaire to each affiliate secretary to help determine what kind of direct billing would most benefit the membership.

The Council suggested that the program committee for next year's Annual Meeting work on sequencing of presentations so as to give better continuity in information offered throughout the course of the meeting. Consideration should also be given to avoid scheduling two papers at the same time on the same subject in different sessions.

Leon reported that the Executive Board is studying the possibility of producing two journals, one directed to practicing sanitarians and fieldmen and the other to continue to be a scientific publication.

Pete Benedetti recommended and the Council unanimously approved the suggestion that the Executive Board make the Chairman of the Affiliate Council a voting member of the Board.

TECHNICAL SESSIONS

For the highlights of this year's technical presentations, please see abstracts in the October, 1979 issue of the *Journal of Food Protection*.

BUSINESS MEETING

Approximately 150-200 persons attended the 1979 Business Meeting. Reports given at that meeting which have previously been outlined and will not be reiterated here include: Local Arrangements for the 1980 meeting, Paul Pace; Executive Secretary's Report, Earl Wright; Report on New IAMFES Publications, Jan Richards; Journal Management Committee, R. B. Read, Jr.; Report on *Journal*, Elmer Marth (this has not previously been detailed but will be included in the next issue of the Journal); Affiliate Council, Clair Gothard; Applied Laboratory, Methods, A. Richard Brazis; Sanitarians' Joint Council, Harry Haverland; Sustaining Membership, Dale Termunde. Other reports presented, written reports of which, when submitted, will be printed in future issues of the Journal, included the following.

Communicable Diseases Affecting Man. Dr. Frank Bryan noted that the Committee's new pamphlet, "Procedures to Investigate Waterborne Disease Outbreaks," is just recently available through the IAMFES office. The publication is the result of the committee's work for the past few years and is a companion publication to the "Procedures to Investigate Foodborne Disease Outbreaks," also the result of the Committee's work. He noted that the Committee will begin work to revise the foodborne publication.

Farm Methods Committee. Dale Termunde highlighted work of this Committee, noting that a final report will be submitted in 1980. The twelve Sub-Committees of the Farm Meth-

ods Committee have been given new charges.

Food Equipment Committee. This report was presented by Karl Jones. The Committee urges all sanitarians to obtain a complete set of the NSF Food Equipment Standards and Criteria and a copy of the NAMA Automatic Merchandising Health-Industry Council's Vending Machine Evaluation Manual and related materials and to evaluate each piece of food equipment and vending machine in the field to determine its compliance with applicable sanitation guidelines. They also recommended that all sanitarians let the Committee and appropriate agencies know of manufacturers failing to comply with guidelines.

Journal Food Service Committee. C. Dee Clingman presented the report of this Committee, noting that K. J. Baker has developed an index of foodservice articles from the past five years' issues of the *Journal of Food Protection.* This index has been submitted to the *Journal* and will be used when space allows. The Committee has established a new award, to be funded by the National Institute for the Foodservice Industry, to honor the outstanding article in foodservice food protection each year. Guidelines were submitted for the establishment of the award and selection of the winner. Projects for the coming year include finalizing the NIFI award procedure, developing a system to solicit articles in the area of foodservice, promoting research and encouraging submission of manuscripts to the *Journal of Food Protection* in a variety of foodservice areas.

3A Symbol Council Report. Earl Wright presented the highlights of the Council's submitted written report. The Symbol Council has been closely watching the program being considered by the Federal Trade Commission to develop equipment standards and certification. The proposed takeover by the FTC would increase considerably the cost to the government of monitoring equipment, where now there is no government cost. On another issue, the 2B finish on dairy equipment was accepted, which allows the 2B finish on all equipment except 33-00 tubing. The butterfly valve under the 08-17 fitting standard was also accepted. Earl noted that the Symbol Council set up a booth at the Dairy and Food Expo in Anaheim and will have a booth at the Expo in Chicago this November.

Dee Clingman, Chairman of the Journal *Foodservice Committee, and Patricia Franks of the National Institute for the Foodservice Industry (NIFI) help plan guidelines for a new award, to be funded by NIFI and presented at the 1980 IAMFES Annual Meeting. The award will go to the author of the most outstanding foodservice food protection article to appear in the* Journal of Food Protection.

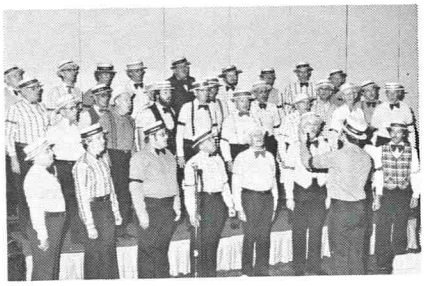
Dr. Mahfouz Zaki, of the Suffolk County, New York, Health Department addresses the Annual Awards Banquet following his acceptance of the Crumbine Award, offered by the Single Service Institute.

BISSC Committee. Earl Wright presented this report for Martyn Ronge. Among the actions of the Baking Industry Sanitation Standards Committee (BISSC) this year were the addition of one standard, approval of another, and referral of three proposed standards to task committees until the fall meeting. Martyn was requested to and will continue to serve on the BISSC Certification Board. A recommendation was made that Martyn be appointed the group's liason between the 3A Committee and BISSC to help avoid conflict between standards for similar equipment used in both the bakery and dairy industries. The recommendation will be referred to the BISSC Executive Board.

International Dairy Federation Representative. Earl Wright presented this report, also, for Harold Wainess. An interim US/IDF Committee, the first step toward U.S. involvement in the IDF, is being developed by representatives of several dairy associations and industries. Temporary officers were chosen to guide the organization until a permanent group is formed, and these officers are as follows: Chairman, Fred Greiner, DFISA, Treasurer, Gregory Farnham, Dairyland Food Laboratories, and Secretary, Harold Wainess, IAMFES. The secretary has sent a letter to the secretary-general of the IDF indicating that the interim committee has been formed and would like formal recognition at the IDF Annual Meeting in Montreux, Switzerland in September. Due to short notice and conflict with meetings in the United States, there will be a small delega-





Scenes from the 1979 Annual Meeting social events: top left and middle, and left, middle, from Early Bird Reception. Top right, above, and left bottom photos from Annual Awards Banquet.

Social events add sparkle to 1979 Meeting

tion to that meeting, but it appears that six or seven U.S. representatives will attend.

Resolutions Committee

The following resolutions were presented by Dave Fry. Both were accepted.

RESOLUTION I.

WHEREAS:

The Florida Association of Milk, Food and Environmental Sanitarians and Local Arrangements Committee labored long and diligently, with exceptional success, to host the Sixty-six Annual Meeting of the International Association of Milk, Food and Environmental Sanitarians in Orlando, Florida, and

WHEREAS:

The facilities for both the technical sessions and the social occasions were anticipated and provided with the usual generosity and style by the Florida Association of Milk, Food and Environmental Sanitarians and Local Arrangements Committee, and

WHEREAS:

These same hosts exercised the highest standards of the International Association of Milk, Food and Environmental Sanitarians in coordinating the efforts of their Industry, Educational and Regulatory members towards the success of the Association's Annual Meeting, and

WHEREAS:

The 1979 meeting was in every respect "Par Excellence" that will long be remembered;

THEREFORE, BE IT RESOLVED:

That the International Association of Milk, Food and Environmental Sanitarians adopt this resolution of appreciation and gratitude to the Florida Association of Milk, Food and Environmental Sanitarians and further that a copy of the resolution be sent to the Florida Association of Milk, Food and Environmental Sanitarians and be published as well in the Journal of Food Protection.

RESOLUTION II.

WHEREAS:

The Sheraton Twin Towers, Orlando, Florida was the site of the 1979 International Association of Milk, Food and Enviromental Sanitarians Annual Meeting, and

WHEREAS:

The personnel of the Sheraton Twin Towers Hotel were most accommodating to the needs of the members and their families of the International Association of Milk, Food and Environmental Sanitarians, and

WHEREAS:

The facilities for the program sessions and the members' and the families' personal comfort were outstanding;

THEREFORE, BE IT RESOLVED:

That an appropriate expression of gratitude be sent to the management and staff of the Sheraton Twin Towers.

1979 Awards

Bob Dawson of the Babson Brothers Company presents Joseph Edmondson, right, the Educator Award plaque. The award includes a $1000 cash award, sponsored by the Farm and Industrial Equipment Institute. Right, Dr. Bailus Walker accepts the Sanitarian's Award for 1979. This award also includes a $1000 cash prize, sponsored by Pennwalt Corporation, Klenzade Products, and Diversey Chemicals.

Edmondson Wins Educator Award

Joseph E. Edmondson, Professor of Food Science and Nutrition at the University of Missouri, Columbia, MO, is the 1979 winner of the IAMFES Educator Award. It was presented at the 66th Annual Meeting of the Association.

Dr. Edmondson won the $1000 award for outstanding academic contributions he has made to the field of food safety and sanitation. A nationally recognized authority in food sanitation, quality, and microbiology, Dr. Edmondson has taught at least ten different college courses during his career. He has also taught extension courses related to food processing and sanitation throughout the state of Missouri. He began one course, for bulk milk handlers, in 1954 and more than 1200 persons had attended the course through 1975.

The organizer and director of the area program in sanitary science at the University of Missouri, Dr. Edmondson has directed a number of master's degree candidates in that program. He also helped organize the area of microbiology program at the University of Missouri. He has served as director of graduate studies for the department of food science and nutrition, from 1962-77, and currently serves as director of undergraduate studies in food science and nutrition. Dr. Edmondson served as

Edmondson, p. 861

Walker Named "Sanitarian of the Year"

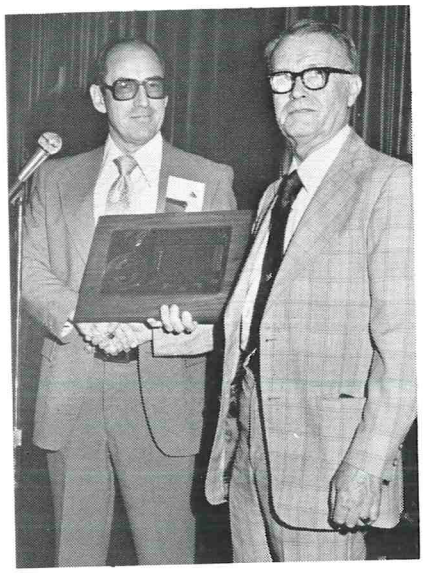
Bailus Walker, Jr., Director of the Environmental Health Administration for the District of Columbia, was named "Sanitarian of the Year."

Dr. Walker received the $1000 award in recognition of outstanding contributions he has made to the health and welfare of his community.

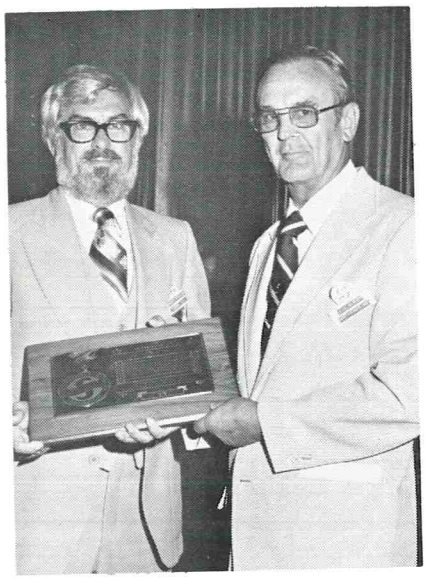
Previously Director of Health and Welfare for Newark, NJ, and before that, Deputy Health Commissioner for Environmental Health in Cleveland, OH, Dr. Walker has held his current position since 1972. Among his first steps in reorganizing and revitalizing the DC program was to ask the DC Council to amend the food code to provide a stronger legal basis for food protection as well as to require training and certification of food service personnel. The DC program was one of the nation's first mandatory training and certification programs. It has since served as a model for other communities.

In addition to work with food service establishments, Dr. Walker pioneered in developing and applying environmental health and epidemiological methods to the study of physical, chemical, and biological hazards in U.S. jails and prisons. An environmental health consultant to the U.S. Department of Justice in the early 1970's, he began a comprehensive study of environmental conditions in correctional institutions. These studies provided the basis for organized environmental health programs in jails and prisons and directly influenced improvements which reduce the chance of disease transmission and injury in these

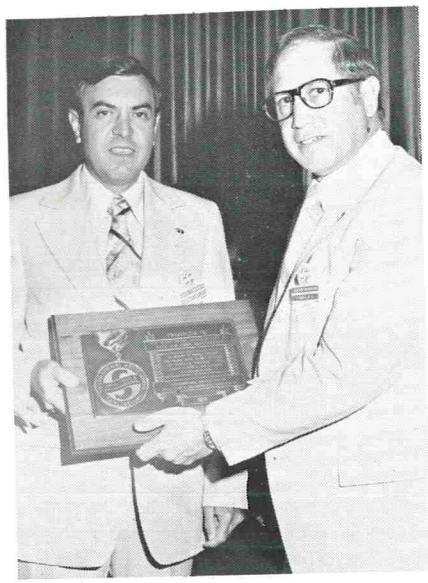
Walker, p. 871



Harold Thompson accepts the 1979 IAMFES Citation Award from Dave Fry, IAMFES Senior Past President.



Joseph Olson is presented the Honorary Life Membership plaque by Howard Hutchings, IAMFES Junior Past President.



Dave Bandler, accepts the Shogren Award plaque from Leon Townsend, Chairman of the IAMFES Affiliate Council.

Thompson Receives Citation Award

IAMFES named Harold E. Thompson, Jr., winner of its 1979 Citation Award.

Harold, "Tommy," is a native of Massachusetts. A graduate of the University of Maine, he received a B.S. degree in dairy technology in 1941. Following a year's work as assistant superintendent of production in an ice cream plant, he served four years as an officer in the Army Medical Corps.

In 1946 Thompson joined the Virginia State Department of Health as a county sanitarian, conducting programs in all phases of environmental health. The following year he became assistant state milk sanitarian with the Virginia State Department of Health with responsibility for implementation of a statewide milk sanitation program.

Thompson was commissioned by the U.S. Public Health Service and entered as a regional milk and food consultant in Boston. He served in New York and Washington D.C. until attending the University of Minnesota where in 1959 he received a Master of Public Health degree.

Thompson, p. 861

IAMFES Awards Life Membership to Olson

Joseph C. Olson, Jr., was named recipient of the 1979 Life Membership Award of IAMFES.

Dr. Olson, recently retired from his position as Director of the Division of Microbiology, Bureau of Foods, Food and Drug Administration, received the award for long and outstanding service to IAMFES.

He has served the Association in many capacities, including that of editor of the *Journal* from 1954-67 when it was known as the *Journal of Milk and Food Technology*. He has been a member of IAMFES and the Minnesota affiliate for 31 years, having served the latter group as secretary-treasurer from 1947-54, and president in 1965.

As Director of the Division of Microbiology for the Bureau of Foods, Dr. Olson managed the research and operation of the Division. He served a special assignment from July, 1976 to January, 1979, as Deputy Assistant to the Director, Bureau of Foods, for International Programs. This position took him around the world to meetings as a member of such groups as the

Olson, p. 876

New York Association Wins Shogren Award

The New York State Association of Milk and Food Sanitarians was the winner of the Shogren Award as IAMFES' top affiliate for 1979.

The New York Association membership has averaged almost 750, including 79 sustaining members, the last five years. Twenty-one per cent of the members belong to IAMFES and sixteen work on IAMFES committees.

Attendance at the Annual Conference of the New York Association runs about 400 members with 70 or more attending a spring meeting which is also held. The Association produces and distributes an eight-page newsletter five times a year. Among Committee activities, the Laboratory Practices Committee holds a day-long workshop each year for laboratory personnel.

Officers of the New York Association this past year have been: William K. Jordan, President; Charlotte W. Hinz, President-Elect; Albert, J. Lahr, Past President; Richard P. March, Executive Secretary; and Board Members, H. I. Cobb, A. R. Place, A. R. Boehlecke, and G. B. Smith.

News and Events

Marth Receives ACDPI Nordica Award

Elmer Marth, University of Wisconsin-Madison food scientist, received this year's Nordica Award from the American Cultured Dairy Products Institute, (ACDPI). He was presented a plaque and a check for $1000 at the organization's annual meeting recently in Madison, WI.

Dr. Elmer Marth

Marth, a UW-Madison faculty member since 1966, was cited for research excellence on cultured dairy products during the past 10 years.

A specialist in food microbiology, Marth has performed several studies aimed at assuring the safety and quality of cultured dairy products. One important finding was that disease-causing bacteria which occasionally make their way into food products do not normally survive in such cultured products as yogurt and buttermilk.

Marth also traced off flavors in milk products to "psychrotrophic," or cold-dwelling, bacteria that grow in chilled, raw milk. The bacteria must be controlled in the milk to avoid problems with the milk products, he found.

In other research, he determined the best growth conditions for buttermilk and cottage cheese starter bacteria.

Currently, Marth is studying aflatoxins in milk products. Produced by certain molds, aflatoxins have been found to cause cancer in laboratory animals.

In addition to research and teaching activities, Marth is editor of the *Journal of Food Protection.* He edited the 14th edition of "Standard Methods for the Examination of Dairy Products," a reference book published in 1978.

He is author or co-author of more than 250 scientific articles and of two books on food microbiology.

In 1975, he received the Pfizer Award from the American Dairy Science Association, and in 1977, the Educator Award from IAMFES, as well as a citation from the American Public Health Association.

Before joining the UW-Madison faculty, Marth was a research microbiologist at Kraft, Inc. for nine years. The Jackson, WI, native received B.S., M.S. and Ph.D. degrees from UW-Madison.

Shoemake Presented Commendation Medal

James L. Shoemake, Sr. Regional Specialist, Milk and Food Programs, Seattle, WA was awarded a Public Health Service Commendation Medal by Donald Kennedy, Commissioner of Food and Drugs, at the Food and Drug Administration Honor Awards Ceremony in Rockville, Maryland June 22, 1979. As announced by Commissioner Kennedy the Commendation Medal was awarded for "sustained superior performance in assisting state and local regulatory officials achieve a high level of consumer protection concerning milk and food products".

Shoemake is a Sanitarian Director in the Commissioned Corps of the United States Public Health Service and provides technical assistance to Federal, State and Local officials in Region X concerning retail foods and state dairy programs.

Shoemake has been a long time member of both IAMFES and NEHA.

Donald Kennedy presents the Commendation Medal to James Shoemake.

Red Lobster Funds Research Grant

Red Lobster, the nation's largest seafood restaurant company, has presented a $5,000 research grant to Texas A&M University in appreciation for a quality control seminar developed and conducted recently at the University for Red Lobster personnel.

The grant, which will be used for a seafood research project, was presented by William Hattaway, Red Lobster president, to Dr. Zerle Carpenter of Texas A&M.

More than 50 Red Lobster personnel attended the seminar July 11-12, including regional directors, regional operations managers, the quality control department, members of the purchasing and menu planning staffs and corporate executives.

Nominations Due for Secretary-Treasurer

It is required by the constitution and by-laws of IAMFES that a secretary-treasurer be elected by mail ballot each year.

Nominations for that office are now open. A biographical sketch and photograph of each nominee should be sent to the Nominating Committee as soon as possible and no later than December 1, 1979. To maintain proper balance on the Executive Board, the nominee this year should be a regulatory representative..

Send nominations and information to:

Orlowe Osten, Chairman
Nominating Committee
IAMFES
1375 Fairmount Ave.
St. Paul, MN 55105

Awards Candidate Nominations Open

The success of the IAMFES awards program depends not only on the organizations which generously and regularly fund the program, but on you, for nominating persons worthy of the awards.

Persons are recognized by IAMFES awards for outstanding contributions to the field of milk, food and environmental sanitation, as well as to the Association.

Give thought to persons with whom you've worked in the Association who are deserving of the honors listed below, and nominate them. Contact Dave Fry, Chairman of the IAMFES Recognition and Awards Committee, T.G. Lee Foods, Inc., P.O. Box 3033, Orlando, FL 32802.

The Awards, to be given at the 1980 IAMFES Annual Meeting, are as follows:

1. *The Sanitarian's Award*--$1000 presented to a state or federal sanitarian who has made outstanding contributions during the past seven years to the health and welfare of his area.
2. *Educator/Industry Award*--$1000 will be presented to an industry employee who has shown outstanding service to food safety and sanitation.
3. *The Citation Award*--An award presented to an IAMFES member who has given outstanding service to the Association in helping fulfill its objectives.
4. *The Shogren Award*--The award is presented to the affiliate organization which has the best state or regional program.
5. *Honorary Life Membership*--An award given to a member who has shown long and outstanding service to the Association.

U.S. POSTAL SERVICE

STATEMENT OF OWNERSHIP, MANAGEMENT AND CIRCULATION

(Required by 39 U.S.C. 3685)

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3. FREQUENCY OF ISSUE	A. NO. OF ISSUES PUBLISHED ANNUALLY	B. ANNUAL SUBSCRIPTION PRICE
Monthly	12	$32.00 1979

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EDITOR *(Name and Address)*
Dr. E. H. Marth, Babcock Hall, Univ. of WI, Madison, WI 53706

MANAGING EDITOR *(Name and Address)*
Earl O. Wright, 413 Kellogg, Ames, Iowa 50010

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NAME	ADDRESS
International Association of Milk Food & Environmental Sanitarians, Inc.	413 Kellogg Ave. PO Box 701 Ames, Iowa 50010

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2. MAIL SUBSCRIPTIONS	3600	3693
C. TOTAL PAID CIRCULATION *(Sum of 10B1 and 10B2)*	3600	3693
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E. TOTAL DISTRIBUTION *(Sum of C and D)*	3685	3759
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2. RETURNS FROM NEWS AGENTS	0	0
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SIGNATURE AND TITLE OF EDITOR, PUBLISHER, BUSINESS MANAGER, OR OWNER
Earl O Wright Managing Editor

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In accordance with the provisions of this statute, I hereby request permission to mail the publication named in Item 1 at the phased postage rates presently authorized by 39 U. S. C. 3626.

SIGNATURE AND TITLE OF EDITOR, PUBLISHER, BUSINESS MANAGER, OR OWNER
Earl O Wright Managing Editor

PS Form 3526 Aug. 1978 *(Page 1)* (See instructions on reverse)

NSF Offers Promotional Seals

The National Sanitation Foundation (NSF) is offering to manufacturers of authorized equipment a book of seals to be used in promotional, packaging, and advertising materials.

The brochure may be obtained by writing:

NSF Building
Ann Arbor, MI 48105
313-769-8010

Harmon Receives ADSA Award of Honor

The 1979 recipient of the ADSA Award of Honor was born in Mountain Grove, MO. He received the Bachelor of Science degree in Dairy Industry from Kansas State University in 1936. His Master of Science degree, also in dairy industry, was from Texas Tech University in 1940 and his Ph.D. degree in Dairy Microbiology from Iowa State University in 1954.

From 1936 until 1954 Dr. Harmon was associated with the Department of Dairy Industry at Texas Tech. From 1954 to 1960 he was a member of the Dairy Department at Michigan State University and from 1960 to 1970 he was Professor of Food Science at Michigan State. In 1970 he became Professor of Food Science and Human Nutrition and in 1971 he was named Associate Chairman of the Department of Food Science and Human Nutrition. His professional career has been principally devoted to food processing and food micro-

con't p. 854

Sandine Receives DRINC Award

The recipient of the 1979 Dairy Research (DRINC) Foundation Award was born in Des Moines, Iowa. He acquired his education at Iowa, North Carolina and Oregon State Universities, along with post-doctoral training at the University of Illinois and sabbatical leaves at the Canadian Food Research Institute and the New Zealand Dairy Research Institute. As a graduate student he received the Phi Sigma Award as the outstanding student in biological sciences. He has served as a faculty member in the Microbiology Department of Oregon State University since 1960 where he and his colleagues have published over 100 manuscripts oriented toward the microbiological aspects of dairy products, particularly cultures and cul-

con't p. 854

1979 ADSA AWARD WINNERS, above, left to right: Dr. L. G. Harmon, Dr. W. E. Sandine, Dr. S. E. Gilliland, Dr. W. J. Harper. Right, Beverly A. Friend.

Friend Receives Hoyt Memorial Award

The Richard M. Hoyt Memorial Award is presented each year by the National Milk Producers Federation to a graduate student whose research efforts have direct application to the solution of significant problems facing the dairy industry.

Beverly E. Friend of the University of Nebraska was born in Milwaukee, WI. She entered the University of Wisconsin in 1965 where she received the B.S. in Biochemistry and Food Science in 1969. She earned the M.S. degree in Food Science at the University of Nebraska in 1971 where her M.S. thesis involved physiological and biochemical similarities between bovine and human milk lysozymes.

con't p. 854

Milk Industry Foundation Teaching Award to Harper

The successful teacher in today's university classroom is a person of many talents, particularly extensive command of subject matter coupled with the ability to ignite students with enthusiasm for the subject. Skill in the use of traditional teaching mechanisms — the formal lecture or laboratory — is augmented by interest in new mechanisms to help students learn. "Outstanding teachers" over the years have excelled in many ways, but a trait common to all is accessibility to students. Classes are described as open and relaxed, and the teacher always was ready to help each student, individually or collectively, academically or personally.

con't p. 857

Gilliland Receives Pfizer Award

The recipient of the 1979 Pfizer, Inc. Award is a native of Minco, Oklahoma. He received his B.S. and M.S. degrees in Dairy Manufacturing from Oklahoma State University in 1962 and 1963, respectively, and his Ph.D. in food science from North Carolina State University in 1966.

Gilliland began his professional career in 1965 at North Carolina State University as an instructor, engaged in Food Science research and teaching. He advanced to the rank of associate professor by 1972 and remained until 1976, when he returned to his native state of Oklahoma as associate professor at Oklahoma State University. He is presently in charge of teaching and research in the Dairy Food Section of Food Science. Dr. Gilliland has an impressive record in research, demonstrating innovation, resourcefulness, and soundness in planning and methods.

His work on concentrated lactic cultures is highly significant as extensive use is made of concentrated cultures in food plants. His discovery of methods for improving

con't p. 857

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ODORLESS—TASTELESS

NON-TOXIC

This Fine Mist-like **HAYNES-SPRAY** *should be used to lubricate:*

SANITARY VALVES
HOMOGENIZER PISTONS — RINGS
SANITARY SEALS & PARTS
CAPPER SLIDES & PARTS
POSITIVE PUMP PARTS
GLASS & PAPER FILLING MACHINE PARTS
and for ALL OTHER SANITARY MACHINE PARTS which are cleaned daily.

The Modern HAYNES-SPRAY Method of Lubrication Conforms with the Milk Ordinance and Code Recommended by the U. S. Public Health Service

The Haynes-Spray eliminates the danger of contamination which is possible by old fashioned lubricating methods. Spreading lubricants by the use of the finger method may entirely destroy previous bactericidal treatment of equipment.

PACKED 6-12 oz. CANS PER CARTON
SHIPPING WEIGHT—7 LBS.

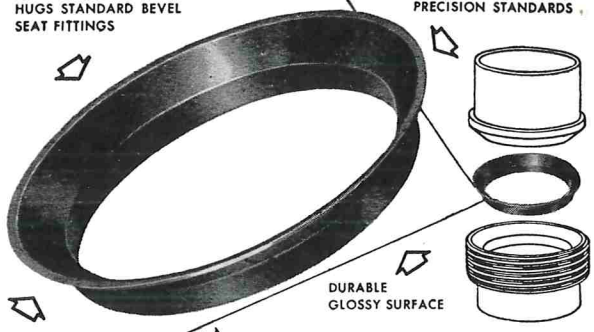
THE HAYNES MANUFACTURING CO.
4180 Lorain Ave. • Cleveland, Ohio 44113

HAYNES-SPRAY INGREDIENTS ARE APPROVED ADDITIVES AND CAN BE SAFELY USED AS A LUBRICANT FOR FOOD PROCESSING EQUIPMENT WHEN USED IN COMPLIANCE WITH EXISTING FOOD ADDITIVES REGULATIONS.

HAYNES SNAP-TITE GASKETS

"FORM-FIT" WIDE FLANGE HUGS STANDARD BEVEL SEAT FITTINGS

MOLDED TO PRECISION STANDARDS



DURABLE GLOSSY SURFACE

DESIGNED TO SNAP INTO FITTINGS

LOW COST...RE-USABLE

LEAK-PREVENTING

NEOPRENE GASKET for Sanitary Fittings

Check these SNAP-TITE *Advantages*

- Tight joints, no leaks, no shrinkage
- Sanitary, unaffected by heat or fats
- Non-porous, no seams or crevices
- Odorless, polished surfaces, easily cleaned
- Withstand sterilization
- Time-saving, easy to assemble
- Self-centering
- No sticking to fittings
- Eliminate line blocks
- Help overcome line vibrations
- Long life, use over and over

Available for 1", 1½", 2", 2½" and 3" fittings.
Packed 100 to the box. Order through your dairy supply house.

THE HAYNES MANUFACTURING CO.
4180 Lorain Avenue • Cleveland 13, Ohio

HAYNES SELF-CENTERING SNAP-TITE® *Gaskets*

SIZES 1" - 1½" 2" - 2½" - 3" - 4"

*MADE FROM **TEFLON®**

"The Sophisticated Gasket"

THE IDEAL UNION SEAL FOR BOTH VACUUM AND PRESSURE LINES

Gasket Color . . . slightly off-white

SNAP-TITE self-centering gaskets of TEFLON are designed for all standard bevel seat sanitary fittings. They SNAP into place providing self-alignment and ease of assembly and disassembly.
HAYNES SNAP-TITES of TEFLON are unaffected by cleaning solutions, steam and solvents. They will not embrittle at temperatures as low as minus 200° F. and are impervious to heat up to 500° F.

FOR A FITTING GASKET THAT WILL OUT-PERFORM ALL OTHERS...

Specify . . . HAYNES SNAP-TITES of TEFLON

- *TEFLON ACCEPTED SAFE FOR USE ON FOOD & PROCESSING EQUIPMENT BY U. S. FOOD AND DRUG ADMINISTRATION*

*Gaskets made of DuPont TEFLON® TFE-FLUOROCARBON RESINS

THE HAYNES MANUFACTURING COMPANY
4180 LORAIN AVENUE • CLEVELAND, OHIO 44113

A HEAVY DUTY SANITARY LUBRICANT

Available in both

SPRAY AND TUBE

All Lubri-Film ingredients are approved additives and can be safely utilized as a lubricant for food processing equipment when used in compliance with existing food additive regulations.

ESPECIALLY DEVELOPED FOR LUBRICATION OF FOOD PROCESSING AND PACKAGING EQUIPMENT

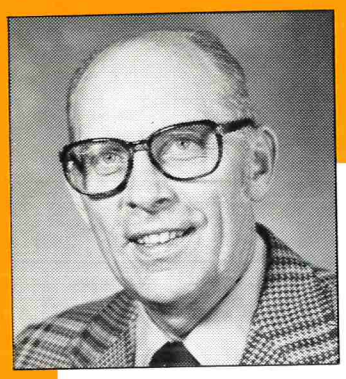
For Use in Dairies — Ice Cream Plants — Breweries — Beverage Plants — Bakeries — Canneries — Packing Plants

SANITARY • NON TOXIC • ODORLESS • TASTELESS

SPRAY — PACKED 6 — 16 OZ. CANS PER CARTON
TUBES — PACKED 12 — 4 OZ. TUBES PER CARTON

THE HAYNES MANUFACTURING CO.
CLEVELAND, OHIO 44113

SOMEONE YOU SHOULD KNOW IN THE DAIRY INDUSTRY



Dr. Clarence C. Olson, Extension Dairyman

Professor Olson is a member of the Department of Dairy Science, University of Wisconsin, Madison. Born and raised on a Wisconsin dairy farm, and a graduate of the University of Minnesota, he began his extension career in North Dakota, taking responsibility in extension programs with both dairy producers and industry. Under his direction, the state saw a rebirth and expansion of Dairy Herd Improvement Association testing programs, the development of membership organizations to provide AI services, and a nearly-complete changeover from farm separated cream to bulk sale of high quality whole milk. He returned to his native state in 1961 to direct training programs for Dairy Herd Improvement Supervisors and Lab Technicians and, since 1973, has led membership promotion and educational programs aimed at helping dairymen and the industry make more profitable use of the Dairy Herd Improvement program.

DHIA Is The Key To Progress

"The role of the extension specialist in the scheme of American agriculture is to make things happen—to take an idea from the research lab and get it into daily use on the farm. That is called progress.

"For dairymen, we believe DHIA is the key to greater progress. The unique partnership with extension through this program can result in benefits to the individual dairyman and for the entire dairy industry. A team effort makes this possible.

"County agents work directly with dairymen through educational programs to help improve feeding, breeding and management practices. We provide special training for agents and help them in meetings and with development of teaching and reference materials; but more specifically, by analyzing herd data. Frequently, this involves a research idea that must survive the challenge of field testing in cooperating DHIA herds. For example, haylage and high moisture corn became staple items in diets of Wisconsin cows after DHIA records proved their greater nutritional value. The same process uncovers problems needing attention by researchers; then DHIA records provide measures of success in solving the problem. The benefits of this team-up of extension-research with cooperating dairymen through DHIA are very evident to the industry.

"Perhaps the best known benefit of DHIA has been the pooling of performance data to identify genetically superior sires and top cows who are chosen to become mothers of future sires. The use of better and better sires by our dairymen has resulted in improved productivity of dairy cattle to the extent that the nation's milk supply is now provided with half as many cows. It would be interesting to speculate what the consumer would have to pay for milk and other dairy products if it were not for greater and more efficient milk production by the nation's dairy cows.

DHIA Is More Than You Think

"It is easy for a program that has been around as long as DHIA to become stereotyped—not true, it is changing all the time. As new research suggests, we work with our state DHI committee to make changes in the DHIA program. These dairymen help evaluate these new options to determine how well they fit the needs of members.

"Through this evolutionary process, DHIA records are now more than just measures of cow performance. The current emphasis is on management guidelines for feeding, breeding, selection and for complete records of identification and ancestry.

"The newest option available to DHIA members is somatic cell testing to evaluate udder health. This effort at improving milk quality along with quantity is very appropriate. Not only is it a valuable tool for the herd owner and the veterinarian but it also monitors the cows environment in order to help prevent mastitis.

"Feed testing and ration balancing are other new options available to members. We do our best to encourage these practices so that the benefits of superior genetics and the investments made in raising a heifer calf to producing age may be realized in more net income via the bulk tank.

"In summary, if we have been effective in promoting 'progress' in our DHIA herds, then faster adoption of new research should result in greater production by DHIA cows. A look at the data shows this has happened because cows on DHIA are currently outproducing cows not on test by over 4000 lbs. of milk yearly. Therefore, the bottom line reads only one way—we believe that if you milk cows and you want to make your cows worth more, then DHIA will insure your future."