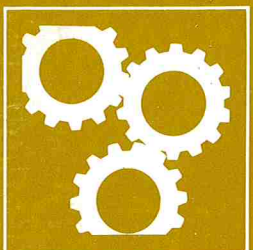
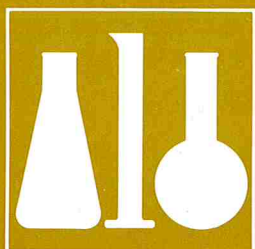
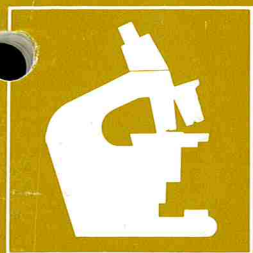


November, 1977
Vol.40, No.11
Pages 741-816
CODEN: JFPRDR 40(11):741-816(1977)
ISSN: 0362-028X



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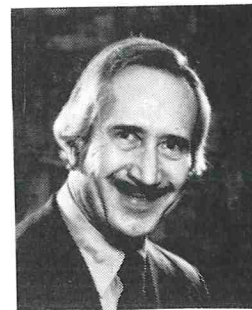
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*A tribute to America's sanitarians by Robert M. Brown,
president of the National Sanitation Foundation.
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Americans are gravely concerned about the staggering cost of health care... most of which is spent in getting well. A ray of sunshine in this business of survival is how little we spend for the services of environmental specialists... mostly in government service... who are paid to keep us healthy.

They are the professionals who usually work under the direction of public health officials and are known as *environmental health specialists* or *sanitarians*. Many are employed in federal agencies such as the EPA, HEW or Department of Agriculture. Some work for state agencies or safeguard the health of employees in industry. Others teach in our schools of public health.

But most of them serve in our own back yards—sometimes quite literally—as registered representatives of city, county and regional health departments.

More particularly, these public servants represent *you* as they inspect the equipment and sanitary conditions of restaurants, hotels, motels, mobile home parks, camps, hospitals, jails, butcher shops, bakeries, dairies and food processors. These are men and women who have spent from four to six years in college earning degrees in health-related curricula that include such subjects as chemistry, bacteriology, epidemiology and sanitary engineering. They are health scientists who know how to test the quality of well water, how to evaluate septic fields and how to defend streams, lakes and subsoil water from pollution by sewage, animal waste, chemicals and radioactive discharges.

Your environmental health specialists represent you and your children in the protection of swimming pools. They are officially concerned with air pollution, noise pollution and many forms of decay. They are your expert

defenders against the spread of disease by rats, flies, mosquitoes, parasites, bacteria and billions of other fearsome microorganisms which are the source of such ills as salmonellosis, gastroenteritis, typhoid and dysentery.

Sanitarians carry basic scientific instruments with them into the "field", which often means streets, alleys, sewers and waste sites. They have an array of thermometers, as well as velometers, pH and residual chlorine testers, sling psychrometers, phosphatase testers, pipettes, radiation counters, reagents and bacteriological sampling tubes. They obtain specimens which are often submitted to other highly trained colleagues for analysis in environmental health laboratories.

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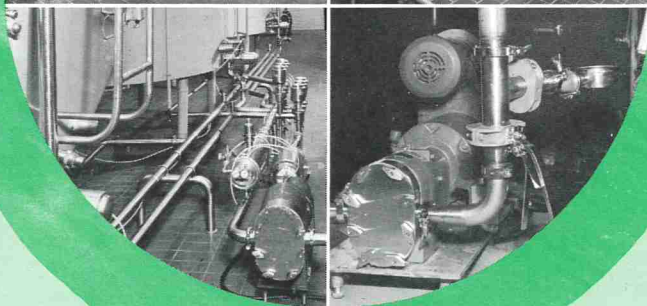
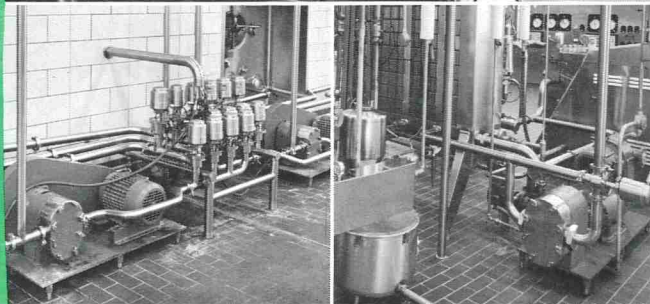
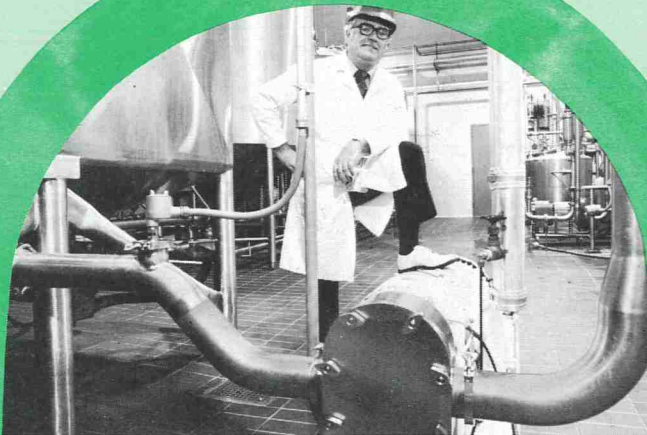
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A handwritten signature in dark ink, appearing to read "Robert M. Brown". The signature is fluid and cursive.

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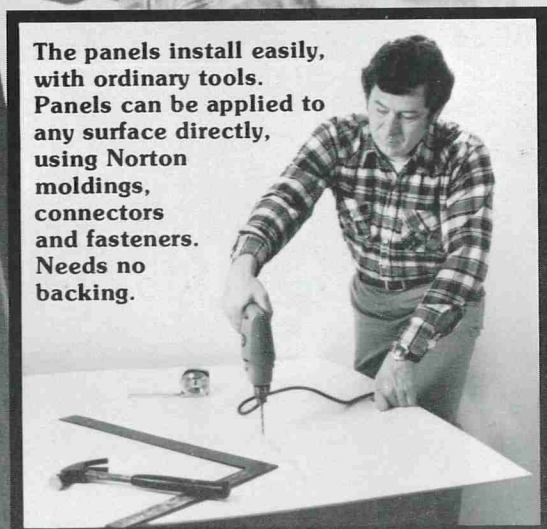
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2nd Class postage paid at Ames, Ia. 50010.

Journal of Food Protection

(formerly Journal of Milk and Food Technology)

Official Publication

International Association of Milk, Food, and Environmental Sanitarians, Inc.,

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The 3M Automatic Colony Counter in the Bacterial Evaluation of Manufacturing-Grade Milk

W. S. LaGRANGE, N. J. MOON, and W. REED¹

Department of Food Technology
Iowa State University, Ames, Iowa 50011

(Received for publication April 25, 1977)

ABSTRACT

Twenty-seven raw milk samples were analyzed for their bacterial quality by the Standard Plate Count (SPC) and the plate loop count (PLC). All resultant plates were counted by three different technicians either manually or with a 3M Brand Automatic Colony Counter, Model 630. Results emphasize the superiority of the SPC over the PLC for samples with high bacterial numbers. The automatic colony counter tended to underestimate the number of colonies on a plate. Correction factors for conversion of PLC counts to SPC and automatic to manual counts were calculated. It is recommended that the PLC and the automatic colony counter be used with appropriate correction factors for routine analysis of large numbers of samples.

Quality evaluation of manufacturing-grade raw milk frequently involves use of the Plate Loop Count (PLC) to determine bacterial numbers. Laboratory workers analyzing large numbers of samples have found the PLC a convenient procedure for evaluating raw milk samples. Counting colonies on resulting plates is a time-consuming task that follows 2 days after preparing the plates.

The PLC uses a 0.001-ml loop to obtain the milk sample. This quantity of sample is discharged into the petri plate, and Standard Methods Agar is mixed with the milk sample. After incubation of plates for 48 ± 3 h at 32 ± 1.0 C, colonies are counted, and the count is multiplied by 1000 to determine the PLC/ml.

The bacterial content of manufacturing-grade milk is highly variable ranging from a few thousand to several million per milliliter. Because the 0.001-ml loop serves as the method of sample dilution, the resulting number of colonies on the plate, particularly with some samples of manufacturing-grade milk, may exceed 300. Indeed, a plate may have more than 3000 colonies for high-count milk.

The general practice in the manufacturing-grade milk industry for plates exceeding 300 colonies is to count the colonies in 1 cm² of the plate and to multiply by 58 to obtain the number of colonies in the plate. If the colonies are too crowded to obtain an accurate count in 1 cm², the

practice is to count one-ninth of a square centimeter of the plate and multiply by 9 and 58 to obtain the number of colonies per plate. This procedure assumes an even distribution of colonies throughout the plate.

It can be a tedious, time-consuming task to count large numbers of colonies on a crowded plate and to calculate the count per milliliter. Also, accuracy may be lowered in the routine of counting a number of crowded plates. Finally, counting a large number of crowded plates in a limited time is a valid concern.

Packard and Ginn (3) completed a study in 1974 of the precision of the 3M Brand Automatic Colony Counter, Model 620, and determined that there was a very good relationship between results from automatic and manual counting methods over the range of 30-300 colonies per plate. Our concern is use of an automatic colony counter in a laboratory when the PLC is used on highly variable count manufacturing-grade milk. Under these conditions, the PLC dilution factor is 10⁻³, and the bacterial content of such milk covers a very wide count range.

In an effort to determine the accuracy of an automatic colony counter, we investigated use of the 3M Automatic Colony Counter and compared results obtained with those obtained by human colony counters on manufacturing-grade milk samples.

MATERIALS AND METHODS

Twenty-seven samples of manufacturing-grade raw milk were analyzed for numbers of bacteria by using the Standard Plate Count (SPC) and the PLC. Samples were selected to give an even distribution of counts between 9×10^3 and 12×10^6 per ml.

All plates were prepared according to the procedures of *Standard Methods for the Examination of Dairy Products* (1). Plastic plates were used to minimize the influence scratches on the plate have on the resulting count using the automatic colony counter. Duplicate plates were prepared for each sample dilution for the SPC and PLC. Dilutions for the SPC were 10⁻³ and 10⁻⁴, and plates with 30-300 colonies were counted when possible. The PLC incorporates a 10⁻³ dilution factor, so the number of colonies on a plate can have any value.

Two different methods were used to enumerate colonies on plates. Counts by both methods were completed within an hour. Each plate was counted in triplicate by three technicians using a 3M Brand, Model 630, Automatic Colony Counter (3M Company, New Business Venture

¹Iowa Department of Agriculture, Des Moines, Iowa 50319.

Division, St. Paul, Minnesota 55101). After each automatic count, plates were rotated about 60° to obtain a different counting position. Each plate was then counted visually by each of the technicians, and the results were separately recorded. When counts exceeded 300 colonies per plate, technicians used Standard Methods (1) technique, and counted a fraction of the plate area and then multiplied by a factor to adjust the result to the total area. Two of the technicians participated in the entire study. The third technician became ill and was replaced by another halfway through the testing.

Results were analyzed for significant differences between the two methods of plating, three individuals, and two counting methods. The split-plot statistical model (4) was used to determine the main effects of each of the variables.

The counts were placed into subgroups of fewer than 30, 30 to 300, and more than 300 colonies per plate for the PLC. These numbers were chosen for convenience only; the comparison of optimum counts per plate for greatest accuracy was not performed. These groups would constitute samples of low, medium to high, and very high count milk and correspond to counts of less than 3×10^4 , 3×10^4 to 3×10^6 , and greater than 3×10^6 /ml. The SPC plates for each sample had 30 to 300 colonies where possible. A separate split-plot analysis of these subgroups indicated the influence of low-, acceptable, and high-count milk samples on the analysis.

RESULTS AND DISCUSSION

The SPC results would be expected to equal the PLC if each plating method gave the same estimate of the numbers. This line of equality is illustrated in Fig. 1. The SPC results were significantly higher than those of the PLC, as shown in Fig. 1, over the entire range of counts, with an average difference of 5.28×10^4 . The prediction equation for these data in $PLC = (0.5761) SPC + 2.0623$. It is probable that the greater shaking involved in preparation of sample dilutions for SPC than for PLC accounts for these differences. Greater shaking would be expected to break up clumps of psychrotrophic bacteria in manufacturing-grade milk (2). Previous studies (5, 6, 7) also have indicated that the SPC and PLC give similar results for low-count but not for high-count milk.

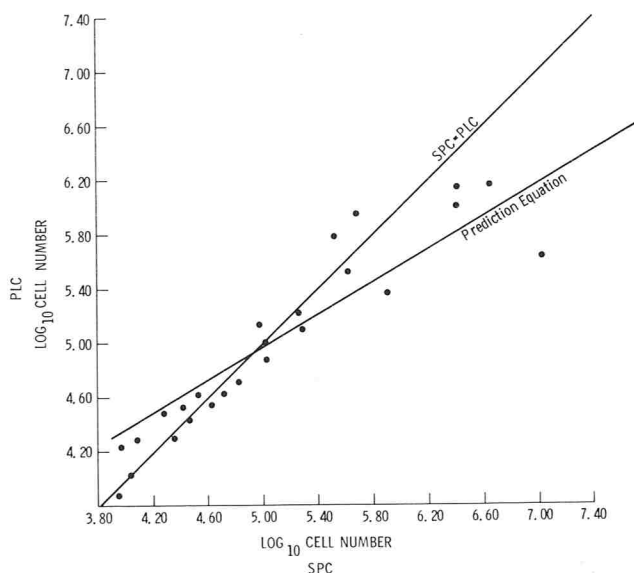


Figure 1. Comparative recovery of organisms on the PLC and SPC, with line of equality and prediction equation line.

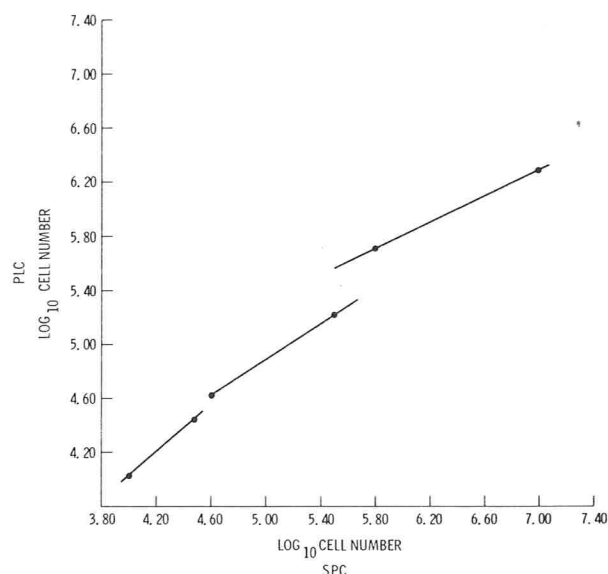


Figure 2. Comparative recovery of organisms on PLC and SPC. Lines are from prediction equations for the subgroups of fewer than 30 per plate, 30 to 300 per plate, and more than 300 colonies per plate.

An analysis of the subgroups of samples indicated that the number of organisms per milliliter had a large effect on the agreement of the PLC with the SPC (Fig. 2). The two methods agreed most closely at the low counts, per milliliter when the slope of the best-fit line was nearly 1.0. As the number per milliliter increased, the slope decreased from 0.865 to 0.475, indicating that the PLC underestimated the count/milliliter more severely for samples with high counts. The intercept changed from 0.585 to 2.958 at large numbers per milliliter, indicating the flatness of the curve at high values. Once again, the number of clumps broken in dilution preparation may be a major factor in determining how closely the PLC approximates the SPC.

These prediction equations could be used to predict the PLC from the SPC or vice versa. The error in estimation of the PLC or SPC for these equations is minimized by using the appropriate equation for the subgroups. The overall prediction equation is less accurate because of the poor agreement between SPC and PLC at high values. This causes the overall prediction equation to be too flat and, thus, over- or under-estimates the PLC from the SPC.

For example, if there were 60 colonies on the SPC plate and the dilution was 1:10,000, the SPC would be 600,000/ml. By using the equation, $PLC = SPC (0.475) + 2.958$ or $PLC = 5.693 = 490,000$ /ml.

In spite of these differences in test method results, the economics of material and labor and the ease of performing the PLC have made it an obvious choice as a testing method for laboratories testing large numbers of raw milk samples.

The counts by the individual were significantly higher than those by the machine in the entire range of counts (Fig. 3). The slightly lower count by the machine (human counts = 1.0523 machine + 0.2281) have been due to

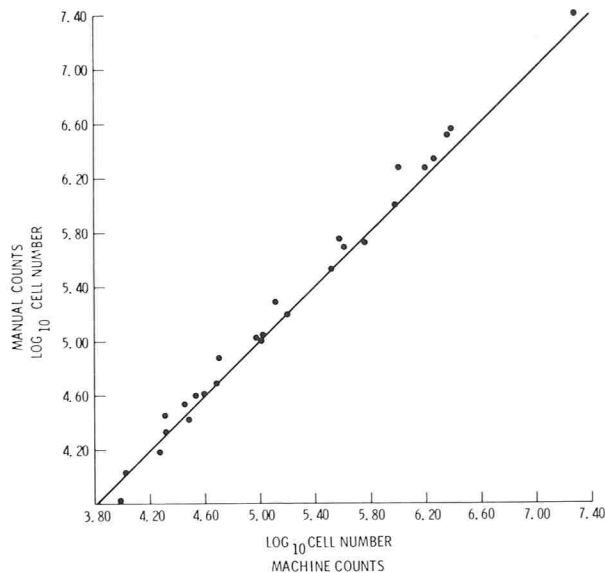


Figure 3. Comparative enumeration of an automatic colony counter and manual counts. (—) is line of equality.

counting two overlying colonies as one, or to an inaccurate count of the colonies along the edge of the plate. We had expected that the manual counts might have been lower than the machine counts at high counts because of the estimation procedure used for plates with > 300 colonies. In every instance, however, the machine counts were slightly lower than the manual counts.

The analyses of the subgroups indicated that the number per milliliter also has an effect on the linear relationship between manual and machine counts (Fig. 4), although it is less than the effect of plating method

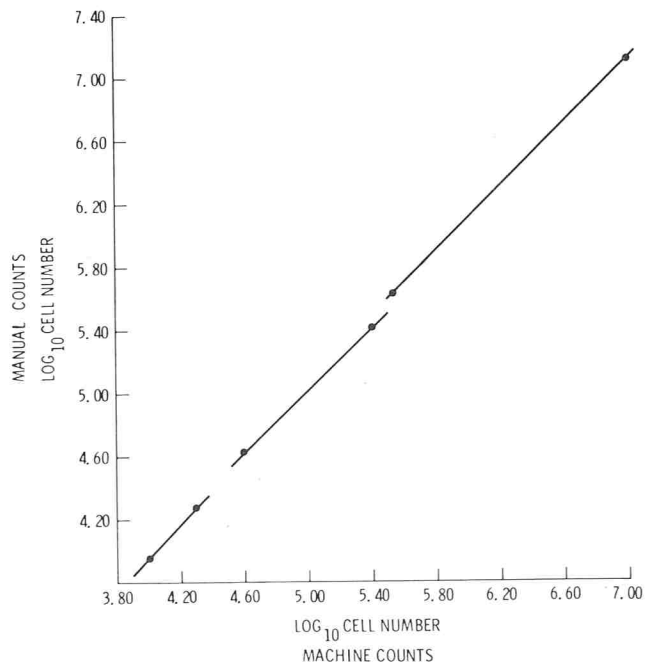


Figure 4. Comparative enumeration of an automatic colony counter and manual counts. Lines are from prediction equations for the subgroups of fewer than 30 per plate, 30 to 300 per plate, and more than 300 colonies per plate.

(Fig. 3). When there were fewer than 30 colonies per plate, the prediction equation was manual count = (1.102) machine - 0.445. When the count was 30 to 300 colonies per plate, manual count = (0.984) machine + 0.104. At higher counts per plate, the equation was manual count = machine (1.019) - 0.0198. These slopes differ significantly, but are very nearly 1.0. These equations could be used to predict the manual count from the machine count or vice versa, as demonstrated earlier.

The machine counted about 98% of the colonies that were counted manually. This is well within the 10% difference for counting methods suggested by Standard Methods (1).

Even though machine counts were slightly lower than manual counts, the automatic colony counters offer several advantages. Samples that contain large numbers of microorganisms per milliliter result in crowded plates. Counting these plates can result in eyestrain and fatigue besides being a time-consuming and boring task. The automatic counter is rapid and not tiring to use. It is likely that its use will increase, particularly in labs that use the PLC to evaluate raw milk. In conclusion, we recommend that when the PLC and automatic counter are used, appropriate correction factors should be included for accurate estimations of bacterial numbers.

ACKNOWLEDGMENT

This work was supported through the use of an automatic counter of the 3M Co., St. Paul, Minnesota. We thank the personnel of the Iowa State Department of Agriculture Food Analysis Laboratory for technical assistance and Don Hotchkiss, Professor, Department of Statistics, Iowa State University, for his statistical guidance.

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Detection of Tracer Dyes in Milk

A. MacINTOSH and A. VILIM¹

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 Health and Welfare Canada, Ottawa, Ontario, Canada, K1A 0L2*

(Received for publication April 25, 1977)

ABSTRACT

An improved ion-exchange resin method to detect low levels of dye marker Brilliant Blue F.C.F. (C.I. No. 42090) in milk was developed. Sensitivity of 0.02 mg of dye per liter (corresponding to 0.008 I.U. of penicillin per milliliter), and semi-quantitative adaptation of the method is described.

The problem of penicillin contamination of milk for human consumption is of great concern because of its health hazard potential. The usual microbiological methods used to test for presence of antibiotic contaminants in milk, require too much time to effectively prevent contaminated dairy products from reaching the market. One way to improve the current situation would be use of dye markers in mastitis treatment products. The infusion of dye-marked mastitis preparations results in production of colored milk, which in turn acts as a strong deterrent to the producer to include contaminated milk in his shipment.

Use of dye markers was first reported by Hargrove et al. (4) and months later by Smitasiri et al. (5). Several types of coloring substances were tested, and in 1960 a food dye, Brilliant Blue F.C.F. was found to be the most suitable by Dawson et al. (2). It showed a reasonably parallel rate of excretion for the dye and penicillin. Dye detection methods were all based on visual techniques except one semi-quantitative dilution method reported by Dawson et al. (2). The first method for sub-visual concentrations of the dye (< 0.125 mg/l) was developed by Rasmussen and Simesen (6). Ion exchange resin was used, and a sensitivity of 0.03 mg of Green S per liter was achieved. In 1965, Feagan et al. (3) reported an improved test for Brilliant Blue F.C.F. using an ion exchange resin.

The first rapid test for presence of dye marker (employing the syringe technique) was reported by Dalgaard-Mikkelsen and Rasmussen in 1962 (1). This found acceptance in Australia, South Africa, and France as an analytical method for on-the-spot detection of contaminated milk.

This report covers further improvements of the ion-exchange method resulting in higher sensitivity and also in simplification of manipulations.

MATERIALS AND METHODS

Apparatus

Small glass columns, made from large volume, 4 ml, Pasteur pipets (Fisher Scientific Co. Limited, Ottawa, Ontario), having the tapered end cut off to leave an opening of about 4 mm and plugged with a wad of fine glass wool. A filtering flask and trap to be attached to a line or pump giving a vacuum of 30-40 cm of mercury, (see Fig. 1).

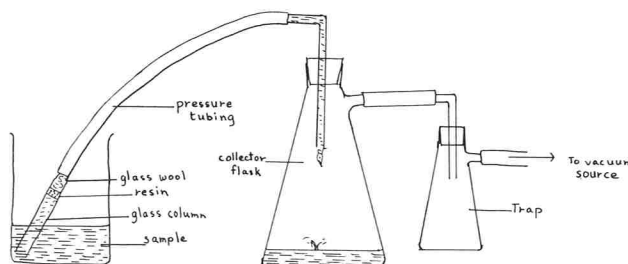


Figure 1. Apparatus used to detect tracer dye.

Chemicals

Dowex AGI-X2 resin (Bio-Rad Laboratories (Canada) LTD., Mississauga, Ontario), 200-400 mesh, in chloride form. Brilliant Blue F.C.F. (C.I. No. 42090), (supplied under the name erioglaucine from Canadian Laboratory Supplies, Ottawa, Ontario).

Procedure

About 200 mg of new Dowex resin were added to a 100-ml sample of milk in a beaker (250 ml) and stirred for 5 min. The restricted end of the glass column was inserted into a heavy-wall flexible tubing attached to a vacuum system. Milk-resin suspension was aspirated through the column allowing the resin to form a bed on the plug of glass wool. The column was inverted, carefully rinsed with about 2 ml of deionized water without disturbing the resin and when the water level reached the top of the resin bed, the vacuum was turned off. The moist column was examined, and a greenish color of the resin bed indicated the presence of dye. At the low levels a blank may be prepared for comparison.

RESULTS AND DISCUSSION

To obtain optimum results, mixing time of the milk-resin suspension was established to be at least 5 min (Table 1). Shorter times reduced the intensity of

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the color by about half, thus reducing the sensitivity of the test.

If clogging or unreasonably slow filtering time occurred, samples were diluted before testing with 100 ml of warm water giving a mixture of about 40-50 C. Deionized water should be used to prevent discoloration of the resin and subsequent difficulty in detecting the dye.

The lowest detectable level of the test was established to be 0.02 mg/1 (3). This was confirmed (Table 2), but

TABLE 1. *Effect of milk-resin mixing time on test sensitivity*

Conc. of dye (mg/1)	Mixing time in minutes			
	1	2	5	10
0.05	+	+	++	++
0.02	tr	tr	+	+
0.01	-	-	tr	+
Blank	-	-	-	-

++ Intensely Colored

+ Colored

tr Trace

- No Color

TABLE 2. *Sensitivity of the small column method^a: a representative trial on one day*

Conc. of dye (mg/1)	Replicate	Observer		
		1	2	3
0.05	1	+	+	+
	2	+	+	+
	3	+	+	+
0.02	1	+	+	+
	2	+	+	+
	3	+	+	+
0.01	1	+	+	tr
	2	tr	+	+
	3	+	+	+
Blank	1	-	-	-
	2	-	-	-
	3	-	-	-

+ Positive

tr Trace

- Negative

^aRepeated on three different days

trained observers could detect levels as low as 0.01 mg/1 since observer No. 2 could detect that level consistently. All replicates at 0.05 mg/1 were classed as positive. This test was done in triplicate on 3 different days. Dye levels of 0.1 and 0.2 mg/1 produced intensive coloration of the resin, but these concentrations were also detectable by the naked eye in the milk before testing.

Semi-quantitative results for the dye levels were obtained by making a series of 1:1 dilutions with water. Samples were analysed visually, and at the sub-visual range by the resin method, until the dye was no longer detectable on the column (Table 3). The number of dilutions indicated the original concentration of the dye.

TABLE 3. *Illustration of the semi-quantitative adaptation of the small column method at the sub-visual level*

Conc. of dye (mg/1)	Sample	Dilutions (1:1 with H ₂ O)					
		1	2	3	4	5	6
0.2	+*	+*	+	+	+	tr	-
0.1	+*	+	+	+	tr	-	-
0.05	+	+	+	tr	-	-	-
0.02	+	+	tr	-	-	-	-
0.01	+	-	-	-	-	-	-
Blank	-	-	-	-	-	-	-

* Visible before analysis

+ Positive

tr Trace

- Negative

As developed by Dalgaard-Mikkelsen and Rasmussen (1), the column technique used a syringe to force the sample through a pre-packed resin bed. Smaller samples were used. When attempting to use their technique in the present studies, the column plugged, and a much longer time was required to force the sample through the column. The sensitivity was also lower.

There are several advantages to the modifications herein proposed. The method is rapid (it requires less than 10 min, including mixing time); increased sensitivity can be realized because much larger samples can be employed without encountering problems of column plugging; sensitivity is also enhanced by better resin-sample contact. The total cost of column and resin is less than five cents for each test.

In terms of practical application, our modification enables us to detect safely dye levels of 0.02 mg/1 corresponding to 0.008 I.U. of penicillin/ml. The method just described has been successfully used in a field trial establishing milk-out curves for six healthy cows treated with dye-marked mastitis cream containing 125 mg of dye and 100,000 I.U. of procaine penicillin G.

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Inhibition of Enteropathogenic *Escherichia coli* by Homofermentative Lactic Acid Bacteria in Skimmilk

I. Comparison of Strains of *Escherichia coli*

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ABSTRACT

Behavior of enteropathogenic and nonpathogenic strains of *Escherichia coli* was determined when they were grown in skimmilk with and without 0.25 or 2.0% added lactic starter and incubated at 21 or 32 C for 15 h, and then after refrigeration at 7 C. With no lactics present, generation times for *E. coli* ranged from 28 to 35 min at 32 C and from 66 to 109 min at 21 C. At 32 C, after an initial 1- to 3-log increase in numbers and 6 to 9 h of incubation, *E. coli* was completely inhibited by both concentrations of starter culture. Complete inhibition of growth by *E. coli* occurred earlier at 32 than at 21 C, but smaller numbers of *E. coli* were obtained at 21 C; some strains virtually did not grow. The final pH of fermented milks ranged from 4.4 to 4.6. The longest survival time for *E. coli* in refrigerated fermented milk was about 17 days when milk was fermented at 32 C with 0.25% starter. A combination of lower incubation temperature (21 C) and higher starter concentration (2.0%) was most effective in controlling growth of *E. coli* in fermented skimmilk.

Presence of *Escherichia coli* in certain dairy products may indicate post-pasteurization contamination. Growth of *E. coli* in dairy products can cause defects in flavor and texture (3). Recently there has been additional concern about the presence of *E. coli* in dairy products and other foods because of the ability of some strains to cause foodborne illness (2,7,12). These strains, designated as "enteropathogenic" (EEC), can cause either a toxigenic or an invasive type of illness (16).

There have been several investigations into the incidence of *E. coli* and EEC in pasteurized and fermented dairy products (8,11,15,22,25). Results of these studies have shown that presence of *E. coli* in dairy products usually results from post-pasteurization contamination (1,11,25), but whether or not such contaminants are pathogenic is a question which has not yet been adequately answered because of problems in isolating and identifying EEC in food (14), and the difficulty of determining potential pathogenicity of isolates (21,23). Until these questions have been answered, it must be assumed that any food contaminat-

ed with *E. coli* may be a potential health hazard, especially if conditions allow growth of this bacterium.

In dairy products and many other foods, fermentation with lactic acid bacteria has been used to control growth of microorganisms responsible for foodborne illness or spoilage. The lactic acid fermentation in milk controls growth of *E. coli* mainly through lowering of pH (19), although production of antibiotics or other growth inhibitors is also possible (9, 13). Survival of *E. coli* in fermented dairy products is highly variable, depending on starter culture used, pH, temperature of storage, and composition of the product (6,19,20,26). Use of a lactic acid fermentation does not necessarily guarantee the safety of a dairy product, as evidenced by growth of EEC in soft-ripened cheese (4), and occurrence of EEC-induced foodborne illness in persons who consumed soft-ripened cheese (12).

The purpose of this study was to help clarify the interaction between *E. coli* and a homofermentative lactic starter culture when both were grown together in sterile skimmilk and when using initial populations and growth temperatures which could occur during production of a fermented dairy product. Also of interest was the effect which pathogenicity or strain variability might have on ultimate survival of *E. coli* during the fermentation.

MATERIALS AND METHODS

Cultures

Cultures used in this study, and their sources are listed in Table 1. *E. coli* cultures used to inoculate skimmilks were incubated at 35 C for 22 to 24 h in nutrient broth. One EEC culture, strain 1624, is a slow lactose fermenter. The lactic starter culture used was a commercial, mixed strain, homofermentative type. It was incubated in sterile skimmilk for 20 to 24 h at 21 C, and transferred at least twice before use as inoculum.

Preparation and fermentation of skimmilk

Screw-cap bottles (8 oz) were filled with 156 ml of skimmilk, and then autoclaved for 15 min at 121 C. The milk was held overnight at 21 C and 32 C, and then inoculated with 0.25 or 2.0% lactic starter, and

TABLE 1. Cultures used in this study.

Culture	Abbreviation	Source
<i>Enteropathogenic E. coli</i>		
Toxigenic		
B ₂ C	B ₂ C	FDA, Washington, D.C.
H 10407	H-1	FDA, Washington, D.C.
Invasive		
1624	A-1	FDA, Washington, D.C.
4608	A-4	FDA, Washington, D.C.
<i>Nonpathogenic E. coli</i>		
K-12	K-12	Dept. of Bacteriology, University of Wisconsin
B	B	Dept. of Bacteriology, University of Wisconsin
<i>Mixed strain lactic starter, O.D.</i>		
	O.D.	Marschall Div., Miles Lab., Madison, Wisconsin

enough *E. coli* to give approximately 1000 cells/ml. Inoculated milks were then incubated for 15 h at either 21 or 32 C, after which they were refrigerated at 7 C for up to 17 days. Samples were taken at 3-h intervals during the first 15 h, and at various times thereafter. Experiments were done in duplicate and results reported are average values.

Enumeration of *E. coli*

E. coli was enumerated using Trypticase Soy Agar (TSA, Difco) pour plates with incubation at 37 C for 24 h. Violet Red Bile agar pour plates and TSA surfaced-spread plates with a VRB overlay (24) were also used and were incubated at 37 C for 24 h. A comparison of these methods is presented in another paper.

pH measurements

The pH of skim milk was measured with a Corning Model 10 pH meter using a miniature combination glass electrode.

RESULTS

Growth of *E. coli* in sterile skim milk

Growth curves for each of the six *E. coli* strains used in this study are in Fig. 1-6 with the specific curve identified as "0.00% starter culture." Generation times for *E. coli* ranged from 28 to 35 min at 32 C (mean = 31 min) with strain B₂C having the shortest generation time and strain A-1 the longest. At 21 C, generation times ranged from 66 to 109 min (mean = 89 min), a much wider range than occurred at 32 C. Strain K-12 had the shortest generation time and strain H-1 had the longest at 21 C. It was not possible to distinguish between pathogenic and non-pathogenic strains on the basis of their growth rates.

Beyond 15 h of incubation, when samples were refrigerated, cultures of *E. coli* which had grown at 32 C maintained relatively constant numbers. However, when *E. coli* was grown at 21 C, there were some strains which grew during refrigeration (H-1, B, and K-12), and one strain (B₂C) which decreased in number after 3 days of refrigeration. These different responses to refrigeration may have resulted from variability in the sensitivity of log-phase cells to the colder temperature, whereas cells grown at 32 C would have reached the stationary phase of growth at the time of refrigeration.

Inhibition of *E. coli* by a lactic starter culture at 32 C

The extent of inhibition of *E. coli* by the starter culture at 32 C can be seen in Fig. 1-6 by comparing the solid

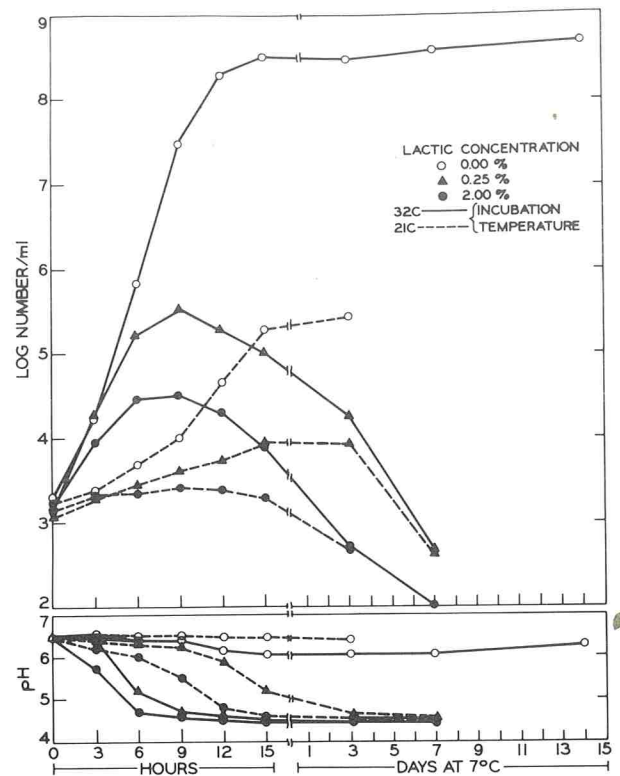


Figure 1. Changes in numbers of *Escherichia coli* A-1 when grown in skim milk with and without an added lactic starter culture, and changes in pH of milk during fermentation.

lines showing the change in numbers of *E. coli* with 0.0, 0.25, and 2.0% added starter culture. With either concentration of lactic culture all *E. coli* strains were completely inhibited from further growth after 6 to 9 h of incubation and at a pH of 5.0 to 5.5. Partial inhibition occurred at 3 to 6 h at higher pH values, especially when 2.0% lactic culture was added. Of the strains, H-1 was most markedly inhibited and reached a maximum population of about 3×10^4 /ml with 0.25% lactic culture and 4×10^3 /ml with 2.0% added lactic culture. Strain K-12 was inhibited least, reaching a maximum of 2.5×10^6 /ml with 0.25% added lactic culture and 1×10^5 /ml with 2.0% added lactic culture.

Inhibition of *E. coli* by a lactic starter culture at 21 C

All strains of *E. coli* tested were inhibited more by the starter culture at 21 than 32 C, even though the pH declined at a much slower rate at the lower temperature. Three strains (H-1, A-1, and A-4) were completely inhibited by 2.0% starter at 21 C and grew only slightly with 0.25% lactic starter, staying below 10^4 /ml. The more resistant strains (B₂C, B, and K-12) reached 3 to 5×10^4 /ml with 0.25% lactic culture, but did not exceed 10^4 /ml when 2.0% lactic culture was added. At 21 C and with 0.25% lactic culture, complete inhibition of growth by *E. coli* did not occur until about 15 h and pH values of 4.9 to 5.2. However, partial inhibition occurred from 6 to 12 h at pH values ranging from 5.4 to 6.2. Strains A-1 and A-4 were partially inhibited at pH values close to 6.0.

A comparison of growth patterns at the two

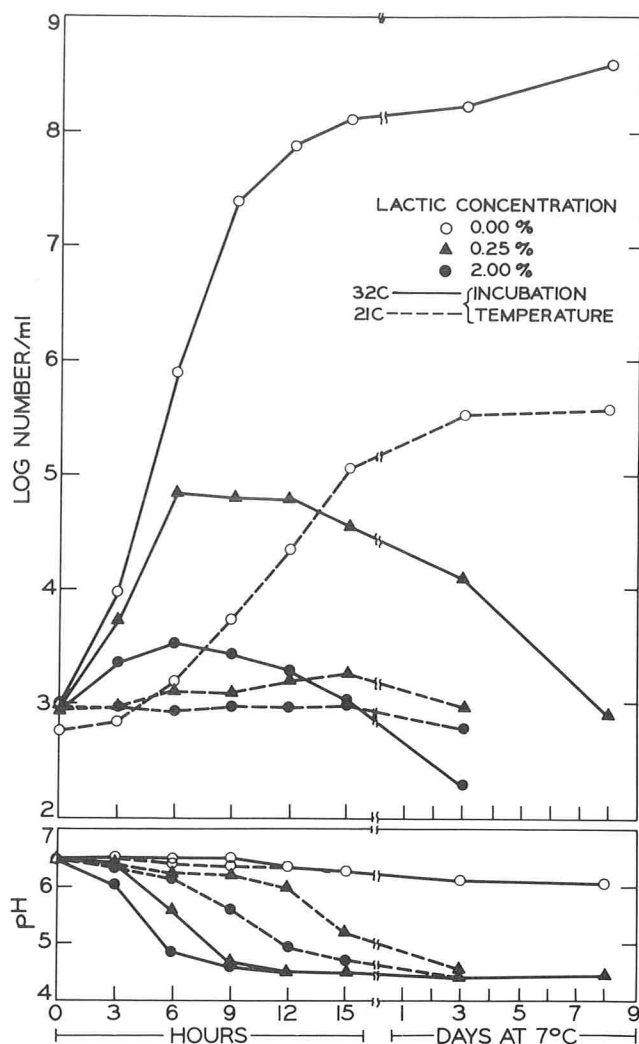


Figure 2. Changes in numbers of *Escherichia coli* A-4 when grown in skim milk with and without added lactic starter, and changes in pH of milk during fermentation.

temperatures shows that complete inhibition more often than not occurred earlier at 32 than at 21 C, however smaller numbers of *E. coli* were obtained at 21 than at 32 C. Also, partial inhibition occurred at higher pH values at 21 than at 32 C.

Survival of *E. coli* in refrigerated fermented milks

The final pH of all fermented milks ranged from 4.4 to 4.6. Milks fermented at 21 C with 0.25% added lactic culture were the only samples not close to their lowest pH after 15 h of incubation (see curves for pH, Fig. 1-6). The longest survival times for *E. coli* in refrigerated fermented milks were 16 and 17 days with strains B₂C and K-12. These were in milk fermented at 32 C with 0.25% starter. Longer survival occurred with these strains because they reached higher numbers early in the fermentation, and after being inhibited from further growth, they seemed more resistant to acid conditions. Strains A-1 and B, although reaching about the same number as strain B₂C, were inactivated more rapidly and this accounted for a shorter survival time. Strain K-12 was inactivated more rapidly than B₂C, but since it had

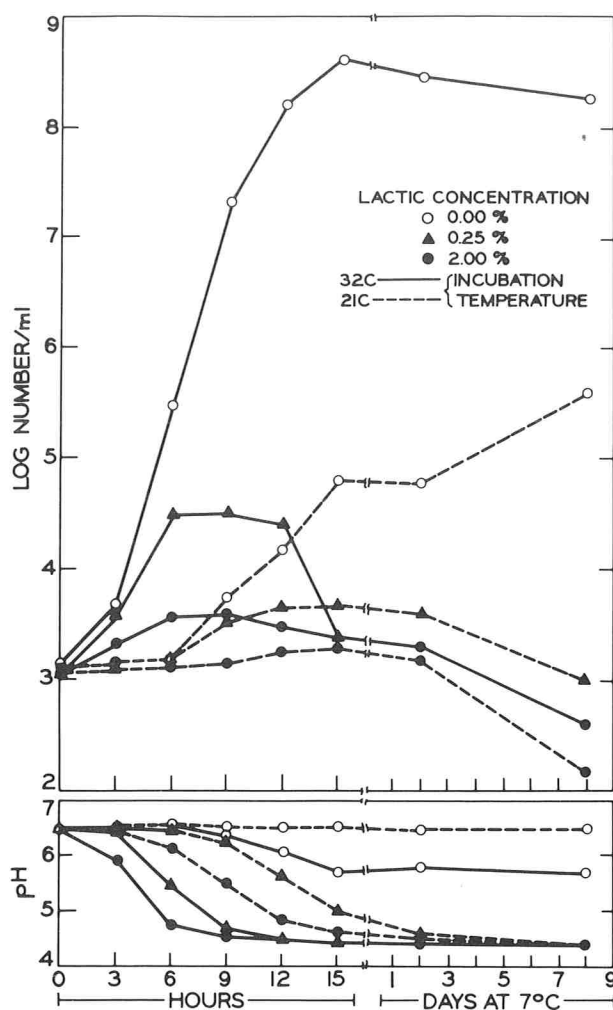


Figure 3. Changes in numbers of *Escherichia coli* H-1 when grown in skim milk with and without added lactic starter, and changes in pH of milk during fermentation.

attained larger numbers during the fermentation at 32 C, survival times were comparable. Not enough values were obtained to calculate accurate "D" values for inactivation of the different strains in fermented milks, but an estimate is from 2 to 3 days for strain H-1 and 8 days for strain B₂C. The coefficient of variation for TSA counts on samples taken from 0 to 15 h was 11% and on refrigerated samples it was 21%.

DISCUSSION

These data emphasize the importance of two variables in inhibiting growth of *E. coli* in fermented dairy products. These are temperature of fermentation and initial amount of lactic starter. Although production of acid by a starter culture is of major importance in the ultimate destruction of *E. coli*, in the first 9 to 15 h of fermentation use of a lower incubation temperature or a higher concentration of lactic bacteria appeared to increase inhibition at higher pH values. This was especially true when a combination of lower temperature (21 C) and higher starter concentration (2.0%) were used. Similar conclusions regarding inoculum size and

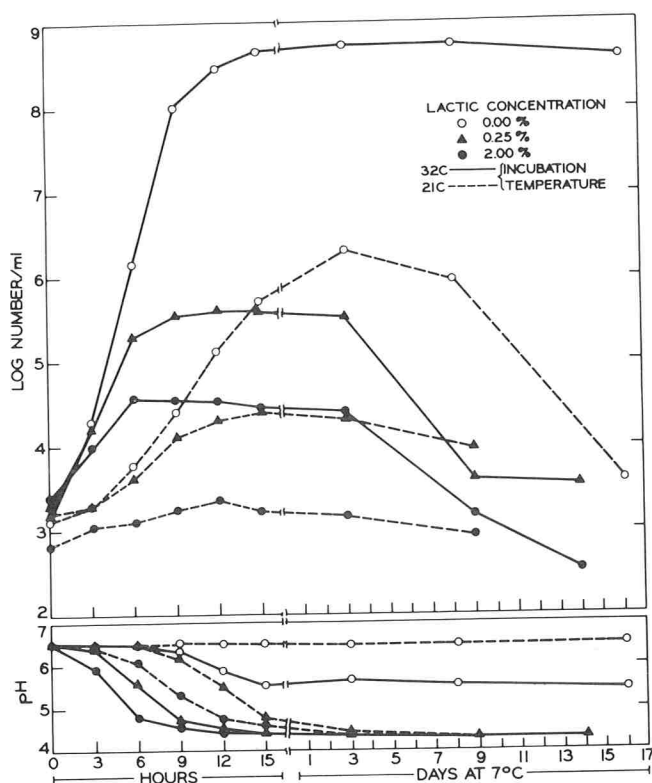


Figure 4. Changes in numbers of *Escherichia coli* B₂C when grown in skimmilk with and without added lactic starter, and changes in pH of milk during fermentation.

incubation temperature were arrived at by Park and Marth (17) when they studied inhibition of *Salmonella typhimurium* by lactic acid bacteria in skimmilk. They also found that growth of *Salmonella* was inhibited at 21 C with only a slight decrease in pH. Possible presence of antibiotics produced by some lactics (13) or production of volatile compounds inhibitory to growth (10) may be responsible for inhibition not attributable to acid production. Inhibition of bacterial growth resulting from production of hydrogen peroxide by lactic cultures may also be possible, as reported by Juffs and Babel (9). Occurrence of pH-independent inhibition of staphylococci and salmonellae through growth of lactic streptococci has been reported by Gilliland and Speck (5), but the cause of the non-acidic inhibition has not been determined. Park et al. (19) previously demonstrated that acid production by a lactic starter was related to inhibition and inactivation of EEC during manufacture of Camembert cheese.

Survival of *E. coli* in refrigerated fermented dairy products has been studied previously (6,19,20,26). Survival depends on storage temperature, type of product, type of lactic bacteria used, and pH of the product. Both Goel et al. (6) and Potashnik et al. (20) found coliforms were rapidly inactivated in *S. lactis*-fermented milks similar to our fermentation products. Inactivation of *E. coli* in cultured buttermilk at a pH in the range of 4.1 to 4.8 and stored at 7.2 C was studied by Goel et al. (6) and their results were similar to ours. Our study was somewhat different because inoculation with

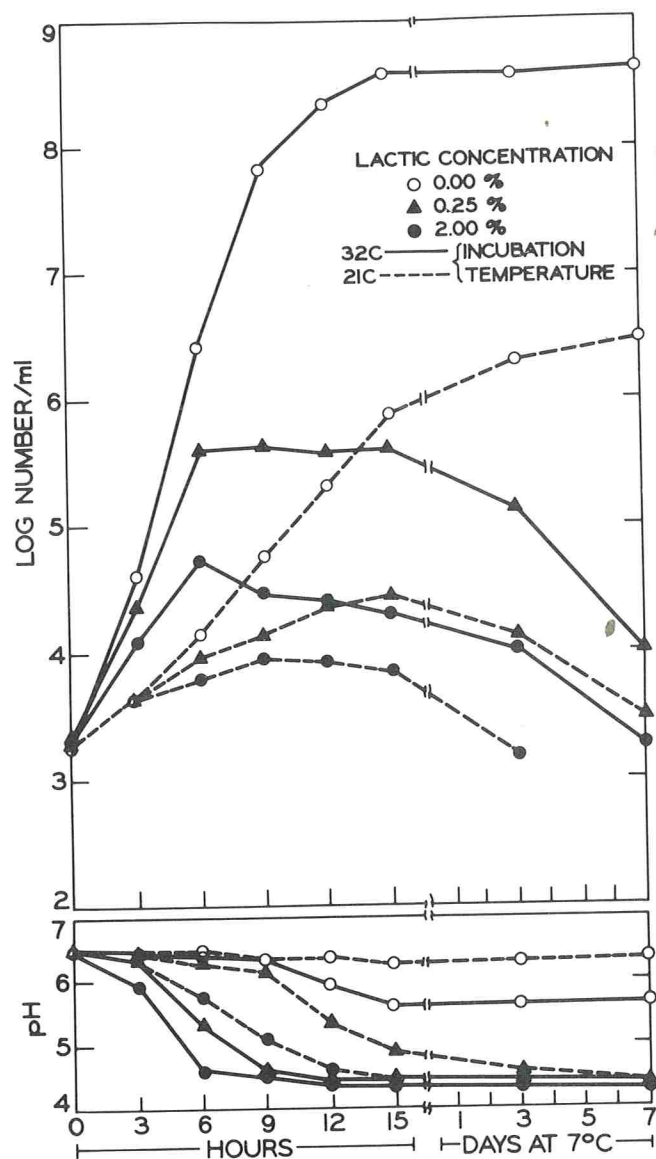


Figure 5. Changes in numbers of *Escherichia coli* B when grown in skimmilk with and without added lactic starter, and changes in pH of milk during fermentation.

E. coli was done before fermentation, not after, which could reduce the initial shock of acid conditions to the cells, and thus allow for longer survival of bacteria. Park and Marth (18) studied behavior of *Salmonella typhimurium* in refrigerated cultured milks and found that survival was lengthened when milks were cultured with a small inoculum of lactics, even though final pH values were similar. This could also be true with *E. coli* but our data are not complete enough to draw that conclusion.

Even though EEC cultures are able to increase in numbers as much as three log cycles during the initial stages of milk fermentation, the finished product is relatively safe from mishandling by the consumer because the final product will not support multiplication of EEC and will probably be bactericidal to any likely to enter the food. However there should be concern about dairy products in which the acidity decreases upon

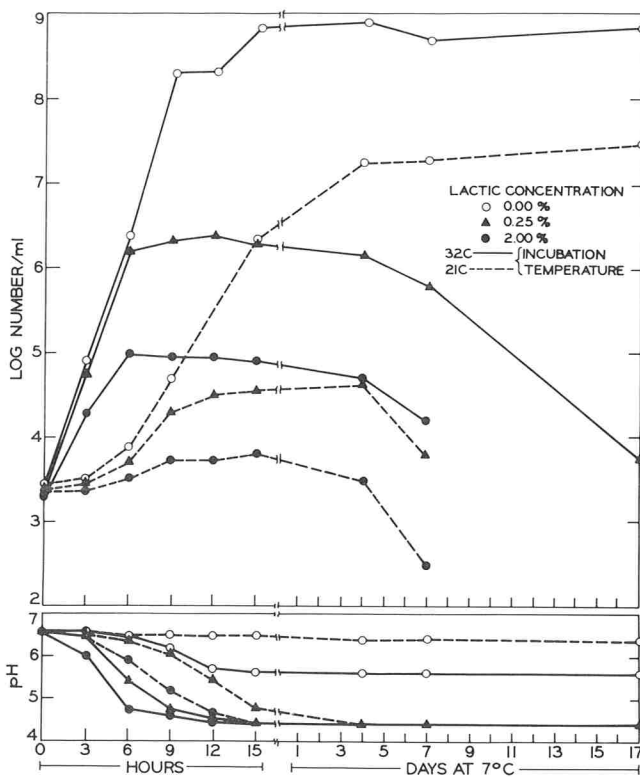


Figure 6. Changes in numbers of *Escherichia coli* K-12 when grown in skim milk with and without added lactic starter, and changes in pH of milk during fermentation.

storage, thus allowing survival or growth of EEC. Certain surface-ripened cheeses fit this description, and since foodborne illness caused by EEC has been associated with such products (12), further research is being done in this area.

ACKNOWLEDGMENTS

Research supported by the College of Agricultural and Life Sciences, University of Wisconsin-Madison, and by the Food and Drug Administration through contract 223-74-2089.

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Inhibition of Enteropathogenic *Escherichia coli* by Homofermentative Lactic Acid Bacteria in Skimmilk

II. Comparison of Lactic Acid Bacteria and Enumeration Methods

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(Received for publication April 22, 1977)

ABSTRACT

Inhibition of enteropathogenic *Escherichia coli* in skimmilk at 21 and 32 C by 0.25 and 2.0% of added *Streptococcus lactis*, *Streptococcus cremoris*, or a mixed strain starter culture was studied. After 15 h of fermentation, fermented milks were refrigerated at 7 C and then were tested periodically for survival of *E. coli*. Three methods for enumeration of *E. coli* during these fermentations were compared. They included trypticase soy agar (TSA) pour plates, violet red bile agar (VRB) pour plates, and TSA surface plating with a VRB overlay. Lactic cultures had similar inhibitory properties at 32 C, but there were differences at 21 C, with *S. lactis* being least inhibitory and the mixed strain culture most inhibitory. The VRB pour plate method gave poorest recovery of *E. coli* when fermentation was at 32 C and when fermented milks were refrigerated. The TSA surface plating method apparently allowed for recovery of injured *E. coli* cells and gave results similar to the TSA pour plate method.

Lactic acid fermentations are commonly used in food processing and preservation, especially in the dairy industry. The ability of lactic acid bacteria to inhibit bacteria able to cause foodborne illnesses such as *Staphylococcus aureus*, *Salmonella*, and enteropathogenic *Escherichia coli* (EEC), is well known (6,9,10). Previous studies have shown that different species and strains of homofermentative lactics may differ in their ability to inhibit *Salmonella* in milk (8). In this study we wanted to learn if three closely related cultures of homofermentative lactic acid bacteria, *Streptococcus lactis*, *Streptococcus cremoris*, and a commercial mixed strain starter culture, would vary in their inhibition of EEC in skimmilk.

One of the major problems in recovery of microorganisms present in an adverse environment, such as an acidic dairy product, is occurrence of sublethal injury to cells. When this occurs, the number of bacteria recovered on selective media decreases and stressed cells may be unable to repair themselves. However, repair will occur on non-selective media. This subject has been reviewed recently by Busta (1) and Ordal et al. (7). Enumeration of injured pathogens in foods is important since they might recover and again become infectious (12). Concern about the presence of sublethally injured EEC in fermented dairy products is justified by the report of Roth and

Keenan (11) that acid conditions can cause injury to *E. coli*. Thus results from use of Violet Red Bile agar (VRB) for enumeration of coliforms, as recommended by *Standard Methods for the Examination of Dairy Products* (5), may be misleading if conditions in the product have stressed the bacteria.

Speck et al. (13) and Hartmen et al. (4) have described plate count procedures to enumerate injured coliforms. The method of Speck et al. (13) was used to compare numbers obtained with this procedure with those from the VRB pour plate method and trypticase soy agar pour plates, thus obtaining information on the amount of acid injury occurring during fermentation and storage, and on the efficiency of an enumeration method which allows for repair of injured cells.

MATERIALS AND METHODS

Preparation of skimmilk fermentations

Cultures used in this study and their sources are listed in Table 1. Preparation of cultures and procedure used for fermentation experiments were as described in a previous paper (3).

TABLE 1. Cultures used in this study.

Culture	Abbreviation	Source
Enteropathogenic <i>E. coli</i>		
Toxigenic		
B ₂ C	B ₂ C	FDA, Washington, D.C.
H10407	H-1	FDA, Washington, D.C.
Invasive		
4608	A-4	FDA, Washington, D.C.
Nonpathogenic <i>E. coli</i>		
K-12	K-12	Dept. of Bacteriology, University of Wisconsin
<i>Streptococcus lactis</i> 4175	—	Marschall Div., Miles Lab., Madison, Wisconsin
<i>Streptococcus cremoris</i> C13	—	Marschall Div., Miles Lab. Madison, Wisconsin
Mixed strain starter O.D.	—	Marschall Div. Miles Lab., Madison, Wisconsin

Enumeration of *E. coli*

Three methods were used to enumerate *E. coli*. The first used Trypticase Soy agar (TSA, Difco) pour plates, the second used Violet Red Bile (VRB) agar (Difco) pour plates, and the third consisted of spreading 0.5 ml of diluted samples on the surface of a TSA plate, incubation of the plate for 1 h at room temperature, and then overlaying with VRB agar. This method has been described by Speck et

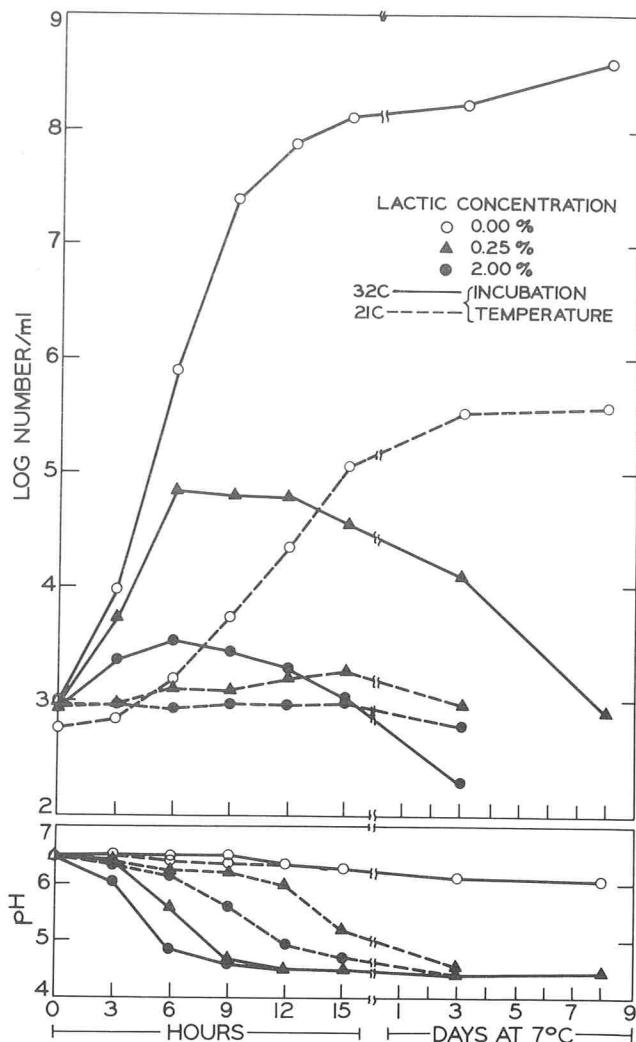


Figure 1. Changes in numbers of *E. coli* A-4 when grown in skim milk with and without lactic starter (O.D.), and changes in pH of skim milk during fermentation.

al. (13) for enumeration of freeze-injured *E. coli*. All plates were incubated at 37 C for 24 h and all experiments were done in duplicate.

pH measurements

The pH of milk was measured with a Corning Model 10 pH meter using a miniature combination glass electrode.

RESULTS

Comparison of lactic cultures

Figures 1 to 3 give data showing inhibition of *E. coli* A-4 by a commercial starter, *S. lactis*, and *S. cremoris*. Comparison of data in these figures shows little difference among the lactic cultures in their ability to inhibit this strain of *E. coli* at 32 C. At 21 C, however, there were differences in the pattern of inhibition, these being most apparent when 0.25% culture was used. Under these conditions, *S. lactis* and *S. cremoris* (Fig. 2 and 3) allowed approximately one log cycle of growth by *E. coli* in 15 h, whereas the commercial starter culture allowed only minimal growth (Fig. 1). Changes in pH were similar in all fermentations.

Data in Fig. 4 to 6 show inhibition of *E. coli* B₂C by the

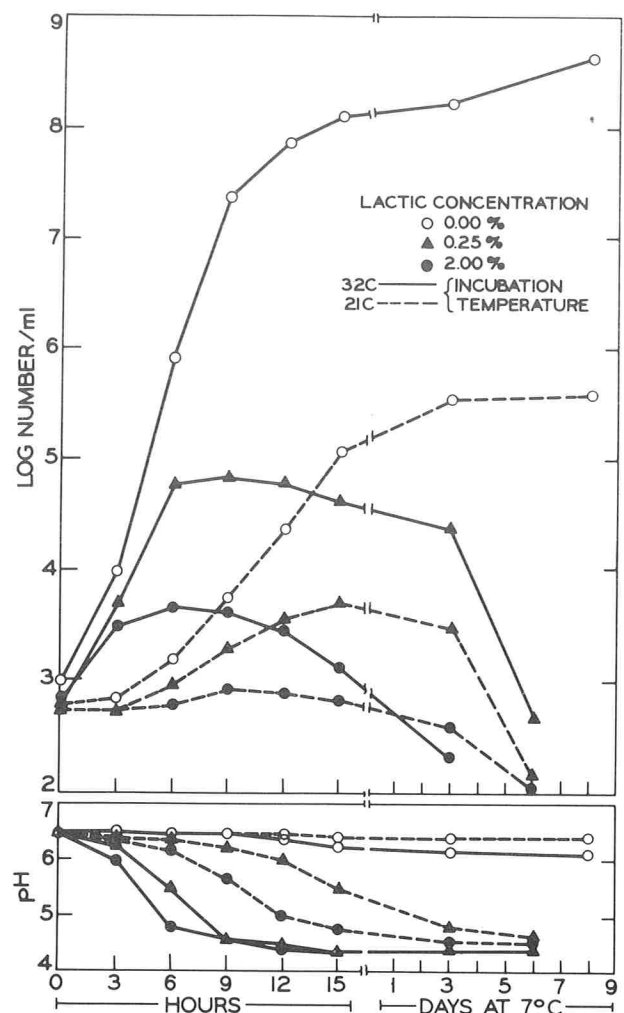


Figure 2. Changes in numbers of *E. coli* A-4 when grown in skim milk with and without *Streptococcus lactis*, and changes in pH of skim milk during fermentation.

lactic cultures. As with strain A-4, results obtained at 32 C were similar for the different lactic cultures. However, again there were differences when incubation was at 21 C. The *S. lactis* culture (Fig. 2), when used at either 0.25% or 2.0%, allowed more growth of *E. coli* than did either *S. cremoris* or the mixed strain starter culture (Fig. 1 and 3). Again, pH values were similar during these fermentations.

Recovery of *E. coli* on VRB agar and on TSA with VRB agar overlay

Tables 2 and 3 give data on recovery of *E. coli* during the first 15 h of fermentation using VRB pour plates and the VRB agar overlay of TSA (VRB + TSA). The TSA pour plate counts were considered as 100% recovery, and results obtained with the two other methods are given as a percentage of the TSA count. All the TSA counts for these fermentations were given in an earlier paper (3). The coefficient of variability was 11% for TSA counts, 12% for TSA + VRB counts, and 15% for VRB pour plate counts during the first 15 h of fermentation. When

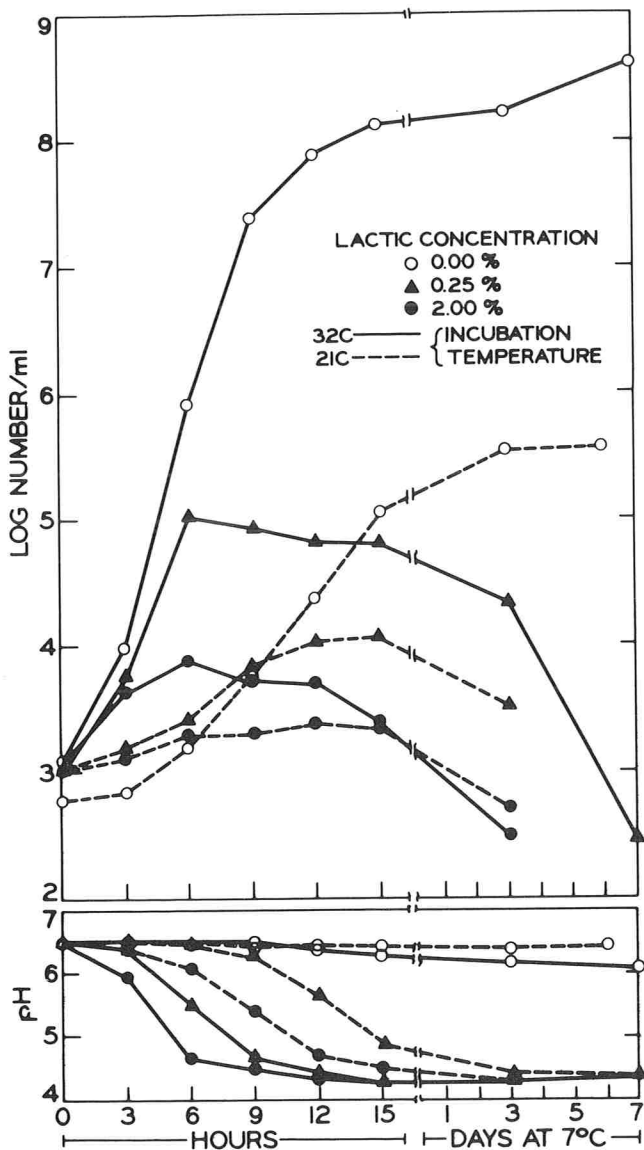


Figure 3. Changes in numbers of *E. coli* A-4 when grown in skim milk with and without *Streptococcus cremoris*, and changes in pH of skim milk during fermentation.

tests were made on refrigerated fermented milks, the coefficient of variability for all methods was about 21% if counts were above 300/ml.

When fermentation was at 21 C with 2.0% commercial starter culture, the mean VRB recovery was 96.6% and the mean VRB + TSA recovery was 102%. These mean values are from pooled data for four *E. coli* strains, and show that during the first 15 h of fermentation at 21 C there was no significant difference in results from the different plate count methods (*t* test, *P* < .05). The average percentage of recovery for each strain during the first 15 h at 21 C ranged from 86% (strain A-4) to 113% (strain B₂C) for the VRB pour plates, and 95% (strain A-4) to 110% (strain K-12) for the VRB + TSA overlay method. Also, there was less variability in percentage of recovery from fermented milks incubated at 21 and 32 C when the overlay method rather than when VRB was used (*s*_{VRB} = 21, *s*_{VRB + TSA} = 17).

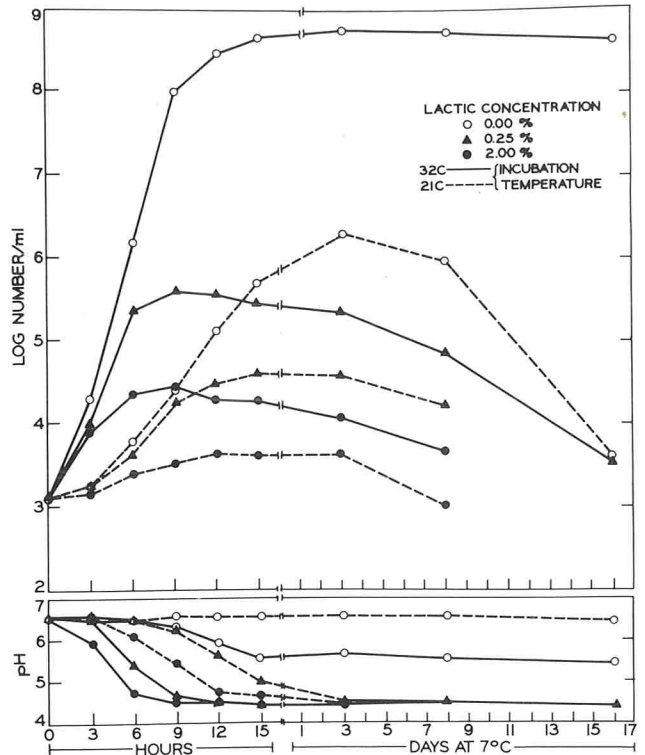


Figure 4. Changes in numbers of *E. coli* B₂C when grown in skim milk with and without lactic starter (OD) and changes in pH of skim milk during fermentation.

TABLE 2. Recovery of *E. coli* from skim milk inoculated with 2.0% starter (O.D.) and incubated at 21 C.

Time (h)	Strain of <i>E. coli</i>	VRB	VRB + TSA ^a	pH
0	B ₂ C	96	78	6.5
	A-4	60	59	6.55
	H-1	96	99	6.55
	K-12	40	71	6.5
3	B ₂ C	102	108	6.4
	A-4	79	83	6.35
	H-1	95	100	6.4
	K-12	107	128	6.4
6	B ₂ C	104	108	6.1
	A-4	103	102	6.15
	H-1	96	109	6.1
	K-12	94	114	5.9
9	B ₂ C	142	89	5.45
	A-4	96	106	5.6
	H-1	107	89	5.5
	K-12	107	137	5.2
12	B ₂ C	126	103	4.8
	A-4	96	116	4.95
	H-1	98	115	4.8
	K-12	126	124	4.65
15	B ₂ C	113	122	4.65
	A-4	84	103	4.7
	H-1	78	102	4.6
	K-12	74 ^b	77 ^b	4.65

^aTSA surface plating with VRB overlay.

^bMean % recovery for pooled data was 96.6 with VRB and 102.2 with VRB + TSA. There is no significant difference in these means.

At 32 C there was a significant difference in the percentage of recovery by the two methods, the mean VRB recovery equaling 91%, and the mean recovery of the overlay method equaling 104% (Table 3). The lower

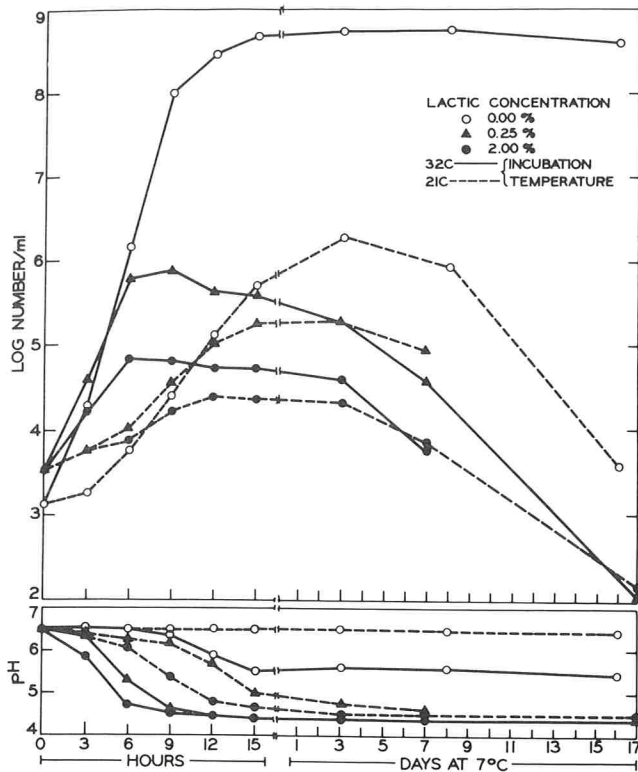


Figure 5. Changes in numbers of *E. coli* B₂C when grown in skim milk with and without *Streptococcus lactis*, and changes in pH of skim milk during fermentation.

TABLE 3. Recovery of *E. coli* from skim milk inoculated with 2.0% lactic starter (O.D.) and incubated at 32 C.

Time (h)	Strain of <i>E. coli</i>	VRB	VRB + TSA ^a	pH
0	B ₂ C	101	105	6.55
	A-4	102	89	6.5
	H-1	96	105	6.5
	K-12	92	116	6.55
3	B ₂ C	108	97	5.95
	A-4	108	119	6.05
	H-1	77	92	6.0
	K-12	74	56	5.9
6	B ₂ C	155	128	4.75
	A-4	85	85	4.85
	H-1	103	88	4.75
	K-12	72	140	4.7
9	B ₂ C	101	96	4.5
	A-4	63	107	4.6
	H-1	79	86	4.55
	K-12	103	111	4.55
12	B ₂ C	82	121	4.5
	A-4	67	112	4.55
	H-1	69	72	4.45
	K-12	100	80	4.45
15	B ₂ C	105	124	4.4
	A-4	52	94	4.5
	H-1	76	91	4.45
	K-12	118 ^b	126 ^b	4.4

^aTSA surface plating with VRB overlay.

^bMean% recovery for pooled data was 91.2 with VRB and 103.7 with VRB + TSA. The difference in these means was significant at the 95% level.

recovery with the VRB pour plate method could have been caused by either increased acid production by the lactics or increased susceptibility of *E. coli* to injury at a

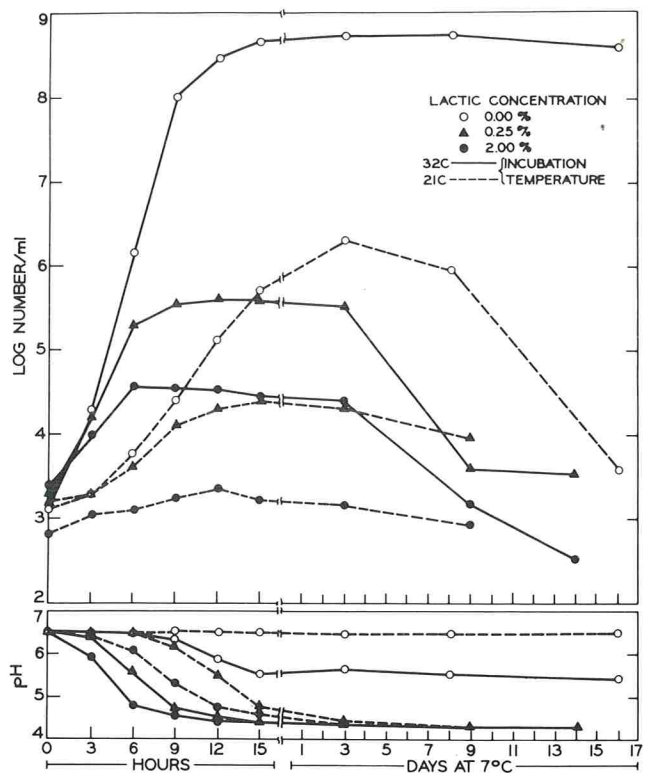


Figure 6. Changes in numbers of *E. coli* B₂C when grown in skim milk with and without *Streptococcus cremoris*, and changes in pH of skim milk during fermentation.

higher temperature. At 32 C, the strain with poorest recovery on VRB was A-4 at 79% and the strain with best recovery was B₂C at 109%. There was a significant difference between the percentage of recovery for these two strains at the 95% level of significance, but not between any of the other strains (new Duncan multiple range test). The VRB + TSA overlay method had a range for recovery of individual strains of 91 to 112% when the fermentation was at 32 C, with no significant differences between any of the strains in average recovery. Thus, during the first 15 h of the lactic fermentation at 21 C, all three methods for enumeration of *E. coli* yielded similar results, but at 32 C, the VRB pour plate method was less efficient than the VRB + TSA overlay or the TSA pour plate, with some strains of *E. coli* responding to differences in methods more than others. It may be more than a coincidence that the strain having poorest recovery on VRB (A-4) is also a strain rather sensitive to

TABLE 4. Recovery of *E. coli* from refrigerated skim milk which had been incubated at 21 C with 2.0% lactic starter (O.D.)

Time (days)	Strain of <i>E. coli</i>	TSA count (x 10 ⁶ /ml)	VRB	VRB + TSA ^a	pH
2	H-1	15	92	99	4.5
8	H-1	15	110	207	4.4
3	B ₂ C	42	56	79	4.55
8	B ₂ C	10	58	108	4.5
3	A-4	5	32	95	4.45
4	K-12	30	48	50	4.4
7	K-12	3	42	115	4.35

^aTSA surface plating with VRB overlay.

inhibition by lactic cultures, whereas the strain having high recovery on VRB (B₂C) is one of those most resistant to inhibition by lactic acid bacteria (3).

Recovery of *E. coli* from refrigerated fermented milks

Recovery of *E. coli* from refrigerated milks using VRB and VRB + TSA methods is shown by data in Tables 4 and 5. Results from all three enumeration methods were somewhat erratic, possibly because of the combination

TABLE 5. Recovery of *E. coli* from refrigerated skim milk which had been incubated at 32 C with 2.0% lactic starter (O.D.).

Time (days)	Strain of <i>E. coli</i>	TSA count (x 10 ² /ml)	VRB		pH
			VRB	VRB + TSA ^a	
			(% of TSA count)		
2	H-1	20	72	100	4.4
8	H-1	4	12	70	4.4
3	B ₂ C	120	42	104	4.45
8	B ₂ C	47	3	33	4.45
3	A-4	2	28	127	4.45
4	K-12	500	44	47	4.4
7	K-12	160	1	100	4.35

^aTSA surface plating with VRB overlay.

of lower cell numbers and a higher number of stressed cells being present. With milks fermented at 21 C (Table 4), recovery of *E. coli* on VRB in four of seven instances was about half that recovered on VRB + TSA plates. There were no discernable trends on VRB recovery with respect to cell number or time samples were stored (Table 4).

Milks fermented at 32 C gave more interesting results, the VRB counts nearly always being significantly lower than either the TSA or VRB + TSA counts (Table 5). This decreased recovery on VRB might have resulted from greater acid production by the starter at the higher incubation temperature and hence a larger number of stressed cells. There also appeared to be poorer recovery on VRB than on VRB + TSA as storage of milk increased and as cell numbers decreased. However, the VRB + TSA method gave counts closer to the TSA pour plate method, although not as consistently with the acidic, refrigerated samples as with the fermenting samples.

DISCUSSION

It is difficult to say if the variation observed in inhibition of *E. coli* by the three lactic cultures is of practical importance. The survival time for contaminating *E. coli* in a fermented milk product probably would not differ by more than a few days with use of different starter cultures if our cultures are representative in terms of inhibitory property and usage in the industry. Similar experiments have been done by Park and Marth (8) in a study on inhibition of *Salmonella typhimurium* by lactic acid bacteria. They found little difference in the ability of *S. lactis*, *S. cremoris*, and mixed strain starter cultures to inhibit *Salmonella* at 21 C, and some differences at 30 C explainable by rates of acid production. Park and Marth (9) did find different rates of inactivation for *S. typhimurium* during refrigerated storage of cultured

milks prepared by use of several lactic starter cultures.

Presence of sublethally injured *E. coli* in fermented milks appears to be related to temperature of incubation, *E. coli* strain, and pH of the milk. The higher incubation temperature may have resulted in lower VRB counts because of the greater shock to cells of a sudden decrease in pH and thereby producing injured cells, or a greater susceptibility of cells to acid-injury at a higher temperature. Decreased recovery of some strains of *E. coli* from refrigerated milks fermented at 32 C may be a result of the same factors. The only previous report on acid injury of *E. coli* was by Roth and Keenan (11) who reported poor recovery on VRB after incubation of *E. coli* in tryptic soy broth acidified with lactic acid to a pH of 4.2. The VRB pour plate method has been found accurate for recovery of coliforms from cheese (2), and is currently recommended for examination of dairy products for coliforms (5). The VRB pour plate method may be acceptable for most dairy products since most would have a higher pH than occurred in our fermentations. However, in dairy products with low pH values, TSA surface plating with a VRB overlay may be better than VRB for recovery of *E. coli*. Since the microbial flora in our samples was limited, the selective nature of this enumeration method was not judged.

ACKNOWLEDGMENTS

Research supported by the College of Agricultural and Life Sciences, University of Wisconsin-Madison and by the Food and Drug Administration through Contract 223-74-2089.

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Enumeration and Identity of Lactobacilli in Dietary Products¹

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(Received for publication April 11, 1977)

ABSTRACT

A medium was developed for selectively enumerating bile-resistant lactobacilli by adding 0.15% oxgall to lactobacillus selective agar (BBL). Lactobacilli were not found in many products sold as sources of *Lactobacillus acidophilus*, whereas others contained widely different numbers of lactobacilli capable of forming colonies on the bile medium. Of seven products from which lactobacilli were isolated, only three contained *L. acidophilus*. One of these products was from a pharmacy and the other two were from dairy processor(s).

A number of dried and liquid products are offered as sources of *Lactobacillus acidophilus* by health food stores and pharmacies. Labels on some of these products claim that other *Lactobacillus* species are also present in addition to *L. acidophilus*. However, most of them are described as sources of "specially cultured" or selected strains" of *L. acidophilus* that help restore and/or maintain a favorable intestinal flora. Examination of such products in England (4) revealed that only a small percentage actually contained *L. acidophilus*.

One characteristic of *L. acidophilus* and other intestinal microorganisms which enables them to survive or establish in the intestinal tract is their ability to grow in the presence of bile salts (10,11). Rogosa et al. (8) developed a selective medium for isolation and enumeration of lactobacilli in feces. Subsequently, a dehydrated form of this medium (LBS) has been made available (BBL, Cockeysville, Md). Ellis and Sarles (2) added antibiotics to the medium and used it to isolate antibiotic resistant lactobacilli selectively from fecal samples.

The present study was undertaken to modify LBS agar by incorporating bile salts to make it selective for bile resistant lactobacilli. Another objective was to use the

medium to evaluate commercial products sold as sources of *L. acidophilus*.

MATERIALS AND METHODS

Source and maintenance of cultures

L. acidophilus NCFM, *L. acidophilus* CNRZ 216, *L. acidophilus* CNRZ 218, *Lactobacillus plantarum*, *Lactobacillus bulgaricus* HWD, *L. bulgaricus* NCSI, *Lactobacillus casei* 7469, *Lactobacillus lactis* and *Lactobacillus fermentum* 36 were from the departmental stock culture collection. The cultures were propagated in lactobacillus MRS broth (Difco, Detroit, Michigan) or in sterile 10% non-fat milk solids (NFMS) using 1% inocula and 18 h incubation at 37 C. They were stored in a refrigerator between transfers.

Enumeration media

LBS agar was prepared from the commercially available dehydrated product or was made from individual ingredients according to the manufacturer's formulation. The desired concentrations (0.05, 0.10, or 0.15%) of oxgall (BBL) were added before pH adjustment and heating. These media will be referred to as LBSO-.05, LBSO-.10, and LBSO-.15 respectively. The prepared LBS media were stored in a refrigerator (3 C) until used. MRS agar (MRS broth plus 1.5% agar) was used as a nonselective plating medium.

Enumeration procedures

Cultures or samples were diluted in sterile dilution blanks containing 0.1% NFMS and 0.01% silicon antifoamer (Sigma Chemical Co., St. Louis, Mo.). Duplicate plates were prepared for each medium from the required dilutions. Each plate was overlaid with the same medium originally used for plating. MRS agar plates were incubated at 37 C without further adjustment. Plates prepared with LBS or LBSO agars were placed in plastic bags, flushed with CO₂ for 1 min, sealed and incubated at 37 C. All plates prepared from stock cultures and dairy case products were incubated 72 h and those prepared from health food store, pharmacy, and animal feed samples were incubated 5 days; 5 days were required for the later because colonies were too small to count accurately. Colonies were counted with the aid of a Quebec colony counter.

Isolation and identification of lactobacilli

Predominating colony types were isolated from LBS, LBSO-.10 or LBSO-.15 agar plates prepared from some of the commercial samples. When sufficient colonies were present, 10 were picked into tubes of sterile MRS broth from each of the three selective media. The isolates were incubated at 37 C for 24-48 h (until growth was evident). Purity was established by streaking the isolates on MRS agar. All isolates that were catalase-negative and gram-positive non-sporeforming rods were identified using the Minitek System (BBL) as described by Gilliland

¹Paper No. 0000 of the journal series of the North Carolina Agricultural Experiment Station. 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 one not included.

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and Speck (5) for identifying lactobacilli. Characteristics for the lactobacilli presented in the 8th edition of *Bergey's Manual of Determinative Bacteriology* (1) were used as bases for identification of isolates.

Procurement and care of samples

Commercial products sold as sources of *L. acidophilus* for humans were obtained from local pharmacies, supermarkets and health food stores. Two animal feed supplements purportedly containing *L. acidophilus* were obtained from a commercial supplier. The analyses for determining numbers of lactobacilli were done within 24 h of purchase. All products were purchased and examined before the expiration date appearing on labels. Each product was stored as recommended by the manufacturer until analyzed.

RESULTS

Of the cultures tested, *L. bulgaricus* NCSI and *L. lactis* were most sensitive to oxgall since they did not grow in LBSO-.05 (Table 1). One tenth percent oxgall (LBSO-.10) was required to prevent colony formation by *L. bulgaricus* HWD. All strains of *L. acidophilus* grew equally well on LBS, LBSO-.05, LBSO-.10, and LBSO-.15. Similar results were observed for *L. brevis*, *L. casei* 7469, *L. plantarum*, and *L. fermentum* 36. Use of crude sodium taurocholate (Sigma Chemical Co., St. Louis, Mo.) in place of oxgall in similar experiments provided comparable results. Attempts to use deoxycholate as the bile salt failed because it precipitated when added to the acidic LBS agar.

Fifteen samples of different products sold as sources of *L. acidophilus* were obtained from local health food stores. There was a wide range in the total numbers of lactobacilli (LBS agar) in these products (Table 2). The count for one product (H-2) was $< 10^2/g$ on all media including a nonselective medium (MRS). Two others shown in the Table (H-4 and H-5) contained no viable lactobacilli. In addition, six other samples from health food stores examined in earlier experiments (data not shown) contained no viable lactobacilli. Of the nine samples presented in Table 2 from health food stores, only three contained lactobacilli that were resistant to 0.1% bile (LBSO-.10) and only two contained ones resistant to 0.15% bile (LBSO-.15). Neither of the two animal feed supplements (A-1, A-2) contained viable lactobacilli. Both products from pharmacies (P-1 and P-2) contained bile-resistant lactobacilli. Sample P-1 contained fewer than did sample P-2. Milk with added *L. acidophilus* (D-1 and D-2) obtained from dairy cases in supermarkets contained bile resistant lactobacilli, although numbers in D-2 were lower than in D-1.

Isolates were obtained from seven of the products listed in Table 2 that contained organisms capable of forming colonies on LBS, LBSO-0.10, or LBSO-.15 agars. Unfortunately, we did not obtain isolates from the other three samples (H-1, H-3, and H-6) which contained lactobacilli. All the isolates were catalase-negative,

TABLE 1. Enumeration of *Lactobacillus* species on LBS agar with and without oxgall.

Culture	Colony forming units/g			
	LBS	LBSO-.05	LBSO-.10	LBSO-.15
<i>L. acidophilus</i> NCFM	2.1×10^9	2.5×10^9	2.3×10^9	2.1×10^9
<i>L. acidophilus</i> 218	4.0×10^8	6.0×10^8	6.5×10^8	4.6×10^8
<i>L. acidophilus</i> 216	1.1×10^9	9.9×10^8	1.1×10^9	1.0×10^9
<i>L. bulgaricus</i> HWD	6.3×10^8	3.3×10^8	N.G. ^a	N.G.
<i>L. bulgaricus</i> NCSI	3.7×10^8	N.G.	N.G.	N.G.
<i>L. brevis</i> RNC	1.7×10^9	1.8×10^9	1.7×10^9	1.6×10^9
<i>L. casei</i> 7469	1.6×10^9	1.6×10^9	1.6×10^9	1.2×10^9
<i>L. plantarum</i>	2.0×10^9	2.0×10^9	2.5×10^9	2.1×10^9
<i>L. lactis</i>	8.9×10^8	N.G.	N.G.	N.G.
<i>L. fermentum</i>	1.6×10^9	1.5×10^9	1.5×10^9	1.0×10^9

^aNo growth on lowest dilution (10^{-5})

TABLE 2. Enumeration of bile resistant lactobacilli in commercial products offered as sources of *Lactobacillus acidophilus*.

Source	Product	Colony forming units/g or ml			
		MRS	LBS	LBSO-.10	LBSO-.15
Pharmacy	P-1 (dry) ^a	6.2×10^8	6.8×10^8	4.7×10^8	$< 10^5$
	P-2 (dry)	2.0×10^9	1.8×10^9	1.8×10^9	1.4×10^8
Animal feed supplement	A-1 (liquid)	$< 10^2$	$< 10^2$	$< 10^2$	$< 10^2$
	A-2 (dry)	$< 10^2$	$< 10^2$	$< 10^2$	$< 10^2$
Health food stores	H-1 (liquid)	4.4×10^3	1.8×10^3	$< 10^2$	$< 10^2$
	H-2 (dry)	$< 10^2$	$< 10^2$	$< 10^2$	$< 10^2$
	H-3 (liquid)	7.7×10^6	5.8×10^6	3.6×10^6	6.7×10^6
	H-4 (dry)	2.5×10^8	$< 10^2$	$< 10^2$	$< 10^2$
	H-5 (liquid)	3.8×10^7	$< 10^2$	$< 10^2$	$< 10^2$
	H-6 (dry)	3.7×10^8	2.0×10^8	$< 10^2$	$< 10^2$
	H-7 (liquid)	2.5×10^8	9.4×10^7	4.5×10^7	$< 10^2$
	H-8 (liquid)	3.6×10^6	3.3×10^6	2.7×10^6	4.1×10^6
	H-9 (dry)	4.1×10^5	1.1×10^4	$< 10^2$	$< 10^2$
Milk with <i>L. acidophilus</i> added	D-1 (liquid)	6.0×10^6	5.9×10^6	—	5.8×10^6
	D-2 (liquid)	3.3×10^6	2.5×10^6	1.6×10^6	1.0×10^6

^aForm in which was obtained.

gram-positive non-sporeforming rods and were considered to be typical lactobacilli. Speciation of the isolates obtained from the seven samples showed that only three (P-1, D-1, and D-2) contained *L. acidophilus* (Table 3). Furthermore, two of these (P-1 and D-1) were the only ones that contained the species listed on their labels. None of the health food store products from which isolates were obtained contained *L. acidophilus*. One of the products from the pharmacy contained *L. brevis* rather than *L. acidophilus*.

TABLE 3. Identification of lactobacilli isolated from commercial products offered as sources of *Lactobacillus acidophilus*

Product	Lactobacillus Species in Product	
	Advertised ^a	Isolated ^b
P-1	<i>acidophilus</i> <i>bulgaricus</i>	<i>acidophilus</i> <i>bulgaricus</i>
P-2	<i>acidophilus</i>	<i>brevis</i>
H-7	<i>acidophilus</i> <i>caucasicus</i>	<i>fermentum</i> <i>lactis</i>
H-8	<i>acidophilus</i> <i>bulgaricus</i>	<i>casei-rhannosus</i>
H-9	<i>acidophilus</i>	<i>lactis</i> <i>fermentum</i> <i>delbruckii</i>
D-1	<i>acidophilus</i>	<i>acidophilus</i>
D-2	<i>acidophilus</i>	<i>acidophilus</i> <i>casei</i> <i>plantarum</i>

^aFrom information on product label.

^bIncludes isolates from LBS, LBSO-.10, and LBSO-.15 agars.

DISCUSSION

Bile acids are generally considered to play a role in controlling the types of bacteria that will grow in the intestinal tract (3). Results from this study have shown that *L. bulgaricus* and *L. lactis*, which do not occur normally in the intestinal tract (1) are inhibited by oxgall in LBS agar. On the other hand, *L. acidophilus* and the other species of lactobacilli included in this study have all been isolated from intestinal sources (1). It is not surprising, therefore, that they grew on the LBS agar containing oxgall. Based on the species included in this study, it appears that the non-intestinal lactobacilli do not form colonies on LBSO-.15 agar; therefore, products containing lactobacilli capable of establishing in the intestinal tract can be evaluated quantitatively on this medium. This should be a more meaningful evaluation than inoculation of a large number of lactobacilli into a liquid medium containing bile where very few cells may initiate growth. Since all strains of intestinal lactobacilli grew equally well on the LBS and LBS containing 0.15% oxgall, this medium (LBSO-.15) is recommended for use in enumerating bile-resistant intestinal lactobacilli. The incubation time had to be increased to 5 days when analyzing some of the products because the lactobacilli in them were apparently in a debilitated state and thus grew slowly; this was particularly noticeable in the dry products.

LBS agar containing oxgall should be useful in selecting

cultures of lactobacilli for use in products designated as sources of intestinal lactobacilli. It should be useful for monitoring the stability of the bile resistance in the lactobacilli during storage — a characteristic that we have found to be storage-dependent. It was used (6) to show that consumption of non-fermented milk containing cells of *L. acidophilus* resulted in significant increases in the numbers of bile resistant lactobacilli in the feces of human test subjects.

Most products sold as sources of *L. acidophilus* that were analyzed in this study did not contain viable *L. acidophilus*. Some did contain other lactobacilli which have been associated with the intestinal tract (1), but of these only *L. casei* (7) has been reported to be useful as a dietary adjunct for establishment in the intestinal tract. The importance of *L. acidophilus* in helping control the intestinal flora has received much attention (for review see reference 9). Our study suggests that more care must be given to the identity of lactobacilli in products offered as sources of given species. Furthermore, care should be taken during preparation and storage of such products to insure that the culture maintains its viability and ability to perform specified functions for reasonable periods of storage.

ACKNOWLEDGMENT

This work was supported in part by funds from the National Dairy Council.

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Comparison of the Elevated Temperature Plate Count (ETPC) and MPN Procedure to Estimate the Densities of Fecal Coliforms and *Escherichia coli* in Soft-Shell Clams

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(Received for publication April 4, 1977)

ABSTRACT

The Elevated Temperature Plate Count procedure of Hefferman and Cabelli was assessed to measure the sanitary quality of soft shell clams. The method, compared to the standard MPN procedure, underestimated the densities of fecal coliforms by about 10%. The estimated cell densities of *Escherichia coli* and *Enterobacter aerogenes* in saline suspension were about 20% lower than the estimate obtained on nutrient agar plates. Thus, the fecal coliform standard used in the assessment of sanitary quality of clams would need to be modified when the analysis is conducted by the Elevated Temperature Plate Count procedure.

Depuration of soft-shell clams (*Mya arenaria*) from moderately contaminated water (231-700 coliforms/100 ml) has been of interest in Nova Scotia in recent years. A pilot plant jointly-supported by industry, Federal, and Provincial Government agencies was operated in Digby, Nova Scotia in 1976 (5). The bacteriological quality of clams before and following depuration was determined by the standard MPN fecal coliform procedure (1) which requires 72 h to complete. During the analyses of samples, depurated shellfish must be stored. This delay is costly; hence a faster method of assessing the sanitary quality of clams was of interest.

Since some rapid procedures to estimate fecal coliform densities in samples were described by other workers (2, 3, 4); our laboratory was requested to assess the usefulness of the elevated temperature plate count technique (ETPC) of Hefferman and Cabelli, (4) with the hope that this procedure might be used to estimate the sanitary quality of depurated clams.

MATERIALS AND METHODS

Samples of contaminated clams were taken from the estuary of the Cornwallis River in Nova Scotia. Clams were placed in polyethylene bags, chilled with ice in a cooling box, and transported to the laboratory for microbiological examination within 24 h of harvesting.

For analysis, clams were thoroughly washed and scrubbed in running potable water. A 100-g quantity of meat and liquor was homogenized for 2 min with sterile saline (ratio of sample and saline 1:9). The fecal

coliform densities in the samples were estimated by the standard 5-tube MPN procedure of APHA (1) in triplicate. Pre-enrichment was in Laulry Sulphate Tryptose (LST) broth for 48 h at 35 C. The positive tubes were cultured in EC broth for 24 h at 44.5 C.

The ETPC of fecal coliforms were obtained in modified (4) MacConkey's agar after 24-h incubation in air at 44.5 ± 0.5 C. Only brick-red colonies >0.5 mm in diameter were counted and the fecal coliform density/100-g sample was calculated. Each sample was plated in duplicate. The modified single strength MacConkey agar consisted of: proteose peptone 20 g, sodium deoxycholate 0.75 g, lactose 10 g, neutral red 0.03 g, crystal violet 0.001 g, agar 13.5 g, distilled water 1000 ml. The pH of the agar adjusted to 7.1. For plating, a 6-ml aliquot of homogenate was mixed with 54 ml of prewarmed (45 C) saline which in turn was added to 60 ml of double-strength tempered (45 C) agar. After mixing, the contents were poured into eight petri plates and incubated in air at 44.5 ± 0.5 C for 24 h.

Each counted colony from the agar plates was subcultured into lactose broth. After 24 h of incubation at 35 C, the cultures were streaked on Eosin Methylene Blue (EMB) agar. Typical *Escherichia coli* or coliform colonies from the EMB agar were purified on nutrient agar (NA) and subsequently they were typed by the IMVIC procedure.

The fecal coliform and *E. coli* counts were calculated for each sample. The log mean of replicate analyses for each sample was obtained and the results from the MPN and ETPC procedures were correlated with the least sum of squares method.

To estimate the effect of temperature shock of ETPC procedure, suspensions of typical *E. coli* (++- -) and *Enterobacter aerogenes* (- - ++) (able to ferment lactose at 44.5 C) were enumerated on nutrient agar plates at 35 C and by the ETPC technique described. The mean and the confidence limits of the mean estimates at P 0.05 were calculated for the purposes of comparison. The results are described in Table 1.

RESULTS AND DISCUSSION

The regression correlation of the paired log means of fecal coliform density estimates for 35 samples of soft shell clams by the MPN and ETPC procedures is shown in Fig. 1. The percent correlation is significant at $p < 0.05$. The standard error, however, is rather high. The mean fecal coliform estimate of samples by the ETPC is 6% lower than the mean of the MPN procedure.

Figure 2 illustrates the regression correlation between the paired log means of MPN and ETPC estimates of *E. coli* densities from the 35 soft shell clam samples. The

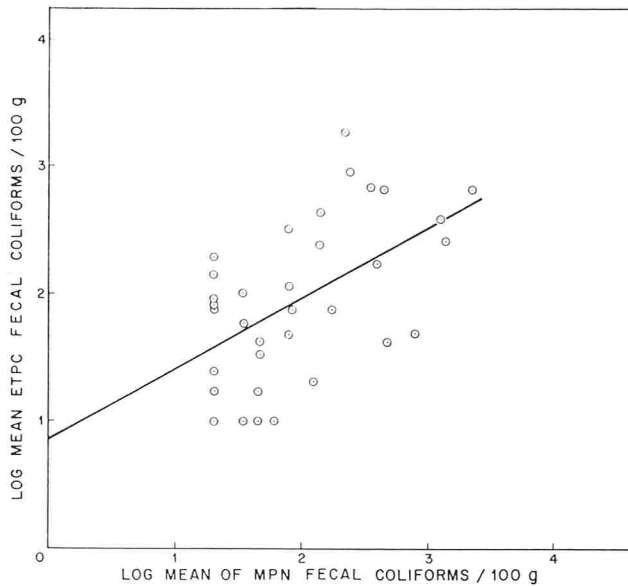


Figure 1. Correlation between the log means of fecal coliform estimates obtained by the MPN and ETPC techniques from the soft shell clam samples. $Y = 0.827 + 0.567X$; Log mean of X : 1.966.

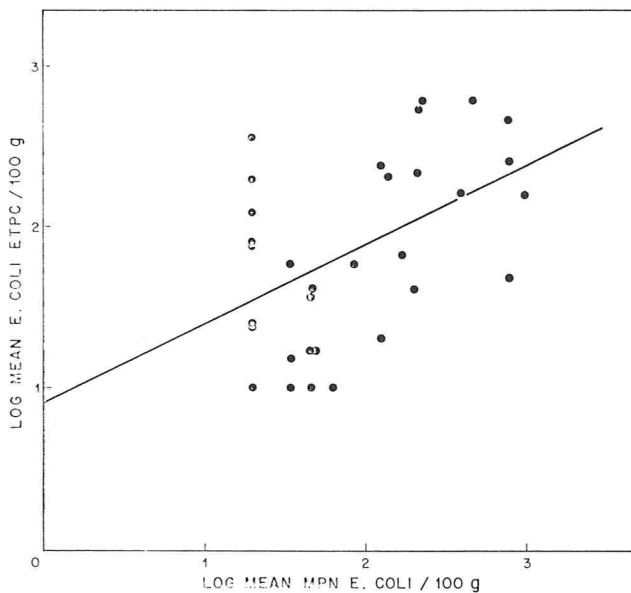


Figure 2. Correlation between the log means of *E. coli* estimates obtained by the MPN and ETPC techniques from the soft shell clam samples. $Y = 0.921 + 0.486X$; Log mean of X : 1.888.

percent correlation between the means is significant at $p < 0.05$, the standard error about the regression line is high. The mean of ETPC *E. coli* counts is about 10% lower than the mean counts of *E. coli* obtained by the MPN procedure. The mean of MPN *E. coli* estimates is 17% lower than the mean of MPN fecal coliform estimate. The mean of ETPC *E. coli* estimate is 19% lower than the mean of ETPC fecal coliform results.

Table 1 describes the estimate of cell densities of *E. coli* (+ + - -) and *E. aerogenes* (- - + +) in saline suspensions by the ETPC and standard plate count

TABLE 1. Densities of *E. coli* (+ + - -) and *E. aerogenes* (- - + +) in saline suspension estimated by ETPC and surface inoculation of nutrient agar plates at 35 C incubation. (Confidence limit at p 0.05)

Culture suspension	Replicate count	Mean count on nutrient agar and conf. limit of mean	Mean ETPC and confidence limit of mean	% recovery:
				Mean ETPC X 100 / Mean NA Count
<i>E. coli</i>	6	3.5×10^6 $2.6 \times 10^6 - 4.5 \times 10^6$	2.7×10^6 $1.9 \times 10^6 - 3.5 \times 10^6$	77.1
<i>E. aerogenes</i>	6	8.2×10^6 $4.9 \times 10^6 - 1.1 \times 10^7$	6.6×10^6 $5.3 \times 10^6 - 7.9 \times 10^6$	80.5

TABLE 2. IMVIC types of cultures isolated from samples analyzed by the MPN and ETPC procedures respectively

Source of cultures	No. of cultures typed	% <i>E. coli</i>	% Intermediates	% <i>E. aerogenes</i>
		+ + - - - + - -	- + - + + + - +	- - + ± ± - + +
E.C. tubes	186	91.9%	2.2%	5.9%
ETPC plates	605	77.0%	3.3%	19.7%

(surface inoculation) technique. Table 2 illustrates the IMVIC types of isolated cultures.

The results from Table 1 and Fig. 1 and 2 indicate that the heat shock and the ensuing selective growth environment employed by the ETPC procedure leads to an underestimation of the actual density of fecal coliforms in samples. This underestimation on the basis of these results appears to be about 10-20%. Thus use of the ETPC procedure for assessment of sanitary quality of soft shell clams would result in applying 10-20% lower acceptance criteria. This could be rectified however, by multiplying the ETPC value by a factor of 1.2. Considering the speed of the ETPC technique, this option would be quite attractive. An MPN procedure employing LST or even EC broth at 44.5 C incubation without pre-enrichment would probably give a better estimate of fecal coliforms than ETPC method. This alternative procedure, however, should first be thoroughly evaluated on soft shell clam samples and its comparability to the standard procedures established.

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Quality of Canned Potatoes, Carrots, and Beets After Long-Term Fresh Product Storage

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(Received for publication April 27, 1977)

ABSTRACT

Quality and nutritional data were obtained for potatoes, carrots, and beets initially and after the raw product was stored for 6 months. Yields of peeled vegetables during 6 months of storage stayed constant for beets but decreased for potatoes and carrots. Proximate analyses for all vegetables remained constant over the storage period with the exception of carbohydrates which decreased slightly. Vitamin C content decreased in all crops while β -carotene in carrots and riboflavin content in beets remained the same over the test period. The nutrient values of products canned immediately or canned after the raw product was stored for 6 months were considered similar. Sensory evaluation results indicate that storage of beets before canning caused changes in flavor and texture characteristics. Storage of carrots before canning had no measurable effect on sensory quality. Sensory evaluation results and Hunter color reflectance values indicated a color change toward yellow, and preference for a white-fleshed potato.

Carrots, beets, and potatoes are vegetables which can be held in a fresh state for extended periods. Vegetable canners could lengthen their processing season if canning quality can be maintained during fresh product storage. Investigators are in general agreement on storage conditions needed to maintain fresh product quality. Recommendation for optimum storage conditions for carrots and beets are 0-2 C and 98-100% relative humidity (RH) (4,6,8,10,11,17,18,19). Potatoes are held at 95-100% RH with sufficient ventilation to maintain product temperature at 7 C (3,5,9,13,14,15,16). Storage below 7 C results in an undesirable accumulation of reducing sugars (3,5,16).

The purpose of this study is to provide canners with information on the quality, including nutritional data, of canned carrots, beets, and potatoes when the raw product was stored for 6 months. A nutritional quality evaluation is of particular interest when considering present nutritional labeling requirements. Loss of ascorbic acid during fresh storage of potatoes has been demonstrated (2,21) and therefore the nutritional quality of potatoes canned immediately after harvest or potatoes canned after the raw product has been stored for 6 months may vary.

MATERIALS AND METHODS

Storage conditions

Beets (*Beta vulgaris* L.) var. Ruby Queen, carrots (*Daucus carota* L.) var. Danvers and potatoes (*Solanum tuberosum* L.) var. Norgold were obtained from commercial sources. Approximately 1200 lb. of product were stored in wooden bins (3 ft \times 3 ft \times 4 ft high). Air ducts were located at the bottom of bins and equipped with 55 c.f.m. fans. Ventilation is needed to keep temperature uniform and prevent "hot spots" due to respiration of vegetables. Sensing bulbs located in the center of the product controlled the fan operation to supply air at 42 ± 1 F only when product temperature was above 42 F. Therefore, fans were operated intermittently to minimize dehydration. Relative humidity of the air was maintained at $98\% \pm 1$ with water sprays.

Canning procedures

Products were canned at 0 and 24 weeks of storage. Potatoes, before canning, were held at room temperature to decrease reducing sugar content. All products were steamed followed by abrasion-peeling. Carrots were diced, beets sliced, and potatoes were packed whole. Each product was packed in 303 \times 406 C-enamel cans with brine containing 0.5% NaCl and 0.1% CaCl₂. The cans were steam exhausted to 170 F and closed. Carrots and beets were retorted in a continuous agitating retort (6.25 rpm) at 254 F for 12 min. Potatoes were processed in a still retorted at 250 F for 20 min. All canned products were stored at ambient temperature.

Nutrient analysis

Nutrient analyses were made on products initially, and after fresh and canned product storage for 6 months.

Proximate analyses (protein, fat, moisture, ash, fiber, and carbohydrates) were done on all products. In addition, carrots were analyzed for Vitamin C, beta-carotene, and Vitamin A, beets for Vitamin C and Riboflavin, and potatoes for Vitamin C and Vitamin B₁. References for methods used are in Table 1.

Quality evaluation

Quality evaluation on all products included bacteriological analyses, drained weight, Hunter color, weight losses during storage, peeling losses, firmness (shear pressure), and flavor. In addition, beets were analyzed for pigment content and potatoes were analyzed for total sugar content (7,12) and specific gravity.

Firmness was determined with a Texture Test System Model TP2A (Food Technology Corporation). The test conditions were: range 0-300 lb., down stroke 15 sec, sample size 100 g, and a test ring 3000 lb. Results are expressed as shear lb.

Objective color was determined with the Hunter Color and Color Difference Meter Model #D25. Standards used were white #025-1300, yellow #025-1304, and pink #025-1302 for potatoes, carrots, and beets,

TABLE 1. *References for analytical methods used.*

Analysis	Reference
Protein	A.O.A.C., 16, (1970) 11th ed. (% N × 6.25)
Moisture	A.O.A.C., 122 (1970) 11th ed. — Vacuum oven
Ash	A.O.A.C., 123 (1970) 11th ed.
Fat	A.O.A.C., 128 (1970) 11th ed.
Fiber	A.O.A.C., 129 (1970) 11th ed.
Carbohydrates	By difference
Calories	By calculation
Vitamin C	J. Biol. Chem. 147:399 (1943)
β -Carotene	Moore and Ely, Ind. Eng. Chem., Anal. Ed. 13:600 (1941)
Riboflavin	A.O.A.C., 789 (1970) 11th ed.
Vitamin B ₁	A.O.A.C., 771 (1970) 11th ed.

respectively.

Total bacterial count on the surface of the products was determined using malt extract agar and plate count agar. A plug 15 mm in diameter weighing and 10 g was aseptically removed, blended with sterile phosphate buffer, serially diluted, and inoculated into the corresponding medium. Malt agar plates were incubated for 5 days at 21 C and PCA plates were incubated for 48 h at 35 C.

Triangle difference tests and preference test (7 point hedonic scale: 1 = dislike extremely, 7 = like extremely) done under controlled conditions were employed to determine significant flavor differences and preferences between individual samples (1). Twenty to 30 semi-trained persons were used.

Preference tests were conducted under fluorescent light while difference tests were conducted under fluorescent light and in the dark.

Pigment analyses in beets were by the method by von Elbe et al. (20). The method by Somogyi and Nelson was used to determine sugar content in potatoes. Specific gravity of potatoes was determined with a potato hydrometer (International Potato Institute).

RESULTS AND DISCUSSION

Raw products used in this study were obtained directly from the growing areas and only product free from damage was stored. Sorting losses before storage were 14, 14.6, and 11.3% for potatoes, carrots, and beets, respectively. Peeling losses were determined at each processing time. Since only small lots (less than 100 kg) of each product were processed, peeling losses or yield are subject to errors. The results can only be indicative of trends. Peeling losses for beets stayed relatively constant with an average of approximately 21%. For carrots and potatoes, peeling losses increased with storage time from 42 and 40% to 67 and 60%, respectively. This increase, in

part, may be attributed to the increase in yeast and mold counts (Table 2). Aerobic plate count remained constant during the 6-months storage period.

TABLE 2. *Aerobic and yeast and mold counts of potatoes, carrots, and beets initially and after storage for 6 months.*

Vegetable crop	Storage time			
	0 Month		6 Months	
	APC ^a	Y&M ^b	APC	Y&M
Potatoes	2.8 × 10 ⁶	2.7 × 10 ²	9.3 × 10 ⁶	7.5 × 10 ³
Carrots	6.2 × 10 ⁵	1.9 × 10 ²	3.3 × 10 ⁶	8.8 × 10 ³
Beets	3.9 × 10 ⁶	1.7 × 10 ²	1.1 × 10 ⁷	1.2 × 10 ⁴

^aAerobic plate count

^bYeast and mold count

Nutritional data for the raw products initially and after 6 months of storage are summarized in Table 3. Proximate analysis values for all vegetables remained constant over the 6-months storage period, with the exception of carbohydrates which decreased slightly in all vegetables. Vitamin C content decreased in all crops over the storage time while β -carotene content in carrots and the riboflavin content in beets remained constant.

Table 4 presents nutritional data for each vegetable canned immediately after harvest, canned after the vegetables were stored for 6 months and after the canned product was stored for 6 months. Nutritional quality of these vegetables was not affected by long-term raw product storage before canning.

Table 5 gives quality data Hunter color reflectance values, pigment content, firmness expressed in shear lb. and percent drained weight. Color differences were noted in potatoes as measured by Hunter reflectance values. a_L Value changed from -0.9 at zero time storage time to 1.4 after 6 months of storage. This change is indicative of a loss in green color making the yellow more predominant. a_L Value and the saturation value $(a^2 + b^2)^{1/2}$ in beets decreased slightly. These values reflected the slight decrease in pigment loss observed during the storage period. Hunter color reflectance value as well as pigment content remained constant in carrots over the 6-month storage period. Percent drained weight increased in all vegetables when canned after 6 months of raw storage. This increase in drained weight will compensate for moisture losses reported to occur

TABLE 3. *Nutritional data of raw potatoes, carrots, and beets initially and after 6 months of storage^a*

Nutrient	Product initially and after 6 months					
	Potatoes		Carrots		Beets	
	0	6	0	6	0	6
Total solids, %	23.8	23.5	8.4	9.0	13.5	13.1
Protein, g/g	0.11	0.13	0.07	0.08	0.12	0.15
Moisture, %	76.2	76.5	91.6	91.0	86.5	86.9
Ash, g/g	0.037	0.038	0.08	0.12	0.059	0.076
Fat, g/g	0.004	0.004	0.02	.01	0.007	0.008
Fiber, g/g	0.017	0.017	0.11	0.12	0.067	0.069
Carbohydrates, g/g	0.84	0.81	0.71	0.68	0.75	0.70
Riboflavin, mg/g	—	—	—	—	0.006	0.006
Total Vit. C, mg/g	0.63	0.52	0.44	0.34	0.19	0.12
Thiamine, mg/g	0.0038	0.0038	—	—	—	—
β -Carotene, mg/g	—	—	1.34	1.26	—	—
Vitamin A, IU/g	—	—	2200.	2100.	—	—

^aData expressed on dry weight basis.

during storage of vegetables.

Results of the flavor evaluations are summarized in Table 6. Preference mean scores for potatoes indicated no preference for flavor, texture or overall preference for potatoes canned immediately after harvest or potatoes stored for 6 months before canning. Color preference score for potatoes was significantly greater when potatoes were canned immediately. Based on Hunter reflectance values obtained for these two samples, the consumer prefers a whiter or less yellow (low b_L value) potato. Triangle difference tests conducted with and without the presence of light showed significant difference between the samples but gave no preference indication for the less yellow fleshed potato.

Triangle difference test on carrots conducted with and without the presence of light showed a significant difference between samples, but again no preference indication could be detected. The preference mean score for all attributes indicated no significant difference from each other. It may be concluded that storage of carrots before canning was not detrimental to quality.

Triangle difference tests on beets conducted with or without the presence of light were great, showed statistical significance between samples, and indicated a definite preference toward the sample canned immediately after harvest. Mean preference scores for

flavor, texture, and overall preference were significantly different from each other at the 5% level of probability, with a preference toward the beet sample canned immediately after harvest, while mean preference scores for color were not significantly different from each other. It would appear that changes in texture and flavor characteristics were the major influence in determining beet quality. The flavor differences noted may be attributed to the reduction in carbohydrate content (Table 3) which occurred during storage and could be corrected by the addition of small amounts of sucrose to the brine. The slight pigment loss measured after the beets were stored did not appear to be of significant magnitude to affect the visual color preference score.

From the data obtained in this study, if raw product storage is economical, it would appear that quality, including nutritional value of the products studied, was unaffected by storage for 6 months.

ACKNOWLEDGMENT

Research supported in part by the College of Agricultural and Life Sciences, University of Wisconsin-Madison and by funds from Krier Preserving Co., Belgium, Wisconsin. The assistance of the Sensory Evaluation Laboratory, Department of Food Science is acknowledged.

TABLE 4. Nutritional data of canned potatoes, carrots, and beets^a.

Nutrient	Product initially and after 6 months								
	Potatoes			Carrots			Beets		
	A ^b	B	C	A	B	C	A	B	C
Protein	120	150	130	80	70	110	150	190	120
Ash	40	50	40	120	140	170	80	120	70
Fat	< 10	< 10	< 10	20	20	30	10	< 10	< 10
Fiber	10	20	10	120	110	150	50	90	50
Carbohydrate	840	810	800	710	730	580	750	650	720
Calories per gram	3.8	3.8	3.7	3.3	3.2	2.8	3.6	3.3	3.4
Riboflavin	—	—	—	—	—	—	0.0038	0.0062	0.0049
Ascorbic acid	0.45	0.47	0.39	0.21	0.18	0.20	0.14	0.24	0.15
Thiamine	0.0027	0.0023	0.0030	—	—	—	—	—	—
Carotene	—	—	—	1.2	0.8	1.0	—	—	—
Vitamin A, IU/gram	—	—	—	2000	1400	1600	—	—	—

^aData expressed in mg per gram total solids.

^bA = Initial analyses, canned immediately after harvest.

B = Analyses of canned product after vegetable storage for 6 months.

C = Analyses of canned product stored for 6 months.

TABLE 5. Quality of potatoes, carrots, and beets canned before storage and after 6 months of raw storage.

Product	Storage interval (months)	Hunter color reflectance values					Pigment, ^a mg/g dry wt	Shear lb	Drained wt., %
		L	a _L	b _L	tan ⁻¹ b/a	(a ² + b ²) ^{1/2}			
Potatoes	0	69.3	-0.9	18.0	-2.9	18.0	—	118	104.5
	6	64.9	1.4	19.2	4.1	19.3	—	—	108.6
Carrots	0	45.1	26.8	26.3	44.5	37.9	1.34	58	96.5
	6	43.0	25.8	26.2	45.4	36.8	1.26	62	102.7
Beets	0	16.0	15.2	2.3	8.6	15.4	7.9	109	98.6
	6	15.8	14.6	2.3	9.0	14.8	6.1	166	103.2

^aBeets = mg betacyanine; Carrots = mg carotene

%Drained weight = Drained weight - fill-in weight × 100

TABLE 6. Canned vegetable preference and triangle difference tests by technical panel.

Vegetable	Storage time (months)	Preference mean score ¹				Triangulation and sample preference level ²			
		Flavor	Texture	Color	Overall	With light	Sample preference	Without light	Sample preference
Potatoes	0	4.40 ^a	4.56 ^a	5.41 ^a	4.61 ^a	—	—	—	—
	6	4.12 ^a	4.50 ^a	3.76 ^b	4.26 ^a	0.1% ⁴	12 pref P ₀	0.1%	6 pref P ₀
		F-Value	ns ³	ns	s	(27/30) ⁵	3 no pref	(15/22)	3 no pref
		LSD (5%)	—	—	0.47	—	12 pref P ₆	—	6 pref P ₆
Carrots	0	3.82 ^a	3.93 ^a	4.79 ^a	3.80 ^a	—	—	—	—
	6	3.73 ^a	3.93 ^a	4.34 ^a	3.71 ^a	0.1%	7 pref C ₀	1.0%	4 pref C ₀
		F-Value	ns	ns	ns	(23/30)	6 no pref	(15/25)	6 no pref
		LSD (5%)	—	—	—	—	10 pref C ₆	—	5 pref C ₆
Beets	0	4.33 ^a	4.58 ^a	4.73 ^a	4.45 ^a	—	—	—	—
	6	3.22 ^b	3.82 ^b	4.69 ^a	3.24 ^b	0.1%	15 pref B ₀	0.1%	19 pref B ₀
		F-Value	s	s	s	(22/24)	4 no pref	(23/30)	4 no pref
		LSD (5%)	0.42	0.38	—	0.46	3 pref B ₆	—	0 pref B ₆

¹Pref scale: 1 = Dislike extremely; 7 = Like extremely; samples evaluated under fluorescent lighting.

²Triangle difference test: Each sample compared to the control (vegetable stored 0 month, P₀, C₀, B₀).

³ns = not significant.

⁴Significantly different from the control sample at the given level of probability.

⁵Correctly identified over total triangle difference tests.

^{a,b}Mean scores with similar superscripts are not significantly different from each other at the 5% level of probability.

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Growth and Enterotoxin A Production by *Staphylococcus aureus* in Fluid Dairy Products¹

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(Received for publication September 11, 1975)

ABSTRACT

Staphylococcus aureus strain 100 grew better in skim milk and whole milk (3.5% fat) than in half and half (10.5%), and whipping cream (30% fat) at 37 C. Enterotoxin A production was 1.14, 0.88, 0.24, and 0.18 µg per 100 g of skim milk, whole milk, half and half, and cream, respectively. Sufficient cell numbers were not obtained for enterotoxin production after 16 h at 22 C in these same media.

Enterotoxin A has been implicated in more food poisoning cases than other enterotoxins of *Staphylococcus aureus*, but conditions necessary for enterotoxin A production have not been well defined. Tatini et al. (9) found that a population of 2 to 3 million cells per ml was associated with detectable enterotoxin A in milk relatively free from competing microorganisms. Donnelly et al. (3) observed that *S. aureus*, strain MF24, grew in both raw and pasteurized milk. Tatini et al. (8) and Donnelly et al. (3) further showed that production of enterotoxin A in milk was associated with an increase in cell numbers, but the quantity of enterotoxin produced was not determined. Minor and Marth (7) reported that both growth of *S. aureus* and enterotoxin A production can occur in cream at 37 C.

Since the concentration of milkfat varies greatly in different fluid dairy products, it is pertinent to determine enterotoxin A production in such products with different amounts of milk fat.

MATERIALS AND METHODS

Fluid dairy products

Pasteurized skim milk, whole milk (3.5%), and cream were obtained from the Washington State University Creamery. Whipping cream and half and half were standardized to 30.0 to 10.5% fat, respectively.

Source and preparation of cultures

S. aureus strain 100, known to produce enterotoxin A, was obtained from M. S. Bergdoll, Food Research Institute, University of Wisconsin, Madison, Wisconsin. The culture was carried on slants of Trypticase Blood Agar Base and prepared by inoculating Trypticase Soy Broth

(TSB) after touching a loop to a stock slant. After 18 h of incubation at 37 C, a loopfull of inoculum was transferred to a second tube of TSB and incubated 18 h at 37 C before inoculating the product under test.

Product inoculation

In the growth studies, 1 ml of *S. aureus* culture was added to give a 10³ cell concentration per ml in each of the products tested. The inoculated products were incubated 24 h at 37 C. One-ml portions were removed and plated, using appropriate dilutions, with *Staphylococcus* 110 medium containing sodium azide and incubated at 37 C for 48 h.

Enterotoxin extraction

In the enterotoxin production studies, test products were inoculated as described above. After 16 h of incubation at 37 C, all samples were extracted according to the procedure described by Mahnke and Rathman (5) with the following modification of the initial steps. One hundred ml of skim milk or milk were blended with 1.2 g of NaCl in a Waring Blendor at high speed for 3 min. When cream was analyzed, 300 ml of 0.2 N NaOH was added before blending. The blended mixture was adjusted to pH 7.5 with 5 N NaOH and centrifuged at 36,400 × g for 10 min at -5 C. The sediment was re-extracted by adding 100 ml of 0.2 N NaCl, adjusted to pH 7.5, and mixed with a Sorvall omnimixer at 8000 rpm for 1 min. The slurry was centrifuged at 36,400 × g for 10 min at -5 C and the supernatant fluid from the two extractions was pooled. To the pooled supernatant fluid, in a separatory funnel, 10 ml of chloroform was added and shaken vigorously 10 times through an arc of 90°. This solution was centrifuged at 36,400 × g at -5 C for 10 min. The supernatant fluid was collected and concentrated by over-night dialysis against 50% polyethylene glycol at 5 C. The concentrate was removed and dialysis tubing was washed with 0.01 M phosphate buffer at pH 7.5. The concentrate was centrifuged at 48,200 × g for 10 min at -5 C. The supernatant fluid was re-extracted with 2 ml of chloroform and the mixture centrifuged at 48,200 × g for 10 min at -5 C. The supernatant fluid was generally clear to slightly cloudy. Occasionally the supernatant fluid was definitely cloudy with a definite separation between the chloroform and aqueous phases. When this occurred, extraction with chloroform was repeated to remove more of the solids that would interfere with absorption of the enterotoxin onto the carboxymethyl cellulose column. Once a clear supernatant fluid was obtained, the pH was adjusted to 4.6 with 6 N HCl while mixing with a magnetic stirrer. This solution was then centrifuged at -5 C for 10 min at 48,200 × g. The pH of the supernatant fluid was adjusted to pH with 1 N NaOH while mixing with a magnetic stirrer. The remaining portion of the extraction procedure was the same as described by Mahnke and Rathman (5), starting with Step IID. The extracted material was also tested for presence of enterotoxin by the procedure of Mahnke and Rathman (5).

¹Scientific Paper No. 4490, College of Agriculture Research Center, Pullman, Washington 99164. Project No. 0108.

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Efficiency of enterotoxin A extraction.

Efficiency of extraction and assay by the slide immunodiffusion test was determined by adding 1 ml of saline solution containing 10 μg of enterotoxin A/ml to 100 ml of pasteurized skim milk, whole milk, half and half, and whipping cream. The "spiked" samples were extracted using the procedures of Casman and Bennett (2) and Mahnke and Rathman (5).

RESULTS AND DISCUSSION

Staphylococcus aureus growth in fluid dairy products

Figures 1 and 2 show the growth curves obtained at 37 and 22 C with *S. aureus* strain 100 in skim milk, whole milk, half and half, and whipping cream. Best growth

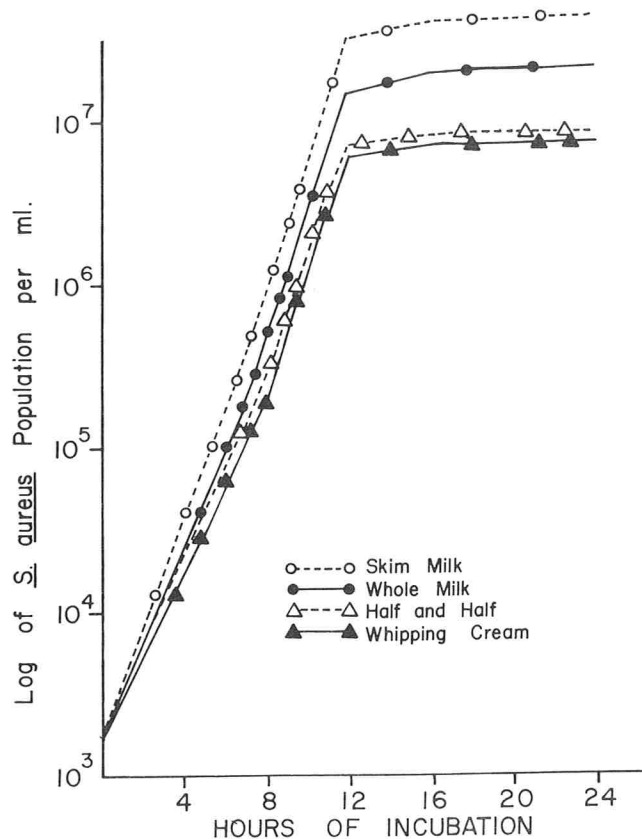


Figure 1. Growth of *Staphylococcus aureus* in various fluid milk products at 37 C.

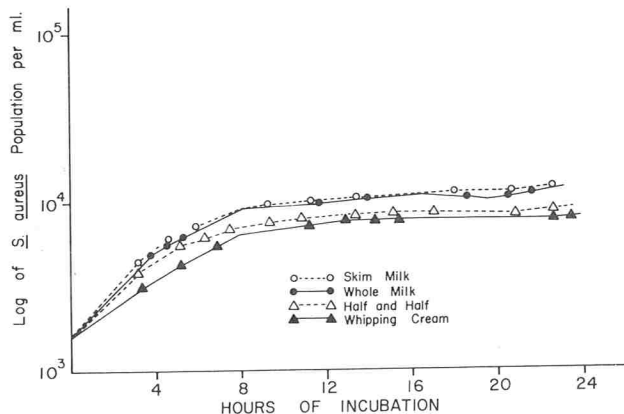


Figure 2. Growth of *Staphylococcus aureus* in various fluid milk products at 22 C.

was observed at 37 C in skim milk and whole milk. Maximum cell concentration was reached at 16 h of incubation at 37 C regardless of the medium employed; however, the cell concentration in the high-fat products was less than the level found in the low fat products (Fig. 1). Comparable results have been reported by Minor and Marth (7). According to Vadehra and Harmon (10), the lipolytic activity of the staphylococci may limit their growth in cream. They reported that the lipolytic enzyme system of *S. aureus* was very active on milkfat and the release of 0.05% capric acid and 0.1% caprylic acid completely inhibited growth of *S. aureus*.

Enterotoxin production in fluid dairy products

The mean values for enterotoxin produced in skim milk, whole milk, half and half, and whipping cream were 1.14, 0.88, 0.24, and 0.18 μg per 100 g, respectively. The mean values are the result of examining three samples per product and nine micro samples per product. The efficiency of the extraction procedure and assay by the slide immunodiffusion test was found to be 40 to 60% depending on the product analyzed. An analysis of variance by Duncan's multiple range test indicated that the amount of enterotoxin produced between the low-fat and the high-fat products was statistically significant at the 5% level of probability. Markus and Silverman (6) reported that production of enterotoxin A occurred during the logarithmic growth phase and was correlated with cell counts (6). Since less growth occurred in the high-fat products, less enterotoxin was expected.

In any discussion on growth of staphylococci in food, the number of cells required to produce sufficient enterotoxin to cause a typical syndrome of food poisoning must be considered. Jones et al. (4) found staphylococcal counts in milk or cheese in proportion to the amount of inoculum, suggesting that staphylococci inoculated in low numbers would have less chance of reaching the cell density required to produce enterotoxin. An inoculum of 10^3 cells per ml was selected in this study to simulate counts approximating those frequently found in commercially produced raw milk (11). However, the *S. aureus* cells added were known to be enterotoxigenic, whereas this may not be true with commercially produced raw milk.

For food poisoning to occur in humans, approximately 1 μg of enterotoxin A must be consumed; however this will vary with individuals (1). The data from this study show that only 0.24 μg and 0.18 μg of enterotoxin were produced per 100 g of half and half and whipping cream, respectively.

Assuming an extraction efficiency of 50%, these quantities do not appear sufficient to cause food poisoning since approximately 200 to 300 g of product would have to be consumed to provide 1 μg of enterotoxin A. Normally 200 to 300 g of half and half or whipping cream are not consumed at one time. However, *S. aureus* growth in cream must be considered a potential health hazard if the cream is held at a suitable growth

temperature and then made into a manufactured product such as butter. One case of food poisoning has been traced to butter made from cream containing enterotoxin A. Milk could be consumed in quantities greater than 400 g and could provide sufficient toxin to cause food poisoning. However, milk has been involved in few outbreaks of staphylococcal food poisoning because sufficient cell concentrations to produce enterotoxins probably would occur only under prolonged storage conditions at elevated temperatures. Another safety factor is the inhibitory effect of other competing microbes against *S. aureus* (8).

Based on the cell populations attained at 22 C and previous reports (4,7,9), these products were not analyzed for enterotoxin. The results reported herein suggest that the low temperatures used to preserve fluid dairy products are sufficient to prevent staphylococcal growth and enterotoxin production whenever the original count is 10^3 cells per ml or less and the holding temperature is 22 C or less.

ACKNOWLEDGMENTS

Appreciation is expressed to Dr. M.S. Bergdoll, Food Research Institute, University of Wisconsin, Madison, Wisconsin, for providing *Staphylococcus aureus* strain 100, known to produce enterotoxin A, and the toxins and antitoxins used in this investigation.

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Retention of Chemical Contaminants by Glass, Polyethylene, and Polycarbonate Multiuse Milk Containers

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ABSTRACT

Glass, polyethylene, and polycarbonate multiuse milk containers were treated with 29 common household chemicals to simulate consumer abuse. Of the three container materials, glass was the easiest to clean and was most resistant to retention of treatment contaminants. The in-line contamination detection device allowed nine treated polyethylene containers and five treated polycarbonate containers to pass undetected. These each held sufficient contaminant to produce an off-odor in milk later placed in the treated container. Gas chromatographic analyses of volatile constituents present in treated, emptied, and washed polyethylene and polycarbonate containers showed compounds that were not present in the untreated containers. Virtually no volatile compounds were detected in either the treated or untreated glass containers. Chlordane residues of 0.007, 24 and 14.6 ppm were recovered from milk placed in glass, polyethylene, and polycarbonate containers, which had been contaminated with full strength chlordane, rinsed, washed, and refilled with milk. Neither the polyethylene nor the polycarbonate multiuse milk container appear to comply with the *Grade "A" Pasteurized Milk Ordinance — 1965 Recommendations of the United States Public Health Service*.

Milk for retail sale is packaged in glass, coated paper, and plastic containers of various compositions and constructions. Both single service and multiuse (refillable) containers are used. Reuse of milk containers presents environmental advantages, yet poses several problems to the consumer and to the industry. The industry has no way of controlling what a consumer may place in the container before it is returned for refilling, nor does the consumer have any way of knowing how a container may have been misused before being returned to the milk plant.

Each year the Oregon Department of Agriculture receives a significant number of complaints against milk packaged in multiuse polyethylene (PE) milk containers dealing with off-odors, off-flavors, and foreign objects. In 1970, regulatory officials in Oregon, Washington, and Idaho received 102 consumer complaints regarding multiuse PE milk containers and one complaint

(involving a foreign object) against a multiuse glass container (3). Fourteen of the complaints against PE containers involved alleged illness. These complaints received by regulatory officials probably represent only a small portion of those cases of consumer dissatisfaction. Many complaints are attributed to the milk itself, when the offense may be caused by the container.

Some known point sources of abuse of multiuse milk containers are: to mix garden sprays for home use, to store common garage chemicals, as refrigerator containers for fruit juices and fruit flavored drinks, to mix and store cleaning solutions for household purposes and as urinals on sport boats. Containers are known to be discarded into garbage cans and public dumps probably due to misuse, yet are scavenged and returned for deposit and reuse. A popular newspaper column directed towards homemakers recommends the use of PE milk containers for storage of iced tea and juice drinks (4). Such use can lead to contaminated containers which produce off-flavors when the container is later used for milk.

The USPHS has published seven safeguards (7) which "must be met before a system utilizing multiple-use plastic milk containers could be considered as complying with the applicable provisions of the *Grade "A" Pasteurized Milk Ordinance — 1965 Recommendations of the USPHS*." These safeguards state in part:

"4. A device shall be installed in the filling line capable of detecting in each container before it is filled, volatile organic contaminants in amounts that are of public health significance...

"7. The container shall not impart into the product, pesticide residue levels or other chemical contaminants in excess of those considered acceptable by the FDA.

If further data become available which would indicate that the use of plastic containers for fluid milk may constitute a public health hazard, such containers will no longer be considered as meeting the applicable provisions of the *Grade "A" Pasteurized Milk Ordinance — 1965 Recommendations of the U.S. Public Health*

¹Polycarbonate (Lexan) containers, General Electric, Pittsfield, MA.

²PBS, Pennwalt Corporation, Philadelphia, PA.

Service."

Problems associated with the multiuse PE milk container system were critically evaluated by Bodyfelt et al. (1). They found the required in-line contamination detector able to determine only five out of 16 PE containers which had been contaminated with various chemical substances. The detector did not react to 11 containers treated with household chemicals. Milk stored in five of those 11 detector-accepted containers had either pesticide residues in excess of legal tolerance limits or had objectionable off-flavors.

The intent of this study was to compare retention of chemical contaminants by polyethylene, glass, and the relatively new polycarbonate (PC) multiuse milk containers. Potential consumer abuse of the various

containers was simulated to determine the effects of contamination upon the various container types and the effects upon milk subsequently placed in a contaminated container.

MATERIALS AND METHODS

Container preparation

New, unused polycarbonate¹, high-density polyethylene and flint glass multiuse milk containers were treated with chemical contaminants to simulate possible abuse by the consumer.

Twenty-nine chemical substances (Table 1) were selected to treat the containers. Selection was based on availability to the consumer, toxicity, and/or their ability to impart off-odors or flavors. Chemicals selected included 10 pesticides, seven herbicides, six household chemicals, five beverages, and urine. Liquid substances both with and without petroleum solvents, emulsions, and wettable powders were represented.

TABLE 1. *Compounds selected for treatment of containers*

Compound and manufacturer	Treatment concentration	Active ingredients (% by wt)
<i>Pesticides</i>		
Isotox Insect Spray, Ortho	purchased ^a and usage ^b	Carbaryl (5), Meta-systox-R (5), Kelthane (2), Aromatic petroleum derivative solvent (18)
Malathion 50 Insect Spray, Ortho	purchased and usage	Malathion (50), Aromatic petroleum derivative solvent (33)
Fruit and Vegetable Insect Control, Ortho	purchased	Diazinon (25), Aromatic petroleum derivative solvent (57)
Liquid Sevin, Ortho	purchased and usage	Carbaryl (27)
Ortho-Klor 74 Chlordane Spray	purchased and usage	Technical chlordane (74), Petroleum distillate (21)
Lindane Borer and Leaf Miner Spray, Ortho	1:2 spray: water and usage	Lindane (20), Aromatic petroleum derivative solvent (59)
Vapona Insecticide Emulsible Concentrate, Shell	1:3 spray: water	2,2-dichlorovinyl dimethyl phosphate (21.8), related compounds (1.6), Petroleum hydrocarbons (65.6)
Methoxychlor Mosquito Insect Spray, Black Leaf Garden Spray, Black Leaf 40	purchased usage	Technical methoxychlor (25), Xylene (72)
Sevin Garden Spray, Ortho, WP ^c	usage	Nicotine expressed as alkaloid (40) Carbaryl (50)
<i>Herbicides</i>		
Triox Liquid Vegetation Killer, Ortho	purchased and usage	Pramitol (1.86), Pentachlorophenol (0.68), Other chlorinated phenols (0.08)
Contax Weed and Grass Killer, Ortho	purchased	Sodium diethylarsenate (10.40), Dimethylarsenic acid (1.77)
Amitrol-T, Amchem	purchased	3-amino-1, 2, 4-triazole (21.0)
Dowpon M Grass Killer, Miller's, WP	purchased	Sodium salt of 2,2-dichloropropionic acid (72.5), Magnesium salt of 2,2-dichloropropionic acid (12.0)
Blackberry and Brush Killer, Miller's	purchased	2, 4, 5-trichlorophenoxyacetic acid, isooctyl ester (12.0) 2, 4-dichlorophenoxyacetic acid, isooctyl ester (25.1)
Phaltan Rose and Garden Fungicide, Ortho, WP	usage	Folpet (75)
Maneb Fungicide, Black Leaf, WP	usage	Manganese ethylenebisdithiocarbamate (80)
<i>Household chemicals</i>		
Prestone II Winter/Summer Concentrate	purchased	Ethylene glycol base (* ^d)
Penta, Pioneer	purchased	Pentachlorophenol (4.3), Other chlorophenols and related compounds (0.9), Mineral spirits (78.8)
Miracle Sander Paint Remover	purchased	Methanol (*), Benzol (*), Methylene chloride (*)
Pine-Sol	usage	Pine oil (30.0), Isopropanol (10.9), Soap (10.0)
Lysol Disinfectant Deodorizing Cleaner	usage	Soap (5.5), o-benzyl-p-chlorophenol (3.2), Isopropanol (1.1)
Crystal White Liquid Detergent, Lemon Fresh	usage	*
<i>Beverages</i>		
Funny Face Goofy Grape, Pillsbury	serving	*
Funny Face Choo-Choo Cherry, Pillsbury	serving	*
Country Time Lemonade Drink Mix, General Foods	serving	*
Grape Juice Frozen Concentrate, Welch's	serving	*
Orange Juice Frozen Concentrate, Minute Maid	serving	*
<i>Other</i>		
Urine, human, pooled sample		*

^aPurchased = Full strength as purchased.

^bUsage = Diluted to recommended usage concentration.

^cWP = Wettable powder.

^d* = Concentration of active ingredients not known.

Simulation of container abuse was conducted as follows: The containers were treated with the contaminants at full strength to simulate storage of the contaminant as purchased and/or diluted to the recommended usage concentration (Table 1). The treatment compounds were placed in the containers and stored at room temperature for 10 days and were then removed. The containers were rinsed until visually clear and were then washed in a simulated bottle washing procedure. The special cleaning compound, PBS², recommended for polycarbonate containers was used. The containers were filled with a 3% solution of PBS at 65.5 C and soaked for 8 min. The cleaning solution was removed and the containers rinsed three times at 50 C. The containers were subjected to a properly functioning, U.S.I. Polytrip in-line contamination detection device, model A-I, serial 003, calibrated to respond to 60 ppm methane, and the response recorded.

Immediately before filling, the containers were sanitized with 200 ppm of Cl and allowed to drain for 30 min. They were then filled with homogenized milk and stored for 10 days at 1 C. Samples of milk were removed and frozen for future residue analysis. Containers and the remainder of milk therein were tempered to 15 C for evaluation of odor by the five trained judges. Samples for sensory evaluation were coded and judged for odor differences against a reference. Untreated coded samples were provided as internal controls. The odor difference from the reference was noted first on a sample of milk poured into a snifter-shaped glass. A second odor evaluation was made on the milk remaining in the coded container, again against a reference in the same material container. Judges knew the containers had been treated with a variety of substances; they did not know which samples were internal controls. (Milk samples were not tasted because of the toxic nature of some of the treatments.) Containers were then emptied, rinsed twice, and washed using the above washing procedure to remove traces of milk before gas chromatographic analyses were initiated.

Two separate studies were carried out in which the sensory evaluation was conducted on the washed containers rather than on milk samples. In these studies the containers were treated with usage level concentrations of selected pesticides and one herbicide, or with various concentrations of cleaner contaminant solutions. The containers were then rinsed and washed according to the above procedure. The empty, coded containers were presented to the judges for evaluation of odor different from the reference. An untreated, coded control was included.

Analysis of volatile compounds within containers

Volatile compounds within the containers were collected for subsequent gas chromatographic analyses essentially as described by Miller et al. (5). The empty containers were purged with purified nitrogen (50 cc/min) for 30 min while being held in a 95 C water bath. The volatile compounds in the effluent were collected on a 102 × 6 mm o.d. stainless steel tube packed with Porapak Q. The Porapak trap was removed from the sampling device and water vapor expelled from the trap by heating with a thermostatically controlled heat gun to 55 C for 15 min under a nitrogen flow of 30 cc/min. The Porapak trap was reversed and the volatile organic compounds eluted with nitrogen (12 cc/min) at 135 C for 30 min and condensed in a 250 × 0.8 mm i.d. coiled stainless steel tube immersed in dry ice snow. This coiled trap was transferred to a modified inlet system (6) on a Varian Aerograph 1200 gas chromatograph and was heated to flash the organic compounds onto the 153 m × 0.8 mm i.d. stainless steel column coated with SF-96. The column was held at 70 C for 5 min and then programmed to 160 C at 2°/min.

Analysis for chlordane residue

Chlordane residue was analyzed using electron capture gas chromatography. The pesticide-containing lipid fraction was extracted from 100-g milk samples (2). Recovery of chlordane from spiked milk samples was from 98 to 101%. Hexane was used instead of pentane and an additional step to remove water from the solvent fraction was found necessary. After the residual water had been drawn off from the separatory funnel and discarded, the solvent layer was transferred to a 500-ml Erlenmeyer flask and about 20 g of anhydrous sodium sulfate was added. The separatory funnel was washed with hexane (3 × 15 ml).

²PBS, Penwalt Corp. Philadelphia, PA

The solvent layer was allowed to remain in contact with the sodium sulfate for about 5 min and then the solvent layer was decanted and passed through the 4-inch glass filter prepared with a glass wool plug and a layer of sodium sulfate (about 25 g). The solvent was evaporated to dryness and the chlordane-containing lipid fraction was retained. The extracted lipid material was stored in the freezer if the analysis was interrupted for more than overnight.

The pesticide-containing lipid fraction was purified before gas chromatographic analysis on a 25 g alumina column using 5% H₂O deactivated alumina. A portion (about 0.35 g) of the lipid fraction was weighed, dissolved in hexane, evaporated to about 3 ml, and placed on the column. Hexane was used to elute the pesticide from the column. The chlordane was found in the first 75 ml of eluate. This eluate was evaporated to 2 ml and then analyzed against a 0.1 µg/ml chlordane standard on a Hewlett Packard 5700A GC with a nickel 63 detector. The column was 1.22 m × 3.175 mm stainless steel packed with 7% 4/1 OV-210/OV-17 on 100-120 Chromasorb HPW. The injector, column, and detector temperatures were 300, 205, and 300 C, respectively. The carrier gas flow rate was 28 cc/min, 5% argon/95% methane.

RESULTS AND DISCUSSION

The new, untreated polyethylene containers had a hot-paraffin-like, smoky or plastic odor. Neither the polycarbonate nor the glass containers had an intrinsic odor.

The response of the in-line contaminant detector to the treated, washed containers is compared with the sensory evaluation of the milk and container in Table 2. None of the glass containers had sufficient volatile contents to trigger a positive response from the detector. No off-odors were found in the milk-container combinations for the glass container with the exception of one container which had been treated with a full strength pesticide containing diazinon.

Fifteen treated polyethylene containers did not trigger a positive response from the detector. There were off-odors in the milk later stored in nine of those 15 containers. A wettable powder pesticide, an emulsified herbicide, two wettable powder herbicides, lemon scented dishwashing detergent, three beverages, and urine had been stored in the PE containers which were undetected by the contamination detection device, yet the combination of container and milk contents had off-odors. These substances are mainly water based, have low volatility, and do not contain a petroleum derived carrier which would activate the detector. The pesticides and herbicides have potential public health significance; the other compounds produce an off-odor in the milk which varies from being a nuisance to being repugnant. The detector did register a positive response for those PE containers which had been contaminated with full strength substances which contained a petroleum derivative solvent.

Nine of the polycarbonate containers were cracked, dissolved, disintegrated, or clouded by the treatment substances and further testing of these compounds was not necessary for such a container could not be reused. The chemicals which damaged the PC containers had in common a hydrocarbon solvent carrier or a chlorinated organic constituent. Five PC containers that had been treated with either an emulsifiable liquid or wettable powder herbicide, a beverage, or urine were not detected

by the in-line detector, yet imparted off-odors to the milk contained therein. Again, as with PE containers, the containers which passed the detector without a positive response yet produced off-odors in milk had contained non-volatile, non-hydrocarbon water based substances.

Five of the pesticides and herbicides tested at full strength were selected for additional testing diluted to usage concentration (Table 3) with the sensory evaluation made on the containers themselves. With the compounds diluted to usage strength, all the treated, washed glass containers passed the detector without response. The panel of trained judges noted an off-odor in the glass container that had been treated with lindane. Two of the five PE containers passed the detector without a response; both of these containers, which had held liquid Sevin or chlordane diluted to the recommended concentration, were judged to have off-odors by the trained panel. At full strength both of these treatments were sufficient to elicit positive detector response, yet at usage strength the

containers went undetected. Two PC containers passed the detector and had off-odors as determined by the panel. Again, as with PE, the chemical substances involved were Sevin and chlordane. Triox, an herbicide which contained a hydrocarbon solvent, dissolved the PC container even at usage level.

Gas-liquid chromatographic analysis of container volatile constituents

The containers which were not detected by the contamination sensing device yet had off-odors in the milk later placed in the container were selected for gas-liquid chromatographic (GLC) analysis. The number of peaks detected in each of the containers examined is shown in Table 4.

The control glass containers produced no significant peaks when analyzed by GLC. Two peaks were observed in the volatile constituents from the container which had held grape flavored punch. No peaks were detected in the volatile constituents from any of the other glass

TABLE 2. Detector response to treated, washed containers and sensory evaluation of milk-container combination

Treatment & (concentration)	Container material					
	Glass		Polyethylene		Polycarbonate	
	Detector response	Sensory evaluation	Detector response	Sensory evaluation	Detector response	Sensory evaluation
Isotox (full strength)	0 ^a	— ^b	* ^c	+++ ^d	damaged ^e	
Malathion (full strength)	0	—	*	+++	damaged	
<i>Insect Control</i> ^f (full strength)	0	+	*	+++	damaged	
Sevin liquid (full strength)	0	—	*	+	0	—
Chlordane (full strength)	0	—	*	++	*	+
Lindane (1:2)	0	—	*	+++	damaged	
Vapona (1:3)	0	—	*	+++	damaged	
Methoxychlor (full strength)	0	—	*	+++	damaged	
Black Leaf 40 (usage)	0	—	0	—	0	—
<i>Sevin (WP)</i> ^g (usage)	0	—	0	++	0	—
Triox (full strength)	0	—	*	++	damaged	
Contax (full strength)	0	—	0	—	0	—
<i>Amitrol-T</i> (full strength)	0	—	0	+	0	+
Dowpon (usage)	0	—	0	—	0	—
Blackberry & Brush Killer (full strength)	0	—	*	+++	damaged	
<i>Phaltan</i> (WP) (usage)	0	—	0	+	0	+
<i>Maneb</i> (WP) (usage)	0	—	0	++	0	++
Prestone (full strength)	0	—	0	—	0	—
Penta (full strength)	0	—	*	++	0	—
Miracle Sander (full strength)	0	—	*	+	damaged	
Pine-Sol (usage)	0	—	*	+++	0	—
Lysol (usage)	0	—	0	—	0	—
<i>Crystal White</i> (usage)	0	—	0	+	0	—
<i>Goofy Grape</i> (serving)	0	—	0	++	0	++
Choo-Choo Cherry (serving)	0	—	0	—	0	—
Lemonade (serving)	0	—	*	+	0	—
<i>Grape Juice</i> (serving)	0	—	0	+	0	—
<i>Orange Juice</i> (serving)	0	—	0	+	0	—
<i>Urine</i>	0	—	0	+	0	+
Controls	0	—	0	—	0	—
	0	—	0	—	0	—
	0	—	0	—	0	—

^a0 = No positive detector response for a contaminant.

^b— = No off-odor in milk-container combination.

^c* = Positive detector response for a contaminant.

^d+ = Slight off-odor in milk-container combination.

++ = Definite off-odor in milk-container combination.

+++ = Pronounced off-odor in milk-container combination.

^eContainer dissolved, disintegrated, cracked or otherwise damaged by treatment.

^fTreatment = Container passed detector without response yet milk-container combination had off-odor.

^g(WP) = Wettable powder.

TABLE 3. *Detector response and sensory evaluation of usage-level treated, washed containers*

Treatment	Container material					
	Glass		Polyethylene		Polycarbonate	
	Detector response	Sensory evaluation	Detector response	Sensory evaluation	Detector response	Sensory evaluation
Isotox	0 ^a	— ^b	* ^c	+++ ^d	*	++
Sevin, liquid ^e	0	—	0	+	0	+
chlordane	0	—	0	++	0	+
lindane	0	+	*	+++	*	++
Triox	0	—	*	+++	damaged ^f	—
Control	0	—	0	+ ^g	0	—

^a0 = No positive detector response for a contaminant.

^b— = No off-odor in washed container.

^c* = Positive detector response for a contaminant.

^d+ = Slight off-odor in washed container.

++ = Definite off-odor in washed container.

+++ = Pronounced off-odor in washed container.

^etreatment = Container passed detector without response yet had off-odor.

^fContainer softened, clouded or cracked by treatment.

^gContainer had smoky, hot-paraffin-like, plastic odor.

TABLE 4. *Number of peaks obtained by gas chromatographic head space analysis*

Treatment	Container material		
	Glass	Polyethylene	Polycarbonate
Control 1	no significant peaks	53	5
2	no significant peaks	57	7
Chlordane usage full strength	NDO ^a	18 ^b overwhelmed the method	+6 +17
Sevin liquid at usage	NDO	NDO	+7
wettable powder at usage	NDO	+2	NDO
Fruit and Vegetable Insect Control, full strength	NDO	overwhelmed the method	NA ^c
Amitrol-T, full strength	NDO	+4	+4
Phaltan, usage	NDO	+6	+4
Maneb, usage	NDO	+4	NDO
Goofy Grape, serving	+2	NDO	+2
Urine	NDO	NA	NDO

^aNDO = No difference observable from the control.

^b+18 = 18 Peaks in addition to those present in the control.

^cNA = Data not available.

containers.

The two PE control containers yielded 53 and 57 peaks, respectively, indicating the large number of volatile compounds found within the new, unused PE container. In all but one of the treated PE containers, additional peaks were found. Chlordane at full strength and Fruit and Vegetable Insect Control containing diazinon, overwhelmed the method indicating the high absorbency of polyethylene.

The control PC containers demonstrated five and seven peaks, respectively, indicating the presence of a few volatile compounds. The PC container treated with chlordane produced the largest number of peaks, perhaps indicative of the affinity of PC for chlorinated organic compounds. Additional peaks were found in several of the other containers, indicating that PC did absorb components from non-chlorinated compounds.

Chlordane residue analysis of milk from treated containers

Containers holding the following pesticides and herbicides did not elicit a positive detector response, yet produced an off-odor either in the empty, washed container when treated with diluted, usage level contaminant or in the milk-container combination when treated with the full strength contaminant: Fruit and Vegetable Insect Control (diazinon) (full strength), Sevin wettable powder (WP) (usage), Sevin liquid (usage), Amitrol-T (full), Phaltan WP (usage), Maneb WP (usage), chlordane (usage), and lindane (usage). Chlordane was selected from the substances listed above for pesticide residue analysis.

Chlordane residues were found present in milk (Table 5) which had been in the polyethylene container treated with full strength chlordane (24 ppm) and in the polycarbonate container treated at both usage level (0.630 ppm) and with full strength chlordane (14.6 ppm). Surprisingly, no chlordane was detected in milk from the usage level treated polyethylene container. At full strength treatment, the milk absorbed more chlordane from the polyethylene container than from the polycarbonate container. The PE and PC containers treated with full strength chlordane contained sufficient solvent to trigger a positive response from the in-line detector. Such containers would not be refilled providing the detector was functioning properly. As there has been no tolerance established by the FDA for chlordane in milk, the presence of this pesticide places it in excess of the legally established limits.

TABLE 5. *Chlordane recovered from milk from treated containers*

Treatment	Container material		
	Glass	Polyethylene	Polycarbonate
	(ppm)		
Full strength	0.007	24.0	14.6
Usage strength	<0.005*	<0.005*	0.630
Control	<0.005*	—	<0.005*

* = Residue value below detection limits of the method.

Detector response to cleaning solutions

Treatments with both cleaning solutions produced off-odors described as piney or lemony, respectively, in all of the treated containers (Table 6). The in-line contamination detector responded to the pine-scented cleaner at all concentrations but did not detect the lemon-scented detergent at any level. The above results demonstrate that a refillable PE container could conceivably become contaminated simply by soaking it in a solution of dishwashing detergent.

TABLE 6. *Sensory evaluation and detector response to polyethylene containers treated with cleaning solutions*

Treatment	Concentration ml/qt.	Relative concentration	Detector response	Sensory evaluation
Lemon-scented dishwashing detergent	13.2	recommended concentration	0 ^a	+ ^b
	16.5	1.25 ×	0	++
	19.8	1.5 ×	0	++
	26.4	2 ×	0	++
Pine-scented cleaner	0		0	- ^c
	4.73	1/3 ×	* ^d	+++
	9.74	2/3 ×	*	++
	14.2	recommended concentration	*	+++

^a0 = No positive detector response.

^b+ = Slight off-odor in the washed container.

++ = Definite off-odor in the washed container.

+++ = Pronounced off-odor in the washed container.

^c- = No off-odor in the washed container.

^d* = Positive detector response for a contaminant.

CONCLUSIONS

The *Grade "A" Pasteurized Milk Ordinance — 1965 Recommendations of the USPHS (PMO) (8)* states in part "...that all milk contact surfaces of multiuse containers shall consist of materials which are nontoxic, fat resistant, relatively nonabsorbent, and do not impart flavor or odor to the product..." The seventh safeguard of the USPHS PMO (5) states "7. The container shall not impart into the product, pesticide residue levels or other chemical contaminants in excess of those considered acceptable by the FDA."

Both the polyethylene and the polycarbonate container have been shown to absorb contaminants and to impart off-odors into the product. Pesticide residues in excess of legal tolerances established by the FDA were found.

A properly functioning in-line contamination detector appears capable of detecting containers which had been contaminated with petroleum-derived substances. The detector was not capable of detecting those test containers which had held water based, non-hydrocarbon substances of low volatility. The detector does not appear to give adequate consumer protection to the multiuse polyethylene or polycarbonate milk container system.

Neither the polyethylene nor the polycarbonate multiuse containers appear to comply with the *Grade "A" Pasteurized Milk Ordinance — 1965 Recommendations of the USPHS*.

ACKNOWLEDGMENTS

The authors thank Matthew V. Andrews, Graduate Research Assistant, Department of Food Science and Technology, for his cooperation and technical assistance and Max L. Deinzer, Donald A. Griffin, Jack Lamberton, Anna B. Marin, and Betsy Shepard of the Environmental Health Sciences Center for their assistance with the pesticide residue analysis. This research was supported, in part, by a grant from the Paperboard Packaging Council. Technical Paper No. 4502. Oregon Agricultural Experiment Station, Oregon State University, Corvallis, OR 97331.

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Differences Between *Aspergillus flavus* Strains in Growth and Aflatoxin B₁ Production in Relation to Water Activity and Temperature

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(Received for publication April 15, 1977)

ABSTRACT

The optimum and limiting conditions of water activity (a_w) and temperature for growth of and aflatoxin B₁ production by various *Aspergillus flavus* strains were determined. Agar media were used in which the a_w was adjusted by addition of sucrose or glycerine. Optimum temperatures for aflatoxin B₁ production at high a_w varied with the strain tested being 13-16, 16-24, or 31 C. Strains with a low temperature optimum for aflatoxin B₁ production showed fast growth at 37 C without aflatoxin B₁ production. A reduced a_w (0.95 and less) together with a moderate or low temperature inhibited toxin production more than growth. However, at a high temperature one strain showed stimulation of aflatoxin B₁ production on the glycerine medium at reduced a_w . No differences were noted between aflatoxin-positive and aflatoxin-negative strains with respect to growth under various conditions.

Water activity (a_w) and temperature are the most important factors controlling fungal growth. A_w and temperature optima and limits for growth of several fungi, including *Aspergillus flavus*, were studied by Ayerst (1). No significant differences between the *A. flavus* strains examined by this worker were shown. In a previous study, we described optima and limits for both growth and aflatoxin production by a strain of *Aspergillus parasiticus* (5). The optimum temperatures for aflatoxin B₁ production were 24 and 32 C, depending on the substrate. Studies by other investigators showed optima ranging from 20 to 35 C (3,4,7,8,9). In one such study a substrate effect on the optimum temperature for aflatoxin B₁ production was also demonstrated (4). Therefore, differences in results of the various investigations could be explained by differences in substrate. With respect to the optimum a_w for aflatoxin B₁ production, we found an a_w value of 0.99 (5), whereas Diener and Davis (4) found a_w values of 0.95 and 0.99 depending on the substrate.

Another explanation for the differences in optimum

temperature and a_w may be sought in the use of different fungal strains. However, this has received little attention, except that in one study optimum temperatures of two strains were compared and no notable difference was found (8).

Since results from an investigation with one strain are too easily considered to be representative for a species, this study was undertaken with three aflatoxin-producing strains of *A. flavus* cultivated on two substrates to determine the environmental conditions necessary for aflatoxin B₁ production. Besides, two non-toxicogenic strains were used to compare their growth characteristics with those of toxicogenic strains.

MATERIALS AND METHODS

Three aflatoxin-producing *A. flavus* strains (RIV 103, RIV 104, ATCC 15517, further indicated by the number) and two aflatoxin-negative *A. flavus* strains (RIV 111, RIV 113, further indicated by the number) were maintained as lyophilized cultures. Cultures grown for 1 week at 24 C on malt extract agar (Oxoid) were washed with an aqueous solution of 0.6% sodium heptadecylsulphate (Tergitol-7, BDH) to prepare a spore suspension.

Various a_w conditions in malt extract agar were achieved by adding sucrose (MES-series) or glycerine (MEG-series). Preparation of agar plates, inoculation, and a_w measurement have been previously described (5). For each determination of rate of growth and aflatoxin B₁ production, two inoculated agar plates with three colonies each and one non-inoculated agar plate, which served as control of the a_w after incubation, were used. They were enclosed in a 0.3-liter polyethylene bag (gauge 0.04 mm). The growth rate of the mycelium was determined by daily measurement of two right-angled diameters of a colony. The regression lines of colony diameter on days after inoculation were calculated for each a_w -temperature combination. The germination time was obtained by extrapolating the regression line to the X-axis (5). Until extraction, the bags were placed at -18 C on the day that the average diameter of the six colonies reached 3 cm or on the 35th day of incubation when they did not reach this size. Extraction and analysis of aflatoxin B₁ have been described before (5). The detection limit of aflatoxin B₁ was 0.1 µg for the six colonies on the two agar plates used for each analysis.

In the first experiment the toxicogenic strains 103, 104, and 15517 and the non-toxicogenic strains 111 and 113 were grown at combinations of 10, 13, 16, 24, 31, 37 ± 0.3 C and five different a_w values on MES and

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Figures 1-8. Growth of and aflatoxin B₁ production by three strains of *Aspergillus flavus* on MES and MEG agar under various conditions of a_w and temperature (rate of growth — white column; aflatoxin B₁ production — black column, the 1-mm black columns represent amounts of 0.1-10 μg aflatoxin B₁).

fig. 1 strain 103 on MES

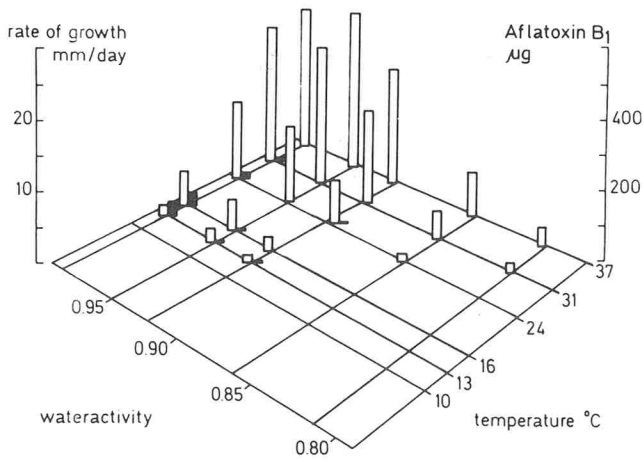


fig. 2 strain 103 on MEG

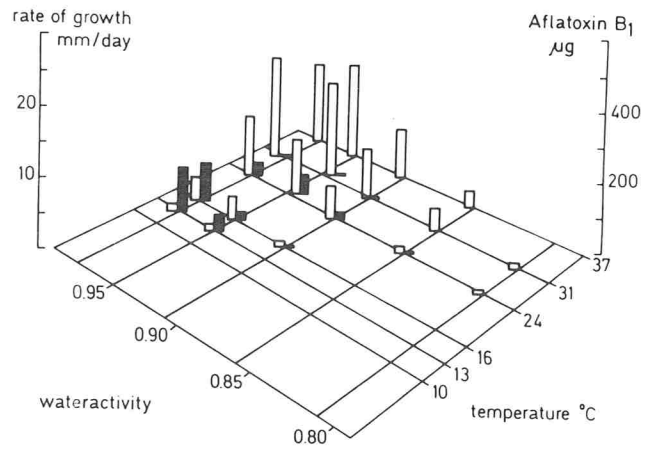


fig. 3 strain 104 on MES

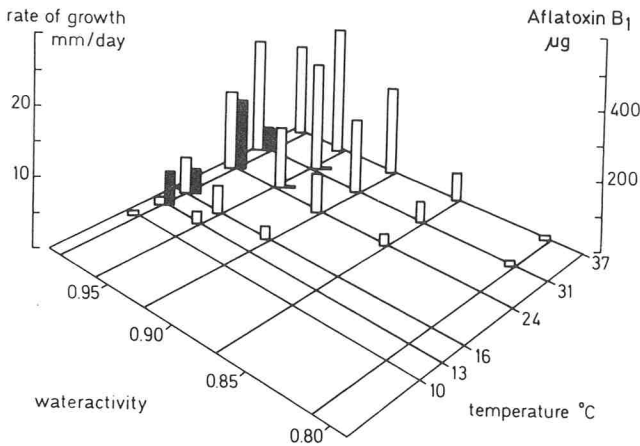


fig. 4 strain 104 on MEG

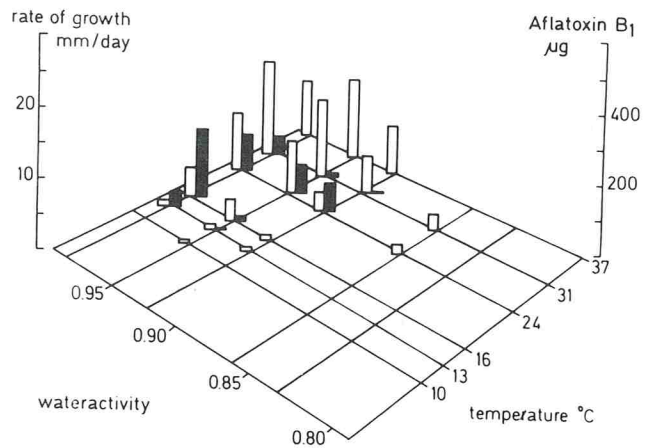


fig. 5 strain 15517 on MES (1st)

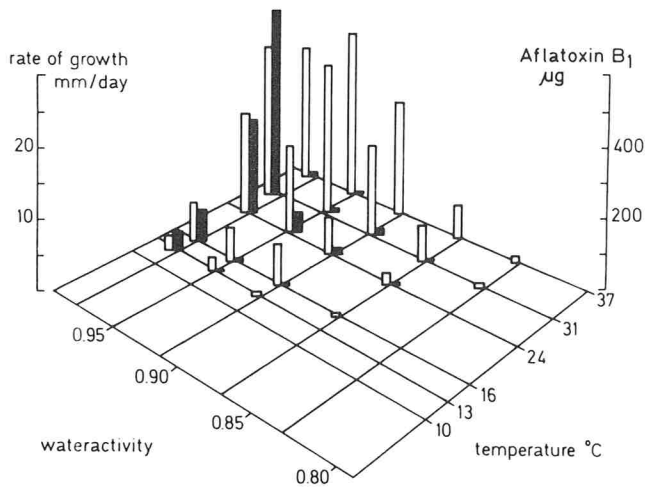
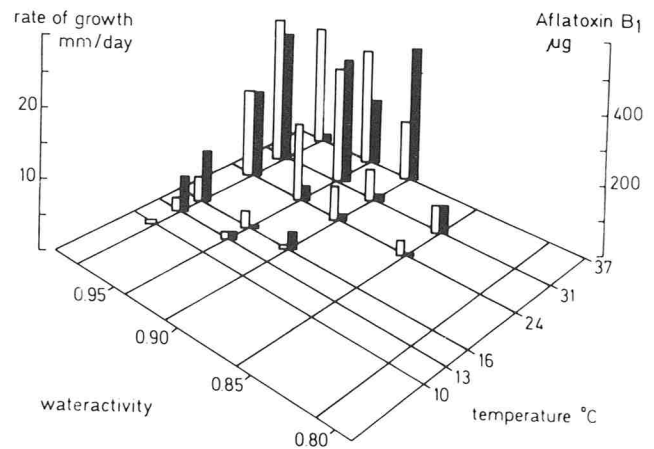
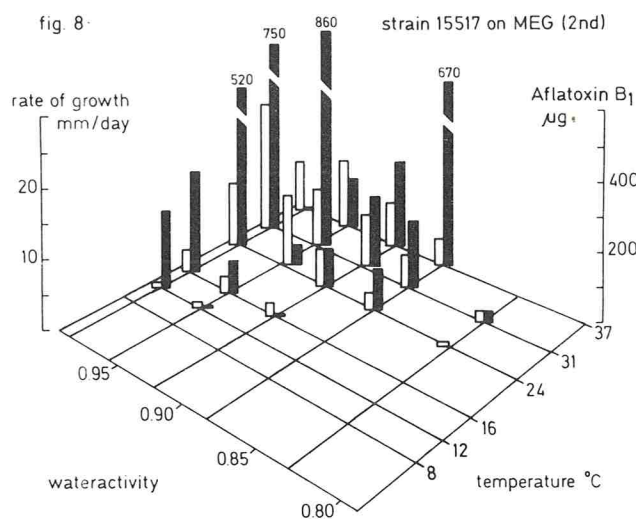
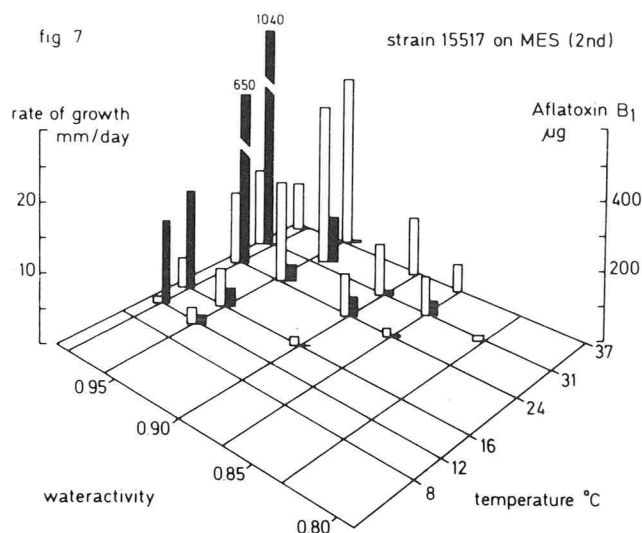


fig. 6 strain 15517 on MEG (1st)





MEG. To assess the repeatability of growth and toxin production a second experiment was carried out, in which strain 15517 was repeated at combinations of 8, 12, 16, 24, 31, 37 ± 0.3 C and five different a_w values on MES and MEG. Moreover, four bags containing MES agar of 0.99 a_w and four bags containing MEG agar of 0.99 a_w both inoculated with strain 15517, were incubated at 24 C. To reproduce the differences in optimum temperature of strains found in the first experiment, a third experiment was carried out in which strains 103, 104, and 15517 were repeated twice at 10, 13, 16, 24, 31, 37 ± 0.3 C on 0.99 a_w MES.

RESULTS

In the first experiment toxigenic and non-toxicogenic strains were tested. Results of the toxigenic strains are shown in Figure 1-8. Results of the non-toxicogenic strains are not shown, because their optimum and limiting conditions for growth were similar to those of the toxigenic strains. The toxigenic strains showed optima for toxin production at 13-16, 16-24, and 31 C. The optimum a_w for toxin production was 0.99 for strains 103 and 104 (Fig. 1-4). Strain 15517 had an optimum a_w on MEG which greatly depended on temperature and which varied from 0.90 to 0.99 (Fig. 6). Table 1 shows the calculated germination times in connection with the rate of growth, depending on the various conditions of a_w and temperature.

In the second experiment, results obtained with strain 15517 were repeated qualitatively (Fig. 7 and 8). The repeatability of the method was shown by comparing the

aflatoxin B_1 contents of replicate cultures on 0.99 a_w MES and 0.99 a_w MEG. These differed not more than 12% from the average.

In the third experiment, the difference in optimum temperatures for toxin production was reproduced (results are not shown). Cultures of strains 103, 104, and 15517, grown at 0.99 a_w and different temperatures, contained maximal amounts of toxin at the same temperatures as in the first experiment.

DISCUSSION

This study was undertaken to investigate whether differences in fungal strains may explain the different optimum temperatures for toxin production reported by various investigators (3,4,7,8,9). This hypothesis has been confirmed by the results of our experiments. The other factor that determines the optimum temperature is the substrate used. The results demonstrate that a high concentration of glycerine rather than a_w did increase the optimum temperature for toxin production. This effect was most striking with strain 15517 (Fig. 6 and 8), whose optimum temperature increased from 31 to 37 C. A similar effect was also found by Diener and Davis (4) in a study with peanuts. They found that the optimum temperatures for aflatoxin B_1 production on damaged and sound mature peanut kernels at high a_w were 20 and 35 C, respectively.

According to the latter study, the a_w optima for aflatoxin B_1 production in damaged and sound mature kernels were 0.95 and 0.99 respectively. In our study not only substrate but also fungal strain and temperature determined the optimum a_w (Fig. 6 and 8). In a previous study (5) with *A. parasiticus* NRRL 2999, we concluded that a reduced a_w inhibits toxin production more than growth. This conclusion still holds true for a moderate temperature, but the present results indicate that at a higher temperature substrate and fungal strain have a greater influence.

The temperature limit for toxin production lies

TABLE 1. Germination time of cultures of three toxigenic *Aspergillus flavus* strains grown on MES or MEG agar in relation to rate of growth

Rate of growth ¹	Agar medium	Germination time (days)		
		103	Strain 104	15517
Maximum	MES	0.8	0.5	0.5
	MEG	0.3	0.3	0.6
10 mm/day	MES	1	1	1-2
	MEG	0.5	0.4	1-2
1 mm/day	MES	9-10	3	6-10
	MEG	4-8	3-5	3-9

¹Rate of growth under various a_w -temperature conditions, as shown in Fig. 1-8

between 10 and 13 C, and equals that for growth. Only a few cultures grow at 10 C without toxin production. In other studies, with rice (3) and peanuts (4) the limits for toxin production were 15 and 12 C, respectively. The a_w limit for toxin production lies between 0.83 and ca. 0.87, whereas in other studies the a_w limits were 0.70 - 0.75 with rice (2) and 0.85 with peanuts (4). However, it is difficult to compare the results because of the different and wide intervals of temperatures and a_w values used in this and the other studies.

The data in this study indicate that foodstuffs contaminated with *A. flavus* spores may contain large amounts of aflatoxin after a short period, because our results demonstrate that six colonies of a potent *A. flavus* strain could accumulate 500 μg aflatoxin B₁ in one and a half days.

Summarized, it can be stated that differences between *A. flavus* strains exist not only with regard to maximal amount of aflatoxin produced, but also with regard to the optimum conditions for aflatoxin production.

ACKNOWLEDGMENT

The authors thank Dr. J. Harwig for his criticism and suggestions.

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Cook/Chill Foodservice Systems: Temperature Histories of a Cooked Ground Beef Product During the Chilling Process¹

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(Received for publication April 22, 1977)

ABSTRACT

Temperature histories during the chilling process under actual operating conditions in a school cook/chill foodservice system and in laboratory experiments show it was not possible to chill cooked entrees to 45 F (7 C) or below within 4 h when entrees were stored in walk-in refrigerators. Consideration should be given to equipment designed for the chilling process before converting to cook/chill foodservice systems.

Rising costs and the shortage of a reliable workforce have contributed to development of the cook/chill concept in school and hospital foodservice systems. In a cook/chill foodservice the entree is cooked 1 to 3 days in advance of service and chilled in bulk to refrigerator temperature. On the day of service, the chilled entree is portioned into individual servings in a central assembly unit, stored in refrigerated holding equipment, and transported to the point of service where convection or microwave ovens are used to reheat the entree to desired serving temperature.

During the chilling process in a cook/chill system the entree should be cooled rapidly to 45 F (7 C) or below to retard bacterial growth. Longree (3) recommends that the internal temperatures of food should reach 45 F (7 C) within 4 h and should not remain between 60-120 F (16-49 C) for more than 2 h. Only a few researchers have reported temperature histories of cooked entrees during the chilling process in cook/chill foodservice systems (1,2,4). These investigators recorded temperatures histories at only one location in the food product; temperature histories are often reported as incidental to the major aim of the research.

Questions have been raised about the capability of refrigeration equipment currently used in schools and hospitals to chill entrees to 45 F (7 C) or less within a 4-h

time period. This study was conducted to observe the length of time required for a cooked ground beef product to cool to 45 F (7 C) when chilled according to procedures which may be used in a school cook/chill foodservice system.

MATERIALS AND METHODS

The study consisted of two parts: I, temperature data collected under actual operating conditions in a school cook/chill foodservice system; and II, temperature data collected in a laboratory under conditions simulating procedures used in the school foodservice system.

Study I

Temperature data were collected in a school cook/chill foodservice system where about 3800 Type A lunches were processed daily. Preparation and chilling procedures for entrees were observed on 9 days during a 4-week period. On each day of the field study, 10 pans of food were selected to be monitored for temperature change during the chilling process; five pans from each of two racks (24-10 × 18 × 4½-inch pans/rack). Temperature at the geometric center of the food mass (10 × 18 × 3 inch) was recorded at hourly intervals from the time of initial chilled storage in the walk-in refrigerator until the temperature at the geometric center of the food mass was approximately 45 F (7 C). Temperature of the food mass was measured by a thermometer (Taylor Bi-Therm Pocket Dial. 0-200 F., Sybron Corp., Arden, NC). Ambient temperature in the refrigerator was read from an integral thermometer attached to the refrigeration unit.

Study II

Procedures used to prepare and chill entrees in the school cook/chill foodservice system were simulated in a laboratory under controlled conditions. A review of the 5-week cycle menu used in the school foodservice system showed that a ground beef product appeared most often as the entree. A recipe for barbeque ground beef was modified to provide the entree for the experimental study. Procedures for preparation and chilling of the ground beef product were standardized to minimize variation among three trials of the experiment.

Preparation. Forty-eight hours before preparation, 25 lb (11.3 kg) of ground beef were removed from frozen storage (-22 F; -30 C) to thaw in a walk-in refrigerator. On the day of preparation, the ground beef was browned for 45 min in a 5-gal steam-jacketed kettle (Groen, Model TDC/2-20, Elk Grove Village, IL). Prepared mustard (1.13 lb; 0.5 kg) and ketchup (2.0 lb; 0.9 kg) were added and the mixture was simmered an additional 30 min to a temperature in excess of 175 F (79 C). The product was poured into a 12 × 20 × 4-inch counter pan to a depth of 2 inches. The product in the pan remained at room temperature (80 F; 27 C) approximately 5 min until the temperature at the geometric

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center the food mass (12 × 20 × 2 inch) reaches 170 F (77 C) as recorded by a potentiometer (Honeywell, Model 153x64-P8H-II-III-42, Minneapolis, MN). The pan of ground beef product was transferred immediately to the walk-in refrigerator adjacent to the preparation area.

Chilling. The pan of ground beef product was placed in the refrigerator on a shelf opposite the refrigerator fan. Five Type T, copper-constantan, thermocouples were positioned in the product to measure temperature at five layers in the center of the product in the pan. Thermocouples, 1 to 5, monitored the temperature of the product at locations 1.8 inches (4.5 cm); 1.4 inches (3.5 cm); 1.0 inch (2.5 cm); 0.6 inch (1.5 cm); 0.2 inch (0.5 cm) from the top surface of the product in the pan, respectively. Thermocouples were fastened to a plastic guide stick to prevent movement of the thermocouples in the food mass during the chilling process. Ambient temperature in the refrigerator was monitored by two thermocouples positioned 6 inches above the pan of product in the refrigerator.

RESULTS AND DISCUSSION

Study I

Data in Table 1 show that the initial temperature of several entrees was 100 F (38 C) or less. It was not a practice of the cooks in the foodservice operation to allow entrees to cool at room temperature before being transferred to the walk-in refrigerator. In most instances, prepared ingredients were removed from refrigerated storage and canned ingredients were removed from the storeroom to be combined with browned ground beef in a steam-jacketed kettle. In other instances, cold ingredients were combined in the unheated steam-jacketed kettle and portioned into 10 × 18 × 4-1/2-inch counter pans.

Results of the time-temperature measurements during the chilling process (Table 1) show that the time required for the average temperature at the geometric center of the food mass to reach 45 F (7 C) ranged from 7.0 to 11.0 h. The average temperature of the entree did not reach 45 F (7 C) within 4 h on any of the 9 days of the field study. Temperatures recorded in the walk-in refrigerator varied during the field study (Table 1).

Study II

Mean temperatures of the product and the refrigerator during 24-h chilled storage for three trials are shown in Fig. 1. The average temperature of the refrigerator varied slightly among the three trials; 38 ± 4 F (3 ± 2 C) for Trial I and 36 ± 4 F (2 ± 2 C) for Trials II and III, with an average of 37 ± 5 F (3 ± 3 C) for three trials.

Data in Fig. 1 show that the thermocouples located near the bottom of the food mass consistently recorded higher temperatures than thermocouples at other locations in the product. Four and one-half hours were required for temperatures near the surface of the product (thermocouple #5) to reach 45 F (7 C) or less which was 30 min longer than the time recommended for cooling (3). At the end of 6 h mean temperatures at all locations in the center of the product were ≤ 45 F (7 C). Findings in Fig. 1 show that it was not possible to chill the ground beef product through the 120-60 F (49-16 C) temperature range within 2 h, nor was it possible to chill the ground

TABLE 1. Time-temperature relationships and load in refrigerator during chilling of nine entrees under actual operating conditions in a school cook/chill foodservice system

Entree	Temperature range	Cooling ^a time	Refrigerator temperature	Load (No. of pans) ^b
	($\frac{F}{C}$)		($\frac{F}{C}$)	
Turkey barbecue	93-45	7.0	39 ± 5	72
	34-7		4 ± 3	
Lasagna	90-46	7.0	39 ± 5	72 ^c
	32-8		4 ± 3	
Macaroni and cheese	68-46	8.0	42 ± 8	48
	20-8		6 ± 4	
Chili II	94-45	8.0	44 ± 11	96
	34-7		7 ± 6	
Chili I	84-48	9.0	42 ± 6	96
	29-9		6 ± 3	
Barbecue ground beef	126-46	9.5	38 ± 6	48
	52-8		3 ± 3	
Hot beef sandwich	162-48	10.0	46 ± 13	72
	72-9		8 ± 7	
Spanish hamburger casserole	100-46	10.5	39 ± 8	72
	38-8		4 ± 4	
Beef stew	125-48	11.0	44 ± 11	70
	52-9		7 ± 6	

^aTime required for the temperature at the geometric center of the entree to pass through the temperature range. Average of 10 pans of the entree.

^b10 × 18 × 3-inch food mass in an 10 × 18 × 4-1/2-inch pan.

^c72 (23-1/4 × 15-1/2 × 2 inch) pans.

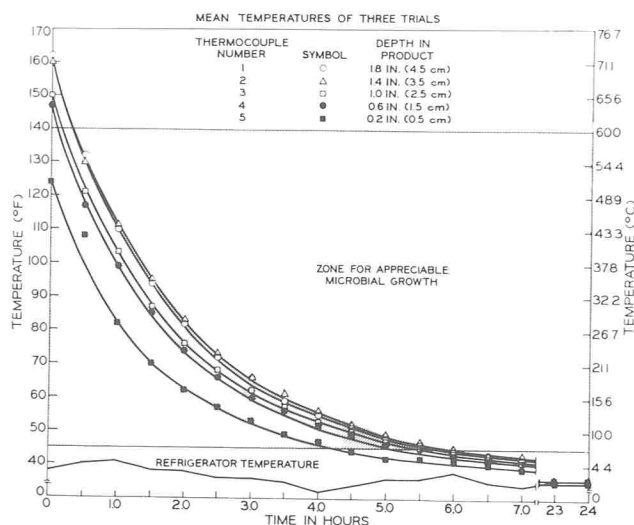


Figure 1. Effect of time on temperature of ground beef during chilling process: Mean temperatures recorded at five depths in the center of product during three trials. Thermocouples #1 and #5 show mean temperatures from only two trials.

beef to 45 F (7 C) or less in 4 h in a typical walk-in refrigerator (37 ± 5 F; 3 ± 3 C).

Factors influencing cooling of the ground beef product include the temperature in the entree before cooling, dimensions of the food mass, product load in the refrigerator, and temperature in the refrigerator. The

internal temperatures of the ground beef product, recorded at the beginning of chilled storage, ranged from 124-161 F (51-72 C) and averaged 149 F (65 C) for the five depths in the center of the product. Although changing the dimensions of the food mass, i.e., depth of the product in the pan, may speed cooling of the product, decreasing depth of the product in the pan would increase the number of pans to be chilled. Depending upon facilities available and number of pans, storage problems could occur when large quantities of food were being chilled.

Product load in the refrigerator during this experiment was one 12 × 20 × 4-inch counter pan, the standard size pan routinely used in foodservice operations. Under normal operating conditions, the number of pans of food to be chilled would be considerably greater than one. A large number of pans of hot food placed in the refrigerator can increase the temperature in the refrigerator and slow the rate of cooling of food products placed in the refrigerator to cool. Although refrigerator temperature could be lower than 37 ± 5 F (3 ± 3 C), it is doubtful that a reduction in refrigerator temperature alone could achieve a temperature of 45 F (7 C) in food within the recommended 4-h period, especially with an

increased product load. When refrigerator temperature is too low, freezing may occur near the surface of the product and may damage the quality of food.

Special equipment is needed to rapidly chill hot entrees to 45 F (7 C) or less without adversely affecting food quality. Such equipment should be evaluated under actual operating conditions in a cook/chill foodservice system before it is advertised as available for the foodservice industry.

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A Comparison of Several Assay Procedures to Detect Penicillin Residues in Milk¹

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ABSTRACT

Milk samples from six healthy cows that alternately received intramuscular injections and intramammary infusions of penicillin were analyzed for the presence of penicillin residues using the disc assay, cylinder plate, and Delvotest-P³ methods. Time required for complete removal of penicillin from milk of treated cows was determined with each of the three tests. The disc assay antibiotic test detected penicillin in only 75% of the milk samples in which penicillin was detected by the Delvotest-P method. The cylinder plate method detected penicillin in only 83% of the milk samples in which penicillin was detected by the Delvotest-P method. Also, the Delvotest-P method detected penicillin residues for a significantly longer ($P < .01$) time than either the disc assay or cylinder plate methods in milk from cows following intramuscular injections. Only once following intramuscular injection was penicillin residue found in milk beyond the manufacturer's recommended withholding time of four milkings, and that did not persist beyond the fifth milking. In milk from cows receiving intramammary infusions, the Delvotest-P method detected penicillin residues for a significantly ($P < .01$) longer period than the disc assay method, but not longer than the cylinder plate method. No antibiotic residue was ever detected by any of the three tests beyond the manufacturer's recommended milk withholding time of six milkings.

Several methods to detect antibiotics residues in milk have been developed over the years. Still no completely acceptable method is available which can easily be applied under industry conditions. A rapid, simple test which will detect antibiotic concentrations in milk within minutes is needed.

Although such a test is not available, much has been accomplished in antibiotic testing since the discovery of penicillin. Florey et al. (5) first described a cylinder plate method for the assay of penicillin. Their method underwent several modifications until Juncher et al. (7) suggested using *Sarcina lutea* as the test organism.

Subsequently, the Food and Drug Administration (FDA) adopted a modification of the cylinder plate method (4) as described by Schmidt and Moyer (13) as the official test to be used for penicillin assay in FDA laboratories.

A disc assay method was described by Foster and Woodruff in 1943 (6). Instead of metal cylinders, filter paper discs were used. Several researchers modified and evaluated this method and reported its reliability for detecting penicillin concentrations as low as 0.1 unit per ml (u/ml) (14). Arret and Kirshbaum (3) developed a rapid disc assay procedure (2.5 h) which detected 0.05 u/ml, and Marth et al. (9) provided further modification to detect a concentration of as little as 0.03 u/ml within 3-4 h.

Kosikowski and Ledford (8) had developed an agar diffusion or reverse phase disc assay test as early as 1960. In 1975, Van Os et al. (15) published an agar diffusion procedure using *Bacillus stearothermophilus* var. *calidolactis* as the sensitive organism. Tablets containing nutrients and a pH indicator (bromocresol purple) are added to ampules containing spores of the test organism which have been seeded in an agar growth medium. A milk sample is then added, and the ampule is incubated for 2.5 h in a water bath at 63-66 C. The nutrients, pH indicator, and antibiotic (if present in milk) diffuse into the agar medium. The color of the medium is purple because of bromocresol purple. If no antibiotic is present, *B. stearothermophilus* grows, lowering the pH of the medium, and causing the color of bromocresol purple to change to yellow in the agar medium. Antibiotics in concentrations sufficient to inhibit growth of the test organism cause the medium to remain purple indicating a positive antibiotic test.

The purpose of this study was to evaluate the sensitivities of the cylinder plate, disc assay, and agar diffusion test (Delvotest-P) for detecting penicillin in milk following intramammary infusion and intramuscular injection of penicillin into lactating cows.

¹Published with the approval of the Director, South Dakota Agricultural Experiment Station, as Publication No. 1492 of the Journal series.

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³Trade name for the agar diffusion test manufactured by Gist-Brocades nv, Postbus 1-Delft, The Netherlands. Distributed in USA by Enzyme Development Corp., 2 Penn Plaza, NY, NY 10001.

MATERIALS AND METHODS

Intramuscular and intramammary treatment

Six lactating Holstein dairy cows were treated alternately, by intramuscular injection and intramammary infusion, with a commercial antibiotic preparation. Treatment by intramuscular injection consisted of one 10-ml injection into the neck or shoulder muscle each day for five consecutive days. Treatment by intramammary infusion consisted of infusing the contents of one 25-ml disposable syringe into the left rear quarter each day for three consecutive days immediately after the morning milking. The treatment schedule was established so that each treatment would consist of treating each cow with the maximum dosage for the maximum number of consecutive treatments that were recommended by the manufacturer of the antibiotic preparation. Each of the six cows was treated twice with each type of treatment over a period of 12 consecutive weeks.

The formulation for the intramammary infusion contained the following ingredients per 25-ml disposable syringe: procaine penicillin G, 1×10^5 units; neomycin sulfate equivalent to neomycin base, 100 mg; sulfamethazine, 1500 mg; and 20 mg of hydrocortisone acetate in homogenized system with a specially prepared base containing 50 mg of chlorobutanol added as a preservative. Each 10-ml dose of the antibiotic preparation used for intramuscular injection consisted of 3×10^6 units of procaine penicillin G with 0.13% methylparaben and 0.02% propylparaben as preservatives, 5% lecithin, 1% sodium citrate, 0.5% polyvinylpyrrolidone, not more than 0.01% sodium formaldehyde sulfoxylate, and 0.075% sodium carboxymethylcellulose.

Milk samples from all four quarters of each cow were collected from each milking after the initial treatment in a treatment series. Samples were collected from all successive milkings until two consecutive samples did not show the presence of antibiotics by any of the assay procedures.

Assay procedures

Milk samples from cows treated by either intramuscular injection or intramammary infusion were tested for antibiotic residues using the *Bacillus subtilis* overnight disc assay method (1) and the *S. lutea* cylinder plate method (4). The last positive sample and succeeding negative milk samples tested by the cylinder plate method were also tested for antibiotic residues using the Delvotest-P method (15) until two succeeding samples were negative. All milk samples were tested within 48 h of being taken. Samples were stored at 4 C until analyzed.

To determine if the zones of inhibition resulted from penicillin, penicillinase-impregnated discs were placed near the disc or cylinder with the test sample (1). If the zone of inhibition around the test sample was decreased near the penicillinase-impregnated disc, penicillin was considered present. If no change was observed in the zone of inhibition around the test sample, the inhibitory substance was considered not to be penicillin. Once the inhibitory substance in milk samples from cows given intramammary or intramuscular treatments was confirmed to be penicillin, the practice of using the penicillinase-impregnated disc on all samples from cows given these two treatments was discontinued. Samples taken after the last intramammary infusion were always checked with penicillinase since the infusion products did contain antibiotics other than penicillin.

Disc assay method. The overnight disc assay procedure was applied as outlined in *Standard Methods for the Examination of Dairy Products* (1). Spore suspensions of *B. subtilis* (ATCC 6633) were obtained from Difco Laboratories. The 1-ml vials of the spore suspension were diluted 1:10 with potassium dihydrogen phosphate buffer, pH 7.2, (34 g of KH_2PO_4 in 500 ml of distilled water, adjusted to pH 7.2 with 1 N NaOH solution, and made up to 1 liter with distilled water) (1).

Plates were prepared by inoculating 1 ml of the 1:10 dilution of spore suspension into 100 ml of Antibiotic Medium No. 1 (Difco) at a temperature of 55 C. Six-ml portions of the seeded agar were then pipetted into 100-mm glass, flat bottomed petri dishes. After agar had solidified, plates were stored at 4 C until they were used. No plates were used immediately after preparation but were stored at least overnight at 4 C before using.

With clean, flamed, tweezers, the edge of a nonsterile 12.7 mm filter

paper disc (S&S No. 740-E) was touched into a well-mixed sample of milk. The milk was allowed to completely wet the disc. Excess milk was always removed by touching the disc to the side of the sample bottle. The disc was then immediately placed on the agar surface. Three to six discs could be placed on each petri dish depending upon the expected size of the zones of inhibition. After plating was completed, plates were inverted and incubated at 32 C for 12-14 h. After the incubation period, the diameters of the zones of inhibition were measured with a divider to the nearest 0.5 mm. Inhibition zones larger than 13 mm were considered as positive tests.

Sarcina lutea cylinder plate method. The *S. lutea* cylinder plate method was applied as outlined by Carter (4) with slight modifications. The test organism used throughout the analysis was derived from *S. lutea* (ATCC 9341) obtained from the Land O'Lakes Laboratory in Volga, South Dakota. The original culture was streaked on an agar slant of Antibiotic Medium No. 1 (Difco), and allowed to incubate for 24 h at 32 C. The growth was washed from the slant with sterile physiological saline (0.85% NaCl) and transferred to a small Roux bottle containing 12 ml of solidified Antibiotic Medium No. 1, and incubated for 24 h at 32 C. After incubation the growth was washed from the agar surface with 100 ml of sterile physiological saline. The entire suspension was poured into a sterile bottle and stored at 4 C for no longer than two weeks (4). This stock culture suspension served as the inoculum for the next stock culture prepared, instead of using the slant culture as suggested by Carter (4). New stock cultures were prepared every 10 days.

The contents of test tubes containing 12 ml of sterilized Antibiotic Medium No. 1 were poured into 100 × 15 mm, glass, flat-bottomed petri dishes. After the agar had solidified, petri dishes (with covers off) were dried in an incubator at 39 C for 50 min. Covers were replaced after drying. One hundred and fifty ml of sterilized Antibiotic Medium No. 4 (Difco) were inoculated with enough stock culture to obtain a concentration in the agar of about 7.5×10^5 organisms per ml. Four ml of the inoculated agar were then added to each plate, making sure that the agar was evenly distributed over the base layer of Antibiotic Medium No. 1. After the agar had solidified, petri dishes (with covers off) were dried in an incubator at 39 C for 25 min. Covers were replaced after drying and the plates were used immediately.

Five stainless steel cylinders were dropped in an upright position onto the agar surface of each plate from a height of about 4 mm with tweezers. The cylinders were not flamed before dropping them onto the plate. The outside diameter of the cylinders was 8 mm; the inside diameter was 6 mm; and the length was 10 mm. Only five cylinders were dropped on a plate to avoid overlapping zones. Pasteur pipettes were used to fill three cylinders with the test sample. The other two cylinders on opposite sides of the plate were filled with a standard concentration of penicillin. Plates were incubated with covers off in a 32 C incubator for 16-18 h. The atmosphere in the incubator was kept moist with a sponge set in a pan of water. The moist atmosphere kept the plates from drying excessively. After incubation, diameters of the zones of inhibition were measured to the nearest 0.5 mm with a divider. Inhibition zones larger than 8.5 mm were considered as positive tests.

Due to the large number of cylinders and pipettes required, they were reused. Both the cylinders and pipettes were first washed or rinsed thoroughly to remove noticeable milk residues and then were boiled in 15% nitric acid solution for at least 45 min. After boiling, pipettes and cylinders were rinsed several times with tap water and then with several rinsings of distilled water. After drying both were ready for use.

Agar diffusion method (Delvotest-P). Materials for the Delvotest-P procedure were obtained from Enzyme Development Corporation, 2 Penn Plaza, New York, NY 10001. The testing method followed was that indicated in instructions supplied with the test kit materials. With a disposable syringe, 0.1 ml of mixed milk sample was added to an ampule of clear, solid agar medium, seeded with *B. stearothermophilus* var. *calidolactis*. To each ampule was added one nutrient-indicator tablet which turned the agar purple. The ampules were placed in a suitably sized beaker with enough water added to the beaker to bring the level of the water at least 13 mm above the level of the agar surface. The top of the beaker was covered with aluminum foil. The ampules

thus prepared were incubated in a water bath at 64 C (\pm 2 C). As a control, an ampule containing antibiotic-free milk was always incubated with the ampules containing test samples. When the agar in the ampule containing the antibiotic-free milk turned yellow, indicating a negative test, incubation of all ampules was stopped. All samples that remained purple were considered positive. A doubtful test was indicated when the agar was neither totally yellow nor totally purple. The purple color had to be at least 4 mm below the agar surface for the test to be considered as doubtful. Incubation time was always between 2.5 and 2.75 h.

Preparation of standard curves. Sodium penicillin G was used as the standard for penicillin. A small quantity of the antibiotic was weighed and dissolved in 100 ml of 1% phosphate buffer, pH 6.0 \pm .1 (8.0 g of monobasic potassium phosphate, 2.0 g of dibasic potassium phosphate diluted to one liter with distilled water and adjusted to pH with 18 N phosphoric acid or 10 N potassium hydroxide) (2). A portion of the penicillin stock solution was diluted with 1% phosphate buffer to give a concentration of 10 u of penicillin/ml. Further dilutions were made in milk containing 2% milkfat. The 2% milk used for the dilutions was steamed for 2 h to caramelize the lactose and give the standard solutions a darker color than the samples that were tested. The darker color of the standard solutions made it easier to identify cylinders with the reference concentration in the cylinder plate test.

The various concentrations of the antibiotic standard solutions were tested by both the cylinder plate and disc assay methods using the same techniques as with the unknown samples. Zone diameters were measured and recorded as with the unknowns. The standard concentrations of penicillin used to establish the standard response line for the cylinder plate method were .2, .1, .05, .125, and 0.125 u/ml (13). The standard concentrations of penicillin used to establish the standard response line for the disc assay method were .4, .2, .1, .05, and 0.25 u/ml. A regression equation was calculated for each standard curve. These equations were used to determine the concentration of antibiotic in the unknown samples.

RESULTS AND DISCUSSION

The main objective of this study was to determine whether the time penicillin could be detected in milk from cows alternately treated via intramuscular injection and intramammary infusion would be increased with the use of the Delvotest-P method over the disc assay and cylinder plate methods of penicillin assay. The withholding time as defined in this study was the time from the last injection or infusion to the last recorded milk sample taken from any quarter of the treated animal in which penicillin was detected. Although penicillin, neomycin, and sulfamethazine were in the preparation used for intramammary infusion, only penicillin was detected in these experiments.

Results using each of the three antibiotics tests are given in Tables 1 and 2. The data from the Delvotest-P are divided into two categories; positive and doubtful. In the positive column, only definitely positive tests are considered in determining the withholding time (doubtful tests are considered as negative tests in this column). In the doubtful column, doubtful tests are considered as positive tests.

The data in Table 1 show that the Delvotest-P method detected penicillin residues after intramuscular injection for a longer time than either the disc assay or cylinder plate methods (33.8 h versus 19.8 and 25.0 h, respectively). The difference in length of time penicillin was

TABLE 1. Detection of penicillin in milk following intramuscular injection of 3,000,000 units of procaine penicillin G as determined by the disc assay, cylinder plate, and Delvotest-P methods

Penicillin detection	Type of antibiotic assay			
	Disc assay	Cylinder plate	Delvotest-P	
			Positive	Doubtful
Time, mean (h)	19.8 ^a	25.0 ^a	33.8 ^b	40.7 ^c
Time, range (h)	10-34	10-34	24-48	34-58
No. milkings ^d	1-3	1-3	2-4	3-5

abc ($P < .01$) Means with the same superscript are not significantly different.

^dManufacturer's recommended milk withholding time is 48 h (4 milkings).

detected was statistically significant ($P < .01$). No significant difference was determined when comparing the disc assay with the cylinder plate method. It is evident that penicillin was detected somewhat longer when considering doubtful tests as positive tests than when considering doubtful tests as negative tests.

The data in Table 1 also show that considerable variation occurred in the length of time penicillin could be detected with each of the three antibiotics tests. A more meaningful representation of the results is made by converting the range of time into the number of milkings it would take for complete removal of the penicillin from the milk. The manufacturer's recommended milk withholding time for the penicillin preparation used for intramuscular injection was 48 h or four milkings. If, when using the Delvotest-P, doubtful tests are considered as positive tests, the manufacturer's recommendation would be exceeded by one milking. Since doubtful tests sometimes result when using the Delvotest-P to detect antibiotics residues in milk, an interesting question is raised. Should doubtful tests be considered as positive or as negative tests? The answer will determine if the recommended milk withholding time will need to be changed.

Studies reported by Marth and Ellickson (10) indicated that a penicillin concentration of 0.002-0.004 u/ml would probably not inhibit the growth of lactic cultures. Doubtful tests occur with the Delvotest-P in the range of 0.002-0.004 u/ml of penicillin. Clearly, if a milk sample gave a doubtful test, the milk probably would not inhibit growth of a lactic culture. However, the minimum concentration of penicillin which can induce hypersensitivity to penicillin in humans is not known. The main reason milk is checked for penicillin residues is that some humans are hypersensitive to penicillin or could become hypersensitive to penicillin (11). Therefore, since penicillin residues in milk in low concentrations can cause doubtful Delvotest-P results, doubtful tests must be considered as positive tests from the standpoint of human hypersensitivity.

Before making a recommendation that the milk withholding time for the penicillin preparation used for intramuscular injection be increased, several factors must be considered. It must be noted that only milk from

one quarter of one cow gave doubtful Delvotest-P results at 58 h (five milkings). It is probable that if a composite milk sample from all four quarters was assayed with the Delvotest-P, no detectable penicillin residue would have been observed because of the dilution factor. However, dilution of milk that contains penicillin is illegal even though the assay of diluted milk might show no detectable penicillin residues (11). Milk from a single quarter would probably never be offered for sale, so the medical importance appears negligible.

The data in Table 2 show that the Delvotest-P method did detect penicillin residues after the last intramammary infusion for a longer time than did the disc

TABLE 2. Detection of penicillin in milk following intramammary infusion of 100,000 units of procaine penicillin G as determined by the disc assay, cylinder plate, and Delvotest-P methods

Penicillin detection	Type of antibiotic assay			
	Disc assay	Cylinder plate	Delvotest-P	
			Positive	Doubtful
Time, mean (h)	35.5 ^a	38.3 ^{ab}	42.7 ^b	43.8 ^b
Time, range (h)	24-58	24-58	34-72	34-72
No. milkings ^c	2-5	2-5	3-6	3-6

^a^b($P < .01$) Means with the same superscript are not significantly different.

^cManufacturer's recommended milk withholding time is 72 h (6 milkings).

assay method. No significant ($P < .01$) difference in antibiotic retention times was observed in comparing data obtained with the Delvotest-P with data from the cylinder plate method. Also, no significant ($P < .01$) difference in retention time was noted when comparing the positive and doubtful columns under the Delvotest-P method.

As with intramuscular injection, considerable variation occurred in the range for detection of penicillin administered by intramammary infusion. The manufacturer's recommended milk withholding time for the intramammary penicillin preparation was 72 h (six milkings). As can be seen in Table 2, the manufacturer's recommendation of six milkings was never exceeded when the milk from cows that had received intramammary infusion was checked with each of the three antibiotic tests.

A comparison of the relative sensitivity of the disc assay, cylinder plate, and Delvotest-P methods for detection of penicillin in raw milk is shown in Table 3. The total number of positive tests for the presence of penicillin detected by the Delvotest-P method was 588 (including doubtful tests considered as positive). The disc assay antibiotic test detected penicillin in 75% of the milk samples in which penicillin was detected by the Delvotest-P method. The cylinder plate method detected penicillin in 83% of the milk samples in which penicillin was detected by the Delvotest-P method. These results agree essentially with those reported by Packard et al. (12).

TABLE 3. Comparison of the relative sensitivity of the disc assay, cylinder plate, and Delvotest-P methods for detecting penicillin in raw milk

Type of treatment	Type of antibiotic assay			
	Disc assay	Cylinder plate	Delvotest-P	
			Positive	Doubtful
(Number of positive tests)				
Intramuscular injection	359	402	469	29
Intramammary infusion	82	85	89	1
TOTAL	441	487	558	30
Sensitivity as % of Delvotest-P	75	83	95	5

Results of the comparison of the sensitivity of the three antibiotic tests used in this study show that a larger number of positive identifications of penicillin-adulterated milk would be anticipated if the Delvotest-P method was used to assay milk samples instead of the disc assay or cylinder plate methods. In addition, an assay by the Delvotest-P method is simpler and faster to complete than either the disc assay or cylinder plate tests. The cylinder plate method is also more sensitive to penicillin residues in milk than the disc assay. However, for routine laboratory analysis of a large number of samples, the disc assay continues to be preferred over the cylinder plate method.

ACKNOWLEDGMENT

The authors express appreciation to Fenton Ludens, SDSU Dairy Research Unit Manager, for administering the antibiotic preparations and for assistance in collecting the milk samples.

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A Bacteriological Survey of Raw Ground Beef¹

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(Received for publication April 25, 1977)

ABSTRACT

The microbiological quality of 150 units of raw ground beef obtained from a local retail store was determined. The range of aerobic plate counts was from 6.9×10^4 to 8.3×10^7 /g. By using the most probable number method 96.7% of the 150 units were positive for coliforms, 94.7% for *Escherichia coli* and 61.3% for *Staphylococcus aureus*. By the plate methods, 99.3% of the units were positive for fecal streptococci and 56% were positive for *Clostridium perfringens*. No salmonellae were isolated. Aerobic and anaerobic organisms were isolated and identified. *E. coli* was the most frequently isolated aerobe followed by organisms in the *Klebsiella-Enterobacter* group. Among the anaerobic isolates, *C. perfringens* was the organism most frequently encountered.

The fresh meat industry is currently undergoing changes in processing, packaging, and distribution of raw ground beef. This change is the result of not only imposition of bacterial standards by some governmental agencies but also the desire by meat processors to increase the shelf-life of raw meat products. To accomplish these goals many retail food chains have centralized processing and distribution of fresh meat products which has resulted in little or no meat

processing at the retail level.

During processing bacterial contamination present on the meat surface is distributed throughout the final product. Therefore the bacterial flora present in ground beef is dependent upon the bacterial flora present on the meat and trimmings, sanitary conditions during processing, temperature, and storage time before sale. Rogers and McClesky (25) found that numbers of bacteria in market samples of ground meat are clearly indicative of the history of the product. The literature reveals that bacterial levels found in ground beef 63 years ago are similar to those found more recently (Table 1). This would indicate that the bacterial quality of ground beef has changed little in the past six to seven decades.

It has been determined that approximately 50% of beef consumed in the US is in the form of ground beef or hamburger and much of this is consumed in a partially cooked state (6). A recent review (28) of epidemiological data pertaining to ground beef indicated that this product was involved in 3.6% of the identified foodborne

TABLE 1. Aerobic Plate Count means, ranges and incubation time and temperatures of various ground beef surveys

Reported by	Year reported	Number of samples	Incubation C/h ^a	Mean	Range
Weinzirl and Newton (30)	1914	44	—	1.7×10^7	$2.6 \times 10^5 - 8.8 \times 10^7$
Geer (14)	1933	10	25/72	1.1×10^7	$3.6 \times 10^6 - 2.2 \times 10^7$
Elford (10)	1936	41	37/48	3.1×10^6	$1.0 \times 10^5 - 2.0 \times 10^7$
Kirsch et al. (18)	1952	20	30/72	1.5×10^7	$1.4 \times 10^6 - 9.5 \times 10^7$
Rogers and McClesky (25)	1957	96	37/48	3.2×10^7	$1.6 \times 10^6 - 2.3 \times 10^8$
Duitschaever et al. (8)	1973	87 ^b	32/48	7.7×10^7	$2.0 \times 10^6 - 7.4 \times 10^8$
Duitschaever et al. (8)	1973	13 ^c	32/48	9.7×10^7	$7.0 \times 10^5 - 2.7 \times 10^8$
Al-delaimey and Stiles (1)	1975	61	32/48	3.8×10^6	$6.0 \times 10^4 - 1.4 \times 10^8$
Westhoff and Feldstein (29)	1976	140	35/48	1.9×10^6	$1.5 \times 10^4 - 1.9 \times 10^8$
This study	1977	150	32/72	6.4×10^6	$6.9 \times 10^4 - 8.3 \times 10^7$

^aIncubation temperature/time.

^bHamburger packaged.

^cHamburger bulk.

¹The opinions or assertions contained herein are the private views of the authors and are not to be construed as official or as reflecting the views of the Department of the Army or the Department of Defense.

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outbreaks from 1967 to 1973. Ground beef has also been implicated in outbreaks of toxoplasmosis and salmonellosis resulting from consumption of raw or grossly undercooked products (12,21). This report presents the results of the analyses of 150 units of ground beef

obtained at the retail level.

MATERIALS AND METHODS

A total of 150 units of ground beef, approximately 1 lb (5 kg) each, were tested over an 11-month period. Three to five units were purchased weekly from a local retail establishment. The units were maintained in the chilled state (4 C) before testing. Analyses were made within 48 h after the ground beef was received in the laboratory.

Sample preparation

A 25-g sample of each unit was weighed into a sterile 1-liter blender. Following addition of 225 ml of sterile phosphate buffered water, the sample was blended at high speed for 2 min. Serial dilutions from 10^{-2} to 10^{-6} were prepared.

Analyses

Aerobic plate count (APC). Duplicate plates for dilutions 10^{-1} through 10^{-7} were prepared and poured in accordance with *Recommended Methods for the Microbiological Examination of Foods* (2). Plates were incubated at 32 C for 48 ± 2 h.

Total coliform and *Escherichia coli* analyses. Total coliform and *E. coli* Most Probable Number (MPN) determinations were made by using the techniques described in the *Bacteriological Analytical Manual for Foods (BAM)* (3).

***Staphylococcus aureus* analyses.** *S. aureus* MPN determinations were made in accordance with the AOAC method (4) except that tellurite polymyxin egg yolk (TPEY) agar was substituted for Vogel Johnson agar. The tube coagulase test (4) was done as needed on isolations from the TPEY agar plates.

***Clostridium perfringens* analyses.** The presence of *C. perfringens* was determined by use of sulfite polymyxin sulfadiazine (SPS) agar and the nitrite-motility reaction. Duplicate pour plates, inoculated with 1 ml each of the original food homogenate were prepared and incubated anaerobically at 35 C for 24 ± 2 h. Black colonies on SPS agar were counted as presumptive *C. perfringens*. Representative colonies were transferred into indole-nitrite medium and incubated at 35 C. After 24 ± 2 h of incubation, tubes showing non-motile and nitrate positive reactions were reported as *C. perfringens*.

Fecal streptococcus analyses. The fecal streptococcus analyses were done in accordance with the procedures outlined in *BAM* (3). In addition, representative colonies from KF streptococcal agar plates were inoculated into ethyl violet azide broth. After incubation at 35 C for 48 ± 2 h, tubes exhibiting a yellow color and sediment were reported as confirmed fecal streptococci.

Aerobic gram-positive isolation procedure. Tubes containing 10 ml of double and single strength trypticase soy broth (TSB) were inoculated with 10 ml and 1 ml, respectively, of the original food homogenates. All TSB tubes contained 4.0 mg of potassium tellurite/ml to inhibit gram-negative organisms. After incubation for 24 ± 2 h at 35 C, each tube was streaked to a blood agar plate for colony isolation. Plates were incubated overnight at 37 C and representative colony types were subcultured to blood agar. Cultures were gram stained to assure purity and identified according to procedures outline by Oetjen and Harris (23).

Aerobic gram-negative isolation procedure. Gram-negative organisms were isolated from the original sample by inoculating 10 ml of the food homogenate into 10 ml of double strength GN broth. After 24 ± 2 h of incubation at 37 C, these tubes were streaked to Hektoen, Brilliant Green Sulfadiazine, and McConkey's agars for isolation. After re-streaking and gram staining representative colonies to assure purity, each isolate was identified biochemically following procedures described by Edwards and Ewing (9) and Oetjen and Harris (23).

Anaerobic isolation procedure. Approximately 10-g samples of each unit were combined with an equal amount of diluent (wt/vol) and then homogenized with a sterile mortar and pestle. One to 2 ml of this homogenate were subsequently added to 10 ml of fluid thioglycollate broth containing 0.5 ml of rabbit serum and to 3 tubes, each containing 10 ml of cooked meat medium (CMM). Two of the 3 CMM tubes were subsequently heated at 100 C for 5 min to destroy any vegetative

organisms. The third tube of CMM was used to cultivate nonspore forming anaerobes. All tubes were incubated anaerobically at 35 C. After 48 h of incubation, each tube was streaked to blood and McLung-Toabe egg yolk agar plates and incubated anaerobically for 48 h at 35 C. Colonies were then subcultured to duplicate blood agar plates, one plate was incubated aerobically and the other anaerobically at 35 C for 48 h. Cultures which grew aerobically were discarded. All remaining isolates were gram stained and identified by procedures outlined in *Laboratory Methods in Anaerobic Bacteriology* (8).

RESULTS AND DISCUSSION

The APC distributions are presented in Table 2. Individual APC determinations ranged from 6.9×10^4 to 8.3×10^7 organisms per gram with a mean of 6.4×10^6 organisms per gram. By comparing the data obtained in this study to data reported in the literature since 1913 (Table 1), one can make some interesting observations. Any comparison of APC data must include a consideration of incubation time and temperature. There were six different incubation time/temperature combinations used in these analyses; these different combinations could easily account for the variations in APC means. Goepfert (16) reported a 0.98 average \log_{10} difference in APC from the same samples by comparing APCs for raw ground beef incubated at 35 C for 48 h to APCs at 20 C for 72 h. These findings would suggest that the bacterial quality of raw ground beef has not changed appreciably in the past 63 years.

The prevalence of wider and lower ranges reported in 1975 as compared to the 1973 study where incubation time/temperature are the same would suggest that raw ground beef with reduced bacterial loads can be produced (Table 1). Emswiler et al. (11) reported that 94% of the raw ground beef samples obtained at the time of manufacture had APCs of 10^5 or less. None of their samples exceeded the Oregon microbiological standard for ground beef of 5×10^6 organisms per gram. If realistic microbiological standards for raw ground beef are to be established they must be founded upon an extensive data base. This data base must be obtained by using consistent analytical procedures (i.e. incubation time/temperature) and from samples exposed to similar post-processing conditions (i.e. storage time/temperature).

Coliform MPN distributions (Table 2) show individual determinations ranged from < 3 to 1.1×10^5 organisms per gram, with an overall mean count of 4.9×10^3 per gram. These data are similar to those reported by Al-delaimy and Stiles (1). Currently, five states have microbial guidelines based upon coliform counts (20). A recent study evaluating 119 samples of raw ground beef indicated that coliform counts were not totally effective for predicting the presence or absence of pathogenic organisms. This study showed that in samples with coliform counts $< 100/g$, *Salmonella*, *C. perfringens*, and *S. aureus* were found in 2, 4, and 36% of the samples, respectively. Likewise, in samples with coliform counts $> 100/g$, *Salmonella*, *C. perfringens*, and *S. aureus* were identified in 9, 23, and 56% of the samples. However, a

correlation between the presence of coliforms and the degree of sanitation in processing facilities has been reported (25, 27).

The distributions for the *E. coli* MPN estimates, given in Table 2, show individual counts ranging from < 3 to 4.6×10^4 organisms per gram with a mean of 1.9×10^3 per gram. Although the *E. coli* test has long been used as an indicator of fecal contamination, presence of *E. coli* does not mean that there are feces in the product. *E. coli* is an organism which is normally found in the intestinal tract of man and other vertebrates; however, it is an organism which is widely distributed in nature. Attempts

the mean *C. perfringens* count was calculated to be 55 organisms per gram. Isolation of *C. perfringens* from 56% of the samples is not unexpected when the ubiquitous nature of this organism is considered. Many of these organisms probably originate from carcass contamination at the time of slaughter. However, contamination of the product could have occurred at any one of the several processing stages from slaughter to final grinding and packaging. The likelihood of proliferation of this organism in refrigerated raw ground meat is minimal (15,20). However, if this product is left unrefrigerated or at an elevated temperature for

TABLE 2. Microbiological analyses of 150 units of raw ground beef

Count range	Aerobic plate Counts X 10^5 /g		Coliform MPN counts X 10^1 /g		<i>E. coli</i> MPN counts/g		<i>S. aureus</i> MPN counts/g		Fecal strep. plate counts X 10^1 /g		<i>C. perfringens</i> plate counts/g	
	Units	CF ^a	Units	CF	Units	CF	Units	CF	Units	CF	Units	CF
<3	18	12.0	24	16.0	8	5.3	59	39.3	8	5.3	66	44.0
3-10	40	38.7	15	26.0	16	16.0	41	66.7	20	18.7	40	70.7
11-20	19	51.4	6	30.0	6	20.0	6	70.7	16	29.3	10	77.3
21-30	9	57.4	16	40.7	5	23.3	9	76.7	11	36.7	7	82.0
31-40	18	69.4	0	—	4	26.0	0	—	9	42.7	4	84.7
41-50	10	76.0	15	50.7	10	32.7	2	78.0	7	47.3	2	86.0
51-60	7	80.8	0	—	0	—	0	—	8	52.7	0	—
61-70	3	82.8	0	—	0	—	1	78.7	9	58.7	0	—
71-80	4	85.4	2	52.0	1	33.3	7	83.3	9	64.7	0	—
81-90	4	88.0	0	—	1	34.0	0	—	5	68.0	0	—
91-100	3	90.0	12	60.0	11	41.3	5	86.7	5	71.3	0	—
>100	15	100.0	60	100.0	88	100.0	20	100.0	43	100.0	21	100.0
% Positive	—		96.7		94.7		61.3		99.3		56.0	

^aPercent Cumulative Frequency.

to correlate presence of *E. coli* to presence of pathogenic organisms in raw meat products have resulted in minimal success (16,29). Miskimin et al. (22) found that the *E. coli* count was suitable as an indicator of the microbiological quality of foods, but to assure safety of a food product, specific pathogen testing is necessary.

The *S. aureus* MPN values (Table 2) ranged from < 3 to 1.1×10^3 organisms per gram with a resulting mean of 5.7×10^1 per gram and are similar to those reported by Pivnick et al. (24) and Surkiewicz et al. (26). Although *S. aureus* is recognized as a potential food poisoning organism, its ability to compete with the microbial flora of refrigerated raw ground beef is questionable (1,15). There have been no reported food poisoning cases where *S. aureus* was implicated from ground beef. However, if salt were added and the product inadequately refrigerated, the potential would exist for rapid cell replication of enterotoxin producing *S. aureus*. Such a product would remain a potential hazard even after cooking, since some *S. aureus* enterotoxins have been characterized as heat stable (5).

In the analyses for fecal streptococci (Table 2) individual determinations ranged from < 1 to 2.1×10^4 organisms per gram. The mean fecal streptococci count was 1.8×10^3 per gram. The concentrations of enterococci are similar to those previously reported by Duitschaever et al. (8) and Goepfert and Kim (15).

The *C. perfringens* plate count distributions (Table 2) ranged from < 1 to 2.7×10^3 organisms per gram, and

an extended time, *C. perfringens* can multiply to a level capable of causing food poisoning (13). In fact, a recent report (28) stated that in two cases of foodborne disease due to ground beef, *C. perfringens* was identified as the etiologic agent.

The analysis of samples in this study yielded no *Salmonella* isolates, which is in agreement with the results presented in several other reports (8,19,28,29). *Salmonella* is unable to proliferate in raw ground beef maintained under proper refrigeration (15). The fact that no salmonellae were found could be due to their inability to compete at 4 C or ideally to their absence. However, the possibility of *Salmonella* contamination in ground beef exists. If *Salmonella* is present, improper cooking, handling, or storage could result in a hazardous product. This is exemplified by the fact that *Salmonella* was implicated in four of the nine outbreaks of foodborne disease from ground beef where the etiological agent was determined (28).

The results of aerobic and anaerobic analyses are found in Table 3. *E. coli* was the most frequently isolated aerobe followed by organisms in the *Klebsiella-Enterobacter* group. Among the anaerobic isolates, *C. perfringens* was the organism most frequently encountered.

Some individuals support the need for microbiological standards which require the producer to supply a product with a lower bacterial content (17,24). Others favor health education of the homemaker and the

TABLE 3. Bacterial Isolates from Raw Ground Beef

	Number of isolations	Percent of samples positive
AEROBIC ORGANISMS		
<i>Gram-negative isolates</i>		
<i>Arizona</i> sp.	1	0.6
<i>Citrobacter</i> sp.	57	38.0
<i>Edwardsiella tarda</i>	2	1.4
<i>Escherichia coli</i>	78	52.0
<i>Klebsiella-Enterobacter</i> group	75	50.0
<i>Proteus</i> sp.	15	9.0
<i>Providencia</i> sp.	2	1.4
<i>Pseudomonas</i> sp.	3	2.0
<i>Serratia</i> sp.	1	0.6
<i>Gram-positive isolates</i>		
<i>Bacillus cereus</i>	2	1.4
<i>Staphylococcus aureus</i>	32	21.0
<i>Streptococcus</i> sp.	3	2.0
ANAEROBIC ORGANISMS		
<i>Bacteroides</i>	4	2.7
<i>C. bif fermentans</i>	3	2.0
<i>C. butyricum</i>	2	1.4
<i>C. capitovale</i>	2	1.4
<i>C. cochlearium</i>	8	5.3
<i>C. chauvoei</i>	2	1.4
<i>C. difficile</i>	6	4.0
<i>C. fallax</i>	1	0.6
<i>C. perfringens</i>	14	9.3
<i>C. septicum</i>	2	1.4
<i>C. sordellii</i>	2	1.4
<i>C. sphenoides</i>	1	0.6
<i>C. tetanomorphum</i>	2	1.4
<i>Peptococcus</i>	4	2.7
<i>Peptostreptococcus</i>	8	5.3
<i>Veillonella alcalescens</i>	1	0.6

foodservice industry in conjunction with active foodborne disease surveillance and investigation to protect the consumer rather than the imposition of microbial standards, especially for low risk food items such as raw ground beef (28).

This study has shown that raw ground beef is a product with large and varied microbial flora which frequently include a number of potentially pathogenic organisms. To improve the bacterial quality and safety of raw ground beef, measures must be taken to reduce the microbial load. Microbiological limits founded upon an extensive data base, which has been derived from standardized testing procedures, could result in a product of reduced microbial content, longer shelf-life and reduced potential for transmission of pathogenic organisms. Health education at all levels, coupled with well conceived microbial limits, could eventually benefit both consumer and producer.

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Microtiter Technique for Enumeration of Mesophiles, Psychrotrophs, and Coliforms in Raw and Pasteurized Milk

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(Received for publication April 11, 1977)

ABSTRACT

The Microtiter Count Method was compared with the Standard Plate Count (SPC) method in evaluating mesophile, psychrotroph, and coliform counts for raw and pasteurized milk samples. Statistical analysis showed that the Microtiter Count Method was reliable when compared with the SPC for making viable cell counts on these products. The Microtiter Count Method is advantageous because it saves time, space, and material; this method should be useful for developing countries where availability of testing materials, manpower, and costs are limiting factors in surveillance of microbial quality.

Accurate and efficient techniques for enumeration of microorganisms in foods are urgently needed in developing countries where the amount of work required exceeds the economic support designated for these essential public health, quality control, and sanitation programs. In this regard, a microtechnique was described by Fung and co-workers (2,4,5) which seems to have potential in increasing work load efficiency for determining viable cell counts while at the same time decreasing the cost per test. The basic procedure of Fung and co-workers was tested in a collaborative study which showed that the Microtiter Count Method was reliable when compared to the Standard Plate Count method in making viable cell counts on raw milk (3). The purpose of this report is to ascertain the accuracy of the Microtiter Count Method as compared to the Standard Plate Count method in enumeration of mesophiles, psychrotrophs, and coliforms in raw and pasteurized milk.

MATERIALS AND METHODS

Samples

Forty-five raw milk samples from the cooling tanks of 15 dairy farms as well as 77 samples of pasteurized milk with 0 to 3 days of storage from six local plants were collected. These samples were tested without further treatment.

Evaluation procedure

Standard Plate Counts were made using the procedure described in *Standard Methods for the Examination of Dairy Products* (1). The

Microtiter Count Method followed procedures described previously (4). All equipment used in the Microtiter Count Method was obtained from Dynatech Laboratory Inc., Alexandria Va. A 0.025-ml sample was withdrawn by use of a 0.025-ml size microdilutor and placed into the first well of the sterile Microtiter plate (8 × 12 wells) which was previously charged with 0.225 ml of sterile buffer. The loops were rotated rapidly in the diluent 30 times before being introduced into the next well. The procedure was repeated until a suitable dilution was reached, usually no more than eight wells in the same series. Each transfer was a 10× dilution. After dilution, two separate 0.05-ml aliquots were deposited on the surface of a suitable agar [Tryptone-Glucose-Yeast extract-Agar (TGY agar) for mesophiles and psychrotrophs and Violet-Red-Bile-Agar (VRB) or Desoxycholate-Lactose-Agar (DLA) for coliforms] and immediately overlaid with 3 to 4 drops of the same melted medium; all media were purchased from E. Merck, Darmstadt, Germany. After the overlay medium had hardened, plates were incubated at 32 C for the mesophiles and coliforms, counting the colonies at 18, 24, 36, and 48 h; and at 7 C for the psychrotrophs, in this instance enumeration was done at 7, 8, 9, and 10 days of incubation.

RESULTS AND DISCUSSION

The accuracy of the Microtiter count as compared to the SPC method is presented in Fig. 1 and 2 for raw and pasteurized milk, respectively. The statistical analysis shows a linear regression and correlation between the two methods. The values found for the coefficients are: $b = 0.935$, $r = 0.882$ ($n = 45$) for the mesophilic count; $b = 0.895$, $r = 0.816$ ($n = 43$) for the psychrotrophic count; $b = 0.974$, $r = 0.876$ ($n = 45$) for coliforms, in raw milk samples; $b = 0.977$, $r = 0.923$ ($n = 77$) for the mesophilic count; $b = 0.923$, $r = 0.938$ ($n = 64$) for the psychrotrophic count; and $b = 0.991$, $r = 0.965$ ($n = 25$) for coliforms in pasteurized milk. The significance of these results are found to be at the 1% level (6).

In the original publication of Fung and Kraft (4) no agar overlay procedure was described after depositing diluted samples on solidified agar. We found, however, that (a) the overlaying of the drop of diluted sample is necessary because it takes too long (about 1 h or more) for the agar to absorb the diluted sample and an agar overlay stabilized the sample quickly; (b) because of humidity wetting the colonies, plates incubated at 7 C

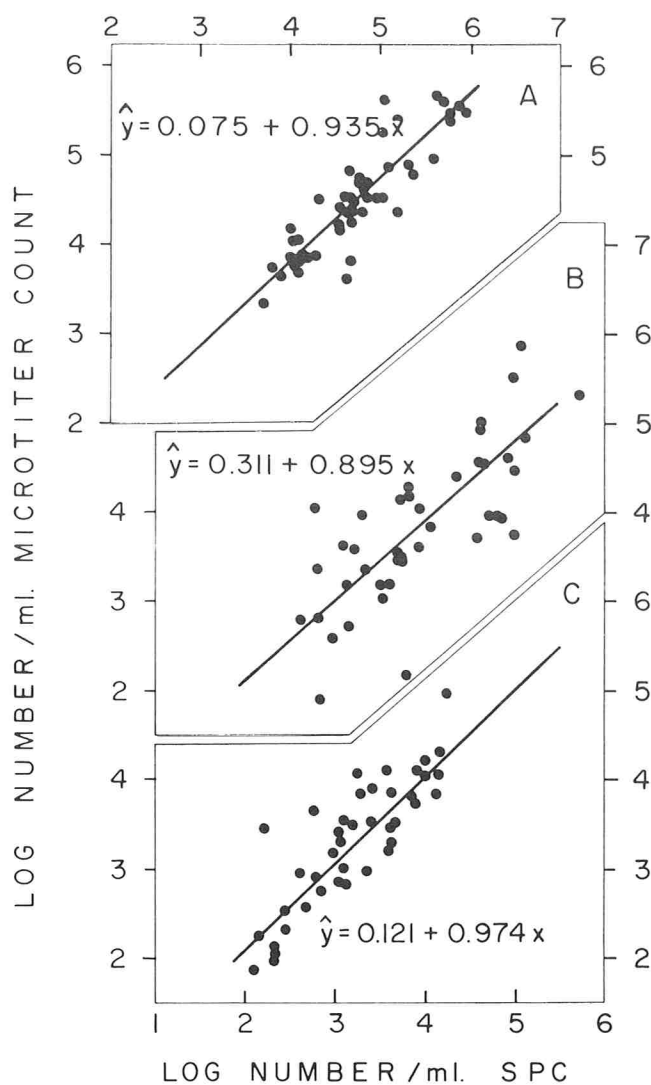


Figure 1. Regression line of microtiter count vs. the SPC in raw milk. (A) *Mesophiles*, (B) *psychrotrophs*, (C) *coliforms*.

spread out easily and lost their definition, with the result that enumeration of colonies became difficult and inaccurate; and (c) when VRBA was used it was noticed that the drop of diluted sample spread out rather quickly on the surface of the agar due to surface active agents. Although sodium desoxycholate is a constituent of DLA, spreading of samples was found to be less rapid. After some practice it is possible to overlay the drop of the melted agar on top of the sample with no inconvenience.

With the Microtiter Count Method, enumeration of the colonies at different time intervals showed that this could be done at 18-24 h for mesophiles and coliforms and as early as 7 days for psychrotrophs. Fung and LaGrange (5) have reported 15 to 20 h as a convenient incubation time for the mesophilic count.

In conclusion, we suggest that the Microtiter Count Method with our modifications may be used for enumeration of mesophiles, psychrotrophs, and coliforms in raw and pasteurized milk samples. The benefits of the Microtiter method can be summarized as follows:

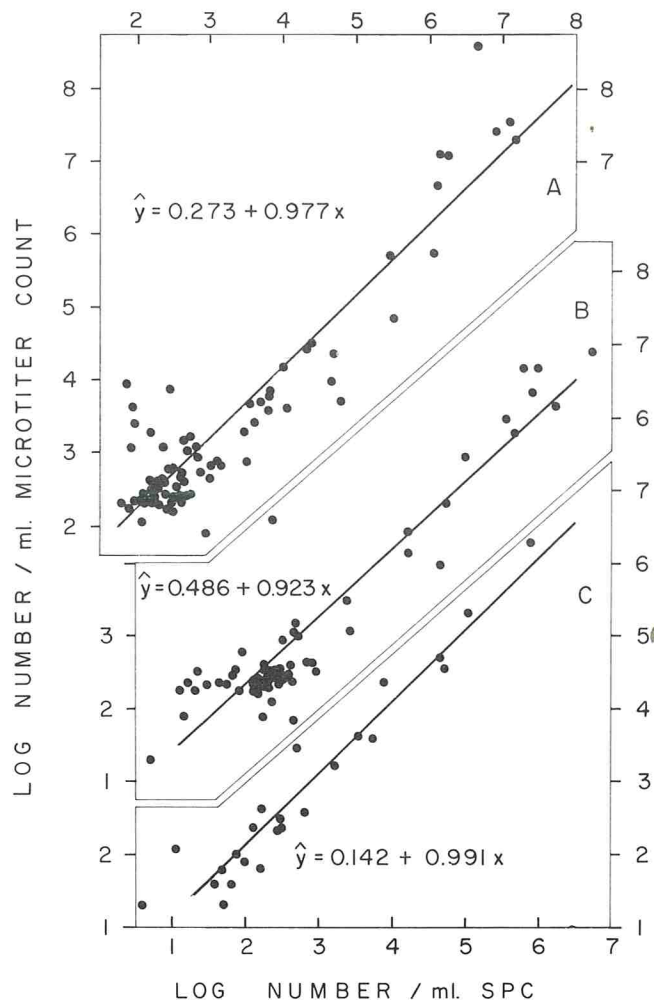


Figure 2. Regression line of the microtiter count vs. the SPC in pasteurized milk. (A) *Mesophiles*, (B) *psychrotrophs*, (C) *coliforms*.

its accuracy is comparable to the SPC, less time is required for processing of a given number of samples, and the amount of media and incubation space needed are greatly decreased.

ACKNOWLEDGMENTS

This investigation was carried out with the support of the Consejo de Desarrollo Científico y Humanístico and the Facultad de Ciencias Veterinarias, Universidad del Zulia, Maracaibo, Venezuela

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3. FREQUENCY OF ISSUE Monthly	A. NO. OF ISSUES PUBLISHED ANNUALLY 12	B. ANNUAL SUBSCRIPTION PRICES \$32.00 per 1977 \$25.00 agy sub. \$16.00 Membership \$14.00 Affil. Mem.
4. LOCATION OF KNOWN OFFICE OF PUBLICATION (Street, City, County, State and ZIP Code) (Not printers) 413 Kellogg, Ames, Iowa 50010 Story County		
5. LOCATION OF THE HEADQUARTERS OR GENERAL BUSINESS OFFICES OF THE PUBLISHERS (Not printers) 413 Kellogg, Ames, Iowa 50010 Story County		
6. NAMES AND COMPLETE ADDRESSES OF PUBLISHER, EDITOR, AND MANAGING EDITOR		
PUBLISHER (Name and Address) International Association of Milk, Food & Environmental Sanitarians, Inc.		
EDITOR (Name and Address) 413 Kellogg, Ames, Ia. 50010 (Box 701)		
Dr. Elmer H. Morth, Editor, Babcock Hall, Univ. of Wisconsin 53706		
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Trends in Foodborne Salmonellosis Outbreaks: 1963-1975

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ABSTRACT

Since 1963 651 outbreaks of human salmonellosis have been reported to the Center for Disease Control. The vehicle of transmission was identified in 463 (71%) of the reported outbreaks. The three most important vehicles have been poultry, meat, and eggs, which accounted for 99 (21%), 69 (15%), and 53 (11%) of the outbreaks respectively. Egg-associated outbreaks decreased markedly during the study period while meat-associated outbreaks (particularly beef) gradually increased and poultry-associated outbreaks showed no constant trend.

In 1963 the *Salmonella* Surveillance Program was established jointly by the Center for Disease Control and the Association of State and Territorial Epidemiologists and Laboratory Directors. The program participates in the investigation of state, local, and interstate outbreaks, solicits and receives reports on *Salmonella* outbreaks throughout the United States, and periodically summarizes reported data. Before 1960 some of the foodborne *Salmonella* outbreaks investigated by state and the local health departments were reported to the National Office of Vital Statistics and summarized annually in Public Health Reports. Reporting was evidently less complete before the new system was instituted because of the median number of outbreaks and the median number of cases reported per year more than doubled under the new system. Thus, it is difficult to determine the trends in salmonellosis before 1963. This study compares the vehicles of transmission before and since the new reporting system was instituted in 1963 and documents changes that have occurred in the relative importance of the major vehicles of transmission implicated in reported salmonellosis outbreaks since 1963.

SURVEILLANCE EMPHASIZED

The new emphasis placed on *Salmonella* surveillance after 1962 was accompanied by a marked increase in the number of commercial food items incriminated as vehicles of transmission — approximately twice as many

items were incriminated in 1963-1975 as in the previous 13 years (Table 1). Since 1963 the three most important vehicles identified were poultry, meat (beef, pork), and eggs. There were 651 reported outbreaks involving 38,811 cases in 1963-1975, and the vehicle of transmission was identified in 463 (71%) reported outbreaks; of these, poultry accounted for 99 (21%), meat for 69 (15%), and eggs for 53 (11%). Together, the three major vehicles caused approximately half of the outbreaks of known cause, with little sustained change in their combined importance over the 13-year period (Fig.

TABLE 1. *Non-typhoid salmonella outbreaks related to commercial products available in USA (1,2,3,9)*

1951-1962	1963-1975
Egg products	Egg products
Cake mixes	Dairy products
Cream-filled cookies	Certified raw milk
Dairy products	Non-fat dry milk
Certified raw milk	Ice cream
Non-fat dry milk	Synthetic ice cream
Soybean milk substitute	Cheddar and cup cheeses
Poultry	Poultry
Pre-packaged chicken salad	Turkey rolls
Fish	Pre-cooked barbecued chickens
Smoked fish	Meat (beef, pork)
Pets	Hamburger
Easter chicks	Pre-cooked, packaged roasts of beef
Misc.	Prepared beef sandwiches
Yeast	Sausage
Gelatin	Beef jerky
Pickles	Fish
Hollandaise sauce	Whitefish chubs
	Chocolate products
	Cocoa mix
	Chocolate candy
	Diagnostic agents
	Carmin dye
	Food supplements
	Pets
	Turtles
	Easter chicks and ducklings
	Misc.
	Yeast
	Gelatin
	Egg noodles
	Apple cider

¹Send request for reprints to Dr. Cohen.

1). However, their relative importance has changed appreciably (Fig. 2).

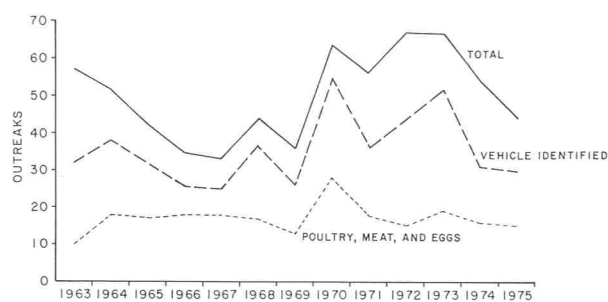


Figure 1. *Salmonellosis* outbreaks reported annually, by number with an identified vehicle and number caused by poultry, meat, and eggs, United States, 1963-1975.

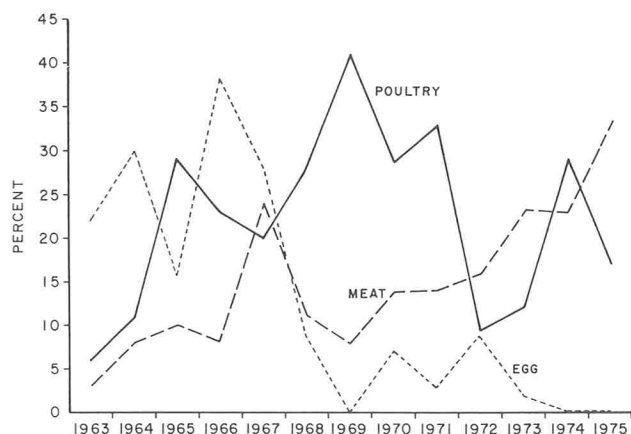


Figure 2. Percentage of salmonellosis outbreaks (outbreaks caused by unidentified vehicle are eliminated) caused by poultry, meat, or eggs, United States, 1963-1975.

The proportion of reported outbreaks associated with poultry showed no consistent trend over the last 13 years. A decrease in the early 1970s was followed by an increase in 1974. Fifty-eight (70%) of the 84 poultry-associated outbreaks reported since 1966 were attributed to turkey products, and this proportion did not change substantially during the decade.

The percentage of outbreaks caused by eggs has decreased markedly since 1966, and in 1974 and 1975 there were no outbreaks attributed to eggs. The available data for 1963-1968 are insufficient to determine if shell

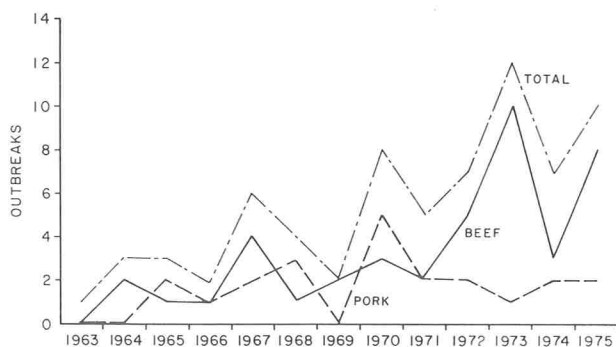


Figure 3. Meat-associated salmonellosis outbreaks, United States, 1963-1975.

or bulk eggs caused the 43 egg-related outbreaks; of the 10 egg-related outbreaks since 1968, nine appear to have been caused by shell eggs. The available data are insufficient to determine if these nine outbreaks were caused by graded or ungraded shell eggs.

Outbreaks associated with meat have increased in relative importance since 1969; there has been little change in the annual number of outbreaks associated with pork, the second most important meat vehicle, and the increase is almost solely attributable to a rise in the proportion of outbreaks caused by beef products (Fig. 3). Roast beef and, since 1974, hamburger have caused most of the beef-related outbreaks (Fig. 4).

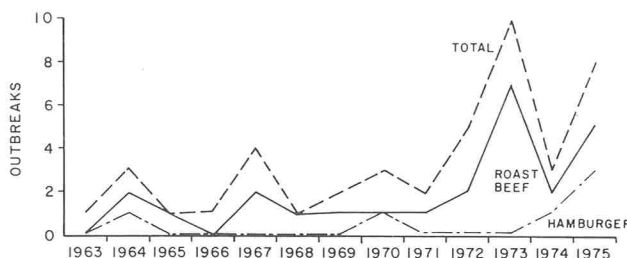


Figure 4. Beef-associated salmonellosis outbreaks, United States, 1963-1975.

INTERPRETING THE DATA

Several factors must be considered in interpreting the preceding data. First, not all *Salmonella* outbreaks are detected, investigated, and reported. Second, while poultry, meat, and eggs were the predominant vehicles of transmission, they may have been contaminated by other foods and not necessarily have been the vehicles that introduced the organism into the kitchen or factory. However, it seems likely that these three items in fact have been the source of contamination of other implicated food items. Third, the investigators may have been biased by their knowledge about which of these vehicles were most commonly incriminated. This bias should exaggerate the incrimination of the vehicles that had been most important in the past such as eggs, and minimize incrimination of vehicles that were relatively unimportant, such as beef; thus, the observed increases in uncommon vehicles and decreases in common vehicles occurred despite this presumed investigator bias and take on added importance.

The major decrease in egg-associated outbreaks over the last 10 years may reflect the public health measures that have been taken during the decade. In the 13 years prior to *Salmonella* surveillance only four of the 266 reported outbreaks were felt to be egg-associated. However, a large interstate outbreak of *Salmonella derby* in 1963 (8) stimulated awareness of an association between eggs and salmonellosis. Public concern and investigations of other bulk egg-associated *Salmonella* outbreaks in the mid-1960s resulted in a series of regulations in 1965 that made pasteurization of bulk eggs mandatory. The 1965 regulations applied to only 80% of the bulk egg production (conversation with Anderson GR, Egg

Production Section, USDA, December 1976) and did not regulate the shell egg industry, and there was no immediate drop in the number of outbreaks attributed to eggs. The gradual decline in the late 1960s of egg-associated outbreaks may have resulted from consumer education and voluntary improvements by egg producers and processors. The Egg Products Inspection Act of December 29, 1970, placed all egg products, bulk as well as shell, under supervision of USDA. The second part of this act, which became effective in July 1972 eliminated human consumption of high-risk shell eggs. Since 1973 there has been only one reported egg-related *Salmonella* outbreak.

The USDA and consumer organizations have made extensive efforts to warn consumers that since raw poultry may be contaminated with *Salmonella*, they should cook it thoroughly and avoid recontaminating it after cooking. Despite this, poultry continues to be an important vehicle of outbreaks of salmonellosis. This suggests there is continued *Salmonella* contamination of raw poultry and that food-handling errors continue to occur.

The causes of the increase in beef-related outbreaks are not clear. Increases in the incidence of salmonellosis have been attributed to such factors as mass production, mass distribution, and *Salmonella* in animal feeds (4), and these factors may have contributed to the increase in meat-associated salmonellosis. The handling of meat may also contribute to this increase. Meat is often eaten after being cooked at temperatures that are not lethal for

salmonellae. Several recent salmonellosis outbreaks have been attributed to the consumption of raw, contaminated hamburger (5) and pre-cooked roasts of beef served in delicatessens and sandwich shops (6,7).

The problem of salmonellae in eggs was approached by hygienic processing, pasteurization, and quality control, but the solution to the problem of salmonellae in poultry and meat products is not as simple. Further studies are necessary to identify the contributions of *Salmonella* in feeds, production hygiene, and food handling to meat and poultry-associated salmonellosis.

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Exotic Fermented Dairy Foods

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(Received for publication April 29, 1977)

ABSTRACT

Cultured dairy products are on the ascendency in the United States. The yogurt market has grown almost 600% over the last decade. There is also renewed interest in products containing *Lactobacillus acidophilus*. For innovative development to be competitive in the U.S. food market, the cultured dairy products industry has to look to exotic fermented dairy products which could be adapted for the American palate. Three such products are discussed, which with slight modifications and imaginative flavoring may have a very good potential in the American market.

Compared to peoples of Eastern Europe, parts of Western Europe, the Middle East and Asia, inhabitants of the North American Continent consume very few fermented or cultured food products. However, slowly but surely interest in fermented foods is growing in the United States. I selected three different exotic fermented dairy products for discussion because these products show good promise for growth in the American food market.

KEFIR

The first product is kefir. In California and the Mid-West, currently, there is a lot of interest in a product called "Low Fat Kefir Milk."

Traditional kefir is a very old product from Eastern Europe, and has its origins in the Caucasus Mountain region in Russia (2). The Caucasus mountain range runs East-West between the Black Sea and the Caspian Sea, and divides the Georgian Republic from the Russian mainland. In the Soviet Union, per capita annual consumption of kefir is 10 lb.

The distinctive feature of kefir is use of kefir grains as inoculum to initiate the characteristic fermentation. The grains are used several times to make several batches of the product. The grains are gelatinous, whitish or yellowish irregular granules varying in size from that of a wheat grain to a walnut. Granules are made up of a polysaccharide called kefiran which is largely insoluble in water, but swells up on hydration like gums, agar, and

similar hydrocolloids. Upon soaking in water, the granules have a slimy, jelly-like feel. Within the folds or involutions of granules, bacteria and yeasts that form the characteristic flora of kefir are found. Yeasts and the bacteria exist in partnership or what is generally referred to as "symbiosis."

The flora of traditional kefir consists of two species of yeasts called *Saccharomyces kefir* and *Torula kefir*, and certain lactobacilli named *Lactobacillus caucasicus*. There are also certain lactic streptococci and aroma producing *Leuconostoc* sp. associated with kefir granules. Depending upon sanitation used in straining and storing of granules, kefir grains may be contaminated with undesirable bacteria such as micrococci, coliforms, and spore forming bacilli.

Kefir is made by adding kefir grains to cow's milk that has been cooled to 74-76 F after heat-treatment at 185 F for 30 min. The milk is incubated overnight at 74 F in a dry, clean place. By morning a smooth, soft curd forms, which when agitated foams and fizzes like beer. The major end-products of the kefir fermentation are lactic acid (about 0.8%) ethyl alcohol (about 1%), and carbon dioxide. Other minor components are traces of acetaldehyde, diacetyl, and acetone. The flavor of kefir is mildly alcoholic, yeasty-sour with tangy effervescence. When kefir curd is agitated, kefir grains rise upwards with the evolving gas (carbon dioxide) and float on the top. Grains are strained out and placed in a clean container with chilled water and stored at 40 F. Grains when stored thus, remain active for 8 to 10 days. Grains also may be placed on a clean piece of cheese cloth and dried in a warm oven or in a desiccator containing CaCl_2 or P_2O_5 . After drying, grains are stored wrapped in aluminum foil. Properly dried grains are active for 12 to 18 months. Dried grains should be transferred at least thrice in milk before they are fully activated, and can be used for producing good quality kefir.

The product marketed in California is not authentic kefir because it does not undergo the alcoholic yeast fermentation. Instead, it is labelled as a fermented

low-fat milk product containing *Lactobacillus bulgaricus*, *Lactobacillus acidophilus*, and *L. caucasicus* with a consistency similar to a milk shake. At present, the kefir sold in California contains added fruit flavors. The State of California has spelled out the standards for this product. Unfortunately, the standard specifies inclusion of the species *L. caucasicus*. An authentic strain of this so called species is not available now. Further, the species name *caucasicus* is no more recognized in bacterial taxonomy because the original culture deposited at the American Type Culture Collection was found to be a mixture of different lactobacilli. This poses a problem for commercial culture manufacturers to provide an authentic starter to meet the regulatory standards unless the standards can be modified.

VILIA OR FILIA

The second product that is of interest is called vilia or filia or pitkapiima. This product originated in Finland, where it is consumed in large quantities every day. In the United States, where there are pockets of Scandinavian settlers this product is still made in the homes. One such area is the Tillamook County in Oregon. While I was going to school in Oregon, we obtained a sample of this product and isolated the lactobacilli and streptococci associated with this fermented milk. In Indiana, there appears to be a brisk ethnic market for this product, and the Kroger Supermarket chain offer a food similar to authentic vilia.

Vilia is made by fermenting cow's milk with a special mixture of lactobacilli and lactic streptococci. Milk is heated to destroy undesirable bacteria, cooled to lukewarm temperature, and is then inoculated with a milk starter of vilia bacteria, usually a portion saved from a previous day's batch. The inoculated milk is kept overnight near a warm stove or the fireplace. By morning, a smooth, uniform coagulum is formed, which when poured produces long ropy or stringy strands as it falls from the lip of the vessel. The product has a slimy mouth-feel very similar to the white of raw egg. The curd has a clean, tart taste and when sweetened with sugar and flavored with fruit extracts makes a refreshing snack.

The bacteria involved in the vilia fermentation are slow-acid producing variants of *Streptococcus lactis* and *Streptococcus cremoris* and long chain producing variants of *L. bulgaricus* and capsule-forming *Lactobacillus helveticus*. These bacteria form the stringy texture (partly due to the enormously long-chained *L. bulgaricus* and capsule of *L. helveticus*, and the slow acid

producing variants of the lactic streptococci) as a result of symbiotic growth. The ropy texture is obtained only in milk and only when the associated bacteria are cultured between 80 and 98 F; at higher temperatures, the ropy characteristic is retained, but the fermentation takes longer. The ropy characteristic is intensified if the product is refrigerated at about 40 F for a day or two.

Currently an excellent frozen concentrated culture suitable for large scale manufacture of vilia is available. A 170-g container of frozen culture will coagulate 200 to 300 gal of milk.

PROGURT

This is an interesting product that comes from Latin America (3). The name progurt is a combination of the words protein and yogurt. This cultured milk was developed in Chile to provide a highly nutritious supplementary food product to upgrade the diet of the common people. The fermentation step was included in formulation of the product so it suits the taste of the local populace. The basic ingredients used are skim milk, buttermilk, and cream.

Skim milk is heated for 30 sec at 205 F, cooled to about 80 F, and inoculated with a one-to-one mixture of *S. cremoris* and *Streptococcus diacetylactis*. Within 12 h, the acidity reaches 0.8% to 0.9%, when it is gently warmed up to 104-110 F. At this point, the product is standardized to 5% milkfat using pasteurized cream and buttermilk, and then is homogenized. It is immediately packaged in styrofoam containers and cooled. In some instances, just before homogenization, a mixed culture of *L. acidophilus* and bifidobacteria may be added. The final product has a pH of 4.4 to 4.5 and has not less than 6.2% protein, 3.0% lactose, and 0.7% ash. Because of the renewed interest in the United States for cultured products and their nutritive properties, progurt has a potential market value.

ACKNOWLEDGMENT

Presented at the Annual Educational Conference, Florida Association of Milk, Food, and Environmental Sanitarians, Orlando, Florida, March 15, 1977.

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Presidential Address

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(Received for publication August 17, 1977)

It has been a real pleasure for me to review the activities of our Association in preparation for these comments. I look fondly at our activities of the past year that I have been privileged to serve as your President and those of the past 4 years since joining the Executive Board. The friendships that have been made and the fine cooperative efforts that are so common will last in my thoughts long after this tenure on the Board has been completed. The opportunities you have given me in this Association will be long remembered.

I continue to be amazed, but proud, of what we as an Association have been able to do during this 4-year period. We moved our Executive Offices from Indiana to Iowa. We have a new Executive Secretary with a completely new support staff. Our Journal has a new name and a new publisher.

All this has been accomplished without any interruption in services to our Affiliates or members. In fact, there is general agreement these have been improved noticeably. We have maintained our membership and have enjoyed excellent programs and attendance at our annual meetings. This happened in spite of budgetary problems that have affected all our work loads and travel plans. IAMFES means a lot to a lot of people. The future is bright for us.

My primary responsibility is to inform you of Association happenings this year. However, before I do that, I want to express my appreciation to all members of the Executive Board who have eagerly shared our work load to keep us moving steadily forward. Likewise, our Executive Secretary has been so reliable in supervising all those chores which may seem so "every day" but which create chaos if timetables and payrolls and all the other functions we take for granted are not handled properly. And certainly I want to thank our hosts, the Iowa Association, for the excellent facilities and support activities that we will remember so fondly long after we leave Sioux City.

FINANCES

Perhaps the most outstanding accomplishment this year has been the careful management of Association resources. Despite the increased costs of publication and postage for our Journal, as well as familiar inflationary pressures in most other areas, we are able to continue

with the same dues structure for another year.

Our financial picture is sound although additional funds would permit us to expand our services in other needed areas. Able management in Ames has been supplemented by valuable assistance from our Budget Committee, headed by Past-President Skulborstad. This year, for the first time, our Financial Statement shows an income from Sustaining Membership. Your Executive Board has recognized the need for such funds to expand our activities. We are indebted to Ken Harrington and the people who worked with him to develop such a program and then make it work.

THE JOURNAL

We passed an important milestone in January when our Journal became the *Journal of Food Protection*. Acceptance of the new name has been overwhelmingly favorable.

All of us are indebted to Elmer Marth for his dedicated service as Editor of our Journal. Thanks to him, his Editorial Board, and our Journal Management Committee, the *Journal of Food Protection* stands as a most respected technical publication in the world. It has made IAMFES familiar to many, both in this country and abroad. At the same time, publication of additional papers of general interest has received special attention for the past several years. It is sad to report that further expansion in this area has been limited by the inability to get papers of this type submitted for publication.

With sadness I report the passing of Dr. Ken Weckel who has given incalculable service to IAMFES and to the Journal. He was a long-time member of our Journal Management Committee and the Editorial Board. Of course, he was a Past-President of IAMFES and was always ready to support any activity of International.

COMMITTEES

Committee activity continues to be a major service by and for our members. Over 200 of us serve on the 25-30 committees important to our Association. Several committees have new leadership and all are reviewing their respective roles and areas of responsibility. Annual reports and special publications of these committees are valuable contributions to the continuing education of all workers in the dairy and food fields.

On behalf of the Executive Board and all our members, I want to thank all those members who contribute so much of their time, their knowledge, and actually, since we are not in a position to reimburse anyone for travel or other costs, their financial support in our behalf. They keep us abreast of developments in their respective areas and identify the need for new research or practices to keep the food and dairy industries strong. At the same time, I would encourage anyone who sees the necessity for new committees or committee attention to make the Executive Board aware of this need.

MEMBERSHIP

It is gratifying to report a moderate increase in membership in all categories. Yet we must recognize we are far from our potential. All too many who should belong to IAMFES do not join any national organization. Affiliates suffer also from these inactive professionals. All of us need to pay more attention to this problem. The vitality of Affiliates and IAMFES alike depends on continued growth with new people and new ideas.

We welcome two new Affiliates to our family. The Texas Association of Milk, Food, and Environmental Protection and the North Texas Association of Milk, Food, and Environmental Sanitarians have met all requirements for affiliation and will be so recognized at this meeting. Each has conducted very successful membership enrollments and have sponsored valuable educational programs for those members. We welcome both of these affiliates to our growing family.

Membership co-chairmen Ray Belknap and Harry Haverland have explained the value of affiliation with IAMFES to several additional groups of fieldmen and sanitarians. Interest is high and speaks well for the future of our Association.

NEW ASSISTANT EXECUTIVE SECRETARY

We welcome a new member to our Management team. David Rodgers joined our staff in May as Assistant Executive Secretary and Associate Editor of the Journal. Dave has an excellent background in the biological sciences and in journalism. He will continue to develop the programs started by Barbara Lee, our first Assistant Executive Secretary. Barbara resigned when her husband accepted a position in another state.

You will recall the position of Assistant Executive Secretary was created by the Executive Board to better serve state and national affiliate groups and to supervise production of the Journal. This has been a most valuable use of our limited resources. Dave is eager to work with the Affiliates so I hope many of you will get to know him better at this meeting. Then you can make your folks aware of his interest in serving your needs. My personal best wishes to David and "welcome aboard".

NEHA

I am pleased to report a general improvement in working relationships with the National Environmental Health Association (NEHA) during the past year. It is my hope that these two important Associations will continue to explore areas of mutual interest and concern. I was a guest of the NEHA Board at its annual meetings in Nashville last year and in San Diego this year. President Frank Arnold of NEHA was with us in Illinois and President Henry Drake and Board Member Harry Steigman are with us this year to enjoy the hospitality of our friends in the Iowa Association. I hope each of you will take this opportunity to get to know both of these gentlemen and the fine organization they represent. Present plans call for us to explore areas of mutual interest for joint committee response with a possible joint meeting of the two Associations in 1980.

As we get to know each other better, I am confident we will find far more areas of mutual interest than of conflict. Present discussions suggest cooperation rather than the often expressed concept of merger of the two organizations. I believe the former is highly probable and desirable. Our success in this may indicate the desirability of some form of united activity at some later time. That decision must be left to our successors. Hopefully, our Executive Boards can meet together from time to time to review our progress toward mutually desirable goals.

OTHER AFFAIRS OF THE ASSOCIATION

At this time I would like to announce the election of William Arledge of Dairymen, Inc., Louisville, Kentucky to the Executive Board of IAMFES. Bill has had a distinguished career in quality control and dairy sanitation. We look forward to his contributions to our discussions. I am pleased to welcome a good friend to the Board.

The Executive Board continues to seek ways to better meet the needs of those who serve our industry in field positions. Last year we welcomed the National Association of Dairy Fieldmen as an affiliate of IAMFES. Our Program Committee has given us an excellent balance of technical and field topics during this annual meeting. Our Journal Editor is actively seeking reports which discuss solutions to field situations in both the dairy and food fields. At the same time, technical papers are presented in a manner that the non-scientist can appreciate the significance of research in solving field problems.

In these remarks I have attempted to make you aware of Association affairs discussed by your Executive Board. I believe we serve the needs identified some 66 years ago by those who formed our Association. But times change and procedures must be reviewed to be sure we do our best for our members and society.

THE FUTURE

To be effective, the Executive Board must have the expressed attitudes of our members as individuals and as affiliates. We must have an active Affiliate Council. You must elect officers and delegates who will bring us your ideas and concerns. This year members of the Executive Board attempted to have one or more of the IAMFES Officers at Affiliate annual meetings. We plan to continue this valuable opportunity to improve communications.

Our organizational structure is that of a relatively weak central management that seeks guidance from strong local affiliates. Yet, a recent request for information from your Affiliate Council Chairman got only seven responses from 28 inquiries. We have to do better than that.

There are many important steps to take and decisions to make if we develop as we should. In closing, I would exercise my privilege before you to note some of the areas where the Executive Board needs Affiliate comment.

1. Some have suggested we change the name of our Association as well as the Journal. Should we? If so, to what?
2. We have a rapidly developing food industry with thousands of workers who do not belong to any organization such as ours. What steps can we take that will be mutually advantageous?
3. We have not sought out new members as we should. It bothers me greatly to see so few young people, women, or members of national minorities at all our meetings. As a University Professor I see

many excellent young men and an equal number of highly qualified young women eager to enter our field. We must develop programs to involve them and provide them with continuing education in their chosen profession.

4. I am painfully aware that my Affiliate and many others are not promoting membership in IAMFES as they should. We need new members with new ideas. They, in turn, need our committee activity, our publications, and the sharing of information which such a group as ours can give them.

IN CONCLUSION

The International Association of Milk, Food, and Environmental Sanitarians and our *Journal of Food Protection* are valuable to all of us in far more ways than we can list here. The Association is well managed by an Executive Board, Executive Secretary, and a Council of Affiliates. Extensive changes have occurred in the central office and in our whole economy in recent years, yet finances are stable and basic objectives of service to the Affiliates and the dairy and food industries continue along traditional concepts.

I want to express my appreciation at this time to all of you who gave me this opportunity to serve on your Executive Board and as your President this past year. It has been a wonderful experience. May we work together in the future to continue these friendships and insure that IAMFES is an ever better Association for us to enjoy.

IAMFES Executive Board: (standing, l-r) → W. Kempa, D. Rodgers, W. Arledge, P. J. Skulborstad, E. O. Wright, E. H. Marth (seated) H. Hutchings, D. Fry, H. V. Atherton, R. P. March.



64th Annual Meeting of IAMFES

The 64th Annual Meeting of the International Association of Milk, Food, and Environmental Sanitarians began in hospitable Midwestern fashion. An Early Bird reception sponsored by the Iowa Association featured a hot buttered corn roast atop the Sioux City Hilton in Sioux City, Iowa.

The Meeting, held August 14-18, 1977 at the Sioux City Hilton, mixed business with just the right amount of social interaction, leaving those attending with a little more knowledge and a few more friends.

Items important to the IAMFES were the focal point of Executive Board meetings held throughout the Meeting. Early on the list of priorities was the newly formed IAMFES Foundation.

The IAMFES Foundation is the dream of many, who over the years

have seen the need for a method of funding research and education in the field of food hygiene. P. J. Skulborstad, IAMFES Senior Past President, presented a rough draft of the Foundation Constitution to the Executive Board for consideration. In addition, members of the Board compiled a list of highly reputable individuals to serve on the IAMFES Foundation Board. Final decisions were postponed for further consideration.

Funds for the IAMFES Foundation come from the Sustaining Membership Program. The Executive Board noted that adequate recognition should be given those corporations or individuals supporting the Sustaining Membership Program.

Another item of high priority considered by the Board was a



proposal from the National Environmental Health Association. The Board entertained discussion from 3 NEHA representatives concerning an IAMFES-NEHA jointure. Jointure, in this case is a broad term used to denote some type of functional relationship between the two organizations.

The discussion was an ice breaking session but spawned the following actions. The Board appointed two ad hoc committees. The first committee will look into the advantages and disadvantages of jointure. The second committee will study factors involved in holding both organization's 1980 Annual Meetings in close proximity. The Affiliate Council



Corn Roast and Receptions

later approved the formation of two such committees. The IAMFES will hold its 1980 Annual Meeting in Milwaukee.

Much of the positive groundwork laid by the IAMFES is done so through its committees. Many of those committees reported to the IAMFES Executive Board throughout the course of the meeting.

First to report was the Awards Committee chaired by P. J. Skulborstad. Skulborstad noted excellent nominational response to all awards except one. The Citation Award yielded only two nominations; both were submitted too late for ample consideration. Skulborstad expressed his regrets that the award could not be given and encouraged much stronger response next year.

Harold Irvin, vice chairman of the Committee on Sanitary Procedures,

... Continued on page 808



Members of the Intersociety Council on → Standard Methods for the Examination of Dairy Products received certificates in recognition of their services. (top row, l-r) Dr. R. A. Marshall, Dr. R. B. Read, Jr., and Dr. E. H. Marth (bottom row, l-r) Dr. W. S. Clark, Jr., Dr. G. H. Richardson, and Dr. A. R. Brazis.



IAMFES Past President and Past Executive Secretary, H. L. Thomasson at the Awards Banquet. ↓



said, the CSP will consider new processing technology in future committee meetings. Examples of such technology are wet collectors, membrane processing and plastic vessels, Irvin said.

Tangible success arose from Dr. Frank Bryan's report on the Committee on Communicable Diseases Affecting Man. Bryan said, the third edition of *Procedures to Investigate Foodborne Illnesses* compiled by the CCDAM was published in January 1977. In the following 9 months over 14,000 copies were distributed. Bryan also presented the first draft of *Procedures to Investigate Waterborne Illnesses*.

The Professional and Educational Development Committee is working on an updated version of the IAMFES promotional brochure, according to Ron Richter, committee chairman. The badly needed materials will be ready sometime next year.

Dale Termunde, chairman of the Farm Methods Committee, gave the FMC two-year report. He expressed need for two subcommittees; one for standardization of bulk tanks, the other for establishing uniform mastitis control practices.

Reporting for the Committee on Food Equipment Sanitary Standards, Chairman Karl Jones said, two members of the committee have retired. He entertained recommendations for replacements. Earl Wright proposed looking to new affiliates for possible replacements.

The Membership Committee plays a vital role in IAMFES affairs. Co-chairmen Ray Belknap and Harry Haverland reported increased membership for 1977. In addition, they announced the formation of three new affiliates; Texas, North Texas and the National Association of Dairy Fieldmen.

Another on the list of very active committees is the Journal Management Committee. Chaired by Dr. R. B. Read, the committee proposed several mechanical changes in the *Journal of Food Protection*. These

changes, including a label for journal shelf identification and use of larger type in the table of contents, will be functional beginning with the January 1978 issue.

On the Advice of Dr. Elmer Marth the Journal Management Committee backed the formation of four subcommittees. The subcommittees will serve as appendages of the editorial staff identifying problem areas relating to food hygiene, for editorial consideration.

The Sanitarian's Joint Council is working on some interesting developments. First involves the Model Registration Act. The Act for registering sanitarians was published but needs continual updating. The Sanitarian's Joint Council has given serious consideration as to how the Act should be used and enforced. In addition, the Council is compiling a code of ethics, serving as guidelines for practicing sanitarians.

Indeed, the IAMFES committees structure is functional and productive. It has become a many faceted network serving the needs of continuing food safety. In order to keep it in productive fashion Junior Past President Henry V. Atherton moved there be a Committee on Committees. The CC would overview works of the "very important" committee structure. As approved by the Executive Board the CC will review the

... Continued on page 810



(far left) David D. Fry, President Elect, hands President's Award to Henry V. Atherton. (immediate left) Senior Past President, P. J. Skulborstad hands Shogren Award for the outstanding Affiliate to Minnesota Sanitarians Association representative O. M. Osten.

Food Sanitation Section Speakers: (l-r, → seated) R. Brackett, H. Hutchings, M. J. Nakamura (standing) G. Prince, M. M. Pullen, and N. A. Cox.

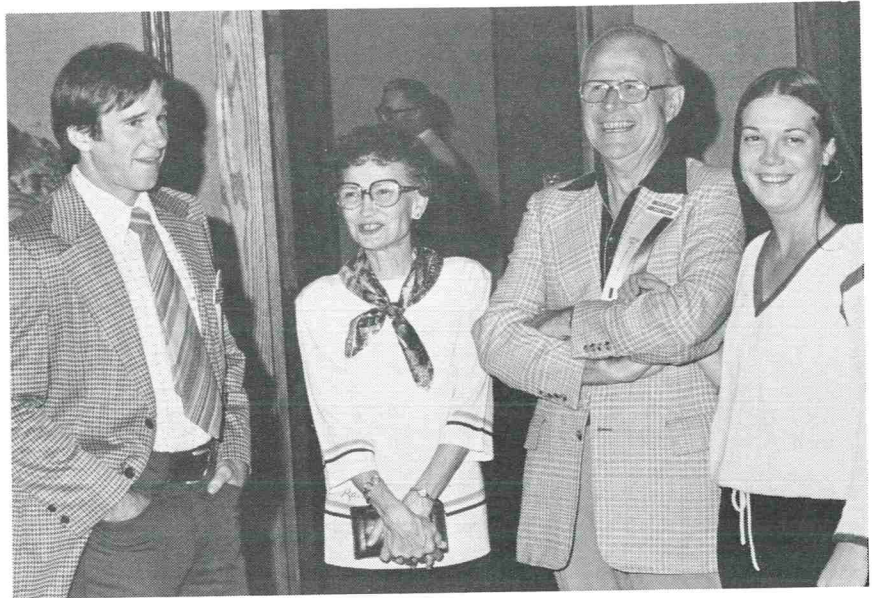


(lower right corner) Dale Termunde, Chairman of the Farm Methods Committee, presents his committee report.

Food Sanitation Section Speakers: (l-r, seated) E. Todd, M. Doyle, D. Clingmann (standing) R. Rust, H. Haverland, T. Corothers. ↓



(l-r) David Rodgers, IAMFES Assistant Executive Secretary; Mrs. Earl Wright; Earl Wright, IAMFES Executive Secretary; and Esther Wright, waiting for a reception to begin.



Display and members of the 1978 Local Arrangements Committee. ↓



progress of various committees and review whether or not they merit continuation.

The first issue of concern to the CC will be to disband non-functional committees. Also, the CC will examine areas where problems in food and dairy safety exist, thereby fostering need for new committees where manpower will be well spent. This decision came out of need to keep the vast committee works on an official bases. Some committees have, over the years, disbanded unofficially. Although they are still on file as functional, these committees no longer serve the IAMFES membership.

Aside from Association business, numerous papers were presented during technical sessions occurring throughout the meeting. Contemporary problems in the dairy and food industry were dealt with in an educative manner. Abstracts of papers presented were published in Volume 40 No. 10 of the *Journal of Food Protection*.

The success of the 64th Annual Meeting can be attributed to all those attending. However, certain individuals went to great lengths to make the 64th Annual Meeting fruitful. The Executive Board voted unanimously to accept the following resolutions.

RESOLUTIONS I.

WHEREAS:

The Iowa Association of Milk, Food and Environmental Sanitarians and Local Arrangements Committee labored long and diligently, with exceptional success, to host the sixty-fourth annual meeting of the International Association of Milk, Food and Environmental Sanitarians in Sioux City, Iowa, and

WHEREAS:

The facilities for both the technical sessions and the social occasions were anticipated and provided with the usual generosity and style by the Iowa Association of Milk, Food and

Environmental Sanitarians and Local Arrangements Committee, and

WHEREAS:

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

WHEREAS:

The 1977 meeting was in every respect "Par Excellence" that will long be remembered;

THEREFORE, BE IT RESOLVED.

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

RESOLUTION II.

WHEREAS:

The Sioux City Hilton Inn, Sioux City, Iowa, was the site of the 1977 International Association of Milk, Food and Environmental Sanitarians Annual meeting, and



← Milk Production Section Speakers: (l-r, seated) S. H. Beale, W. G. Bickert (standing) E. A. Kaeder, and K. Kirby.

Milk Sanitation Section Speakers: (l-r, seated) J. H. Martin, J. F. Frank, H. H. Vaux (standing) P. Cady, R. Brazis, R. Redfern, and R. March. ↓

WHEREAS:

The personnel of the Sioux City Hilton Inn were most accomodating to the needs of the members and their families of the International Association of Milk, Food and Environmental Sanitarians, and

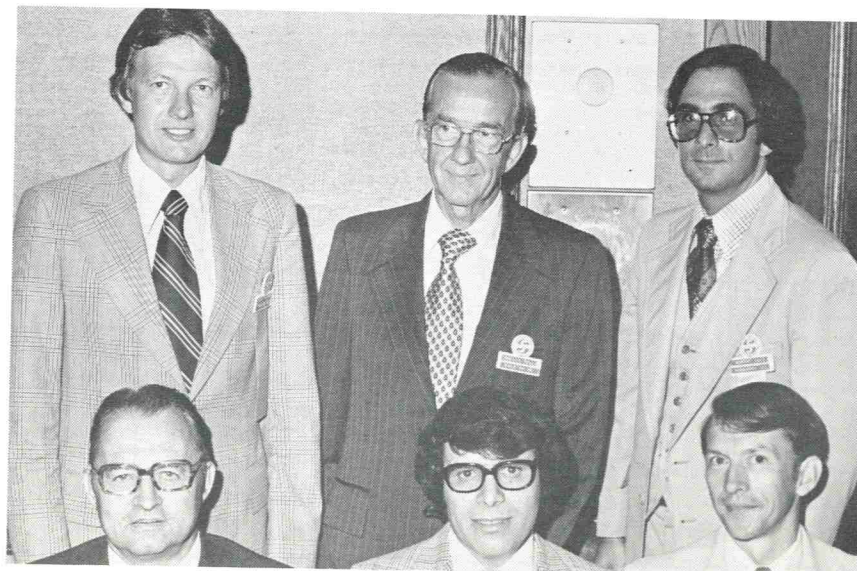
WHEREAS:

The facilities for the program sessions and the member's and their families personal comfort were outstanding;

THEREFORE, BE IT RESOLVED.

That an appropriate expression of gratitude be sent to the management and staff of the Sioux City Hilton Inn.

Plans are already underway for the 65th Annual Meeting of the IAMFES which will be held in Kansas City, Missouri, August 13-17, 1978. If it is anything like the 64th it's sure to be a success.



Milk Sanitation Section Speakers: (l-r, → seated) C. Webster, S. O. Gnan, R. T. Marshall (standing) D. Lund, M. Speck, R. Katz.

Government Institutes Conducts Toxic Substances Control Seminar

The growing concern over toxic substances in food, drugs and the environment will be subject of national discussion at the Toxic Substances Control Conference in Washington, D.C., December 8 and 9, 1977.

Bringing together top government regulators with the business and chemical manufacturing community, the Toxic Substances Control Conference is the second in a series of forums aimed at sorting out the myriad of problems brought about by the many new laws and regulations controlling toxic substances in their use and manufacture.

The impact of these laws and new testing programs affects not only the multi-billion dollar chemicals market, but all businesses using substances that may be considered toxic.

President Jimmy Carter has given

"top priority" to enforcement of these toxic substances control programs. In addition to the Toxic Substances Control Act itself, several other new laws have given chemical-related industries considerable concern, including a major new regulatory program to track hazardous substances from production through distribution to ultimate "approved" disposal.

Some 600 executives, lawyers, and scientists from government and industry are expected to attend the high-powered conference. The program will highlight regulatory developments by the Environmental Protection Administration, the Occupational Safety and Health Administration, the Food & Drug Administration, and other federal agencies. Toxicologists, public health officials and scientists from industry, academia and government will describe the latest progress in identification, classification and testing of hazardous substances. International and industrial concerns will be key topics at the Conference.

The Toxic Substances Control Conference will be held in the Shoreham Americana Hotel in Washington, D.C., December 8 and 9. Conducted by Government Institutes, Inc., the Conference will be taped and proceedings made available for sale.

Additional details are available from Government Institutes, Inc., 4733 Bethesda Avenue, N. W., Washington, D.C. 20014 (telephone: 301/656-1090).

U.S.D.A. Publishes Purchasing Guide for Schools

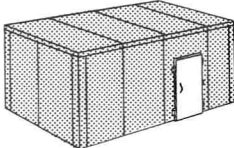
A new U.S. Department of Agriculture publication, "Food Purchasing Pointers for School Food Service," is designed to help local schools and school districts buy food in quantity, according to Assistant Secretary Carol Tucker Foreman.


Although schools receive some commodities from the Department of Agriculture (USDA), they purchase many items directly from vendors. Previously, USDA had no guidelines for such purchases. This publication is intended to fill that void by suggesting procedures and specifications that buyers can adapt to fit individual school purchasing needs.

The two-part publication outlines procedures for sound purchasing practices, gives information on inventory systems and stock control, and includes sample forms and contracts. Part two suggests specifications and buying tips for selected food items used in school food programs. The publication is loose-leaf so that as specifications are developed or changed new sheets may be inserted.

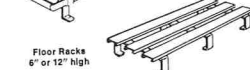
Copies of the publication (Program Aid No. 1160) have been sent to state agencies that administer school food programs for distribution to local schools. Other interested persons can purchase copies for \$3.00 each from the Superintendent of Documents, U.S. Government Printing Office, Washington, D.C. 20402.

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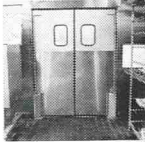


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Los Angeles County Health Department Wins 1977 Samuel J. Crumbine Award

The Department of Health Services of Los Angeles County, California, which is responsible for providing a wide range of health services to 7,000,000 county residents, has been named the winner of the 1977 Samuel J. Crumbine Consumer Protection Award.

The Award is presented annually by the Single Service Institute to honor a local health authority for outstanding achievement in protecting consumers through a public program of food and beverage sanitation. The selection is made by a special jury of public health professionals and consumer representatives.

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

The Los Angeles County Department of Health Services won the award for an entry which, in the jury's opinion, developed the most effective program of information and education aimed at both food service managers and consumers in an effort to fulfill food service sanitation objectives.

"Since food service managers need satisfied consumers and consumers need safe and satisfying food service, the active and informed participation of both groups is crucial to the success of food service sanitation programs," according to Charles W. Felix, Director, Environment, Health and Communications of the Single Service Institute. "Effective communications among food service managers, consumers and the public health agency are essential, and this is why programs must focus on information and educational activi-

ties along with inspection and enforcement efforts."

The Crumbine Award was presented November 1 at the annual meeting of the American Public Health Association in Washington, D.C. Accepting the Award on behalf of the winning organization will be Morrison E. Chamberlin, Acting Director of the Los Angeles County Department of Health Services.

In announcing its decision, the Crumbine Award jury pointed to two aspects of the winning agency's information and education program as being particularly effective or innovative. One is the Department's Food Sanitation Committee, an advisory body that includes representatives of the food service industry, labor groups and consumer organizations. The Food Sanitation Committee actively assists the Department in developing policies and regulations for food and consumer protection programs, and further aids in the dissemination of information among food service managers and consumers.

The jury characterized as especially innovative the Department's program to prevent misrepresentation of foods listed in menus by restaurants. To avert possible fraudulence in menu "puffery," the program sets forth clear policies for the guidance of food service establishments, trains an inspection staff to enforce menu guidelines, and even provides for

laboratory analysis of suspect foods. In addition, consumers are urged to aid the program by reporting to the Department instances where they believe menu practices to be misleading.

This year's Crumbine Award jury included Dr. Frank Arnold, Silver Spring, Maryland; Henry V. Atherton, University of Vermont, Burlington, Vermont; Mary Ellen Burris, Director of Consumer Affairs, Wegman's Food Markets, Inc., Rochester New York; Larry Gordon, Administrator, Health and Environmental Programs, Health and Social Services Department, Santa Fe, New Mexico; Dr. George Kupchik, Professor and Director, Environmental Health Sciences Program, School of Health Sciences, Hunter College of the City University of New York; Vinson R. Oviatt, Environmental Safety Branch, Division of Research Services, National Institute of Health, Bethesda, Maryland; and Dr. Bailus Walker, Jr., Director, Environmental Health Administration, government of the District of Columbia, Washington, D.C.

Requirements for the 1978 Crumbine Award have already been set by the Award jury. Unlike past years, in which entries have focused separately on specific elements of food and beverage sanitation programs, the 1978 Award will be given for a comprehensive effort which combines these elements.

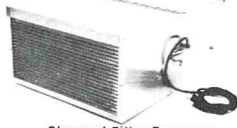
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American Dietetic Association Supports Dietary Goals for U.S.

The American Dietetic Association (ADA) has given qualified support to the "Dietary Goals for the United States," a report released earlier this year by the Senate Select Committee on Nutrition and Human Needs. The Committee recommends broad changes in American eating habits to prevent and control through dietary means several diseases.

In its statement in the September Journal of The American Dietetic Association, the ADA observes:

"At some point between complete adoption and outright rejection of the Goals, there is evidence that Americans . . . would do well to look closely at present eating practices. While data are incomplete to assure that everyone would necessarily benefit from adopting this plan for eating, it is sufficient to expect that many people, particularly those now at high risk with regard to the degenerative diseases and obesity, would certainly benefit.

"Because eating habits are based on cultural, social, and economic factors, as well as nutrient content of foods, we believe that such changes cannot be brought about by legislation or governmental regulation alone. The American Dietetic Association believes that acceptance of a prescribed way of eating by the American public will be attained only if a suitable variety of foods is available; if the people are well informed and motivated to change; and if qualified professional educators, such as registered dietitians, are available to provide counseling and support."

The Senate Select Committee "goals" recommend that Americans eat more fruits and vegetables and whole grains; eat proportionately less red meat and more poultry and fish; cut down on foods high in fat and partially substitute polyunsaturated fat for saturated fat; substitute nonfat milk for whole milk; eat less

butterfat, eggs, and other high-cholesterol foods; and greatly decrease consumption of both sugar and salt and foods high in either.

In response to the report, The American Dietetic Association's recommendations included:

- Continuous re-evaluation of the specificity of the goals as new scientific knowledge emerges

- Comprehensive investigation of the role of diet in preventing diseases and restoring health in disease conditions

- A study of the impact of the recommended Dietary Goals on Institutional and home food services, as well as on food production and distribution

- Integration of nutrition education into preventive and health care programs as a prime effort of the Association, Congress, and other agencies

- Adoption of a Nutrition Policy to establish a framework for implementation of programs to promote the optimal nutritional health of the public.



James R. Welch, President-National Mastitis Council.

Milking Machine Symposium Program of National Mastitis Council Meeting

The Third International Symposium on Machine Milking will be held February 20-23, 1978, in the Executive Inn, Louisville, Kentucky. The symposium will be the program for the Seventeenth Annual Meeting of the National Mastitis Council. Eminent speakers from around the world will provide comprehensive coverage of all aspects of machine milking in the two and one-half day program.

Major topics include:

- Stimulation of the milk ejection reflex

- Physical response of the teat to milking

- Operational characteristics of conventional milking units

- Operational characteristics of non-conventional milking units

- Milking machine effects on mastitis infection

- In-line milk testing for health and production

- Equipment maintenance and evaluation

- Increasing milking efficiency through automation

- Milking barn and parlor design and performance

Advanced registration should be made by sending name, firm represented, and check for \$20 to National Mastitis Council, 910 Seventeenth Street, Washington, D.C. 20006. Hotel reservations should be made directly with The Executive Inn, Watterson Expressway at Fairgrounds, Louisville, Kentucky, 40213, telephone 502-367-6161. Indicate that you will be attending the National Mastitis Council meeting.

A Proceeding including all papers presented at the meeting will be published and distributed to all meeting registrants. Advance orders for additional copies of the Proceedings received before January 10 will be honored at \$6 per copy.

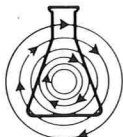
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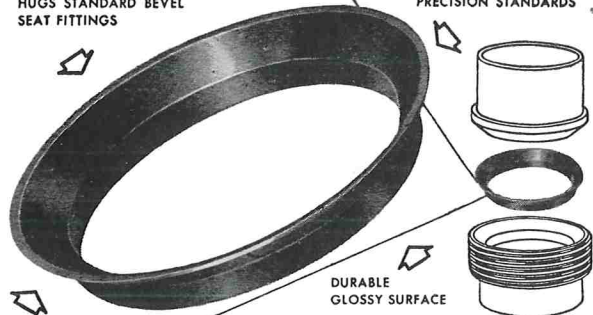
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*Dr. George C. Fisher, Head
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3. Cow Performance
4. Equipment Maintenance
5. Herd Health Program
6. Record Keeping

Though some areas may appear more important than others, the exclusion or neglect of any one will result in less profit, or as a sudden problem in production or herd health—a problem easily avoided with proper management and teamwork.

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Total Management provides the farmer with

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• PRODUCTION GOALS

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