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August 21, 22, 23, 24, 1972
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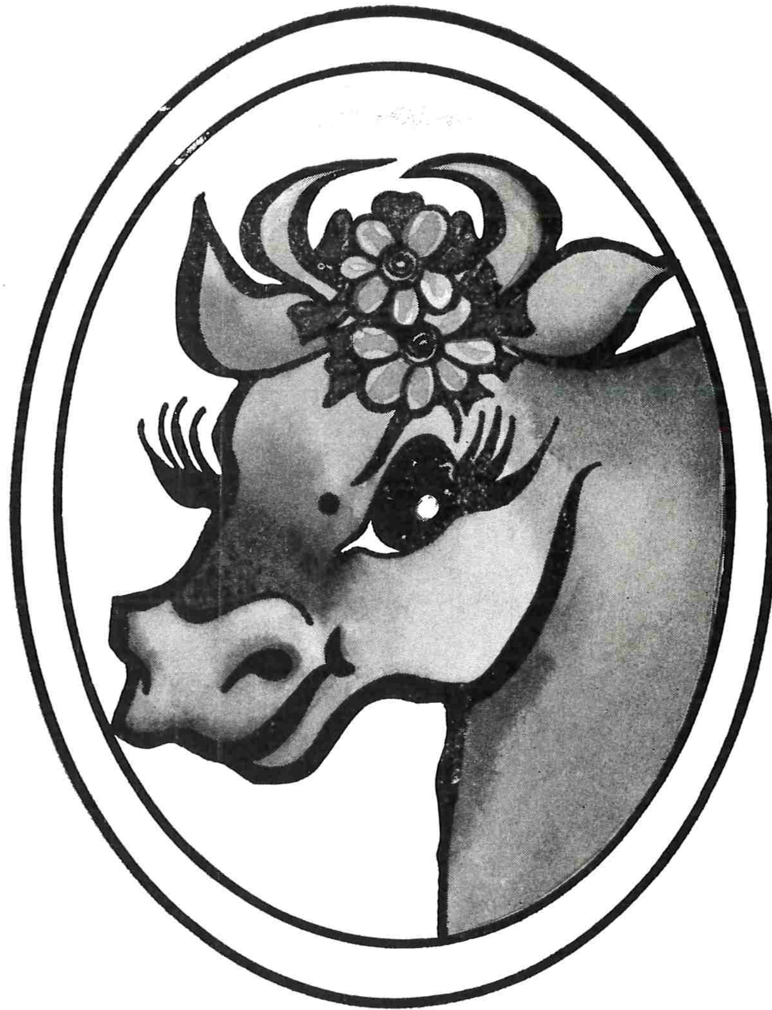

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USE OF ELECTRICAL CONDUCTIVITY FOR MONITORING MILK RESIDUE REMOVAL FROM PIPES DURING RINSING[1]

E. L. RUIZ AND D. B. BROOKER
Department of Agricultural Engineering
AND
M. E. ANDERSON AND J. R. FISCHER
USDA, ARS, TF
AND
R. T. MARSHALL
Department of Food Science and Nutrition
University of Missouri
Columbia, Missouri 65201

(Received for publication October 27, 1971)

ABSTRACT

A system was designed and assembled for use in determining the amount of solids in a water-milk mixture. Data were collected for three different fluid flow velocities and two different temperatures of the rinse water. There was no significant difference in the amount of milk residue removed from a stainless steel pipe when rinse water temperature was either 35 C or 51.7 C and when the flow velocity was varied between 3.27 and 9.80 ft/sec. Results also indicated that the sensitivity of electrical conductivity was approximately 0.02 mg milk solids/ml and was limited by the rate of ionization of the milk salts when the milk film was dried on the surface.

Automated cleaned-in-place (CIP) cleaning systems are used in modern food processing plants. CIP systems use less labor and less cleaning materials than systems that require disassembly of equipment, and in addition, eliminate the possibility of contamination which may occur when cleaned equipment is reassembled *(1)*. Cleaning time could be further reduced if the CIP systems were regulated by monitoring and control devices that automatically discontinued a phase of the cleaning cycle when the cleaning fluid was no longer removing soil.

Fischer *(2)* determined that the concentration of milk in a water-milk mixture could be continuously measured with a cell that monitored the electrical resistance of the mixture. His theory was that the electrical resistance of the solution was a function of the quantity of milk salts ionized in the solution. Fischer's work was centered about the rinsing of milk from a stainless steel plate with water. The work reported here was concerned with removal of milk residue from the interior surface of a sanitary stainless steel pipe. Use of pipe flow permitted a more rigorous analysis of the effect of fluid flow parameters on soil removal.

[1]Contribution from the Missouri Agricultural Experiment Station, Journal Series No. 6226. As approved by the Director.

MATERIALS AND METHODS

The mechanical system

A schematic diagram of the equipment and piping system is shown in Fig. 1. The piping unit which consisted principally of 1 1/2-inch sanitary stainless steel pipe was insulated with a rubber material to prevent heat loss. The pumping unit consisted of a sanitary centrifugal pump driven by a 7 1/2 hp electrical motor equipped with a variable speed controller. A turbine flow meter, able to measure flow rates within a range of 15 to 150 gpm with an accuracy of 0.5% of the measured value, was installed in the line.

The electrical system

A conductivity cell (fabricated by authors; calibrated using standard cell, K = 1.0, Yellow Springs Instr. Co., Inc.) measured the electrical resistance of the flowing solution. The cell consisted of stainless steel probes fixed to a plastic cylinder. In one experiment the cell was placed in one arm of a Wheatstone bridge. Two cells were used in the second experiment and were placed in adjacent bridge arms.

The standard curves

The resistance of a water-milk solution varies with the concentration of milk solids in the solution and with temperature. The relationships are shown as standard curves in Fig. 2. Data were obtained by injecting, at a known rate, solutions of various milk solids concentrations into the water flowing through the test sections and past the conductivity cell. Fluid flow rates used were 15, 30, and 45 gpm, resulting in average flow velocities of 3.27, 6.53, and 9.80 ft/sec. Flow velocities of these magnitudes did not affect resistance when milk solutions were injected. Fluid temperatures of 35 C (95 F) and 51.7 C (125 F) were used. For these flow rates and for both temperatures the Reynolds Numbers indicated turbulent flow.

An equation of the following form best fit the data:

$$\log Y = A + B (\log X) + (\log X)^2$$

where Y = concentration (mg/ml)
X = change in resistance (ohm)
A, B, and C are constants (Fig. 2)

Correlation coefficients for the curves for 35 C and 51.7 C were 0.999 and 0.999, respectively. Data were tested for a first order log-log relationship and correlation coefficients for each temperature were 0.988.

Change in resistance at a given time interval obtained from curves such as those shown in Fig. 3 was substituted into the appropriate standard curve equation (Fig. 2). From this

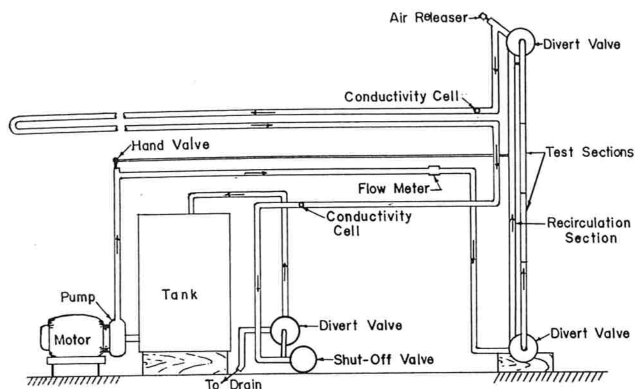

Figure 1. A schematic diagram showing the flow of fluid during an experiment.

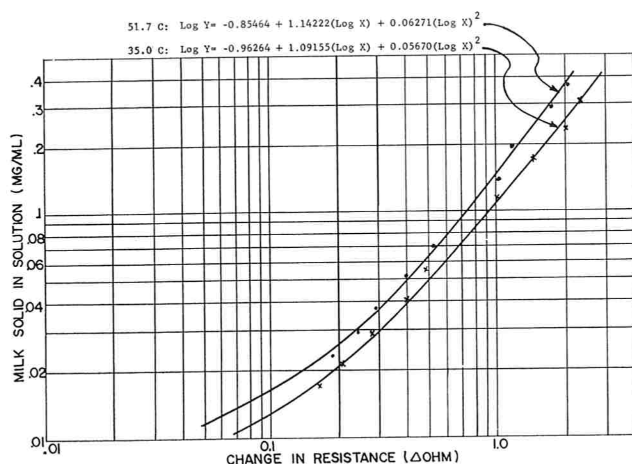

Figure 2. Standard curves for the relationship of milk solids concentration in solution to change in resistance. Curves are shown for rinse water temperatures of 35 C and 51.7 C.

calculation the concentration of milk solids (mg/ml) was obtained. Then this value was substituted into the following formula to obtain the flow rate of milk solids in mg/sec that passed the conductivity cell:

$$FRS = C \times V$$

where, FRS = flow rate of milk solids passing the cell (mg
C = concentration of milk solids (mg/ml)
V = flow rate of the rinse water (ml/sec)

The total milligrams of milk solids was evaluated by numerically integrating the milligrams of milk solids per second over the length of time that the change in resistance occurred.

Experimental procedure

Test sections of pipe were prepared for the experiments by cleaning with 3.0% each of alkali (HC-41, Klenzade Products, Beloit, Ws.) and acid (AC-3, Klenzade Products, Beloit, Ws.) solutions, rinsing and then drying in an oven at 37.8 C (100 F) for 15 min. After drying, 2 ml of the 10% milk solution (200 mg of milk solids) were pipetted and evenly spread onto the inner surface of each of two test sections of pipe. The test sections were dried at 37.8 C for 1 hr and then installed in the system.

A test was begun by routing the rinse water through the test sections which were connected so that the fluid flowed from one test section to the other and then past the conductivity cell. A trace was started on a strip chart recorder as soon as water was diverted to the test sections and was continued until the resistance of the solution returned to that of clean water. For this portion of the tests, only one conductivity cell was used, and it was located near the outlet end of the test sections. Tests were done at temperatures of 35 C and 51.7 C and at flow velocities of 3.27, 6.53, and 9.80 ft/sec.

After a trace was recorded, the test sections were removed from the apparatus. Twenty-five milliliters of 1.0 N hydrochloric acid were placed in each section and the section rotated and shaken by hand for 1 min. The resulting milk and acid mixture was collected in a plastic bag and analyzed for calcium content using the method devised by Heinz (3).

RESULTS

Before the experiment wherein milk was dried onto the interior surfaces of test pipes, a test was conducted to determine whether the apparatus could be used to test the amount of milk solids passing through the pipe in a given time period. Four milliliters of 10% reconstituted nonfat dry milk (400 mg of milk solids) were injected into the water passing through the system. Table 1 shows the average amounts of milk solids in 6 replications (entitled "milk injected") for the different flow rates and temperatures. No significant differences among treatments were noted indicating that the apparatus

TABLE 1. TOTAL MILK SOLIDS (MG) AS DETERMINED BY CONDUCTIVITY MEASUREMENT WHEN 400 MG OF MILK SOLIDS WERE INJECTED INTO THE TEST SECTION AND WHEN DRIED ONTO THE INTERIOR SURFACES OF THE TEST PIPES.

Temperature (C)	Flow velocity (ft/sec)	Reynolds number[1] (10^4)	Quantity of milk solids detected (mg)[2]	
			Milk injected[3]	Milk dried on pipes[4]
	3.27	4.7	398	230
35.0	6.53	9.5	407	219
	9.80	14.3	402	180
			402	246
	3.27	6.4	406	213
51.7	6.53	12.8	393	221
	9.80	19.2		

[1]Viscosity of water at 35 C and 51.7 C are 1.76 and 1.30 lb_m/hr ft, respectively.
[2]6 replications per entry.
[3]Grand mean = 401; standard deviation = 14.
[4]Grand mean = 218; standard deviation = 29.

TABLE 2. DIFFERENCES IN QUANTITIES (MG) OF IONIZED MILK RESIDUE WHEN THE MILK PARTICLES WERE GIVEN VARYING PERIODS OF TIME TO IONIZE. FLOW VELOCITY WAS 6.53 FT PER SEC

Temperature (C)	Time elapsed between the two measurements (sec)	Replication 1	2	3	4	Mean
				(mg)		
35.0	5.82	45.49	32.76	36.16	36.77	37.80
	8.88	60.26	39.58	69.66	31.30	50.20
	14.40	69.05	101.13	103.60	72.88	86.42
51.7	5.82	52.07	37.27	34.15	79.57	50.76
	8.88	78.58	37.73	88.93	112.31	79.39
	14.40	103.97	99.81	116.45	114.55	108.70

could be used to find the amount of milk solids passing the conductivity cell.

The amount of milk removed, of the 400 mg dried on pipe surfaces, was calculated (Table 1) from the resistance data recorded by the strip chart recorder, as explained earlier. The means for temperatures of 35 C and 51.7 C, averaged over the three flow rates, were not significantly different. Means representing quantities removed at the three flow rates, averaged over temperature, were different only when the lowest velocity was compared to the highest ($P<0.05$). and more solids were apparently removed at the lower flow rate.

The amount of solids remaining on the pipe sections after each test was determined by the residual calcium method. The mean for all the data was 48.07 mg with a standard deviation of $\pm$ 7.51 mg. There was no significant difference between the respective means of 47.07 and 49.08 mg obtained for temperatures of 35 C and 51.7 C. Means of 45.36, 48.93, and 49.93 mg were determined for the flow velocities of 3.27, 6.53, and 9.80 ft/sec, respectively. No significant differences ($P<0.05$) were found among these mean quantities.

DISCUSSION

It was evident, when data from Table 1 were examined, that a substantial amount of the milk solids which had been dried on the pipes was not accounted for. Also, it is surprising to note that more solids were apparently removed when the flow velocity was 3.27 ft/sec than when it was 9.80 ft/sec.

It is hypothesized that some of the milk particles that were swept from the pipe surface by the turbulent flow were not dissolved when they passed the conductivity cell; the resistance of the solution is dependent on the ions present. Therefore, failure of dislodged milk to dissolve would have resulted in the low values.

The longer the milk particles are in the solvent (rinse water) the more apt they are to be ionized. Since the particles removed from the pipe surface at the fluid flow velocity of 3.27 ft/sec took longer to reach the conductivity cell, (on the average 4.12 sec compared to 1.52 sec for the highest flow rate) it is likely that more of the milk residue had ionized before reaching the cell than when flow rates were higher.

TESTS OF IONIZATION HYPOTHESIS

To test the hypothesis that resistance measurements would decrease (more milk solids would be

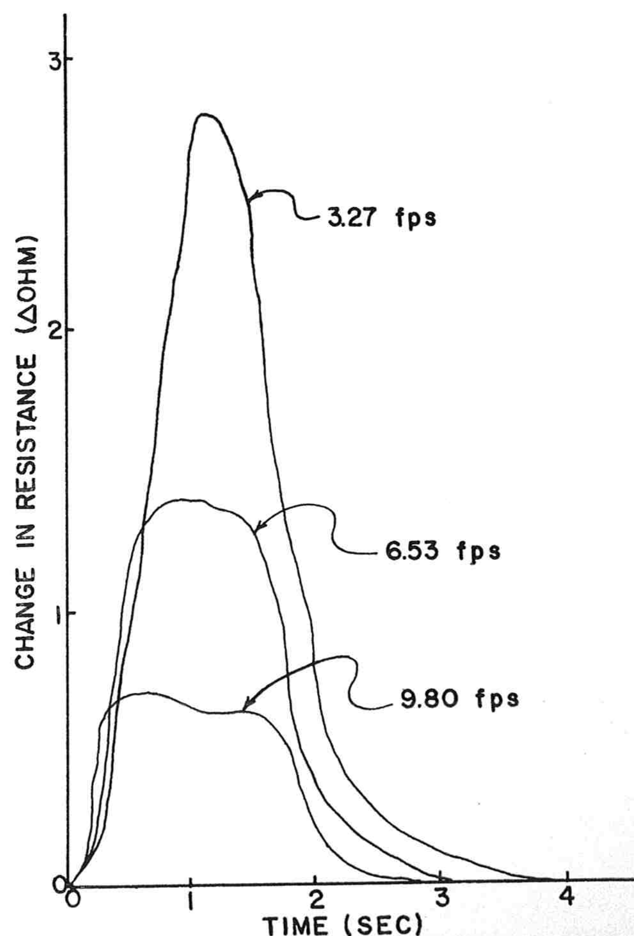

Figure 3. Typical curves obtained at 35 C for the three flow rates as plotted by the X-Y recorder.

detected) if the milk particles were given more time to ionize, two conductivity cells were placed in the system, one immediately downstream from the test section and one several feet downstream, and the tests were repeated at a flow rate of 6.53 ft/sec. Piping was added to the system so that the cells were separated by 38, 58, and 94 ft. Tests were made at 35 C and 51.7 C.

A preliminary test was made with injected milk, and there was no significant difference between the amounts of milk solids measured at the two cells even when separated by 38 ft. This indicated that when milk solids were injected as fluid milk, there was no change in the amount ionized during passage from the first to the second cell.

When dried-on milk solids were removed from the test sections by the flowing fluid, as was done in the previous tests, there were significant differences in the resistance tracings of the two cells. Ionization apparently occurred during flow between the two conductivity cells (Table 2). The greater the distance between cells, the larger the quantity of solids detected at the second cell. Therefore, if an accurate measurement of the amount of solids removed during a rinse cycle is desired, the conductivity cell must be located a considerable distance from the surface being cleaned. We did not measure the total time to reach equilibrium because additional piping was not available.

SUMMARY AND CONCLUSIONS

There was no significant difference in the amount of milk residue removed from a pipe when rinse water temperatures were either 35 C or 51.7 C. Based on residue of calcium recovered from rinsed test sections there was no significant difference in the amount of milk residue removed when the rinse water velocity was varied between 3.27 and 9.80 ft per sec.

The factors which limit the application of electrical conductivity in monitoring the rinsing of milk from equipment surfaces are: *(a)* rate of ionization of milk salts from dried on films, and *(b)* sensitivity of the instrument which, in these experiments, was approximately 0.02 mg of solids per milliliter.

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The Milk Industry Foundation is cooperating with the National Reading Center in a project to encourage improved reading habits by Americans.

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The Center points out that an estimated one of every 20 children is held back a grade each year because of a reading problem, and that 30 percent of children in America suffer reading difficulties. Moreover, a large percentage of the unemployed persons in the U. S. have serious reading difficulties, which contributes to their problem.

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NORMAL MILK SOMATIC CELL COUNTS

R. P. NATZKE AND R. W. EVERETT
Department of Animal Science
New York State College of Agriculture

AND

D. S. POSTLE
New York State Veterinary College
Cornell University
Ithaca, New York 14850

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ABSTRACT

Samples were collected during various stages of lactation from cows in commercial dairy herds in which the infection status was known. The average cell count of all cows uninfected at the time of sampling was 214,000/ml. For each quarter infected there was an approximate doubling of cell counts of the composite milk. Uninfected cows at the time of sampling had a low cell count and showed little upward trend through the first five lactations. Cell counts from these cows tended to be highest in the first two weeks post calving, lowest in mid-lactation, and intermediate at drying off. Cows with lactations of more than 305 days had higher cell counts probably because of undetected new infections. Cell levels from cows which were uninfected for three years showed no significant change ($P < .05$) caused by lactation age or stage.

Many values have been given for the normal cell count of milk from individual cows. These values range from 900,000/ml as reported by Cooledge *(3)* to as low as 20,000/ml as reported by Chu *(2)*. Much of the variation in reported values may be traced to differences in cell measurement techniques and in definition of a normal udder. Brown et al. *(5)* suggest a cell level of up to 100,000/ml as being normal.

Smith and Schultze *(10)* found that 46 second-lactation cows which were negative bacteriologically at the time of sampling had an average cell count of 765,000/ml. This compared to an average of 178,000/ml in 68 normal quarters of 22 first lactation cows. Ward and Horton *(11)* reported an increase in milk leucocyte levels from the first through the sixth lactation. Afifi *(1)* found an average leucocyte count of 208,000/ml for cows in their fourth lactation with no record of previous clinical mastitis. Cows with previous mastitis produced a cell level of 729,000/ml in their fourth lactation. Miller and Finkner *(4)* noted increases in leucocyte numbers through the first 4 lactations, after which the level remained constant.

It is generally reported that the leucocyte concentration in milk is high during the first weeks of lactation, then drops to its lowest level, and increases throughout lactation until drying off *(1)*. Schalm and Lasmanis *(8)* suggest that the increase in cells toward the end of lactation resulted from new infections.

The purpose of this study was to determine the normal somatic cell concentration in milk from cows with bacteriologically negative quarters at various ages and stages of lactation. This normal value was compared to that for cows with infections.

MATERIALS AND METHODS

Observations were made on milk samples from cows which were part of a 3-year experiment in which routine bacteriological testing was being conducted. In the 3-year experiment all quarters of all cows were sampled initially, as cows entered the herd, before and after lactation therapy, at drying off, after calving, and annually. A quarter was considered infected if two consecutive samples contained the same mastitis pathogen. For the purpose of the present study, if quarters were negative at each sampling during the 3-year project, they were considered free of infection for the entire study. New infections which recovered spontaneously between sampling would not have been detected. Mastitis pathogens were isolated and identified according to procedures previously described *(7)*.

The present study was conducted during the final five months of the 3-year project described above. Composite milk samples were collected from cows as they entered one of four stages of lactation. Samples from one milking were collected during the first 2 weeks after calving, approximately 30 and 45 weeks after calving, and at drying off. Samples were transported to the laboratory under refrigeration. Somatic cell concentration was determined by the direct microscopic somatic cell count (DMSCC) *(6)*. The film component of variance was 38% of the mean, and the counting error was 75.9% of the mean. The mean strip count was 70.8. These variances compared favorably with guidelines suggested for the DMSCC *(9)*.

Cell counts were recorded and transferred to IBM tape for integration with information on the infection status of these cows from the original project. Means of DMSCC scores were compared by using the variance of the difference between means.

Data obtained were divided into three groupings for analysis. The first contained information from cows with one or more infected quarters at the time of sampling. Group 2 contained data from cows uninfected at the time of sampling but which may have been infected at some time during the

TABLE 1. AVERAGE SOMATIC CELL VALUES FOR COMPOSITE MILK SAMPLES FROM 824 COWS, BY NUMBER OF INFECTED QUARTERS

No. quarters infected	No. of cows	Cells/ml	$\sigma_{\bar{X}}$
0	574	214,000	23,800
1	160	507,000	94,908
2	67	701,000	150,457
3	23	1,470,000	557,210

TABLE 2. AVERAGE SOMATIC CELL NUMBERS FROM COMPOSITE MILK SAMPLES FROM 574 COWS CONSIDERED BACTERIOLOGICALLY NEGATIVE AT THE TIME OF SAMPLING

Lactation age	No. of cows	Average somatic cell/ml	$\sigma_{\bar{X}}$
1	225	148,000	16,167
2	138	197,000	40,176
3	79	268,000	68,729
4 + 5	83	209,000	41,073
6 +	49	488,000	219,068

TABLE 3. EFFECT OF STAGE OF LACTATION ON SOMATIC CELL LEVEL FOR COWS CONSIDERED UNINFECTED IN ALL FOUR QUARTERS AT THE TIME OF SAMPLING

Stage of lactation	No.	Cells/ml	$\sigma_{\bar{X}}$
Week 1 + 2 post calving	142	241,000	42,570
Week 30 post calving	161	149,000	38,225
Week greater than 45 post calving	82	312,000	43,114
At drying off	189	208,000	56,440

TABLE 4. AVERAGE CELL COUNT OF COWS UNINFECTED FOR ENTIRE TIME ON THREE YEAR TRIAL, BY LACTATION AGE

Lactation age	No. cows	Somatic cells/ml	$\sigma_{\bar{X}}$
1st lactation			
0 - 24 weeks	38	136,000	34,374
25 - 50 weeks	23	317,000	96,561
2nd & 3rd lactation	14	112,000	40,345
4th lactation and over	20	153,000	62,619

TABLE 5. AVERAGE CELL COUNT OF COWS UNINFECTED FOR ENTIRE TIME ON THREE YEAR TRIAL, BY LACTATION STAGE

Lactation stage	No. cows	Somatic cells/ml	$\sigma_{\bar{X}}$
Weeks 1 + 2 post calving	70	196,000	38,316
Week 30 post calving	16	129,000	67,889
Week 45	1	361,000	0
At drying off	8	118,000	54,262

3-year observation period. Group 3 included information from cows which were uninfected for the entire time they were on the 3-year project.

RESULTS AND DISCUSSION

Infected cows

In Table 1 data from group 1 are summarized. Cows in which all 4 quarters were pathogen free at the time of sampling had an average somatic cell level of 214,000/ml. If a single quarter contained a pathogen, the cell count of all milk from that cow was more than twice as high at 507,000/ml. The estimated average infection level for cows in the U. S. is 25% of the quarters or one quarter per cow. This could be the cell count for an average cow. As the number of infected quarters increased to three the somatic cell content increased, reflecting the increased response to inflammation. The standard deviation of individual samples ranged from 570,000/ml with no infected quarters to 2,816,000/ml in 3 infected quarters. The differences between no infected quarters and 1, 2, or 3 infected quarters were significant at the 5% level of probability. The difference between 1 and 3 infected quarters and 2 and 3 infected quarters was significant at the 11 and 20% probability level, respectively. The standard errors in Table 1 clearly demonstrate the normal biological variation in the cell numbers of cows' milk.

Uninfected cows

In this portion of the study all cows (group 2) uninfected at the time of sampling were divided by lactation age; data are presented in Table 2. A slight increase in cell numbers was noted as the age increased. The increase in cell numbers on cows 6 years and older may have been a result of infections which were present before the 3-year project was begun. These infections would have been eliminated through therapy but the cell levels remained high. The variance also increased with age. The standard deviation of individual observations increased from 242,000/ml in first lactation cows to 1,533,000-/ml in sixth lactation cows. None of the differences between any of the means in Table 2 were significantly different at the 5% level of probability.

The cell levels of uninfected cows at the time of sampling are presented in Table 3 according to stage of lactation. Average cell numbers for all stages were low. The sample taken shortly after calving contained somewhat more cells than mid-lactation samples. This difference coincides with the increase of other blood constituents shortly after calving. The 82 cows which had extended lactations of more than 305 days yielded significantly more cells than the 161 cows sampled at week 30 ($P < .05$). This increase may have resulted from cows that became infected but were not detected because of the bacteriological survey schedule. However, the 189 uninfected cows sampled on the day of drying off produced a cell count of only 208,000/ml. These data indicated that if a physiological increase in cells occurred during late lactation, it was not detected. It is suggested that udders in late lactation, free of infection, do not contribute to elevated cell numbers in bulk milk.

The final series of analyses was conducted using data from group 3 cows whose udders were appar-

ently free of infection for at least 3 years before the latest sampling. Cows in their first 3 lactations were negative for their productive lives. Cows in the fourth or greater lactation were infection-free for at least the last 3 years.

Results presented in Table 4 show that cows uninfected for at least 3 years produced milk with low cell counts even in the older age groups. None of the differences between means in Table 4 were significantly different at the 5% level of probability.

The slightly higher cell count in cows between lactation stages of 25 and 50 weeks may have resulted from the sampling schedule. Bacteriological samples were not drawn in mid-lactation unless treatment was required or the routine annual sample was collected at that time.

Cell counts from the 95 cows in the uninfected grouping were divided by lactation stage and presented in Table 5. Again it was noted that the average cell count of the milk during the first 2 weeks post calving was higher than at the time of drying off. This further substantiates the observation that the uninfected cows do not have elevated cell counts at the end of lactation. Because of the large variation in DMSCC scores, none of the differences in Table 5 were significantly different at the 5% level of probability.

ACKNOWLEDGEMENTS

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THE PENNSYLVANIA STATE UNIVERSITY COLLEGE OF AGRICULTURE 30TH ANNUAL DAIRY FIELDMEN'S CONFERENCE JUNE 6 AND 7, 1972

The annual Dairy Fieldmen's Conference is going to be held at The Pennsylvania State University on June 6 and 7, 1972. All meetings will be held in the auditorium of the J. Orvis Keller Building and the banquet will be held on Tuesday evening at the Nittany Lion Inn.

The fee is $15.00 per person and includes: Registration, conference proceedings, banquet, and Dairy Fieldmen's Conference Scholarship. Please send in a check payable to The Pennsylvania State University. If more than one individual from a company or other association is attending, a preregistration form should be completed for each individual, but one check will suffice for the entire group. All preregistration forms and checks (payable to The Pennsylvania State University) should be sent to: Agricultural Conference Coordinator, Room 410 J. Orvis Keller Building, The Pennsylvania State University, University Park, Pennsylvania 16802. A large crowd is expected, so advance registration is very important.

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EFFECT OF FERMENTATION TEMPERATURE ON CHANGES IN MEAT PROPERTIES AND FLAVOR OF SUMMER SAUSAGE[1]

J. C. Acton, J. G. Williams[2], and M. G. Johnson
Department of Food Science
Clemson University
Clemson, South Carolina 29631

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Abstract

Changes in several parameters during summer sausage fermentation by *Pediococcus cerevisiae* at 22, 30, and 37 C were followed over a 72 hr period. A decrease in meat pH from 5.9 to 4.6 was significantly correlated with the increase of lactic acid. Significantly less acid was produced at 22 C than at 30 and 37 C. The meat water-holding capacity, as determined by extract release volumes, was significantly affected by time and temperature of fermentation. Lactic acid production was correlated with growth of the added starter culture but maximal amounts of lactic acid were not produced until approximately 24 hr after maximal cell populations were reached. Panel analysis of summer sausage fermented at 22 and 37 C showed that fermentation temperature within this range did not significantly affect product flavor. The importance of this latter finding to meat processors is discussed.

Total sausage production in federally inspected meat plants in 1969 was 3.2 billion pounds. Of this total, 189 million pounds were dried and semi-dried products *(2)*. Fermented sausage products, i.e., thuringer, cervelat, summer sausage, Lebanon bologna, and pepperoni, comprise the largest group of semi-dry or dry sausages. Summer sausage and cervelat originated in Germany and are the most popular of the semi-dry sausages in this country *(17)*.

Traditional processes for manufacture of fermented sausage require approximately 150 hr for fermentation and processing before drying. Fermentation by this method is accomplished by the lactic acid microorganisms present in the flora of the meat constituents as well as those introduced from equipment *(6, 14)*. Product failures because of uncontrolled fermentations are not uncommon *(6, 12)*. Drying sausage to the semi-dry stage (20% shrink) requires 10 to 25 days and to the dry stage (35-40% shrink) requires 60 to 90 days *(16)*.

Starter cultures for fermented sausage manufacture were introduced in 1940 and employed species of the genus *Lactobacillus (16)*. *Pediococcus cerevisiae* was proposed as a starter culture in 1958 by the American Meat Institute Foundation. This culture has since achieved wide acceptance by the meat industry *(5, 16)*. Cultures of this organism are available in lyophilized form or as a frozen concentrate *(5, 6)*. Use of starter cultures has significantly reduced the time required for fermentation from 150 hr for the traditional process to 12 to 15 hr for the process using the frozen concentrate *(5)*. These starter cultures have also aided processors in maintaining uniform product quality from batch to batch.

This study was conducted to evaluate the effect of three fermentation temperatures, 22, 30, and 37 C, on meat pH, acidity, and water-holding capacity over a 72 hr period. Plate counts of total viable bacteria and total lactic acid bacteria in meat fermented at each temperature were determined. Semi-dried product samples from six time-temperature treatments were evaluated for flavor.

Materials and Methods

Sausage fermentation and processing

A summer sausage formula (Table 1) was used in this study. Fresh pork trimmings were coarsely ground through an 8 mm plate and completely mixed into fresh ground beef (4 mm plate) with a Hobart H-600 mixer. Pork trimmings contained approximately 25% fat and ground beef approximately 20% fat. Cure and seasonings were thoroughly blended into the meat mixture before adding the starter culture, *P. cerevisiae,* to a level of 2×10^6 cells/g meat. Total mixing time was 12 min.

The initial meat mixture contained 58.3% moisture, 15.6% protein, and 24.6% fat. This mixture was divided into three batches for fermentation at 22, 30, or 37 C. Each batch was further subdivided into 6 portions: 3 portions of 500 g and 3 portions of 1500 g. These quantities were placed in polyethylene bags, vacuumed, and stored at their respective batch temperature. At 12 hr intervals, one portion from each fermentation temperature was removed for pH, lactic acid, extract release volume, and plate count determinations. The 1500-g packages were sampled at 0, 24, 48, and 72 hr periods; the 500-g packages were analyzed after 12, 36, and 60 hr of storage.

Meat remaining from the 1500-g samples was stuffed into 50-mm diameter fibrous casings (Visking, Union Carbide). These sausage sticks, each weighing approximately 1.3 kg, were stored at 0 C to prevent further fermentation before heat processing 12 to 48 hr later.

The sausage sticks were removed from 0 C storage and

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[2]Participant of Clemson University's Student Science Training Program of 1971 sponsored by the National Science Foundation.

TABLE 1. SUMMER SAUSAGE INGREDIENTS

Ingredient	Quantity
Meats:	
lean beef, boneless	9.072 kg
lean pork trimmings	9.072 kg
Cure:	
sodium nitrite	1.4 g
sodium chloride	498.9 g
sodium erythorbate	8.5 g
Seasonings:	
ground black pepper	45.4 g
ground white pepper	34.0 g
ground mustard	11.3 g
sucrose	90.7 g
dextrose	181.4 g
Starter culture[1]:	
Pediococcus cerevisiae (suspended in 30 ml water)	11.34 g

[1]Registered as Accel by Merck Chemical Division, Merck & Company, Rahway, New Jersey.

TABLE 2. FLAVOR RATINGS OF SUMMER SAUSAGE FERMENTED AT 22 C AND 37 C[1]

Temperature (C)	Fermentation time, Hr 0[2]	24	48	72
22	3.4	4.3	6.0	8.0
37	3.4	4.1	5.9	8.4

[1]Flavor rating scale: 9 = extremely tangy flavor; 1 = no tanginess in flavor. Scores are averages for seven panelists.
[2]Flavor scores for 22 C and 37 C are from the same sample at 0 hr.

initially heated (no smoke) at 93 C for 20 min and then at 98 C until an internal temperature of 68 C was obtained. The product was next placed in a 10 C drying room having 10 to 15 air changes/hr. The air relative humidity ranged from 75 to 85%. After 12 hr of cooling at 10 C, the product was held in a 5 C still air cooler for 48 hr and then returned to the 10 C drying room for 10 days. Composition of the summer sausage and flavor analyses were conducted at the end of this drying period.

pH and lactic acid determinations

At each time interval duplicate 10-g samples of meat from each fermentation temperature were blended for 60 sec with 100-ml quantities of distilled water in an Osterizer. The pH values of homogenates were recorded with a pH meter. The initial meat mixture (0 hr) had a pH of 5.90 and this value served as the titration endpoint in total acidity titrations. Meat homogenates having a pH below 5.90 were titrated with 0.1 N NaOH. Developed acidity was assumed to be due to lactic acid production. The mEq of alkali required to raise the pH to 5.90 were converted to percent lactic acid by multiplying the mEq of NaOH added by 0.902.

Extract release volume

The extract release volume was used as an indicator of the change in the meat water-holding capacity. A procedure modified from Jay *(11)* was followed. Triplicate 25 g samples of meat tempered at 4 C were finely blended with 100 ml amounts of distilled water. Each homogenate was immediately transferred to a 100 mm funnel containing one thickness of Whatman #1 filter paper. The funnel was inserted in a 100 ml graduate cylinder and the homogenate allowed to filter at 4 C for 30 min. The filtrate volume (extract release volume) that had accumulated in the cylinder was measured and recorded.

Plate counts

Counts of total viable bacteria and of lactic acid bacteria were made at 12 hr intervals on samples from each of the three fermentation temperatures. Ten gram samples of meat were blended for 1 min with 90 ml quantities of 0.9% saline and subsequent decimal dilutions were prepared with the same diluent. Duplicate 1 ml samples of the appropriate dilutions were mixed with standard plate count agar *(1)* or lactic agar *(7)*. Plates were incubated at 30 C for 48 to 72 hr before counting.

Sausage composition

Percentages of moisture, fat, and protein were determined for the initial meat mixture (uncooked) and the processed sausage that had been fermented for 24, 48, or 72 hr. Moisture was determined by the AOAC *(3)* method. Ether extractables (Soxhlet) were used to calculate percent fat. The Kjeldahl nitrogen method following AOAC *(3)* was used for protein determinations.

Panel analysis for flavor

Seven panelists familiar with the flavor of fermented meat products rated the degree of flavor development using a 9-point hedonic scale. Flavor was described on the rating scale in degrees of "tanginess," a descriptive term commonly used for fermented meats *(4, 13, 16)*. Panelists were informed that acidity or sharpness of flavor could be described as tanginess. The control sample (0 hr) was evaluated first followed by product samples fermented at 37 C and 22 C for 24, 48, and 72 hr.

RESULTS AND DISCUSSION

pH and percent lactic acid

Fermentation time was a significant factor affect-

TABLE 3. CORRELATION COEFFICIENTS BETWEEN VARIABLES

	pH	% Lactic acid	ERV	TPC	TLAC	Flavor
Time	−0.92**	0.95**	0.80**	0.88**	0.89**	0.98**
Temperature	−0.16	0.16	0.35	0.11	0.16	0.01
pH		−0.95**	−0.82**	−0.88**	−0.89**	−0.90**
% Lactic acid			0.78**	0.83**	0.84**	0.97**
ERV				0.87**	0.88**	0.80*
TPC					0.96**	0.93**
TLAC						0.89**

**Highly significant ($p \leq 0.01$).
*Significant ($p \leq 0.05$).

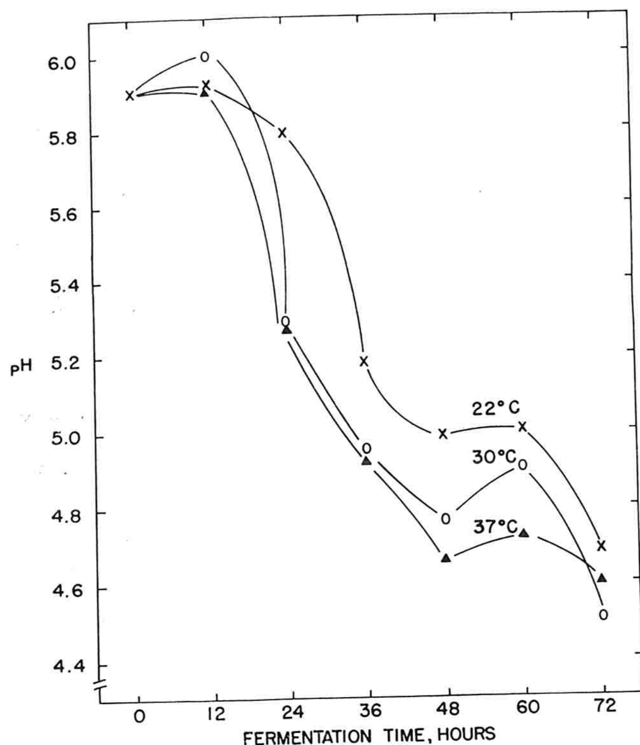

Figure 1. Change of meat pH during fermentation of summer sausage.

ing both the rate of decrease in pH (Fig. 1) and the rate of increase in percent lactic acid (Fig. 2). That the lactic acid produced was responsible for the lowering of meat pH is supported by a significant ($p <$ .05) correlation between these two variables (Table 3). Fermentation of the sausage at 30 or 37 C yielded similar pH values and amounts of lactic acid. However, significantly less acid was produced and pH reduction was not as great when fermentation was conducted at 22 C. The acidities developed after 72 hr at each temperature were within the range of 0.5 - 1.5% generally reported for summer sausage, cervelat, and thuringer *(13)*. The final pH values of 4.5 to 4.7 are within the range of 4.5 to 5.4 usually obtained in these types of products *(16)*.

Extract release volume

As shown in Fig. 3, the extract release volume (ERV) values rapidly increased for the first 36 hr for meat fermented at each of the three temperatures. Both time and temperature of fermentation were significant ($p <$ .01) in their effect on ERV. Overall ERV means for fermentation at 30 and 37 C were significantly higher than the ERV mean for fermentation at 22 C. The fact that protein denaturation is both temperature- and time-dependent *(8, 15)* accounts for the greater water-holding capacity of the meat fermented at 22 C. Subjectively, this denaturation of protein was followed visually by noting the decrease in concentration of soluble muscle pigments. A clearing of the filtrate occurred as the pigment concentration decreased.

A significant ($p <$ .01) correlation also occurred between ERV and pH (Table 3). The continual decrease of pH toward the approximate isoelectric point of actomyosin, pH 5.0, with the simultaneous increase in ERV is in agreement with the water-holding capacity studies reported by Hamm *(9, 10)*.

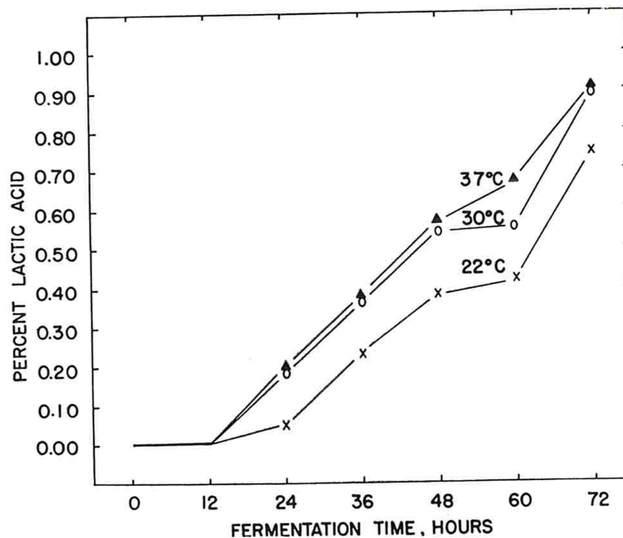

Figure 2. Change in lactic acid concentration of meat during fermentation of summer sausage.

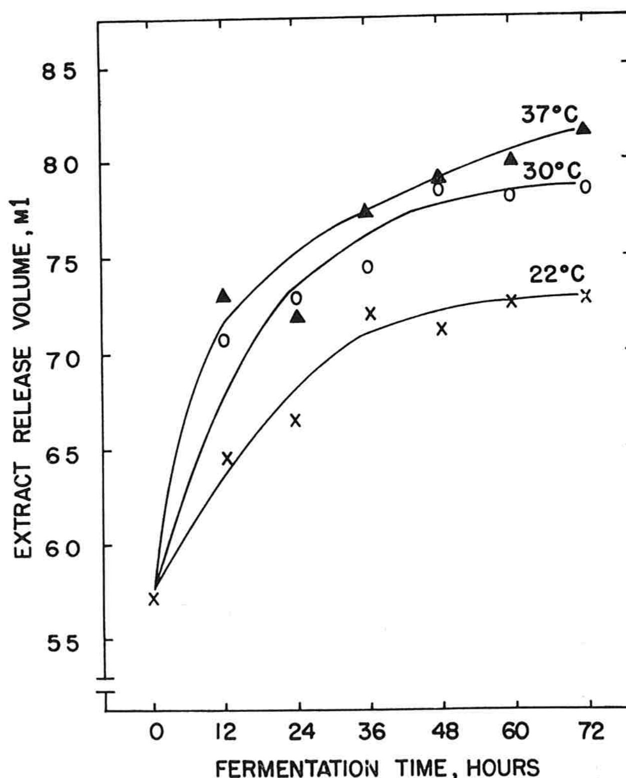

Figure 3. Change of extract release volume of meat during fermentation of summer sausage.

Plate counts

The recoveries of total viable bacteria and total lactic acid bacteria at each fermentation temperature and time interval are shown in Fig. 4 and 5, respectively. Comparison of the data in Fig. 2 and 5 for the 22 C fermentation shows that the delay in lactic acid production correlates well with the delay in growth initiation of lactic acid bacteria at this temperature. The data also indicate that these organisms produced substantial amounts of lactic acid in the 24 hr period after reaching maximal cell populations at about 48 hr. After 72 hr, the organism in colonies isolated from the highest meat dilution plated with either medium was similar to that isolated directly from the starter culture, *P. cerevisiae*, indicating that the latter organism had gained predominance in the sausage meat microflora.

Though the numbers of lactic acid bacteria recovered after 72 hr were essentially the same for each of the fermentation temperatures, substantially less acid was produced at 22 C (0.74%) than at 30 or 37 C (0.89%). This suggests that at 22 C, it would be necessary to extend the fermentation time beyond 72 hr to permit greater lactic acid production by this particular starter culture.

Product composition and flavor analysis

The processed summer sausage had the following composition: 46.9% moisture, 19.4% protein, and 27.3% fat. The reduction in moisture content during the heat processing and drying periods collectively resulted in an increase in both protein and fat content. A shrink or weight loss of 19.6% occurred. Sausage having a 20% shrink is classified as "new sausage" and is ready for marketing 10-25 days after heat processing and drying *(16)*. A moisture loss of approximately 30% would be required to classify the product as semi-dry or medium dry sausage *(16)*.

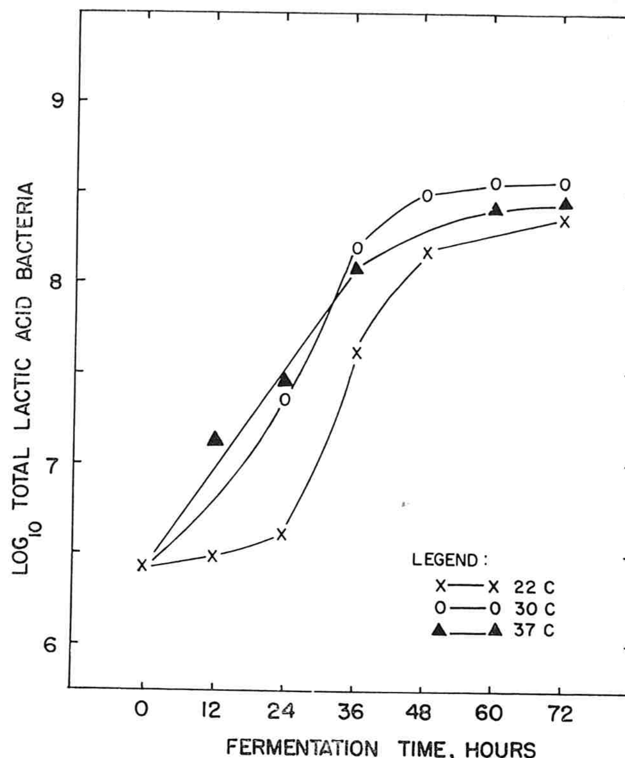

Figure 5. Total lactic acid bacteria counts in meat during fermentation of summer sausage.

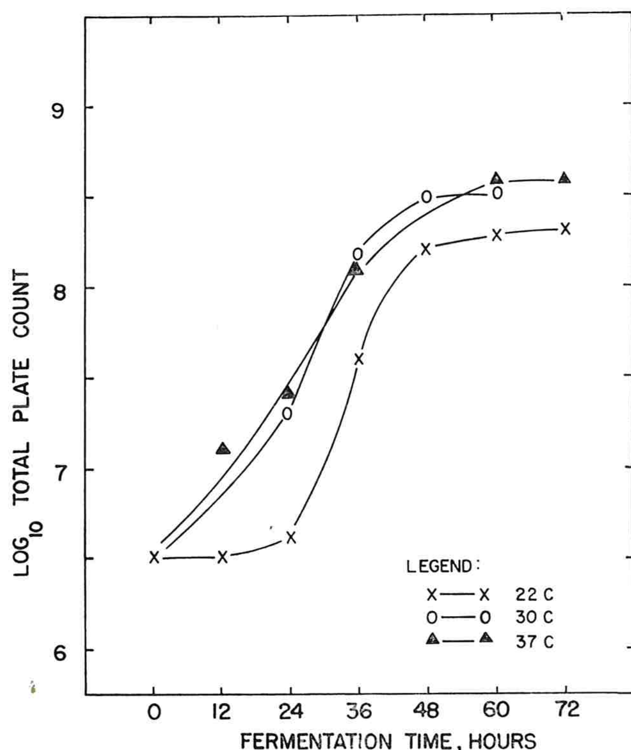

Figure 4. Total plate counts of meat during fermentation of summer sausage.

The degree of product tanginess as rated by panelists is presented in Table 2. The flavor intensity increased as fermentation time increased although there appeared to be little difference between products fermented at 22 or 37 C. It is possible that a trained panel would be necessary to detect minor changes in flavor intensity. A uniform flavor can be achieved with controlled batch fermentations *(14, 16)*. An aging period must follow the fermentation to allow the flavor to "mellow," reducing the harshness found in freshly processed sausage *(13)*.

Correlation between variables

The interrelationships among variables are shown by the correlation coefficients in Table 3. Although many significant correlations were found, the most important observation is that there was little correlation between temperature of fermentation (a factor) and the resultant flavor of the product (variable). This correlation coefficient was low and nonsignifi-

cant. From a practical viewpoint, our data indicate that a meat processor may be able to prepare fermented sausage products at room temperature (22-24 C) and thus eliminate the requirement for heating rooms or excessive time in the smokehouse. However, further research must be conducted to ascertain product safety with regard to the possible growth of undesirable and potentially harmful microorganisms at this lower temperature.

ACKNOWLEDGMENT

The authors thank the Merck Chemical Division of Merck & Company for providing samples of their starter culture, Accel, for this project.

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GROWTH OF *LEUCONOSTOC CITROVORUM* IN SKIMMILK FORTIFIED WITH COBALT, MANGANESE, AND IRON COMPOUNDS[1]

M. C. Goel and E. H. Marth

Department of Food Science and The Food Research Institute
University of Wisconsin, Madison, Wisconsin 53706

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Abstract

Cobalt chloride, manganous chloride, ferric citrate, and ferrous sulfate were tested for their effects on growth of and chain formation by four strains of *Leuconostoc citrovorum*. Chemicals were added to sterile skimmilk before inoculation with test cultures. Numbers of *L. citrovorum* were determined with plate count procedures at periodic intervals during 30 hr of incubation at 22 or 30 C. Smears made concurrently were stained and examined microscopically to determine numbers of cells in chains.

Cobalt chloride at a concentration of 0.1% completely inhibited growth of all strains of *L. citrovorum*, whereas 0.01% retarded growth of three strains, and 0.001% was essentially without effect. Chains of *L. citrovorum* strains exposed to 0.1% cobalt chloride remained fairly constant in length throughout the incubation, probably because the organism failed to grow. Lower concentrations of cobalt chloride had no marked effects on chain length of *L. citrovorum*. Manganous chloride (0.00001-0.001%), ferric citrate (0.001-0.1%), and ferrous sulfate (0.001-0.1%) were essentially without effect on growth of most *L. citrovorum* strains at 22 or 30 C. One strain produced markedly longer chains in the presence of manganous chloride, whereas others behaved similarly but less dramatically. Ferric citrate at the higher concentrations caused somewhat longer chains to be formed when *L. citrovorum* grew at either 22 or 30 C. The organism also tended to form longer chains when skimmilk contained 0.01 or 0.1% ferrous sulfate. This chemical also markedly reduced the generation time of *L. citrovorum* when incubation was at 22 C.

Reports are available on vitamin, amino acid, and mineral requirements of lactic acid bacteria *(1, 3, 5, 10, 13-19, 21-24)*, but only a few experiments have been concerned specifically with effects of minerals on growth of leuconostocs *(10, 13, 18, 22)*. Macleod and Snell *(10)* observed that of eight different lactic acid bacteria, including two strains of *Leuconostoc mesenteroides*, only *Lactobacillus casei* and *Streptococcus faecalis* grew in a manganese-deficient substrate. Growth of one or more lactic acid bacteria examined by these investigators was enhanced by one of the following ions when added to the medium at an appropriate concentration: calcium, manganese, magnesium, iron, nickel, and cobalt. Other reports summarized by Marth *(11)* indicated that: *(a)* acid production by *Streptococcus lactis* and *Streptococcus cremoris* suffered when the medium was deficient in iron, *(b)* cobalt and zinc can replace iron, *(c)* acid production was enhanced by addition of 0.5% sodium chloride to the medium, *(d)* growth of *S. cremoris* was unaffected by potassium nitrate or potassium chlorate but was retarded by potassium persulfate, potassium bromate, and potassium iodate, *(e)* growth of *Streptococcus thermophilus* was somewhat inhibited by low concentrations (up to 16 ppm) of copper, and *(f)* acid production by *Streptococcus diacetilactis* and *Leuconostoc dextranicum* was enhanced when skimmilk contained 1-2% sodium chloride. Fortification of raw or pasteurized milk with potassium chloride and manganese chloride and inoculation with *L. casei* has been claimed to improve the quality of cheese produced from either type of milk *(12)*.

Although growth of *Leuconostoc citrovorum* can be enhanced by adding certain animal by-products or an extract of *Streptococcus lactis* cells to the medium *(8, 9)*, relatively little is known about the response of this bacterium in skimmilk fortified with cobalt, manganese, or iron compounds. All of these substances are known to affect growth of other lactic acid bacteria.

To provide some of the information which is lacking, experiments were conducted to: *(a)* determine how several strains of *L. citrovorum* grow in skimmilk fortified with cobalt chloride, manganous chloride, ferric citrate, and ferrous sulfate, and *(b)* learn if these chemicals cause changes in the length of chains produced by the bacterium.

Materials and Methods

Cultures

Four strains of *L. citrovorum* were used in this study. Sources of the cultures were reported earlier by Goel and Marth *(7)* who also observed that two of the strains (CAF-B and Da 3) formed long chains and the other two (9 and 14) produced short chains when grown in skimmilk at 22 or 30 C. Cultures were handled as previously described by Goel and Marth *(7)*.

Test samples

Skimmilk from the University of Wisconsin dairy plant was autoclaved at 121 C for 15 min; cooled to 22 or 30 C;

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TABLE 1. CHANGE (IN PERCENT[1]) IN GENERATION TIMES OF FOUR STRAINS OF *Leuconostoc citrovorum* WHEN GROWN AT 22 AND 30 C IN SKIMMILK WITH ADDED SALTS.

		Incubation temperature and strain of *Leuconostoc citrovorum*							
		22 C				30 C			
Compound	Concentration (%)	CAF-B	Da 3	9	14	CAF-B	Da 3	9	14
		(%)							
Cobalt chloride	0.001	+6.1	+0.9	−16.3	+41.2	+29.8	+2.3	−2.7	+7.5
	0.01	+21.1	+48.8	+218.8	+271.1	+38.2	+16.0	+212.6	+89.5
	0.1	+976.6	+904.1	+1457.5	+3390.3	+755.4	+464.1	+1185.5	+1445.6
Manganous chloride	0.00001	−2.9	NC	−26.8	−12.2	+2.8	+4.9	−4.3	+18.3
	0.00005	−4.4	+7.9	−20.3	−12.2	−11.2	−6.7	+2.9	+19.0
	0.0001	−14.7	+15.9	−13.9	−28.5	−11.6	+13.2	+3.6	−8.5
Ferric citrate	0.0001	—	—	—	+17.0	—	—	—	−9.4
	0.0005	—	—	—	−3.7	—	—	—	−17.4
	0.001	—	—	−3.6	−4.1	—	—	NC	−5.1
	0.01	—	—	NC[2]	—	—	—	NC	—
	0.1	—	—	−9.7	—	—	—	−4.4	—
Ferrous sulfate	0.001	—	—	—	−4.6	—	—	—	−6.2
	0.01	—	—	—	−14.6	—	—	—	−28.7
	0.1	—	—	—	−31.1	—	—	—	−2.1

[1]Minus value indicates reduction in generation time from that of the control; plus value indicates an increase in generation time over that of the control.
[2]NC = No change.

TABLE 2. CHAIN LENGTH OF *Leuconostoc citrovorum* WHEN GROWN AT 22 AND 30 C IN SKIMMILK WITH 0.0, 0.001, 0.01, AND 0.1% ADDED COBALT CHLORIDE.

		Strain of *L. citrovorum* and percent of additive							
		CAF-B				Da 3			
Incubation temp.	Hours	0.0%	0.001%	0.01%	0.1%	0.0%	0.001%	0.01%	0.1%
		(Avg. no. cells/chain)							
22 C	0	5.2	5.2	5.2	5.2	5.6	5.6	5.6	5.6
	6	8.6	5.2	4.0	6.4	8.2	6.2	10.0	10.2
	10	14.6	8.2	8.0	6.0	8.6	8.8	9.8	8.4
	14	8.2	10.0	8.2	6.6	13.8	8.6	11.6	7.0
	18	8.4	8.0	8.8	6.6	13.0	14.2	15.8	7.0
	22	8.0	7.2	8.4	8.8	13.4	16.6	15.4	7.4
	26	6.2	6.0	8.2	9.0	11.8	11.6	14.6	7.2
	30	6.2	6.2	6.6	8.2	10.4	9.0	9.8	6.8
30 C	0	3.6	3.6	3.6	3.6	5.2	5.2	5.2	5.2
	6	11.0	12.8	9.8	9.2	10.2	11.0	6.4	6.6
	10	16.6	17.6	19.6	10.8	10.2	14.2	11.0	9.2
	14	19.6	15.0	15.0	12.4	10.8	18.0	15.0	8.6
	18	12.6	13.4	9.0	10.6	10.8	12.0	13.0	8.8
	22	8.0	10.0	7.2	9.2	15.6	12.4	13.2	8.6
	26	5.8	6.8	6.4	6.4	8.8	10.4	10.4	7.6
	30	5.0	7.0	5.0	6.4	8.6	9.0	10.4	7.4

fortified separately with sterile aqueous solutions of cobalt chloride, manganous chloride, ferric citrate, or ferrous sulfate to provide desired concentrations; inoculated with 0.25% of a 48-hr old culture of the strain of *L. citrovorum* being investigated; and then incubated quiescently for 30 hr at 22 or 30 C. Samples were removed periodically and viable numbers of *L. citrovorum* were determined using Elliker's lactic agar *(4)* and an incubation of 96 hr at 22 or 30 C; the same temperature was used both for milks and plates. Earlier work by Goel and Marth *(6)* indicated that conventional plate counts satisfactorily estimated numbers of leuconostocs, especially at the higher dilutions. Generation times were calculated from these data as described earlier *(7)*.

Smears of milk samples also were prepared each time a sample was taken for plating. They were stained by the Levowitz-Weber procedure *(20)* and were examined microscopically to determine the number of cells in chains formed by *L. citrovorum*. Cells in ten randomly selected chains from different microscopic fields of a smear were counted and the mean chain length was calculated. Results were analyzed statistically by analysis of variance.

RESULTS

Effects of cobalt chloride

Data which detail the effects of cobalt chloride on growth and chain formation by *L. citrovorum* are shown in Fig. 1 and Tables 1, 2, and 4. Information in Fig. 1 shows that growth of all *L. citrovorum* strains was virtually completely inhibited by 0.1% cobalt chloride and that growth by three of the four strains was markedly reduced when 0.01% of the chemical

TABLE 3. CHAIN LENGTH OF *Leuconostoc citrovorum* WHEN GROWN AT 22 AND 30 C IN SKIMMILK WITH 0.0, 0.00001, 0.00005, AND 0.0001% ADDED MANGANOUS CHLORIDE.

		Strain of *L. citrovorum* and percent of additive							
		CAF-B				Da 3			
Incubation temp.	Hours	0.0 %	0.00001 %	0.00005 %	0.0001 %	0.0 %	0.00001 %	0.00005 %	0.0001 %
		(Avg. no. cells/chain)							
22 C	0	4.0	4.0	4.0	4.0	10.8	7.6	12.4	10.8
	6	5.4	6.0	8.0	7.6	8.4	7.2	9.8	10.8
	10	10.2	9.4	9.4	7.4	11.2	12.0	12.4	11.8
	14	16.6	12.6	11.0	11.2	12.6	11.6	17.6	15.4
	18	17.2	13.0	8.8	11.0	15.4	12.8	15.4	15.4
	22	13.4	11.2	6.6	6.8	16.8	13.4	15.4	14.0
	26	10.6	9.0	6.6	6.0	13.2	13.2	13.0	14.0
	30	7.6	8.4	6.6	6.0	9.8	10.0	9.8	12.0
30 C	0	4.0	4.0	4.0	4.0	12.8	12.8	12.8	12.8
	6	10.8	9.0	9.2	12.4	12.8	12.2	15.4	11.0
	10	13.4	10.2	12.0	15.8	13.6	17.6	28.2	27.4
	14	15.2	13.2	12.2	16.6	13.4	24.2	37.6	34.6
	18	13.6	9.8	11.2	16.2	18.2	24.6	34.4	39.2
	22	9.2	8.2	8.6	11.8	13.8	24.8	33.2	35.2
	26	9.6	8.4	8.2	10.0	12.0	26.8	33.0	31.2
	30	8.2	7.2	7.4	7.8	9.4	25.0	29.4	22.4

was present. Use of 0.001% cobalt chloride caused minimal, if any, changes in growth of the bacteria. Tests done at 30 C (data not shown) yielded results similar to those obtained at 22 C except that the degree of inhibition caused by the intermediate concentration of chemical was less pronounced at the higher temperature. None of the concentrations of cobalt chloride were stimulatory to *L. citrovorum*. Changes in generation times, as recorded in Table 1, further verify that *L. citrovorum* did not prosper when cobalt chloride was present in skimmilk.

Results of an analysis of variance (Table 4) indicated that chain length of *L. citrovorum* was not affected significantly by cobalt chloride. In spite of this, some comments are in order. Chains of all four strains were far more uniform in length when the highest (0.1%) rather than other concentrations of chemical was studied (data for only two strains are in Table 2). Undoubtedly, this occurred because the bacteria failed to grow under these conditions (Fig. 1, Table 1). There was a tendency for several strains to form longer chains at 30 C when the low concentration of cobalt chloride was present (Table 2) but these differences were without statistical significance (Table 4).

Effects of manganous chloride

Concentrations of manganous chloride used in these tests generally enhanced growth of *L. citrovorum* (Fig. 2, Table 1), although some strain variation existed. Growth of the organism, as reflected by generation times, was stimulated more by the chemical at 22 than at 30 C (Table 1). Stimulation at 22 C was directly proportional to the amount of manganous chloride present in the skimmilk but this did not occur when incubation was at 30 C (Table 1).

This compound had a statistically significant effect on the length of chains formed by *L. citrovorum* (Table 4). To be sure, other factors also exerted a significant influence on this parameter of growth. Of the four strains, chain formation by Da 3 at 30 C was most influenced by manganous chloride (Table 3). Longer chains occurred at all concentrations of manganous chloride tested. A less dramatic ten-

TABLE 4. ANALYSIS OF VARIANCE OF RESULTS ON CHAIN LENGTH OF *Leuconostoc citrovorum* WHEN GROWN IN SKIMMILK FORTIFIED WITH COBALT CHLORIDE OR MANGANOUS CHLORIDE.

Source of variation	Degrees of freedom—either additive	Mean square: Cobalt chloride	Mean square: Manganous chloride
Additive (A)	3	1.47	41.59*
Incubation temperature (B)	1	24.53**	690.05**
Incubation time (C)	7	29.13**	76.65*
Strain (D)	3	239.11**	1828.99**
A × B	3	4.41**	116.28**
A × C	21	0.89	18.80
A × D	9	9.58**	68.19**
B × C	7	9.82**	48.81**
B × D	3	8.71**	313.50**
C × D	21	9.88**	64.25**
A × B × C	21	0.702	9.36
A × B × D	9	0.645	36.31*
A × C × D	63	1.30	14.15
B × C × D	21	2.45**	18.68
A × B × C × D	63	2.03	15.47
Experimental error	63	2.03	15.47

*Significant at 0.05 level
**Significant at 0.01 level

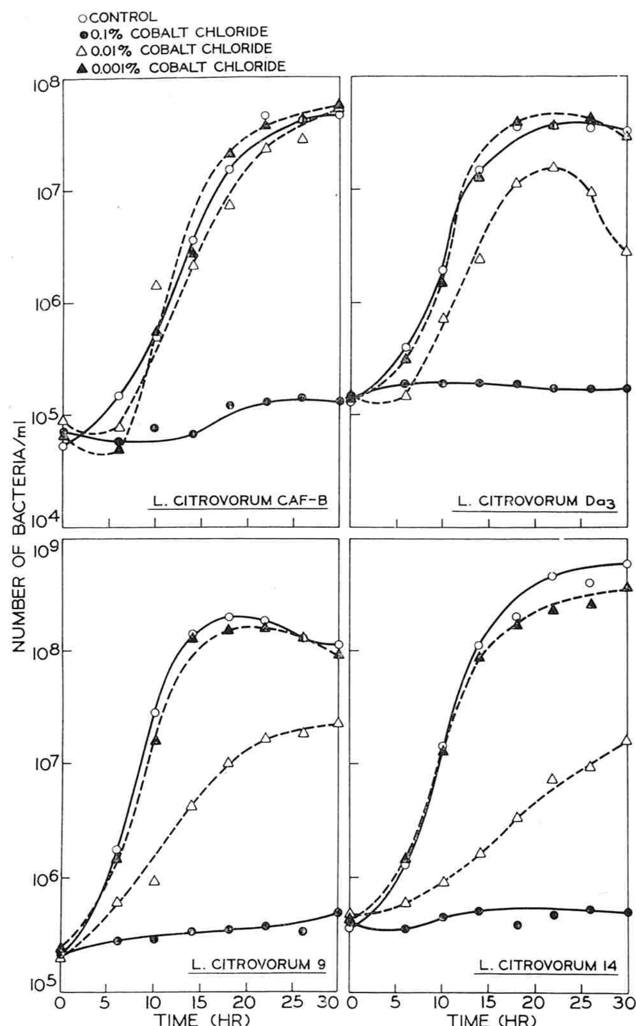

Figure 1. Growth of four strains of *Leuconostoc citrovorum* at 22 C in skimmilk fortfied with various concentrations of cobalt chloride.

dency to form longer chains was exhibited by the other strains at 30 C when the higher concentrations of manganous chloride were used (data for two strains not shown). The tendency of the bacteria to form longer chains was minimal at 22 C, even when the higher concentrations of chemical were present (Table 3).

Effects of iron compounds

Addition of 0.0001 to 0.1% ferric citrate to skimmilk was essentially without effect on growth of two strains of *L. citrovorum* (Fig. 3, Table 1) at 22 C or 30 C (growth curves for 30 C not shown). The additive did have a statistically significant effect on the length of chains produced by *L. citrovorum* (Tables 5 and 6). Chains tended to be longer at 22 and 30 C when the higher concentrations of ferric citrate were used.

Only one strain, 14, of *L. citrovorum* was used to test ferrous sulfate for its effects on growth and chain formation. Data in Fig. 4 and Table 1 suggest that this additive was stimulatory, particularly at 22 C, when the highest concentrations were present. Ferrous sulfate also caused a statistically significant increase in the length of chains formed by the organism (Tables 7 and 8). Formation of longer chains was more evident at 30 C than at 22 C.

DISCUSSION

Data from these experiments indicate that cobalt chloride is unsuitable as an additive to skimmilk if growth of *L. citrovorum* is to be enhanced. In contrast, use of manganous chloride or ferrous sulfate may have some merit, particularly when incubation is at 22 C. Both of these additives were more stimulatory to *L. citrovorum* at 22 C than were either liver concentrate or pancreas extract *(12)*. The same cannot be said for incubation at 30 C. None of the

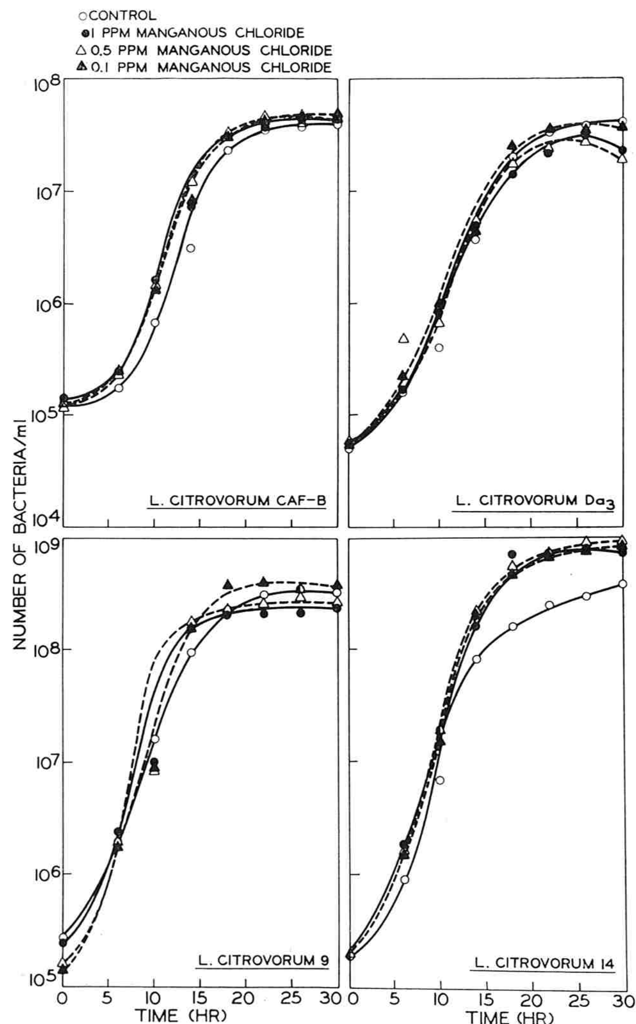

Figure 2. Growth of four strains of *Leuconostoc citrovorum* at 22 C in skimmilk fortified with various concentrations of manganous chloride (0.1 ppm = 0.00001%, 0.5 ppm = 0.00005%, 1 ppm = 0.0001%).

TABLE 5. CHAIN LENGTH OF *Leuconostoc citrovorum* WHEN GROWN AT 22 AND 30 C IN SKIMMILK WITH 0.0 - 0.1% ADDED FERRIC CITRATE.

		Strain of *Leuconostoc citrovorum*							
		9				14			
Incubation temp.	Hours	0.0%	0.001%	0.01%	0.1%	0.0%	0.0001%	0.0005%	0.001%
		(Avg. no. cells/chain)							
22 C	0	3.6	4.8	3.6	3.6	4.8	4.8	4.4	6.0
	6	5.2	5.2	6.2	3.6	7.0	5.6	4.2	7.8
	10	5.2	4.8	6.2	5.8	4.8	4.4	4.6	7.0
	14	4.8	5.0	7.2	6.2	4.2	3.6	4.2	6.2
	18	3.8	3.4	5.8	6.2	4.0	3.4	3.6	3.2
	22	4.0	3.6	6.0	6.2	2.8	3.0	3.2	3.4
	26	3.6	3.2	4.8	4.6	2.8	2.4	2.6	3.2
	30	3.2	3.2	3.6	4.0	2.6	2.4	2.8	3.2
30 C	0	6.0	6.0	6.0	6.0	6.0	5.2	5.2	6.0
	6	9.0	5.6	7.0	8.4	7.0	6.4	8.2	8.2
	10	7.2	6.6	8.0	10.6	8.2	7.6	7.2	10.4
	14	6.8	5.8	6.8	6.6	7.8	6.4	6.4	8.6
	18	5.0	4.6	7.0	5.8	4.6	5.6	6.6	7.8
	22	5.4	4.6	6.8	6.0	4.2	4.6	6.6	4.8
	26	4.0	4.2	6.2	6.2	3.8	3.8	5.2	3.6
	30	4.2	4.2	5.8	5.2	3.8	3.8	4.0	3.6

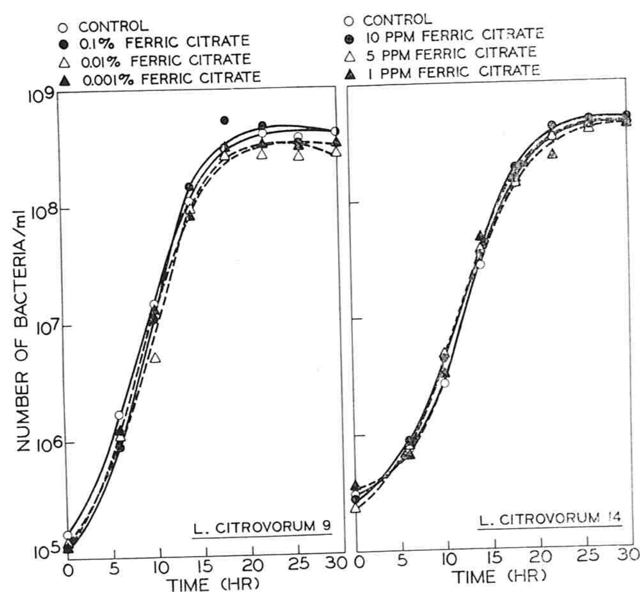

Figure 3. Growth of *Leuconostoc citrovorum* 9 and 14 at 22 C in skimmilk fortified with different concentrations of ferric citrate (1 ppm = 0.0001%, 5 ppm = 0.0005%, 10 ppm = 0.001%).

compounds used in this investigation enhanced growth of *L. citrovorum* as much as was observed when skimmilk was fortified with an extract of *Streptococcus lactis* cells *(9)*.

Manganous chloride, as reported in this paper, and pancreas extract, as reported earlier *(8)*, more effectively caused longer chains to be produced by *L. citrovorum*, particularly strain Da 3, than did liver concentrate *(8)*, an extract of *S. lactis* *(9)*, cobalt chloride, ferrous sulfate, or ferrous citrate. Chain formation, of course, is also affected by the strain of *L. citrovorum*, incubation temperature, and time of incubation.

It is generally recognized that lactic acid bacteria grow better in the presence of manganese than in its absence. In spite of this, only a minimal improvement in growth was observed in these experiments when manganese was added to skimmilk. This does not necessarily mean that *L. citrovorum* can grow adequately without manganese. Instead, it suggests that skimmilk contains virtually enough manganese to supply the needs of the organism. The manganese content of milk ranges from 7 to 34 μg/liter with 20 to 30 μg/liter as the most frequently reported values *(2)*.

Since lactic acid bacteria grow anaerobically, do not contain cytochrome, and are catalase-negative,

TABLE 6. ANALYSIS OF VARIANCE OF RESULTS ON CHAIN LENGTH OF *Leuconostoc citrovorum* 9 AND 14 WHEN GROWN IN SKIMMILK FORTIFIED WITH FERRIC CITRATE.

Source of variation	Degrees of freedom—either strain	Mean square	
		L. citrovorum-9	*L. citrovorum-14*
Additive (A)	3	7.27**	1.78*
Incubation temperature (B)	1	35.10**	68.06**
Incubation time (C)	7	6.48**	14.26**
A × B	3	0.58	0.98
A × C	21	0.73	0.52
B × C	7	1.24	1.99**
A × B × C	21	0.63	0.51
Experimental error	21	2.24	0.51

*Significant at 0.05 level
**Significant at 0.01 level

TABLE 7. CHAIN LENGTH OF *Leuconostoc citrovorum*-14 WHEN GROWN AT 22 AND 30 C IN SKIMMILK WITH 0.0, 0.001, 0.01, AND 0.1% ADDED FERROUS SULFATE.

Incubation (Hr)	22 C				30 C			
	0.0%	0.001%	0.01%	0.1%	0.0%	0.001%	0.01%	0.1%
	(Avg. no. cells/chain)							
0	3.6	3.6	3.6	3.2	4.4	5.4	2.8	4.0
6	4.6	5.2	5.2	5.4	5.8	6.8	6.8	8.2
10	6.2	4.6	5.4	5.6	6.0	9.0	8.6	20.0
14	3.2	4.6	7.0	6.6	6.0	9.0	8.2	14.2
18	3.2	3.8	6.4	5.6	6.4	7.8	10.4	12.0
22	2.6	3.6	3.8	5.6	7.0	8.0	6.2	10.0
26	2.6	3.8	3.0	4.8	5.2	7.6	5.8	10.0
30	2.8	3.4	2.6	4.6	4.0	7.8	5.4	7.2

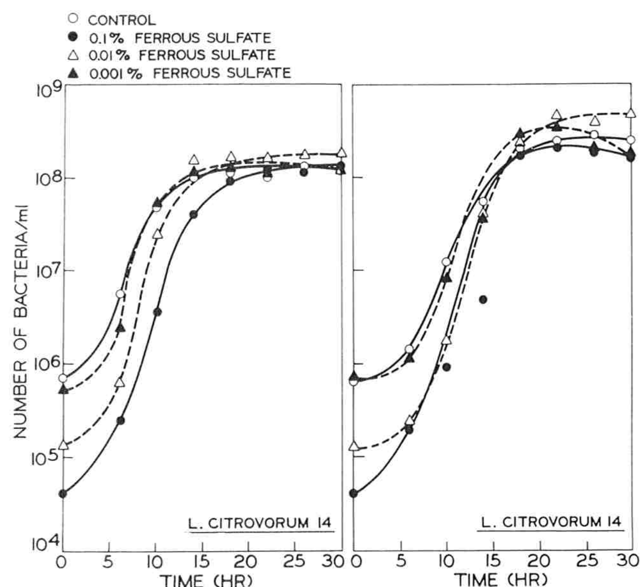

Figure 4. Growth of *Leuconostoc citrovorum* 14 in skimmilk fortified with different concentrations of ferrous sulfate. Curves on the left drawn from data obtained when incubation was at 30 C; those on the right from data obtained at 22 C.

it might be expected that iron, beyond a certain minimal requirement, would have little effect on the growth of *L. citrovorum*. This certainly was true when iron was supplied as ferric citrate. However, addition of ferrous sulfate enhanced the rate at which the bacterium grew even though maximum populations in skimmilk fortified with the additive were not too different from those attained in plain skimmilk.

TABLE 8. ANALYSIS OF VARIANCE OF RESULTS ON CHAIN LENGTH OF *Leuconostoc citrovorum*-14 WHEN GROWN IN SKIMMILK FORTIFIED WITH FERROUS SULFATE.

Source of variation	Degrees of freedom	Mean square
Additive (A)	3	31.02**
Incubation temperature (B)	1	176.22**
Incubation time (C)	7	16.29**
A × B	3	10.73**
A × C	21	2.93
B × C	7	4.67
A × B × C	21	2.36
Experimental error	21	2.36

*Significant at 0.05 level
**Significant at 0.01 level

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EATON E. SMITH

Eaton E. Smith has announced his retirement from the State of Connecticut, Department of Consumer Protection, with an effective date of July 1, 1972.

Mr. Smith has been an employee of the State of Connecticut for approximately thirty years, having entered State service in 1943 with the then Dairy and Food Commission which was succeeded by the Food and Drug Commission in 1947; and later the Department became the first "Department of Consumer Protection" in the country, in 1959.

Mr. Smith was a Field Food Inspector from 1943 to 1956; Senior Food Inspector from 1956 to 1962; Chief of the Food Unfair Sales Practices Division from 1962 to 1968; and he was appointed Director of the Food and Drug Division in 1968.

During several years Mr. Smith was assigned to the inspection of wholesale frozen desserts plants, not only within the State of Connecticut, but also those plants who shipped into Connecticut from such states as Massachusetts, New York, New Jersey, Pennsylvania, Rhode Island, New Hampshire, Vermont, and Maine.

Eaton E. Smith is a long-time member of the Connecticut Association of Dairy and Food Sanitarians, and has served as President. He is also a member of the International Association of Milk, Food and Environmental Sanitarians, and served on committees of International and also appeared on the programs of International's annual meetings.

Mr. Smith served in the Connecticut Department of Consumer Protection under eight Commissioners. He is a Registered Sanitarian, a Past President of the Association of Food and Drug Officials of the United States, and a member of the New York Conference of Health Officers and Food and Drug Officials. He served as Secretary for many years in the New England Association of Food and Drug Officials.

Mr. Smit is a member of the Connecticut Environmental Health Association, the Connecticut Advisory Committee of Food and Drugs, the Institute of Food Technologists—Nutmeg Section, New York Milk and Food Sanitarians, Rhode Island Milk and Food Sanitarians and Public Trustee of the Food and Drug Law Institute.

Eaton E. Smith served as a member of the New Foods Panel, White House Conference on Food, Nutrition and Health, and served for several years on the Food Standards Committee of the U. S. Food and Drug Administration.

Mr. Smith has been active in community affairs in the town where he resides, East Hampton, Connecticut, having served that community as Town Treasurer for three terms during 1937 to 1943. He is a 32nd degree Mason, and a member of Christ Church Episcopal.

Mr. Smith resides with his wife, Gladys, in East Hampton, Connecticut, and they have three sons—one, Warren (married), resides in Sherwood, Oregon, and is presently the Mayor in that community, and he is in the insurance business in Portland, Oregon. Herbert is a career man in the Air Force, residing in Honolulu, Hawaii. Son Leonard is with I.T.T. in Chicago, Illinois, with his wife and son—Mr. Smith's grandson.

Mr. Smith expects to do some traveling with his wife Gladys, and also plans to do some part-time consultant work during his retirement.

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COMPOSITION CONTROL IN CONTINUOUS BUTTERMAKING OPERATIONS[1]

VERNAL S. PACKARD, JR.

Department of Food Science and Industries
University of Minnesota, St. Paul 55101

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ABSTRACT

In a survey of four continuous churning operations, composition of butter from the printer was found to average 0.11% lower in moisture and 0.18% higher in butterfat than butter from the churn. Curd content as measured by difference, averaged 1.2%. When operating under constant adjustment, churn-induced composition variability, as expressed by standard deviation, was, ± 0.087%, for moisture, and ± 0.058% for salt. Expressed in the same terms, analytical error was found to be ± 0.074% and ± 0.015%, respectively. The test procedure used was a modified Kohman analysis. Suggestions for improved composition control are presented.

Continuous churning of butter at high production rates has placed new stresses on conventional methods of composition control. Earlier work *(1)* showed that some improvement in method precision could be obtained by a slight modification of techniques. Even so, the need for continuous monitoring devices is great. In the absence of such devices improved control must be achieved through statistically validated sampling and testing techniques. Development of this type of control program requires statistical verification of analytical error and machine variability as well as an understanding of the nature and extent of stress periods. In continuous butter manufacture stress periods occur immediately following shutdowns, both for cleaning and sanitizing and for mechanical failure either in the churn or elsewhere in the process. They also occur during the shift from an emptied storage tank of cream to a full one.

Beyond the above considerations, there is also the question of the influence of soft butter printing on composition. It is the finished, packaged product that must meet legal standards and a composition control program must, therefore, take into account compositional changes that occur during packaging.

The work reported herein was done in an attempt to analyze the above factors with thought to improving composition control in continuous buttermaking.

METHODS AND MATERIALS

Sampling procedure

On-line samples of butter were collected directly from the churn and from the printer at four commercial continuous buttermaking operations. Churn samples were taken by spatula, the column of butter sliced open and "core" butter scooped from the interior of the column. This butter was placed in screw-cap glass containers, the caps were tightened solidly, and masking tape was wrapped around the caps in several thicknesses to secure the seal.

When churn samples were taken, a quarter-pound stick of printed butter was obtained from the printer. These sticks were overwrapped several times with heavy aluminum foil to prevent dehydration.

All samples were refrigerated at 35-40 F until removed for test purposes. As an added precaution, samples, once cooled, were stored in an air-tight insulated container.

In all instances samples were taken at 10-min intervals and in two operations the sampling schedule bracketed known periods of anticipated stress, i.e., a conversion to a fresh tankful of cream, and a brief shutdown for cleanup of processing equipment other than the churn.

It should be noted that samples obtained from the churn and printer cannot be secured as paired samples. A hopper (from which the printer is fed) is located between the two processing units and may contain as much as 400 lb. of butter. At production rates of 4000 lb. per hour, butter in the hopper would clear the equipment in approximately 6 min. Since samples were collected at 10-min intervals over periods of 1.5-4.5 hr, the influence of this lag period on averages reported herein should be slight.

Kohman procedure

Butter in screw-cap jars was tempered and sampled from the jars. Quarter-pound prints were first sectioned, a sufficient quantity for three analyses placed in a screw cap jar, and the lid tightened solidly for tempering purposes. All samples were allowed to temper at 20 C in a constant temperature room. They were tempered overnight or for at least 4 hr before mixing with a spatula to a semi-plastic consistency, and sampling. Each lot was mixed only once, initially, and not between extractions of analytical size portions.

Three replicate determinations were made on each sample by the USDA modified Kohman procedure for butter analysis *(2)*. Samples were weighed on a torsion balance (Torbal ET-1, The Torsion Balance Co., New York) having a sensitivity of ± 1 mg on a 10-g sample. Butterfat was extracted three times using 50-ml portions of petroleum ether and the entire residue of salt titrated using 0.4277 N silver nitrate and 10 drops of 5% potassium chromate indicator.

Duplicate salt determinations were also made by the direct titration method on 5-g samples. The butter, after weighing, was melted in approximately 75 ml of warm distilled water, 6 drops of 5% potassium chromate (or 5 drops of a 0.1% solution of dichlorofluorescein in 70% ethyl alcohol) were added, and the mixture titrated with 0.4277 N silver nitrate. In all instances, silver nitrate was dispensed from a 10 ml burette.

[1]Paper No. 7864 Scientific Journal Series, Minnesota Agricultural Experiment Station, St. Paul.

TABLE 1. GRAND MEANS[1] OF MOISTURE, BUTTERFAT, SALT, AND CURD[2] ANALYSES OF CHURN AND PRINT BUTTER SAMPLES FROM FOUR CONTINUOUS CHURNING OPERATIONS

Plant	Moisture (%)			Butterfat (%)			Salt (%)			Curd (%)		
	Churn	Print	(Diff.)	Churn	Print	(Diff.)	Churn	Print	(Diff.)	Churn	Print	(Diff.)
A	16.35	16.29	(−0.06)	80.38	80.47	(+0.09)	2.06	2.01	(−0.05)	1.24	1.18	(−0.06)
B	16.33	16.21	(−0.12)	80.22	80.48	(+0.26)	2.07	2.05	(−0.02)	1.45	1.26	(−0.19)
C	16.45	16.24	(−0.21)	80.56	80.80	(+0.24)	1.92	1.90	(−0.02)	1.07	1.08	(+0.01)
D	16.11	16.04	(−0.07)	80.58	80.71	(+0.13)	2.10	2.07	(−0.03)	1.20	1.17	(−0.03)
Grand Avg.	16.31	16.19	(−0.11)	80.43	80.61	(+0.18)	2.03	2.00	(−0.03)	1.24	1.17	(−0.06)

[1]Means were calculated from triplicate analyses of samples taken at 10-min intervals. The total number of samples analyzed, including both churn and print samples, was: plant A (60), plant B (70), plant C (30), plant D (14).
[2]Curd values were obtained by difference.

RESULTS AND DISCUSSION

Differences in composition between butter at the churn and printed butter for the four commercial operations are shown in Table 1. In all instances some moisture loss occurred during printing, resulting in higher butterfat percentages. Loss in moisture varied from 0.06% to 0.21% and averaged, for the four operations, 0.11%. For this reason, composition changes during printing should be considered in a composition control program.

Figure 1 represents graphically the kind of composition control exhibited in one plant before, during, and after conversion to a new batch of cream. In particular, it may be seen that the butterfat test increased markedly and more than an hour transpired before the system was brought under reasonable control. The same kind of problem developed at a second plant during a 45-min interruption in processing as shown in Fig. 2. It seems evident that conditions during an interruption of flow tend generally to cause low moisture and high butterfat test results.

It must be emphasized that part of the variability in control indicated in Fig. 1 and 2 is caused by analytical error, not entirely by machine variability. To distinguish between the two variables, time periods were selected when the machines were running under constant adjustment and an analysis of variance determined on the data. A total of nine such periods, with five observations per period, was selected. These statistics are summarized in Table 2.

The most meaningful values in Table 2 are those shown for moisture and salt. It is these two components which are measured in commercial practice. (Curd is assumed constant at 1.4%, and butterfat is obtained by difference.) First, it may be noted that machine variability and test error are nearly the same for moisture. As related to composition control, adjustment of machines based upon one moisture analysis would appear unjustified. This would be

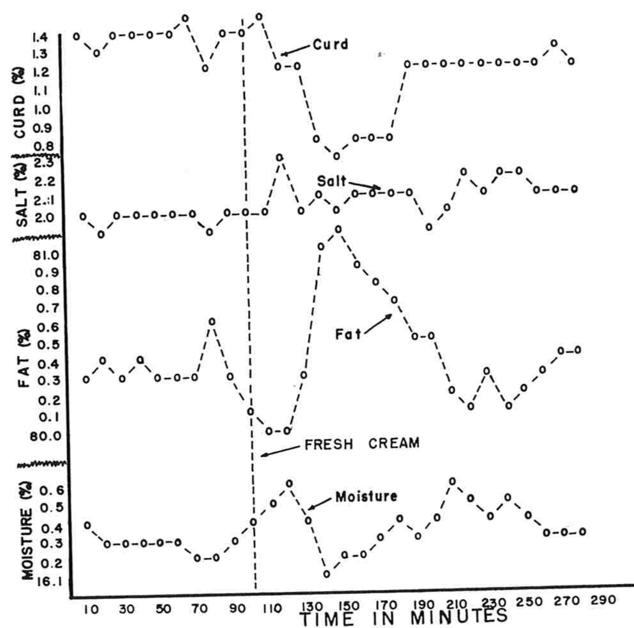

Figure 1. Composition control in continuous buttermaking before, during, and after conversion to a new batch of cream.

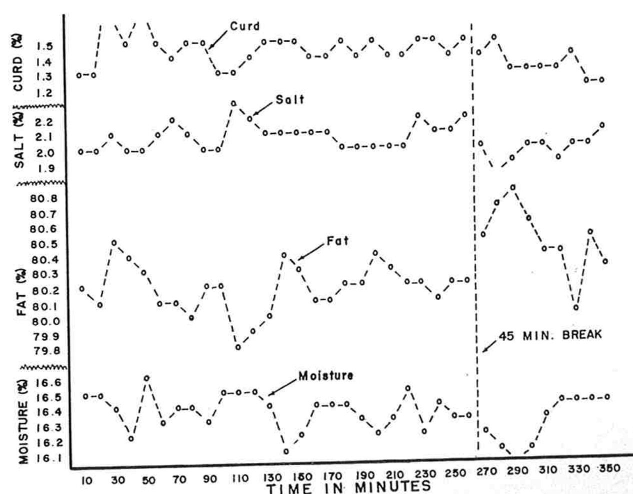

Figure 2. Composition control in continuous buttermaking before and after an interruption in cream flow.

TABLE 2. MEAN SQUARE VALUES OF AN ANALYSIS OF VARIANCE OF CONTINUOUS BUTTERMAKING MACHINE VARIABILITY AND ANALYTICAL ERROR[1]

Butter component	Machine variability (Mean square)	Standard deviation	Method variability (Mean square)	Standard deviation
Moisture	0.0077	±0.087	0.0055	±0.074
Butterfat	0.0268		0.0083	
Salt[2]	0.0034	±0.058	0.00023	±0.015
Salt[3]			0.00017	±0.013
Curd	0.0164		0.0058	

[1]A total of 45 samples taken from four commercial butter plants was analyzed in triplicate.

[2]Salt determinations were made by the conventional USDA modified Kohman procedure.

[3]Salt determinations were made by direct titration, in butter-water mixture, using potassium dichromate as indicator.

especially true if the Kohman balance were used. Previous work *(1)* has shown the standard deviation of moisture analyses to be approximately 0.14% for this balance, or more than one and one-half times as variable as the Torbal ET-1 balance. To put it another way, the buttermaking machine itself is a significantly smaller variable than the test now being applied for control purposes.

While a direct salt analysis may be biased *(3)*, data in Table 2 indicate that the test procedure is at least more precise than the system being monitored. To that extent a single analysis made routinely would likely suffice, and machine adjustments based on that analysis would be justified.

In this work duplicate direct titration salt analyses of 172 samples of both churn and print butter, using potassium dichromate as indicator, averaged 0.065% higher than salt analyses by the conventional procedure. When dichlorofluorescein indicator was compared with potassium dichromate on 48 samples of butter analyzed by direct titration, results averaged 0.022% lower.

This and earlier work seem to suggest some ways for improving composition control in continuous buttermaking. First, a balance more sensitive than the Kohman balance should provide better precision in both moisture and salt analyses in which sample weight is a variable. Secondly, because test error is nearly as great, if not greater (depending upon the balance used) than machine variability in moisture control, duplicate moisture determinations should be made, the results averaged, and adjustments on the machine made only when averages indicate the necessity to do so. Adjustments based on single analyses are not justified. On salt analysis a single direct titration of a 5-g sample would appear to be adequate, although the positive bias of direct titrations should be accounted for, and some improvement could be expected from use of dichlorofluorescein indicator. Thirdly, testing should be concentrated around known stress periods. Whenever new cream is being introduced, or an interruption in flow has occurred, moisture analyses, in duplicate, made continuously, followed by appropriate machine adjustments, could help reduce excessive butterfat overages. Moreover, the machine should not be considered in adjustment until at least two series of moisture tests indicate the desired moisture level. If a good job of adjustment is done, the machines are able to hold reasonably uniform composition for at least one-half hour, if not 1 hr, depending on hydraulic oil temperature changes that can take place during initial start-up. After the systems are brought under control, routine testing can continue, preferably at 0.5-hr intervals, and certainly at hourly intervals. Routine testing, at a minimum, should include duplicate moisture analyses and single direct salt titrations. Also, tighter control appears possible, after the machines are brought into adjustment, when sampling includes printed butter as well as butter at the churn.

ACKNOWLEDGEMENTS

The author is indebted to Dr. Frank Martin of the University of Minnesota for his counsel in programming the statistical data. He is also appreciative for the assistance by Mrs. Elizabeth Jangula and Mr. Mark Titus in the analytical work performed in this investigation.

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AUTOMATION IN THE DAIRY LABORATORY

G. H. Richardson
Department of Nutrition and Food Sciences
Utah State University
Logan, 84321

Abstract

Recent developments in dairy laboratory instrumentation are reviewed. Microbiological, physical-chemical, and mastitis testing instrumentation, potentially valuable in providing milk payment and quality information, are receiving regulatory approval. The economics associated with some of these devices suggest the need for development of third party, central milk and food testing laboratories.

Automation involves the technique of making an apparatus, a process, or system operate automatically thus freeing human operators for other tasks. We pursue dairy laboratory automation in order to save time and obtain more accurate data at less expense in the long run. Modern automation usually suggests computerization. Many of the systems available for use in laboratories can be interfaced with small, low cost computers or, through data handling systems, to larger or remote computers.

Complete automation, wherein all functions are handled by instrumentation in the laboratory is yet in the future. Proper sampling, mixing, and preparation still require manual steps. Samples still need to be transferred manually to sampling devices and identified to the instrumentation through data keyboard or tape reading systems. Some modules on new instruments increase the manual steps required for assay and can therefore be eliminated from the original purchase. The systems described herein must therefore be considered partially automated.

There are mixed emotions when automation is discussed (Fig. 1). One individual, fearful of losing his job, carefully flooded the electronics of one dairy test instrument to assure its failure! Relative cost figures cause the greatest concern. How many samples, at what cost, are needed to make the investment practical? How long should we amortize? What personnel changes will this create?

The cost of many instruments now suggests the need for central, third party testing facilities capable of handling thousands of samples from dairy herd improvement, dairy processing, and regulatory sources. This concept has worked beautifully in Ontario, Canada where virtually all milk in the province is now tested in a central laboratory *(30)*.

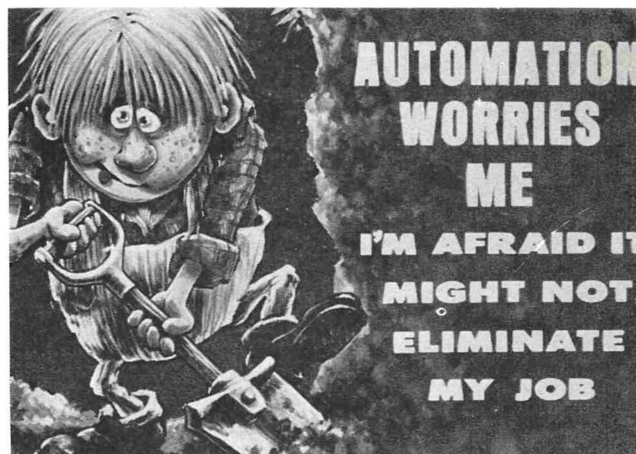

Figure 1. One reaction to the threat of automation.

Microbiological and physical-chemical assays are routinely conducted in dairy laboratories. These are discussed in separate sections.

Microbiological Assays

The plate loop count method *(33, 34)* for determining viable bacterial counts of raw milk has received good acceptance. This method provides an estimate from 3,000 to over 300,000 *(34)* organisms per milliliter while eliminating the need for numerous transfer pipettes and dilution blanks. By substituting a 0.01-ml cylinder *(15)* for the 0.001-ml loop a lower estimate can be obtained on pasteurized milk products. These devices have been incorporated into an automatic system recently described by Bradshaw et al. *(9)*. Milk containers and divided petri plates are alternately positioned onto a turntable (Fig. 2) and the petri dish lid is removed. The loop and cylinder are dipped three times at a fixed speed into the milk samples then the sample is rinsed into the divided petri sections using a 1.5 ml rinse measured by a Brewer[2] pipetting machine. Tempered agar is similarly added, the lid is replaced and the plate is cooled in 72 sec on a cold plate. This device automatically plates milk samples at 5 per minute. The authors reported a log variance of 0.0018 for the standard plate count and 0.0031 for the automated

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[1]Presented at the 58th Annual Meeting of the International Association of Milk, Food and Environmental Sanitarians, San Diego, California, August 15-19, 1971.

[2]Mention of products or companies does not constitute endorsement by Utah State University over comparable products.

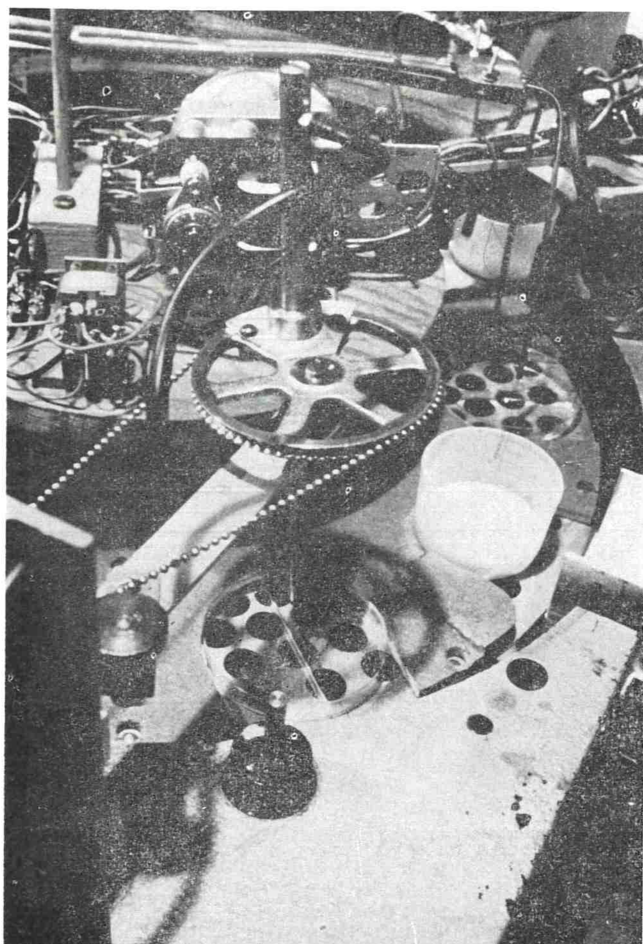

Figure 2. Automatic device for preparing plate counts from milk samples.

procedure.

Buchler Instruments has announced the Buchler Autofill 600 *(10)*. This device can dispense media into petri plates, cool, and stack the plates at 600 per hour. Such a prepoured plate would be valuable for use in the instrumental method for plating and counting aerobic bacteria recently described by Campbell and Gilchrist *(12)*. This system employs a mechanical sample distribution apparatus that resembles a small phonograph where the needle moves from the center to the outside edge of the record. The special "needle" tube contains the milk sample and the "record" is the open petri dish containing pre-cooled agar. The milk is dispensed from the sample tube onto the dry agar surface in an Archimedes spiral as the petri plate is rotated. Following incubation high count milk will produce crowded colonies near the center of the plate, however, an estimate can be obtained by counting < 100 colonies near the edge. (Fig. 3). Between sample contamination is eliminated in this system, therefore, it is possible to obtain good estimates from 10^2 to 10^7 organisms per milliliter. A modified counting grid on a Quebec colony counter allows rapid enumeration, however, a laser beam system is being evaluated to automate the counting step.

American Instrument Co. recently introduced the Petri-Scan *(1)*. This device substitutes a sensitive videcon system for the human eye (Fig. 4). It counts petri dish or membrane colonies down to 0.2 mm in diameter and the results are digitally displayed in 2 sec. Advantages claimed for this instrument include; shorter incubation times are possible, more colonies per plate can be counted, fewer dilutions are required to obtain a countable dish, instrument adjustments can be made to reduce the counting of artifacts, and the $5,000 unit can also count plaques.

Burns *(6, 11)* reported on Abbott's automatic zone reading system for antibiotics assay work. This will probably find more application in those laboratories routinely screening hundreds of antibiotics daily.

Microbiologists at the Brigham Young University have recently been awarded a grant to evaluate colony counting instrumentation using energy emission from the developing colonies. The release of $^{14}CO_2$ from glucose has also been correlated with count *(6)*.

Grace Picciolo reported on a spin-off development from the Goddard Space Flight Center life detection research. *(4, 23)*. Using the firefly luciferinase reaction it is possible to measure very small quantities of adenosine triphosphate (ATP). The reaction involves production of light which can be measured and correlated with bacterial count. Dr. Picciolo described the possibility of measuring ATP from bacterial cells in urine, exclusive of the ATP in other body cellular material. Similar claims have been made for the Du-Pont Luminescence Biometer *(16)*. This raises the possibility that total ATP from leucocytes and bacterial cells can be quantitated, then the ATP from the bacterial cells quantitated alone, and the difference would be an indication of leucocyte ATP con-

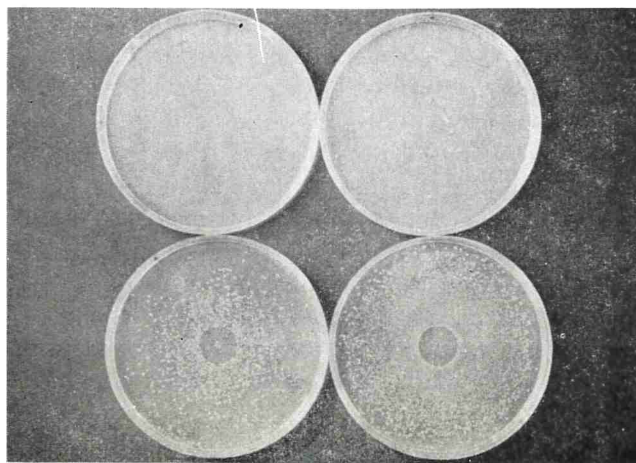

Figure 3. Typical plates prepared using the Bradshaw and Gilchrist apparatus *(12)*. Upper left, control; upper right, 1,000 organisms/ml; lower left, 50,000/ml; lower right, 1,000,000/ml.

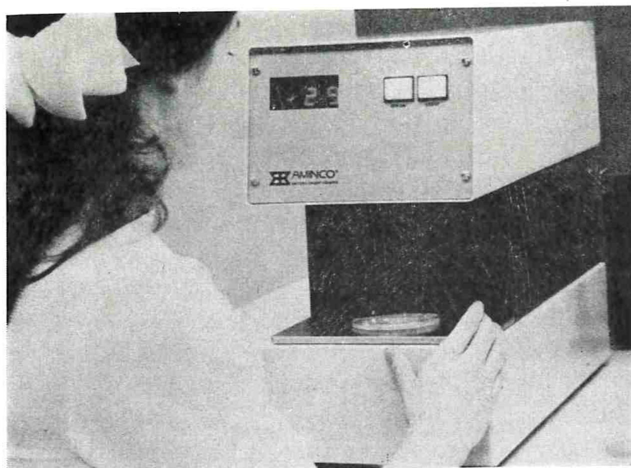

Figure 4. The Petri-Scan in operation.

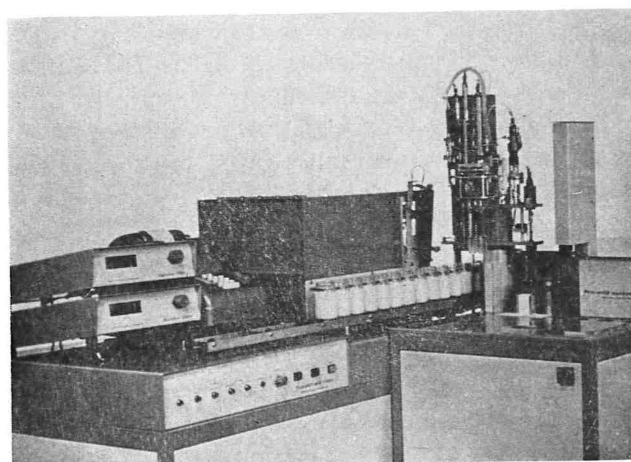

Figure 5. The Milko-Tester Automatic.

tent.

Technicon has recently entered the automated microbiology field with the Automatic Anti-Microbial Susceptibility System (TAAS) *(27)*. The unit is designed for hospital clinic laboratories and will predict the best possible antibiotic treatment for a patient within 3 hr of colony isolation of the organism involved. A specially designed laser differential photometer has been developed by Science Spectrum Inc. which can produce similar results in minutes *(7)*. These devices should provide diagnostic help for veterinarians concerned with more effective mastitis therapy.

The Microtiter system has been successfully applied to preparing milk dilutions, enumerating viable bacteria in milk, and to the MPN technique *(18, 19, 20)*.

Physical-Chemical Assays

Automation of the Babcock and Mojonnier tests has gone about as far as it can go. Newer test devices hold much more promise than these classical fat tests.

The Milko-Tester turbidometric approach *(31)* to fat measurement has received AOAC approval and approximately 200 instruments are being used in the United States. Shipe *(28)* is working with Milko-Gel-like preparations which are needed to assure proper reproducibility of this system. A similar device is now marketed through Fiske Associates Inc. and is called the Fiske Fat Tester Lactronic (F. E. Wing Jr., *personal communication*).

Protein tests are simplified through partially automated dye binding methods. The Udy acid orange-G test *(21)* has AOAC approval. Foss Electric Co. of Denmark has an amido black dye binding system called Pro-Milk II. Fiske Associates Inc. have also recently introduced a similar system, the Fiske Prot-O-Mat *(17)*, which replaces the filtration step with centrifugation. The Fiske devices are currently under study by W. F. Shipe at Cornell University.

The Pro-Milk and Milko-Tester concepts have been combined in the Milko-Tester Automatic (MTA) (Fig. 5). This unit takes dichromate preserved and tempered samples, mixes them and evaluates them for protein and fat at 180 samples per hour. H. F. MacRae *(28)* reported that two units have tested over one million samples in the Canadian Dairy Herd Anaylsis Service (DHAS). The two units can assay over 50,000 samples per month with a laboratory staff of four technicians and one supervisor. A 1969 comparative study revealed a ±0.03% instrument standard deviation. The difference between the MTA and Mojonnier method was ± 0.07%. With equipment amortized in 5 years, and including labor, the fat and protein costs per sample for the MTA were slightly over 10 cents per sample.

Winder has revealed the development of the Mark V Darison Solution Analyzer *(29)* (Fig. 6). Using ultrasonic energy, this unit can test fat and solids-not-fat non-destructively. The device is much smaller than previous models, thanks to space-age electronics. Fifty units are being assembled for sale at approximately $9,000 each. This unit has additional potential for in-line analysis and process control. It has already found several applications in non-dairy plant operations.

Continuous flow analysis, as developed by the Technicon Corporation, has been applied to over 500 wet chemistries. Units are available for testing blood for over 18 simultaneous parameters. Over 20,000 Auto Analyzer systems are in use in hospital clinical laboratories. The possibilities for use in the food laboratory are limited mainly by the imagination of the designing chemist. Figure 7 shows the Auto Analyzer II on loan for milk fat collaborative studies at Utah State University. Through addition of two more channels, the unit could measure protein

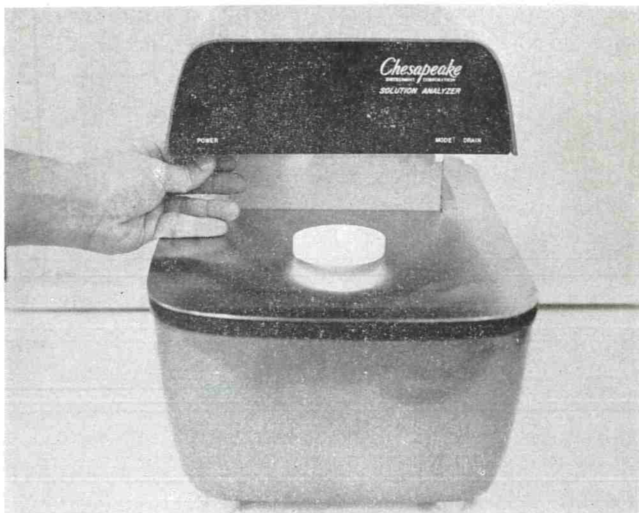

Figure 6. The Mark V Darison Solution Analyzer.

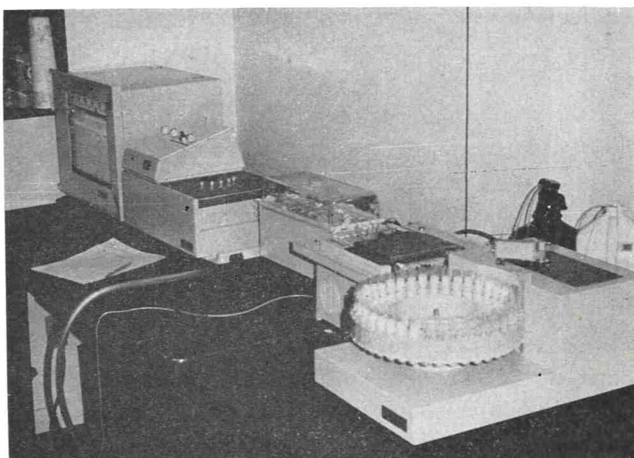

Figure 7. The Auto Analyzer II milk fat system.

and lactose simultaneously, and provide digital printout for a $25,000 capital investment. Systems are also available for testing alkaline phosphatase and somatic cells *(5)*. This equipment has been adapted to run continuous Kjeldahl tests at 20 per hour in some food laboratory applications! It can also test for milk minerals. While severe bias problems prevent its' use for broad spectrum milk fat samples, the unit has found application for testing 40 mixed herd samples per hour *(2)*. A 9 ml cream pipette is used to transfer mixed and 38 C tempered milk into the sample tube. The milk is then automatically mixed with reagents and evaluated turbidometrically using the chemistry reported by Nakai and Le *(22)*. A data printout unit automatically records the fat content on paper tape. Sample identity can be read automatically from a magnetic tag on the sample tube holder and printed onto the tape.

Shultze *(3)* has found that the Auto Analyzer II somatic cell counter is much faster than manual methods. It operates at 60 samples per hour and the screening and confirmatory assays are combined. Read et al. *(24, 25)* used a Coulter Counter in collaborative studies and demonstrated the value of this device in mastitis programs *(26)*. DeMedereiros *(14)* claimed an increased milk yield resulted through application of the Coulter Counter and good management practices. One 250-cow herd produced an annual increase of over $19,000 upon reduction of the somatic cell count from 900,000 to 500,000 per milliliter. Other somatic cell counters are reportedly being developed which will cost approximately $1,000 (J. C. Bruhn, *personal communication*).

Perhaps the most promising instrumentation on the dairy laboratory horizon is the Grubb Parsons Infra Red Milk Analyzer (IRMA) manufactured in England. This instrument can evaluate milk for fat, protein, and lactose at over 90 samples per hour without use of any reagents *(8)*. The IRMA takes advantage of the infra red energy absorption by various organic chemical bonds to quantitate milk constituents. Most of the milk in the province of Ontario is currently tested in the Department of Agriculture laboratory in Guelph using eight Mark I IRMAs *(30)*. These models require external homogenization. The Mark II IRMA (Fig. 8) provides built-in ultra-sonic homogenization. Several of these are now in use in the United States and Canada. The unit in Fig. 8 is currently in use at the Dairymens Cooperative Creamery Association (DCCA) in Tulare, California.

One technician can operate two Mark II instruments for more than 180 samples per hour. The repeatability of the instrument is better than ± 0.035, ± 0.06, ± 0.06, and ± 0.09 for fat, protein, lactose, and solids-not-fat. It has been demonstrated that the calibration slope can be maintained so as to assure an over-all accuracy of ± 0.02% or better at all levels of individual component percentage in whole milk *(28)*. The calibration knobs on the DCCA IRMA in Tulare have been sealed by the California state regulatory officials and have not been touched since the instrument installation in February 1971!

Biggs estimated the instrumental cost of IRMA analysis, (assuming 80% of potential instrument productivity and a technician cost of $3.00 per hour) to be less than 3.5 cents for fat, protein, lactose, and solids-not-fat *(28)*. California has legalized the IRMA for milk payment purposes. Collaborative studies have been completed and IRMA received AOAC approval in October 1971.

SUMMARY AND CONCLUSIONS

An IRMA combined with a somatic cell counting system appears most practical at present. This would combine milk payment, solids accounting, and animal

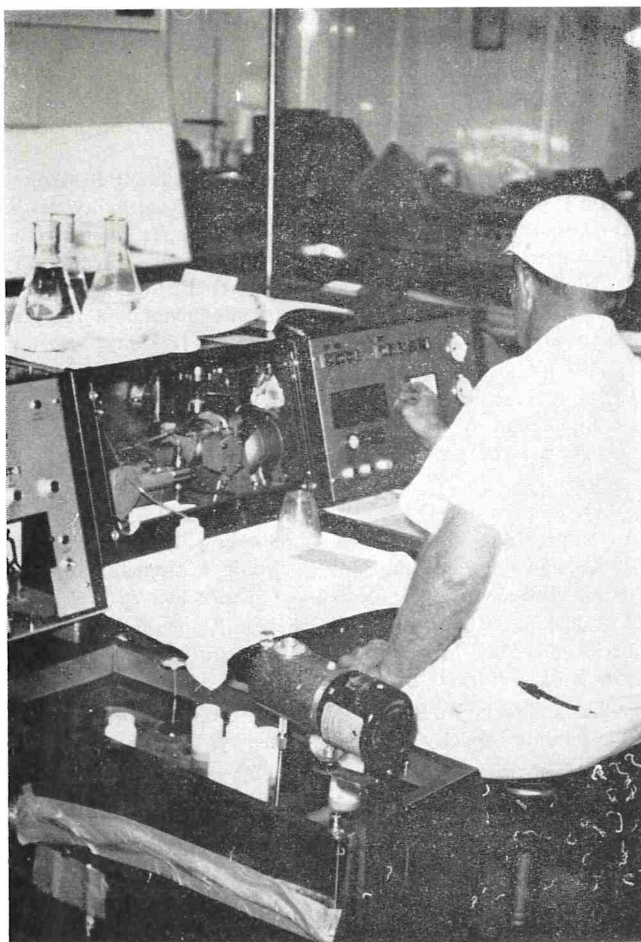

Figure 8. The Mark II IRMA in operation.

health information. Manual presentation to the instruments is more compatible with the manual steps required for sample identification than is automatic sampling. Dye binding systems have the inherent problem of producing higher apparent protein tests with abnormal milk samples *(13, 32)*. Thus breeding for higher protein production might result in breeding for mastitis susceptibility! The IRMA can reduce this possibility because lactose reduction in abnormal milk can be detected and the computer could indicate suspected abnormal samples for further testing. The somatic cell unit would confirm and indicate the degree of problem. Dye binding tests will prove more valuable in mixed herd and bulk samples than with individual animal breeding and DHIA programs.

It is evident that better instrumentation is now available and being perfected for the dairy laboratory. The question always reverts to one of economics. We have learned to accept the need for larger producer units and processing plants. It seems logical that we recognize the need for larger, better equipped, centralized laboratory facilities and unify our efforts to accelerate their establishment. Perhaps it may prove possible to bring the laboratory to the samples *(2)!* A third person testing facility has merit and should be considered. The idea of dumping sulfuric acid in milk, centrifuging, and paying the dairy farmer according to the results seems inconsistent in a society that can send three men to drive around on the moon and then bring them back without a mid-course correction!

Louis Pasteur gave us the challenge when he said, "Take interest, I implore you, in those sacred dwellings which one designates by the expressive term: laboratories. Demand that they be multiplied, that they be adorned. These are the temples of the future-temples of well-being and of happiness. There it is that humanity grows greater, stronger, better."

It is hoped we can see adornment of our laboratories with automated devices which will help us to do a better job in quality control, milk payment, animal health, and breeding practices than we are presently doing.

ACKNOWLEDGMENTS

The author is indebted to United Card Company, Dr. W. C. Winder, Dr. J. E. Campbell, American Instrument Co., Dr. H. F. MacRae, and Dr. T. Kasamdjaiff for permission to use the photographs; to members of the ADSA Committee on Instrumentation for Analysis of Gross Composition, which the author has chaired for three years, for their encouragement and support; and to the director of the Utah Agricultural Experiment Station for approval to publish this paper No. 1210.

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PROGRAM FOR ISM 15TH ANNIVERSARY EDUCATIONAL CONFERENCE AND EXPOSITION

A stimulating and eye-catching program is planned for the Institute of Sanitation Management National Environmental Sanitation & Maintenance Management Conference and Exposition, October 15-19, 1972, at the Sheraton Hotel, Philadelphia, Pennsylvania.

Opening day, Sunday, October 15, features an Educational Conference Session with a nationally known keynoter from the U. S. Environmental Protection Agency, "Toward A Healthy Environment" ISM President's Report, the theme setter, "Epilogue/Prologue, Out of te Past Comes the Future." An Anniversary Reception to be hosted by the Florida Citrus Commission and Clearwater, Florida, site of the '73 Conference and Exposition.

Monday, October 16, will feature "A Day With the U. S. Environmental Protection Agency." This will include the why's and wherefore's of the federal government environmental standards, i.e., water, air, solid waste, noise, radiation and the permit programs. Also included will be, up-to-the-minute environmental planning, technical and financial assistance as well as enforcement and surveillance programs. The Environmental Sanitation and Maintenance Exposition opens for a three day run, free of charge to the trade, along with a Film Festival featuring the latest in film management techniques. Four Cross-Fertilization Roundtables dealing with environmental problems of the day, round out the day's program.

(Continued on Page 301)

THE THIRTEENTH EDITION OF *STANDARD METHODS FOR THE EXAMINATION OF DAIRY PRODUCTS*[1, 2]

E. H. Marth

Department of Food Science and the Food Research Institute
University of Wisconsin
Madison, Wisconsin 53706

Abstract

An Intersociety Council was appointed late in 1968 to develop the 13th edition of *Standard Methods for the Examination of Dairy Products* (SMEDP). The Council was made up of 9 persons who represented professional societies, regulatory agencies, the dairy industry, and the academic community. The Council enlisted the help of 58 other persons who are experts in various fields and jointly prepared the 21 chapters and 2 appendices in the 13th edition of SMEDP.

Major features of the 13th edition of SMEDP include: *(a)* a detailed review of pathogens which have occurred in milk and milk products, *(b)* a separate chapter on sampling methods of all kinds, *(c)* a separate chapter on media and reagents and on methods for their preparation, *(d)* a chapter on screening and confirmatory tests for abnormal milk, *(e)* expansion of the chapter on chemical methods, and *(f)* inclusion of supplemental microbiological and chemical methods in the appendices, which will be on paper of a color different from that of the chapters. Numerous minor changes, both editorial and in technical matters, have been made in all chapters and appendices.

Milk and milk products available to the American consumer are among the safest foods which can be obtained. Furthermore, the quality, as reflected by flavor, odor, appearance, and shelf-life, is generally excellent. Safety, quality, and nutritive value are all expected, and rightfully so, by the consumer when these foods are purchased.

No single factor has been responsible for this development in the dairy industry. Instead, a number of factors have, over the years, contributed to this progress. Among the more significant are: eradication of diseases from dairy cattle, compulsory pasteurization, improvements in sanitation on the farm and in the processing plant, improvements in refrigeration and distribution, and adequate and uniform laboratory control of raw and finished products and of the environments in which they are produced and processed. Achievement of adequate laboratory practices is to a large degree the result of 12 editions of *Standard Methods for the Examination of Dairy Products* (SMEDP).

The origin of SMEDP can be traced back to 1905 when Dr. S. C. Prescott of the Massachusetts Institute of Technology reported on "The Need for Uniform Methods in the Sanitary Examination of Milk." The report was given to the Laboratory Section of the American Public Health Association (APHA) which met in Boston *(7, 9)*. In his report, Professor Prescott suggested that a committee be established to study the methods used for bacteriological testing of milk and then to recommend a uniform procedure for this important task. The committee was appointed and, with Prescott as chairman, labored until 1909 when a final report was submitted to APHA. The report was published in 1910 and, in effect, was the first edition of SMEDP *(7)*.

Prescott and his committee not only prepared the first edition of SMEDP but also established a procedure which served to prepare 11 other editions. Development of these editions has recently been reviewed by Reinbold *(7)* and will not be discussed in this paper.

The procedure used to prepare the first 12 editions involved: *(a)* appointment of a chairman who was responsible to APHA, *(b)* appointment of committee members selected by the chairman, and *(c)* consultation between chairman and committee members to develop an edition of SMEDP. To be sure, for recent editions the committee really consisted of a series of subcommittees each of which had the specific responsibility to update and otherwise revise one or several chapters in SMEDP. For example, 49 persons contributed to the 12th edition of SMEDP *(9)*.

Much progress was made by use of this procedure but there were some built-in limitations. In 1960, just before the 11th edition appeared, J. C. Olson, Jr. *(6)* editorialized on the procedure used to prepare SMEDP. He indicated the need for a continuing effort between editions and suggested that expenditure of public funds to prepare SMEDP would be in order because the book and its contents serve consumers, industry, and regulatory agencies. When the 12th edition was completed, its chairman, W. G. Walter, asked the question, "Should there be a 13th

[1]Contribution from the College of Agricultural and Life Sciences, University of Wisconsin, Madison.

[2]Presented in part at the 58th Annual Meeting of the International Association of Milk, Food, and Environmental Sanitarians, San Diego, California, August 17-21, 1971. A similar report also was presented at the 66th Annual Meeting of the American Dairy Science Association, East Lansing, Michigan, June 20-23, 1971.

edition of SMEDP?" *(8)*. In his discussion of the question, Walter indicated the need for: *(a)* support of applied research on methodology; *(b)* a mechanism to keep SMEDP up-to-date; *(c)* meetings of committees when parts of SMEDP are being revised; *(d)* a mechanism for collaborative testing of methods; *(e)* an advisory committee made up of representatives from industry, government, and universities; and *(f)* financial support to do the things needed for further improvement of SMEDP.

Concerned individuals in APHA and in the Public Health Service [now in the Food and Drug Administration, (FDA)] recognized the validity of comments by Olson, Walter, and others and undertook to make some of the changes needed before preparation of the 13th edition of SMEDP was begun. A contract was developed between APHA and FDA (formerly Public Health Service) so that public funds (as suggested by Olson in 1960) could be made available to develop the manuscript for the 13th edition of SMEDP. Availability of funds was contingent on appointment of an advisory council (as suggested by Walter in 1967) which was to oversee development of the 13th edition, and thereby relieve a single individual from being responsible for the total task.

Intersociety Council

The advisory council or Intersociety Council, as it was later designated, was appointed in the fall of 1968 and was made up of the following persons:

Dr. W. J. Hausler, Jr., State Hygienic Laboratory, University of Iowa, Iowa City; chairman of the Council and a representative of APHA.

Dr. W. S. Clark, Jr., American Dry Milk Institute, Chicago, Illinois; representative of the dairy industry.

Dr. J. L. Dizikes, Dairy Division Laboratory, U. S. Department of Agriculture, Chicago, Illinois; representative of the U. S. D. A.

Dr. E. H. Marth, Department of Food Science, University of Wisconsin, Madison; representative of the American Dairy Science Association.

Mr. J. N. Murphy, Jr., Division of Laboratories, Texas Department of Health, Austin; representative of the International Association of Milk, Food, and Environmental Sanitarians.

Dr. V. H. Nielsen, Department of Food Technology, Iowa State University, Ames; representative of academia.

Dr. C. H. Okey, Division of Laboratories, Maine Department of Health, Augusta; representative of the Association of State and Territorial Public Health Laboratory Directors.

Dr. J. C. Olson, Jr., Division of Microbiology, FDA, Washington, D. C.; representative of FDA.

Dr. W. G. Walter, Department of Botany and Microbiology, Montana State University, Bozeman; chairman and editor of the 12th edition of SMEDP and a representative of academia.

Two other persons regularly participated in deliberations by the Council. They are:

Dr. G. J. Kupchik, Institute of Health Sciences, Hunter College, City University of New York, who served as APHA Project Director.

Dr. R. B. Read, Jr., Division of Microbiology, FDA, Washington, D. C., who served as the Project Officer.

Activities of the Council

The Council met three or four times each year during 1969, 1970, and 1971. Its first meetings were devoted to identifying deficiencies which existed in the 12th edition of SMEDP. In addition to its own deliberations, the Council arranged for Round Table discussions at the 1969 annual meetings of the American Dairy Science Association; the International Association of Milk, Food, and Environmental Sanitarians; and the American Public Health Association and the 1970 annual meeting of the American Society for Microbiology. These sessions enabled interested individuals who attended the annual meeting of any of these societies to express their opinions about changes needed in the 13th edition of SMEDP.

After evaluating the many suggestions generated both inside and outside of the Council, it was evident that some research should be done to provide answers for questions which could not be resolved by information in the literature. Studies have been completed on the temperature at which the pH of agar should be measured and on survival of coliforms in some cultured milk products. Results of this research has been reported in papers by Walter et al. *(10)* and Goel et al. *(1)*. A rather extensive study on plating and incubation conditions for testing nonfat dry milk involved 15 laboratories and has been completed although results have not yet been published. The Council has also concerned itself with studies on: grading sediment discs, evaluating aureomycin rose-bengal agar for enumerating yeasts and molds, coliform methods, stains for the direct microscopic methods, and evaluation of the *Bacillus megaterium* test to detect inhibitory substances in milk. Some of these studies were undertaken by investigators independent of the Council and others remain to be done. Both time and funds available to the Council limited the number and scope of studies that could be undertaken and completed before the 13th edition of SMEDP went to press.

A subcommittee of the Applied Laboratory Methods Committee of the International Association of Milk, Food, and Environmental Sanitarians was particularly active while the Council deliberated on the 13th edition of SMEDP. Although this subcommittee operated independently of the Council there was reasonably close liaison because most of the subcommittee members also actively participated in preparing the 13th edition of SMEDP. The subcommittee devoted its efforts to the standard plate count (methods to shake dilution bottles, plastic versus glass petri dishes, holding time for dilution blanks

before plating, and two-versus three-day incubation) and reported the results of its efforts in four papers by Huhtanen et al. *(2-5)*.

Late in 1969 the Council developed committees for the different chapters which were to be included in the 13th edition of SMEDP. An attempt, although not always successful, was made to structure each committee so that regulatory, industrial, and university interests were represented. Appointment of committees and their chairmen was completed early in 1970. A list of all committees appears at the end of this paper. Chairmen of committees were invited to meet with the Council in the summer of 1970 and at that time consider the subject matter to be included in each chapter. A preliminary draft of some chapters was available for consideration at that time.

Committee chairmen and the committees then proceeded with the chapters and appendices and at intervals made results of their efforts available for review by the Council. At least two drafts, and sometimes three or four, of each chapter and appendix were prepared before the material was accepted by the Council. As a result of this procedure, each chapter in the 13th edition probably was more thoroughly reviewed than was true of previous editions of SMEDP.

All chapters and appendices were in final form by the fall of 1971 and some were in the hands of the printer during the summer of 1971. Maintenance of this schedule enabled publication of the 13th edition of SMEDP in the spring of 1972.

THE THIRTEENTH EDITION

Early during its deliberations, the Council adopted the philosophy which prevailed for the 12th edition of SMEDP. This was stated by Walter *(9)* as follows: "No new method or modification of an old method should be introduced unless it has undergone careful comparative testing in several laboratories, with the data available to the committee and to any other interested parties, preferably by publication in a recognized scientific journal." To be sure, the Council was not able to adhere to this philosophy in all instances. Development of several more editions of SMEDP on the same basis as the 12th and 13th editions will make it possible for this philosophy to prevail throughout the entire book.

The thorough review given each chapter resulted in many minor changes which should make all chapters more readable and should serve to remove inconsistencies which existed between some chapters. Also, the organizational structure of chapters, hopefully, has been further improved. Details of these changes cannot be given here but some of the major changes will be highlighted.

Chapters which remained essentially unchanged from their counterparts in the 12th edition include: 1 (Quality Tests), 7 (Thermoduric, Thermophilic, and Psychrotrophic Bacteria), 10 (Microbiological Methods for Concentrated and Dry Milk), 11 (Microbiological Methods for Butter), 12 (Microbiological Methods for Cheese and Other Cultured Products), 13 (Microbiological Methods for Ice Cream and Related Frozen Products), 14 (Direct Microscopic Methods), 15 (Reduction Tests), 17 (Sediment in Fluid Milk), 20 (Radionuclides in Milk), and 21 (Simplified Technics for Viable Counts of Raw Milk).

Chapter 2, Significant Pathogens in Dairy Products, has been updated and expanded to include information previously not available. Greater emphasis has been given to the hazards to laboratory workers and food processing plants when pathogens are handled. Methods to isolate and identify pathogens are not given but references to sources of such information are included.

A major change is evident in Chapter 3, Sampling Dairy Products. All information on sampling of milk, milk products, equipment, water, etc. is now included in this chapter. Sampling for bacteriological and chemical testing is discussed. This rather significant change was made because persons who do the sampling often are not involved with laboratory work and hence find it awkward to page through the entire book until a desired method is found. Chapter 3 will be reprinted and made available separately at modest cost to interested individuals.

Chapter 4, Culture Media and Preparation, is new although much of the material in this chapter appeared in the Appendix of the 12th edition. The Council believed that formulae for media and methods to prepare media are sufficiently "standard" and are an integral part of many of the methods in SMEDP so the information should be presented in a chapter. Several new media are included because they were added to procedures elsewhere in the book. The distilled water suitability test also was added to this chapter.

The Standard Plate Count Method is detailed in Chapter 5. Important changes include the requirements that milk be at or below 4.4 C when it arrives in the laboratory and that samples must be tested within 36 hr after collection. Changes in specifications for pipets were made so they reflect current practices.

The only major changes to occur in Chapter 6, Coliform Bacteria, are elimination of desoxycholate lactose agar as a testing medium and imposition of time limits by when cultured products must be tested for these bacteria.

Chapter 8, Detection of Abnormal Milk, is new and, in part, represents upgrading to "standard" of methods which appeared in the Appendix of the 12th edition. Screening tests that appear in this chapter are: modified Whiteside, California Mastitis Test, Wisconsin Mastitis Test, and the direct microscopic single strip reticle procedure. Confirmatory tests given are: direct microscopic field counting, single strip counting, and strip reticle counting procedures and the electronic somatic cell counting procedure.

A few changes were made in Chapter 9, Detection of Inhibitory Substances in Milk. Included are specification of the proper number of *Bacillus subtilis* spores to be used as inoculum and reference to FDA and AOAC methods which may be applicable in some instances.

Chapter 16, Microbiological Tests for Equipment, Supplies and Water, remained essentially unchanged except that information on testing of air was moved to the Appendix. The Council believed that methods dealing with air should not be considered as "standard" at this time.

Methods to determine phosphatase are given in Chapter 18. There have been substantial editorial changes in this chapter to make it more readable. The dialysis phosphatase method has been eliminated from the chapter and moved to the Appendix.

The nineteenth chapter, Chemical Methods, has been expanded and now includes: the thermistor cryoscope method for water in milk; the thiosulfate titration method for available chlorine; Babcock fat test methods for homogenized milk, chocolate milk or drink, cream cheese, natural cheese, process cheese, process cheese food, liquid skimmilk, and whey; Gerber fat test methods for milk cream, and chocolate milk; the Milko-Tester method for raw milk; the Roese-Gottlieb (Mojonnier) method for fat; and methods for organochlorine pesticides.

Appendix A in the 13th edition lists some useful or supplemental microbiological methods which cannot yet be considered as "standard." Included are: the aureomycin-rose bengal agar method to detect molds and yeasts, the *Bacillus megaterium* method to detect inhibitory substances in milk, the enterococcus test for sanitary quality of butter, the disintegration method for microbiological testing of paper materials, a microbiological screening method for retail milk containers, microbiological tests for air, the Moseley keeping quality test, the roll tube method to estimate numbers of bacteria (removed from Chapter 21), swabbing methods using calcium alginate and dacron swabs, and sterility tests for products in flexible packages.

Supplemental chemical methods appear in Appendix B. Included are: acidity tests for whey, whey concentrate, whole milk, and skimmilk; the dialysis phosphatase method; a method to detect heated milk in raw milk; the Gerber test for fat in frozen desserts; the modified Kohman method for fat, moisture, and salt in butter; measurement of pH of cheese; a dye binding method to measure protein in milk; measurement of hydrolytic rancidity in raw milk; and the lactometer method to determine solids in milk. Both Appendix A and B will appear on paper of a different color than that used for the chapters. The Council believes this will help to identify the Appendix so its contents will not be confused with the "standard" methods in the 21 chapters.

List of Committees That Prepared the 13th Edition of SMEDP

Chapter 1, Quality Tests

W. J. Hausler, Jr., *Chairman,* State Hygienic Laboratory, University of Iowa, Iowa City 52240.

W. G. Walter, Department of Botany and Microbiology, Montana State University, Bozeman 59715.

Chapter 2, Significant Pathogens in Dairy Products

F. P. Koontz, *Chairman,* State Hygienic Laboratory, University of Iowa, Iowa City 52240.

I. S. Snyder, Department of Microbiology, College of Medicine, University of Iowa, Iowa City 52240.

Chapter 3, Sampling Dairy Products

A. R. Brazis, *Chairman,* Division of Microbiology, Food and Drug Administration, 1090 Tusculum Avenue, Cincinnati, Ohio 45226.

E. W. Cook, Quality Control Laboratory Division, Industrial Highway, Southampton, Pennsylvania 18966.

E. F. Schaffer, Pennsylvania Department of Agriculture, 2301 N. Cameron Street, Harrisburg 17120.

H. K. Hansen, Iowa Department of Agriculture, East 7th and Court Avenue, Des Moines 50319.

K. W. Whaley, Division of Laboratory Services, Tennessee Department of Health, Nashville 37219.

Chapter 4, Culture Media and Preparation

C. H. Okey, *Chairman,* Division of Laboratories, Maine Department of Health, Augusta 04330.

W. G. Walter, Department of Botany and Microbiology, Montana State University, Bozeman 59715.

Chapter 5, The Standard Plate Count

A. R. Brazis, *Chairman,* Division of Microbiology, Food and Drug Administration, 1090 Tusculum Avenue, Cincinnati, Ohio 45226.

W. S. Clark, Jr., American Dry Milk Institute, 130 N. Franklin Street, Chicago, Illinois 60606.

W. E. Sandine, Department of Microbiology, Oregon State University, Corvallis 97331.

Chapter 6, Coliform Bacteria

J. J. Jezeski, *Chairman,* Department of Botany and Micro-

biology, Montana State University, Bozeman 59715.

R. T. Marshall, Department of Food Science and Nutrition, University of Missouri, Columbia 65201.

E. B. Collins, Department of Food Science and Technology, University of California, Davis 95616.

CHAPTER 7, THERMODURIC, THERMOPHILIC, AND PSYCHROTROPHIC BACTERIA

C. Vanderzant, *Chairman,* Department of Animal Science, Texas A and M University, College Station 77843.

C. K. Johns, Retired, Formerly with the Dairy Technology Research Institute, Canada Department of Agriculture, Ottawa, Ontario.

CHAPTER 8, DETECTION OF ABNORMAL MILK

J. C. Olson, Jr., *Chairman,* Division of Microbiology, Food and Drug Administration, 200 C Street S.W., Washington, D. C. 20204.

A. R. Brazis, Division of Microbiology, Food and Drug Administration, 1090 Tusculum Avenue, Cincinnati, Ohio 45226.

R. T. Olson, Spokane County Health District, 819 N. Jefferson, Spokane, Washington 99201.

D. I. Thompson, Wisconsin State Laboratory of Hygiene, 437 Henry Mall, Madison 53706.

W. W. Ullman, Laboratory Division, State Department of Health, P. O. Box 2340, Hartford, Connecticut 06101.

CHAPTER 9, DETECTION OF INHIBITORY SUBSTANCES IN MILK

E. H. Marth, *Chairman,* Department of Food Science, University of Wisconsin, Madison 53706.

C. W. Christensen, Difco, Inc., 920 Henry Street, Detroit, Michigan 48201 (Deceased).

C. W. Griffin III, BioQuest, Division of Becton, Dickinson and Company, 250 Schilling Circle, Cockeysville, Maryland 21030.

A. Kirshbaum, Consultant to the Pharmaceutical Industry, 11013 Gainsborough Road, Potomac, Maryland 20854.

J. W. Messer, Division of Microbiology, Food and Drug Administration, 1090 Tusculum Avenue, Cincinnati, Ohio 45226.

M. Weber, New Jersey Dairy Laboratories, 222-226 Easton Avenue, New Brunswick 08903.

CHAPTER 10, MICROBIOLOGICAL METHODS FOR CONCENTRATED AND DRY MILK

G. W. Reinbold, *Chairman,* Department of Food Technology, Iowa State University, Ames 50010.

C. W. Bohrer, National Canners Association, 1133 20th Street N.W., Washington, D. C. 20036.

W. S. Clark, Jr., American Dry Milk Institute, 130 N. Franklin Street, Chicago, Illinois 60606.

J. L. Dizikes, Dairy Division Laboratory, U. S. Department of Agriculture, 1819 W. Pershing Road, Chicago, Illinois 60609.

CHAPTER 11, MICROBIOLOGICAL METHODS FOR BUTTER

J. M. Jensen, *Chairman,* Department of Food Science and Human Nutrition, Michigan State University, East Lansing 48823.

C. H. Harper, Jr., Beatrice Foods Company, 1526 S. State Street, Chicago, Illinois 60605.

H. F. Long, Research and Development Division, Kraftco Corporation, 801 Waukegan Road, Glenview, Illinois 60025.

CHAPTER 12, MICROBIOLOGICAL METHODS FOR CHEESE AND OTHER CULTURED PRODUCTS

G. W. Reinbold, *Chairman,* Department of Food Technology, Iowa State University, Ames 50010.

R. F. Anderson, National Cheese Institute, Inc., 110 N. Franklin Street, Chicago, Illinois 60606.

L. G. Harmon, Department of Food Science and Human Nutrition, Michigan State University, East Lansing 48823.

CHAPTER 13, MICROBIOLOGICAL METHODS FOR ICE CREAM AND RELATED FROZEN PRODUCTS

H. L. Wildasin, *Chairman,* H. P. Hood and Sons, 500 Rutherford Avenue, Boston, Massachusetts 02129.

J. H. Hetrick, Department of Food Science, University of Illinois, Urbana 61801.

M. Purko, Research and Development Division, Kraftco Corporation, 801 Waukegan Road, Glenview, Illinois 60025.

CHAPTER 14, DIRECT MICROSCOPIC METHODS

D. I. Thompson, *Chairman,* Wisconsin State Laboratory of Hygiene, 437 Henry Mall, Madison 53706.

G. Sherman, Dairy Laboratory, U. S. Department of Agriculture, 1819 W. Pershing Road, Chicago, Illinois 60609.

R. W. Mykleby, Land O'Lakes, Inc., 614 McKinley Place N.E., Minneapolis, Minnesota 55413.

M. Weber, New Jersey Dairy Laboratories, 222-226 Easton Avenue, New Brunswick 08903.

W. A. Moats, Market Quality Research Division, U. S. Department of Agriculture, Beltsville, Maryland 20705.

CHAPTER 15, REDUCTION TESTS

R. Dabbah, *Chairman,* Ross Laboratories, 625 Cleveland Avenue, Columbus, Ohio 43216.

J. C. Olson, Jr., Division of Microbiology, Food and Drug Administration, 200 C Street S.W., Washington, D. C. 20204.

J. J. Jezeski, Department of Botany and Microbiology, Montana State University, Bozeman 59715.

J. C. Flake, Evaporated Milk Association, 910 Seventeenth Street N.W., Washington, D. C. 20006.

CHAPTER 16, MICROBIOLOGICAL TESTS FOR EQUIPMENT, SUPPLIES AND WATER

R. Y. Cannon, *Chairman,* Department of Animal and Dairy Sciences, Auburn University, Auburn, Alabama 36830.

T. I. Hedrick, Department of Food Science and Human Nutrition, Michigan State University, East Lansing 48823.

D. Vesley, School of Public Health, University of Minnesota, Minneapolis 55455.

H. W. Walker, Department of Food Technology, Iowa State University, Ames 50010.

E. A. Zottola, Department of Food Science and Industries, University of Minnesota, St. Paul 55101.

CHAPTER 17, SEDIMENT IN FLUID MILK

E. O. Wright, *Chairman,* Department of Food Technology, Iowa State University, Ames 50010.

J. C. Flake, Evaporated Milk Association, 910 Seventeenth Street N.W., Washington, D. C. 20006.

F. E. Fenton, Dairy Division, Consumer and Marketing Service, U. S. Department of Agriculture, Washington, D. C.

20250.

C. H. Harper, Jr., Beatrice Foods Company, 1526 S. State Street, Chicago, Illinois 60605.

W. S. Clark, Jr., American Dry Milk Institute, 130 N. Franklin Street, Chicago, Illinois 60606.

Chapter 18, Phosphatase Methods

G. K. Murthy, *Chairman*, Food and Drug Administration, 1090 Tusculum Avenue, Cincinnati, Ohio 45226.

V. H. Nielsen, Department of Food Technology, Iowa State University, Ames 50010.

R. W. Weik, Division of Food Technology, Food and Drug Administration, 200 C Street S.W., Washington, D. C. 20204.

Chapter 19, Chemical Methods

L. J. Bianco, *Chairman*, Kraft Foods, Division of Kraftco Corporation, 500 Peshtigo Court, Chicago, Illinois 60690.

P. B. Manning, Department of Food Science and Industries, University of Minnesota, St. Paul 55101.

J. A. Burke, Division of Chemical Technology, Food and Drug Administration, 200 C Street S.W., Washington, D. C. 20204.

W. Roth, Washington State Food and Drug Laboratory, College of Pharmacy, University of Washington, Seattle 98105.

R. W. Mykleby, Land O'Lakes, Inc., 614 McKinley Place, N.E., Minneapolis, Minnesota 55413.

Chapter 20, Radionuclides in Milk

R. L. Morris, *Chairman*, State Hygienic Laboratory, University of Iowa, Iowa City 52240.

G. K. Murthy, Food and Drug Administration, 1090 Tusculum Avenue, Cincinnati, Ohio 45226.

L. R. Setter, Retired, Formerly with the Bureau of Radiological Health, Environmental Protection Agency, Rockville, Maryland.

R. G. Graybeal, Iowa Electric Light and Power Company, P. O. Box 351, Cedar Rapids 52406.

Chapter 21, Simplified Technics for Viable Counts of Raw Milk

H. B. Warren, *Chairman*, Research and Development Laboratory, Fairmont Foods Company, 4123 S. 67th Street, Omaha, Nebraska 68117.

J. C. Boyd, Department of Animal Science, Montana State University, Bozeman 59715.

W. S. LaGrange, Department of Food Technology, Iowa State University, Ames 50010.

J. W. Messer, Division of Microbiology, Food and Drug Administration, 1090 Tusculum Avenue, Cincinnati, Ohio 45226.

Appendix A, Supplemental Microbiological Methods

R. W. Stachwick, *Chairman*, Carnation Research Laboratories, 8015 Van Nuys, Van Nuys, California 91412.

E. H. Marth, Department of Food Science, University of Wisconsin, Madison 53706.

G. W. Ronald, State Hygienic Laboratory, University of Iowa, Iowa City 52240.

R. T. Russell, Food Protection Laboratory, Research Corporation, Syracuse University, Syracuse, New York 13210.

Appendix B, Supplemental Chemical Methods

The committee listed for Chapter 19 also prepared Appendix B.

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MISSION 5000: A NATIONAL PROGRAM TO ELIMINATE DUMPS[1]

THOMAS J. SORG

Special Waste Technology Branch,
Processing and Disposal Division
Office of Solid Waste Management Programs
Environmental Protection Agency
Cincinnati, Ohio 45268

ABSTRACT

Results of a national survey of over 17,000 land disposal sites are presented. Open dumps predominate, 94%, and contribute to our environmental problems of air, water, and visual pollution. Only 6% of the land disposal sites met the very minimum requirements for a sanitary landfill. MISSION 5000, a national program to eliminate 5000 open dumps during a 2-year period, is described in detail. The Environmental Protection Agency's activities of program direction, training, and technical assistance are discussed. Activities and support of State government, civic, trade and professional organizations, and the public are also outlined. Solid waste management solutions and alternatives to open dumps are offered with the basic operational differences between open dumps and sanitary landfills highlighted. The paper concludes with a first year progress report on MISSION 5000.

The Environmental Protection Agency recently released a public service television film spot that tells of a MISSION 5000 success story in Lawton, Oklahoma. Before 1971, when Lawton disposed of its solid waste in an open-burning dump, the people of the community were objecting; the county sanitarian was critical; the Oklahoma Solid Waste Director had indicated that corrective action was overdue. Pressure was building from all sources to eliminate the environmental problems of this solid waste disposal site. Even with this, Lawton might have continued to use this burning dump for years if the State had not passed a law prohibiting open burning by cities of over 10,000 population by January 1, 1971. But the law was in effect and Lawton was on notice to do something about its dump.

The city council started to investigate other landfill operations and identify possible new sites; the city attorney was authorized to obtain land purchase options. Today, the dump is closed and the city is operating a sanitary landfill, an acceptable solid waste disposal method. The solution did not occur easily or overnight. Six landfill sites were considered, and public hearings were held to select the best. As commonly occurs, nearby residents vigorously objected to having a "dump" near them. The county sanitarian satisfied some objections and assured them that the site would be operated as a sanitary landfill and not as a dump. Landfill specifications and procedures were established, and the site was fenced and made to look attractive. The site is expected to last 12 to 14 years at which time it may be converted into an industrial or recreational park. Lawton, Oklahoma, made a switch from an obnoxious burning dump to an acceptable method of solid waste disposal —a MISSION 5000 success story.

MISSION 5000

What is MISSION 5000? MISSION 5000 is a national program to improve the quality of our environment by eliminating 5,000 of America's open and burning dumps during a 2-year program ending June 30, 1972.

Why MISSION 5000? Open and burning dumps are a part of our Nation's environmental crisis. Dumps contribute to air, water, and visual pollution problems; provide harborage for insects and rodents; and may be a source of accidents and disease. Unfortunately, open and burning dumps are also the most common means of solid waste disposal in the United States.

A national survey of land disposal practices conducted between 1967 and 1970 by 41 State agencies in cooperation with the Environmental Protection Agency found that only 6% of the 17,000 sites surveyed met minimal requirements for a sanitary landfill. The other 94% failed to meet one or more of the three most important factors characterizing a sanitary landfill—no burning, no water pollution, and daily earth cover. For the purpose of definition, sites that did not meet all three criteria were classified as dumps. Nearly three-fourths of the dumps reported on contributed to air pollution, and one-half had an existing or potential water pollution problem.

MISSION 5000 is aimed at helping to solve today's environmental problems with the use of existing and proven technology and is advocating immediate action to eliminate dumps. A dump and its pollution problems can be eliminated in one of two ways: *(a)* the dump can be converted to a sanitary landfill or *(b)* it can be eliminated and replaced with an acceptable alternative disposal method such as a new sanitary landfill or an incinerator. The sanitary landfill method of disposal will play a major role in MISSION 5000 because many dump operations

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can be converted to acceptable sanitary landfill operations. Furthermore, when a dump is eliminated, some immediate operation must take its place; cost frequently dictates that the replacement be a sanitary landfill.

SANITARY LANDFILL

Contrary to the opinion of many people, the sanitary landfill is an acceptable method of waste disposal and is not an open or burning dump. This misconception stems from the fact that most people do not know the difference between a dump and a sanitary landfill, and many dump sites have been mislabeled sanitary landfills. Preliminary reports of a psychrometric study of attitude factors and problems of solid waste revealed that only 7.5% of over 1,600 residents surveyed in 10 cities could name one distinguishing characteristic of a sanitary landfill.

The principal difference between the two types of operations is engineering; whereas a dump is a disposal site in which wastes are deposited with little or no regard for pollution control or aesthetics, a sanitary landfill is an *engineering* method for disposing of solid waste on land without polluting the environment. From the large number of existing dump sites, it is readily apparent that engineering has not been extensively applied to the design and operation of land disposal sites.

A more specific definition of a sanitary landfill, and the most commonly used one, is that developed by the American Society of Civil Engineers. "A sanitary landfill is a method of disposing of refuse on land without creating nuisances or hazards to public health or safety, by utilizing the principles of engineering to confine the refuse to the smallest practical volume, and to cover it with a layer of earth at the conclusion of each day's operation, or at such more frequent intervals as may be necessary."

A sanitary landfill is a well-controlled and truly sanitary method of disposal of solid waste upon land. There are four basic operations: *(a)* the solid wastes are deposited in a controlled manner in a prepared portion of the site; *(b)* the solid wastes are spread and compacted in thin layers; *(c)* the solid wastes are covered daily or more frequently, if necessary, with a layer of earth; and *(d)* the cover material is compacted daily.

There are many distinguishing characteristics of a sanitary landfill operation—the three most important are no burning, no water pollution, and daily earth cover. All wastes are compacted with heavy machinery and covered with earth; the wastes are not burned and therefore do not contribute to air pollution. Solid wastes are not placed in water, and all precautions are taken to prevent ground or surface water pollution. All wastes are unloaded in a controlled manner, compacted, and covered daily to maintain a clean and orderly site, for vector control, and to minimize the amount of water entering the fill. Dust and odors are controlled, and blowing paper is picked up each day. When completed, a well designed and operated site can become a community asset such as a golf course, playfield, or garden.

CONVERSION OF OPEN DUMPS

Not all dump sites will be converted to or replaced by sanitary landfills, however. Some communities will develop plans for conventional incinerators or will look to other, newer concepts of solid waste disposal such as pyrolysis, high-temperature incineration, or resource recovery. Most of the recent waste disposal developments are still in the research or demonstration phases, however, and most communities will not take steps to adopt these methods until they have been demonstrated and proven economically and technically feasible. Moreover, the time required for planning, designing, and constructing these types of disposal facilities takes many months, often several years, and are not an overnight cure for the open and burning dump problem. For communities considering such facilities, the MISSION 5000 program recommends the dump sites be upgraded to sanitary landfills or be eliminated and replaced by one or more sanitary landfills until the community adopts and constructs its proposed disposal facility. Communities with several dumps should consider converting one or more sites to sanitary landfills and eliminating all others.

The minimum requirements to properly eliminate a dump consist of: *(a)* exterminating rodents to prevent migration from the dump to the surrounding community; *(b)* correcting all water pollution problems; *(c)* extinguishing all fires to halt air pollution; and *(d)* covering all waste. Does a site closed off to the public need to be covered with earth? Yes. An uncovered dump site continues to be a potential source of accidents, a community eyesore, and an enticement for nearby residents and collectors. Furthermore, an uncovered site can easily be set on fire again, and there is high risk of surface and groundwater pollution. Such a dump has not been truly eliminated; it is only a question of time before it will be reopened.

OPERATION OF MISSION 5000

How is MISSION 5000 operating? The Office of Solid Waste Management Programs of the Environmental Protection Agency lauched the 2-year project

on July 1, 1970. The role of this Federal office, though important, is limited. Its primary responsibilities and activities are program direction and coordination; project promotion; technical information and assistance; and training. The Federal government has no regulatory authority over the disposal of solid waste nor does MISSION 5000 have financial assistance available for communities. Regulatory authority to ensure proper disposal of solid waste is strictly a State or local responsibility, and funds needed to eliminate dumps must come totally from the community itself. Consequently, the success of MISSION 5000 depends on the dedicated action of officials at the Federal, State, and local levels; the encouragement of civic, trade, and professional organizations; and the understanding and support of every citizen.

Program direction and coordination are handled by the technical staff at our headquarters and a team of MISSION 5000 project officers in the Environmental Protection Agency regional offices. Technical information on the proper methods of eliminating open dumps and operating acceptable disposal methods has been developed and published and is available upon request. Technical assistance at the field level on various problems of eliminating open dumps is also available from our regional project officers and headquarters staff.

The 1-day package training course, developed for technical and semi-technical personnel by our Training staff, was conducted 55 times by 40 State agencies, with the assistance of our Office during fiscal year 1971. As a result of these courses, over 3,700 people were trained in methods of eliminating open dumps and operating acceptable alternative disposal techniques and learned of the existing State rules and regulations. Most attendees have been local and State sanitarians involved in solid waste disposal.

Promotion of the MISSION 5000 project is taking various forms. Television film spots designed to educate and develop public support are being sent to television stations throughout the time of the project. The series of six films describes various problems of and solutions to the open and burning dump; one is about the Lawton, Oklahoma, success story. In addition to the film spots, a 21-min, sound, color film "5000 Dumps" has been produced. The film was designed for use by community action groups and local officials and is also appropriate for use in the classroom. It shows how a number of communities are solving the practical problems of closing dumps and are successfully establishing sanitary landfills. The film (Order No. M-2119-X) can be borrowed from the National Medical Audiovisual Center (Annex), Station K, Atlanta, Georgia 30324.

The support of various national, civic, trade, and professional organizations has been sought, and over 25 national organizations have publicly announced their support. Some of these organizations are encouraging their membership to participate actively, several local and State chapters have adopted MISSION 5000 as a special project.

Importance of State Programs

Because regulatory authority over land disposal operations is a State and local responsibility, strong and effective State solid waste programs are essential to the success of MISSION 5000. Most important are the State rules and regulations and enforcement programs governing land disposal operations. Before about 1968, few States had effective regulations governing the proper operation of land disposal sites. During the past several years, however, several States have enacted legislation that prohibits open and burning dumps and requires a State permit or license for all sanitary landfill operations. Unfortunately, few States presently have strong enforcement programs to ensure compliance with their rules and regulations. If MISSION 5000 efforts, however, can lead to enactment of land disposal regulations in most of our States and the States can develop strong enforcement programs, the problem of open and burning dumps should ultimately be solved in its entirety.

The State solid waste agencies are working closely with the Environmental Protection Agency on MISSION 5000. In addition to conducting the training courses, the States are providing technical assistance and information and are responsible for reporting the number of dumps eliminated within their State. A survey form is completed for each site that is eliminated or converted to a sanitary landfill, and the actions taken to correct the environmental problems are described. The State agencies submit the completed forms to the regional project officers who conduct periodic inspections of these sites to ensure that the basic requirements are met.

Progress During the First Year

MISSION 5000 passed the halfway mark on July 1, 1971, and has less than 1 year to go. State agencies reported that as of September 1, 1971, more than 2,100 open and burning dumps had ceased operation in the past several years; however, only about 800 of these sites were eliminated or converted to sanitary landfills according to Environmental Protection Agency requirements since MISSION 5000 began. All other sites were eliminated or converted before MISSION 5000 or did not meet the minimum re-

quirements for stopping the environmental problems associated with their operation.

Of the sites meeting MISSION 5000 requirements, 51% were eliminated and 49% were converted to sanitary landfills. The data survey also revealed that the waste that would have gone to the then eliminated dump sites was sent to existing sanitary landfills. This indicates that consolidation or the regional approach was the most frequent solution selected.

SUPPORT OF CITIZENS NEEDED

Private citizens frequently ask what they can do for MISSION 5000. Because MISSION 5000 needs the support of all the people, a few suggested ways to help are

- Determine (go and see) how solid waste is disposed of in the community.
- Determine if the city's disposal methods meet acceptable standards.
- Find what State and local laws are needed to improve solid waste disposal.
- Express concern to responsible officials, community action groups, and the local press.
- Encourage your firm, organization, or other community group to take a stand against open and burning dumps.
- Support community projects that will foster the development of acceptable disposal methods and that will eliminate dumps.

The sanitarian profession can provide valuable help to MISSION 5000. This profession has more day to day contact with solid waste disposal operations than any other profession involved in solid waste management. Sanitarians at the State and local level frequently inspect land disposal operations and, therefore, are in an excellent position to influence the type of operation. Pressure, persuasion, and technical knowledge used effectively can encourage and assist communities and private operators to eliminate their open dumps and to provide proper waste disposal methods.

Although MISSION 5000 progressed slowly during the first year, we are optimistic that the goal to eliminate 5,000 dumps will be reached by July 1, 1972. A time lag of several months to a year normally exists between the decision to eliminate a dump and the time to carry out such plans. Consequently, greatest progress is anticipated during the last few months of the program—after communities have had sufficient time to carry out their decision.

Space-age technology has produced many improvements in modern day life, but unfortunately, it has not significantly affected the use of open dumps. This oldest method of waste disposal continues to be the most commonly used method. Although the initial cost is cheap and the initial effort, minimal, the long-term cost and effort are dear. With present technology, however, there is no reason why dumps cannot be eliminated in favor of a pollution-free and aesthetically acceptable method. Research promises many future improvements in the field of solid waste management, but the environmental crisis is immediate and we need not await the results of research to improve waste disposal practices. To do less than present know-how and technology permits is unworthy of a Nation that takes pride in its technological excellence. Accomplishing MISSION 5000 will not come easy. It will take hard work and money. But the reward is great—a cleaner, healthier, and more beautiful land.

THE PROPIONIC-ACID BACTERIA–A REVIEW

I. GROWTH[1, 2]

D. H. HETTINGA AND G. W. REINBOLD
Department of Food Technology
Iowa State University
Ames, Iowa 50010

(Received for publication November 11, 1971)

ABSTRACT

This review, appearing in three parts, is concerned with the growth, metabolism, and miscellaneous metabolic activities of propionibacteria. The first section, presented here, deals primarily with nutritional requirements, substrate and product inhibition, physical factors, associative action of other microorganisms, and enumeration and isolation of propionibacteria. The nutrition of propionic-acid bacteria has been extensively investigated and the absolute requirements for their nutrition are known. Since propionibacteria play an important role in the fermentation of Swiss cheeses, development of a completely selective medium and continued study of agents inhibitory and stimulatory to propionibacteria would be a valuable aid in solving many of the practical problems associated with Swiss cheese manufacture. Part two will discuss formation of propionate and acetate, the carboxylation, decarboxylation, and transcarboxylation reactions, the roles of biotin and vitamin B_{12}, and the enzymes involved in the propionic-acid fermentation. The third and final section concerning miscellaneous metabolic activities will deal with erythritol metabolism, formation of diacetyl-acetoin and vitamin B_{12}, production of previously undiscussed volatile compounds, propionin, lipids and phospholipids, and the production of slime. The third section also will include concluding comments to provide a brief summary of the important points developed in this review.

Propionibacteria may be characterized, in general, as being Gram-positive, catalase-positive, nonspore-forming, nonmotile, facultative anaerobic, rod-shaped bacteria. Catalase production by propionibacteria is a phenomenon contradictory to the generally observed fact that anaerobic bacteria are catalase-negative; in spite of this contradiction it has even been shown that catalase production can be increased *(243)*. Eleven species of propionibacteria are presently recognized; their complete characterization can be found in a thesis by van Niel *(231)* and in Bergey's Manual of Determinative Bacteriology *(21)*. These organisms play important roles in several industrial processes. For instance, they are important in development of the characteristic flavor and eye production in Swiss-type cheeses; they produce as a by-product of their fermentation large quantities of propionic acid that can be utilized commercially. Since they synthesize relatively large amounts of vitamin B_{12}, they can be utilized for commercial production of this vitamin.

The relationship between the rate of enzymatic reactions and concentration of reactants can be described by simple equations of the Michaelis-Menten type and by Lineweaver-Burk plots. Since growth kinetics and regulation for propionibacteria have been investigated *(83, 136, 205)*, the relationship between the composition of the medium and vital functions of microorganisms, including growth of cells, provides some insight into developments of physical cultivation of propionibacteria.

The nutrition and metabolism of propionibacteria have been extensively investigated. There is, however, no single, up-to-date, comprehensive review that has brought together all the fragments of knowledge on the nutrition and metabolism of these microorganisms. An attempt is made in this paper to fill this void.

NUTRITIONAL REQUIREMENTS

The nutritional requirements of the propionibacteria have long been regarded as complex. Amino acids, although beneficial, are not essential *(261)*, whereas, certain vitamins *(43)*, minerals *(15, 171)*, and unknown constituents of yeast extract *(5, 54, 261)* are required for growth and metabolism.

An early report by Berger et al. *(20)* demonstrated that propionibacteria contain the necessary peptidases that may be required for producing essential amino acids. Antila and Antila *(10)* determined the free amino acid content in several varieties of cheeses. They found that proline was generally in high concentration in Gruyére and Emmental cheese. Production of this amino acid in cheese is generally associated with growth of large numbers of propionic-acid bacteria. Wood et al. *(261)* determined that amino acids were beneficial, but not essential, and that certain cultures grow with difficulty in their absence while others thrive. Fromageot and Chaix *(70)* reported that the sulfur-bearing amino

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[1]Journal Paper No. J-7027 of the Iowa Agriculture and Home Economics Experiment Station, Ames, Iowa. Project No. 1838.

[2]This review will appear in three parts: I. Growth; II. Metabolism; and, III. Miscellaneous Metabolic Activities. Literature citations will follow part III.

acids are growth stimulatory. According to Tatum et al. *(224)*, aspartic and glutamic acids could replace ammonium nitrate as a growth factor for propionibacteria. Antila *(5, 6)* reported that when one of the two amino acids was omitted from a mixture containing asparagine, glutamic acid, and ammonium chloride, a reduction of growth resulted. Simultaneous absence of the three constituents severely affected adaptation to a substrate such as lactate. This reduction in growth was clearly caused by the removal of the nitrogen source required by the propionibacteria. Antila *(6)* also found three strains of propionibacteria able to synthesize alanine, valine, serine, tyrosine, aspartic acid, glutamic acid, arginine, cystine, and methionine. An important observation made by Peltola and Antila *(159)* was that nitrates retard the amino-acid deamination activity of propionibacteria during cheese ripening. Although Tatum et al. *(224)* found that propionic-acid bacteria are able to utilize ammonical nitrogen, it was Wood et al. *(261)* who first grew these organisms satisfactorily through numerous transfers in an ammonium sulfate medium. Previously, Wood et al. *(274)* had observed inconsistent growth in ammonium sulfate during serial transfers. These observations supported and proved the contention that propionibacteria are able to survive and grow in the absence of amino acids.

Thiamine had been reported by Tatum et al. *(224)* as stimulatory to certain species of propionibacteria. Quastel and Webley *(172)* reported that the presence of thiamine at low concentrations greatly increased the rate at which acetic acid was oxidized by cells of propionibacteria grown in thiamine-deficient medium. Silverman and Werkman *(194, 195)* found, however, that *Propionibacterium pentosaceum* could be adapted to grow vigorously in its absence. Ostensibly, this vitamin is synthesized by this microorganism. Wood et al. *(261)* found that riboflavin stimulates growth but is not an obligatory requirement nor is it a necessary constituent in the culture medium.

An early report by Snell et al. *(200)* showed pantothenic acid to be an essential growth factor for *P. pentosaceum*. Delwiche *(43)* used a large inoculum and found that repeated transfers of 25 cultures representing 9 different species could be made in a vitamin-free basal synthetic medium. In this medium all cultures had an absolute requirement for pantothenic acid and biotin; a few required *para*-aminobenzoic acid (PABA) or thiamine for growth beyond the fifth or sixth serial transfer. All cultures grew abundantly and rapidly through 15 serial transfers on media of known B-vitamin content, thus discounting the possibility that growth factors were transferred through the inoculum *(43)*. These results supported earlier findings of Thompson *(225)* that only pantothenic acid and biotin were required by propionibacteria. *Propionibacterium freudenreichii* utilizes coenzyme A (CoA) in place of pantothenic acid *(137)*. The response to CoA was approximately one-third of its response to an equivalent amount of calcium pantothenate on a microgram concentration basis. Zodrow et al. *(288)*, in investigating effects of calcium pantothenate and biotin on growth and biosynthesis of corrinoids (precursors to vitamin B_{12}) by propionibacteria, found a synergistic action of calcium pantothenate and biotin. Only calcium pantothenate, however, was essential for biosynthesis of corrinoids.

In preparation for studies on the metabolic role of biotin in species of propionibacteria, Lichstein *(125)* found it necessary to determine the exact requirement for biotin and the activity of several of its analogs. This, the first detailed study of biotin requirements of propionibacteria, revealed that this vitamin could be replaced by either oxybiotin or dethiobiotin in the nutrition of these organisms. He found that, on a microgram concentration basis, dethiobiotin seemed more active than biotin. A straight line response was obtained when the arithmetic concentration of biotin, oxybiotin, or dethiobiotin was plotted against turbidimetric measure of growth. This is in direct contrast to other microorganisms commonly employed for the assay of biotin where a linear response is obtained only when the logarithm of the concentration of vitamin is employed *(125)*.

Shtikkel' et al. *(192)* demonstrated that prolonged culture of strains of *Propionibacterium shermanii* in media containing ascorbic acid produced a stable increase in the amount of growth and vitamin B_{12}-synthesizing properties of these strains under aerobic conditions; the activity of strains in the production of propionic acid, however, was not increased by such treatments.

The carbon source plays an important role in the nutrition of propionibacteria. Antila *(5)* found that formation of lactic acid during cheesemaking provides a suitable fermentable substrate for propionibacteria. He determined that these bacteria will best ferment lactic acid in the presence of yeast extract. Antila and Hietaranta *(9)*, however, stated that a heavy lactate concentration in certain instances hinders growth and fermentation by propionibacteria. Hietaranta and Antila *(78)* determined yeast extract to be definitely stimulatory, but greatest stimulation came from cell-free filtrates of milk cultures of *Streptococcus thermophilus* and *Lactobacillus* spp. According to El-Hagarawy et al. *(54)*, 1% sodium lactate in a basal broth caused a more rapid rate of

TABLE 1. THE FERMENTATION OF VARIOUS CARBOHYDRATES BY SPECIES OF THE GENUS *Propionibacterium*[a]

	Propionibacterium species										
Substrate	*freudenreichii*	*shermanii*	*zeae*	*technicum*	*raffinosaceum*	*peterssonii*	*jensenii*	*arabinosum*	*pentosaceum*	*thoenii*	*rubrum*
Glycerol	+	+	+	+	+	+	+	+	+	+	+
Erythritol	+	+	——	——	——	——	+	——	+	+	+
Adonitol	+	+	——	——	+	——	+	——	+	+	+
Arabinose	−	+	+	+	−	−	−	+	+	−	−
Arabitol	——	+	——	——	+	——	+	——	+	+	+
Rhamnose	−	−	+	——	−	−	−	−	+	−	−
Xylose	−	−	−	−	−	−	−	−	——	−	−
Dulcitol	——	−	−	−	−	−	−	−	−	−	−
Fructose	+	+	+	+	+	+	+	+	+	+	+
Galactose	+	+	+	+	——	+	+	+	+	+	+
Glucose	+	+	+	+	+	+	+	+	+	+	+
Mannitol	−	−	+	+	+	——	+	——	+	−	+
Mannose	+	+	+	+	+	+	+	+	+	+	+
Sorbitol	——	−	——	——	−	−	−	+	+	+	−
Cellobiose	——	——	+	——	——	−	−	+	+	——	——
Lactose	−	+	+	+	——	+	+	——	+	+	+
Maltose	−	−	+	+	——	+	+	+	+	+	+
Melibiose	−	−	——	——	−	——	——	+	——	−	−
Sucrose	−	−	+	+	+	+	+	+	+	+	+
Trehalose	——	−	——	——	+	——	+	——	+	+	+
Melezitose	−	−	——	——	——	——	——	——	——	−	+
Raffinose	−	−	——	+	+	−	+	+	——	−	+
Dextrin	−	−	−	+	−	——	−	——	−	−	−
Esculin	+	+	——	+	——	+	+	——	+	+	+
Glycogen	−	−	−	+	−	−	−	——	−	−	−
Inulin	−	−	−	−	−	−	−	−	−	−	−
Salicin	——	−	+	+	+	+	−	−	+	+	+
Starch	——	−	−	+	−	−	−	——	+	−	−

[a]Data here presented compiled from 7th ed. of Bergey's *(21)*.
+ = positive fermentation
− = negative fermentation
—— = data not available

acid production than when lactose was used in the same concentration. Acid production by *P. shermanii* was considerably increased by addition of sodium lactate, yeast extract, and (or) protein hydrolysates to the medium. Biotin, thiamine, pantothenic acid and *para*-aminobenzoic acid were with out effect *(51, 52, 53, 54)* when yeast extract is provided in the medium. The ability to ferment various carbohydrates differs with each species, as shown by data in Table 1.

Field *(59)* and Field and Lichstein *(61, 62, 63)* found that, when glucose was autoclaved with the medium, it enhanced growth more than when it was added aseptically to the basal broth after autoclaving. They also noted that N-D-glucosylglycine, glycyl-L-asparagine, asparagine, and several dicarboxylic acids could stimulate growth similar to autoclaved glucose media and that acid-hydrolyzed casein stimulated glucose utilization. According to Tatum et al. *(224)*, potato extract, orange juice, and yeast water stimulate glucose fermentation and acid production by propionibacteria. The effect of potato extract probably resulted from essential growth factors and not primarily from its available nitrogen or buffering capacity *(224)*.

Emilsson and Sjöström *(57)* stated that propionibacteria cannot ferment the citric acid in cheese, but according to Antila *(5)*, this observation was probably made with a strain that grew poorly in cheese. Antila *(5)* found that citrate decomposition will occur in cheese if lactate is present. Hietaranta and Antila *(78)* proved that propionibacteria are able to break down citrate; the organisms grow abundantly in yeast water with citrate as the carbon source. They *(79)* found that *Propionibacterium peterssonii* ferments citric acid faster than *P. pentosaceum* and

that the rate of growth in citrate broth was slower than that in lactate broth or lactate-citrate broth. Fermentation of citrate was retarded by the presence of lactate.

Kiuru *(105)* established that metallic iron and iron ions stimulate production of propionic acid, and, according to Pulay et al. *(171)*, *P. shermanii* requires a minimum amount of iron for production of CO_2. Magnesium and manganese ions in fairly large concentrations, accelerate decarboxylation of succinate by *Propionibacterium arabinosum (93)*, and these trace elements along with cobalt are necessary as cofactors in several enzymatic reactions in propionate fermentation *(36, 148, 173, 185)*. Quastel and Webley *(173)* reported that acetate and propionate oxidation by cells of propionibacteria grown in thiamine-deficient medium is accelerated in the presence, but not in the absence, of thiamine by both Mg^{++} and K^{+} and that their effect was synergistic. They noted that, in the absence of added thiamine, Mg^{++} and K^{+} accelerate the oxidation of succinate, fumarate, lactate, ethyl and propyl alcohols, and glucose and that K^{+} augments the effect of Mg^{++}, perhaps by increasing the permeability of the cells to Mg^{++}. Barker and Lipmann *(15)* were two of the early investigators who determined the metabolic role of phosphate among propionibacteria. Propionibacteria require the presence of phosphate and, in some instances, specific types of phosphates for certain enzymatic reactions. This will be discussed in a later section in detail. A further role of phosphates in propionibacterial metabolism including the formation of ATP, phospholipids, RNA, and DNA has been studied and for further information the reader is referred to the paper by Kanopkaite et al. *(92)*. A general article on the nutrition of the propionic-acid bacteria has been written by Krane *(110)*.

SUBSTRATE AND PRODUCT INHIBITION

Growth and fermentation of propionibacteria are stimulated by a variety of compounds utilized in their nutrition. It, therefore, is likely that they can be inhibited by the same or like substances. Growth of five strains of propionibacteria was inhibited to various degrees in media containing up to 4% calcium or sodium propionate *(8)*; the sodium salt was more inhibitory than the calcium salt. Lactate (calcium and sodium salts) also inhibited growth of propionibacteria *(9)*; inhibition depended on the growth medium rather than on the presence of undissociated acid. According to Antila *(5)*, acetate, in certain instances, can stimulate growth of propionibacteria; at a relatively low concentration it, however, could suppress growth.

Field and Lichstein *(60, 61, 62)* studied the effects of different treatments of glucose added to the medium on growth patterns of propionibacteria. They found that, when glucose was separately autoclaved and aseptically added to a synthetic medium, growth was suppressed or delayed, but, when the glucose was mixed with the medium and then autoclaved or autoclaved separately and added to a synthetic medium, there was stimulation of growth. The authors suggested that reducing carbohydrates such as glucose probably react with phosphates and amino acids during heating to produce a factor(s) that replace(s) the CO_2 required for initiation of growth from a small inocula. Rodzevich et al. *(181)* examined use of starch enzymic hydrolysate in nutrient media for *P. shermanii* in place of glucose as a carbon source and found no appreciable difference.

Recently, Neronova and associates *(140, 141)* investigated the growth inhibitory effect of fermentation products of propionibacteria. They reported that propionic acid suppressed growth less than acetic acid and that the magnitude of over-all inhibition was not additive when both acetic and propionic acids were present. When the two acids were present in the medium, the one at the highest relative concentration was responsible for controlling growth. By using lactate as the primary nutrient source, concentration and conditions at which the substrate was inhibitory also were determined.

Since sodium chloride is used in the making and curing of Swiss cheese varieties, it is important to understand what effect it has on growth and metabolism of propionibacteria. According to Peltola *(158)*, sodium lactate is fermented in a nutrient solution containing 4% salt. The beginning of gas formation was retarded by higher concentrations of salt. Rollman and Sjöström *(182)* experimented with different levels of salt ranging from 0 to 8% in a lactate substrate. They found that, with a fast growing strain of *Propionibacterium*, concentrations of 6% salt were required to impede growth at pH 7.0 and 3% at pH 5.2; whereas, a slow growing strain had greater salt tolerance at pH 5.2 than at 7.0.

PHYSICAL FACTORS

Many investigators have utilized 30 C, considered to be the optimum growth temperature, for culturing propionibacteria *(21)*. Orla-Jensen *(151)* stated that propionibacteria grow at temperatures between 15 and 40 C, and Kurmann *(113)* found that the best cheese-plant storage temperatures for cultures range between 5 and 15 C when held 1 month between transfers. Since it was generally believed that propionibacteria are able to grow at temperatures not lower than 10 to 15 C, Park et al. *(154)* set out to

determine the minimum growth temperature of these organisms. They found that the previous lower limit of 10 C was incorrect and that propionibacteria will grow at temperatures between 2.8 and 7.2 C during a 4-month incubation period. Such low-temperature growth patterns have a special significance in the manufacture of Swiss-type cheeses. When growth of various strains of propionibacteria associated with Swiss-type cheeses occur at low temperatures in this range, defects or problems may result for the manufacturer. Zodrow and Stefaniak *(289)* investigated the effect of temperature on growth and corrinoid (vitamin B_{12} precursor) production of *P. shermanii*. They found that the highest yield of corrinoids was obtained at temperatures between 18 and 27 C and maximal propionic-acid production at 24 C when the cultures were incubated for 16 days.

In studying the influence of hydrogen-ion concentration on growth of propionibacteria, Tittsler *(226)* found that the optimum pH was 6.5 to 7.0. Whittier and Sherman *(253)* studied numerous factors affecting the propionic-acid fermentation. The relation of hydrogen-ion concentration to the rate of propionic-acid production distinctly showed that the optimum level is pH 7.0 and that at pH 5.0 there is practically no growth and, consequently, little production of propionic acid. Later, Tittsler and Sanders *(227)* reported that, with the decrease in pH from 6.0 to the "critical point" (pH 5.0), initiation of growth was increasingly delayed and rate of growth was reduced. The bacteria lost viability at pH values below the critical level for growth. Kurtz et al. *(115, 116)* investigated interrelationships between pH and population levels in Swiss cheese and determined that an increase in pH from 5.0 generally correlated with increases in populations of *P. shermanii* in the cheese.

Vorob'eva et al. *(244)* studied the effect of light on growth, fermentation, and oxidizing ability of propionibacteria. They found that a light intensity above 60×10^3 erg sm^2 sec inhibited their growth and decreased the oxidizing ability of propionibacteria and shifted the ratio of fermentation products to favor propionate formation. Light also caused a decrease of vitamin B_{12} and flavin content in the organisms, whereas the cytochrome *b* content underwent no changes.

Vorob'eva *(241, 242)* showed that oxygen can participate in the metabolism of propionibacteria. Gas analysis showed that O_2 is consumed by the bacteria grown on different carbon sources and that O_2 changes the character of the fermentation toward greater pyruvic-acid accumulation.

Associative Action of Other Microorganisms

Growth and fermentative ability of propionibacteria can be stimulated or inhibited by associative growth of other microorganisms. As early as 1921, Sherman and Shaw *(189, 190)* and Whittier and Sherman *(253)* extensively studied the stimulating effect by associative growth of bacteria on the propionic-acid fermentation. They found that propionibacterial fermentations were stimulated during interaction with different types and species of bacteria, some of which were unable to ferment the available carbohydrate. They did not offer any explanation for this phenomenon. Similar observations were made by von Freudenreich and Orla-Jensen *(239)*. Several conflicting reports have since appeared concerning the stimulatory-inhibitory role of the lactobacilli on growth and fermentation by propionic-acid bacteria. Kiuru *(106)* found that a strain of *Lactobacillus lactis* had an appreciable delaying effect on the fermentation reactions of *P. peterssonii*. Winkler *(257)* tested 12 strains of lactobacilli and found 2 strains of *L. lactis*, 2 of *Lactobacillus helveticus*, and 1 of *Lactobacillus acidophilus* inhibited growth of certain strains of propionibacteria. He determined that two inhibitory agents, one heat-labile and the other heat-stable, were involved. Recently, Nieuwenhof et al. *(145)* reported data supporting the contention that lactobacilli stimulate growth of propionibacteria. They offered no explanation for the stimulating effect, but discounted the possibility that variations in the pH of the cheese were responsible. Hietaranta and Antila *(78)* found cell-free chalk-milk culture-filtrates of *L. helveticus* stimulatory, and Hunter and Frazier *(82)* determined that *L. helveticus* produced metabolic products with stimulating activity. These metabolic products could contain excreted biotin and pantothenate, which have been proven stimulatory.

Ritter et al. *(180)* investigated the stimulatory or inhibitory effects of micrococci on propionibacteria. In their study, relative quantitative differences in the volatile acids produced by the propionibacterial test strain in pure culture and in association with micrococci in a test medium were used to measure stimulation or inhibition. Ritter and Schwab *(179)* in later work reported that the presence of micrococci caused approximately a 20% increase in CO_2 production by propionibacteria in cheese.

Enumeration and Isolation

Enumeration and isolation of propionibacteria present certain difficulties because of their strong tendency toward anaerobiosis. Ordinary plating procedures and media generally are of no value for

TABLE 2. THE COMBINATION AND PREPARATION OF A COMPLETE SYNTHETIC CULTURE MEDIUM FOR PROPIONIBACTERIA *(112)*

Component	Amount
I. Minerals	
CH_3COONH_4	4.00 g
$Na_2HPO_4 \cdot 2H_2O$	1.20 g
KH_2PO_4	1.20 g
$MgSO_4 \cdot 7H_2O$	0.60 g
$FeSO_4 \cdot 7H_2O$	0.02 g
$MnCl_2 \cdot 4H_20$	0.01 g
Distilled water	100.00 ml
II. Glucose	5.00 g
Distilled water	80.00 ml
III. Caseinhydrochloride	0.20 g
Distilled water	20.00 ml
IV. Amino acids (A)	
glycine	0.05 g
DL-alanine	0.20 g
DL-phenylalanine	0.20 g
DL-valine	0.20 g
DL-leucine	0.50 g
L (+) arginine	0.20 g
DL-aspartic acid	0.20 g
L (+) glutamic acid	0.50 g
L (−) tryptophane	0.20 g
Distilled water	400.00 ml
Amino acids (B)	
DL-isoleucine	0.20 g
L (+) lysine-dihydrochloride	0.20 g
DL-proline	0.20 g
L (−) oxyproline	0.20 g
DL-histidine-monohydrochloride	0.20 g
L (−) tyrosine	0.20 g
DL-methionine	0.20 g
Distilled water	300.00 ml
Amino acids (C)	
DL-cystine	0.05 g
DL-serine	0.20 g
DL-threonine	0.20 g
Distilled water	100.00 ml
V. Vitamins or growth factors	
thiamine	0.40 g
riboflavin	0.40 g
nicotinic acid	0.40 g
biotin	0.40 g
meso-inositol	0.40 g
Ca-pantothenate	0.40 g
p-aminobenzoic acid	0.40 g
pyridoxyl-PO_4	0.40 g
folic acid	0.40 g
ascorbic acid	0.40 g
cholinchlorine	0.40 g
Distilled water	40.00 ml

Because of the durability of solutions I, II, III, and IV, they are prepared separately. They are brought to pH 7.0 with sodium hydroxide and autoclaved for 20 min at 121 C. The growth-factor solution V is filter-sterilized. Solutions I to IV are mixed together (yielding 1 liter) and 2 ml of the growth-factor solution V is added. The substrate is filled aseptically into sterile tubes (3-5-10 ml, etc.) and is thus ready to use.

enumeration because propionibacteria do not grow under conventional plating conditions and do not compete well with other microflora in general-purpose counting media. Many investigators have worked on these problems, but even though several suitable growth media have been developed, a completely satisfactory selective medium is still unavailable.

Von Freudenreich and Orla-Jensen *(239)*, who first isolated these organisms from Swiss cheese in 1906, employed gelatin plates and shake cultures. Sherman and Shaw *(190)* added dilutions of cheese to tubes containing nutrient agar made with peptone, yeast extract, lactic acid, and agar and then sealed the tubes with the seeded nutrient medium. Until 1941, the shake-culture method of growing and counting was used almost exclusively for propionibacteria. At this time, Demeter and Janoschek *(47)* and, a little later, Dorner *(49)* found that a medium containing peptone, sodium lactate, and yeast extract was suitable to isolate and enumerate propionibacteria in cheese. With this medium, Demeter and Janoschek *(47)* recommended that, before pouring plates, 5 ml of a 0.5% sodium sulfite solution be added to each 100 ml of agar. They also cautioned to make deep pour plates covered with 3-4 mm of paraffin oils, which, in combination with sodium sulfite, will provide a suitable anaerobic environment. This type of medium was later altered by Kambar et al. *(87)*, who added sodium thioglycollate instead of sodium sulfite to the medium and covered the agar with a thick, water-agar layer to maintain a reduced oxidation-reduction potential.

Niethammer and Hitzler *(144)* investigated the suitability of various media for growth of different strains of propionibacteria isolated from cheese and other materials. They found that best results were obtained with Difco "Brewer Anaerobic Agar 279" *(48)*. After incubation at 22 to 24 C, growth begins in a few days. The organisms remain viable on this medium for 4 months. A 1.0% solution of Vitambact (Hameln) with 1.0% glucose is recommended for liquid cultures. According to Stolp *(213)* Vitambact contains normal ingredients for growth, enzyme-di-

gested yeast, and amino acids as well as trace elements.

Kurmann *(112)*, in 1960, developed a completely synthetic medium for growth of propionibacteria. The composition of this medium is shown on Table 2. Later, Kurmann *(114)* attempted to develop a selective medium to detect propionibacteria in milk. He modified a lactate agar by adding one or a combination of formalin, sodium sulfite, sodium taurocholate, sodium tellurite, sodium thiosulfate, phenol, thallus sulfate, 2,3,5-triphenyltetrazolium chloride, and hydrogen peroxide. These additions did not improve its selectivity.

Recently developed methods for enumeration and isolation of propionibacteria include the candle-oats jar procedure of Vedamuthu and Reinbold *(232)* and the pouch method of Hettinga et al. *(77)*. With the use of a candle-oats jar, Vedamuthu and Reinbold *(232)* produced a humid semianaerobic, high CO_2 tension atmosphere. Such an environment with lactate agar provided ideal conditions for good development of surface colonies of propionibacteria, necessary for observation of colonial characteristics and for pure culture isolation. The pouch method technique involved the use of a 2% lactate agar placed in a pouch made of a clear triple-laminated film of low gaseous diffusibility to obtain sufficient anaerobiosis. This method has reduced the incubation time and space needed for conventional plating methods *(77)*. Development of a selective enumeration medium for the propionibacteria by use of the H_2S-producing ability of the genus as a differentiating character also has been investigated (*unpublished data,* ISU). Results have shown that this approach is unreliable since there is considerable variation in H_2S production by different species and strains of propionibacteria. For certain strains and species, use of iron-sulfite, sodium-lactate medium was adequately selective.

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PROGRAM FOR ISM 15TH ANNIVERSARY CONFERENCE

(Continued from Page 284)

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FATE OF *STAPHYLOCOCCUS AUREUS* IN CULTURED BUTTERMILK, SOUR CREAM, AND YOGURT DURING STORAGE[1]

T. E. Minor and E. H. Marth

Department of Food Science and The Food Research Institute
University of Wisconsin
Madison, Wisconsin 53706

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Abstract

Five brands each of commercially produced cultured buttermilk, sour cream, and unflavored yogurt were inoculated with *Staphylococcus aureus* to contain initial numbers of approximately 10^2 and 10^5 organisms per gram. Inoculated products were stored at 7 and 23 C and were tested daily to determine numbers of viable staphylococci. Generally, products which contained initial numbers of 10^2 organisms per gram were free of viable staphylococci, or nearly so, within 24 hr regardless of storage temperature. When products were inoculated with higher numbers of the organism, survival was greatest in sour cream which was followed in order by buttermilk and yogurt. Yogurt samples with approximately 10^5 organisms per gram, initially, were free of viable staphylococci by the 2nd to 4th day of storage, whereas the organisms persisted in sour cream until the 4th to 7th day. Survival was markedly higher in one brand of sour cream and somewhat so in another. Temperature of storage had no obvious effect on survival of staphylococci in buttermilk or yogurt but survival was greater in sour cream stored at 7 C than at 23 C. The pH values of the products remained reasonably stable throughout storage.

It is not uncommon for cheese to be contaminated with *Staphylococcus aureus* and this cultured food occasionally has been associated with staphylococcal intoxications *(7)*. When cheeses became toxic they were made from raw milk containing staphylococci and/or staphylococcal enterotoxin, from toxic raw milk which was pasteurized (pasteurization inactivates staphylococci but not enterotoxin), or from non-toxic raw milk which was pasteurized and then contaminated with staphylococci. It is well known that staphylococci can grow and produce enterotoxin in milk during cheesemaking, particularly if acid development by the lactic acid bacteria is inadequate *(7)*. Earlier work in our laboratory *(4)*, indicated that growth of *S. aureus* in pasteurized milk gradually acidified with different acids was related to the change with time in hydrogen ion concentration.

Other cultured dairy products such as buttermilk, sour cream, and yogurt are not likely vehicles for staphylococcal food poisoning and this is borne out by the lack of reported outbreaks attributed to these foods *(7)*. Cultured milks customarily receive a rigorous heat treatment (e.g. 85-91 C for 0.5 to 1.0 hr) in the initial stage of their manufacture. This heat treatment not only inactivates staphylococci but might inactivate a part or all of the enterotoxin, at least type A, if some were present *(6)*. In order for staphylococci to occur in cultured milks they must enter heated milk before or during fermentation or they must enter the finished product before it is consumed. Although it is commonly assumed that staphylococci do not retain viability for prolonged periods when they are in these acid foods, there are no data in the literature to substantiate this conclusion. Survival of staphylococci in these foods is of concern since some of them, particularly sour cream and buttermilk, often serve as ingredients in other foods, where the protective effect of a low pH may be lacking. Consequently, the present study was made to determine the fate of *S. aureus* when the bacterium was added to commercially prepared buttermilk, sour cream, and unflavored yogurt.

Materials and Methods

Experimental design

Five different brands each of cultured buttermilk, sour cream, and unflavored yogurt were inoculated with approximately 10^2 and 10^5 *S. aureus* per gram and then were stored at 7 and 23 C for up to 7 days. Two replicates for each variable and a single control (no *S. aureus*) were prepared of each brand for each storage temperature and were tested simultaneously.

Culture

A culture of *S. aureus* strain 100 was obtained from Dr. K. F. Weiss (The Food Research Institute, University of Wisconsin). The organism was stocked on Brain Heart Infusion (BHI) agar (Difco) slants and refrigerated. Before each experiment, the organism was transferred from a stock slant to BHI broth, incubated at 37 C for 24 hr, transferred to BHI broth a second time, and used after 18 hr of incubation at 37 C. The broth culture was used directly to establish a population of 10^5 organisms per gram in the test product and a 1:1000 dilution [in sodium phosphate buffer (pH 7.4)] of culture was employed for the smaller inoculum.

Preparation of test samples

Samples of commercial cultured products were obtained

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TABLE 1. SURVIVAL OF *S. aureus* IN CULTURED BUTTERMILK STORED AT 7 AND 23 C FOR UP TO 6 DAYS

		No. *S. aureus*/gram after days							
	Initial	Small inoculum			Large inoculum				
Brand	pH	0	1	2	0	1	2	3	4
					7 C				
1	4.3	400	80	<10	300,000	70,000	20,000	5,000	700[1]
2	4.3	400	<10	—	700,000	1,000	300	40	<10
3	4.4	400	20	<10	200,000	5,000	1,000	300	<10
4	4.4	400	<10	—	200,000	1,000	70	<10	—
5	4.1	400	30	<10	400,000	800	60	<10	—
					23 C				
1	4.3	600	200	200[2]	100,000	10,000	3,000	1,000	90[3]
2	4.3	500	<10	—	500,000	1,000	400	10	<10
3	4.4	700	30	<10	200,000	3,000	1,000	500	40[3]
4	4.4	600	20	<10	300,000	6,000	800	50	<10
5	4.1	500	<10	—	300,000	1,000	400	30	<10

[1]Days 5, 6 = 20, <10
[2]Days 3, 4, 5 = 100, 80, <10
[3]5th day = <10

TABLE 2. SURVIVAL OF *S. aureus* IN SOUR CREAM STORED AT 7 AND 23 C FOR UP TO 7 DAYS

		No. *S. aureus*/gram after days							
	Initial	Small inoculum		Large inoculum					
Brand	pH	0	1	0	1	2	3	4	5
					7 C				
1	4.4	400	<10	40,000	2,000	600	200	10	<10
2	4.3	400	<10	300,000	20,000	3,000	700	50	<10
3	4.4	400	200[1]	90,000	400,000	400,000	300,000	200,000	80,000[2]
4	4.3	800	<10	100,000	6,000	1,000	500	40	<10
5	4.4	600	<10	900,000	50,000	60,000	20,000	100	20[3]
					23 C				
1	4.4	400	<10	30,000	50	20	<10	—	—
2	4.3	400	<10	300,000	3,000	900	500	50	<10
3	4.4	400	400[4]	200,000	200,000	200,000	80,000	10,000	8,000[5]
4	4.3	900	<10	100,000	7,000	4,000	2,000	100	<10
5	4.4	900	<10	900,000	50,000	3,000	100	20	<10

[1]Days 2, 3, 4 = 100, 60, <10
[2]Days 6, 7 = 40,000, 7,000
[3]6th day = <10
[4]Days 2, 3 = 50, <10
[5]Days 6, 7 = 3,000, 900

from local retail outlets. Buttermilk was acquired in one-quart quantities and inoculated in its container by adding 1 ml of inoculum through a small hole pierced in the top of the carton. The hole was sealed with adhesive tape and the container was shaken vigorously 25 times. Samples of the other dairy products were obtained in 8- or 16-oz. quantities and 400 g of the product were weighed into each of two sterile 1-liter beakers. The product plus 0.5 ml of inoculum was stirred vigorously with a spatula. Each of the inoculated materials was distributed equally among four sterile 8-oz round wide-mouth jars (one-half of the jars were stored at one temperature and the remainder at the other). Controls were prepared from untreated product.

Determination of cell survival

At appropriate intervals, an 11-g aliquot was aseptically removed from each jar after the contents were briefly stirred with a sterile spatula. Appropriate dilutions were made in sterile buffered water and pour plates were prepared according to recommendations of *Standard Methods for the Examination of Dairy Products (11)*. Plates were poured with Mannitol Salt agar (Difco) and incubated at 37 C for 48 hr. Mannitol-fermenting colonies of proper size and appearance were recorded as *S. aureus* and occasionally were confirmed as staphylococci by microscopic examination. The average cell population of two replicates was reported for each variable. For all counts of <100 per gram, the number

TABLE 3. SURVIVAL OF S. *aureus* IN UNFLAVORED YOGURT STORED AT 7 AND 23 C FOR UP TO 4 DAYS

Brand	Initial pH	No. S. *aureus*/gram after days						
		Small inoculum		Large inoculum				
		0	1	0	1	2	3	4
7 C								
1	3.9	200	<10	30,000	40	10	<10	—
2	4.0	200	<10	30,000	50	30	<10	—
3	3.9	300	<10	40,000	100	<10	—	—
4	4.1	200	<10	200,000	1,000	200	50	<10
5	3.7	200	<10	100,000	5,000	600	100	<10
23 C								
1	3.9	200	<10	20,000	30	10	—	—
2	4.0	300	10[1]	40,000	300	80	40	<10
3	3.9	100	<10	40,000	20	<10	—	—
4	4.1	200	<10	100,000	700	50	10	<10
5	3.7	200	<10	200,000	2,000	700	100	<10

[1]2nd day = <10

of "staphylococcal-like" colonies per gram obtained from the control plates (usually <30) was subtracted to obtain the final result. Because of the opaque nature of dairy products, staphylococcal counts of <10 per gram were unobtainable.

RESULTS

Inactivation of S. aureus in buttermilk

Table 1 depicts the survival of S. *aureus* in cultured buttermilk. Staphylococci were rapidly inactivated in brands 2-5 and neither brand, storage temperature, size of inoculum, nor initial pH appeared to have any substantial effect on the rate of inactivation. Survival of S. *aureus* in brand 1 was greater and longer than in the other brands, although the pH value was similar to those of the other buttermilks.

With the exception of data obtained from brand 1 stored at 23 C, staphylococci were not detected after 24-48 hr of storage when the buttermilks initially contained 400-700 organisms per gram. Buttermilks inoculated with 2-7 $\times$ 10^5 S. *aureus* per gram were free of detectable staphylococci after 3-6 days of storage. Approximately 99% of staphylococci present initially in buttermilks 2-5 failed to survive 24 hr of storage at either temperature, regardless of the size of the inoculum.

Fate of S. aureus in sour cream

The fate of S. *aureus* in sour cream is shown in Table 2. Results obtained with this product were somewhat different than those reported for buttermilk (Table 1).

Recovery of initial numbers of organisms varied substantially between some of the products when the larger inoculum was employed but this problem did not exist when the smaller inoculum was used. Except for brand 3, no staphylococci were detected after 24 hr of storage in sour cream inoculated with 400-900 organisms per gram. A population of 400 staphylococci per gram persisted in brand 3 for 3-4 days. Survival of organisms was markedly greater in sour cream 3 and somewhat so in sour cream 5 when the larger inoculum was used. Survival of S. *aureus* was greater in sour creams 1, 2, and 4 stored at 7 C (large inoculum) than when these products were stored at 23 C. Survival of organisms in sour creams 1, 2, and 4 stored at 23 C (large inoculum) followed the trend observed with buttermilk.

There was very little difference in initial pH between brands of sour cream and the pH values approximated those observed when buttermilk samples were tested.

Inactivation of S. aureus in yogurt

Data on survival of S. *aureus* in yogurt are detailed in Table 3. Inactivation of staphylococci was greater in yogurt than in buttermilk or sour cream (Tables 1, 2). Again, some variation occurred in initial recovery of staphylococci (yogurts 1-3) when products were treated with the large inoculum.

Staphylococci were completely inactivated within 24 hr in nearly all yogurts inoculated with 100-300 cells per gram. No organisms were detected after 2-4 days of storage when yogurt received the large inoculum of S. *aureus*. In most instances, 99% or more of the organisms were inactivated within 24 hr of storage (large inoculum). The brand, initial pH, storage temperature, and size of inoculum had little, if any, effect on inactivation of staphylococci in the yogurts. The pH values of these products were in the range of 3.7-4.1 and were lower than

those of buttermilk and sour cream samples.

Product stability

The pH of all products remained stable over the storage periods, never changing >0.2 unit. Changes in pH were, for the most part, upward.

The nonstaphylococcal flora of buttermilk samples was monitored throughout the storage periods. Initial numbers of organisms varied from 1×10^6 to 3×10^8. The numbers remained quite stable during storage at 7 C and declined about 10-fold during storage at 23 C.

DISCUSSION

Data obtained in this study indicate that, in general, cultured dairy products which might be contaminated with staphylococci in the plant subsequent to fermentation should be free of the organisms by the time they reach the consumer. The data also show that inactivation of S. *aureus* in these foods is not uniform and in some instances viable staphylococci could reach the consumer albeit in low concentrations. Growth of staphylococci did not occur in any of the foods tested. This suggests that they would not become hazardous even if staphylococci were present and if the product were abused by failure to store it in a refrigerator. The only concern then would center around the use of these products, if they were to contain viable staphylococci, as ingredients in other foods where growth of and toxin production by staphylococci would be possible.

Fate of coliforms (10^3/g) in yogurt, buttermilk, sour cream, and cottage cheese during storage at 7.2 C was studied by Goel et al. *(1)*. Many of the yogurts were free of viable coliforms within 24 hr, whereas survival was extended for 3-10 days in the other products. In one sour cream sample, extended survival of *Escherichia coli* was noted. Several samples of cottage cheese supported substantial growth of coliforms and extended survival occurred in others. Studies by Mickelsen et al. *(3)* and Tuckey et al. *(10)* indicate growth or extended survival of staphylococci in cottage cheese during storage is unlikely, particularly if the cheese is made by the short-set method.

We studied inactivation of S. *aureus* (10^8/ml) in an artificial medium containing lactic acid and observed a 10,000-fold decline in numbers after 24 hr of incubation at 37 C when the pH of the medium was 4.3 *(9)*. Initial inactivation of S. *aureus* in cultured dairy products was, therefore, not as great as that observed in the artificial medium. Survival of staphylococci in the artificial medium appeared to be primarily a function of hydrogen ion concentration, whereas the behavior of S. *aureus* in cultured dairy products appears to be related to factors other than just pH. It is well known that other microorganisms can produce substances which stimulate or inhibit growth of staphylococci *(5, 7, 8)*, but their influence on survival is unknown. Different strains, species, and genera of microorganisms are utilized as starter cultures to manufacture cultured milks. Since products of microbial growth can vary even between two related strains, the type of starter culture used by a manufacturer may, in part, determine the suitability of a fermented food for survival of staphylococci.

The medium used in this investigation to recover staphylococci (Mannitol Salt agar), like most selective-differential media, has a low plating efficiency *(5)*. We tested the efficiency of Trypticase Soy agar with and without 7% added NaCl in recovering S. *aureus* exposed to several hydrogen ion concentrations for 24 hr in Trypticase Soy broth. A 10-fold reduction in the number of recoverable organisms was observed regardless of exposure to acid when the salt agar was used (unpublished data). This may account for the low initial recoveries sometimes encountered in this study. Recovery was not impaired initially in two-thirds of the samples, perhaps because the food, in some instances, afforded greater protection to the organism. McDivitt and Topp *(2)* reported that staphylococci grown in milk were better able to withstand the selective action of some media.

Results of this study suggest that there is at least one unanswered question worthy of further investigation. The variability of staphylococcal survival in acidic media which normally are very deleterious to the microorganisms may have significant consequences for human health as related to consumption of certain foods.

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INTERACTIONS OF FOOD STARTER CULTURES AND FOOD-BORNE PATHOGENS: AN INTRODUCTORY STATEMENT[1]

M. L. SPECK
Department of Food Science
North Carolina State University
Raleigh, North Carolina 27607

(Received for publication November 3, 1971)

[1]A symposium on interactions between food starter cultures and food-borne pathogens was held at the 71st Annual Meeting of the American Society for Microbiology in Minneapolis, Minnesota, May 2-7, 1971. The symposium consisted of an introductory statement by Dr. M. L. Speck and papers by Drs. S. E. Gilliland, A. Hurst, W. E. Sandine, C. E. Parmelee, and K. Shahani. Most of the papers presented at the symposium will appear in this and in subsequent issues of the *Journal of Milk and Food Technology*.

Use of starter bacteria to manufacture cultured foods has been practiced for so many years that many of the resultant bioprocessed foods are now considered a normal, if not essential, part of man's diet. While the exact composition and flavor of a given type of food may vary from country to country, food production through mediation by microbial actions is world-wide.

A primary appeal of cultured foods has been their unique flavor. This has not always been the basis for their appeal. Before foods could be preserved by many of our modern techniques, the biological conversions by microorganisms were used to advantage. Consumption of milk preserved by a lactic acid fermentation led Metchnikoff into his interesting research and provocative conclusions regarding the exceptional nutritional qualities of Bulgarian milk. Although efforts were made to renew interest in this fascinating field in the 1920's and 1930's, microbial technology as well as medical and scientific interest were not prepared to explore the merits of the research.

Today, there is a renewed interest in the place that food bioprocessing microorganisms can have in improving human health. A number of factors have contributed to this, such as: (*a*) a greater awareness of the extensive human morbidity resulting from food-borne illnesses caused by salmonellae, staphylococci, *Clostridium perfringens,* and shigellae; (*b*) the need for longer shelf-life of foods caused by centralized food processing and modern merchandising methods, and corollary observations that many bioprocessed foods do possess excellent shelf-life; (*c*) a revived interest in the functions of the intestinal tract as influenced by its microbial flora; (*d*) research during recent years that has revealed marked antagonisms by conventional lactic streptococci, lactobacilli, and leuconostocs for various food-borne pathogens and food-spoilage microorganisms; and (*e*) the sophisticated technology now available whereby large quantities of food starter microorganisms can be developed for use in the controlled preparation of bioprocessed foods, or even as an individual component of the human diet.

During the 1969 meeting of the American Society for Microbiology in Miami Beach, the Food Section mentioned this seminar as a likely candidate for scheduling at a forth-coming annual meeting. At the 1970 meeting in Boston, your convenor was asked to organize the seminar which is now being presented. The participants have been actively engaged in research dealing with antagonisms of the lactic acid bacteria for food-borne pathogens or spoilage microorganisms. They will attempt to present primarily the high-lights of information available on the subject in their individual presentations. It is hoped that these brief presentations will help encourage research by more of our colleagues in these most fascinating and challenging fields of microbial ecology and food production.

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INTERACTIONS OF FOOD STARTER CULTURES AND FOOD-BORNE PATHOGENS: LACTIC STREPTOCOCCI VERSUS STAPHYLOCOCCI AND SALMONELLAE[1]

S. E. Gilliland and M. L. Speck
Department of Food Science
North Carolina State University
Raleigh, North Carolina 27607

(Received for publication November 3, 1971)

Abstract

The lactic streptococci have been utilized to produce certain fermented foods for many years. In addition to producing a cultured food having new characteristics, these organisms aid in preserving the food. Growth of salmonellae and staphylococci is retarded in foods cultured with lactic streptococci. This antagonistic action is caused by factors in addition to the acidic environment created by the streptococci since pathogens can be repressed even when the milk is maintained at pH 6.6 during starter growth. The intensity of the antagonistic action cannot be predicted by the rate at which acid is produced by the streptococci.

Historically the lactic streptococci have been important in the production of certain fermented foods. For many years presence of these organisms was dependent on the microbial flora occurring in the raw food or on those entering the food from its immediate environment. Originally food was fermented for preservation, however, as technology advanced the demand for higher quality cultured foods resulted in use of starter cultures for food bioprocessing. Efforts to improve or standardize the quality of cultured foods have been directed toward controlling the kinds of microorganisms effecting the changes desired in the foods. Flavor and texture are usually the primary qualities by which cultured foods are evaluated and cultures for bioprocessing are selected for their ability to develop these qualities. An added role of certain cultures is being recognized as that of inhibiting food-borne pathogens. There has been a striking absence of food-borne illnesses caused by properly cultured dairy products. This indicates that the starter cultures might have a definite relationship to human health by their restrictive effects on food-borne pathogens. The purpose of our investigations has been to study the nature of the interactions between some of the lactic acid starter cultures and species of salmonellae and staphylococci. A better understanding of such interactions should enable more satisfactory manufacture, storage, and distribution of semi-preserved foods.

[1]Presented at the 71st Annual Meeting of the American Society for Microbiology, Minneapolis, Minnesota, May 2-7, 1971.

Growth of salmonellae and staphylococci has been shown to be retarded in certain cultured milk products. Studies reported by Goepfert and Chung *(3)* indicated that salmonellae were capable of some growth during the manufacture of fermented sausage. However, the number present decreased during the curing of the cheese. Their results suggest that the lactic streptococci exert a repressive effect on salmonellae. There are a number of reports in the literature indicating that staphylococci added to milk grow some during manufacture of Cheddar or Colby cheese but decrease in number as the cheeses are aged. It is, however, unclear whether or not the starter cultures exert antagonistic actions on the staphylococci. Reiter et al. *(8)* reported that staphylococci were inhibited during production of Cheddar cheese and the number of staphylococci decreased as the cheese was aged. Repression of the staphylococci was decreased when the starter was infected with bacteriophage. Similar findings were reported by Jezeski et al. *(5)*.

Addition of *Streptococcus lactis* cultures to ground beef was reported by Reddy et al. *(7)* to suppress growth and action of psychrotrophic spoilage bacteria resulting in extended shelf life for the product.

Interactions between Lactic Cultures and Pathogens

In an effort to understand these antagonisms more completely, we have studied interactions among commercial lactic streptococcus cultures and different food-borne pathogens. When lactic streptococci were inoculated into milk containing salmonellae, growth of salmonellae was repressed. Results of such an experiment are shown in the lower portion of Fig. 1. For this experiment two containers of sterile reconstituted non-fat milk solids were inoculated with 600-700 *Salmonella gallinarum*/ml. One container was then additionally inoculated with 1% of a milk culture of a commercial lactic streptococcus cheese starter (VT_3). Both containers were incubated statically at 32 C and populations of salmonellae determined by plating on brilliant green agar. As can

be seen, growth of S. *gallinarum* was repressed in the presence of the streptococci. The repression of salmonellae was not due to the low pH created by the streptococci, as illustrated by the data shown in the upper portion of this slide. In this experiment the milk cultures were contained in New Brunswick fermentors and the constant pH maintained by automatic addition of ammonium hydroxide. Even though the pH of both samples was maintained at the initial pH of uninoculated milk, the antagonistic action of the streptococci toward the S. *gallinarum* was still very evident.

Figure 2 shows the results of a similar experiment involving *Staphylococcus aureus* and Cheddar cheese starter VT_5. The pH of both samples was maintained at 6.6 to eliminate any effect from a low pH. The staphylococci were enumerated on mannitol salt agar. Results show that the staphylococci are also susceptible to inhibition by streptococci and that the antagonism is not entirely from the low pH. In experiments involving staphylococci in which the pH was not controlled, inhibition was greater.

A primary function of the lactic streptococci in manufacturing cultured milk products is to produce acid; however, the effectiveness of the streptococci in repressing salmonellae and staphylococci does not necessarily coincide with the rate of acid production. A total of 15 commercial Cheddar cheese starter cultures were evaluated for their rapidity of acid production in milk and their inhibition of salmonellae and staphylococci. A container of sterile reconstituted non-fat milk solids was inoculated with 1×10^3 *Salmonella typhimurium* and *Staphylococcus aureus* per ml. The inoculated milk was then aseptically dispensed into sterile tubes. One tube served as the control and the remaining tubes were further inoculated with 1% using the lactic streptococcus starter cultures. All tubes were incubated at 32 C for 6 hr after which the salmonellae were enumerated on brilliant green agar and the staphylococci on mannitol salt agar. The percent inhibition for each pathogen was calculated using the following formula:

$$100 \left[\frac{\text{(Population of pathogen alone)-} \\ \text{(Population in presence of starter)}}{\text{Population of pathogen alone}} \right] = \text{\% Inhibition}$$

All the streptococcus cultures evaluated were antagonistic toward the pathogens. Results from the evaluation of six of the streptococcus cultures are presented in Table 1. The times required for the cultures to reach pH 5.0 are shown and the streptococcus cultures (A-F) are arranged in order of decreasing activity with respect to acid production. The staphylococcus culture was inhibited to about the same degree (98.1 to 98.9%) by all six streptococcus cultures and was more sensitive than S. *typhi-*

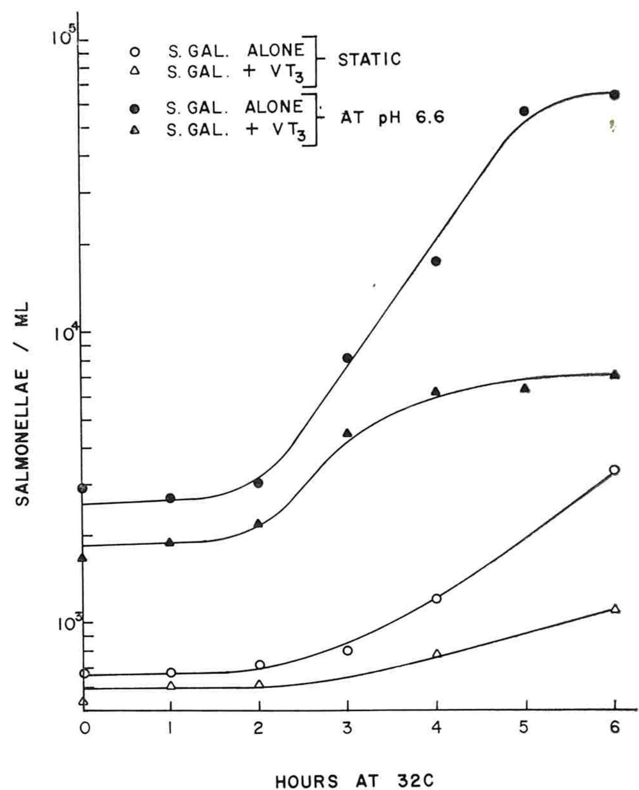

Figure 1. Inhibition of *Salmonella gallinarum* by lactic streptococcus VT_3 in milk with and without pH control.

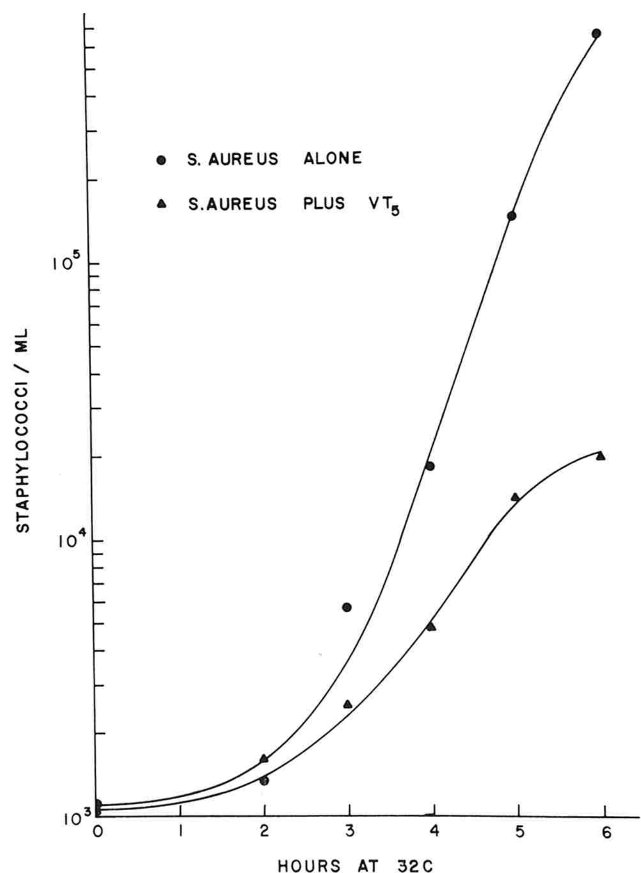

Figure 2. Inhibition of *Staphylococcus aureus* by lactic streptococcus VT_5 in milk at pH 6.6.

TABLE 1. INHIBITION OF *Salmonella Typhimurium* AND *Staphylococcus aureus* BY LACTIC STREPTOCOCCI IN MILK.

Streptococcus culture	Minutes to reach pH 5.0	Percent inhibition	
		S. typhimurium	*S. aureus*
A	351	92.0	98.9
B	360	88.2	98.3
C	375	93.4	98.6
D	381	91.2	98.6
E	387	86.3	98.1
F	435	89.1	98.5

murium. The amount of inhibition of the *Salmonella* ranged from 86.3 to 93.4%. These data show that streptococcus cultures which produce acid rapidly, such as A and B, are not necessarily most antagonistic toward the pathogens. The degree of antagonism toward the pathogens cannot be predicted by the rate at which acid is produced by the streptococci in milk.

MODE OF ACTION

When considering the mode of action of the antagonism produced by the streptococci, we must include such possibilities as production of antibiotics, hydrogen peroxide, and volatile fatty acids. Inhibition of food-borne pathogens by several other types of food bioprocessing organisms has been attributed to these substances. Certain lactobacilli have been shown in our laboratories to produce sufficient hydrogen peroxide to inhibit coagulase positive staphylococci. Streptococci also produce hydrogen peroxide. Results from studies in our laboratory *(2)* have shown that the lactic streptococci produce auto-inhibitory levels of hydrogen peroxide. Milk cultures produced acid faster in the presence of catalase which corresponded to a lack of hydrogen peroxide accumulation. The peroxide accumulated in the early stages of acid production then dissipated. Since the streptococci produce sufficient levels of peroxide to limit their acid production, it is possible that they produce a sufficient amount to affect growth of food-borne pathogens. Preliminary studies have indicated that hydrogen peroxide alone is not responsible for the antagonism of the streptococci toward the salmonellae and staphylococci, however, it may contribute to the antagonism particularly during the early periods of growth.

The leuconostocs, which are component strains of certain mixed strain starter cultures, have been shown to be inhibitory to *S. gallinarum* and certain other gram-negative bacteria. The main inhibitor produced was identified as acetic acid which was much more antagonistic than lactic or mineral acids at comparable pH values *(9)*. Growth of the salmonellae was completely inhibited in broth containing 0.012 *M* acetic acid (pH 4.5). The broth containing lactic acid alone (pH 4.5) permitted some growth, even though this was at a lower level than the control (broth adjusted to pH 4.5 with HCl). Presence of acetic acid-producing leuconostocs or related species in starter cultures would be expected to enhance the inhibition of food-borne pathogens in bioprocessed foods manufactured with these cultures. Naturally, these strains must be compatible with the other component strains of bacteria and not adversely affect the final flavor of the finished food.

Baribo and Foster *(1)* reported production of an inhibitor by *Streptococcus lactis* active against *Lactobacillus casei*. It was most active at an acid pH. Certain of the streptococci produce antibiotics characterized as proteins or peptides and named diplococcin *(6)* and nisin *(4)* which might also be responsible for inhibition of food-borne pathogens. Other unidentified antibiotics might also be involved.

In some instances, repression of food-borne pathogens by food-bioprocessing bacteria has been attributed entirely to depletion of nutrients required by the pathogens. This does not appear to be true with the lactic streptococci. The antagonism was evident during the early periods of incubation and it does not seem reasonable that the streptococci would deplete the milk of vital nutrients in such short periods. Furthermore, the salmonellae and staphylococci are usually far less nutritionally fastidious than the lactic streptococci.

The cause of the antagonism toward salmonellae and staphylococci produced by the lactic streptococci is far from being completely elucidated. The antagonism may be the result of one or more of the aforementioned possibilities. Additional unknown inhibitors are suspected to be present.

To further insure the absence of food-borne pathogens from bioprocessed foods, those starter cultures that exhibit maximum antagonism toward the pathogens should be given preferential selection for use in food manufacture. This is especially true in view of the desire of regulatory and health agencies to eliminate the dissemination of food-borne pathogens.

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PROGRAM

FIFTY-NINTH ANNUAL MEETING
INTERNATIONAL ASSOCIATION OF MILK, FOOD AND ENVIRONMENTAL SANITARIANS, INC.
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Pfister Hotel **Milwaukee, Wisconsin**

August 21-24, 1972

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TOPICS FOR AFFILIATE AGENDA AT ANNUAL MEETING

1. Review of objectives of the Affiliate Council.
2. Affiliate participation of the development of the annual program.
3. Discussion of criteria for and recommendation of nominations for Sanitarian of the Year Award.

SUNDAY, AUGUST 20, 1972

1:30-5:30 P.M.—Executive Board—Mirror Room
8:00-11:00 P.M.—Executive Board—Mirror Room

MONDAY, AUGUST 21, 1972

NATIONAL MASTITIS COUNCIL REGIONAL MEETING

(See separate program for time schedule and subjects)
8:00 A.M.-1:00 P.M.—Registration—Grand Foyer, 7th Floor

CHECK BULLETIN BOARD

FARM METHODS COMMITTEE—To be announced Louis XIV Room

SPECIAL MEETINGS:

8:00 A.M.-12:00 Noon—Executive Board—Mirror Room
1. Report on Local Arrangements
2. Report of Executive Secretary
3. Report of Sanitarians Joint Council

1:30 P.M.-5:00 P.M.—Executive Board—Mirror Room
1. Report of Journal Management Committee
2. Regular Agenda

1:30 P.M.-5:00 P.M.—Individual Committee Meetings (See Bulletin Board)

7:30 P.M.-9:00 P.M.—Affiliate Council—Henry VIII Room

7:30 P.M.-10:00 P.M.—Executive Board—Mirror Room
1. Committee Chairman
2. Meet with Past Presidents
3. Reports of Affiliate Council Chairman

5:30 P.M.-6:30 P.M.—Cocktail Reception Imperial Ballroom

TUESDAY, AUGUST 22, 1972

8:00-5:00—REGISTRATION—
Grand Foyer, 7th Floor

MORNING—GENERAL SESSION—IMPERIAL BALLROOM

WALTER F. WILSON, *President-Elect, Presiding*

9:30—INVOCATION
REV. EARL W. DUNST,
St. Paul's Church

9:35—ADDRESS OF WELCOME
DONALD I. WILKINSON,
Sec. Wisconsin Dept. of Agriculture

9:50—PRESIDENTIAL ADDRESS
ORLOWE M. OSTEN

10:15—"THE FOOD INDUSTRIES IN THE SEVENTIES"
WILLIAM O. BEERS

11:00—"FOOD REGULATORY ACTIVITIES"
VIRGIL O. WODICKA

11:45—NOMINATIONS-1973
ORLOWE M. OSTEN

AFTERNOON—MILK SANITATION SECTION—IMPERIAL BALLROOM

PARNELL J. SKULBORSTAD, *Presiding*

1:30—DOOR PRIZE DRAWING

1:45—"ANALYSIS OF ABNORMAL MILK PROGRAM"
WALLACE C. LAWTON

2:30—"REVERSE OSMOSIS WHEY SEPARATION"
J. R. BRUCE

3:15—MILK BREAK

3:30—"LAGOONS: A TREATMENT SYSTEM FOR MILKING CENTER WASTES."
DR. ROBERT R. ZALL

4:15—"SOLIDS – LIQUID SEPARATION – AN IMPORTANT STEP IN RECYCLING CATTLE WASTES"
DR. ALVIN C. DALE

AFTERNOON—FOOD AND ENVIRONMENTAL SANITATION—GRAND BALLROOM EAST

EARL O. WRIGHT, *Presiding*

1:30—DOOR PRIZE DRAWING

1:45—"POLYCHLORINATED BIPHENYLS IN FOOD AND THE ENVIRONMENT"
ROBERT L. BRADLEY, JR.

2:30—"CONSUMER FOOD PROTECTION"
DR. JOHN BRUHN

3:15—MILK BREAK

3:30—TO BE ANNOUNCED

4:15—"QUALITY CONTROL IN THE BREWING INDUSTRY"
DONALD G. BERGER

TUESDAY, AUGUST 22, 1972

BUSINESS MEETING—IMPERIAL BALLROOM

5:00—Wisconsin Association of Milk and Food Sanitarians

EVENING DISCUSSION GROUPS

7:30-9:30—These discussion groups are for the benefit of our members who have special questions or problems which they wish to discuss informally with others. Selected individuals have agreed to answer questions and otherwise assist in discussions.

7:30—FOOD SANITATION
Henry VIII Room
LOUIS KING, *Moderator*
JOHN BRUHN
CARL VANDERZANT
PAUL ELLIOTT

7:30—MILK
Kennedy Room
WALLY LAWTON, *Moderator*
J. O. YOUNG
ROBERT ZALL
TRUMAN TORGENSEN

7:30—ENVIRONMENTAL SANITATION
Louis XIV Room
ROBERT BRADLEY, *Moderator*
R. T. WILSON
VICTOR LYONS
DICK WHITEHEAD

WEDNESDAY, AUGUST 23, 1972

MORNING GENERAL SESSION—IMPERIAL BALLROOM

ORLOWE OSTEN, *Presiding*

8:30—DOOR PRIZE DRAWING

8:45—O C E A PROJECT
DR. EARL HEALTH, Speaker

9:30—MILK BREAK

9:45—DOOR PRIZE DRAWING

10:00—IAMFES ANNUAL BUSINESS MEETING
1. Report of Executive Secretary
2. Report of Secretary-Treasurer
3. Committee Reports
4. 3-A Symbol Council Reports
5. Report of Resolutions Committee
6. Report of Affiliate Council
7. Old Business
8. New Business
9. Election of Officers

10:00-11:15—BUSINESS MEETING
Grand Ballroom East
Wisconsin Fieldmans Association
National Association of Dairy Fieldmen

11:15-12:00—Special Fieldmen's report of National Mastitis Council Regional Meeting

12:00—LUNCHEON
Grand Ballroom, Center and West
Wisconsin Sanitarian & Fieldmen
Award Luncheon

AFTERNOON—MILK SANITATION SECTION—IMPERIAL BALLROOM

DOUG BRAATZ, *Presiding*

1:30—DOOR PRIZE DRAWING

1:45—"NEW DAIRY PRODUCTS"
J. ORVILLE YOUNG

2:15—"DAIRY FOOD ALLERGENS AND ALLERGY"
JOSEPH R. SPIES

3:00—MILK BREAK

3:15—"NEW DEVELOPMENTS IN WHEY UTILIZATION"
CLYDE H. AMUNDSON

4:00—"UTILIZATION OF DAIRY INGREDIENTS IN OTHER FOODS"
JOHN J. JONAS

4:30—"WISCONSIN DAIRY INDUSTRY"
TRUMAN TORGENSON

AFTERNOON—ENVIRONMENTAL SECTION—KENNEDY ROOM

1:15—DOOR PRIZE DRAWING

1:30—"FLOOR COVERINGS RELATED TO SANITATION PRACTICES"
VICTOR J. LYONS

2:15—"RECYCLING OF FOOD CANS"
R. T. WILSON

3:00—MILK BREAK

3:15—"*CLOSTRIDIUM BOTULINUM* AND SMOKED FISH PRODUCTION 1963-1972"
PAUL J. PACE

4:00—"NOISE POLLUTION EVALUATION"
STEVE W. SHEPARD

AFTERNOON—FOOD INDUSTRY SANITATION SECTION—GRAND BALLROOM EAST

1:30—DOOR PRIZE DRAWING

1:45—"FLOUR MILLING INDUSTRY"
KENNETH NYBERG

2:15—"QUALITY ASSURANCE OF FOODS"
TOM WOODS

3:00—MILK BREAK

3:15—"MICROBIOLOGY OF GULF COAST AND POND-REARED SHELLFISH"
CARL VANDERZANT

4:00—"MICROBIOLOGY IN REDMEAT AND POULTRY INSPECTION"
R. PAUL ELLIOTT

WEDNESDAY EVENING AUGUST 23, 1972

6:30-7:30—RECEPTION
Hall of Presidents

7:30—ANNUAL AWARDS BANQUET

Grand Ballroom, Center and West

ORLOWE W. OSTEN, *Presiding*

INVOCATION
IVAN PARKIN

MASTER OF CEREMONIES
MYRON C. DEAN

INTRODUCTIONS

PRESENTATION OF AWARDS
MILTON HELD, *Chairman*

1. Past President's Award
2. Citation Award
3. Honorary Life Membership
4. Sanitarian's Award

The Sanitarian's Award is sponsored jointly by the Diversey Corporation, Klenzade Products, Inc., and Pennwalt Chemicals, Inc. and is administered by the International Association of Milk, Food and Environmental Sanitarians

5. National Fieldman's Awards

INSTALLATION OF OFFICERS
ENTERTAINMENT

THURSDAY, AUGUST 24, 1972
MORNING—GENERAL SESSION—IMPERIAL BALLROOM

E. MARTH, *Presiding*

9:00—DOOR PRIZE DRAWING

9:15—"ORGANIC FOODS—ANOTHER CONSUMER HOAX?"
J. H. VON ELBE

10:00—"FOOD DISTRIBUTION IN TODAY'S CONSUMER CLIMATE"
HARRY N. COUDEN

10:45—TO BE ANNOUNCED

ENTERTAINMENT MEN AND WOMEN

MONDAY, AUGUST 21, 1972

5:30 P.M.-6:30 P.M.—RECEPTION
Imperial Ballroom

WEDNESDAY, AUGUST 23, 1972

6:00 P.M.—COCKTAIL HOUR
Hall of Presidents

7:00 P.M.—BANQUET AND ENTERTAINMENT
Grand Ballroom, Center and West

THURSDAY, AUGUST 24, 1972

1:30 P.M.—BREWERY TOUR
(Buses, etc.)

ENTERTAINMENT FOR THE LADIES

TUESDAY, AUGUST 22, 1972

10:00-4:00—
Johnson Wax Co., Racine, Wisconsin
Luncheon will be arranged

WEDNESDAY, AUGUST 23, 1972

10:00-2:00—
Mitchell Park Conservatory—Milwaukee
Ladies' Hospitality Room
Charles I Room

REGIONAL MEETING NATIONAL MASTITIS COUNCIL
Pfister Hotel, Milwaukee, Wisconsin August 21, 1972

MORNING PROGRAM

DR. C. W. BURCH, *Chairman*
Veterinary Science Department
University of Wisconsin, Madison

9:45—WELCOME
DR. D. E. JASPER, President,
National Mastitis Council

10:00—EFFECTS OF A THREE-YEAR MASTITIS CONTROL
PROGRAM ON PRODUCTION AND MASTITIS INCIDENCE
DR. R. P. NATZKE, Animal Science Department, Cornell University, Ithaca, N. Y.

10:30—STREPTOCOCCUS AGALACTIAE INCIDENCE AND ERADICATION IN WISCONSIN
DR. ARLAN SMITH, Animal Health Division, Wisconsin Department of Agriculture, Madison

11:00—OBSERVATIONS ON THERAPY OF STAPHYLOCOCCUS MASTITIS
DR. D. E. JASPER, School of Veterinary Medicine, University of California, Davis

11:30—USE OF MASTITIS SCREENING TESTS IN DAIRY HERD IMPROVEMENT ASSOCIATIONS
DR. L. H. SCHULTZ, Dairy Science Department, University of Wisconsin, Madison

12 to 1 P.M.—Lunch

AFTERNOON PROGRAM

MR. LYLE VINEY, *Chairman*
Evansville, Wis.

1:00—A DAIRYMAN'S VIEWS ON MASTITIS
MR. RUSSELL O'HARROW, Oconto Falls, Wis.
Member, Board of Directors, National Mastitis Council

1:15—MASTITIS CONTROL FROM A PRACTITIONER'S VIEWPOINT
W. JERRY HICKS, D.V.M., Edgerton, Wis.

1:35—DRY THERAPY—ALL COWS VS. SELECTED COWS
DR. GILBERT WARD, Dairy and Veterinary Science Departments, University of Wisconsin, Madison

2:00—OPEN FORUM
Moderator—W. D. KNOX, Hoard's Dairyman, Ft. Atkinson, Wis. Speakers plus other experts will answer questions from the floor.

3:00 to 4:00—PANEL ON PROCEDURES AND PROBLEMS IN MONITORING CELL COUNTS IN BULK MILK
Moderator—DR. J. W. SMITH, USDA, Beltsville, Md.
EXPERIENCES WITH THE TECHNICON
DR. LOIS SMITHIES, Animal Health Division, Wisconsin Department of Agriculture, Madison
USE OF THE WISCONSIN MASTITIS TEST
MR. DON THOMPSON, In Charge of Laboratory Approval Program, Wisconsin Division of Health, Madison
STANDARDIZATION OF SOMATIC CELL CONFIRMATORY TESTING PROCEDURE
DR. A. RICHARD BRAZIS,
Public Health Service, Cincinnati, Ohio

3-A SANITARY STANDARDS FOR PLATE TYPE HEAT EXCHANGERS FOR MILK AND MILK PRODUCTS

Serial #1103

Formulated by
International Association of Milk, Food and Environmental Sanitarians
United States Public Health Service
The Dairy Industry Committee

It is the purpose of the IAMFES, USPHS, and DIC in connection with the development of the 3-A Sanitary Standards program to allow and encourage full freedom for inventive genius or new developments. Plate type heat exchanger specifications heretofore and hereafter developed which so differ in design, material, fabrication, or otherwise as not to conform with the following standards, but which, in the fabricator's opinion are equivalent or better, may be submitted for the joint consideration of the IAMFES, USPHS, and DIC, at any time.

A.

SCOPE

A.1
These standards cover the sanitary aspects of plate type heat exchangers for milk and milk products.

A.2
In order to conform with these 3-A Sanitary Standards, plate type heat exchangers shall comply with the following design, material, and fabrication criteria.

B.
DEFINITIONS

B.1
SURFACES

B.1.1
Product Contact Surfaces: Shall mean all surfaces which are exposed to the product.

B.1.2
Non-Product Contact Surfaces: Shall mean all other exposed surfaces.

C.

MATERIALS

C.1
All product contact surfaces shall be of stainless steel of the AISI 300 series[1] or corresponding ACI[2] types (See Appendix E), or equally corrosion resistant metal that is non-toxic and non-absorbent, except that:

C.1.1
Rubber and rubber-like materials may be used for gaskets. These materials shall comply with the applicable provisions of the "3-A Sanitary Standards for Multiple-Use Rubber and Rubber-Like Materials Used as Product Contact Surfaces in Dairy Equipment, Serial #1800."

C.1.2
Plastic materials may be used for gaskets. These materials shall comply with the applicable provisions of the "3-A Sanitary Standards for Multiple-Use Plastic Materials Used as Product Contact Surfaces for Dairy Equipment, Serial #2000", as amended.

C.2
All non-product contact surfaces shall be of corrosion-resistant material or material that is rendered corrosion-resistant. If coated, the coating used shall adhere. All non-product contact surfaces shall be relatively non-absorbent, durable and cleanable.

D.

FABRICATION

D.1
All product contact surfaces shall be at least as smooth as a No. 4 mill finish on stainless steel sheets. (See Appendix F).

D.2
All product contact surfaces shall be easily accessible for cleaning, either when in an assembled position or when removed. Removable parts shall be readily demountable. Heat transfer plates or gasket frames shall be readily removable from the press. Individual removable heat transfer plates or gasket frames shall be considered to comply with this requirement.

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[1]The data for this series are contained in the following reference: AISI Steel Products Manual, Stainless & Heat Resisting Steels, April 1963, Table 2-1, pp. 16-17. Available from: American Iron & Steel Institute, 633 Third Avenue, New York, New York 10017.

[2]Alloy Casting Institute Division, Steel Founders' Society of America, 21010 Center Ridge Road, Rocky River, Ohio 44116.

D.3

All inside corners on product contact surfaces shall have radii of 1/4 inch except where smaller radii are required for essential functional reasons. In no case shall such radii be less than 1/32 inch.

D.4

There shall be no threads on product contact surfaces.

D.5

Sanitary connections shall conform to the applicable provisions of the "3-A Sanitary Standards for Fittings Used on Milk and Milk Products Equipment and Used on Sanitary Lines Conducting Milk and Milk Products, Serial #0809", as amended and supplements thereto.

D.6

Transfer plate gaskets shall be continuous and shall be removable or shall be bonded to the transfer plate in such a manner that the bond is continuous and mechanically sound, and so that in the environment of its intended use the gasket does not separate from the plate.

D.7

A leak protector groove of sufficient width to be readily cleanable and open to the atmosphere at both ends shall be provided to allow leakage past gaskets to drain to waste.

D.8

Presses (or frames) shall be provided with legs of sufficient length to give a clearance of at least 4 inches between the lowest part of the press and the floor. Legs shall have rounded ends with no exposed threads. If made of hollow stock they shall be effectively sealed.

D.9

Presses (or frames) shall be so constructed that when opened plates and/or terminal frames may be separated to provide a space for cleaning and inspection equal to the lesser of the width of one plate or 15 inches.

D.10

Non-product contact surfaces shall be free of pockets and crevices and be readily cleanable. Surfaces to be coated shall be effectively prepared for coating.

APPENDIX

E.

STAINLESS STEEL MATERIALS

Stainless steel conforming to the applicable composition ranges established by AISI[1] for wrought products, or by ACI[2] for cast products, should be considered in compliance with the requirements of Section C.1 herein. Where welding is involved the carbon content of the stainless steel should not exceed 0.08%. The first reference cited in C.1 sets forth the chemical ranges and limits of acceptable stainless steels of the 300 series. Cast grades of stainless steel corresponding to types 303, 304, and 316 are designated CF-16F, CF-8, and CF-8M, respectively. These cast grades are covered by ASTM[3] specifications A296-68 and A351-70.

F.

PRODUCT CONTACT SURFACE FINISH

Surface finish equivalent to 150 grit or better as obtained with silicon carbide, is considered in compliance with requirements in D.1 herein.

These Standards are effective June 23, 1972, at which time the "3-A Sanitary Standards of Plate Type Heat Exchangers for Milk and Milk Products as of September 1951", published January-February 1952, Serial #1100, and amendments thereto are rescinded and become null and void.

[3]Available from American Society for Testing and Materials, 1916 Race Street, Philadelphia, Pennsylvania 19103.

3-A SANITARY STANDARDS FOR TUBULAR HEAT EXCHANGERS FOR MILK AND MILK PRODUCTS

Serial #1204

Formulated by
International Association of Milk, Food and Environmental Sanitarians
United States Public Health Service
The Dairy Industry Committee

It is the purpose of the IAMFES, USPHS, and DIC in connection with the development of the 3-A Sanitary Standards program to allow and encourage full freedom for inventive genius or new developments. Tubular heat exchangers heretofore and hereafter developed which so differ in design, material, fabrication, or otherwise as not to conform with the following standards, but which, in the fabricator's opinion are equivalent or better, may be submitted for joint consideration of the IAMFES, USPHS, and DIC, at any time.

A.

SCOPE

A.1

These standards cover the sanitary aspects of tubular heat exchangers without agitators for milk and milk products.

A.2

In order to conform with these 3-A Sanitary Standards, tubular heat exchangers shall comply with the following design, material and fabrication criteria.

B.

DEFINITIONS

B.1

Tubular Heat Exchanger shall mean a heat exchanger having one continuous tube, two or more concentric tubes, or two or more tubes in parallel.

B.2

Surfaces:

B.2.1

Product Contact Surfaces: Shall mean all surfaces which are exposed to the product and surfaces from which liquids may drain, drop, or be drawn into the product.

B.3

Non-Product Contact Surfaces: Shall mean all other exposed surfaces.

B.4

Mechanical Cleaning or Mechanically Cleaning: Shall denote cleaning, solely by circulation and/or flowing chemical detergent solutions and water rinses onto and over the surfaces to be cleaned, by mechanical means.

C.

MATERIALS

C.1

All product contact surfaces shall be of stainless steel of the AISI 300 series[1] or corresponding ACI[2] types (See Appendix, Section E.), or equally corrosion resistant metal that is non-toxic and nonabsorbent, except that:

C.1.1

Optional metal alloy may be used but only in applications requiring disassembly and manual cleaning. (See Appendix, Section G for the composition of an acceptable optional metal alloy.)

C.1.2

Rubber and rubber-like materials may be used for bonded or removable gaskets, seals and parts used in similar applications. These materials shall comply with the applicable provisions of the "3-A Sanitary Standards for Multiple-Use Rubber and Rubber-Like Materials Used as Product Contact Surfaces in Dairy Equipment, Serial #1800".

C.1.3

Plastic materials may be used for bonded or removable gaskets, seals and parts used in similar applications. These materials shall comply with the applicable provisions of the "3-A Sanitary Standards for Multiple-Use Plastic Materials Used as Product Contact Surfaces for Dairy Equipment, Serial #2000", as amended.

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[1]The data for this series are contained in the following reference: AISI Steel Products Manual, Stainless & Heat Resisting Steels, April 1963, Table 2-1, pp 16-17. Available from: American Iron & Steel Institute, 633 Third Avenue, New York New York 10017.

[2]Alloy Casting Institute Division, Steel Founders' Society of America, 21010 Center Ridge Road, Rocky River, Ohio 44116.

C.1.4
Single service gaskets of a sanitary type may be used.

C.2
All non-product contact surfaces shall be of corrosion-resistant material or material that is rendered corrosion-resistant. If coated, the coating shall adhere. All non-product contact surfaces shall be relatively non-absorbent, durable and cleanable. Parts removable for cleaning having both product contact and non-product contact surfaces shall not be painted.

D.

FABRICATION

D.1
All product contact surfaces shall be at least as smooth as a No. 4 mill finish on stainless steel sheets. (See Appendix, Section F.)

D.2
All permanent joints in metallic product contact surfaces shall be welded, except that tubes may be either expanded and rolled or welded into tube sheets or return fittings. When tubes are expanded and rolled into tube sheets or return fittings, the resulting joint shall be completely rigid and without pockets or crevices. All welded areas of product contact surfaces shall be at least as smooth as the adjoining surfaces except that welded joints in tubes in tubular heat exchangers designed to be mechanically cleaned need only to be smooth and free from pits, cracks, inclusions or other defects.

D.3
All product contact surfaces shall be easily accessible for cleaning and inspection either when assembled or when removed except that the milk contact surfaces of tubular heat exchangers designed to be mechanically cleaned do not have to be accessible for inspection if the heat exchange surface is one continuous tube. If the heat exchange surface of a tubular heat exchanger is two or more tubes in parallel, the product contact surfaces shall be accessible for manual cleaning and inspection when necessary.

D.3.1
Removable parts shall be readily demountable.

D.4
Tubes shall be supported in a manner that will prevent sagging. In a heat exchanger designed to be mechanically cleaned of the type that incorporates two or more concentric tubes, means shall be provided to keep the tubes equally spaced. The means provided to keep tubes equally spaced shall not interfere with mechanical cleaning.
The construction of a heat exchanger of the concentric multi-tube type designed to be mechanically cleaned shall be such that product and/or cleaning and/or sanitizing solutions will not enter areas that are not readily cleaned and/or rinsed.

D.5
Connections in product contact surfaces shall conform to "3-A Sanitary Standards for Fittings Used on Milk and Milk Products Equipment and Used on Sanitary Lines Conducting Milk and Milk Products, Revised, Serial #0809", as amended and supplements thereto and/or to the applicable provisions for welded sanitary product pipelines found in the "3-A Accepted Practices for Permanently Installed Sanitary Product-Pipelines and Cleaning Systems", Serial #60500, as amended.

D.6
Internal angles of 135° or less on product contact surfaces shall have minimum radii of 1/4 inch, except where smaller radii are required for essential functional reasons. When the radius is less than 1/32 inch, the product contact surface of this internal angle must be readily accessible for cleaning and inspection if the heat exchanger is not designed to be mechanically cleaned. Heat exchange tubing that is not circular in cross section shall have minimum radii of 1/8 inch on all internal angles of 135° or less on product contact surfaces.

D.7
The minimum diameter of circular heat exchange tubing shall be 0.902 inch I.D. except that circular cross section heat exchange tubing used in a heat exchanger may be of smaller diameter if the heat exchanger is designed for mechanical cleaning.

D.8
Gaskets shall be removable or bonded to the surface. Gasket retaining grooves shall be no deeper than their width. The minimum radius of any internal angle in a gasket retaining groove shall be not less than 1/8 inch, except that the radius may be 3/32 inch where a standard 1/4 inch O-Ring is to be used and the radius may be 1/32 inch where a standard 1/8 inch O-Ring is to be used.

D.9
There shall be no threads on product contact surfaces.

D.10
Means of supporting tubular heat exchangers:

D.10.1
If legs are used, they shall be smooth with round-

ed ends and no exposed threads. Legs made of hollow stock shall be sealed. The minimum clearance between lowest part of frame and floor shall be six inches.

D.10.2

Bases, when used, shall have smooth exterior surfaces.

D.10.3

Bases, which because of size or type cannot be mounted on legs, shall be designed for grouting and sealing.

D.11

Non-product contact surfaces shall be readily cleanable and shall be free of pockets and crevices. Surfaces to be coated shall be effectively prepared for coating.

APPENDIX

E.

STAINLESS STEEL MATERIALS

Stainless steel conforming to the applicable composition ranges established by AISI[1] for wrought products, or by ACI[2] for cast products, should be considered in compliance with the requirements of Section C.1 herein.

Where welding is involved the carbon content of the stainless steel should not exceed 0.08%. The first reference cited in C.1 sets forth the chemical ranges and limits of acceptable stainless steels of the 300 series.

Cast grades of stainless steel corresponding to types 303, 304, and 316 are designated CF-16F, CF-8 and CF-8M, respectively. These cast grades are covered by ASTM[3] specifications A 296-68 and A 351-70.

F.

PRODUCT CONTACT SURFACE FINISH

Surface finish equivalent to 150 grit or better as obtained with silicon carbide, is considered in compliance with the requirements of Section D.1 herein.

G.

OPTIONAL METAL ALLOY

An optional metal alloy having the following minimum and maximum composition is deemed to be in compliance with C.1.1 herein.

Zinc	-	8% maximum
Nickel	-	19 1/2% minimum
Tin	-	3 1/2% minimum
Lead	-	5% maximum
Iron	-	1 1/2% maximum
Copper	-	the balance

An alloy of the composition given above is properly designated "nickel silver", or according to ASTM Specification #B 149-70, may be entitled, "leaded nickel bronze".

These Standards are effective June 23, 1972, at which time the "3-A Sanitary Standards for Internal Return Tubular Heat Exchangers for Use with Milk and Milk Products, approved April 29, 1952", published November-December 1962, Serial #1200 and amendments thereto are rescinded and become null and void.

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[3]Available from American Society for Testing and Materials, 1916 Race Street, Philadelphia, Pennsylvania 19103.

E-3-A SANITARY STANDARDS FOR HOMOGENIZERS AND PUMPS OF THE PLUNGER TYPE FOR LIQUID EGG AND LIQUID EGG PRODUCTS

Serial #E-0401

Formulated by
International Association of Milk, Food and Environmental Sanitarians
United States Public Health Service
United States Department of Agriculture
Institute of American Poultry Industries
Dairy and Food Industries Supply Association

It is the purpose of the IAMFES, USPHS, USDA, IAPI and DFISA in connection with the development of the E-3-A Sanitary Standards program to allow and encourage full freedom for inventive genius or new developments. Specifications for homogenizers and pumps of the plunger type heretofore and hereafter developed which so differ in design, material, fabrication, or otherwise as not to conform with the following standards, but which in the fabricator's opinion are equivalent or better, may be submitted for the joint consideration of the IAMFES, USPHS, USDA, IAPI and DFISA at any time.

A.

SCOPE

A.1
These standards cover the sanitary aspects of homogenizers and pumps of the plunger type for eggs and liquid egg products.

A.2
In order to conform with these E-3-A Sanitary Standards, homogenizers and pumps of the plunger type shall comply with the following design, material and fabrication criteria.

B.

DEFINITIONS

B.1
Product: Shall mean liquid eggs and liquid egg products.

B.2
Plunger Pump: A displacement pump that moves the product by the reciprocating motion of a plunger(s) operating in a cylinder(s).

B.3
Homogenizer: A plunger pump which has a specially designed homogenizing valve or valves for the purpose of blending the product ingredients and/or producing homogeneity of the product.

B.4

Surfaces:

B.4.1
Product Contact Surfaces: Shall mean all surfaces which are exposed to the product and surfaces from which liquids may drain, drop, or be drawn into the product.

B.4.2
Non-Product Contact Surfaces: Shall mean all other exposed surfaces.

C.

MATERIALS

C.1

Product Contact Surfaces:

C.1.1
All product contact surfaces shall be of stainless steel of the AISI 300 series[1] or corresponding ACI[2] types (See Appendix, Section E.) or stainless steel that is non-toxic and non-absorbent and which under conditions of intended use is equal in corrosion resistance to stainless steel of the AISI 300 series[1] or corresponding ACI[2] types, except that:

C.1.2
Other corrosion-resistant metals that are non-toxic under conditions of intended use may be used when their properties are required for functional, reasons such as valve parts, valve seats, impact rings and parts used in similar applications.

C.1.3
Rubber and rubber-like materials may be used for gaskets, seals, and parts used in similar applications. These materials shall comply with the applicable provisions of the "E-3-A Sanitary Stan-

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[1]The data for this series are contained in the following reference: AISI Steel Products Manual, Stainless & Heat Resisting Steels, April, 1963, Table 2-1, pp. 16-17. Available from: American Iron and Steel Institute, 633 3rd Avenue, New York, New York 10017.

[2]Alloy Casting Institute Division, Steel Founder's Society of America, 21010 Center Ridge Road, Rocky River, Ohio 44116.

dards for Multiple-Use Rubber and Rubber-Like Materials Used as Product Contact Surfaces in Egg Processing Equipment, Serial #E-1800".

C.1.4
Plastic materials may be used for gaskets, seals, and parts used in similar applications. These materials shall comply with the applicable provisions of the "3-A Sanitary Standards for Multiple-Use Plastic Materials Used as Product Contact Surfaces for Dairy Equipment, Serial #2000", as amended.

C.1.5
Single-service sanitary type gaskets may be used.

C.2

Non-Product Contact Surfaces:

C.2.1
All non-product contact surfaces shall be of corrosion-resistant material or material that is rendered corrosion-resistant. If coated, the coating used shall adhere.

C.2.2
All non-product contact surfaces shall be relatively non-absorbent, durable and cleanable. Parts removable for cleaning having both product contact and non-product contact surfaces shall not be painted.

D.

FABRICATION

D.1
All product contact surfaces shall be at least as smooth as a No. 4 mill finish on stainless steel sheets. (See Appendix, Section F.)

D.2
All permanent joints in product contact surfaces shall be welded or if it is impractical to weld, the joint shall be fitted in a manner that it will be completely rigid and without pockets or crevices. All such areas of product contact surfaces shall be at least as smooth as the adjoining surfaces.

D.3
All product contact surfaces shall be easily accessible for cleaning, either when in an assembled position or when removed. Removable parts shall be readily demountable. There shall be no dead-ended passages.

D.4
When disassembled, all product contact surfaces shall be self-draining except for normal clingage.

D.5
All internal angles of 135° or less on product contact surfaces shall have minimum radii of 1/4 inch except those where for space or functional reasons it is impossible to have a radius of 1/4 inch. When for functional reasons the radius must be less than 1/32 inch, in such applications as flat sealing surfaces, valves, etc., the product contact surface of this internal angle must be readily accessible for cleaning and inspection.

D.6
There shall be no threads in contact with the product.

D.7
Inlet and outlet connections shall conform with the applicable provisions of the "E-3-A Sanitary Standards for Fittings Used on Egg and Egg Products Equipment and Used on Sanitary Lines Conducting Egg and Egg Products, Serial #E-0800".

D.8
Coil springs having product contact surfaces shall have openings between coils including the ends when the spring is in a free position. Coil springs shall be readily accessible for cleaning and inspection.

D.9
Gaskets having product contact surfaces shall be removable. Gasket retaining grooves on product contact surfaces shall be no deeper than their width. The minimum radius of a gasket retaining groove shall not be less than 1/32 inch.

D.10
Non-product contact surfaces shall be free of pockets and crevices and be readily cleanable and those to be coated shall be effectively prepared for coating.

D.11
The means of supporting homogenizers and pumps of the plunger type shall be one of the following:

D.11.1
With legs: Legs shall be smooth with rounded ends, have no exposed threads, and shall be of sufficient length to provide a clearance between the lowest parts of the base and the floor of no less than four inches. The lower ends of legs made of hollow stock shall be closed.

D.11.2
Mounted on the base: The base shall be designed to permit sealing to the mounting surface.

D.11.3
By a flange or shield: When the pump is designed to be inserted into a vessel containing product, the flange or shield shall be designed to protect against the entrance of contaminants into the vessel through the opening through which the pump is inserted.

D.12

The space between the cylinder(s) and the drive shall be readily accessible for cleaning, self-draining and protected so that liquids will not enter the drive. This space shall be provided with a cover or shield. The cover may be designed to permit observation without removing it from the homogenizer or pump.

D.13

Homogenizers or pumps of the plunger type to be used as the timing device in a high-temperature short-time pasteurizing system shall be provided with an easily accessible or externally visible means of sealing to prevent the operation of the homogenizer or pump at a greater capacity than that which gives legal holding time without breaking the seal.

APPENDIX

E.

STAINLESS STEEL MATERIALS

Stainless conforming to the applicable composition ranges established by AISI[1] for wrought products, or by ACI[2] for cast products, should be considered in compliance with the requirements of Section C.1.1 herein. Where welding is involved the carbon content of the stainless steel should not exceed 0.08%. The first reference cited in C.1.1 sets forth the chemical ranges and limits of acceptable stainless steels of the 300 series. Cast grades of stainless steel corresponding to types 303, 304, and 316 are designated CF-16F, CF-8, and CF-8M, respectively. These cast grades are covered by ASTM[3] specifications A296-68 and A351-70.

F.

PRODUCT CONTACT SURFACE FINISH

Surface finish equivalent to 150 grit or better as obtained with silicon carbide, is considered in compliance with the requirements of Section D.1 herein.

G.

Recording or indicating gauges furnished with a homogenizer or a pump of the plunger type should be of the sanitary diaphragm or pressure bulb type. They should comply with applicable criteria of this standard.

These standards are effective June 28, 1972, at which time the "E-3-A Sanitary Standards Covering Homogenizers and High Pressure Pumps of the Plunger Type for Liquid Egg Products, Serial #E-0400" are rescinded and become null and void.

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[3]Available from American Society for Testing and Materials, 1916 Race Street, Philadelphia, Pa. 19103.

ASSOCIATION AFFAIRS

ANNUAL MEETING OF THE ONTARIO MILK AND FOOD SANITARIANS

President, W. G. Johnson and Nancy Brown, Ontario Dairy Princess at the Annual Meeting of the Ontario Milk and Food Sanitarians Association, Holiday Inn, Toronto.

Dr. A. N. Myhr, Past President of IAMFES, Inc. and the Ontario Milk and Food Sanitarians Association, receives the "Sanitarian of the Year," award from Miss Nancy Brown, Ontario, Dairy Princess, and Mr. J. L. Baker, Commissioner of Agriculture, at the recent annual meeting of the Association.

DFISA ANNUAL MEETING ATTRACTS RECORD CROWD

Attendance rose to an all-time high at the April 12-14 Annual Meeting of Dairy and Food Industries Supply Assn. Nearly 250 journeyed to Naples, Fla., for the 53rd gathering, including 157 members representing 113 companies. The previous attendance high was 214, set at the 1970 Naples meeting.

Scheduled at the highly successful meeting were six speakers who discussed industry problems and offered solutions, the impartial assignment of exhibit space for Food & Dairy Expo '72, election of Board of Directors and social events.

Roy E. Cairns, marketing director of the Foundry Division, Waukesha Foundry Co., Waukesha, Wisc., was elected President of the 400-member equipper and supplier group. Gordon A. Houran, vice president-sales for the Milk & Food Equipment Division, De Laval Separator Co., Poughkeepsie, N. Y., was chosen President-Elect.

Five incumbents and one new member were elected to the Board of Directors. At-Large Directors chosen include Ralph Bjorgen (newly elected), director of sales, Plastics & Synthetics Division, Norton Co., Akron; Arthur A. Rogers, president, C. E. Rogers Co., Mora, Minn.; and for the Western Area, Harry R. Goff, president, James Dole Corp., San Francisco.

General Commodities & Services Commodity Group Director is I. T. Swartwood, executive vice president-sales, Sun Industries, and vice president/general manager, Kemp Products, Cleveland.

Elected Ingredients Commodity Group Director was Worth Weed, treasurer, Foote & Jenks, Inc., Jackson, Mich. Hase H. Smith, president, M. G. Newell Co., Greensboro, N. C., was elected Jobbers Commodity Group Director.

Exhibit space was assigned impartially in the traditional space locations lottery. 252 member companies participated in the drawing, for a current total of 161,000 square feet of exhibit space. Ample additional space is still available to exhibitors for the October 1-5, Atlantic City Convention Hall Show.

Speakers at the Beach Club Hotel meeting included William D. Knox, editor, *Hoard's Dairyman*, "The Milk Producer as a Marketing and Political Power"; Truman F. Graf, Dept. of Agricultural Economics, University of Wisconsin, "Changing Patterns in Dairy Marketing"; and Fred Stein, National Industrial Pollution Control Council, Dept. of Commerce, "Added Costs of Environmental Protection."

Other speakers were Sam R. Hoover, Agricultural Research Service, Dept. of Agriculture, "USDA's Food Research Program—Implications for Industry"; Robert S. Wheeler, Best Foods Division, CPC International, "Marketing Strategy for Today"; and Ronald D. Knutson, Consumer & Marketing Service, Dept. of Agriculture, "Government Policy on Mergers, Acquisitions and Consolidations."

All the papers except that of Mr. Knox are available from DFISA, 5530 Wisconsin Avenue, Washington, D. C. 20015.

DAIRY FIELDMEN AND SANITARIANS CONFERENCE N. C. STATE UNIVERSITY

Participants in the Dairy Fieldmen and Sanitarians Conference at N. C. State University were (Left to Right) M. E. Gregory, N. C. State University; Charles Standard, Time Chemical; Pat Muldoon, V.P.I., Blacksburg, Va.; Roger Wilkowske, USDA, Washington, D. C. and G. S. Parsons, N. C. State University.

Nearly 100 dairy industry personnel and dairy sanitarians participated in the annual program. The conference was held in the Food Science Building on the N. C. State University campus. Co-chairman of the program was M. E. Gregory, Food Science Department and Guy S. Parsons, Animal Science Department.

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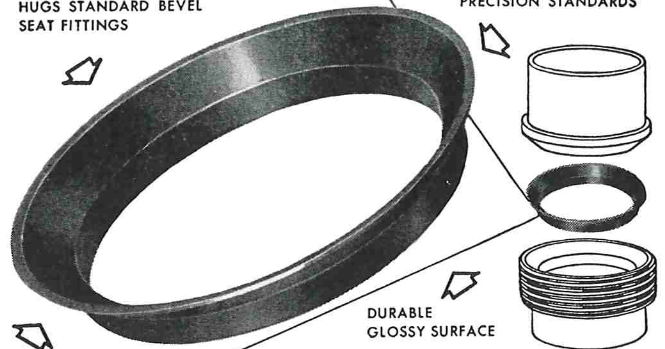

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Dairy authorities speak out on better cow milking.

W. Nelson Philpot/Louisiana State University
North Louisiana Hill Farm Experiment Station/Homer

Mastitis Detection and Control

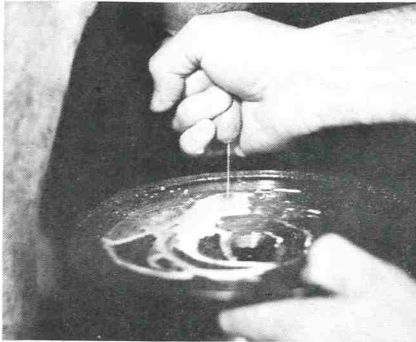
The term "mastitis" generally refers to the clinical form of the disease which occurs relatively infrequently in most herds and is characterized by swelling, redness, heat, and soreness of the udder, and an altered secretion. Research has revealed that for every individual case of clinical mastitis in a herd there are usually 25 to 40 subclinical cases. The subclinical form is important because it usually precedes the clinical form, constitutes a reservoir of organisms that may prompt new infections, reduces milk production, and lowers the quality of milk.

DETECTION

Strip Cup. The strip cup test should be conducted on the fore-milk from each quarter of every cow in the milking string before each milking to detect any prevailing abnormalities such as flakes, clots, pus, or wateriness.

California Mastitis Test. The CMT is conducted by drawing foremilk from each quarter of the udder into shallow cups of a special paddle and adding a test solution that reacts with body cells in the milk to form a gelatinous complex. The amount of gel formed is related to the number of cells present (principally white blood cells) which are, in turn, related to the presence and degree of mastitis. The CMT should be conducted at regular intervals and the scores recorded for current and future reference.

Microbiological Tests. Laboratory culturing of milk samples from individual quarters is necessary to identify the organisms involved in clinical cases of mastitis and, also, to distinguish disease-free animals from those with subclinical infections. These services are available through producer associations, milk plants, veterinarians, or private, state, or institutional laboratories.



More than 20 types of micro-organisms may cause mastitis, but two types—Streptococcus agalactiae and Staphylococcus aureus—account for at least 90% of all mastitis.

PREVENTION

Good milking practices are vitally important in maintaining overall udder health. But infections of the udder can still occur. They are the result of bacteria passing through the teat canal and multiplying inside the udder. Research has shown that this usually occurs during the interval between milkings and the rate of infection is related to the number of mastitis organisms present on the end of the teat. The most effective way to reduce the number of prevailing organisms on the teat is by dipping the teats after each milking in a disinfectant teat dip that is formulated to provide an effective residue between milkings. It is also important that the dip be nonirritating and, preferably, possess tissue-toning qualities. Research has shown that the regular use of such teat dips will reduce new infections by at least 50%.

THERAPY

Clinical Mastitis. The treatment of mastitis at the clinical level requires decisive management. Such treatment, though necessary, is less than desirably effective for restoring the gland to normal function; thus, every effort should be made to prevent the disease from progressing to the clinical stage.

Subclinical Mastitis. Mastitis in the subclinical form among a few animals may spread to other animals and evolve into a general herd problem. The most effective therapeutic approach to subclinical mastitis is via a dry cow treatment program. In herds with a high level of infection each quarter of every cow should be treated at drying off. Only selected quarters of individual animals should be treated in other herds.

Drug therapy has been found to be effective in eliminating streptococcus bacteria, but generally ineffective against staphylococcus bacteria. Animals with well established staphylococcus infections often should be removed from the herd.

The level of mastitis infection in a herd can be reduced approximately 50% within 1 year when teats are dipped following milking and all quarters of each cow are treated at the time of drying off.

Súrge . . . the accent is on YOU!

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