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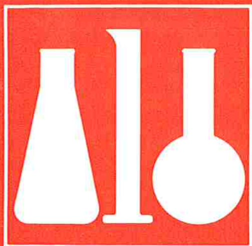

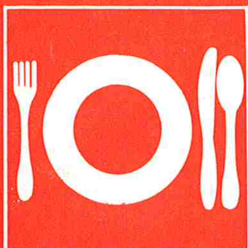

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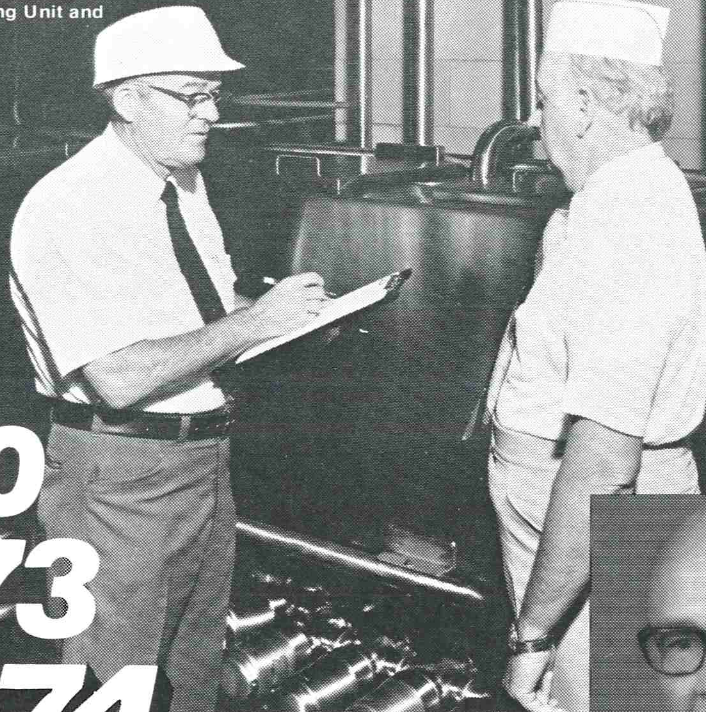

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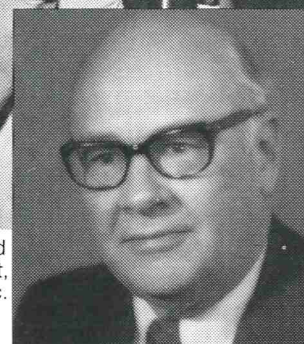

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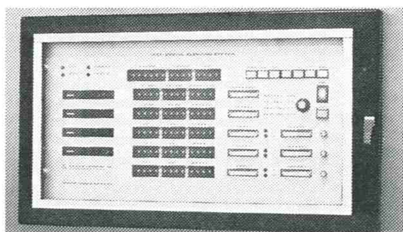

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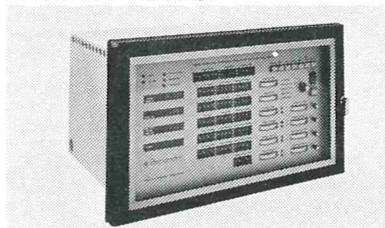

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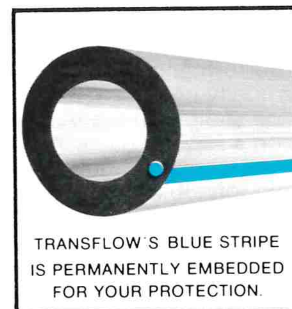

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*Asterisk designates person to whom inquiries regarding the paper should be addressed.

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Antagonistic Action of *Lactobacillus acidophilus* Toward Intestinal and Foodborne Pathogens in Associative Cultures[1]

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(Received for publication May 23, 1977)

ABSTRACT

Lactobacillus acidophilus exerted antagonistic actions on growth of *Staphylococcus aureus, Salmonella typhimurium,* enteropathogenic *Escherichia coli,* and *Clostridium perfringens* when grown with each in associative cultures. *S. aureus* and *C. perfringens* were more sensitive to the inhibition than were *S. typhimurium* and *E. coli.* The amount of the antagonism produced varied among strains of *L. acidophilus* and could not be directly related to amounts of acid produced; hydrogen peroxide produced by the lactobacilli appeared to be partially responsible for the antagonistic interaction. The inhibitory effect was produced also under anaerobic conditions in a pre-reduced medium.

Certain species of lactobacilli appear to be important in helping maintain a proper balance among micro-organisms in the intestinal tract (for recent reviews see references *9* and*10*). *Lactobacillus acidophilus* is the primary species implicated as being involved in this manner. Consumption of large numbers of viable cells of *L. acidophilus* has been used to re-establish normal intestinal flora following oral antibiotic therapy (*9,10*) and to treat patients infected with intestinal pathogens (*11*). *L. acidophilus* produced antibiotic-like substances when grown in laboratory media (*4,8,12,13*) and presence of the inhibitory properties was detected by assaying spent media from the lactobacilli by disc assay or using the cultures in cross streak tests on agar media. Extensive growth of the lactobacilli was required before inhibitory activity was detected. One report (*13*) indicated that the inhibitory material was produced only when *L. acidophilus* was grown for extended periods (2 to 52 weeks) in a solid medium (liver veal agar). Inhibitory material was either not produced or not detected when *L. acidophilus* was grown in liquid media. Vakil and Shahani (*12*) and Hamdan and Mikolajcik (*4*) reported production of antibiotics by *L. acidophilus* in milk at 37 C. In these studies, the inhibitors were extracted from the growth medium after 48 h of incubation, but data were not presented to determine whether or not sufficient inhibitory material was produced during the early stages of growth to inhibit the test organisms in associative cultures.

The objectives of the present study were to determine if *L. acidophilus* produced antagonistic action toward enteric pathogens in associative liquid cultures to determine the influence of various incubation conditions on the antagonism.

MATERIALS AND METHODS

Source and maintenance of cultures

L. acidophilus strains NCFM, 4962, CNRZ 216, CNRZ 218, HA3, and HM6 were from the culture collection of the Food Science Department, North Carolina State University. The original sources and identity characteristics have been previously described by Gilliland et al. (*3*). All strains were maintained by propagation in sterile 10% reconstituted non-fat-milk-solids (NFMS) containing 0.5% thiotone (BBL, Cockeysville, Md.) using 1% inocula and incubation at 37 C for 18 h. The cultures were stored at 5 C between transfers. The cultures were subcultured at least two times in the desired experimental assay medium immediately before use in experiments.

Staphylococcus aureus B925, *Clostridium perfringens* 3624, and *Salmonella typhimurium* were from the culture collection of the Food Science Department, North Carolina State University. Enteropathogenic *Escherichia coli* 0222:B4 was obtained from the Food and Drug Administration, Washington, D.C. Cultures of *S. aureus, S. typhimurium,* and enteropathogenic *E. coli* were maintained by routine propagation on trypticase soy agar (BBL) slants (18 h at 37 C). Slant cultures were stored at 5 C. Loop inocula from the slants were placed into the desired sterile experimental assay media followed by incubation for 18 h at 37 C. At least two additional subcultures (1% inocula, 18 h at 37 C) were made in the same assay media before use in experiments. *C. perfringens* was maintained in the same manner as used for *L. acidophilus,* except that lactobacilli MRS broth (Difco) containing 0.1% sodium thioglycollate was used in place of the milk medium.

Enumeration procedures

Distilled water containing 0.1% NFMS and 0.01% silicone antifoamer (Sigma Chemical Co., St. Louis, Mo.) was used as the

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[1]*Paper number 5155 of the Journal Series of the North Carolina Agricultural Experiment Station, Raleigh, North Carolina. The use of trade names in this publication does not imply endorsement by the North Carolina Agricultural Experiment Station of the products named nor criticism of similar ones not mentioned.*

[2]*Present address: Department of Animal Science and Industry, Oklahoma State University, Stillwater, Oklahoma 74074.*

diluent. Dilutions were prepared following procedures described in *Standard Methods for the Examination of Dairy Products* (*1*). Duplicate plates were prepared for each dilution, and plates were poured with the required medium.

S. aureus was enumerated on mannitol salt agar, *S. typhimurium* on brilliant green agar, and *E. coli* on violet red bile agar. *C. perfringens* was enumerated on egg yolk-free tryptose-sulfite-cycloserine (TSC) agar according to the method of Hauschild et al. (*5*). The TSC plates were incubated in a GasPak anaerobic system (BBL, Cockeysville, Md.). All plates were incubated 48 h at 37 C.

Interaction assay

Several media were used to provide adequate nutrients for associative cultures in which the various strains of *L. acidophilus* and the individual pathogens would grow well. The following media were used: lactobacilli MRS broth (MRS), lactobacilli MRS broth plus 0.5% sodium thoglycollate (MRS-Thiog), 10% NFMS plus 0.5% thiotone (Milk-Thio), and 10% NFMS plus 0.5% yeast extract (Milk-YE). Each was prepared in the desired volumes and sterilized by autoclaving 15 min at 121 C. Pre-reduced Milk-Thio medium was prepared, sterilized, and inoculated according to procedures described by Holdeman and Moore (*6*).

For each experiment, the required volume of sterile assay medium was inoculated with 1×10^3 to 1×10^4 colony forming units (CFU) of the desired pathogen per ml. The inoculated medium was thoroughly mixed and aseptically dispensed into sufficient containers to provide a control and one for each culture of *L. acidophilus* to be tested. The containers designated for *L. acidophilus* were additionally inoculated (1%) with the desired strain of lactobacillus. The control and associative cultures were incubated 6 h in a water bath at 37 C. At the end of the incubation time, samples were removed and placed in an ice bath until analyzed. The number of CFU of pathogens per ml was determined using the appropriate selective medium and in some experiments the pH or titratable acidity of the samples was also measured. Titration values were determined by titrating 9-g samples to pH 8.6 with 0.1 N NaOH using an automatic titrator. Percentages of inhibition were determined using the following formula:

$$\% \text{ Inhibition} = 100 \frac{(\text{CFU/ml in control}) - (\text{CFU/ml in associative culture})}{(\text{CFU/ml in control})}$$

To determine if hydrogen peroxide was responsible for the inhibition, duplicate control and associative culture samples were prepared. Active catalase (Nutritional Biochemical Corporation, Cleveland, Ohio) was added (70 U/ml) to one set of samples and the same amount of heat inactivated (121 C for 15 min) catalase was added to the duplicate set. The samples were incubated 6 h at 37 C and the percentages of inhibition determined as previously described.

To eliminate the effect of decreases in pH resulting from acid produced by *L. acidophilus*, control and associative cultures were placed in 1-liter sterile fermentors equipped with automatic pH controllers. Sodium carbonate (5%) in 5% ammonium hydroxide was used as the neutralizer to maintain both samples at the initial pH of the assay medium.

RESULTS

L. acidophilus NCFM was antagonistic to growth of *S. aureus*, *S. typhimurium*, and *C. perfringens* in associative cultures in MRS broth media (Table 1). MRS-Thio broth was used for the samples inoculated with *C. perfringens*. *S. typhimurium* was the least sensitive to the inhibitory action produced by the lactobacillus. Control cultures of the pathogens were pH 6.2-6.6 and the associative cultures were pH 5.4-5.5. *L. acidophilus* 4962 was inhibitory to *S. aureus*, *S. typhimurium*, and *E. coli* in Milk-Thio medium (Table 2). *E. coli* and *S. typhimurium* were inhibited to about the same degree, but *S. aureus* was inhibited to a greater extent. At the end of the assay period, control cultures of the pathogens were pH 6.2-6.4; the associative cultures were pH 5.7-5.8. The decreases in pH resulting from growth of the lactobacilli in the associative cultures (Table 1 and 2) do not appear sufficient to have been entirely responsible for the antagonistic actions exerted on the pathogens.

TABLE 1. *Inhibition of* Staphylococcus aureus, Salmonella typhimurium *and* Clostridium perfringens *by* Lactobacillus acidophilus *NCFM in associative cultures in MRS broth media*

Test cutlure	Sample	CFU[a] of Pathogens/ml[b]	% Inhibition	pH[b]
S aureus B925[c]	Control	3.1×10^5		6.2
	L. acidophilus	1.0×10^4	96.8	5.5
S. typhimurium[c]	Control	3.1×10^6		—
	L. acidophilus	4.8×10^5	87.4	
C. perfringens[d]	Control	1.9×10^7		6.6
	L. acidophilus	3.6×10^5	98.1	5.4

[a]CFU = Colony forming units.
[b]Determined after 6 h of incubation of associative cultures at 37 C.
[c]MRS broth used as assay medium.
[d]MRS broth plus 0.1% sodium thioglycollate used as assay medium.

TABLE 2. *Inhibition of* Staphylococcus aureus *B925,* Salmonella typhimurium *and enteropathogenic* Escherichia coli *0222:B4 by* Lactobacillus acidophilus *4962 in associative culture in Milk-Thio medium*

Test culture	Sample	CFU of pathogens/ml[a]	% Inhibition	pH[a]
S. aureus B925	Control	1.5×10^7		6.4
	L. acidophilus	2.7×10^5	98.2	5.8
S. typhimurium	Control	1.7×10^6		6.4
	L. acidophilus	2.3×10^5	86.5	5.8
Enteropathogenic	Control	3.3×10^7		6.2
E. coli 0222:B4	*L. acidophilus*	4.3×10^6	87.0	5.7

[a]CFU = colony forming units; determined after 6 h of incubation at 37 C.

The effect of decreasing pH on inhibition of *S. aureus* and *E. coli* by *L. acidophilus* 4962 was eliminated by growing the cultures in fermentors in which the medium was automatically maintained at pH 6.5 (Table 3). Both pathogens were inhibited about equally (61.2% and 62.5%); however, the amount of inhibition was less than observed in the same associative cultures without pH control (Table 2). This suggested that inhibition was caused by a factor(s) in addition to the acid produced or that acidic conditions potentiated the activity of the

TABLE 3. *Inhibition of* Staphylococcus aureus *B925 and enteropathogenic* Escherichia coli *0222:B4 by* Lactobacillus acidophilus *4962 in Milk-Thio medium maintained constantly at pH 6.5*

Test culture	Sample	CFU/ml[a]	% Inhibition
S. aureus B925	Control	1.2×10^7	
	L. acidophilus	4.5×10^6	62.5
Enteropathogenic	Control	8.5×10^8	
E. coli 0222:B4	*L. acidophilus*	2.2×10^8	61.2

[a]CFU = colony forming units; determined after 6 h of incubation at pH 6.5 and 37 C.

inhibitory factor(s).

Different strains of *L. acidophilus* exhibited different inhibitory capabilities when compared on the basis of their ability to produce acid and inhibit *S. aureus* and *S. typhimurium* in associative cultures (Table 4). Milk-YE

TABLE 4. *Comparison of acid production and inhibition of* Staphylococcus aureus *and* Salmonella typhimurium *in associative cultures*[a] *of* Lactobacillus acidophilus

Strain of *L. acidophilus*	ml N/10 NaOH[c]	% Inhibition[b]	
		S. aureus	*S. typhimurium*
4962	3.2[d]	75.8[d]	49.4[d]
216	3.2	84.7	64.4
NCFM	3.1	73.7	66.7
HM6	2.9	55.3	55.0
HA3	2.3	70.0	61.1

[a]Milk-Yeast Extract medium.
[b]Determined after 6 h at 37 C.
[c]ml N/10 NaOH to titrate 9-g samples to pH 8.6 after 6 h at 37 C.
[d]Average values from three experiments.

medium was used for these comparisons since *L. acidophilus* HM6 and HA3 failed to grow well in Milk-Thio medium. All strains of *L. acidophilus* and the two pathogens grew well in the Milk-YE medium. The lactobacilli are arranged in the table in descending order based on acid production (titration values at the end of the 6-h assay period). The amount of antagonistic action toward the pathogens could not be predicted by the amounts of acid produced by individual cultures of *L. acidophilus*. The titration values indicate that little growth of the lactobacilli had occurred in the 6-h period. In all instances except one (*L. acidophilus* HM6), *S. aureus* was more sensitive to the inhibition than was *S. typhimurium.*

To determine the influence of a low oxidation-reduction (O/R) potential on the antagonism, pre-reduced Milk-Thio medium was used as the assay medium. The resazurin in the medium remained reduced throughout the experiments indicating that a low O/R was maintained. Non-pre-reduced Milk-Thio was included for comparison. Both *L. acidophilus* NCFM and 4962 were inhibitory to the *E. coli* in both media (Table 5). Strain 4962 was more inhibitory than strain NCFM in each medium. The amount of inhibition observed in the pre-reduced medium was somewhat less than in control medium, however, the percentages of inhibition produced in the pre-reduced medium by strain NCFM and 4962 were 62.7 and 75.5%, respectively. These results suggested that exposure of the cultures to air during associative growth might enhance the inhibition.

Incorporation of active catalase into the associative cultures generally reduced the intensity of the antagonism produced by three strains of *L. acidophilus* evaluated (Table 6). Again *S. aureus* was more sensitive than *S. typhimurium.* In each instance (except *L. acidophilus* 4962 vs. *S. typhimurium*) less inhibition was observed in the samples containing active catalase than in the ones containing heat inactivated catalase. This suggests partial involvement of hydrogen peroxide in the antagonistic interaction.

TABLE 5. *Inhibition of enteropathogenic* Escherichia coli *0222:B4 by* Lactobacillus acidophilus *in pre-reduced Milk-Thio medium*

Medium	Sample	CFU/ml[a]	% Inhibition
Non-pre-reduced	Control	1.0×10^8	
	L. acidophilus NCFM	2.4×10^7	76.0
	L. acidophilus 4962	1.7×10^7	83.0
Pre-reduced	Control	1.1×10^8	
	L. acidophilus NCFM	4.1×10^7	62.7
	L. acidophilus 4962	2.7×10^7	75.5

[a]CFU = colony forming units; each value represents average counts after incubation for 8 h at 37 C from three experiments.

TABLE 6. *Effect of catalase on antagonism of* Lactobacillus acidophilus *toward* Staphylococcus aureus *and* Salmonella typhimurium

L. acidophilus	% Inhibition[a]			
	S. aureus		*S. typhimurium*	
	Control[b]	Catalase[c]	Control[b]	Catalase[c]
NCFM	73.1	60.3	60.2	48.1
4962	76.2	63.5	57.1	59.3
216	92.3	79.4	75.5	64.4

[a]Determined after 6 h at 37 C; averages from two experiments.
[b]70 units heat inactivated catalase per ml.
[c]70 units active catalase per ml.

DISCUSSION

Vincent et al. (*13*) observed no antibacterial activity when liquid cultures of *L. acidophilus* were tested. They reported that it was necessary to grow *L. acidophilus* on solid agar media for the antibacterial substance(s) to be produced. Furthermore, extended incubation of agar media inoculated with the lactobacilli was necessary before appreciable inhibitory activity was detected. More recent studies (*4,12*) have shown that *L. acidophiles* does produce inhibitory material in liquid media. However, in both these reports the inhibitory material was extracted from 48-h milk cultures with organic solvents; no evidence was presented to indicate that sufficient activity was present in younger cultures to exert inhibitory action toward pathogenic bacteria. For *L acidophilus* to be effective in helping control proliferation of intestinal pathogens in the intestinal tract, it would seem that such inhibition should be produced in its early stages of growth. Furthermore, the inhibition would need to be manifested during associative growth. Our results show that *L. acidophilus* does exert antagonistic actions toward the growth of a variety enteric pathogens during the early stages of growth in associative cultures.

The antagonistic action produced by some species of lactobacilli toward other bacteria has been attributed to their metabolic production of hydrogen peroxide (*2,7,14*). Vincent et al. (*13*) indicated that catalase had no effect on the inhibitory action produced by *L. acidophilus* and,

thus, hydrogen peroxide was not involved. Results from the present study show that the addition of active catalase to the associative cultures reduced, but did not eliminate the inhibitory action produced by *L. acidophilus.* This suggests that hydrogen peroxide is partially responsible for the antagonism. However, it apparently accounts for a small portion of activity.

Other researchers *(8,12)* have indicated that acidity produced by *L. acidophilus* was not the sole cause of its antagonistic action toward other bacteria. Results from the present study confirm this. The antibacterial action produced by *L. acidophilus* is probably due to a combination of factors including acid, hydrogen peroxide, and other inhibitory substances including the antibiotics previously reported *(4,12,13)*.

The gram positive bacteria (*S. aureus* and *C. perfringens*) were more sensitive to the inhibitory action of *L. acidophilus* than were the gram negative species (*S. typhimurium* and *E. coli*). This may be useful in future studies in determining the mechanism whereby the inhibitor(s) functions.

Since the environment in the intestinal tract is anaerobic, *L. acidophilus* must be able to exert an antibacterial effect under such a condition if it is to play a role in controlling enteric pathogens in vivo. Results obtained in the pre-reduced medium show that an antagonistic action toward *E. coli* is produced by *L. acidophilus* under conditions. The ability of *L. acidophilus* to produce its antagonistic effects under the different environmental conditions suggests that *L. acidophilus* may help to restrict growth of various pathogens in the intestinal tract. *L. acidophilus* is beneficial in treating patients infected with certain intestinal pathogens *(11)*.

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Changes of Color of Aqueous Beef Extract Caused by *Pseudomonas fragi*[1]

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ABSTRACT

Growth of *Pseudomonas fragi* had a significant (P < .05) effect on the color of aqueous beef extract stored at 1 ± 1 C. As numbers of *P. fragi* increased from log 5 to 7.9/ml during 10 days of storage at 1 ± 1 C, the pH increased from 5.5 to 6.0. At the end of 10 days there was a 76% loss of oxymyoglobin in samples inoculated with *P. fragi*. There was a 45% loss of oxymyoglobin in sterile-control samples. A possible mechanism for conversion of oxymyoglobin to metmyoglobin is suggested.

Bright, cherry red color is the paramount, overriding quality attribute influencing purchasers of fresh meats (*10*). Microbial growth is one of the major factors which causes discoloration in fresh meats (*7,15,17,19,21*). Although taxonomic studies on distribution of microorganisms reveal the predominance of the genus *Pseudomonas* in fresh beef stored at refrigerator temperatures (*2,3,4,6,13,14,22*), little is known about the effect of growth of these organisms on color stability of beef.

In work reported herein, we have examined the effect of growth of *Pseudomonas fragi* on color, pH, and protein degradation of aqueous beef extract.

MATERIALS AND METHODS

Preparation of culture

Initially, several samples of *P. fragi* ATCC 4973 were lyophilized to ensure an adequate stock in pure culture. Fresh suspensions were prepared in Brain Heart Infusion (BHI) Broth from these freeze-dried cells. After incubation at 21 C for 48 h, cells were collected by centrifugation at 7300 × *g* for 20 min. Cells were washed with sterile deionized water, centrifuged again at 7300 × *g* for 20 min, then suspended in sterile deionized water. Cell concentrations in suspensions, adjusted to 0.02 optical density (600 nm), were determined initially by aerobic plate count (*12*) with incubation at 21 C.

Preparation of sample

The sterile aqueous extract was prepared from the semitendinosus muscle of USDA Good beef containing a minimum amount of intramuscular fat. Replications were obtained from semitendinosus muscles of six different animals. The external fat layer was removed and the muscle was cut into cubes (approximately 2.5 cm^2) with a knife. The cubes were ground through a 0.32-cm plate using a KitchenAid food grinder. One hundred grams of ground meat were mixed with 250 ml of cold sterile deionized water and 12 g of Hyflo Super-Cell. These were blended for 6 sec at low speed in a chilled stainless steel blendor jar. The meat slurry was filtered under vacuum using Whatman No. 1 filter paper. The filtrate was again filtered under vacuum using 47-mm membrane (Millipore type SC) filters. The clear filtrate was cold-sterilized by filtering under vacuum using 0.22-μm membrane filters (Millipore Sterifil Aseptic System Type GS). Inoculated beef extract samples were prepared by mixing with approximately 10^5/ml of *P. fragi* cells. Sterile controls and the inoculated beef extract were aseptically transferred into sterile test tubes (20 ml each) in a transfer room under U.V. lights. During transfer, the beef extract was covered with aluminum foil to prevent deterioration of color by the U.V. light.

Storage

Tubes with sterile and inoculated beef extract were stored in an unlighted cooler at 1 ± 1 C for 2, 4, 6, 8, and 10 days. Solution temperature was monitored by two thermocouples placed in extra tubes. Solutions were oxygenated by shaking five times each day. Following each storage time, samples were analyzed for aerobic plate count, pH, and color. Amount of protein degradation was determined by sodium dodecyl sulfate (SDS) polyacrylamide gel electrophoresis (PAGE) after 10 days of storage.

Color measurement

Absorbancy of the extracts was determined at 473, 507, 573, and 597 nm using a Model 24 Beckman Spectrophotometer. From values obtained, the relative concentrations of myoglobin (Mb), oxymyoglobin (O_2 Mb) and metmyoglobin (MMb) were determined by the absorbancy ratio method of Broumand et al. (*5*).

pH Measurement

The pH of samples of cell-free beef extract was measured with a Fisher Accumet Model 320 Expanded Scale Research pH Meter.

Bacterial plate counts

Aerobic plate counts were made according to Hausler (*12*), except that the incubation temperature was 21 C.

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Protein Determinations

Total protein content of the beef extract, hemoglobin, and myoglobin standards were determined by micro kjeldahl (*1*).

Sodium dodecyl sulfate (SDS) polyacrylamide gel electrophoresis

Electrophoresis was conducted according to procedures described by Weber and Osborn (*25*).

Statistical analyses

Analyses of variance, least significant differences, and correlation coefficients were used to statistically analyze the data (*20*).

RESULTS AND DISCUSSION

Microbial growth

Samples of sterile beef extract (controls) had no microbial growth throughout the 10 days of storage at 1 ± 1 C. Numbers increased significantly ($P < .01$) in samples inoculated with *P. fragi* and stored for 10 days at 1 C (Table 1). The mean aerobic plate counts of inoculated samples increased from log 5/ml at 0 day to log 7.9/ml after 10 days of storage. There was a significant difference ($P < .01$) in bacterial numbers due to *P fragi* inoculation and storage, as well as a significant ($P < .01$) interaction between *P. fragi* inoculation and storage.

Effects of P. fragi on pH

The pH of the sterile extract remained relatively constant throughout the experiment (Fig. 1). Similar results were reported by Ockerman et al. (*18*) and Ockerman and Cahill (*17*). The pH of the inoculated extract increased from 5.5 to 6.0 during the 10 days of storage, but this increase was not statistically significant (Table 1). There was, however, a significantly ($P < .05$) positive correlation between aerobic plate counts and pH (Table 2). Storage time had no significant ($P < .05$) effect on the pH of the beef extract (Table 1). The higher pH in inoculated samples may have resulted from production of ammonia and amines (tyrosine to tyramine, ornithine to putrescine, lysine to cadaverine, etc.) due to decarboxylation and deamination of amino acids in aqueous beef extract by *P. fragi*.

Effects of P. fragi on color.

Color of the beef extract was determined by the relative concentrations of Mb, 0_2 Mb and MMb. Inoculation with *P fragi* and storage time significantly ($P < .01$) affected color (Table 1). There was a significant ($P < .01$) interaction, also between storage time and

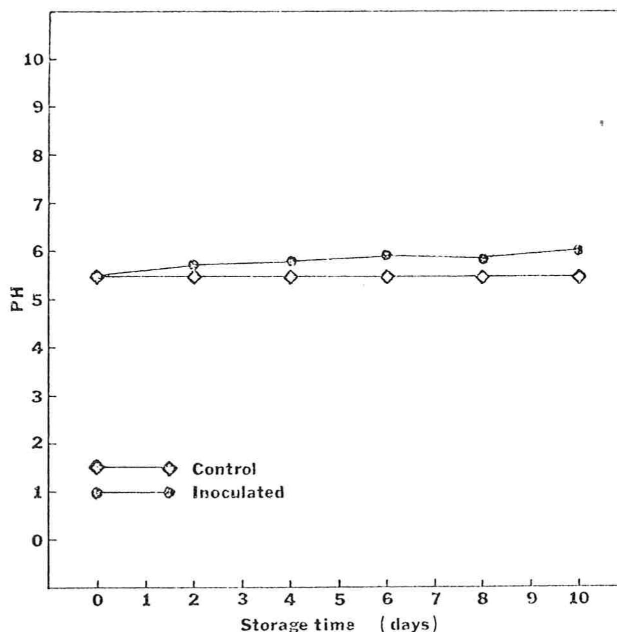

Figure 1. *The pH of control and inoculated beef extract stored at 1 ± 1 C.*

TABLE 2. *Correlation coefficients of aerobic plate counts (APC), %Mb, %0_2Mb, %MMb, and pH values for inoculated beef extract stored at 1 ± 1 C.*

Parameters	%Mb	%0_2Mb	%MMb	pH
APC	0.28	–0.66	0.66	0.76
%Mb		–0.38	0.29	0.20
%0_2Mb			–0.99	–0.81
%MMB				0.82

Number of comparisons = 72.
All correlation coefficients were significant at $P < 0.05$.

inoculation, for %0_2Mb and %MMB (Table 1). significantly ($P < .05$) lower than those in the control (Fig. 2). There was 76% loss of 0_2 Mb in inoculated samples in 10 days; 45% of 0_2 Mb was lost during the same period from the sterile samples. Differences were significant from day 2 through day 10 (LSD = 7.1).

During storage at 1 ± 1 C for 10 days, % MMb concentrations in inoculated samples were significantly ($P < .01$) higher than in controls (Fig. 3). Correlation coefficients of –0.66, between log of bacteria number and %0_2Mb, and of —0.81, between pH and % 0_2Mb,

TABLE 1. *Analyses of variance of microbial growth, %Mb, %0_2Mb, %MMb, and pH values of aqueous beef extract stored at 1 ± 1 C.*

Source	DF	Mean squares				
		Microbial growth	%Mb	%0_2Mb	%MMb	pH
Replication (A)	5	0.48^{ns}	8.12^{ns}	729.65**	637.69**	0.49^{ns}
Storage time (B)	5	3.47**	8.12^{ns}	3863.75**	3792.45**	0.37^{ns}
Inoculation (C)	1	882.90**	28.12*	12194.01**	11050.88**	0.35^{ns}
B × C	5	3.47**	8.12^{ns}	626.48**	631.52**	0.25^{ns}
Error	55	0.88	4.49	37.34	24.29	0.21

** Significant ($P < .01$).
* Significant ($P < .05$).
^{ns}Not significant ($P < .05$).

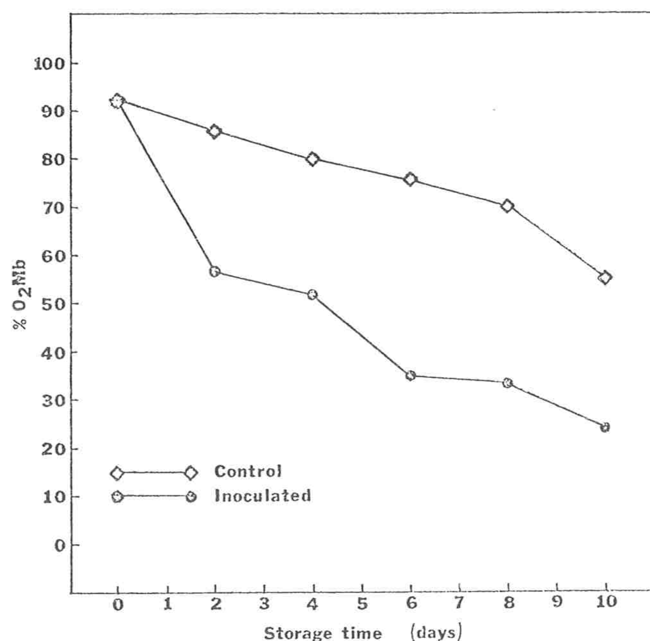

Figure 2. *The %O_2Mb concentration of the beef extract stored at 1 ± 1 C.*

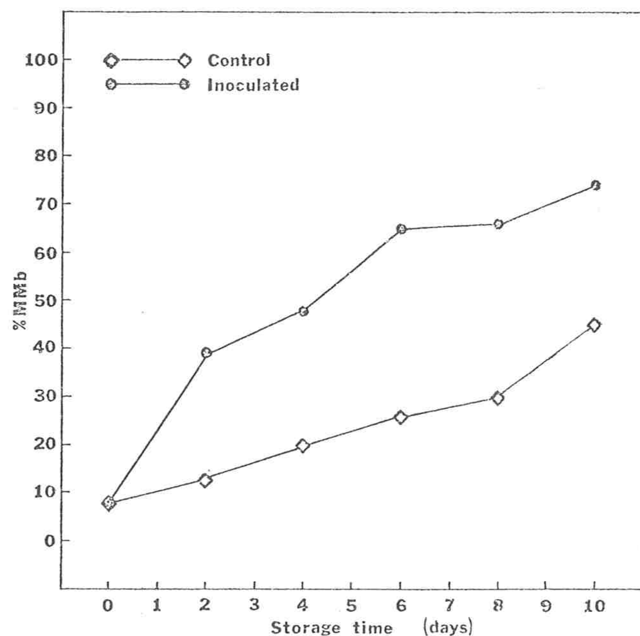

Figure 3. *The %MMb concentration of the aqueous beef extract stored at 1 ± 1 C.*

suggested that both microbial growth and pH were important in discoloration of the extract. These data support the observations of Robach and Costilow (*19*), Solberg (*21*), Lechowich (*15*), and Ockerman and Cahill (*17*) but contradict findings of Lin et al. (*16*). A significant negative correlation coefficient of –0.99 between %O_2 Mb and %MMb was observed (Table 2) which indicated that as the concentration of %O_2 Mb decreased the concentration of %MMb increased.

Effect of P. fragi on proteins

It is evident from SDS-polyacrylamide gel electrophorograms (Fig. 4) that *P. fragi* caused protein degradation in samples of extract stored at 1 + 1 C for 10 days. This is not surprising, since it is known that *P. fragi* has proteolytic activity (*8,11,24*). These observations suggest that proteolysis by *P. fragi* may have been one of the major factors in discoloration of the beef extract. The report of Fox (*9*) that the globin part of the myoglobin molecule is susceptible to attack by protease supports this suggestion. It is also possible that degradation of the peptide chain in the myoglobin molecule by *P. fragi* resulted in alteration of the reactivity of the heme group and eventually complete discoloration. Bovine hemoglobin and myoglobin seem to have the same electrophoretic mobility, therefore, hemoglobin and myoglobin in aqueous solution were not separated by SDS-polyacrylamide gel electrophoresis.

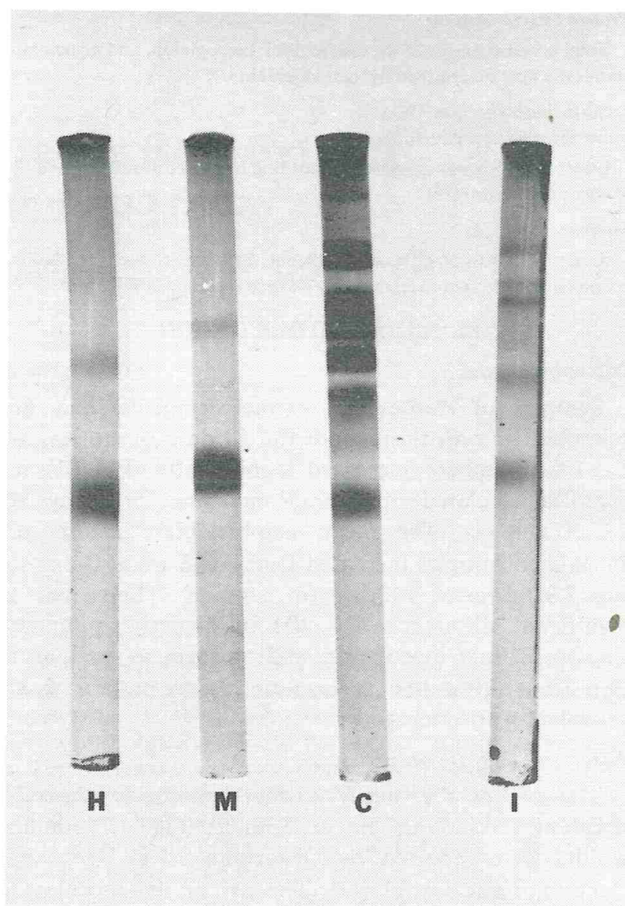

Figure 4. *SDS-polyacrylamide gel electrophotogram of aqueous beef extracts stored 10 days at 1 ± 1 C and bovine hemoglobin and myoglobin standards. Electrophoresis was performed at a constant current of 5 mA/tube for 7 h. Protein concentration: Hemoglobin (H) = 25µg/tube; Protein concentration: Myoglobin (M) = 25 µg/tube, Protein concentration: Control beef extract (C) =45 µg/tube, Protein concentration: Inoculated beef extract(I) = 46 µg/tube.*

CONCLUSION

Growth of *P. fragi* had a significant ($P < .05$) detrimental effect on color of aqueous beef extract stored at 1 ± 1 C. Based on the findings of this study,

proteolysis by *P. fragi* is a major factor in discoloration of beef extract stored at 1 ± 1 C.

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Production of Aflatoxins B and G on Solid and Broth Culture Media

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ABSTRACT

Mycelial growth and production of aflatoxins by *Aspergillus parasiticus* NRRL A-16,462, on whole peanuts, undelintered cottonseed, and shredded wheat was compared to growth and aflatoxin production on 10% broths of the same substrates. The greatest amount of toxins was produced on shredded wheat as a whole substrate. The least amount was produced in shredded wheat broth medium. Intermediate levels of toxin were produced on cottonseed in both types of media. More aflatoxin was produced on peanut broth than on whole peanuts. Mycelial growth on whole shredded wheat was greater than growth on the other solid media though it had the lowest amount of growth of the three broth media. Among whole substrates, growth on fuzzy cottonseed was least but mycelial growth on cottonseed broth was second of the three broth media. Growth on whole peanuts was as great as on shredded wheat; on peanut broth, it was the greatest of the three broth media. On whole substrates, the ratio of aflatoxins B:G produced was highest on shredded wheat and lowest on peanuts. In broth culture the B:G ratio was largest on the cottonseed and lowest on shredded wheat.

Aflatoxins are closely related secondary metabolic products of certain strains of the *Aspergillus flavus* group that cause significant economic and food safety problems by contamination of oilseeds, grains, and nuts. Aflatoxins cause numerous biological responses in a wide range of organisms and are mutagenic as well as carcinogenic and teratogenic (*1*). Considerable reseach has been and is still underway on these toxins (*6*).

Temperature and moisture contents of substrates are major factors in fungal growth and elaboration of the toxins. Diener and Davis (*4,5*) showed that temperature, relative humidity, and kernel moisture were important in aflatoxin production in whole peanuts, and Beuchat and Lechowich (*2*) reported similar results with whole navy, pinto, and kidney beans (varieties of *Phaseolus vulgaris*). Mayne et al. (*9*) showed that these factors also affect aflatoxin production on cottonseed, peanuts, and whole, bite-sized shredded wheat (*8*). Most of these reports were of research conducted on whole seeds for which surface areas of the seeds differed. Differences in surface area of the seeds and accessibility of oxygen to the substrate can affect aflatoxin production (*5*). Use of broths containing ground peanuts was reported in only one study other than the present one. De Vogel et al., (*3*) compared relative rates of mycelial growth on whole peanuts and on peanuts in broth cultures. The purpose of the present research was to compare growth of *Aspergillus parasiticus* (a strain that produces the four major aflatoxins), total aflatoxin production, and the ratio aflatoxins B:G on three products (peanut, cottonseed, and shredded wheat) employed as whole substrates and homogenized in broth cultures to equalize moisture content and surface area of the substrates.

MATERIALS AND METHODS

A. parasiticus NRRL A-16,462, was used as the test inoculum. Potato Dextrose Agar (PDA) slants were inoculated with the fungus and incubated at 30 C for 7 days. Transfers to produce inocula were made from growth on PDA agar plates. After 7 days at 30 C, spores were harvested with a sterile, moistened cotton swab and mixed in sterile .05% Tween 20,[2] to break any clumps of spores.

Substrates for studying growth and aflatoxin production on whole materials were shredded wheat, whole or split blanched peanuts, and undelintered cottonseed at 37, 25, and 25% moisture, respectively. Mayne et al. (*8*) added water to a level of 33% in their study of aflatoxin elaboration on whole cottonseed products (comparable to 37% moisture on an oil-free basis and considered optimum for aflatoxin production). Toxin production on peanuts and cottonseed equilibrated at high relative humidity could then be compared with toxin production on whole shredded wheat.

To test solid substrates, 10 g were placed in tared 250-ml Erlenmeyer flasks plugged with cotton. Appropriate amounts of tap water were added and flasks were weighed before and after autoclaving at 121 C for 15 min. After the flasks were cooled and weighed, sterile tap water was added to replace evaporated water. Each flask was inoculated with 1 ml of the spore suspension and incubated quiescently for 7 days at 30 C for optimum toxin production.

Broth cultures was prepared by modifying the method of De Vogel et al. (*3*). No salts or sucrose were added to the medium. One hundred g of each substrate was ground in an Omnimixer at high speed for 60 sec placed in 1 liter of distilled water, and boiled gently for 10 min, then filtered through two layers of cheesecloth. After cooling to room

[1]*One of the facilities of the Southern Region, Agricultural Research Service, U.S. Department of Agriculture.*

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temperature, 0.2 N NaOH was added to adjust the pH to 7.0, and 100 ml of the broth was transferred to 250-ml Erlenmeyer flasks, autoclaved at 121 C for 15 min, cooled, inoculated with 1 ml of the spore suspension, and incubated for 7 days at 30 C.

Because mold growth on the whole substrates cannot be measured accurately while growing, it was estimated by observing the samples and recording growth as 0, 1+, 2+, 3+, or 4+, the range being from no growth (0) to heavy growth with much sporulation (4+). Dry weights of mycelial pads grown on broths were obtained by separating the pad, washing it with 100 ml of distilled water, and mild drying overnight in a vacuum oven at 44 C.

Before assaying for aflatoxins, the mold was removed from the broths, placed in beakers, and killed by contact with boiling chloroform that was subsequently evaporated on a steam bath. The entire mycelial pad or solid substrate was macerated in a Waring blender with 250 ml of aqueous acetone at slow speed for 2-3 min, allowed to stand for 30 min, then reblended at high speed for 5 min. After filtering, all extracts of the pads, as well as the broth media were analyzed for aflatoxins by the method of Pons et al. (*11*).

Trace metals were determined by atomic absorption spectroscopy in the analytical laboratory of the Center.

RESULTS AND DISCUSSION

Production of total aflatoxins on the two types of media is shown in Tables 1 and 2. The tables reflect the totals of intra-and extracellular aflatoxin produced. Almost all of the toxin produced was intracellular.

TABLE 1. *Aflatoxins produced on whole substrates*

Substrate	Aflatoxins (ppb) B_1	B_2	G_1	G_2	Total	B:G Ratio
Peanut	30,000	ND[a]	20,000	ND	50,000	1.5:1
Cottonseed	225,000	30,000	100,000	33,000	388,000	1.8:1
Shredded wheat	375,000	ND	100,000	ND	475,000	3.8:1

[a]ND = none detected.

TABLE 2. *Aflatoxins produced on broth substrates*

Substrate	Aflatoxins (ppb) B_1	B_2	G_1	G_2	Total	B:G Ratio
Peanut	179,000	3,000	55,000	ND[a]	237,000	3.3:1
Cottonseed	53,000	4,000	13,000	ND	70,000	4.4:1
Shredded wheat	1,200	ND	400	ND	1,600	3.0:1

[a]ND = none detected.

Performance of fungus differed greatly on the three solid substrates. The highest quantity of aflatoxins was produced on whole shredded wheat and cottonseed; the least, on whole peanuts, even though peanuts have been considered the major source of aflatoxins in food and feed products. When the fungus was grown on these same substrates in broths, the order and magnitude of aflatoxin production was reversed. It was greatest on peanut, less on cottonseed, and least on shredded wheat. Shredded wheat, as a whole substrate, yielded the greatest amount of toxin but produced the least amount when the shredded wheat was in broth medium. Growth on cottonseed produced intermediate toxin levels on both types of media but growth on peanut broth yielded more aflatoxin than did solid peanuts. The reason for this is unknown since the broth contained only 10% homogenized peanuts. If peanuts do contain better growth nutrients than the other substrates, the greater dispersal in broths may be responsible for this increased growth.

The ratio of aflatoxins B:G was significantly different between the two types of media (Table 1 and 2). B:G ratios for peanuts and cottonseed were lower on whole substrates than on broths but for whole shredded wheat, it was slightly higher than for shredded wheat in broth culture.

Growth on the various substrates is shown in Table 3. Here, too, there are differences between growth on whole and on broth substrates. Growth on whole shredded wheat was the greatest of the three solid media. Conversely, when shredded wheat was in broth form, the dry weight of the fungal mat produced was the lowest of the three broths. Cottonseed and peanut seeds supported less growth than did the corresponding broths.

TABLE 3. Aspergillus parasiticus *growth on the various media*

Medium	Whole substrate[a]	Broth (grams)[b]
Peanut	4+	2.35
Cottonseed	2+	1.24
Shredded wheat	4+	0.55

[a]Growth on whole substrate is estimated.
[b]Growth on broths is given as dry weight of the mycelial pad.

If growth on the three substrates in two forms is compared, it is noted that *A. parasiticus* produced much more total aflatoxins on whole substrates than it did in broth cultures. Although the relative amounts of toxins produced on peanut and shredded wheat are reversed, depending upon the medium, peanut was the only substrate to yield more total aflatoxins in broth than on solids. Shredded wheat does not contain any toxic materials, and Mayne, et al. (*9*) showed that gossypol (a toxic pigment in cottonseed) had no inhibitory effects on the mold. Wells et al. (*13*) reported that growth of *A. flavus* on whole peanuts was much slower than growth of two other molds used for comparison. Whether this is due to the presence of a natural inhibitor of *A. flavus* in peanuts, such as was reported by Turner et al. (*12*), is not known, but this possibility is being explored further. Marsh et al. (*7*) showed that certain trace metals could affect aflatoxin production by *A. parasiticus*, but free metals were not a factor here since all salts used by De Vogel et al. (*3*) in broth culture were excluded to avoid the effect. Several trace metals (Mn, Cu, Fe, Zn) were assayed in the three substrates to determine if striking differences in their amounts might be a factor. The results in Table 4 show that the difference between these trace metals in the substrates including Zn, were not large enough to be considered a major factor in aflatoxin production.

Aflatoxin production on whole substrates decreased in the order: shredded wheat, cottonseed, and peanut. This agrees with the findings of Mayne et al. (*9*), on whole substrates with water added to 33% moisture, but the order is completely reversed in broth cultures. The large decrease in total toxins and absence of G_2 in all three broths suggests that the increased water content, and decreased surface area of substrates can alter or affect mold growth and toxin production. In addition to the

TABLE 4. *Some trace metal concentrations in the three broth substrates*

Broth	Mn	Cu	Fe	Zn
	(ppm)	(ppm)	(ppm)	(ppm)
Peanut	13.8	15.5	23.0	18.8
Cottonseed	5.1	21.8	30.7	23.9
Shredded Wheat	12.8	10.8	13.0	20.0

increase in total aflatoxins produced in peanut broth, all three substrates in broth culture showed a striking increase in the relative amount of aflatoxin B to G, with no G_2 formed. Whether or not the systems synthesizing the individual aflatoxins are less functional (or absent) in mycelia grown in broth culture can not be determined without an extensive study of the mycelial protein patterns, mycelial growth, and aflatoxin production under varying conditons. This was not the goal of the present investigations. The results, however, do confirm that the aflatoxin B:G ratio and total aflatoxins production on products destined for food or feed uses can be altered by the conditions of the substrate.

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Purification and Activity of *Clostridium perfringens* Alpha Toxin[1]

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ABSTRACT

Clostridium perfringens α-toxin obtained from a commercial source contained at least seven protein bands when examined by SDS polyacrylamide disc gel electrophoresis. The fraction with an R_m of 0.29-0.56 contained 95.6% of the total hemolytic activity and all of the phospholipolytic acitivity. The α-toxin when passed through a Sephadex G-100 column yielded several peaks but only one active fraction. This fraction upon electrophoresis in a SDS polyacrylamide disc gel system contained two protein bands. Only one protein with a R_m of 0.64 possessed hemolytic and phospholipolytic activites. A second passage of the active fraction through Sephadex G-100 yielded only one protein band. This protein exhibited both hemolytic and phospholipolytic activities. For *C. perfringens*, it is apparent that both of these activities reside with the same protein.

Alpha toxin (E. C., 3.1, 4.3, phosphoglyceride, diglyceride-hydrolase, lecithinase C, or phospholipase C) is not the etiological agent responsible for the *Clostridium perfringens* food poisoning syndrome (*4,7*). However, it may serve as an indirect measure of the population level attained by *C. perfringens* in a food system (*5*).

There appear to be two differing views regarding the hemolytic and phospholipolytic activities of the alpha toxin of *C. perfringens*. Mitsui et al. (*12*) have shown that both of these activities are associated with a single metabolic entity having a molecular weight of approximately 49,000. Bernheimer et al. (*2*) failed to separate the two activities by density gradient electrophoresis and isoelectric focusing. They concluded that both activities reside in the same molecule. However, Sabban et al. (*15*) have shown that a *C. perfringens* phospholipase C preparation can under certain conditions induce hemolysis of red-blood cells (rbc) without release of any organic phosphate from the rbc membrane phospholipids. They also observed that heating the toxin in the presence of Ca^{++} caused loss of the hemolytic activity, whereas hydrolytic activity was retained. They concluded that hemolytic activity was distinct from hydrolytic activity. Whether or not these two activities were considered to be associated with two separate metabolites of *C. perfringens* or with one protein molecule which possessed multicatalytic sites was not reported.

For *Bacillus cereus*, there is convincing evidence that the hydrolytic and phospholipolytic activities reside in two different chemical entities. Attempts to separate these two activities from *B. cereus* preparations have been successful (10, 13). Bonventre and Johnson (*10*) using Sephadex gel filtration demonstrated that hemolytic, phospholipolytic, and lethal activities are catalyzed by separate proteins. Molnar (*13*) reported the separation of *B. cereus* toxin into two components (Factors I and II) on columns of calcium -phosphate gel. The phospholipase activity, determined by the egg-yolk reaction, was found in the fraction containing Factor I. Ivers and Potter (*9*) have reported that hemolysin, phospholipase C, and lethal toxin of *B. cereus* are three different metabolites.

This research was undertaken to determine whether both hemolytic and phospholipolytic activities are associated with the same protein.

MATERIALS AND METHODS

Toxin

A standardized commercial preparation of *C. perfringens* alpha-toxin was purchased from Wellcome Reagents, Limited, Beckenham, England. The potency of the preparation of Hemolytic (HU) and Lecithovitellin (LV) units was supplied by the manufacturer.

Enzyme assays

For measurements of hemolytic activity, the Hemolysin Indicator plate test of Duncan and Harmon (*5*) was used with modifications suggested by Park and Mikolajcik (*14*). The hemolytic activity in HU units/ml was read from a standard curve prepared with the Wellcome alpha-toxin preparation.

The *Lecithovitellin Agar* test was used to measure phospholipolytic activity (*5*). The activity in LV units/ml was read from a standard curve prepared with the Wellcome alpha-toxin preparation.

Phospholipolytic activity was also measured by a modified titrimetric method according to the method of Zwaal et al. (*17*). One unit of Phospholipase C was defined as the amount of enzyme which liberated one micromole of titratable H^+ per min.

Gel filtration

Sephadex G-100 was used in a 75 × 1.8-cm column. The elution buffer was 0.05 M Tris-HCl pH 7.6 containing 5.0 mM $CaCl_2$ and 0.1 M KCl. The flow rate was 7.2 ml/h. Numerous 4-ml fractions were

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[1]*Approved as Journal Series Article 82-77, Ohio Agricultural Research and Development Center, Wooster.*

collected. All fractions were checked for hemolytic and phospholipolytic activities and absorbance was read at 280 nm using a UV monitor. Fractions containing the hemolytic and phospholipolytic activities were combined, dialyzed 24-h against distilled water, and concentrated against polyethylene glycol (PEG 20). Dialysis tubing with a molecular weight cutoff of 12,000 was used. The concentrate was checked for purity by double immunodiffusion (*3*), immunoelectrophoresis (*1*), and SDS polyacrylamide disc gel electrophoresis (*11*). Immunoelectrophoresis was done for 1.5 h in 0.1 M barbitone acetate buffer, pH 8.6. Oxoid ID agar tablets (Colab, Glenwood, Il) were used for preparation of the gel.

Polyacrylamide disc gel electrophoresis was done in an alkaline system using Tris-glycine buffer with 0.1% SDS, pH 8.3, and a gel concentration of 10%.

RESULTS AND DISCUSSION

Polyacrylamide disc gel electrophoresis of the Wellcome alpha-toxin preparation revealed the presence of at least seven distinct protein bands (Fig. 1 and 2).

A duplicate unstained gel was placed next to a stained gel to locate areas of interest. The unstained gel was cut into five fractions based upon relative mobility (R_m). The R_m range of the areas cut is shown in Table 1. Each fraction was mascerated in distilled water. One half of the suspension was dialyzed against distilled water at 7 C overnight. The remaining half was not dialyzed. Both the dialyzed and undialyzed fractions were then characterized for protein content by the modified Lowry method (*8*), phospholipase C activity by the titrimetric procedure, and hemolytic activity on HI plates.

Results are shown in Table 1. The undialyzed fractions produced greater hemolytic zones than the dialyzed fractions probably due to the presence of 1% SDS in the running buffer which exhibited a light hemolytic activity (Fig. 3). Therefore, quantification of hemolytic activity was based on the dialyzed fractions. Fraction 5 with a R_m of 0.55-1.0 contained the highest amount of protein (Table 1). However, Fraction 4 with a R_m of 0.29-0.56 contained 95.6% of the total hemolytic activity and was the only one to exhibit phospholipase C activity.

The Wellcome preparation of alpha-toxin was also purified by Sephadex Gel Chromatography (Fig. 4). Hemolytic and phospholipolytic activities were located in tubes 16 through 22. The contents of these tubes were combined and concentrated. The purity of this fractions was checked by immuno-diffusion, immunoelectrophoresis, and SDS disc gel electrophoresis. Two bands were present but only the band with a Rm of 0.64 possessed both hemolytic and phospholipolytic activities and the other band was inactive. (Fig. 5).

For further purification, this fraction was again subjected to gel filtration with a flow rate of 4 ml/h. This time 2-ml fractions were collected. Two peaks were observed: one at tubes 18-24 and the other at tubes 30-42. Hemolytic and phospholipolytic activities were present in the latter tubes (Fig. 6). The contents of the active tubes were combined, dialyzed overnight against distilled water, and concentrated against PEG 20 to a total volume of 2 ml. SDS-polyacrylamide disc gel electrophoresis revealed the presence of one band which possessed both hemolytic and phospholipolytic activities

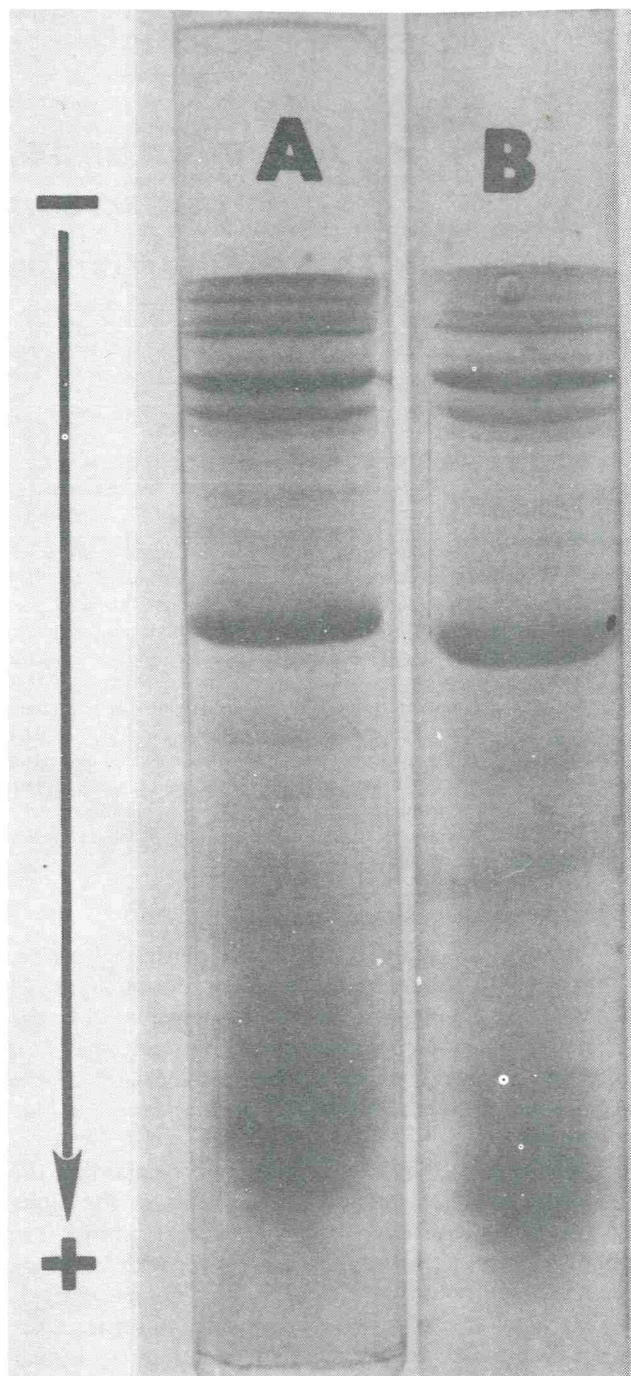

Figure 1. *Polyacrylamide disc gel electrophoretic patterns (duplicate samples) of* C. perfringens *alpha toxin.*

(Fig. 5).

The findings support the premise that hemolytic and phospholipolytic activities of *C. perfringens* alpha toxin are associated with the same protein.

In man and most animals, cell membranes consist of lipoprotein complexes containing lecithin (*16*). It appears that the mechanism of action of phospholipase C as a hemolysin involves degradation of the phospholipids, resulting in a change in the permeability of the membrane and leakage of hemoglobin.

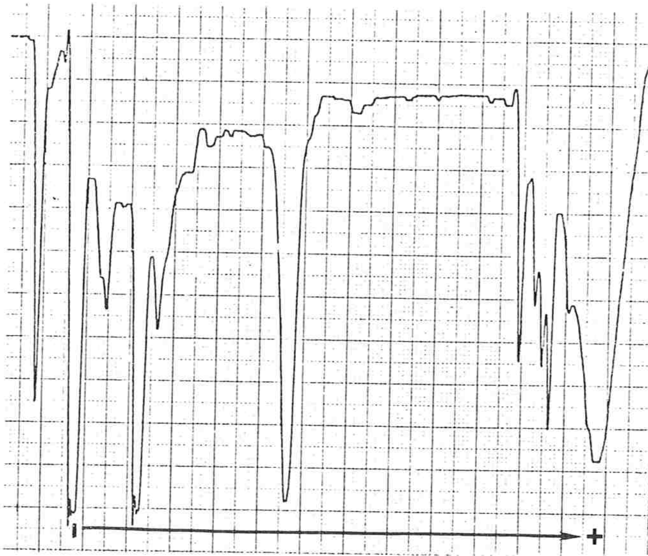

Figure 2. *Densitometer scan of commercial alpha toxin following polyacrylamide disc electrophoresis.*

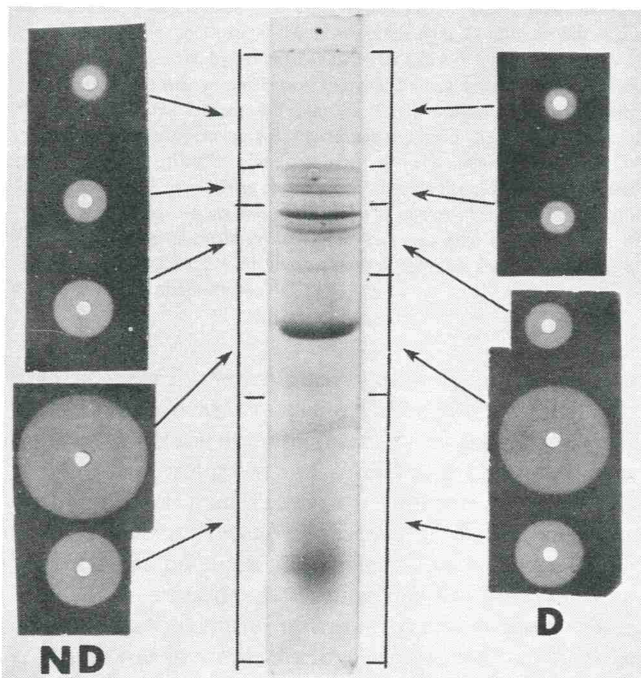

Figure 3. *Hemolytic zones of non-dialyzed (ND) and dialyzed (D) fractions of alpha toxin obtained upon polyacrylamide gel electrophoresis.*

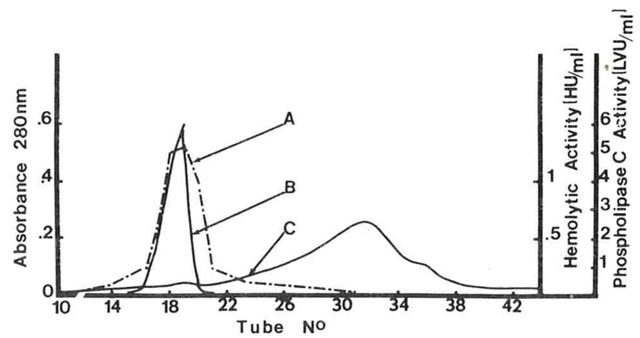

Figure 4. *Separation of alpha toxin on a Sephadex G-100 column. Four-ml fractions were collected. A, hemolytic activity; B, phospholipase C activity; and C, absorbance at 280 nm.*

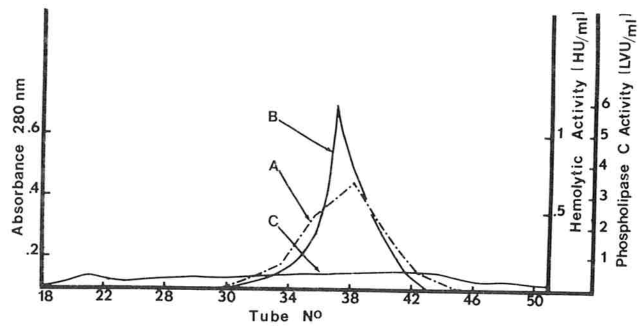

Figure 5. *Disc electrophoreis histograms of commercial alpha toxin purified by Sephadex G-100 column chromatography (left) and upon rechromatography (right) of the partially purified fraction.*

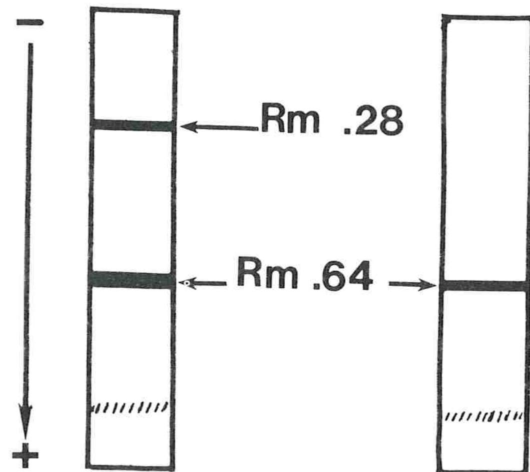

Figure 6. *Separation of partially purified alpha toxin on a Sephadex G-100 column. Two-ml fractions were collected. A, hemolytic activity; B, phospholipase C activity; and C, absorbance at 280 nm.*

TABLE 1. *Selected characteristics of fractions isolated by polyacrylamide gel electrophoresis of alpha toxin.*

Fraction No.	Relative mobility	Area under densitogram	Total protein content	Hemolytic activity	Specific hemolytic activity	Total activity
	(R_m)	(mm^2)	(μg)	(unit)	(unit/mg protein)	(%)
1[a]		5.0	24	0.0008	0.033	0.16
2	0-0.10	8.5	32	0.0100	0.312	1.53
3	0.10-0.29	18.5	116	0.0448	0.386	1.89
4	0.29-0.56	20.6	172	3.3560	19.512	95.62
5	0.56-1.00	40.0	342	0.0556	0.163	0.80

[a]Stacking gel.

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Survival of Enteropathogenic and Non-Pathogenic *Escherichia coli* During the Manufacture of Camembert Cheese

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ABSTRACT

Camembert cheese was manufactured from milk contaminated with pathogenic and nonpathogenic strains of *Escherichia coli. E. coli* was enumerated using Violet Red Bile Agar (VRB) pour plates, a most probable number method, and surface plating on Trypticase Soy Agar (TSA) plates followed by an overlay of VRB (TSA + VRB). Numbers of *E. coli* during cheese manufacture increased about two log cycles during the first 6 h, then decreased during the initial stages of ripening with some strains disappearing from the cheese within the first 2 weeks of ripening, and other strains surviving 4 to 6 weeks. The rate of inactivation of *E. coli* in the cheese decreased as the cheese ripened and the pH increased. No growth of *E. coli* was observed in ripe cheese at a pH of 6.7, however rapid growth of *E. coli* occurred on the surface of the cheese. The TSA + VRB method was acceptable for enumeration of *E. coli* in Camembert cheese.

Presence of *Escherichia coli* in dairy products has been used as an indicator of post-pasteurization contamination (*15*). Cheese manufacturers have for many years sought to control growth of *E. coli* and other coliforms during cheese manufacture to prevent gassy defects (*7,34*). However, recently presence of *E. coli* in dairy products has taken on added significance since a strain (0124:B17) caused gastroenteritis in persons who consumed a certain lot of imported Camembert cheese (*21*). This was probably the first documented outbreak of *E. coli*-caused foodborne illness in the U.S. Outbreaks in other countries have also occurred (*2,11*).

The pathogenicity of certain strains of *E. coli* has been well documented (*3,25*). These strains have been divided into two groups, those producing a shigella-like (invasive) and those producing a cholera-like (toxigenic) illness (*25,29*). There appears to be some relationship between pathogencity of an *E. coli* strain and its serotype (*3*).

Several investigators have surveyed raw foods (*33*) and dairy products (*17,23,26,30*) for serotypes of *E. coli* associated with enteropathogenicity. It appears that wherever fecal coliforms occur there is also a chance that enteropathogenic *E. coli* (EEC) will be found, although the frequency of EEC occurring in foods contaminated with *E. coli* may be highly variable. Methods used in these surveys would not have isolated slow lactose fermenting EEC such as the strain isolated in the Camembert cheese outbreak (*8,22*). Hall and Hauser (*14*) found that 6.4% of food handlers studied, many of them in good health, were carriers of EEC, thus demonstrating that a source of potential contamination exists.

Since the presence of EEC in dairy products and in particular in Camembert cheese is of public health significance, this study was undertaken as a continuation of the earlier study by Park et al. (*27*) to assess the fate of *E. coli* during manufacture and ripening of Camembert cheese, and to evaluate use of an enumeration method designed to recover injured cells from food products.

MATERIALS AND METHODS

Cultures

Sources of cultures used in this study were given by Frank and Marth (*9*). The *E. coli* cultures were grown in nutrient broth at 37 C for 24 h before use as an inoculum. Enough culture was added to coliform-free pasteurized milk to provide about 100 viable cells/ml. The starter culture used was a commercial mixed strain lactic culture. It was incubated in sterile skimmilk at 21 C for 20 to 22 h and used immediately for cheese manufacture. A 2.0% starter inoculum was used in milk for cheese making. The *Penicillium camemberti* culture (from K. B. Raper, Department of Bacteriology, University of Wisconsin, Madison, Wisconsin) was grown on Czapek Agar slants at 21 C for 7 to 9 days. The mold from one agar slant was blended with 50 ml of sterile water and this mixture was added to milk at the time of cheese manufacture.

Manufacture of cheese

Camembert cheese was manufactured from 18.1-kg lots of coliform-free pasteurized milk according to the procedure used by Park et al. (*27*). Milk was adjusted to 33 C; clotted milk was cut 50 min after addition of rennet, starter, mold, and *E. coli*; curd was dipped onto hoops and allowed to drain at room temperature overnight; and the 250-g (approximate) wheels of cheese were dry-salted the next day. Cheese was ripened for 1 week at 15.5 C in an incubator with high humidity to allow mold growth, then was wrapped in foil and stored at 10 C for up to 7 more weeks. The procedure just described is referred to as the standard manufacturing method. Samples analyzed for *E. coli* at time of inoculation and at intervals of 1.5, 6, 24, and 48 h, and 1, 2, 3, 5, and 7 weeks.

An alternate method of manufacture was also used. This involved inoculating pasteurized milk at 30 C with 2.0% starter, mold, and *E.*

coli; allowing 1.5 h of incubation; adding rennet; cutting 50 min later; draining hoops overnight at room temperature; brine-salting for 1 h; and allowing cheese to dry at 17 C for 1 day. Cheese was ripened at 12 C for 9 days, wrapped in foil, held at 12 C for 4 additional days, and then stored at 7 C for 5 weeks. This method is similar to that used by a manufacturing plant in France. All cheese manufacturing trials were done in duplicate and results are reported as average values. All cheese made complied with the Federal standard of 50% milkfat in dry matter. Cheese having slow acid production was made from fresh pasteurized milk, using 0.25% starter and the standard manufacturing method.

Surface inoculations were made when a mat of mycelia covered the cheese (5 days at 15.5 C). The surface on one side of a cheese wheel was marked off into areas of 20 cm^2 and each area was inoculated with 0.1 ml of a suspension of *E. coli* (approximately 500 cells). The inoculum was spread with a glass rod. No liquid remained on the surface of the cheese. At the time of analysis each section was cut deep enough to give 20 g of cheese which was blended with 180 ml of sterile 2.0% sodium citrate solution. Appropriate serial dilutions were made. Two samples were analyzed at each time interval and average values are reported. *E. coli* was enumerated using the surface plating technique of Speck et al. (*31*).

Enumeration of E. coli

Three methods were used to enumerate *E. coli*. The first was a 3-tube Most Probable Number (MPN) method using incubation in nutrient broth for 24 h at 37 C followed by streaking of positive tubes on Eosin Methylene Blue Agar (Difco) to test for typical growth. Only tubes giving typical colonies as compared to a control were counted as positive. The second method employed Violet Red Bile Agar (VRB, Difco) with incubation at 37 C for 24 h. The third method was suggested by Speck et al. (*31*) for enumeration of sublethally injured coliforms. It involves surface plating on Trypticase Soy Agar (TSA, Difco) and a 1-h incubation at room temperature followed by adding an overlay of VRB agar. Incubation was then at 37 C for 24 h. This method is referred to as the TSA + VRB surface plating method.

Cheese samples were diluted by adding 20 g of cheese to 180 ml of sterile 2.0% sodium citrate solution and blending at low speed for 3 min. Serial dilutions were made using 9 ml of sterile sodium citrate solution. Cheese was sampled by taking a pie-shaped cross-section of the wheel. Surface samples were taken by cutting 6.5 mm into the wheel, and center samples were taken from the unripened core of the wheel.

Survival of E. coli at various pH values

Survival curves for *E. coli* in Camembert cheese at a pH of 4.6 were obtained by thoroughly mixing *E. coli* with 1-day old cheese and following the decline in numbers over a period of days at 15.5 C. Survival of *E. coli* at a pH of 5.4 was determined in a similar manner using cheese ripened for 4 weeks which was inoculated after the rind was removed. Incubation was at 10 C. Camembert ripened for 6 weeks was used to obtain a pH of 6.7 and incubation was at 5 and 10 C. The pH of all samples remained constant during the experiments. The TSA + VRB surface plating method was used to enumerate *E. coli*. These experiments were done in duplicate and results are reported as averages.

Measurment of pH, moisture, salt and fat

The pH of cheese, depending on degree of ripeness, was measured with either a Corning Model 10 pH meter having a miniature combination glass electrode or with a saturated calomel half-cell, gold electrode, and a Leeds and Northrup portable potentiometer. For moisture analysis cheese was dried for 16 h in a forced-draft air oven at 110 C. The Volhard method (*16*) was used to determine NaCl in the cheese. The percentage of milkfat was determined by the Babcock method. Chemical analyses were done in triplicate.

Serological typing

Serological typing of EEC isolates was done to confirm the absence of "wild" coliforms in the cheese and to help judge the effectiveness of our enumeration methods. A total of 120 isolates were serotyped by the FDA in Washington, D.C. These isolates were taken from four lots of 2-day old cheese each contaminated with a different EEC strain. A minimum of 10 isolates was obtained from each enumeration method for each strain of EEC.

RESULTS

Composition of cheese

All cheese was compositionally acceptable (50-57% moisture, 1.6-2.0% salt, and 52-55% milkfat in dry matter) and ripened in a manner normal for Camembert cheese. Changes in pH of cheese made by the standard method are given in Fig. 1. Since Camembert ripens from the outside inward, values for ripening cheese shown in the figure were obtained from a cross section of the cheese wheel and indicate an average pH value.

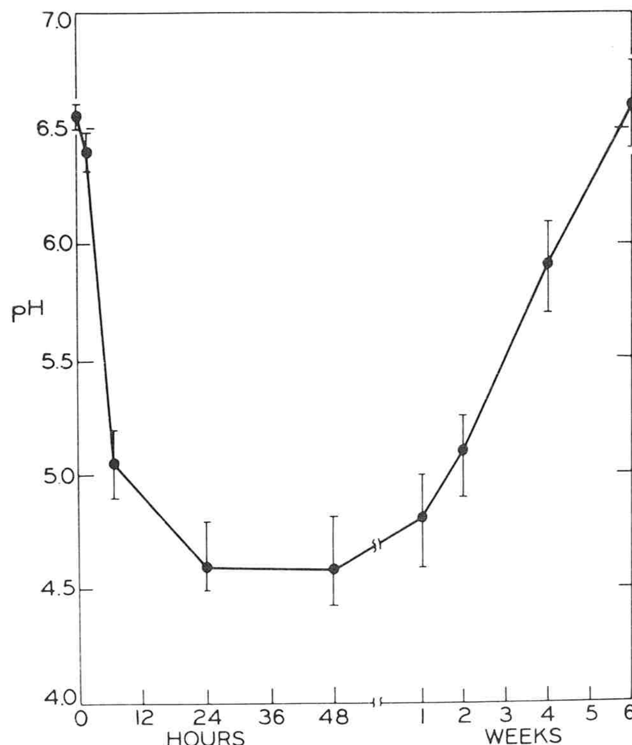

Figure 1 *Changes in pH occurring during the manufacture and ripening of Camembert cheese.*

Survival of E. coli during manufacture and ripening of the cheese

Figures 2 through 5 show growth and survival for seven strains of *E. coli* during manufacture and ripening of cheese. These figures also compare results obtained with three enumeration methods. Each strain of *E. coli* increased in numbers by about two log cycles during the initial stages of manufacture. Some of this increase resulted from concentration by entrapment of cells in curd. When largest numbers occurred, the pH of cheese was between 4.9 and 5.2 and decreasing rapidly. After overnight draining, the pH of cheese was close to its lowest point and the number of *E. coli* had decreased by about one log cycle, except for strains 4608 and 1624 which showed almost no change. After salting of cheese and incubation at 15.5 C for 1 week, a further decrease

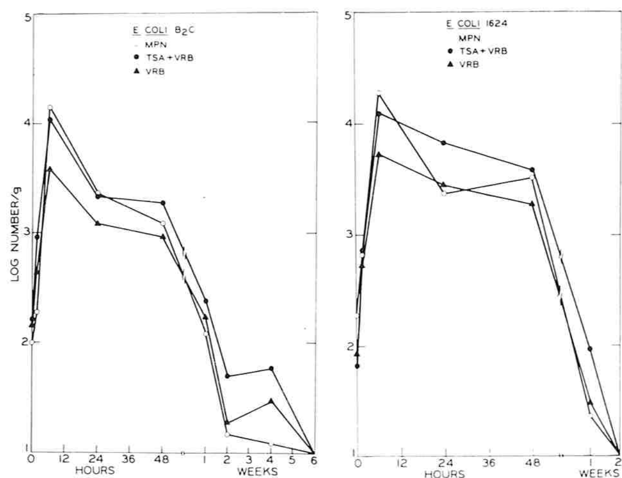

Figure 2. *Changes in numbers of enteropathogenic* E. coli *B2C and 1624 during manufacture and ripening of Camembert cheese.*

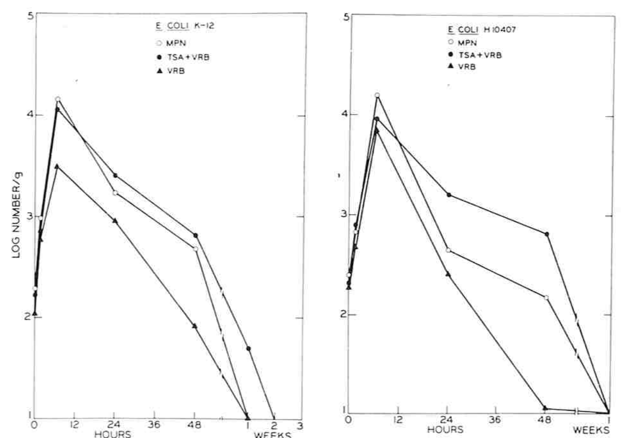

Figure 3. *Changes in numbers of* E. coli *K-12 and enteropathogenic* E. coli *H10407 during manufacture and ripening of Camembert cheese.*

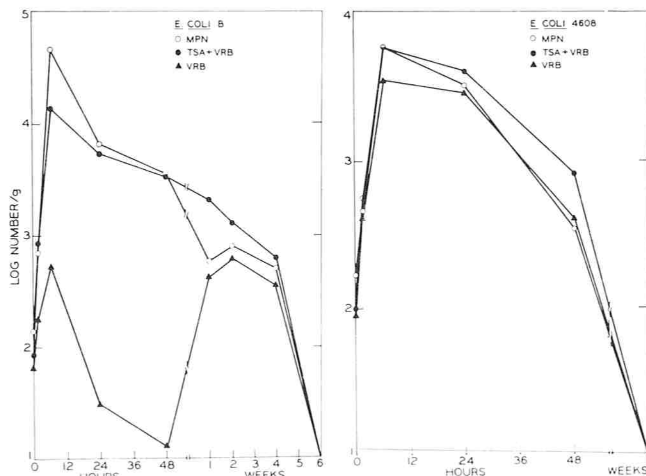

Figure 4. *Changes in numbers of* E. coli *B and enteropathogenic* E. coli *4608 during manufacture and ripening of Camembert cheese.*

occurred in numbers of all strains of *E. coli*, the rate of decrease being highly variable. Three pathogenic strains (1624, 4608, and H10407) and one nonpathogenic strain (K-12) did not survive in numbers greater than 10/g past

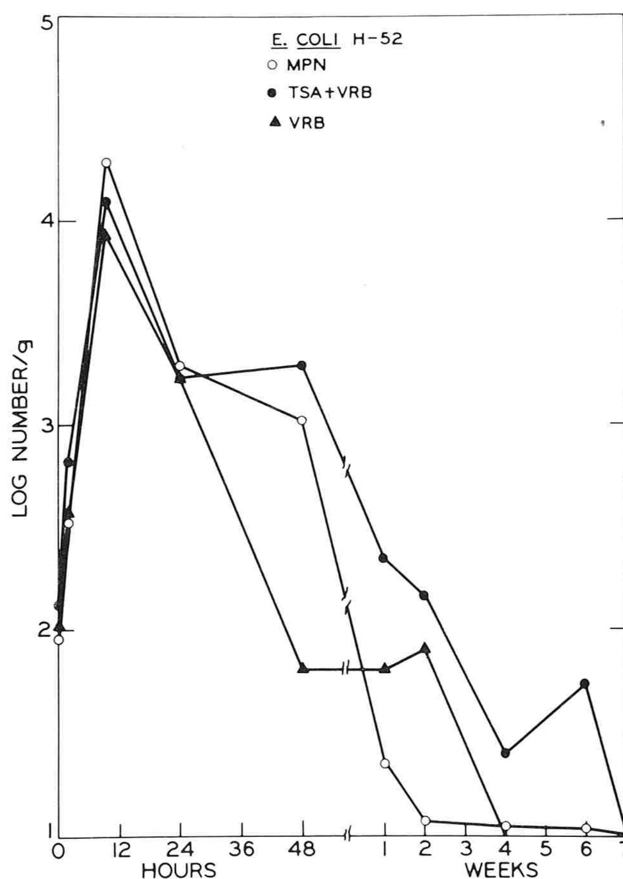

Figure 5 *Changes in numbers of* E. coli *H-52 during manufacture and ripening of Camembert cheese.*

1 to 2 weeks after the date of cheese manufacture. At this point the cheese was not mature enough for consumption. One pathogenic strain (B2C) and two nonpathogenic strains (H-52 and B) survived in numbers ranging from 10 to 1000/g past 4 weeks, and thus could be present in the cheese at the time of consumption. Substantial increases in numbers did not occur in the ripened cheese.

The effect of inoculum size on survival of EEC B2C is shown by data in Fig. 6. With inocula of 10^2, 10^3, and 10^4/ml, growth and survival curves were nearly parallel. As would be expected, the amount of contamination is an important factor in determining the length of survival and numbers of *E. coli* in the final product.

The effect of slow acid production by the starter culture on growth and survival of EEC 1624 and B2C is shown by data in Fig. 7. Use of 0.25% instead of 2.0% starter affected the pH of the cheese only in the initial hours of manufacture, it being lowered only to 6.4 instead of 5.0 after 6 h (Fig. 1 and 7). The cheese had a normal pH thereafter. The slower acid production allowed about 10 times more EEC 1624 to develop in the cheese than when acid production was normal. This strain was inactivated as rapidly as it was when acid production was normal (Fig. 2). Strain B2C increased in numbers by about four log cycles when acid production was slow, as compared to two log cycles during normal manufacture (Fig. 2).

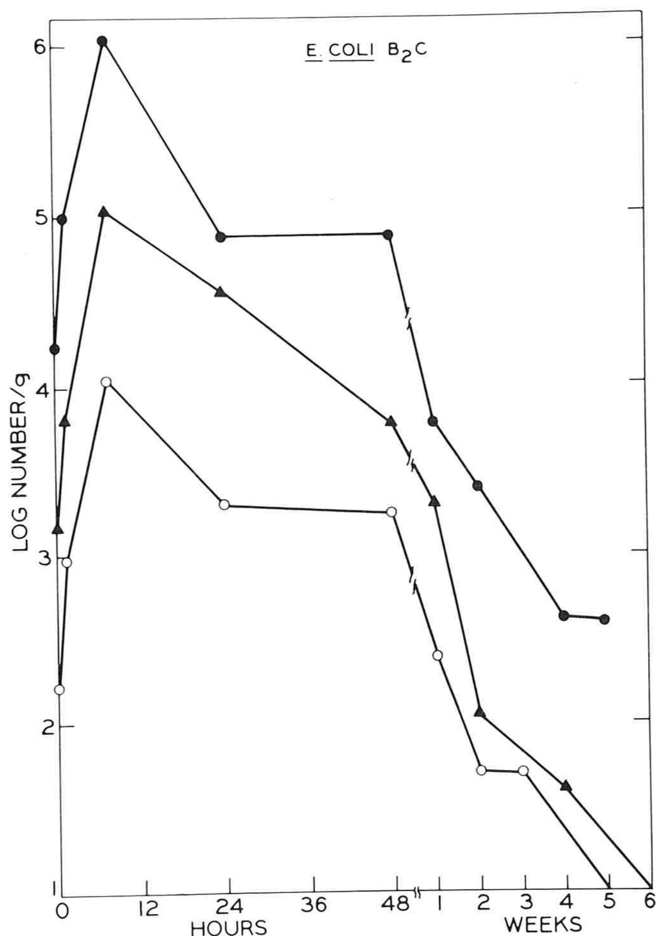

Figure 6. *Effect of three different amounts of initial contamination on numbers of enteropathogenic* E. coli *B2C during manufacture and ripening of Cemembert cheese. Enumeration was with the TSA + VRB surface plating method.*

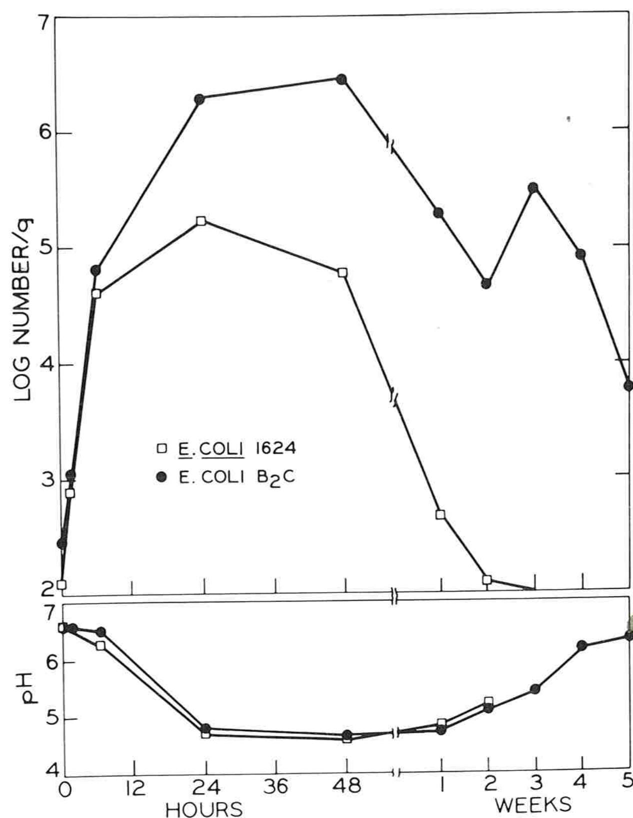

Figure 7. *Changes in numbers of enteropathogenic* E. coli *and pH of the cheese during manufacture and ripening of Camembert cheese made with 0.25% lactic starter.*

During ripening, numbers of strain B2C decreased less when less starter was used than when acid production was normal. After 4 weeks of ripening, 10^5 cells/g remained in the low starter cheese compared to less than 100/g in the normal cheese.

Comparison of enumeration methods for E. coli

Figures 2 through 5 provide a comparison of three enumeration methods for seven strains of *E. coli.* The TSA + VRB surface plating method gave results comparable to those from the 3-tube MPN method in practically all instances. With two nonpathogenic strains (K-12 and H-52), the surface plating technique was more sensitive than the MPN method when low numbers were encountered. With three pathogenic strains (B2C, 1624, 4608), the VRB pour plate method produced results similar to those obtained with the other two methods. In contrast, strains K-12, H-52, and EEC H10407 were not recovered as well with VRB pour plates as with the other methods, especially after cells were exposed to acid conditions. *E. coli* B seemed especially sensitive to VRB during the first 2 days of cheese manufacture. Variability in testing samples from replicate vats of cheese also differed with enumeration method. The VRB pour plate method showed average variability of 46%, the MPN method had 50%, and the surface plating method had 40%. The enteropathogenic isolates recovered by these three methods were serotyped and 95 to 100% were found to match the inoculated strains. This indicates that only the inoculated *E. coli* strains were enumerated with each method and that the cheeses had not become contaminated with other coliforms.

Comparison of manufacturing procedures

Data presented in Fig. 8 deal with behavior of *E. coli* B2C and H-52 during the two manufacturing procedures having normal acid development. Slightly larger numbers were observed with the alternate rather than the normal manufacturing procedure during the initial stages of manufacture, possibly because milk was incubated 1.5 h before addition of rennet. Also, *E. coli* was inactivated sooner in the ripened cheese made with the alternate method, possibly because of the lower ripening and storage temperatures. Although pH changes were similar in cheese made with the two methods, the alternate method produced cheese which ripened more evenly and softened more slowly.

Survival of E. coli in different areas of the cheese wheel

Table 1 gives representative data on number of *E. coli* at the core and on and close to the surface of the wheel of cheese made from milk containing the coliform. Salting of cheese was associated with a reduction in numbers of

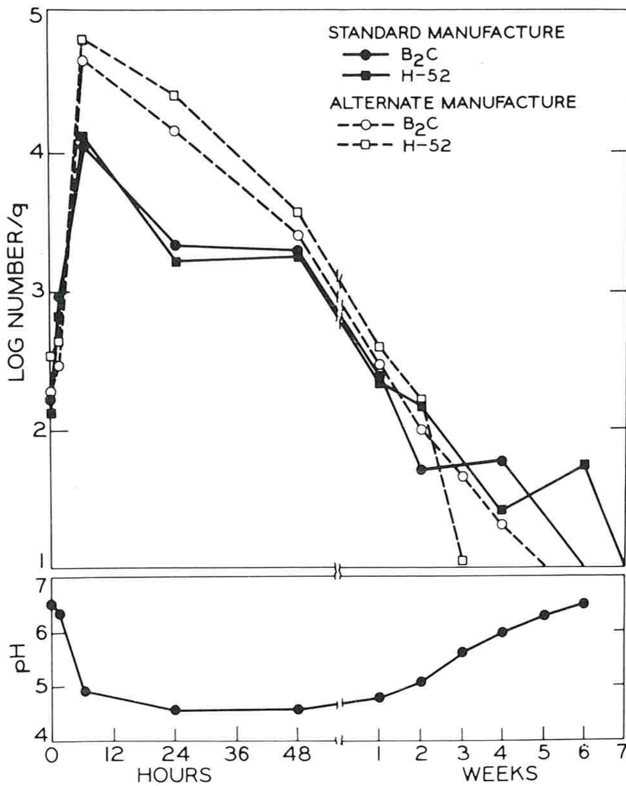

Figure 8. *Changes in numbers of* E. coli *H-52 and enteropathogenic* E. coli *B2C in Camembert cheese when made by two procedures. Enumeration was with the TSA + VRB surface plating method. Changes in pH of cheese manufactured with the alternate procedure are shown.*

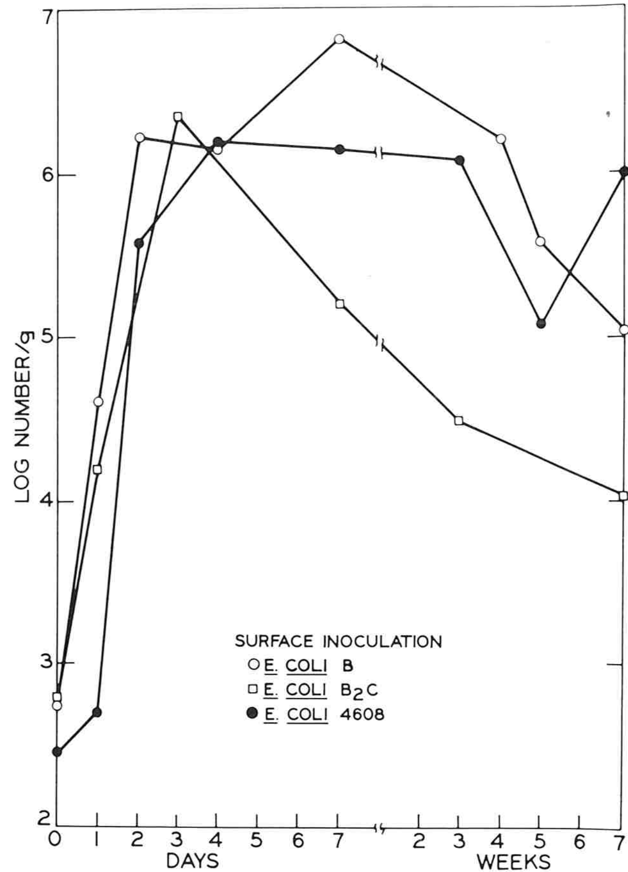

Figure 9. *Growth and survival of enteropathogenic* E. coli *on the surface of Camembert cheese stored for 2 days at 15.5 C and then at 10 C. Enumeration was with the TSA + VRB surface plating method.*

E. coli near the surface. Even though pH near the surface of the cheese rose quickly in the first weeks of ripening, *E. coli* near the surface did not survive as long as near the center of the cheese. However, when *E. coli* was inoculated onto the newly developed mycelial mat, rapid growth occurred, with increases of over three log cycles and subsequent survival of large numbers (Fig. 9). This demonstrates a major difference between behavior of *E. coli* near the surface and on the surface of ripened cheese.

Survival of E. coli in cheese at specific pH values

Because of the heterogenous nature of ripening Camembert cheese, an attempt was made to measure survival of EEC in a more homogeneous environment. During the first week of ripening at 15.5 C most of the cheese wheel is at a pH of 4.6 to 4.7. Survival of EEC in unripened cheese (inoculation after manufacture) at pH 4.6 is shown in Fig. 10. Strains 4608, B2C, and 1624 had approximate "D" values of 1 to 2 days. Erratic results were obtained with strain H10407. EEC inoculated into cheese at a pH of 5.4 had approximate "D" values of 8 to 10 days for strains H10407, 4608, and 1624, and over 14 days for strain B2C (Fig. 11). This longer survival for strain B2C coincides with its longer survival in ripening cheese (Fig. 2) and fermented milk (*9*). Data showing survival of EEC in cheese at pH 6.7 held at 5 and 10 C are in Table 2. Although there may be lengthy survival under these conditions, there does not appear to be growth. Variability of these data (Fig. 10,11, and Table 2) was less than 20%.

TABLE 1. *Populations of* E. coli *at the surface and in the core of Camembert cheese during its ripening.*

		Age of cheese							
		48 h		1 wk		2 wk		4 wk	
Strain	Sample position	No. x 10^2	pH	No. x 10^2	pH	No. x 10^2	pH	No. x 10^2	pH
B	Surface	5.6	4.6	0.5	5.6	< 0.1	6.1	—[1]	6.6
	Core	37	4.5	14	4.7	17	4.7	4.7	5.1
H-52	Surface	6.5	4.6	1.1	5.4	—	6.3	—	6.9
	Core	20	4.5	2.0	4.7	< 0.1	5.0	0.2	5.1
1624	Surface	19	4.7	—	4.9	—	5.7	—	6.1
	Core	31	4.5	1.1	4.6	—	4.8	—	5.6

[1]None detected

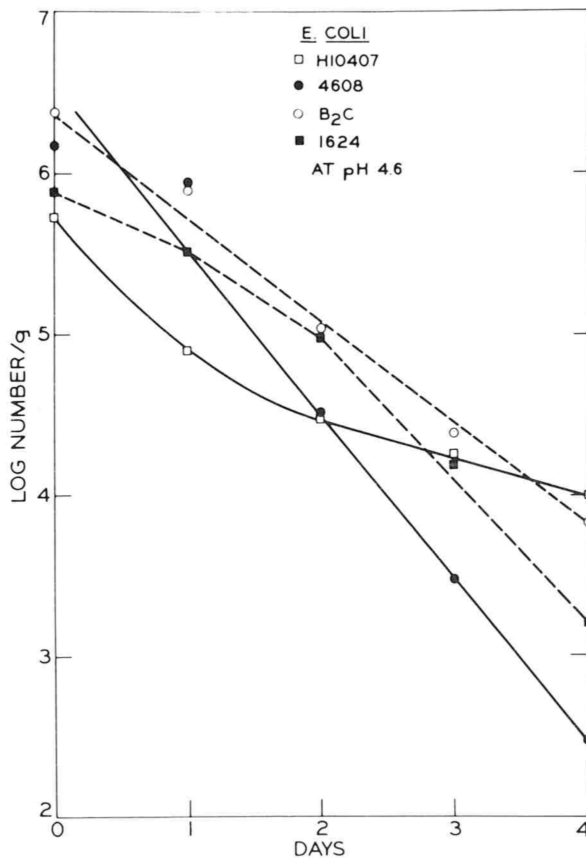

Figure 10. *Survival of enteropathogenic* E. coli *in Camembert cheese at a pH of 4.6.*

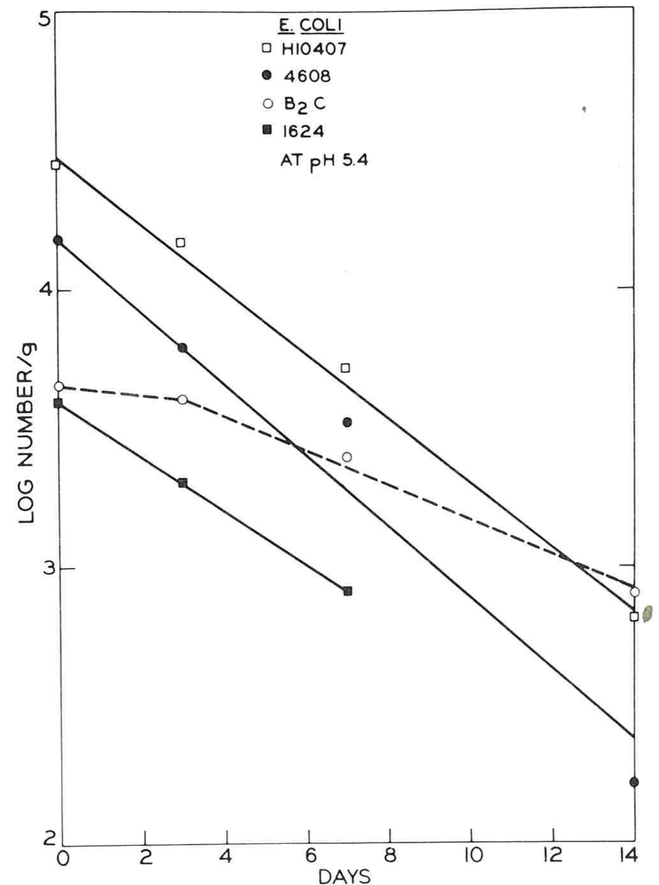

Figure 11. *Survival of entropathogenic* E. coli *in Camembert cheese at a pH of 5.4*

DISCUSSION

Enumeration of E. coli in cheese

Enumeration of coliforms in dairy products using the VRB pour plate method is recommended in *Standard Methods* (*15*). Although this method is useful for most dairy products, previous results have shown low recovery from fermented milks contaminated with certain strains of *E. coli* (*10*). Our data support the theory that some strains of *E. coli* may be stressed in the acidic environment of a fermented dairy product causing poor recovery on selective media. A comparison of *E. coli* counts in the cheese at 48 h shows the effect of acid exposure. Strains K-12, B, H-52, and H10407 all showed some sublethal injury. Susceptibility of *E. coli* to injury by an acidic environment has been demonstrated by Roth and Keenan (*28*). When compared to most other varieties of cheese, unripened Camembert has a low pH so injury is likely in this cheese but may not occur in others.

Advantages and disadvantages of MPN procedures are well-known (*4,6,*). Our data show that the TSA + VRB surface plating method has the sensitivity of the MPN method. With the inherently greater precision of a plate count method, the surface plating method may provide more useful results than does the MPN method. Even though selective agents in VRB agar are diluted in the overlay methd, selectivity of this medium was sufficient in these experiments. To more accurately assess occurrence of acid-stressed coliforms in dairy products, studies with naturally contaminated products must be done.

Behavior of E. coli during cheese manufacture

The general pattern of the growth and survival curves for *E. coli* during manufacture of Camembert cheese are

TABLE 2. *Survival of enteropathogenic* E. coli *at pH 6.7 in ripened Camembert cheese.*

Strain of *E. coli*	Temp. (C)	Time (days) 0	1	2	4	7	12
		(no/g × 10^2)					
H10407	5	30[1]	21	10	5.5	< 1.0	< 1.0
	10	51	13	7.5	7.0	5.0	< 1.0
4608	5	22	14	8.5	6.0	4.5	< 1.0
	10	37	16	20	22	16	< 5.0
B2C	5	24	16	17	15	11	< 5.0
	10	21	15	17	19	17	< 5.0

[1]TSA + VRB enumeration method was used for all counts.

similar to those reported by Park et al. (*27*) who used different strains of EEC. The amount of growth observed also coincides with growth of these strains in cultured milk (*9*) when one substracts the nearly 10-fold increase in numbers caused by concentration in converting milk to cheese. Death of *E. coli* also occurs at similar pH values (4.6-5.0) in cheese and the fermented milks (*9*). The large numbers and long survival observed when the amount of starter was reduced (Fig. 7) are similar to results obtained by Park et al. (*27*) when they added penicillin to milk to inhibit acid production. Results of Nevot et al. (*24*) also emphasize the importance of acidification during the manufacture of Camembert cheese to achieve destruction of pathogens.

Behavior of pathogenic E. coli in ripening cheese

Knowledge of the physical, chemical, and microbiological aspect of the ripening process in Camembert cheese is helpful in understanding the behavior of *E. coli* in this product. Four structural zones can be distinguished in ripening Camembert. These include the surface, rind, ripe zone, and unripened core (*18*). Surface and rind characteristics are a result of rapid growth of yeasts and micrococci after salting (*20*) followed by growth of *P. camemberti*. The ripe zone results from the proteolytic action of enzymes diffusing from the mold on the surface into the cheese (*18*). After hydrolysis of casein into water-soluble nitrogenous compounds, proteolysis continues through action of starter streptococci, *P. camemberti*, and possibly *Lactobacillus casei* causing formation of low molecular weight nitrogenous compounds including amines and ammonia (*5,14,32*). During this ripening process, milkfat remains structurally unchanged (*18*) and lactic acid is degraded (*1*). As ripening proceeds, the unripened core becomes small and the amount of ammonia in the ripe zone and the rind increases (*19*). This results in the increase in pH shown in Fig. 1 and Table 1.

Our results show the effect of the ripening process on behavior of *E. coli* in cheese. The unripened core is bactericidal probably because of its low pH (Fig. 10). The ripe zone is initially bactericidal (Fig. 11) and then bacteriostatic for *E. coli* (Table 2). Inability of *E. coli* to grow in ripened Camembert cheese was also observed by Park et al. (*27*). The surface of the cheese will support rapid growth (Fig. 9). Thus, following the layers of cheese from the center outward, conditions become more favorable for survival and finally growth of *E. coli*. Fantasia et al. (*8*) observed growth of EEC in naturally contaminated Camembert cheese stored at 4 C and room temperature. It was not determined if growth occurred throughout the cheese or only on the surface. It would be possible for *E. coli* surviving in the ripe zone of the cheese to contaminate the surface during packaging and handling, and then to initiate growth. The bacteriostatic effect of ripened Camembert cheese on *E. coli* might be related to the large number of lactic bacteria present (from 10^8 to 10^9/g) (*20*). Lactic streptococci have been observed to partially inhibit growth of staphylococci and salmonellae in milk maintained at a pH of 6.6 (*12*). Production of antibiotics by the internal microflora of the cheese is possible, but Grecz (*13*) did not find antibiotic activity in Camembert cheese. Ammonia in ripened cheese may be partially responsible for inhibition since it constitutes 4.9 to 7.9% of the total nitrogen in the ripened cheese (*19*).

Effective measures that Camembert cheese manufacturers can take to insure the safety of their product include use of an adequate amount of active starter, draining the curd below room temperature to slow growth of *E. coli* while allowing adequate acid production (*9*), and use of stringent sanitation measures during manufacture, especially during ripening and packaging of the cheese. Presence of even small numbers of *E. coli* in ripened Camembert cheese presents a potential health hazard because of their ability to grow when present on the surface and past history of food poisoning associated with this product.

ACKNOWLEDGMENTS

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Evaluation of Rapid Tests for Monitoring Alterations in Meat Quality During Storage

I. Intact Meat

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ABSTRACT

Seven rapid analytical tests (color value, thiobarbituric acid number, extract release volume, pH, "tyrosine" value, pH_t, and redox potential) were evaluated as possible indicators of bacterial contamination in intact meat. Color value is a reflectance value related to hedonic acceptance of the meat. Comparisons of results from these seven tests with determination of bacterial load (plate count) and with time of storage were analyzed statistically to determine the relative contributions of bacterial action and of intrinsic reactions. The color values and "tyrosine" values were the most effect monitors of bacterial contamination. Although the thiobarbituric acid test effectively monitored changes in meat quality, it was not specific for those produced by bacteria. The remaining tests were ineffective under conditions employed.

Proteolysis and lipid oxidation occur in fresh meat during storage. Detectable unfavorable organoleptic changes appear when the number of bacteria exceed $10^6/cm^2$ for intact meat (*8*). Bacteria count is generally a reliable criterion of spoilage. The determination of bacterial levels, however, requires a minimum of 48 h of incubation for an accurate count, while physical and chemical changes caused by high bacterial populations and storage conditions can be measured more rapidly and could frequently be a convenient alternative.

The tests selected for evaluation had to meet the following criteria: they must be rapid with results available within 1 h, they must be accurate with a small (< 50 g) sample of meat, and they must be easy to do with commonly available lab equipment. The seven analytical tests chosen, color value, thiobarbituric acid number, extract release volume, pH, "tyrosine" value, pH_t, and redox potential meet these criteria.

Surface color alteration is the most obvious change during storage. Strange et al. (*11*), using a reflectance spectrophotometric method to follow surface color changes, found a high correlation of reflectance data with consumer acceptability.

Meat pH changes with the increasing bacterial population. Shelef and Jay (*10*) reported that the pH of beef rose with increasing bacterial growth, and described a rapid method to detect spoilage involving measurement of pH following addition of a standard volume of HCl to a filtered homogenate of the meat.

Pearson (*6*) reported that EMF (electromotive force) of spoiled meat may fall below –250 mv. Initially, EMF of fresh high quality meat is high; the effect of this high EMF on microbial growth is to prolong the initial lag phase in the growth curve (*4*).

Another rapid method purported to predict microbial quality of beef is extract release volume (ERV) (*2*). ERV is related to water holding capacity of meat (*3*) which is highly correlated with pH (*12*).

Pearson (*7*) demonstrated that the "tyrosine" value of meat increased with storage time along with total volatile nitrogen until amino acid deamination by the aerobic metabolism of pseudomonads limited formation of free amino acids. He indicated the "tyrosine" value also measured other reductants soluble in trichloroacetic acid such as tryptophan, cysteine, phenolics, sulfhydryls etc.

A deteriorative change not necessarily caused by microbial contamination is lipid oxidation. The thiobarbituric acid (TBA) test measures the carbonyl residues resulting from lipid peroxidation and the method for TBA analysis used was a variation of the procedure devised by Witte et al. (*13*).

The rate of the above changes depends on initial bacterial load, physical and biochemical state, availability of oxygen, temperature of storage, and muscle composition.

We evaluated the selected tests for effectiveness in monitoring microbial quality. Since intrinsic changes occur in meat during storage in addition to changes caused by bacterial action, experiments were designed so that effects of bacterial action alone could be identified.

The various tests have previously been used in studies of storage change in ground meat, not necessarily in

[1]*Agricultural Research Service, U.S. Department of Agriculture.*

relation to bacterial contamination and generally have not been evaluated for use on intact meat. Our studies show that their values on ground and intact meat are strikingly different. The present report concerns applications to intact meat.

EXPERIMENTAL

Materials

Three sides of beef (graded USDA Good) were obtained within 90 min post slaughter. Samples of longissimus dorsi (l.d.) muscle (ca. 100 g) were removed for immediate analysis. The rest of the l.d. was removed and cut into samples the next day. Samples used for color evaluation and bacteria counts were cut across the grain into approximately 50-g portions each with a surface area of approximately 60 cm^2. The remaining l.d. was divided into 35-g samples. Each sample was wrapped in oxygen-permeable meat wrap (PVC stretch film MC-FMC) and randomly assigned to storage at −1 or 7 C.

Methods

All tests were done on samples stored at −1 C and 7 C to obtain insight as to the relative effects of the intrinsic changes in meat and the effects of bacterial growth on meat. An ideal storage temperature for fresh meat is −1 C. At this temperature (−1 C) meat is not frozen but the rate of bacterial growth is greatly retarded. At 7 C, bacterial growth is accelerated. Thus the choice of these temperatures allows differentiation of the effects of intrinsic changes from the effects of bacterial growth.

Bacteria plate counts. Meat samples for bacteria counts were shaken with a sterile 0.1% peptone solution contained in sterile quart Mason jars. Appropriate dilutions were made before spreading on nutrient agar and incubating for 3 days at 20 C. All dilutions were plated in triplicate. The bacteria counts are reported as $\log_{10}$ of the actual count.

Color. The color value, % reflectance at 630 nm minus % reflectance at 580 nm or Δ%R, of the meat was determined according to the method of Strange et al. (*11*) using a Beckman[2] DBG recording spectrophotometer equipped with a diffuse reflectance attachment.

Extract release volume. Fifteen grams of meat were blended with 60 ml of water for 2 min and filtered immediately through a Whatman # 1 filter (15 cm in diameter) folded in the manner described by Jay (*3*). Volume of filtrate, termed ERV, was measured after 15 min of filtration.

pH, pH_t, EMF. The pH of the ERV filtrate was measured with a combination probe glass electrode. The EMF was measured on the same ERV filtrate using a platinum electrode and a silver-silver chloride half-cell. The platinum electrode was standardized with a ferrous-ferric standard EMF solution (*5*). The pH_t, adapted from the method of Shelef and Jay (*10*), was determined by adding 2 ml of 0.05 N HCl solutions to a 10-ml aliquot of the ERV filtrate and noting the changed pH. This new pH was called pH_t.

TCA extract. Twenty grams of meat were blended with 50 ml of cold 20% trichloroacetic acid (TCA) for 2 min. The blender contents were rinsed with 50 ml of water, mixed together, and filtered through a Whatman #1 filter. This filtrate is termed the TCA extract and is used in the TBA and tyrosine tests.

TBA number. The TBA number was determined using a variation of the method described by Witte et al. (*13*). A 5-ml aliquot of the TCA extract was mixed with 5 ml of 0.01 M 2-thiobarbituric acid. Either of two procedures was used for TBA color development. One procedure involved storage for 14 h at room temperature (ca. 20 C) and the other for 30 min at 100 C. Color development, measured as Absorbance (A) at 532 nm, was identical when either color development procedure was used with standard solutions of tetraethoxypropane or with TCA extracts of meat. Absorbance at 532 nm is reported as the TBA number.

[2] *Reference to brand or firm name does not constitute endorsement by the U.S. Department of Agriculture over others of a similar nature not mentioned.*

Tyrosine value. Two and one half ml of the TCA extract were diluted with 2.5 ml of water. To this 10 ml of 0.5 N NaOH were added followed by 3 ml of Folin's Reagent (diluted 1 Folin's:2 water). After mixing, the color was developed for 15 min at room temperature before reading at 660 nm. The "tyrosine" value is reported as mg of tyrosine/g of meat (*7*).

RESULTS AND DISCUSSION

The intact samples of l.d. were stored and analyzed for bacteria counts and the seven physical and chemical changes as described in the experimental section. Bacterial contamination as well as intrinsic changes in meat during storage are causes for the changes measured in the quality tests selected for evaluation.

Data in Fig. 1 indicate that the bacterial population grew more slowly on meat stored at −1 C than at 7 C. Throughout most of the storage period (20 days) there were at least 3 to 4 log differences in bacterial numbers between the two temperatures.

Δ%R and "tyrosine" value versus time for meat stored at both temperatures are shown in Fig. 2. Δ%R decreased more rapidly after 5 days storage and "tyrosine" value increased more rapidly for meat stored at 7 C.

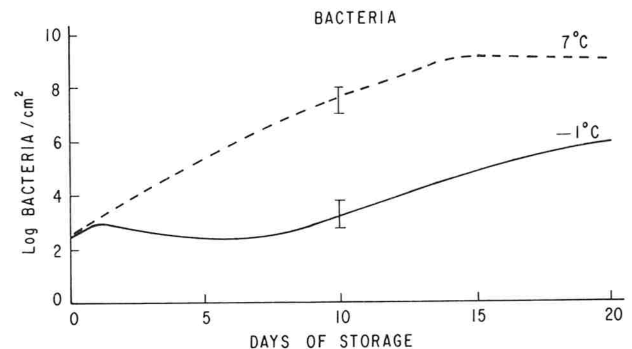

Figure 1. *Bacteria, - - - - Longissimus dorsi stored at 7 C; — Longissimus dorsi stored at —1 C. Vertical brackets in this and in successive figures represent range of determinations for the day indicated.*

Figure 3 shows the TBA number and the pH_t number versus day of storage. The TBA numbers increase during storage but no definite differences were observed between meats stored at the two temperatures.

The pH_t for the meat stored at 7 C increased more than the pH_t for the meat stored at −1 C. This increase occurred near the end of the storage period. However, the size of this increase was smaller than the variations in pH_t among carcasses.

ERV and pH versus time are shown in Fig. 4. ERV increased rapidly and pH decreased rapidly during the first few hours after slaughter. These changes were expected due to onset of rigor. During storage the ERV for meat held at both temperatures decreased slowly but variations among duplicates were larger than the decrease noted. After the rapid initial decrease, pH stayed at approximately the same level until, at extremely high levels of bacterial contamination ($>10^8/cm^2$), it rapidly increased about 0.4 pH unit. EMF values versus

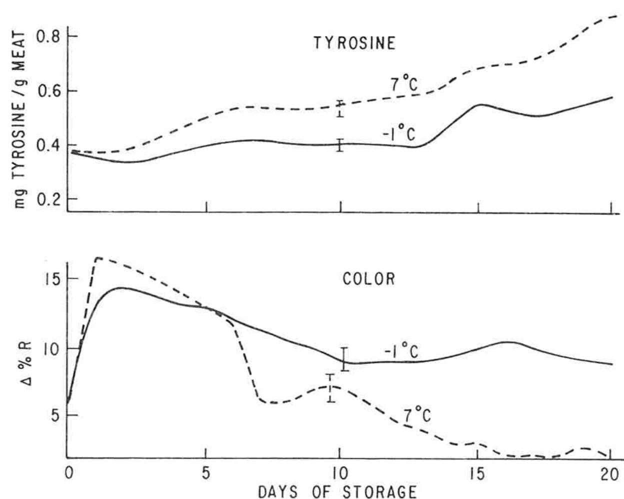

Figure 2. *"Tyrosine" value, - - - - Longissimus dorsi stored at 7 C; — Longissmus dorsi stored at -1 C. Color (Δ %R), - - - - Longissimus dorsi stored at 7 C; — Longissimus dorsi stored at -1 C.*

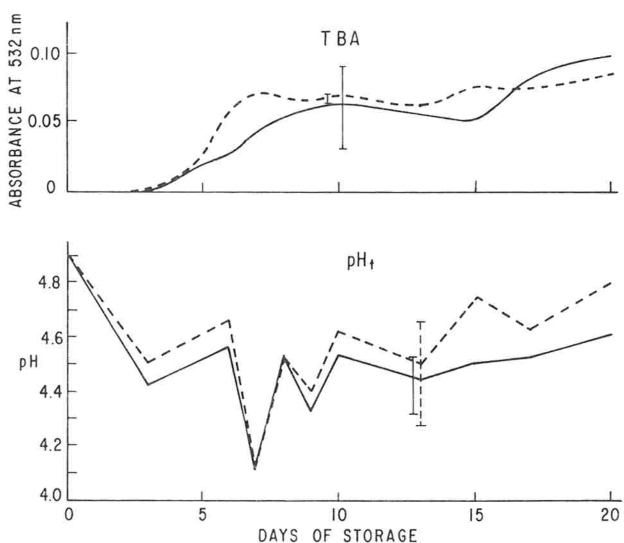

Figure 3. *Thiobarbituric Acid Number, - - - - Longissimus dorsi stored at 7 C; — Longissimus dorsi stored at -1 C. pH_t - - - - Longissimus stored at 7 C; — Longissimus dorsi stored at -1 C.*

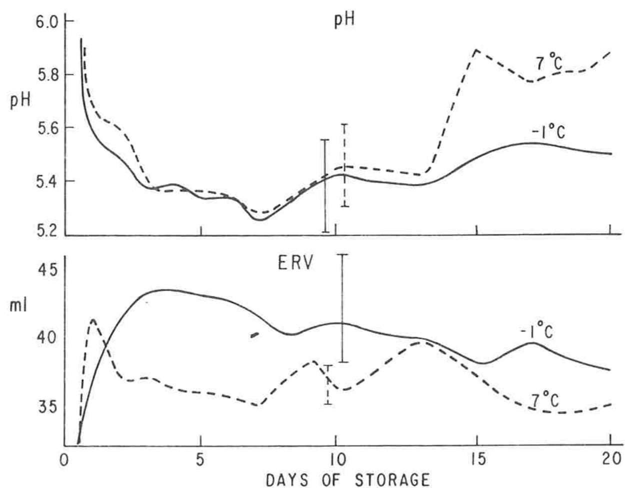

Figure 4. *pH, - - - - Longissimus dorsi stored at 7 C; — Longissimus dorsi stored at -1 C. Extract Release Volume, - - - - Longissimus dorsi stored at 7 C; — Longissimus stored at -1 C.*

time are not shown. These values were so erratic that any trends are meaningless.

Data from meat stored at both temperatures were pooled and statistical analyses carried out on the pooled data for each quality test. Table 1 lists the linear correlation coefficients ("r" values) for the $\log_{10}$ bacteria counts/cm^2 versus the seven quality tests. Correlation with Δ%R, "tyrosine" value, TBA number, and pH_t were significant at the 99% confidence level.

"Tyrosine" value, TBA number, pH and pH_t may be expected to increase as bacteria increase while Δ%R, ERV, and EMF may be expected to decrease according to the literature previously cited. Our results agree with the expected direction of the change.

Intrinsic changes in meat were assumed to occur as the meat aged during storage and would be related to the length of storage. Table 2 shows the "r" values for storage time in days versus eight quality test results, determination of $\log_{10}$ bacteria count/cm^2 being the eighth test. Bacteria count, Δ%R, "tyrosine" value, TBA number, and EMF all had significant, "r" values, the directions of the changes being as predicted in the literature previously cited.

TABLE 1. *Correlation of quality tests with log bacteria/cm^2*

Quality test	Linear correlation coefficient	Number of samples
Color (Δ %R)	–753[a]	103
"Tyrosine" value	.696[a]	72
TBA number	.491[a]	76
Extract release volume	–.223	65
pH	.266	65
pH_t	.348[a]	64
EMF	–.364	29

[a]P > 99%.

TABLE 2. *Correlation of quality tests with storage time in days.*

Quality test	Linear correlation coeffieicent	Number of samples
Log bacteria counts/cm^2	.678[a]	103
Color (Δ%R)	–.512[a]	103
"Tyrosine" value	.528[a]	72
TBA number	.507[a]	76
Extract release volume	–.060	94
pH	.261	94
pH_t	.209	64
EMF	–.497[a]	29

[a]P > 99%.

Δ%R, "tyrosine" value, and TBA number were the only quality tests which correlated closely with both bacteria count and time of storage. Table 3 gives the probabilities that the "r" values for the quality tests versus bacteria count and versus length of storage differ. Δ%R was more highly correlated with bacteria count than with length of storage. "Tyrosine" value was also more highly correlated with bacteria count than with length of

TABLE 3. *Comparison of quality test for predicting log bacteria count/cm², storage time, and color.*

Quality test	Linear correlation coefficient			P^a
	Bacteria count	Storage time	Color	
Color (Δ%R)	–.75	-.51	—	.002
"Tyrosine" value	.69	.53	—	.14
TBA number	.49	.51	—	.88
TBA number	.49	—	-.53	.70
TBA number	—	.51	–.53	.82

[a]Probability the r's are equal.

storage but not to the same extent at Δ%R. This observation is not obvious if Fig. 2 alone is used for evaluation of the quality tests.

TBA number versus bacteria count, versus length of storage, and versus Δ%R all have about the same "r" values. The "r" value for TBA number versus color was calculated. Benedict et al. (*1*) reported that lipid oxidation in ground meat can have a negative effect on color. The effects of length of storage, bacteria counts, and color changes on TBA numbers cannot be separated with these data.

As a further check on the validity of the quality tests as indicators of meat quality the pooled data were divided into two classes: quality test data from meat samples with low bacteria counts $\leq 10^4/cm^2$ and quality test data from meat samples with high bacteria counts $\geq 10^7/cm^2$. Quality test data from meat samples with bacteria counts $> 10^4/cm^2$ and $< 10^7/cm^2$ were not used. The Student's "t" test was used to test the hypothesis that the quality test results on low bacteria count meat are not different from the results of quality tests on high bacteria count meat. Δ %R, "tyrosine" value, and TBA number all had "t" values that did not support this hypothesis. Δ %R's for low bacteria count meat were significantly higher than the Δ %R's for high bacterial count meat. "Tyrosine" values and TBA numbers for low bacteria count meat were significantly lower than for high bacteria count meat (Table 4).

Of the seven quality tests evaluated, Δ%R is the most effective monitor of meat quality during storage. Δ%R is a measure of the degree of meat pigment oxidation. Several precautions should be observed when using this quality test. Meat with insufficient time to bloom fully will give a low Δ %R even though of good quality. Bloom is the conversion of myoglobin (a purple meat pigment) to oxymyoglobin (a red meat pigment) by oxygen. Concentration of myoglobin in the muscle also affects Δ%R. The muscle type and the age of the slaughtered animal will affect myoglobin levels (*9*). Occasionally, meat with an extremely high bacteria count will give a higher Δ%R than expected due to reduction of met-myglobin (a brown meat pigmeat) to myoglobin by the reducing capacity of bacteria present.

"Tyrosine" value was also an effective monitor of meat quality. The "tyrosine" value is an indicator of proteolysis as it measures the amino acids tyrosine and tryptophan present in a nonprotein extract of meat.

TBA number gave significant results in these tests but its correlations with bacteria and with time of storage were less than those of either Δ%R or "tyrosine" value. The TBA number may be a better quality monitor with a meat that is more easily oxidized than intact beef, such as ground beef or pork.

ERV did not predict or monitor meat quality as well as expected, but it had a significant "r" value when compared with pH. The pH of a water extract of the meat was not a sensitive monitor of meat quality. It increased when the number of organisms exceeded 10^8 but not reliably. However, pH measurements on the surface of the meat may be a more effective monitor because with intact meat most alterations occur on the surface.

pH_t had a significant "r" value when compared with bacteria count and it followed the same trends predicted by Shelef and Jay (*10*), but it did not change in intact meat to the extent that they reported with ground meat.

EMF of meat has promise as an effective quality test. However, methodology must be worked out to reduce the large variation in the EMF's measured. We did EMF measurements on only a few of our meat samples because variability was extremely high.

In conclusion, Δ%R was the most effective monitor of bacterial contamination in intact meat. It is nondestructive and convenient to use. The next most effective monitor was "tyrosine" value. As a monitor for bacterial quality, it was effective but interference due to intrinsic changes in meat was more likely to affect the "tyrosine" value than Δ %R.

TABLE 4. *Comparison of mean quality test data on high bacterial count ($\geq 10^7$ / cm²) and low bacteria count ($\leq 10^4$ / cm²) meat.*

Quality test	Low bacteria count meat		High bacteria count meat		t^b
	Mean	N^c	Mean	N^c	
Color (Δ%R)	13.23	45	6.79	31	5.37[a]
"Tyrosine" value	.46 mg/g meat	27	.73 mg/g meat	28	4.67[a]
TBA number	.05 A	30	.16 A	29	4.50[a]
Extract release volume	39.6 ml	27	37.0 ml	16	2.38
pH	5.35	26	5.53	22	2.31
pH_t	4.38	26	4.48	22	1.47
EMF	–154 mv	13	–209 mv	7	1.58

[a]Quality test means for low and high bacteria count meat were different. P < 99.9%.
[b]Student's t value.
[c]Number of samples.

TBA number increase was correlated about equally with bacteria numbers and with storage time. However, changes in the TBA number were small and usefulness of TBA as a monitor for bacterial quality in intact meat appears limited.

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Keeping Quality of Pasteurized Milk for Retail Sale Related to Code Date, Storage Temperature, and Microbial Counts

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ABSTRACT

Keeping quality of milk samples collected in original containers from fillers and stored at 1.7, 5.6, and 10.0 C remained organoleptically acceptable, on the average, 17.5, 12.1, and 6.9 days, respectively. Samples were tested for specific groups of bacteria at collection and when the milk became unacceptable (flavor score < 36). In addition to a total aerobic count the specific groups included pseudomonads, lipolytic, proteolytic, acid-producing, and coliform bacteria, and lipolytic and proteolytic pseudomonads. Keeping quality at any storage temperature was unrelated to the manufacturer's code date (last day product is to be sold). There was a significant correlation between keeping quality at 10.0-C storage and the other two storage temperatures, suggesting a practical test to measure keeping quality at the lower temperatures. Microbial counts, made at bottling and when the sample became unacceptable, were not consistently related to the time required for milk to become unacceptable at any storage temperature. When samples were stratified by flavor defect, certain microbial tests were significantly related to keeping quality.

In Connecticut, milk offered for retail sale must be marked with the last date it can be sold, the "code date." Each processor establishes the length of the code period. We have shown that, on the average, dairies in Connecticut overestimated code periods by about 2 days, but this overestimate varied from 2 to 8 days on code periods that ranged from 7 to 14 days (*9*). Processors point out that milk stored at 1.7 C will keep longer than if stored at a higher temperature, but they do not state what storage temperature reflects the choice of code establishing the length of the code period?

We therefore investigated the following: (*a*) Is the code period actually related to the length of time milk will remain acceptable when stored at either 1.7, 5.6, or 10 C? (*b*) Are microbial counts made at the time of bottling related to the length of time milk will remain acceptable? (*c*) Are specific types of microbial counts (e.g. proteolytic or lipolytic bacteria) related to the length of time milk remains acceptable? (*d*) Are microbial counts made on freshly bottled milk related to microbial counts made when the milk is no longer acceptable? (*e*) Are microbial counts made on the same milk stored at different temperatures statistically related?

MATERIALS AND METHODS

Sampling

At intervals from June, 1975, through November, 1976, 54 samples of pasteurized whole milk were obtained from 23 dairies in Connecticut. No dairy was sampled more frequently than once in 2 months. A third of the dairies were sampled once, another third twice, and the remainder three to five times. Each sample consisted of six subsamples collected in original containers taken directly from the filler, and in almost all instances from the same filling valve. Each sample, in its original container, was packed in ice during transport to the laboratory.

One set of three subsamples was used for microbiological analysis and the other set for organoleptic analysis. Seventy percent of the samples were in quart containers and 22% in half-gallon containers. The remainder were either gallons or half-pints. Paper containers comprised 72%, and glass bottles 28%, of the samples.

Organoleptic analysis

plate technique on previously poured and hardened media (*11*) except that a pour plate technique was used with Violet Red Bile agar. After inoculations, plates were incubated at 30 C for the times designated in the next section.

Test media

Total counts of aerobic and proteolytic bacteria were made on the medium of Martley et al. (*15*). After 48 h of incubation both the total number of bacterial colonies as well as the number producing a protease were counted. The agar surface was then flooded with an oxidase reagent (*7*) (composed of a-naphthol and p-aminodimethylaniline oxalate). Those colonies which turned blue were classified as pseudomonads (*7*). Thus, from a single plate of medium, total aerobic, total proteolytic, total pseudomonads, and proteolytic pseudomonads were enumerated (*11*).

Lipase production was detected on the medium described by Sierra (*16*), with Tween 20 (Fisher Chem. Co., Fairlawn, N.J.) as the lipid source. After incubation for 5 days, the lipase producers were counted and after flooding the agar surface with the oxidase reagent, the lipolytic pseudomonads were enumerated.

Acid-producing bacteria were determined with the medium of Fabian et al. (*3*). Colfiorm bacteria were detected using Violet Red Bile agar (Difco).

Statistical analysis

All microbial counts were transformed as previously described (*7*). Data were statistically analyzed using Data-Text statistical computer programs (*2*).

Definitions

"Code date" is the date marked on the container designating the last day on which the milk may be sold or offered for sale. "Code period" is the number of days between the date of bottling and the code date. "Days to go bad" is the number of days from bottling until the flavor score dropped below 36. Similarly, "keeping quality" is the number of days a sample remained acceptable; that is, with a flavor score of 36 or higher.

RESULTS AND DISCUSSION

Relation of code period and temperature of storage to keeping quality

The code period among the 54 samples ranged from 7 to 15 days and averaged 10.8 days (Table 1). As expected, if the flavor defect was of microbial origin, the days to go bad decreased as the storage temperature increased. At 1.7 C the average number of days to go bad was 17.5, exceeding even the longest code period of 15 days. Nevertheless, 9% of the samples stored at this temperature were judged unacceptable before the expiration of their code period. About 5% of the samples had defects which were probably not of microbial origin; such as oxidized and chemical flavor.

At 5.6 C, the average days to go bad, 12.1, exceeded the code period of 70% of the samples, and nearly 43% of the samples became unacceptable before the expiration of their assigned code period. At 10.0 C only 4% of the samples remained acceptable beyond their code period, the average being 7 days. The time of year of sampling did not affect the length of time required for samples to become unacceptable.

It is clear from data in Table 1 that the lower the storage temperature the longer the milk retains an acceptable flavor and this agrees with the observations of others (*1,4*). At any of the three storage temperatures it is also clear that there is no relation between the assigned code

TABLE 1. *Keeping quality (days to go bad) of 54 pasteurized milk samples in relation to code period (days) and storage temperature*

Code period (Days)	No. samples	Storage temperature (C)		
		1.7	5.6	10.0
7	4	24.8[1]	16.0	8.3
9	3	15.0	11.0	7.0
10	25	15.8	10.6	6.4
11	6	18.7	14.0	7.7
12	8	18.9	14.1	7.1
14	6	18.0	10.8	6.7
15	2	18.5	13.0	7.5
10.8 (Avg)		17.5 ± 5.2[2]	12.1 ± 3.9	6.9 ± 1.6

[1]Average number of days to attain a flavor score of < 36 for the number of samples shown.
[2]Mean ± standard deviation.

period and the length of time the samples remained acceptable. Therefore, among the 23 dairies sampled, code period appears to be arbitrarily assigned without regard to the actual keeping quality of the milk.

Among the 54 samples, the number of days required to go bad at one temperature was closely related to the length of time required to go bad at the other storage temperatures. Although samples stored at 10.0 C quickly became unacceptable (Table 1), the brief time they remained acceptable correlated well with their longer acceptable storage life at 1.7 and 5.6 C (Fig. 1). This

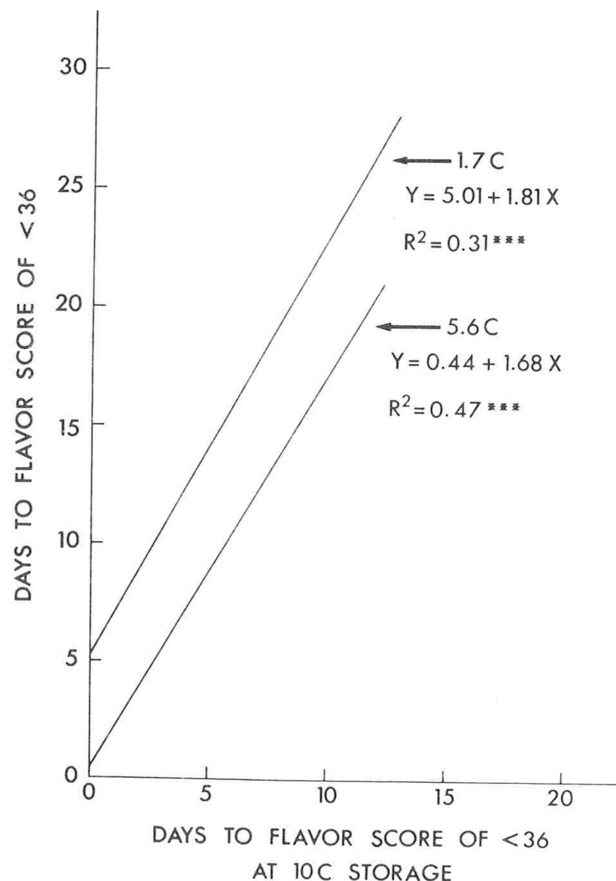

Figure 1. *Relation of days required to attain an unacceptable flavor score (< 36) for samples stored at 1.7 and 5.6 C compared to days required at 10.0 C. *** significant at P ⩽ 0.001.*

suggests that practical, rapid estimates of storage life, hence code period, at lower and more reasonable storage temperatures could be easily obtained from results of storage at higher temperatures. The experimental evidence suggests that such a procedure could give rise to more meaningful code periods than are presently used.

Of considerable importance in the present study is that only 9% of the samples stored at 1.7 C failed to reach the end of their assigned code period. For the samples collected from retail stores in 1974 and 1975, 15% were unacceptable before the end of the code period (*9*). This indicates that processors lose control of the product after it enters the retail store. For example, the temperature of 53% of samples collected from stores in 1974-75 was in the 4.4 to 7.2 C range (*9*). Similarly, in the present study 43% of the samples stored at 5.6 C became unacceptable on or before the last day of the code period. This appears to show that if all milk at the retail market were allowed to reach the end of the code period before sale, almost half the samples would be unacceptable. These results emphasize that a major share of maintaining milk quality rests with the retail market and reaffirms the need for lower storage temperatures in store refrigerators, preferably below 4.4 C. These data also indicate that the flavor of milk stored between 4.4 and 7.2 in home refrigerators will likely also deteriorate to unacceptable levels before the code period expires.

Relation of initial microbial counts to keeping quality

Microbial counts made on the day of bottling (called initial counts) were generally low (Table 2) and negatively correlated with length of time for the sample to become unacceptable at any storage temperature. That is, the lower the initial count, the longer it took for the sample

TABLE 2. *Transformed bacterial counts[1] (means of 54 samples) of pasteurized milk on the day of bottling (initial) and when the flavor score dropped below 36 at different storage temperatures*

Microbial test	Initial	Storage temperature (C) 1.7	5.6	10.0
Total aerobic count	5.1[a2]	11.4[b]	14.1[c]	14.8[c]
Proteolytic bacteria	3.9[a]	11.0[b]	13.6[c]	14.4[c]
Pseudomonads	3.7[a]	10.8[b]	13.5[c]	13.9[c]
Proteolytic pseudomonads	3.5[a]	10.7[b]	13.3[c]	13.7[c]
Lipolytic bacteria	4.3[a]	10.3[b]	12.8[c]	13.5[c]
Lipolytic pseudomonads	3.6[a]	10.2[b]	12.4[c]	12.8[c]
Acid producers	1.9[a]	5.3[b]	8.4[c]	11.2[d]
Coliform bacteria	1.2[a]	1.4[a]	4.3[b]	8.5[c]

[1]For counts less than 10 the assigned code was 1. For any count-x, equal to or greater than 10, the transform was 2n, if $10^n \leqslant x$ but $< 5 \times 10^n$, or $2n + 1$ if $5 \times 10^n \leqslant x$ but $< 10 \times 10^n$. Such transformations on logarithm intervals have been described (*6,11*). The range of the transformed counts shown in the table can be calculated as follows: for odd-numbered transforms, subtract 1 from the transformed value and divide the integer portion by 2. The result is the exponent, n, where the value, x, has the range $5 \times 10^n \leqslant x < 10 \times 10^n$. For example, the transformed count 5.1 indicates that the microbial count lies in the range 500 but less than 1,000. For even-numbered transforms, divide the integer portion by 2; the result is the exponent, n, where x lies in the range $10^n \leqslant x < 5 \times 10^n$. For example, a tranform of 14.1 indicates that the microbial count lies in the range 10 million but less than 50 million.
[2]Means in any row followed by the same letter do not differ significantly at $P \leqslant 0.05$.

to become unacceptable. However, at all storage temperatures only acid producers were significantly correlated with days to go bad (Table 3). For samples

TABLE 3. *Relation (R^2) of microbial counts on the day of bottling with keeping quality (days to go bad) of 54 pasteurized milk samples stored at different temperatures*

Microbial test	Storage temperature (C) 1.7	5.6	10.0
Acid producers	0.144**	0.076*	0.159**
Proteolytic bacteria	0.020	0.072*	0.086*
All 8 microbial tests	0.211	0.143	0.308*

*Significant at P = 0.05.
**Significant at P = 0.01.

stored at 5.6 and 10.0 C, the total count of proteolytic bacteria was also significantly negatively correlated with days to go bad.

However, despite the statistical significance these correlations accounted for 16% or less of the variablility in days to go bad. Indeed, all eight initial bacterial counts combined accounted for only 14 to 31% of the observed variability in days to go bad at any of the storage temperatures. Watrous et al. (*17*) have also shown that initial bacterial counts including Standard Plate, coliform, and psychrotrophic counts on commercially pasteurized samples were of little value in predicting keeping quality. Randolph et al. (*14*) found no correlation between keeping quality and a Standard Plate Count of samples obtained directly from dairies. Further, Patel and Blankenagel (*13*) also clearly point out that milk with low bacterial counts does not necessarily have a long shelf life. Keeping quality, they suggest, is affected primarily by the type of organism and not necessarily by the total number present. Our earlier reports showed all of this statistically (*6,10*). In none of the statistical analyses in the present study did the initial total count appear as an indicator of keeping quality. Thus, initial bacterial counts have little apparent value by themselves for predicting keeping quality of milk at any storage temperature.

We did observe, however, that samples in glass containers became unacceptable sooner than those in paper cartons (Table 4). Initial microbial counts, except for coliform bacteria, were slightly but insignificantly higher in glass than in paper containers. When the samples became unacceptable, nearly all bacterial counts were slightly, but insignificantly, lower in glass containers compared to paper. In essence, although

TABLE 4. *Keeping quality (days to go bad) of pasteurized milk in glass and paper containers stored at different temperatures*

Container	No. samples	Storage temperature (C) 1.7	5.6	10.0
Paper	39	18.8	12.9	7.4
Glass	15	14.2	9.8	5.7
Difference		4.6	3.1	1.7
t-test (d.f. = 52)		3.13**	2.80**	3.75***

**Significant at P = 0.01.
***Significant at P = 0.001.

samples became unacceptable sooner in glass than in paper containers, at the time they became unacceptable there was no difference in the microbial counts.

Relation of final microbial counts to keeping quality

Final microbial counts at all storage temperatures were obviously higher than the initial counts (Table 2). However, unlike the initial counts, all final counts were positively correlated with the number of days to go bad at all storage temperatures. That is, the longer the time required to become unacceptable, the greater the number of bacteria. This apparently reflects the effect of incubation period. It also suggests that no particular group of organisms can be singled out as the cause of unacceptable flavor.

Examination of data in Table 2 reveals that the final counts at 5.6 and 10 C do not differ except for acid producers and coliform bacteria, and that all final counts of milk stored at 1.7 C are significantly less than at 5.6 and 10 C. This further indicates that the number of organisms is not the sole determinant of unacceptable flavor, particularly at colder temperatures where they multiply slowly.

At 1.7 C proteolytic pseudomonads made the greatest relative increase over initial count. At 5.6 C the acid producers and proteolytic pseudomonads showed the largest relative increase while at 10.0 C acid producers and coliform bacteria exhibited the largest relative increases over initial counts. Thus, at each temperature it is likely that a different group of organisms may be responsible for deterioration of flavor score.

Relation of individual flavor defects to keeping quality

The final flavor defects were examined to determine what caused the samples to become unacceptable (Table 5). Initially, three flavor defects; feed, cooked, and cooked and feed were assigned to 94% of the samples. When a sample was declared unacceptable the most common flavor defect was old or lacking freshness, accounting for 61 and 54% of samples stored at 1.7 and 5.6 C, respectively. However, at 10.0-C storage, less than a third were designated as lacking freshness. At 10-C storage, the two off-flavors putrid and curdled, accounted for 31% and acid flavor accounted for 9% of the samples. The number of samples with these off-flavors found at 10-C storage was noticeably increased over that of samples stored at 5.6 C. The proportion of samples with a fruity flavor remained nearly constant at all storage temperatures. Bitter flavor , accounting for 17% of the samples at 1.7 C, dropped to 11% at 5.6 and 10.0 C.

The keeping quality of samples stored at different temperatures and with a specific flavor defect is also given in Table 5. For example, samples stored at 1.7 C and judged as lacking freshness lasted an average of 18.1 days, while those with the same flavor criticism but stored at 10.0 C lasted an average of only 7.4 days. The keeping quality of samples stored at 5.6 C is nearly midway between those stored at 1.7 and 10.0 C. At 10.0-C storage no large differences are seen in number of days to go bad between any of the flavor defects.

Relation of keeping quality to initial flavor score and microbial counts

The initial flavor score was only correlated with the number of days to go bad at 1.7 C, and it accounted for only 9% of the observed variation (Table 6). Initial flavor score was poorly correlated with days to go bad for samples stored at 5.6 and 10.0 C. Results of an earlier study of laboratory-pasteurized milk showed that initial flavor score accounted for 38 to 41% of the variability in days to go bad when the milk was stored at 1.1 to 3.3 C (*10*).

Initial microbial counts also did not correlate with days to go bad at any storage temperature. Combining initial flavor score with one or more initial microbial counts improved the prediction of days to go bad for samples stored at 1.7 and 5.6 C, but not at 10.0 C (Table 6). However, at best only 22% of the observed variability in days to go bad was accounted for by these counts. Use of counts from other microbial tests gave no improvement to the prediction of days to go bad. Thus, neither initial flavor score nor microbial counts appear to be useful as reliable predictors of keeping quality of commercially pasteurized milk.

TABLE 5. *Keeping quality (days to go bad) of pasteurized milk samples stored at different temperatures and classed by final flavor defect*

	Storage temperature (C)					
	1.7		5.6		10.0	
Flavor defect	No. samples	Avg days to go bad	No. samples	Avg days to go bad	No. samples	Avg days to go bad
Rancid	1	21.0	0	—	1	4.0
Acid	0	—	1	10.0	5	7.4
Lacks freshness	33	18.1	29	12.6	17	7.4
Putrid and/or curdled	4	14.8	9	13.4	17	6.8
Bitter	9	21.0	6	13.7	6	7.0
Fruity	4	16.5	4	11.0	5	6.8
Malty	1	7.0	1	4.0	1	7.0
Unclean	0	—	1	15.0	1	9.0
Misc.[1]	2	4.0	3	3.4	1	3.0
Avg days to go bad		17.5 ± 5.2		12.1 ± 3.9		6.9 ± 1.6

[1]Misc. includes oxidized and chemical; not considered microbial defects.

TABLE 6. *Relation (R^2)[1] of keeping quality (days to go bad) with initial flavor score (made at bottling) and inital microbial counts of 54 samples stored at different temperatures*

Variable	Storage temperature (C) 1.7	5.6	10.0
Initial flavor score (IFS)	0.092*	0.072	0.007
IFS + acid producers	0.208*	—	—
IFS + acid prod. + lipolytic bacteria	0.216*	—	—
IFS + lipolytic pseudomonads	—	0.140*	—
IFS + lip. pseud. + acid prod.	—	0.182*	—

*Significant at P = 0.05.
[1]Only selected R^2 values shown since the same variables did not appear at each storage temperature.

Relation of individual flavor defects to microbial counts

The cause of certain flavor defects can be attributed to particular kinds of organisms (*5*). Bitter, fruity, putrid, and stale (lacking freshness) have been attributed to psychrotrophic organisms (*14*). We have seen that neither initial nor final microbial counts correlated with the keeping quality of all milk samples expressed as the number of days to go bad. Therefore, samples were stratified according to the final flavor defect and again examined for the relation of days to go bad to microbial counts (Table 7). For both initial and final microbial counts we expect that a negative correlation of days to go bad with a microbial count to be important. That is, the lower the count, the longer the milk will remain acceptable. A positive correlation, on the other hand, may be interpreted simply as the effect of incubation period. The longer the milk remains acceptable, the greater the opportunity for bacteria to grow. Unfortunately, results obtained with this analysis were extremely variable.

Examples of the relations of initial or final microbial counts with three different flavor defects are given in Table 7. In the group judged as lacking freshness, initial counts did not correlate with days to go bad for samples stored at 1.7 and 5.6 C. At 10.0-C storage, acid producers were significantly negatively correlated with days to go bad, but accounted for only 19% of the variability. At 1.7-C storage all final counts did not correlate with days to go bad although all tended to be negatively correlated. At 5.6- and 10.0-C storage all correlations were positive, with total count being most highly correlated with days to go bad. Similar results are shown in Table 7 for two other flavor defects, putrid and bitter.

Thus, as we and others have shown (*1,3,4,6,10,14,17*) total counts bear little relation to keeping quality. We show here that even when samples are stratified for flavor defect, there is no clear relation between microbial count, but there is some statistical significance between specific microbial groups and keeping quality.

The information presented here and by others (*13,17*), requires one to reflect on the ultimate value of determining keeping quality based solely on bacterial tests, an example being the Mosely keeping quality test (*12*). This test, like others, is not correlated with actual flavor analysis since it has been shown that milk with a flavor defect may or may not have a high total bacterial count. Thus, more discriminative tests for keeping quality need to be developed, and such tests must be uncomplicated enough to be done routinely in any laboratory.

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TABLE 7. *The best correlation (r) of days to go bad with initial or final microbial counts: grouped by final flavor defect*

		Temperature of storage (C)								
		1.7			5.6			10.0		
Final flavor defect	Microbial count	No. samples	Microbial test	r	No. samples	Microbial test	r	No. Samples	Microbial Test	r
Lacks freshness	Initial	33	PrPs[1]	+0.137	29	Lip	–0.204	17	Acid	–0.438*[2]
	Final	33	Acid	–0.197	29	TotCnt	+0.424*	17	TotCnt	+0.765***
Putrid	Initial	4	Acid	–0.802	9	Acid	–0.497	17	Coli	–0.863***
	Final	4	Pr	+0.636	9	Lip	–0.801**	17	PrPs	+0.651**
Bitter	Initial	9	LipPs	+0.436	6	Pr	–0.833	6	Acid	+0.577
	Final	9	Lip	+0.763*	6	TotCnt	+0.986***	6	Ps	+0.845

[1]Abbreviations: PrPs, proteolytic pseudomonads; Lip, lipolytic bacteria; Acid, acid producers; TotCnt, total number of bacteria; Coli, coliform bacteria; Pr, proteolytic bacteria; LipPs, lipolytic pseudomonads; Ps, pseudomonads.
[2]Significance level: *P = 0.05; **P = 0.01; ***P = 0.001.

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Comparison of Three Assays for Peroxidase in the Blue Crab (*Callinectes sapidus*)

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ABSTRACT

Three different enzyme assays which have been used to determine peroxidase activity in food products were compared for their relative sensitivity to measure peroxidase in the blue crab (*Callinectes sapidus*). These included a modified Nagle and Haard, a Nickel and Cunningham, and a Lundquist and Josefsson procedure. The enzyme extracts were tested using a spectrophotometer to measure the ΔOD/min. The modified Nale and Haard procedure was the most sensitive method for detecting peroxidase activity in the blue crab. The assay, which is rapid, can be used with crude extracts and does not require enzyme purification.

An empirically determined relationship exists between peroxidase activity and off-flavors and off-colors in food products (*1,4,5,6,11*). Information concerning the presence and activity of peroxidase in seafood products has not been adequately reported. It is important that the presence, activity, and heat resistance of peroxidase be defined in the lump (backfin), flake, and claw meat of blue crabs (*Callinectes sapidus*) since the meat is pasteurized or frozen and subsequently stored for extended periods.

Previous methods proposed to determine presence of peroxidase activity in animal tissues required purified enzymes rather than crude extracts (*7*). This phenomenon has been explained by the fact that these methods for detecting animal peroxidase were of relatively low sensitivity probably due to the high level of H_2O_2 used for analysis. Additionally, some procedures lack specificity since it has also been shown that some fractions from the hemolymph of the blue crab will stain for peroxidase (*3,8,11*). The objective of this study was to analyze and compare three diferent peroxidase assays routinely used for animal and plant tissues with or without modification for their suitability to detect and measure peroxidase activity in crab meat extracts.

MATERIALS AND METHODS

Samples

Live blue crabs (*Callinectes sapidus*) used in this study were supplied by Graham and Rollins, Inc., Hampton, Virginia, transported to Blacksburg, Virginia, and kept alive until needed for analysis. They were all harvested at the same time and separated for testing on the basis of sex, size, and weight.

Extraction

Ten grams of raw meat were excised from the crabs, rapidly weighed, placed into a blender with 20 ml of cold triple-distilled deionized water, and homogenized for 30 sec at high speed. All extractions were done at 25-26 C. The homogenate was filtered through Whatman No. 42 filter paper. Since only a crude extract was needed for qualitative studies, this procedure was considered adequate.

Enzyme assays

Enzyme activities were measured in triplicate from each of three different samples of a pure horseradish peroxidase standard (P8250, Sigma Chemical Co.) and of raw crab extracts by each of three methods; a modified Nagle and Hard (*9*), Lundquist and Josefsson (*7*), and Nickel and Cunningham (*10*) procedure. For the modified Nagle and Haard (*9*) method, the substrate consisted of 0.1 ml of H_2O_2 (30%) in 100 ml of 0.01 M potassium phosphate (pH 6.0). The cuvette contained 2.8 ml of the substrate, 0.01 ml of a 1% (w/v) solution of o-dianisidine in methyl alcohol plus 0.1 ml of crab extract. The two other assays as described by Lundquist and Josefsson (*7*) and Nickel and Cunningham (*10*) were done without modification. The rate of enzyme reaction was spectrophotometrically determined by measuring the rate of oxidized dye formation at maximum wavelength adsorption, 460 nm for the Nagle and Haard, 675 nm for the Nickel and Cunningham, and 400 nm for the Lundquist and Josefsson assays. The effect of pH on each method of analysis was determined to elicit optimum operating conditions. Protein was precipitated in each procedure to determine if changes in optical density could be affected by other components of crab meat. Of particular interest were ammonia, free amino nitrogen, trimethyamine (TMA), trimethylamine-oxide (TMAO), and other non-protein nitrogen compounds. However these and other water-soluble compounds in crab extracts having molecular weights of less than 5,000 failed to give positive enzyme reactions (*2*). When the protein in the crab extract was precipitated using 8% TCA (trichloroacetic acid) activity was no longer detected.

The enzyme assays were done at 25 C in a double beam Coleman 124 spectrophotmeter equipped with a Perkin-Elmer model 56 recorder (Coleman Instrument Division, Maywood, Illinois). Rates of the reactions were measured as the initial change in absorbance (1 cm light path) at a fixed wavelength and expressed as ΔOD/min.

RESULTS AND DISCUSSION

The comparison of relative sensitivity of each assay procedure tested against a pure horseradish peroxidase standard is illustrated in Fig. 1. The Nickel and Cunningham (*10*) assay proved to be the most sensitive. The modified Nagle and Haard (*9*) method was much less

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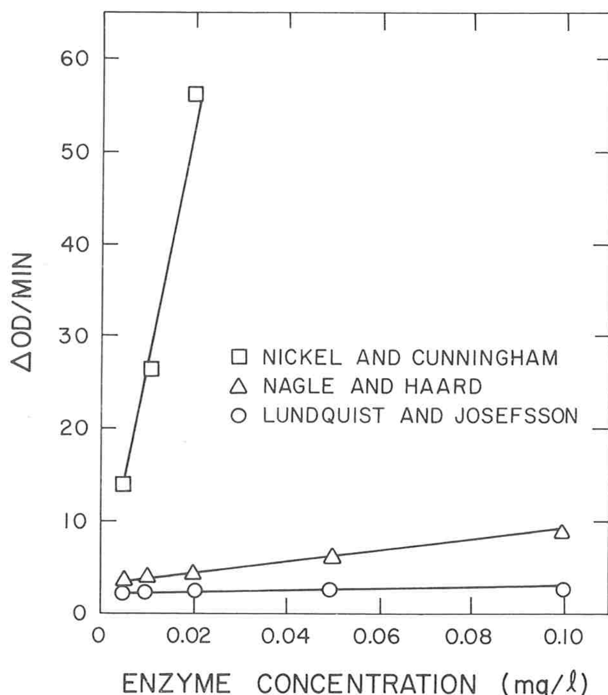

Figure 1. *Comparative sensitivity of three assays for the determination of peroxidase activity in a pure horseradish peroxidase standard.*

sensitive and the method used by Lundquist and Josefsson (*7*) was least sensitive. Lack of activity in the latter procedure was anticipated since there was insufficient free 0_2 in the pure enzyme system to react with in β-D-glucose, glucose oxidase, and H_20 to form H_20_2 which oxidizes to o-dianisidine.

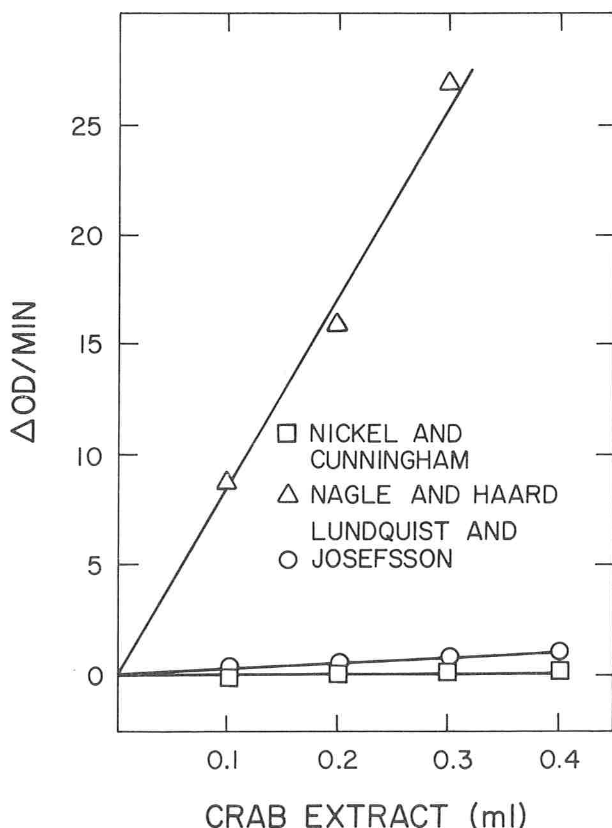

Figure 2. *Comparative sensitivity of three assays for the determination of peroxidase activity in raw blue crab extracts.*

A comparison of the relative sensitivity of each assay procedure tested toward peroxidase present in a mixed raw crab extract is given in Fig. 2. The modified Nagle and Haard (*9*) procedure was the most sensitive. The Nickel and Cunningham (*10*) procedure, even though it was very sensitive to a pure standard, exhibited essentially no change in optical density with the enzyme extract. Nickel and Cunningham (*10*) stated that the reactivity of 2,3',6-trichloroindophenol (leuco-TIP), the substrate in this assay, may be disadvantageous in assaying crude systems; however, no indication was given as to why it does not work well in crude systems. Results obtained in this study agree with their findings. The Lundquist and Josefsson (*7*) procedure indicated only minor changes in optical density with the enzyme extract. Although their assay has been reported to be very sensitive for detection of peroxidase in animal tissue (*7*), the enzyme may need to be highly purified for optimum results with blue crabs.

The modified Nagle and Haard (*9*) procedure provides a sensitive, rapid, and quite reproducible method of measuring peroxidase activity in blue crab meat. This method is advantageous over others reported in the literature since it can be used with crude extracts and does not require enzyme purification. The modified procedure may also be useful in measuring peroxidase activity in other shellfish and finfish products.

Since peroxidase is considered to have an empirical relationship to off-flavors and off-colors in food products (*1,4,5,12*), research needs to be done to determine whether peroxidase is a cause of off-flavor development in pasteurized and cooked crab meat following prolonged storage under refrigerated (32-36 F) and frozen conditions.

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Effect of Heat Treatments on Survival and Growth of a Psychrotroph and on Nitrogen Fractions in Milk[1]

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ABSTRACT

Grade A raw milk which had initial psychrotrophic counts of less than 10^3/ml was inoculated with an antibiotic-resistant *Pseudomonas* sp. to a final cell concentration of 10^2, 10^4, or 10^6/ml. The inoculated milk was held at 4 C for 14 h and then exposed to the following time-temperature treatments: 72 C for 15 sec, 79 C for 15 sec, 88 C for 10 sec, and 95 C for < 5 sec. An uninoculated raw milk control was handled and analyzed along with inoculated samples. Aliquots of milk were analyzed for marked *Pseudomonas* sp., total psychrotrophic counts, numbers of *Pseudomonas*, and for distribution of nitrogen before and after each heat treatment and after storage of non-heat-treated raw milk and heat-treated samples for 7 and 14 days at 7 C. Psychrotrophic counts were significantly affected by heat treatment, initial cell inoculum, days stored, and plating media. Non-casein N, non-casein protein, total albumin, β-lactoglobulin, proteose-peptone, and globulin N were significantly decreased by heat treatment. Non-casein N, non-casein protein, β-lactoglobulin, and proteose-peptone were significantly increased by days of storage.

The trend towards extended storage at refrigeration temperatures of raw and pasteurized milk has resulted in psychrotrophs becoming important determinants of the quality and shelf-life of milk and milk products. Survival of small numbers of psychrotrophs during pasteurization could result in a decreased shelf-life of the finished product. Most research has indicated that psychrotrophs do not survive proper pasteurization and that their presence in processed milk is due to post-pasteurization contamination (*13, 16*). Some investigators have reported that small numbers of psychrotrophs may survive pasteurization (*10, 15, 17*).

Pasteurization may create conditions which are conducive for microbial growth by causing a breakdown of milk proteins into smaller nitrogen fractions; thereby, making nutrients more readily available for microorganisms which survive the heat treatment.

The objectives of this study were to determine: (*a*) if a selected psychrotrophic *Pseudomonas* species could survive different heat treatments and subsequently grow in milk during low temperature storage, and (*b*) the effect that different heat treatments had on nitrogen fractions of milk.

MATERIALS AND METHODS

The microorganism used in this study was isolated from manufacturing grade raw milk. Psychrotrophic isolates were obtained from Pseudomonas F agar (Difco) streak plates which had been incubated at 7 C for 10 days. Colonies which appeared to have characteristics of species in the genus *Pseudomonas* were picked into Trypticase Soy Broth (BBL:TSB), incubated at 25 C for 24 h, transferred to Schaedler agar (BBL:SA) slants, incubated at 25 C for 18 h and stored at 2 ± 1 C until identified.

Standard methods and procedures were used to identify the gram-negative isolates as *Pseudomonas* species (*3, 4*). The species used in this study was obtained using the following procedure. Isolates identified as *Pseudomonas* were streaked onto plates of a glucose-salts minimal agar (MA) (*11*) supplemented with one of the following: (*a*) Keflin (sodium cephalothin, Eli Lilly and Co.)—250 μg/ml; (*b*) Keflin-500 μg/ml; (*c*) streptomycin sulfate—500 μg/ml; (*d*) Keflin—250 μg/ml plus streptomycin sulfate—250 μg/ml; and (*e*) Keflin—500 μg/ml plus streptomycin sulfate—500 μg/ml. Minimal agar was selected since most pseudomonads can grow on this medium while certain other bacteria present in milk microflora cannot. The antibiotics Keflin and streptomycin were chosen because of their selective inhibitory action. Keflin is inhibitory to most bacteria, with most pseudomonads being resistant. However, most pseudomonads are sensitive to streptomycin; therefore, the combination of the two antibiotics and minimal agar should provide a medium selective for *Pseudomonas* species. Isolates resistant to 250 or 500 μg/ml of one or both antibiotics were picked into TSB, incubated at 25 C for 24 h, then restreaked onto MA plus 250 μg Keflin/ml plus 250 μg streptomycin/ml (MAA) and incubated at 25 C for 48 h. Isolates growing on MAA were placed on SA slants, incubated at 25 C for 24 h, then stored at 2 ± 1 C. An isolate was randomly selected from the species resistant to both antibiotics and designated *Pseudomonas* sp. LW. This species was used throughout the study. Periodic checks were made of stock cultures of this species to be certain that its resistance to Keflin and streptomycin had not been lost during storage.

Grade A raw milk obtained from the dairy herd owned and operated by the Department of Animal Sciences, University of Kentucy was used in this study. The milk had initial psychrotrophic counts of 10^3/ml or less.

The inoculum was prepared by growing *Pseudomonas* sp. LW on SA slants or in TSB for 18 h and diluting the growth on the slant or in the broth with phosphate buffered distilled water or TSA. Addidional dilu-

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tions were made using sterile 10% skim milk (Difco), so that final cell concentrations of 10^2/ml, 10^4/ml, and 10^6/ml were obtained when 100 ml of skim milk were added to the Grade A raw milk. Raw milk containing the inoculum and an uninoculated control were agitated for 1 h and then held at 4 C for 14 h to allow *Pseudomonas* sp. LW time to adjust to its new environmental conditions.

A Cherry-Burrell Model Spira-Therm laboratory pasteurizer was used for all heat treatments. After heat treated milk passed through the cooling unit, samples were collected aseptically into sterile Erlenmeyer flasks and flasks were stored at 7 C for 14 days. The collection point and surrounding area were sanitized with a 200 ppm solution of sodium hypochlorite before and after samples from each treatment were collected to minimize contamination of samples.

Each trial consisted of collecting three aliquots of milk per treatment for six treatments (Trt); (*a*) Trt 1-control (uninoculated raw milk); (*b*) Trt 2-inoculated raw milk after storage at 4 C for 14 h; (*c*) Trt 3-milk from Trt 2 after being heated at 72 C for 15 sec; (*d*) Trt 4-milk from Trt 2 after being heated at 79 C for 15 sec; (*e*) Trt 5- milk from Trt 2 after being heated at 88 C for 10 sec; and (*f*) Trt 6-milk from Trt 2 after being heat at 95 C for < 5 sec.

Enumeration of the antibiotic resistant *Pseudomonas* sp., psychrotrophs, and total *Pseudomonas* as well as analysis of the nitrogen fractions were done on each aliquot on the day collected (day 0) and after storage at 7 C for 7 and 14 days. All enumerations were done using the surface spread plate technique. Dilution of 10^0 was obtained by dividing 1 ml of milk among five plates. Additional decimal dilutions were prepared using phosphate buffered distilled water and plating 0.1 ml of the appropriate dilution. All plates were incubated at 7 C for 10 days. MAA was used to enumerate the antibiotic resistant *Pseudomonas* sp. Psychrotrophic counts were determined using SA. This is a general purpose medium which should provide counts of viable cells, injured and uninjured, in the samples and was selected because injured cells may be inhibited by selective media (*9*). The number of *Pseudomonas* was determined using Pseudomonas Isolation agar (Difco:PIA), a medium selective for *Pseudomonas*. Use of these three media should permit enumeration of the antibiotic resistant *Pseudomonas* sp. (MAA), of *Pseudomonas* (PIA), and of total psychrotrophs (SA). Since initial studies indicated that plating efficiency of these three media were similar for *Pseudomonas* sp. LW, differences in counts between SA and MAA should give an estimate of the number of injured cells or MAA-sensitive cells in the samples. The difference between PIA and MAA should indicate the number of antibiotic-sensitive *Pseudomonas* present in the sample.

The procedure of Aschaffenburg and Drewry (*1*) was used to fractionate milk samples into nitrogen fractions. Their nomenclature was used for the fractions obtained. The amount of nitrogen in each fraction was determined using the auto-Kjeldahl.

A split-plot design was used. Data were analyzed using the Statistical Analysis System (*5*). The F-test was used to determine significance at the 1 and 5% levels.

RESULTS AND DISCUSSION

The psychrotrophic mutant *Pseudomonas* sp. LW used in this study had characteristics similar to *Pseudomonas fluorescens* as well as being resistant to both Keflin and streptomycin.

Initial counts (0 day) and counts of samples after storage at 7 C for 7 and 14 days are shown in Table 1 for each treatment. The largest increase in counts occurred during the inital 7 days of storage of uninoculated raw milk samples (Trt-1) and of inoculated, non-heat treated samples (Trt-2). Samples from these two treatments showed little additional increase in counts during the last 7 days of storage. The results obtained for these samples were expected based on previous studies which have shown that psychrotrophs will increase within 7 days 7 C, especially when a sample is inoculated and stored at low temperature. Increases in counts obtained from samples from these two treatments were over 10-fold greater when

TABLE 1. *Total counts of milk samples stored at 7 C for 0, 7, and 14 days*

Treatment	Initial inoculum	Media								
		SA[a]			PIA[b]			MAA[c]		
		Storage time (days)								
		0	7	14	0	7	14	0	7	14
		(log_{10}/ml)								
Uninoculated raw milk	10^2/ml	3.4[d]	8.2[d]	8.5[d]	3.7	7.2	7.9	1.7	5.2	5.5
Inoculated raw milk[f]		3.0	8.0	8.0	3.2	7.0	7.7	2.5	6.0	6.0
72 C for 15 sec[g]		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
79 C for 15 sec[g]		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
88 C for 10 sec[g]		0.0	0.0	0.6	0.0	0.0	0.0	0.0	0.0	0.0
95 C for < 5 sec[g]		0.1	0.7	0.7	0.0	0.0	0.1	0.0	0.0	0.0
Uninoculated raw milk	10^4/ml	2.9[d]	7.5	8.7	2.8	6.6	7.8	1.4	2.9	3.8
Inoculated raw milk		3.8	7.8	8.7	3.6	7.5	8.0	2.6	4.1	5.1
72 C for 15 sec		0.0	0.0	0.2	0.0	0.0	0.0	0.0	0.0	0.0
79 C for 15 sec		0.0	0.0	0.0	0.0	0.2	6.1	0.0	0.1	0.1
88 C for 10 sec		0.1	0.1	2.2	0.1	0.0	0.0	0.0	0.0	0.0
95 C for < 5 sec		0.2	0.8	3.5	0.0	0.2	0.9	0.0	0.1	0.5
Uninoculated raw milk	10^6/ml	3.5[e]	7.8	8.3	3.4	7.3	7.4	2.1	6.2	6.2
Inoculated raw milk		5.2	7.8	8.1	5.3	7.6	7.5	5.1	7.2	6.9
72 C for 15 sec		1.2	3.0	6.1	0.7	3.5	6.4	0.0	2.1	4.7
79 C for 15 sec		1.0	3.8	6.6	0.7	4.6	6.4	0.0	4.6	6.6
88 C for 10 sec		1.2	3.8	6.5	0.6	4.1	6.7	0.0	1.9	4.6
95 C for < 5 sec		1.2	3.9	6.7	0.6	4.0	6.7	0.0	1.6	4.5

[a]Schaedler agar.
[b]Pseudomonas Isolation agar.
[c]Minimal agar containing 250 μg/ml each of Keflin and streptomycin.
[d]Mean of 9 samples.
[e]Mean of 15 samples.
[f]Inoculated raw milk was held at 4 C for 14 h before being used for heat treatments.
[g]Inoculated raw milk (f) used for all heat treatments.

plated with SA than when plated with either PIA or MAA.

The psychrotrophic counts were compared on the basis of initial cell inoculum (concentration), heat treatments, days of storage, and media. The results of analysis of variance using a regression procedure are shown in Table 2. Significant differences ($P < .01$) were found for all factors studied.

TABLE 2. *Analysis of variance of factors affecting psychrotrophic plate counts*

Source	Degrees of freedom	Level of Significance
Concentration (Conc)	2	A[a]
Treatment (Trt)	5	A
Day	2	A
Media	2	A
Conc × Trt	10	A
Conc × Day	4	A
Day × Media	4	A
Conc × Media	4	A
Trt × Media	10	A
Trt × Day	10	A

[a]A = Significant ($P < .01$)

Milk samples which had 10^2 cells/ml added either had no detectable cells after heat treatment or numbers had increased less than one log during the 14-day storage. Samples from Trt-6 showed a slight increase in count after 7 days of storage when plated with SA and after 14 days when plated with PIA. No increase in count was detected for these samples when plated with MAA. These results suggest that the psychrotroph added to raw milk did not survive heat treatment or that survivors were unable to grow on MAA after storage for 14 days.

When initial inoculum was increased to 10^4 cells/ml, all 14-day samples except those from Trt-4, showed increases in counts on SA. A two-log increase in count occurred during the last week of storage for samples from Trt-5 and Trt-6. Samples from Trt-6 showed increases in counts after 7 days when plated with SA and PIA and after 14 days with MAA.

Increasing the initial inoculum to 10^6 cells/ml resulted in growth on the 0-day plates with SA and PIA as well as increases in counts for all samples when plated on all three media after storage for 7 days. The fact that counts were greatest on the SA at 0-day platings yet were similar after 7 days of incubation suggests that some injured cells were present which could not grow on selective media.

These results indicate that the psychrotroph used in this study could survive heat treatments presently used to pasteurized milk, when the initial psychrotrophic concentration in the raw milk is high enough. Most growth in heat-treated samples occurred after 7 days of storage, indicating that this psychrotroph may have been injured and needed time for repair before it could multiply. Another possibility is that survivors needed time to adjust to the new environmental conditions created by the heat treatments. These results are in agreement with those of other investigators (*2, 13*) who indicated that pasteurized milk needed to be stored at 7 C for at least 1 week before heat-resistant psychrotrophs could be detected.

To determine where significant difference existed between treatments, plate counts from three inoculum levels and three media were combined and a mean count determined for each treatment. When the mean counts of each treatment were compared, raw milk counts were significantly higher than populations enumerated from heat-treated milks. After mean counts of raw milk samples were removed from the analysis, populations in heat-treated samples still differed ($P < .01$) from each other. Therefore, the Bayesian Least Significant Difference (B. L. S. D.) (*14*) procedure was used to compare plate counts of heat-treated samples with each other. Analysis showed that counts from samples in Trt-3, Trt-4, and Trt-5 were lower ($P < .05$) than plate counts from samples in Trt-6, but did not differ significantly from each other. Therefore, under our conditions, use of a lower temperature for a longer time appeared more efficient in destruction or injury of this psychrotroph. Another possibility is that higher temperatures may cause greater destruction and/or injury, but also create conditions which favor faster recovery of injured cells compared with lower temperatures.

To determine if a change occurred in nitrogen fractions of milk, milk samples from each treatment were separated into seven fractions: non-casein nitrogen

TABLE 3. *Mean nitrogen values in each fraction for each treatment stored at 7 C for 0, 7, and 14 days for milk containing an initial inoculum of 10^2 cells per milliliter*

		Nitrogen fraction (mg N/100 ml)[a]																				
		NCN			NCPN			TA			L			RA			PP			GN		
		Storage time (days)																				
Treatment	Initial inoculum	0	7	14	0	7	14	0	7	14	0	7	14	0	7	14	0	7	14	0	7	14
Uninoculated raw milk	10^2/ml	106[b]	109	121	74	84	90	59	61	48	21	35	21	38	26	27	17	12	17	7	42	27
Inoculated raw milk		105	114	107	76	86	74	58	62	48	36	39	30	22	24	22	17	17	12	3	27	20
72 C for 15 sec		95	114	106	65	80	79	53	53	56	18	35	34	35	19	22	2	18	11	26	22	15
79 C for 15 sec		93	108	106	64	84	73	50	56	50	26	44	24	24	14	26	16	46	15	8	11	29
88 C for 10 sec		87	80	92	61	52	62	51	50	45	18	22	20	33	28	26	10	6	14	5	6	13
95 C for < 5 sec		84	79	93	58	51	60	48	45	41	20	20	12	29	26	29	1	7	18	22	6	35

[a]NCN = non-casein nitrogen; NCPN = non-casein protein nitrogen; TA = total albumin; L = β-lactoglobulin; RA = residual albumin; PP = proteose-peptone; GN = globulin nitrogen.
[b]Mean of 6 samples.

(NCN), non-casein protein nitrogen (NCPN), total albumin (TA), β-lactoglobulin (L), residual albumin (RA), proteose-peptone (PP), and globulin nitrogen (GN). The amount of nitrogen in each fraction was determined. The mean nitrogen values obtained for each treatment after storage at 7 C for 0, 7, and 14 days are given in Tables 3, 4 and 5.

The mean nitrogen values of the fractions were compared, using a regression procedure to determine the effect of bacterial concentration, heat treatment, and days of storage on these fractions. The results are shown in Table 6. Bacterial concentration had no significant effect on any fraction. As days of storage increased, the amount of nitrogen in NCN, NCPN, L, and PP increased significantly. These results are similar to those of Kanauchi et al. (*8*) who found that NCPN and PP increased as days of storage increased. Part of the increase during storage could be due to increase in microbial protein, especially the NCN, NCPN, and PP. This is indicated by the interaction of concentration and days of storage in these fractions being significant ($P \leqslant .05$). Additional studies will be needed to determine if nitrogen values are affected by microbial proteins, milk enzymes, or microbial enzymes released during destruc-

TABLE 4. *Mean nitrogen values in each fraction for each treatment stored at 7 C for 0, 7, and 14 days from milk containing an initial inoculum of 10^4 cells per milliliter*

		Nitrogen fraction (mg N/100 ml)[a]																				
		NCN			NCPN			TA			L			RA			PP			GN		
	Initial	Storage time (days)																				
Treatment	inoculum	0	7	14	0	7	14	0	7	14	0	7	14	0	7	14	0	7	14	0	7	14
Uninoculated raw milk	10^4/ml	105[b]	122	114	73	89	80	60	68	58	35	38	29	26	32	28	7	21	17	25	5	14
Inoculated raw milk		109	123	115	80	89	83	59	70	71	36	35	28	24	35	43	12	13	15	9	5	19
72 C for 15 sec		109	120	111	78	86	79	62	67	62	29	27	22	33	41	40	12	14	18	7	9	5
79 C for 15 sec		103	116	109	68	82	75	53	60	58	15	23	22	38	38	37	12	17	17	9	4	9
88 C for 10 sec		91	108	102	59	76	67	54	59	52	23	20	18	31	40	34	8	15	28	16	5	3
95 C for < 5 sec		89	102	91	56	70	55	42	50	45	16	21	14	26	30	32	7	6	12	11	23	9

[a]NCN = non-casein nitrogen; NCPN = non-casein protein nitrogen; TA = total albumin; L = β-lactoglobulin; RA = residual albumin; PP = Proteose-peptone; GN = globulin nitrogen.
[b]Mean of 6 samples.

TABLE 5. *Mean nitrogen values in each fraction for each treatment stored at 7 C for 0, 7, and 14 days for milk containing an initial inoculum of 10^6 cells per milliliter*

		Nitrogen fraction (mg N/100 ml)[a]																				
		NCN			NCPN			TA			L			RA			PP			GN		
	Initial	Storage time (days)																				
Treatment	inoculum	0	7	14	0	7	14	0	7	14	0	7	14	0	7	14	0	7	14	0	7	14
Uninoculated raw milk	10^6/ml	97[b]	108	125	69	77	96	61	53	71	25	24	31	36	29	40	7	8	28	8	18	6
inoculated raw milk		97	113	119	67	81	87	60	53	68	21	24	32	39	29	37	8	13	20	3	16	7
72 C for 15 sec		93	108	115	64	78	86	61	65	67	26	26	32	34	39	35	6	13	23	4	6	5
79 C for 15 sec		87	97	111	60	70	81	60	60	63	26	27	27	33	33	35	5	6	23	3	10	3
88 C for 10 sec		80	88	100	45	63	62	47	63	47	17	27	16	32	36	35	4	12	19	4	6	3
95 C for < 5 sec		81	90	98	54	63	61	54	49	50	19	22	16	34	27	37	9	9	19	10	8	3

[a]NCN = non-casein nitrogen; NCPN = non-casein protein nitrogen; TA = total albumin; L = β-lactoglobulin; RA = residual albumin; PP = proteose-peptone; GN = globulin nitrogen.
[b]Mean of 10 samples.

TABLE 6. *Analysis of variance of factors affecting nitrogen fractions in milk*

		Level of significance						
	Degrees of	Nitrogen fractions[a]						
Source	freedom	NCN	NCPN	TA	L	RA	PP	GN
Concentration	2	ns[b]	ns	ns	ns	ns	ns	ns
Treatment	5	A[c]	A	A	A	ns	B[d]	B
Day	2	A	A	ns	A	ns	A	ns
Treatment × Day	10	ns	ns	ns	ns	ns	ns	ns
Concentration × Treatment	10	ns	ns	ns	B	ns	B	ns
Concentration × Day	4	A	A	ns	ns	B	A	B
Concentration × Treatment × Day	20	ns	ns	ns	ns	ns	B	ns

[a]NCN = non-casein nitrogen; NCPN = non-casein protein nitrogen; TA = total albumin; L = β-lactoglobulin; RA = residual albumin; PP = proteose-peptone; GN = globulin nitrogen.
[b]ns = Non-significant
[c]A = Significant ($P < .01$).
[d]B = Significant ($P < .05$).

tion or lysing of cells.

The amount of nitrogen in each fraction decreased as temperature of treatment increased. This same phenomenon was shown by other investigators (*6, 7, 12, 18*) using more severe heat treatments. Our data were analyzed to determine where the significant differences between treatments existed (Table 7). This analysis showed that Trt-1 differed significantly from Trt-2 for fractions NCN, NCPN, and GN. These results suggest that microbial growth had an effect on these three fractions, since the only difference between the two treatments was microbial concentration. This effect apparently was negated in the initial analysis (Table 6) because the mean nitrogen values from all treatments for each concentration were combined for the comparisons. Comparison of nitrogen values of heat-treated samples showed significant differences between samples for all fractions except RA and GN (Table 7).

An additional analysis of results from heat-treated samples was made using the B.L.S.D. procedure to determine where differences existed. The amount of nitrogen in the NCN and NCPN fractions from Trt-3 and Trt-4 was significantly greater than from Trt-5 and Trt-6, but no significant difference existed between Trt-5 and Trt-6. The amount of nitrogen in fractions TA and L decreased as temperature of treatment increased, but a significant difference in nitrogen values was not observed except in samples from Trt-5 and Trt-6. These results are similar to those obtained by Harland et al. (*7*) for milk serum protein. For fraction PP the only significant difference was between Trt-4 and Trt-6.

The results of this study show that as the concentration of a psychrotrophic *Pseudomonas* sp. was increased in raw milk, a greater number of the bacteria survived the heat treatments, and that higher temperatures for shorter holding times were less effective than lower temperatures for longer times in destruction of the psychrotroph. Heat treatment and days stored had a varied effect on the amount of nitrogen in fractions obtained from milk which could possibly influence the microbial growth.

TABLE 7. *Analysis of variance of the effect of treatments on nitrogen fractions*

Source	Degrees of freedom	Nitrogen fraction[a]						
		NCN	NCPN	TA	L	RA	PP	GN
Trt 1[b], 2[c] vs Trt 3, 4, 5, 6[d]	1	A	A	B	A	ns	ns	B
Trt 1 vs Trt 2	1	A	A	ns	ns	ns	ns	B
Trt 3, 4, 5, 6	3	A	A	A	A	ns	A	ns

A = Significant ($P < .01$).
B = Significant ($P < .05$).
ns = Non-sifnificant.
[a]NCN = non-casein nitrogen; NCPN = non-casein protein nitrogen; TA = total albumin; L = β-lactoglobulin; RA = residual albumin; PP = proteose-peptone; GN = globulin nitrogen.
[b]Uninoculated raw milk
[c]Inoculated raw milk
[d]Heat-treated milk

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Report of the Journal Management Committee — 1977

The Journal Management Committee met at 9:00 a.m. on August 8, 1977. The members present were: Dr. C. K. Johns, Dr. Elmer Marth, Mr. Paul Pace, Mr. Donald Raffel, Mr. Robert Sanders, and Dr. R. B. Read, Chairman.

The Committee reviewed its previous recommendations and is concerned that several of these have not been acted upon by the Executive Board. The Committee would like to repeat several recommendations from its previous report and recommends that these be acted upon. These recommendations are:

1. The Committee recommends that the Journal publish condensed announcements of new products and processes of interest to the membership together with means for the membership to request and receive detailed information about these products or processes.

2. The Committee recommends that each affiliate appoint a correspondent to submit reports of (*a*) affiliate activities, (*b*) implementation of new regulations, (*c*) construction of new food plants or the development of new processes for foods, (*d*) programs of affiliate annual meetings, and (*e*) other items of interest to the membership for publication in the Journal.

3. To obtain more material for the Journal of direct interest to the practicing sanitarian, we recommend that the Assistant Managing Editor of the Journal review material in other publications to identify and obtain appropriate articles for reprinting in the Journal.

4. We recommend that a system be developed wherein the issues of the Journal can be partially identified as to date by an identification at the fold of the cover.

In addition to reaffirming the above recommendations, the Committee further recommends:

1. The Executive Board authorize the placement of advertisements for the Journal in such publications as *Food Technology, Food Product Development, Food Processing,* and the *Journal of the American Dietetics Assoc.* provided that this be economically feasible. Similarly, we recommend that suitable exhibits be given at meetings of the IFT, APHA, and the American Dietetics Assoc.

2. We recommend that four committees of three or four persons each be appointed which would report to the Assistant Managing Editor of the Journal. Each committee would be charged with the responsibility of developing a list of topics of current concern that should be discussed in the Journal and with identifying persons who could prepare articles on the topics of concern. The recommended committees are as follows:

(a) A committee of active and knowledgeable sanitarians who are in the field. Membership in this committee would be at the recommendation of the Affiliates.

(b) A committee of active and knowledgeable dairy fieldmen. Membership in this committee would be at the recommendation of the National Association of Dairy Fieldmen Affiliate.

(c) A committee concerned with the foodservice industry. This committee would be appointed by the Journal editor.

(d) A committee of research scientists responsible for identifying subjects and authors for review articles. This committee would be appointed by the Journal editor.

3. We recommend that an asterisk be placed after the Journal article author's name to whom reprint requests should be sent.

4. We recommend that the Table of Contents page be restructured so that the titles of the papers and the authors be in larger type. This can be done by expanding the format to make a 2-page spread with the inclusion of the list of sustaining members to fill out the 2 pages.

5. We recommend that the list of Affiliates be published quarterly instead of monthly.

6. We recommend that an overseas subscription to the Journal carry a two dollar surcharge to cover the increased mailing cost.

7. We recommend that each issue of the Morbidity and Mortality Weekly Report of the CDC and Consumer Reports of the FDA be reviewed by the Assistant Managing Editor for items of interest to the membership and that appropriate articles be reprinted in the Journal.

R. B. READ, Jr.
Chairman
Journal Management Committee

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Heated Yogurt — Is It Still Yogurt?

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ABSTRACT

Traditional yogurt contains viable cells of the culture used as starter for making the product. The culture provides the enzyme lactase in the finished yogurt which has been reported to continue hydrolysis of lactose when the cells are lysed in the intestinal tract. Heating yogurt to inactivate the starter for shelf-life prolongation also inactivates the lactase. This could become an unsuspected problem for lactose intolerant persons who can consume traditional yogurt.

The practice of including large quantities of micro-organisms in the diet probably had its greatest impetus from the studies of Metchnikoff. As a result of his studies on prolongation of life, he theorized that people in the Balkan countries enjoyed long life because of the large quantities of cultured milk (yogurt) which they consumed. He proposed that the lactobacilli in this milk took up residence in the intestinal tract and overgrew the toxin producing putrefactive microorganisms that otherwise would exist in the intestine. Although cultured milk products had been used for centuries, this was the first time that any real effort had been made to attach any nutritional value to the microorganisms used in making such products. Previously, the main interest had been in producing cultured and acidified milk products so that they could be preserved, and because such products often had desirable flavor and texture qualities.

Following Metchnikoff's publications, many researchers began investigating the implantation of lactobacilli in the intestine by drinking milk in which these bacteria had grown. Later it was discovered that the cultures used in making yogurt did not survive or implant and grow in the intestinal tract. However, a closely related microorganism, *Lactobacillus acidophilus,* did possess this property and, thereafter, most of the studies dealing with implantation of bacteria in the intestine dealt with *Lactobacillus acidophilus* (*5*). Nevertheless, over the years many people felt that yogurt did impart certain nutritional qualities to their diet that were not obtained by consuming other foods, but yogurt consumption remained at a relatively low level until the 1960's. The per capita sales of yogurt since 1955 has grown from .11 to 2.33 lb. in 1976. Such phenomenal growth was caused by many factors. Many people had discovered and enjoyed the delightful yogurt flavors, especially those obtained by addition of fruits. It must be suspected also that many people just feel good by inclusion of yogurt in their diet, and that they would not continue to eat it if it caused any type of digestive distress.

BENEFITS OF YOGURT

In spite of the nutritional benefits that consumers ascribe to yogurt, nutritionists have been hard pressed to identify any particular ingredient that may be responsible. Usually yogurt is made from milk which has been fortified with extra milk solids so that the resulting product contains more of the normal milk nutrients, but this is inadequate to explain any particularly unique nutritional property of yogurt. However, Gallagher et al. (*2*) showed that people who were lactose intolerant were able to digest yogurt, buttermilk and cottage cheese without any problems. They recommended that such persons should be encouraged to consume these cultured products as a source of their calcium requirements. The implication from this study suggested that cultured milk products possess some property that would allow lactose intolerant persons to digest such products with no difficulty.

Goodenough and Kleyn (*3*) studied the influence of viable yogurt microflora on the digestion of lactose in rats. Their study showed that animals fed natural yogurt containing viable yogurt culture were able to digest lactose more efficiently than animals fed other experimental diets, including pasteurized yogurt. They attributed this to the viable cells in yogurt that presumably enabled the rats to digest the lactose in the intestinal tract. However, no specific evidence was presented that showed the yogurt culture was able to remain viable in the intestinal tract. Kilara and Shahani (*4*) also studied the lactase activity of cultured and acidified dairy products including yogurt. They concluded that the yogurt culture when disintegrated, as occurs in the intestinal tract, releases the enzyme lactase which continues to break down lactose in the dairy product. These studies point to the importance of the enzyme present in the cells of the yogurt culture. Thus, the lactase would break down the lactose contained in the dairy products and, thereby, allow lactose intolerant people to consume these products with no subsequent problems.

It is becoming increasingly clear that benefits from yogurt cultures derived by consumers are dependent on the contents of the culture rather than the necessity of the yogurt culture being viable in the intestinal tract. Evidence is mounting that the activity of the enzyme lactase within these cultures is of definite value to the consumer. Consequently, our attention should be focused on factors that affect production of this enzyme by yogurt cultures, and on factors which may affect the enzyme's stability in yogurt. This leads us to a consideration of current yogurt manufacturing processes.

MANUFACTURE OF YOGURT

A natural development in the increased production of yogurt has been centralization of manufacturing operations. As a result, yogurt is shipped over much longer distances for distribution. To prevent additional acid formation by the culture during periods when refrigeration may not be adequate or when storage conditions may be too long, there has been an interest in heat-treating yogurt before packaging and distribution. This inactivates the yogurt culture and eliminates further acid development. For some years, there has been disagreement on whether or not such destruction of yogurt starter would have any adverse effects on the constituents of yogurt. Presumably, the relatively mild heat treatment required to inactivate the starter would be suspected of having inconsequential results on any constituents, although conclusive studies on this have not been reported. Considering the importance of lactose intolerance to consumers, we have conducted a number of studies dealing with stability of the enzyme lactase within yogurt after various pasteurization treatments. Our studies have shown that indeed the starter cultures are very easily inactivated by temperatures of about 140 F for as short a time as several minutes. However, treatments that inactivate the culture also inactivate the lactase present in the starter culture and in the yogurt. We have extended our studies to samples of yogurt from the retail market and have found that those samples that contain no yogurt starter are also deficient in the enzyme lactase. When viable yogurt culture and lactase are absent, it can be concluded that some procedure has been practiced to cause such deficiences, whether it is heat treatment or the manufacture of the product without culture. In such instances, however, it must be realized that the product labeled as yogurt has lost some identity with what traditionally has been known as yogurt.

FROZEN YOGURT

Another development in yogurt manufacture has been frozen yogurt. We have studied the levels of starter culture and the enzyme lactase in different steps involved in the manufacture of soft frozen yogurt. No culture or lactase activity is present in any of the products until yogurt is added to the other ingredients. Flavoring has no effect other than that expected by the small dilution caused by its addition. Freezing of the yogurt results in a slight reduction in the total counts of the starter cultures, but causes an increase in the amount of lactase enzyme present in the yogurt. This increase is caused by the leaking of the enzyme from cells of the starter culture which have been affected by the freezing. The yogurt has been stored up to 8 weeks, and while some lowering of culture viability occurs, it is small; but, more important, the lactase activity remains high. Thus, the freezing of yogurt appears to have no harmful effect on the lactase activity contained in the yogurt.

It is becoming increasingly clear that in the manufacture and processing of yogurt, particular attention should be on the fate of the lactase enzyme. Of course, the yogurt culture must have grown sufficiently to produce the needed acid and other metabolic by-products for yogurt manufacture; but, thereafter, the lactase produced takes precedence over culture viability. Freezing can damage the culture somewhat, but this has no adverse effect on the lactase. Heat treatments, on the other hand, which inactivate the starter culture also inactivate the lactase enzyme. This is a situation that could have a very undesirable effect on many consumers.

WHAT ABOUT THE CONSUMER?

There must be many people who have some degree of lactose intolerance who are able to eat yogurt with no adverse effects through the assistance they receive from the lactase activity from the starter. Such persons undoubtedly do not understand why they can enjoy eating yogurt. If such persons consumed yogurt containing no lactase (i.e., heated yogurt) they could no longer eat yogurt with the anticipated benefits normally obtained from the yogurt culture's enzyme. This seems more than an adequate reason for not using a heat treatment of yogurt for extending its shelf-life.

This, then, should lead the industry to consider what it may have at stake concerning the integrity of its product labeled as yogurt. The heating of yogurt to kill the culture tends to become an additional alteration in the composition of the product to remove its identity further from what traditionally has been known as yogurt.

FDA PROPOSAL

The industry is facing a dilemma over the question of whether or not to heat treat yogurt in view of a proposal by the Food and Drug Administration to establish new identity standards for certain cultured milk products (*1*). In this proposal, yogurt could be heat treated after culturing if the product is so labeled. It was stated, however, that "the Commissioner believes it is in the best interests of the consumer to preserve the food in its traditional form, i.e., containing live microorganisms, and to provide for labeling to inform consumers when yogurt has been heat-treated after culturing." In explaining the FDA proposal to allow heat inactivation of the culture to extend shelf-life of yogurt, the proposal

also states "except for destroying the microorganisms, these foods retain essentially the same characteristic attributes." Obviously, the FDA considered it desirable to have the viable culture present in yogurt, but did not have the data of our study before preparation of its proposal to permit heat treatment of yogurt.

The labeling of yogurt as "heated" when heat has been applied to inactivate the culture is not a satisfactory way to inform consumers that the product contains no live culture, or any lactase. Consumers have been taught to construe the heat treatment of milk products as beneficial—which obviously would be just an opposite effect when applied to yogurt. For consumers to be denied a component present in traditional yogurt could reflect unfavorably on the credibility of the product and the industry.

There may be some question as to whether the presence of lactase originating from the yogurt culture can be considered a nutritional component. This seems to be a problem more of semantics rather than one of real importance to the industry and the consumer. Obviously, any constituent of yogurt that contributes to the well being of the consumer is functioning within the broad concept of a nutrient. We must realize that intolerance to lactose is not an all or nothing situation found only among a small minority of ethnic groups; various degrees of lactose intolerance exist in people of most races. Furthermore, there may be additional constituents formed by cultures in yogurt that have benefits that still are not identified. With the information that is available, it appears that yogurt and the presence of viable yogurt starter, or at least the enzymes that these starters contain, can play a very important role in the health and well being of consumers. Heating of yogurt is not consistent with preserving its nutritional attributes.

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Commercially Available Minikits for Identification of *Enterobacteriaceae*: A Review

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ABSTRACT

Most of the research pertaining to evaluation and use of various commercial *Enterobacteriaceae* test kits has been conducted by clinical microbiologists. This paper reviews more than 50 reports of studies in which a number of these diagnostic kits were compared to conventional biochemical tests for characterization and identification of *Enterobacteriaceae*. It is hoped that this information will be of value to food and environmental microbiologists who are concerned with identification of *Salmonella, Escherichia coli,* "coliforms," and other members of this family.

The search for convenient and rapid methods and systems for identification of microorganisms isolated from clinical specimens, and samples of food and environment has motivated development of a variety of miniaturized microbiological techniques and diagnostic kits that aim to save space, material, labor, and cost. Development of miniaturization techniques was reviewed in 1968 (*24*), 1972 (*17*), and 1975 (*18*). The basic approaches of these methods include use of small amounts of media (liquid or solid), convenient vessels with multiple small chambers with or without dehydrated or agar media, paper strips or discs impregnated with media, and development of ingenious inoculation and incubation systems.

Some of the commercial diagnostic kits have designs and concepts that are similar to many of the mini-tests described in the reviews (*24,17,18*). Commercial kits have some advantages over test systems prepared by individual laboratories. The products are (*a*) available in convenient packages, (*b*) easy to store and discard, (*c*) of uniform quality and (*d*) improved through research and development. In addition, consultation is available on interpretation of test results and reduction of costs. We now review results generated by various research groups on comparative evaluation of some of these commercial diagnostic kits to assist clinical and food laboratories or individuals in selecting systems for adaptation in their respective laboratories.

API-20E

This device, manufactured by Analytab Products, Inc., 200 Express street, Plainview, New York, 11803, is based primarily on the research of early workers who in 1968 established many of the physical and chemical requirements of such micromethods (*9*). This device is simply a modification of one of the many "little tube" methods (*24*).

The API system was used in 1971 to effectively identify 522 enteric and 79 *Moraxella* and *Pseudomonas* cultures (*22*). Other investigators (1972) efficiently and accurately identified 671 *Enterobacteriaceae* with the API system which was particularly effective for *Klebsiella, Enterobacter, Proteus* and *Providencia* (*3*). The API also was used to easily differentiate 78 *Salmonella* cultures from 22 non-*Salmonella* cultures (*38*). In 1971, when API was compared to conventional tests for identification of 128 *Enterobacteriaceae,* speciation was 88% correct (*53*). Later when the study was repeated with heavier inocula speciation was 93% correct and the authors concluded that API was the only commercially available system with which *Enterobacteriaceae* could be speciated with 90% accuracy.

Disadvantages mentioned were the time required for preparation and inoculation of the system and the care required in the tedious task of filling each tube (*54*). In a similar study with API, *Enterobacteriaceae* were identified with 96.4% accuracy (*49*). The authors reported that one disadvantage was moderate dehydration of some of the cupules during overnight incubation. They also suggested that perhaps dulcitol or malonate might be used to replace the test for amygdaline. They claimed, however, that advantages of API outweighed any disadvantages.

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In 1974, API was compared to conventional tests for identification of *Enterobacteriaceae.* Agreement was 88.2% initially but increased to 98% when cultures giving equivocal results with the API system were retested. The authors reported that the tests for lysine decarboxylase, citrate, and H_2S were often difficult to read in that it was not easy to differentiate a weak positive from a negative (*8*).

AUXOTAB [INOLEX ENTERIC]

This system is manufactured by Inolex Corporation, 3 Science Road, Glenwood, Illinois 60425. In 1972 researchers compared Auxotab Enteric 1 and 2 cards to conventional biochemical tests for identification of *Enterobacteriaceae* and *Pseudomonas* isolates. They concluded that the Auxotab system was unsatisfactory for identification of *Pseudomonas.* Also *Klebsiella, Enterobacter* and *Serratia* cultures were very difficult to characterize. For the individual biochemical tests, they found greater than 90% agreement except for arginine, ornithine, lysine, and malonate with *Escherichia coli*; ONPG with *Pseudomonas*; and citrate with *Proteus mirabilis.* The authors felt that many of the problems of this system could be eliminated by merely revising the literature included in the package and that contamination of Auxotab during inoculation was difficult to avoid (*11*).

The accuracy of the Auxotab Enteric 1 system was found to be adequate for identification of 160 *Enterobacteriaceae* cultures with 83.8% correct speciation and 90% accuracy at the generic level (*54*). The authors, however, thought the system was not a convenient alternative to the conventional method because it was tedious and would be difficult to begin and complete during an 8-h day. Centrifugation of broth cultures would require several centrifuges if large numbers of cultures were to be tested. Contamination of laboratory benches and laboratory personnel also was a real problem because filled capillaries spilled.

In 1973, others used Auxotab to correctly identify 87% of 417 known cultures of *Enterobacteriaceae* (*43*). They reported, however, that the H_2S test was too sensitive, but the tests for urease, malonate, lysine, and ornithine lacked sensitivity. Because of the inability of Auxotab to consistently produce clear-cut reactions, many reactions were subjectively rated positive or negative. This study indicated a need for product modification in regard to ease of handling, time required, and accuracy of identification for *Enterobacteriaceae.* Later in the year, the same investigators evaluated an improved Auxotab system and reported that it correctly identified 96% of the 200 *Enterobacteriaceae* cultures tested (*44*). Some of the problems of the system had been solved, sensitivity had been increased for the lysine, orithine, malonate, and H_2S tests, and the use of individual plastic incubation trays eliminated the contamination problem. However, the time required was unchanged and the oversimplified flow charts supplied by the manufacturer failed to adequately indicate variations frequently encountered with *Enterobacteriaceae.*

ENTEROTUBE

The manufacturer of this system is Roche Diagnostics Division, Hoffman-LaRoche, Inc., Nutley, New Jersey 07110.

Enterotube was compared, for 242 bacterial strains, to conventional tests and discrepancies were found for some of the tests (lysine, 87.6% agreement and urease, 90.9% agreement), while agreement was high for others, i.e. phenylalanine deaminase, 98.8% agreement and citrate, 97.9% (*15*). According to these investigators, use of the Enterotube without additional tests would result in the occasional misidentification of *Enterobacteriaceae* cultures. When, in another study, Enterotube was compared with routine conventional media for identification of 147 clinical cultures, agreement was 82.3% and the greatest cause for disagreement was the inability to differentiate *Enterobacter* and *Klebsiella.* These organisms could not be separated because this system did not include a test for ornithine decarboxylase (*36*). With regard to the individual tests, other researchers (*33*) reported excellent agreement for H_2S, indole, citrate, glucose, and lactose. However, for urea, dulcitol and phenylalanine deaminase, agreement was 85%. Also, the lysine test proved to be totally unsatisfactory. The authors suggested removal of lactose from the lysine test medium and replacing the tests of dulcitol and lactose with tests of ornithine and arabinose to permit separation of the genera in the tribe *Klebsiellae.* As a result of these 1971 studies, the ornithine test was added to the Enterotube system.

In 1973 agreement of the improved Enterotube system with the conventional tests after 24 h of incubation was 92.4% (*29*) and identification of 414 *Enterobacteriaceae* stock cultures was 96.4% correct (*52*). Accuracy was less than 90% for only two groups, *Salmonella* (89.2%) and *Edwardsiella* (87.5%). The authors reported that the Enterotube occasionally failed to detect weak positive reactions obtained with conventional media. However, they concluded that this system is a convenient, rapid, and accurate means of identifying typically reacting enterics (*52*). Both the Enterotube and conventional systems gave similar results for the identification of 586 clinical and stock cultures (*42*). In fact, results from the Enterotube agreed more closely with the findings reported in the percentage tables (*14*) than results from conventional media for the first approximation of identification. Enterotube was now considered highly useful for identifying enterics in clinical laboratories (*42*).

When the Enterotube was compared to conventional techniques in 1974, there was complete agreement for 97 of the 100 *Enterobacteriaceae* tested (*16*). In another study overall accuracy was 97.6% with 391 enterics (*56*). Most reactions showed an agreement of more than 98% except for lysine (95%), ducitol (94%), and citrate (92%). Identification was correct 84% of the time when the flow

diagram supplied by the manufacturer was used alone, 92% of the time when the flow diagram and the reaction chart were used together and 93% of the time when ENCISE was used (Enterotube Numerical Coding and Identification System for *Enterobacteriaceae*).

Encise II is a large data base that can be approached by a binominal computer system or by a four digit number reference both of which are available from the manufacturer in an accompanying guide. By use of Enterotube coupled with the guidance provided by Encise II, 408 of the 424 *Enterobacteriaceae* cultures tested were correctly identified (*27*).

MINITEK

This, the newest of the miniaturized systems, is manufactured by BBL, Division of Becton, Dickinson and Company, Cockeysville, Maryland 21030. In 1974, high correlations were reported between the Minitek disk reactions and the conventional system with clinical isolates (*7*). The authors stated that this system provides complete speciation of *Enterobacteriaceae* but recommended use of a companion DNase plate. In the same year, agreement was 97% between Minitek and conventional methods for the identification of 461 *Enterobacteriaceae* (*23*). The authors recommended a modification of the color comparator card for interpretation of the lysine reaction and observed that dehydration of unlayered disks could be prevented by maintaining sufficient water in the humidor. They felt that the performance and flexibility of this system were highly satisfactory for the rapid and reliable identification of *Enterobacteriaceae* in their laboratory. With the Minitek 822 out of 904 (91%) *Enterobacteriaceae* were correctly identified (*28*) and the errors were all attributed to false positive reactions on the H_2S disks. With the Minitek system it was possible to speciate *Enterobacteriaceae* within 24 h if a DNase plate was simultaneously inoculated. Incorrect reactions could be avoided by the prompt and careful addition of oil. Mineral oil spillage could cause erroneous results. The Minitek method was found easy to use and required half as much time as the conventional system. Flexibility was one feature of this system which the other systems did not have. In a 1976 study of 720 fermentation reactions involving 60 different *Enterobacteriaceae* cultures, agreement was 100% between Minitek disks and conventional tube tests (*12*) and false positive reactions on H_2S reported earlier (*28*) were not encountered.

PATHOTEC

These reagent-impregnated strips are manufactured by the General Diagnostics Division of Warner-Lambert Company, Morris Plains, New Jersey 07950. In a 1965 study (*19*), each of 170 strains of gram-negative bacteria was individually tested with four PathoTec strips (cytochrome oxidase, lysine decarboxylase, phenylalanine deaminase, and urease). The authors concluded that these reagent-impregnated paper strips were very useful in identification of clinically important bacteria. In 1968, the conventional IMViC tests, which are traditionally used for distinguishing *Escherichia* from *Aerobacter* (*Enterobacter*), and the newly formulated reagent-impregnated strips (citrate, indole, and Voges-Proskauer) were compared (*1*). For 100 strains of bacteria with positive IMViC parameters, agreement with the standard tests was 95% for citrate, 97% for indole, and 90% for Voges-Proskauer. In 1969, PathoTec was compared to standard procedures for 813 bacteria (*5*). Out of a total of 2,063 reactions, PathoTec agreed with the standard tests 2,026 times (98.2% agreement). When the efficacy of seven PathoTec strips was compared to conventional biochemical tests for 291 hospital isolates (*34*), the oxidase, phenylalanine, and Voges-Proskauer strips were accurate and valuable for rapid identification. The indole strip was reliable with one exception: negative indole tests on *Proteus* strains from solid medium should always be confirmed with a conventional method. When compared to conventional methods, the citrate and lysine strips were found to be unacceptable due to a considerable number of discrepancies, and the urease strip offered no time or cost advantage and was less useful in the presumptive identification of *Proteus*. In 1973, the PathoTec system was compared to conventional methods for identification of 191 members of the *Enterobacteriaceae* family (*35*). The overall accuracy of identification to genus was greater than 90% and agreement for individual tests was greater than 90% except for the urease which was not as sensitive as the conventional counterpart.

In another study (*1973*), the PathoTec Rapid I-D System was compared to standard tests in the identification of 193 gram-negative bacilli (*4*). Test agreement was 100% for oxidase and phenylalanine deaminase; 99% for indole, nitrate, and Voges-Proskauer; 98% for malonate; 97% for lysine decarboxylase; 90% for urease; 84% for H_2S; and 75% for esculin hydrolysis. Many of the commonly isolated *Enterobacteriaceae* were correctly identified within 4 h, but false positive H_2S reactions on the PathoTec strips led to errors in identification of some *Proteus*. The authors felt that the PathoTec system differed in three ways from other identification kits for *Enterobacteriaceae*; first, it only required 4 h, second it is very flexible and each test could be a useful addition to any identification system, and third, the substrates on the strips are dry before use, which prolonged stability. Also in 1973, the PathoTec "Rapid I-D System" and two experimental test strips (ornithine decarboxylase and beta-galactosidase) were used to identify 1,252 members of *Enterobacteriaceae* from fresh clinical specimens (*45*). Accuracy of identification was 98% for the 12-strip test on a side-by-side comparison with similar conventional procedures. Also, 103 gram-negative nonfermentors were accurately grouped. Positive blood cultures (308) were tested (1975) by conventional methods and PathoTec

strips (*13*). This group of cultures included 222 strains of *Enterobacteriaceae*, 40 strains of facultative gram-positive cocci, 26 strains of anaerobes and 20 assorted strains. The findings showed 96.5% agreement for *Enterobacteriaceae*, 98% for facultative gram-positive cocci, 100% for the anaerobes and 99% for the assorted strains. These workers concluded that the PathoTec system was an excellent basic choice for the identification of bacteria within and also outside the *Enterobacteriaceae* family in the routine clinical microbiology laboratory. In another study (1975), the PathoTec ornithine decarboxylase strip was evaluated for identification of swarming *Proteus* (*30*). For 181 strains, this strip was 100% accurate, within 4 h, when compared to the conventional ornithine decarboxylase test. Almost 70% of the *Proteus mirabilis* strains were identified within 2 h. Also in 1975, the PathoTec "Rapid I-D System" was compared to conventional procedures for accuracy in identifying 471 *Enterobactericeae* cultures and in individual tests. Pathotec exhibited 94.3% accuracy in identification and 95.5% agreement of the 4,910 individual test comparisons. PathoTec exhibited 94.3% accuracy in identification and 95.5% agreement of the 4,910 individual test comparisons. The authors concluded that PathoTec was highly accurate and versatile (*51*).

R-B

The R-B media (name derived from names of co-inventors William Rollender and Orville Beckford) is manufactured by Corning Diagnostics, 25 Lumber Road, Roslyn, Long Island, New York 11576. In 1970, for identification of 415 clinical isolates, the R-B system completely agreed with conventional methods for 412 (99%) of these organisms (*40*). The motility test in R-B was unreliable, but did not cause incorrect identification since other criteria were available. For example, even though motility is an important test for differentiating members of the *Klebsiella-Enterobacter-Serratia* group, the ornithine test helps differentiate *Enterobacter* from *Klebsiella*. The use of a flexible needle could ultimately lead to improper inoculation with this system. Also the two-tube system could not differentiate *Enterobacter* from nonpigmented *Serratia; Salmonella* from *Arizona; Proteus rettgeri* from *Providencia*.

In the hands of other investigators (1970), the R-B system, when compared to conventional methods, correctly identified only 45% of the enteric organisms tested (*32*). The researchers felt that the reactions of this system were more difficult to read and interpret than conventional tests. Those reactions deemed unreliable were glucose and lactose fermentation, lysine, and motility. The authors reported that the time required for interpretation of results was excessive and that the R-B system was not an acceptable alternative to conventional methods for identification of *Enterobacteriaceae*. In another study (1971) involving 451 *Enterobacteriaceae* cultures, correct identification was 89.6% with the R-B system and 98.2% with the conventional methods (*47*). The R-B system was very accurate for species of *Arizona, Citrobacter, Enterobacter, Escherichia, Providencia* and *Serratia;* moderately accurate for *Edwardsiella, Proteus, Salmonella* and *Shigella;* and very poor for *Klebsiella*. This poor performance with *Klebsiella* was attributed to unsatisfactory results from the lysine, indole, and motility tests. Both the lysine and motility tests were very difficult to read and interpret. The authors felt that this system could be used as an initial step in the identification scheme or as a screening device but not as an alternative to the conventional procedures.

The inability of researchers evaluating R-B to quickly and easily read and interpret results, led to the Beckford modification of the R-B tubes. The lower end of the tubes was constricted to prevent the diffusion of end products and interference of one reaction with another. The modified R-B system was evaluated and was satisfactory in the primary grouping of more than 2,000 *Enterobacteriaceae* cultures freshly isolated from clinical specimens (*25*). The physical modifications of the tubes seemingly eliminated the difficulties and this system was now considered as a real alternative for the first approach in classifying enterics from clinical specimens. In a similar study, this modified system correctly identified 95.5% of the 200 cultures tested and was thought to be a marked improvement over the original product (*48*). In another comparison (1972), correct classification was 93% by genus and 45% by species with the conventional and 90% and 40%, respectively, with the R-B system (*37*). These workers felt that it would be advantageous to do the conventional tests for Simmon's citrate and Christensen's urea along with the R-B to allow identification of *Proteus* and *Providencia* and also permit adequate separation of *Escherichia coli* and *Shigella* sp. from other groups within the family. In a more recent study (1974), the expanded (4-tube) R-B system accurately identified 2,200 hospital isolates of *Enterobacteriaceae* with no problem whatsoever (*26*) and about 250 of these cultures were atypical strains. The authors reported that results were very clear-cut, the system was easy to use and would be satisfactory for routine identification of *Enterobacteriaceae*.

STUDIES IN WHICH TWO OR MORE MULTIPLE TEST SYSTEMS WERE COMPARED

Enterotube was compared to Pathotec in 1969 and agreement was good between the systems (*21*), but Enterotube took less time because subculturing wasn't necessary. When five multiple test systems were compared, in 1972, to conventional methods for identifying clinical isolates (*20*), agreement was best for Enterotube (92%) and next for R-B (84%). The authors stated that with R-B the lysine and motility reactions were difficult to read and that this system was probably more subject to technical errors than other systems. In a 1973 study, API and Enterotube were compared to conventional procedures for 324 *Enterobacteriaceae* cultures (*6*). They reported 95% agreement between API

and conventional methods and a similar agreement for Enterotube except for organisms of the *Klebsiellae* tribe. Therefore, they concluded that both systems offered advantages over the conventional procedure because media preparation and storage were avoided and inoculation and the reading of results were greatly simplified. The API system also had advantages over the Enterotube in that *Klebsiellae* could be easily identified and the API had a longer shelf life. In a comparison of PathoTec, R-B and conventional techniques (1973), 140 strains of enteric bacilli were tested (*41*). The improved PathoTec and R-B systems exhibited an overall accuracy of greater than 95%, both in results of individual tests and in identification of cultures when compared to conventional methods. When R-B and Enterotube were compared to the conventional method for identification of 200 *Enterobacteriaceae* (1973), the number of errors was about equal for these two miniaturized systems (*10*). However, the Enterotube was easier to read, could be inoculated more rapidly, and was more successful than the R-B for identification of isolates without the use of further tests. API, Auxotab, and Enterotube were compared in a 1974 parallel study for accuracy in identifying enterics (*31*). For a group of 133 known *Salmonella* cultures, Enterotube identified 90.2%, Auxotab 93.9%, and API 100%. For 150 unknown cultures isolated from the Ohio River, identification of *Salmonella* was 97% for Enterotube, 98.5% for Auxotab, and 100% for API. For identification of 14 *Enterobacteriaceae* control cultures, Enterotube identified 64.2%, Auxotab, 100% and API, 100%. Five multiple test systems were compared to conventional procedures for accuracy in the identification of 329 strains of *Enterobacteriaceae* (*39*). For individual tests, the API system was the most reliable followed by Enterotube, R-B, Pathotec, and Auxotab. The highest percent of correct identification, as compared to the conventional method was Enterotube (97%), API (94%), R-B (91%), Pathotec (77%), and Auxotab (42%).

In 1975, 214 cultures obtained from clinical material were identified by API, Enterotube, and conventional methods (*55*). With API, 99% were correctly identified and with Enterotube, 82%. On the second testing, 90% were correctly identified with Enterotube.

The R-B and Minitek systems were compared to conventional techniques for identification of 294 isolates (*46*). Both systems were rapid and allowed the correct identification of 85% of the organisms tested. For individual biochemical reactions, the R-B system showed a 92.6% agreement with the conventional and Minitek, 93.1%. The inconsistency of H_2S results led to the misidentification of 30 isolates by Minitek. Prolonged incubation of these two systems caused several false-positives and therefore was counterproductive. In another study comparing Minitek and API to conventional tests for identification of *Enterobacteriaceae*, an overall accuracy was 95.3% for API and 97.6% for Minitek (*2*). Ten percent of the isolates initially identifed with API were identified 24 h later with Minitek due to additional test requirements. The authors concluded that Minitek was less expensive than API, but technically more cumbersome.

Performance of API, Enterotube, Inolex, Minitek, PathoTec, and R/B was evaluated in 1975 (*50*). A minimum of 200 clinical isolates was used with each system and the results show 100% identification accuracy, or very nearly so, for *Klebsiella, Proteus,* and *Shigella* with all six systems. For *Citrobacter,* Enterotube and Minitek were 100%, PathoTec, 96%, R-B, 94%, Inolex, 92.3% and API, 91.3%. For identification of *Edwardsiella,* API and Minitek were 100% accurate, while the other four systems ranged between 80-90%. For *Escherichia coli,* Inolex and Minitek were 100%, while the other four were between 91 and 96%. For *Salmonella*, API and Inolex were 100%, Minitek, 94.7%, PathoTec, 93.8%, Enterotube, 89.3% and R/B, 88%. Some general observations made during the above study were that Enterotube and R/B were the easiest of the six systems to inoculate, while Minitek was the most difficult. API and Minitek required the most manipulation following incubation and PathoTec and R/B the least. Minitek was found to be the most versatile and Enterotube the least. PathoTec required the least incubation time and therefore was the quickest.

All of the systems mentioned in this review have some numerical or computer-assisted system that is available through the manufacturer. Some of these are fully computerized, while others depend on a punch-card procedure. Regardless of the type, these numerical systems are important aids for identification of unusual strains (*50*).

In closing, there is a list of precautions concerning the use of these miniaturized systems that should be brought to the reader's attention. First, no abbreviated system can consistently be as accurate as a complete identification scheme. The user, therefore, must be willing to accept the expected accuracy of the device he has chosen. Second, in addition to biochemical data, a microbiologist should always consider colonial, morphological, and possibly serological information to make an accurate identification. Also it is good to remember that these systems have been designed for use with *Enterobacteriaceae* and the various manufacturers do not claim that they will work satisfactorily with other organisms. And finally, all instructions provided by the manufacturer should always be carefully read and closely followed if the user expects these systems to identify *Enterobacteriaceae* with a high degree of accuracy (*50*).

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Special Reports from IDF

Toxicological Aspects of Packaging: Total Energy Use and Packaging Waste

The International Dairy Federation (IDF) has initiated publication of *Dairy Packaging Newsletter.* This newsletter contains material prepared by experts from the IDF Group dealing with packaging. Two reports from a recent issue appear below. They are being reprinted with permission of P. Staal, Secretary General of IDF, Brussels, Belgium.

TOXICOLOGICAL ASPECTS OF PACKAGING

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Foodstuff is packed primarily to protect it from influences which are harmful to it. But this causes a further problem, namely that the packaging material itself may be harmful. Transfer of constituents of the packaging material itself to the content (migration) can doubtless be limited but cannot be wholly avoided even when packages are made from glass or metal.

To limit migration of individual constituents of the material (specific migration), toxicological considerations are the deciding factors. The EEC and the Council of Europe transferred the task of determining permissible migration limits to a working party on toxicology of the Council for Europe. (The first publication of the working party is expected at the end of 1977). To do this, the so called "no effect level" (mg/kg body weight) of the migrants has to be known. Using a safety factor which, according to the type of migrant, could lie between 100 and 1000, the ADI-value (acceptable daily intake), is determined for human beings (mg/60 kg body weight), from the "no effect level." The so-called PADI value (packaging ADI) is equivalent to the ADI value. It is therefore greater by 10^2 to 10^3 than the "no effect level" obtained by animal experiments.

The calculation of the toxicologically permissible specific migration is based on the PADI value. If, for instance, a daily intake of 1 kg of food is assumed, then the permissible contamination of the foodstuff in ppm is derived directly from the PADI value in mg.

On the average, 1 kg of food stuff is in contact with 6 dm^2 of packaging material. The PADI value divided by 6 therefore gives the permissible migration value per dm^2. By introducing specific factors for food stuffs, it is attempted to take into account the amount of food consumed as well as the eating habits in different countries.

There are particularly strict regulations for carcinogenic materials such as vinyl chloride. In the USA these are based on the Delaney-Clause which states that a substance is deemed unsafe if it is found to induce cancer when ingested by man or animal, or if it is found, after tests which are appropriate for the evaluation of the safety of food additives, to induce cancer in man or animal. According to US law, vinyl chloride must therefore be "undetectable" in food stuffs. The meaning of "undetectable" depends on the state of the analytical technique at the time.

For vinyl chloride, the EEC has preferred to lay down toxicologically, analytically and technically justifiable limiting values. (For packaging materials made from PVC 1 ppm and for foodstuffs packed in it 50 ppb). It has been shown that the limiting values of the specific migration based on toxicological considerations and the methods for their control must match each other.

The experience that, as late as 1970, vinyl chloride was considered a harmless chlorinated hydrocarbon, has led to the re-examination of the toxicity of other monomers which are today still considered harmless (e.g. acrylonitrile, vinylidene chloride and styrene). Results have not yet been obtained. Materials must therefore never be considered as having been finally evaluated. New knowledge can make a new evaluation necessary at any time.

It is difficult to test for migration in individual foodstuffs. Examination of simulated foods in model systems requires effort and expense. There is therefore a tendency today to give more importance to the testing of the composition of packaging materials. In the context of the endeavours for harmonization a close collaboration has developed between public authorities and industry in the EEC. It can be expected that this will soon lead to a harmonization of the regulations in the individual countries and thus to a removal of the present obstacles to the free exchange of goods.

TOTAL ENERGY USE AND PACKAGING WASTE

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In most countries packaging systems for foods have proved their worth in saving considerably more than they cost, saving not only waste and labor, but also energy. There is however, widespread misconception, sometimes deliberately fostered, that a very appreciable part of our communal waste consists of discarded packaging. Such is however not the case. In most Western European countries the total amount of communal waste is in the order of magnitude of 350-450 kilos a head a

year. In Sweden the corresponding figure is 475 kilos, of which approximately 30%, i.e. slightly more than 140 kilos, comprise packaging waste. Of these 140 kilos, approximately 70% are paper and the rest glass, metals and plastics.

Apparently, today it is possible to separate an appreciable part of the paper from the communal waste for recirculation. It should be noted that such recirculated material has a limited technical use, for the time being mainly as liners in cartons, in corrugated cardboard and so forth. Separation of steel is also widely practiced, particularly where the waste has been used as fuel and steel can be recovered from the ashes. It is today technically feasible to separate the plastics from communal waste, but the economy of the process is quite impossible and it leads to severe contamination problems, since the process is based upon flotation.

Glass can be sorted out by hand, but the use of waste glass is technically and economically strictly limited. (It can only be used for products where the color is not critical, and apparently with a total admixture to the cull of at most 30%).

The policy of some West European countries as far as communal waste is concerned is briefly outlined below.

FRANCE. A "Monsieur Recyclage" has been appointed by the government. He is subordinated to the ministry of development and industry and his main mission is to improve the post "Raw Materials" in the French foreign trade. In the Paris area, the TIRU (Service du Traitement Industriel des Résidus Urbains) is subordinated to the Electricite de France. The town refuse treated by TIRU represents 1/7 of the French waste. This is incinerated in four plants for generation of heat and power. The ashes used for landfill and the steel is recovered for reuse. While the total waste volume per capita has increased in France, development of light one-way packages for many different goods has meant that the volume of packaging waste tends to decrease (see table below).

SPAIN. Paper waste is widely recycled. It is realized that communal waste could be used for generation of steam and power, but lack of capital has prevented investments. Only one plant, in Barcelona, converts communal waste to steam-power. This is more in the nature of a demonstration plant.

DENMARK. The local consumption of paper board was 800,000 tons in 1974 and it is estimated that it will be close to 850,000 tons in 1980. Local production is 280,000 and 300-350,000 tons, respectively. In 1974 220,000 tons were recovered of which 145,000 were re-used in the country and 75,000 tons exported. For 1980 the corresponding figures are expected to be 320-360,000 and 180-210,000, respectively. Today a 100,000 tons per year news print mill is planned. It will utilize 60,000 tons of recovered paper waste, 35,000 tons mechanical pulp and 15,000 tons imported bleached sulfite pulp.

Assuming interest on capital, depreciation and maintenance at a total of 30% per annum of the capital cost, an assumption which is not realistic, the figure being much too low, the production cost of news print in such a mill would be 2,800 dkr/ton as compared to a current market price of approximately 2,200 dkr/ton.

GERMANY. The waste policy is formulated in the "Abfallwirtschaftsprogramm 1975 der Bundesregierung," The folowing points can be stressed: 1. Futher rationalization of packaging-and distribution systems. 2. When new packaging materials are designed, the problem of waste disposal should be taken into account. 3. Packaging materials should be recycled if economically feasible. 4. Available packaging material should be efficiently utilized.

In 1973, the total paper waste amounted to about 4.7 millions tons of which approximately 30% consisted of packaging materials. Of the total paper waste approximately 2.8 million tons were recovered for reuse. It has been officially stated that the quality of news print must be decreased, the demands on hygiene in paper must be decreased, sorting of paper waste must be more efficient, utilization of paper waste must become more efficient if more waste paper is to be recoverd.

Recovery of plastic waste from the communal waste is considered economically impossible. The discussion concerning the communal waste now centers around the problem; "Should waste be burnt directly in furnaces or is a pyrolysis process for the production of gas to be preferred?"

NETHERLANDS. The law regarding waste disposal is still under discussion. Today approximately 40% of the paper waste is recovered. 162 municipalities with approximately 4 million inhabitants incinerate the communal waste in 12

INDUSTRIAL TREATMENT OF URBAN REFUSE
1976 TRADING RESULTS
FRANCE

(non-sorted refuse)

	PARIS AREA	PROVINCES
Collect	1,651,112 T.	10,000,000 T.
Treatment	1,477,770 T.	6,500,000 T.
Micellaneous (non-treated)	173,342 T.	3,500,000 T.
Sale of energy:		
— Electricity	104,636,000 KW/hour	non-estimated results
— Steam	2,011,113 T.	
— Clinker	182,933 T.	
— Old-iron	18,205 T.	

localities with 36 furnaces. Only 4 of these incinerators are used for the efficient production of steam: Amsterdam 149,000 megawatthours, Haag 69,000, Rotterdam 68,000, Rotterdam Botlik 55,000. Of the total communal waste for the country about 30% is disposed of by incineration. No further expansion is planned.

UNITED KINGDOM. The Waste Management Advisory Council is responsible for planning of waste handling. As yet, waste is mostly used for land fill. There is hardly any use of waste as fuel. One experimental power station was built in London but has for various reasons not been successful.

SWITZERLAND. Apparently, the Swiss have decided in favor of incineration. On January 1, 1975, 41 incineration plants were actually in operation, 6 were being built and 1 completely planned, 10 combined plants (incineration - composting) were in operation, 2 composting stations were in operation, 14 land filling stations were in operation and 7 were planned. These 67 operating plants served 4,991,310 inhabitants out of a total population of 6,296, 783, handling 1,603,168 tons of communal waste.

SWEDEN. In Sweden incineration for generation of heat is practiced in most of the larger communities. In the smaller communities, waste is still mainly used as land fill. Apparently there is a definite lower limit to the economical size of incineration plants and many of the communities in Sweden as well as in Norway and Finland are too small.

Report of the Editor
Journal of Milk and Food Technology
Journal of Food Protection
1976 - 1977

REVIEW OF VOLUME THIRTY-NINE

The last issue of the *Journal of Milk and Food Technology* appeared in December of 1976. Appearance of this issue also completed publication of Volume 39.

Volume 39 was the largest ever published. Its 948 pages exceeded the number of pages in Volume 38 (1975) by 9% and in Volume 30 (1967) by 85%. Volume 39 contained 134 papers of which 94 reported original research, 28 were technical papers of general interest, and 12 were nontechnical papers of general interest. This distribution of papers is quite similar to that found in Volume 38. Details about the make-up of Volume 39 and of other recent volumes appear in Table 1.

Subject matter of Volume 39 again was varied. It is noteworthy that this volume again contained more papers that considered foods other than dairy products (60%) than dealt with dairy foods (34%). About 6% of the papers in Volume 39 discussed topics that could not properly be classified in either of these two major groups. The prediction was made in the 1974-1975 report of the Editor that future volumes of the journal would exhibit greatest growth in papers dealing with foods other than dairy products. This prediction has come true in only a few years and there is every reason to think that the trend established in the last few years will continue. Lack of funding for dairy-oriented research, profound changes in the dairy industry, organizational changes in universities, and the great need for research on foods of non-dairy origin are factors that account for this rapid change in composition of the journal.

CHANGE IN TITLE OF JOURNAL

The title *Journal of Milk and Food Technology* was dropped at the end of 1976 and the new title, *Journal of Food Protection,* became effective in January, 1977. The following steps were taken to facilitate transition from one title to another: (*a*) both titles appeared on covers of most issues published in 1976 (Volume 39), (*b*) articles were prepared for and appeared in the last issue of the *Journal of Milk and Food Technology* and the first issue of the *Journal of Food Protection;* these articles indicated what would happen and also discussed the evolution of the journal published by IAMFES, and (*c*) an announcement of the change in title was sent to approximately 90 domestic and foreign scientific and trade journals. Most of these publications carried the announcement. Editors of journals in Germany and France translated the announcement and then published it in German and French. It may have appeared in other languages but that was not noted by the Editor.

A new design for the cover of the Journal was instituted with the first issue of the *Journal of Food Protection.* Both the new title and new cover appear to be well received among readers of the Journal.

PRESENT STATUS OF VOLUME FORTY

It was decided not to change the assignment of numbers to volumes when the new title of the Journal was instituted. Hence, the first volume of the *Journal of Food Protection* is Volume 40.

The first six issues of Volume 40 consisted of 436 pages, including

TABLE 1. *Summary of contents of the* Journal of Milk and Food Technology, *1967, 1971-1976*

Item	Volume 30 (1967)	Volume 34 (1971)	Volume 35 (1972)	Volume 36 (1973)	Volume 37 (1974)	Volume 38 (1975)	Volume 39 (1976)
1. Total pages, including covers	512	728	832	752	752	868	948
2. Total papers published	64	102	132	108	102	136	134
3. Research papers							
a. Number	30	67	78	65	72	100	94
b. Pages	137	288	317	284	330	402	400
c. Percent of total pages	26.7	39.5	38.1	37.7	43.9	46.3	42.2
4. General interest papers-Technical							
a. Number	11	24	35	31	21	26	28
b. Pages	47	150	242	208	160	178	188
c. Percent of total pages	9.2	20.6	29.1	27.7	21.2	20.5	19.8
5. Equipment standards							
a. 3-A, pages	9	40	23	17	41	25	32
b. E-3-A, pages	—	30	15[a]	—	—	2	18
c. Percent of total pages—all standards	1.7	9.6	4.5	2.2	5.4	3.1	5.2
6. General interest papers—Nontechnical							
a. Number	23	11	19	12	9	10	12
b. Pages	72	46	76	49	29	46	57
c. Percent of total pages	14.1	6.3	9.1	6.5	3.8	5.2	6.0
7. Association affairs							
a. Pages	64	45	47	84	75	67	65
b. Percent of total pages	12.5	6.3	9.1	11.2	9.9	7.7	6.8
8. News and events							
a. Pages	51	17	7	4	0	26	36
b. Percent of total pages	9.9	2.3	0.8	0.5	0.0	3.0	3.7
Percent of pages-technical material, including standards	37.6	69.7	71.7	67.6	70.5	69.9	67.3
Percent of pages-nontechnical material	36.5	14.9	15.5	18.2	13.7	16.0	16.7
Percent of pages—covers, adds, index, etc.	25.9	15.4	12.8	14.2	15.8	14.1	16.0

[a]Includes 1 page of Baking Industry Equipment Standards.

covers. This compares with 456, 400, and 264 pages in the first six issues of Volumes 39, 38, and 30 (1967), respectively. Contents of the first six issues of Volume 40 included 65 research papers, 13 technical papers of general interest, and 7 non-technical papers of general interest. This compares with 45, 20, and 9 papers in the same categories for the first six issues of Volume 39. The first six issues of Volume 40 contained a total of 85 papers compared to 74 papers in the same issues of Volume 39.

A special type of paper of primary interest to persons in the field appeared in several issues of Volume 40. Several such papers are now awaiting publication. A different format than normally used was adopted for these papers so they can be easily identified.

Awaiting publication on July 1, 1977 were 66 research papers, 11 technical papers of general interest, and 5 non-technical papers of general interest. This compares with 53, 6, and 2 papers in the same categories in July 1, 1976. Additionally, 25 research papers were being reviewed or revised on July 1, 1977. To insure prompt publication of papers, the remaining issues of Volume 40 will contain approximately 85 papers and the total for the year will be approximately 170 papers. This will reflect an increase of approximately 26% over the number of papers in Volume 39 and 165% over the number of papers in Volume 30 (1967).

REVIEW PAPERS

Review papers on timely topics continue to be an important part of the material offered to readers of the Journal. Since developments come about ever more rapidly, review papers take on greater and greater importance if readers want to be well informed on subjects which may not receive their daily attention.

Authors are encouraged to prepare review papers. They will be published promptly and without a manuscript service charge. Prospective authors should contact the Editor if they have questions about the suitability of their material for publication.

INTERNATIONAL CHARACTER OF JOURNAL

Volume 39 contains papers by authors from the following countries outside of the United States: Canada, Germany, India, Iran, Israel, Netherlands, Poland, and Switzerland. Thus far, Volume 40 has papers by authors from Canada, Egypt, India, Ireland, Netherlands, and Spain in addition to the United States. The additional breadth of coverage afforded to the Journal through publication of papers by authors outside of the United States serves to enhance the value of the Journal to all readers. It is hoped that more authors outside of the U.S. will consider the Journal as a suitable medium for publication of their research findings.

EDITORIAL BOARD

The Editorial Board currently consists of 50 U.S. and Canadian scientists in academic, governmental, and industrial laboratories. No changes in the Editorial Board appear necessary at this time. Many members of the Board have given many hours of time to evaluate manuscripts and to help authors improve their communication of scientific information. This time and help given without remuneration is gratefully acknowledged.

The following persons who are not members of the Editorial Board have reviewed manuscripts during the first six months of 1977: S. E. Barnard, R. L. Bradley, Jr., A. L. Branen, R. G. Cassens, S. R. Cecil, C. L. Duncan, W. S. LaGrange, R. C. Lindsay, T. E. Minor, and V. S. Packard. Their help is acknowledged and appreciated.

Respectfully submitted,
ELMER H. MARTH
Editor
Journal of Food Protection

Journal of Milk and Food Technology
Index to Volume 40

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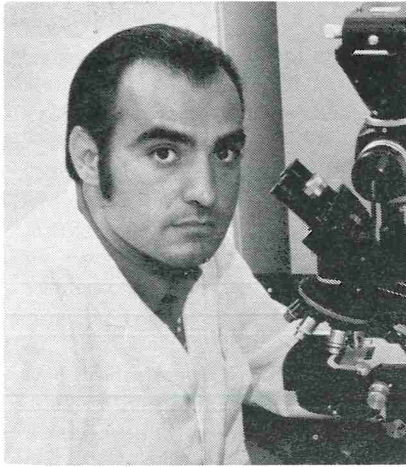

Cox Receives Poultry Award

Dr. Nelson A. Cox, Research Microbiologist, Russell Research Center, U.S.D.A., A.R.S. at Athens, Georgia has been named recipient of the 1977 Poultry and Egg Institute of America Award at the 66th Annual Meeting of the Poultry Science Association held August 8-12, 1977 in Auburn, Alabama. This award consists of a $1000 honorarium and a plaque and is given every other year to a research scientist under 40 years of age to stimulate and reward research in poultry and egg technology.

Dr. Cox, 34 years old, received his B.S. degree in bacteriology in 1966, his M.S. degree in Food Science in 1968, and his Ph.D. in Poultry Science in 1971 all from Louisiana State University. On receiving his doctorate he joined the Animal Products Laboratory at the Russell Research Center, Athens, Georgia.

During the past 6 years his research has included investigations on practical methods to improve the microbiological quality of broiler carcasses with emphasis on destruction of salmonella, microbiology of poultry chilling, and development of improved sampling and cultural methods for detection of salmonellae in poultry and poultry products. Important contributions include determination of the fate of orally inoculated salmonellae in the laying hen, evaluation of hot water and hot acid treatments on the microbiological condition of broiler carcasses, characterization of microbiological changes associated with immersion chilled and air chilled broilers, evaluation of the role of pigmented and non-pigmented pseudomonads in producing spoilage odors in poultry meat and development of rapid, reliable methods for detecting salmonella on broiler carcasses.

He has recently become internationally known for evaluation of various miniaturized kits for identification of *Enterobacteriaceae* isolated from food. In 1976, he was an invited speaker at an international symposium held in Cambridge, England involving rapid methods and automation in microbiology.

Cox has published widely on his research and is author or coauthor of 50 scientific and technical articles. He received the 1971 C. W. Upp Memorial Award, 1972 Ralston Purina Graduate Research Award and presently serves on the Editorial Board of the *Journal of Food Protection*.

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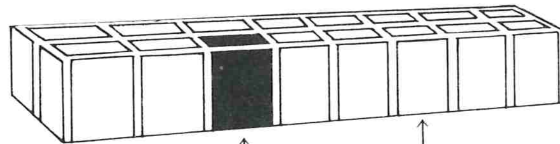

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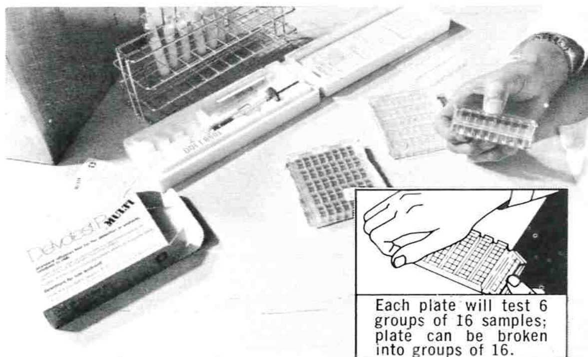

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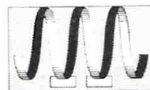

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NATIONAL MASTITIS COUNCIL ANNUAL MEETING

February 21-23, 1978

EXECUTIVE INN — LOUISVILLE, KENTUCKY

Everyone interested in prevention and control of bovine mastitis is cordially invited to attend the 17th annual meeting of the National Mastitis Council.

This year the National Mastitis Council hosts the International Symposium on Milking Machines. Thirty-four speakers from eleven countries will provide in-depth coverage of this pertinent subject.

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INCREASING MILKING EFFICIENCY THROUGH AUTOMATION

Capacity attendance is anticipated at this meeting. Make your hotel reservation now with the Executive Inn, Louisville, Kentucky 40213. Ask for special NMC rates.

Advance registration may be made for $20.00 through the National Mastitis Council office at 910-17th Street, NW, Washington, D.C. 20006. Registration fee at the meeting is $25.00.

JAMES R. WELCH, *President*
National Mastitis Council

IAMFES Officer Nominees for 1978-79 Secretary-Treasure

Harry Haverland and Charles Price

(Notice to membership—ballots will be mailed to paid members as of December 21, 1977)

Harry Haverland Director, Cincinnati Training Facility F.D.A.

Harry Haverland is Director, Cincinnati Training Facility, Food and Drug Administration, USPHS. Harry was born in Hamilton, Ohio, September 9, 1926. He received a B.S. from the University of Cincinnati and a M.P.H. from the University of Minnesota in 1959.

His public health experience includes six years as a local milk sanitarian with the City of Hamilton, Ohio prior to joining USPHS in January 1960. His first assignment was in Dallas, Texas as a Milk and Food Consultant. Subsequent assignments include Regional Milk and Food Consultant, Boston, Massachusetts; Chief, Food Hazards Surveillance Unit, Cincinnati, Ohio; Chief, Interstate Travel Sanitation Branch, Washington, D.C.; Director, Division of Food Service and Milk Sanitation, Washington, D.C.

During World War II, Harry served as a laboratory technician with the Navy in the South Pacific.

Membership in organizations include IAMFES, Inc.; Royal Society of Health; NEHA; American Academy of Sanitarians; Ohio Association of Sanitarians; and the Association of Food and Drug Officials. He is a certified Sanitarian in Ohio and a Registered Sanitarian in West Virginia.

Harry has served several years as one of the IAMFES, Inc., representatives to the Sanitarians Joint Council and has served as Secretary-Treasurer to SJC for the last three years. He serves as Co-chairman, IAMFES, Inc., Membership Committee.

He is married 30 years to Helen Boone Haverland. They have two daughters; Kathy, married and living in Virginia and Alice Ann, a Junior at the University of Cincinnati. Harry and Helen like to talk about their grandchildren, Robbie and Beth.

Harry Haverland

Coming Events

February 12-15, 1978. NCA ANNUAL CONVENTION AND INTERNATIONAL EXPOSITION FOR FOOD PROCESSORS. McCormick Place, Chicago, Contact: Y. Narita, Convention Service Manager, National Canners Association, 1133 20th St., NW., Washington, D.C. 20036.

February 15-16, 1978. DAIRY INDUSTRY CONFERENCE. Center for Tomorrow, Ohio State University, Columbus, OH. Contact: J. Lindamood, Dept. of Food Science and Nutrition, 2121 Fyffe Rd., Ohio State University, Columbus, OH 43210.

February 15-16, 1978. UNIVERSITY OF ILLINOIS DAIRY MARKETING FORUM. Ramada Inn Convention Center, Champaign, Illinois. Contact: James Gruebele, 1978 Dairy Marketing Forum, 305 Mumford Hall, Urbana, IL 61801.

February 26-March 1, 1978. 20th ANNUAL MEAT SCIENCE INSTITUTE. University of Georgia, Athens, GA. Co-sponsored by the University of Georgia and the National Independent Meat Packers Association. Contact: Dr. John Carpenter, Food Science Dept., College of Agriculture, University of Georgia, Athens, GA 30602.

February 26-March 3, 1978. 8th CONFERENCE ON ENVIRONMENTAL ENGINEERING IN THE FOOD PROCESSING INDUSTRY. Asilomar Conference Grounds, Pacific Grove, California. Contact: Sandford Cole, Engineering Foundation, United Engineering Center, 345 East 47 St., New York, NY 10017.

March 20-24, 1978. MIDWEST WORKSHOP IN MILK AND FOOD SANITATION. Center for Tomorrow, Ohio State University, Columbus, OH. Contact: J. Lindamood, Dept. of Food Science and Nutrition, 2121 Fyffe Rd., Ohio State University, Columbus, OH 43210.

March 20-30, 1978. UNIVERSITY OF MARYLAND 38th ANNUAL ICE CREAM SHORT COURSE. Dept. of Dairy Science,

Charles D. Price Senior Milk Sanitation Specialist F.D.A.

Charles D. Price is Senior Milk Sanitation Specialist for the U.S. Food and Drug Administration, Chicago, Illinois. He is responsible for administration of the Region V field operations of FDA in the Cooperative State/FDA Program for Certification of Interstate Milk Shipments. In this capacity he is staff consultant on milk sanitation and quality control procedures to the state, industry and FDA offices located in Illinois, Indiana, Michigan, Minnesota, Ohio and Wisconsin.

Charles D. Price

He was raised on a dairy farm in southwestern Wisconsin and began his employment with FDA following his graduation from Platteville, Wisconsin State University in 1964. He has served as an investigator with FDA at posts in Minneapolis, Chicago and Fort Smith, Arkansas. He has held his present position since 1971.

Charles has been a member of IAMFES and its Illinois affiliate, the Associated Illinois Milk, Food and Environmental Sanitarians since 1972. He served as auditor of the Illinois affiliate in 1973 and progressed in his service though posts as first vice president, president elect and president. In 1976 while president of the Illinois affiliate he also served as local arrangements chairman for the 63rd annual meeting of the IAMFES held at Arlington Heights, Illinois. He is currently the affiliate's junior past president.

Mr. Price has been a registered sanitarian since 1971 and is a member of the Arkansas Society of Registered Sanitarians. In addition, he serves as FDA's field representative on the Sanitarians Career Development Committee of the U.S. Public Health Service.

In recent years Charles has also been active in many other areas related to Public Health and the milk and food industries. He is a subcommittee member of the Farm Methods Committee of IAMFES and a member of IAMFES affiliates in Indiana, Minnesota and Wisconsin. Since 1974 he has served as a member of the Dairy Advisory Council of the Wisconsin Department of Agriculture. He is also a member of the Chicago Dairy Technology Society, AFDOUS, National Mastitis Council, National Association of Dairy Plant Fieldmen and the Northeast Dairy Practices Council.

Charles began an association with the U.S. Jaycees in 1969 and is currently President of the Lombard, Illinois Chapter. In 1976 he was recognized as Outstanding State Director of the Illinois Jaycees and also as Outstanding Spiritual Development Project Chairman. He has been named Jaycee of the Month several times and was named Jaycee of the Year in 1976 by the Lombard Jaycees. He serves as board member of the Lombard United Fund and is a member of his Church board of directors.

Charles and his wife Louise live at 508 S. Brewster in Lombard, Illinois. His two daughters, Christine and Amber, are in elementary school.

Animal Sciences Center, College Park, MD. Contact: J. Mattick, Dept. of Dairy Science, University of Maryland, College Park, MD 20742.

March 27-29, 1978. AMERICAN CULTURED DAIRY PRODUCTS INSTITUTE ANNUAL TRAINING SCHOOL AND JUDGING CONTEST. Stouffer's Inn, Indianapolis, Indiana. Contact: C. Bronson Lane, ACDPI, P.O. Box 7813, Orlando, FL 32804.

March 28-30, 1978. WESTERN FOOD INDUSTRY CONFERENCE, Freeborn Hall, University of California at Davis. Contact: John C. Bruhn, Dept. of Food Science and Technology, University of California, Davis, CA 95616, Phone: 916-752-2192.

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Marth Addresses Food Conference

Dr. Elmer Marth, Professor of Food Science and Bacteriology, University of Wisconsin, addressed the National Conference on Food Protection in Foodservice, September 6-9, in Chicago. Sponsored by the National Institute for the Foodservice Industry, the conference was attended by 150 representatives of the foodservice industry, government and education from 33 states and Canada.

Focus of the meeting was on roles and responsibilities in insuring the protection of the ever-expanding "eating-out public." Participants provided recommendations for national uniformity in foodservice sanitation regulations. In addition, recommendations were proposed for other food protection concerns such as funding and cost/effectiveness of regulatory food protection programs, procedures for public disclosure of official regulatory inspection reports, truth-in-menu regulations, use and advertising of analogue foods, nutritional labeling of menu items, evaluation of consumer "give aways," energy and water conservation in foodservice, pest control, foodborne illness reporting, microbiological standards for menu items and other future concerns relevant to foodservice food protection.

Copies of the conference proceedings may be obtained by writing to C. Dee Clingman, Director, Food Protection Programs, National Institute for the Foodservice Industry, 120 South Riverside Plaza, Chicago, Illinois 60606.

Food Engineering Award

Nominations for the 1978 Food Engineering Award are now being accepted by the award's sponsors, Dairy and Food Industries Supply Association and American Society of Agricultural Engineers. Deadline for nominations is March 1, 1978.

The award is presented biennially for original contributions in research, development, or design or in the management of food processing equipment or techniques of significant economic value to the food industry and the public. The award consists of a gold medal, certificate and $2,000 cash stipend.

Candidates will be evaluated on the application of human performance and progress to engineering and technology, development of machines, processes or methods for the food industry, and leadership in the professional development of the food industry.

Nominations should include a 500-word statement describing the nominee's achievements and recognition in the food industry, how he meets the award criteria, professional and business history, published works, educational background and organizational memberships.

Nomination forms are available from James L. Butt, ASAE executive secretary, 2950 Niles Road, St. Joseph, Mich. 49085. Nominations may also be made in letter form.

Previous winners were Dr. Arthur W. Farrall, professor and chairman emeritus of the agricultural engineering department, Michigan State University; Robert P. Graham, Western Regional Research Laboratory, U.S. Department of Agriculture; and Dr. Walter M. Urbain, professor emeritus, department of food science, Michigan State University.

Raymond Mykleby

Mykleby Named New Drinc Executive Vice President

Raymond Mykleby has been named executive vice president of Dairy Research Inc. (DRINC), the product and process R&D arm of United Dairy Industry Association (UDIA), effective September 1, 1977.

Formerly vice president of research and technology at Land O'Lakes, Inc., Mykleby was responsible for a 32-person professional staff in research product development, consumer affairs and corporate quality assurance programs/analytical laboratories.

He has served on various committees with the National Milk Producers Federation and is a member of the Milk Industry Foundation and 3-A Sanitary Standards and Component Pricing commissions.

Mykleby, has a B.S. degree in Dairy and Food Technology from the University of Minnesota and an M.S. degree in Food Science from Penn State University.

UDIA is an organization dedicated to increasing the sales of U.S. produced dairy products through its total promotion program. In addition to DRINC projects, the program includes the advertising and sales promotion campaigns of American Dairy Association and nutrition research and education programs of National Dairy Council.

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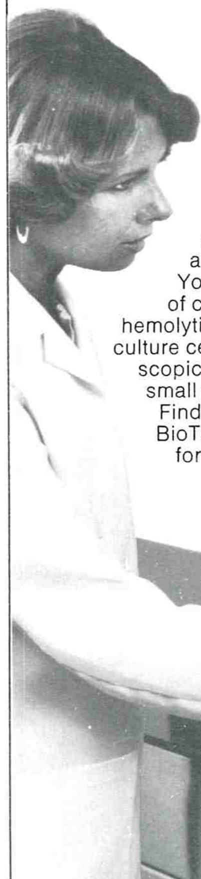
BioTran II counts colonies automatically



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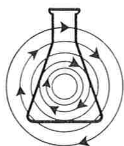

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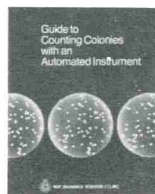

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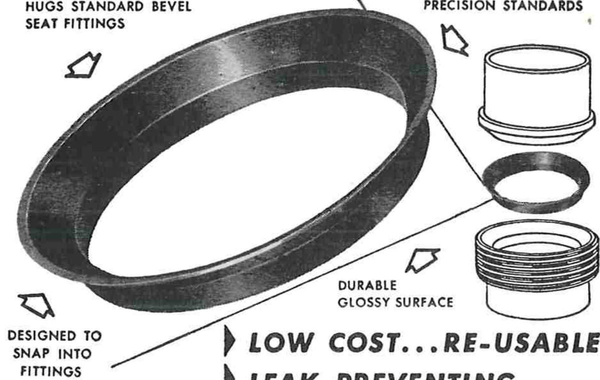

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Stimulation: The First Step Toward A Better Harvest

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

Oxytocin Means Let Down

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

Complementary Milk: Profits Left in the Udder

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

Thirty Seconds of Profitable Time

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

SURGE

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