JANUARY, 1975
Vol. 38, No. 1
Pages 1-60
JMFTA 38(1):1-72 (1975)

Journal of Milk and Food Technology

ABSTRACTS OF RESEARCH PAPERS TO BE GIVEN AT THE 1975 ANNUAL MEETING MUST BE SUBMITTED BY FEBRUARY 15. SEE NOVEMBER, 1974 ISSUE OF JOURNAL FOR DETAILS.

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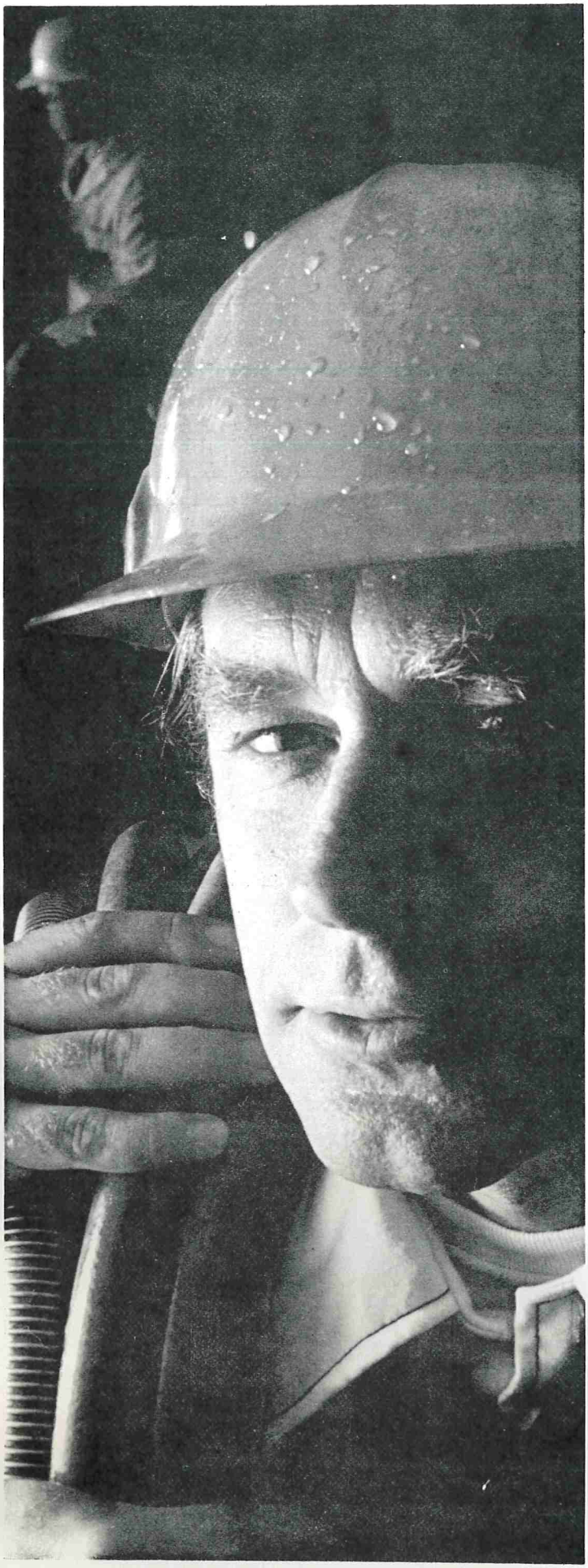

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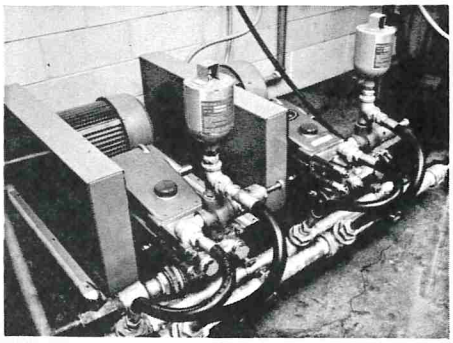

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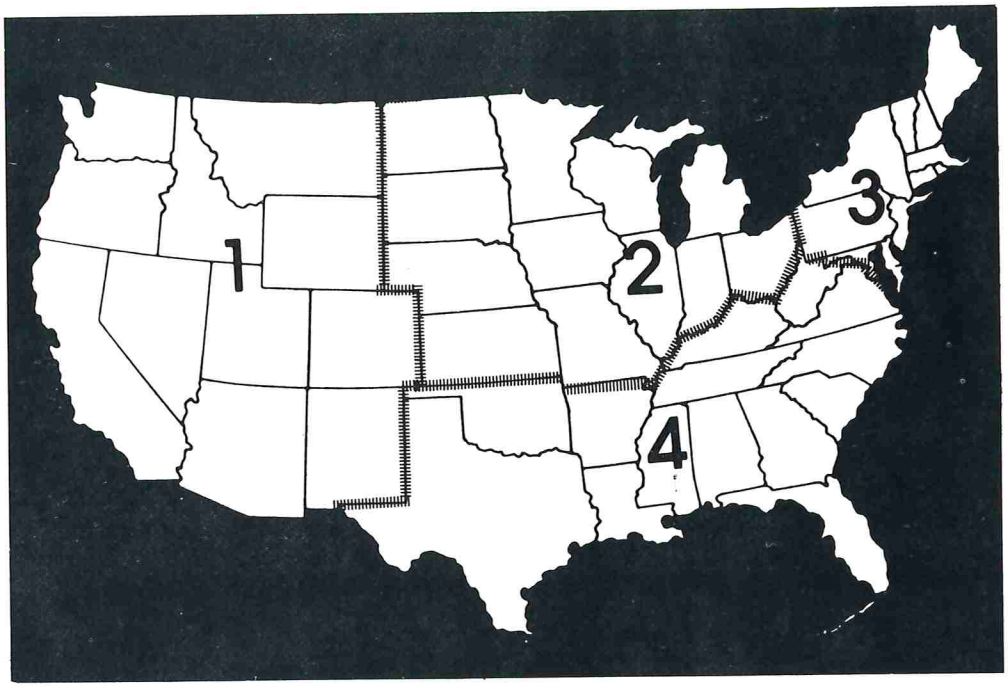

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The Journal of Milk and Food Technology is issued monthly beginning with the January number. Each volume comprises 12 numbers. Published by the International Association of Milk, Food and Environmental Sanitarians, Inc. with executive offices of the Association, 413 Kellogg Ave., P. O. Box 701, Ames, Ia. 50010.

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

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Journal of Milk and Food Technology

INCLUDING MILK AND FOOD SANITATION

Official Publication,

International Association of Milk, Food and Environmental Sanitarians, Inc., Reg. U.S. Pat. Off.

Vol. 38 January, 1975 No. 1

Manuscripts: Correspondence regarding manuscripts and other reading material should be addressed to Dr. Elmer H. Marth, Dept. of Food Science, University of Wisconsin, Madison, Wis. 53706.

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J. Milk Food Technol. Vol. 38, No. 1, Pages 3-7 (January, 1975)

Role of Enterococci in Cheddar Cheese: Proteolytic Activity and Lactic Acid Development[1]

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(Received for publication June 3, 1974)

ABSTRACT

Eight lots of Cheddar cheese were manufactured by using two strains of *Streptococcus faecalis* and *Streptococcus durans* in combination with a commercial lactic culture. Each lot consisted of a control vat of cheese, manufactured with lactic starter only, and an experimental vat of cheese containing the lactic starter and one of the enterococcus strains. Combinations of two curing temperatures (7.2 and 12.8 C) and two early cooling treatments (air vs. brine cooling) were used for cheeses from each vat to determine the effects of these handling procedures, as well as of enterococcus addition, on proteolysis and lactic acid development. These characteristics were monitored from milling to up to 6 months of curing. Cheeses manufactured with *S. faecalis* exhibited more protein breakdown than did the control cheeses and those made with *S. durans,* the latter two being nearly identical in the extent of proteolysis. More proteolysis was consistently observed in those cheeses cured at 12.8 C. No statistical difference was observed inproteolytic activity between air- and brine-cooled cheeses. Cheeses made with *S. durans* had a higher final percentage of lactic acid than did controls and cheeses made with *S. faecalis.* Cheeses manufactured with enterococci exhibited a more rapid initial production of lactate. Cheeses cured at 12.8 C had greater percentages of lactic acid compared with those cured at 7.2 C. Air-cooled cheeses also developed significantly higher levels of lactic acid than did brine-cooled cheeses.

The microbiological response *Streptococcus durans* and *Streptococcus faecalis* when used as supplemental starters during cheese curing has been discussed previously by Jensen et al. (*7*). This article, and the one to follow, will discuss four biochemical parameters monitored at various stages of curing. Proteolytic activity, lactic acid development, lipolysis, and citric acid utilization were the parameters selected because these metabolic activities are collectively the most responsible for development of characteristic Cheddar cheese flavor (*9, 11, 12*). This paper will compare proteolysis and lactic acid levels in control Cheddar cheese with cheeses made with *S. faecalis* and *S. durans* added as supplemental starters. In a future article organoleptic considerations will be discussed.

MATERIALS AND METHODS

Cheese was manufactured and treated as described previously by Jensen et al. (*7*). Proteolysis was determined by estimating free and peptide tyrosine by the method of Vakaleris and Price (*13*), which measures the amount of 280-nm absorbing material in the noncasein fraction of cheese.

Lactic acid was determined by a method adapted from that of Harper and Randolph (*6*). Ten grams of cheese were weighed into a Waring Blendor jar and blended for 5 min with 90 ml distilled water. Ten-milliliter aliquots of the homogenate were taken in duplicate and separately diluted to 100 ml in volumetric flasks. Twenty-five milliliters of this dilution were removed to a 250-ml Erlenmeyer flask, and the following reagents were added in this order: (*a*) 10 ml barium chloride solution (98.8 g c.p. crystals per liter); (*b*) 5 ml of zinc sulfate solution (225 g c.p. crystals per liter); and (*c*) 5 ml of 0.66 N sodium hydroxide.

After thorough mixing, samples were filtered through Whatman No. 40 filter paper. To 10 ml of the filtrate, 1.0 ml of fresh 1.0% ferric chloride solution was added as the chromogen. Readings were taken at 425 nm on a Beckman Model DU Spectrophotometer (Beckman Instrument Co., Fullerton, California) with standardized silica cuvettes. The amount of lactic acid was determined from a standard curve, which was prepared by adding a known amount of lithium lactate (c.p. grade) and then calculating total lactic acid.

RESULTS

Proteolysis

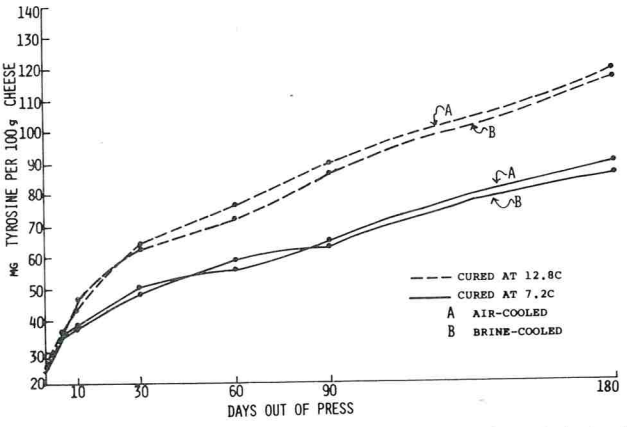

Figure 1. *Increase of noncasein tyrosine during curing of air- and brine-cooled control cheeses.*

Figure 1 shows the appearance of noncasein tyrosine during curing in control cheeses as an average of eight lots. Cheeses cured at 12.8 C exhibited more noncasein tyrosine than those cured at 7.2 C, beginning as early as the 10th curing day, and with the difference increasing more at each successive sampling point. This interaction is corroborated in Table 1, as well as differences caused

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TABLE 1. *Analysis of variance; proteolysis in control cheeses*

Source of variation	d.f.	M.S.	F values	Level of significance
Lot	7	1143.21	48.71	1%
Cooling procedure (C)	1	84.10	3.58	
Curing temperature (T)	1	9729.08	414.53	1%
C × T	1	20.50	0.87	
Error	21	23.47		
Days of curing (D)	6	23307.52	354.92	1%
C × D	6	20.79	0.32	
T × D	6	1000.45	15.23	1%
C × T × D	6	27.79	0.42	
Error	168	65.67		

by the main effects of curing temperature and days of cure. Data in this table also indicate that there is no significant difference in proteolysis when different early cooling procedures are used.

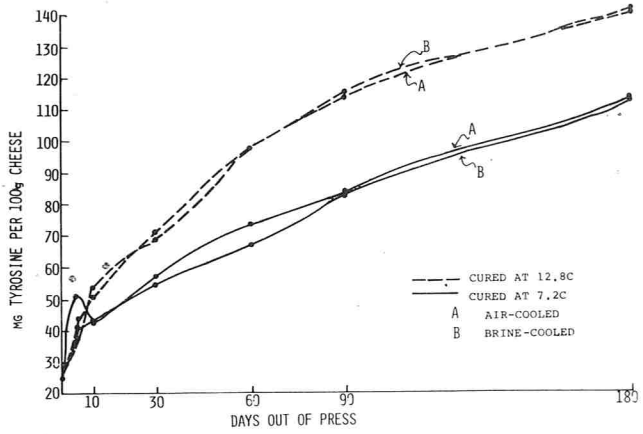

Figure 2. *Increase of noncasein tyrosine during curing of air- and brine-cooled experimental* (S. faecalis) *cheeses.*

Data illustrated in Fig. 2 indicate the average amount of noncasein tyrosine for the four lots of cheese manufactured with *S. faecalis* as a supplemental starter. The pattern is essentially the same as shown in the control cheeses (Fig. 1) with more proteolysis occurring in cheeses cured at 12.8 C than at 7.2 C and no clear difference between air- and brine-cooled cheeses cured at

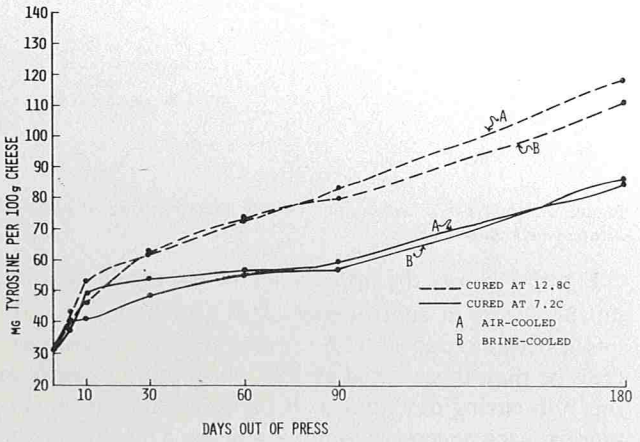

Figure 3. *Increase of noncasein tyrosine during curing of air- and brine-cooled experimental* (S. durans) *cheeses.*

the same temperature. There is, however, considerably more noncasein tyrosine in the cheeses made with *S. faecalis* for each respective treatment combination.

The average amount of noncasein tyrosine liberated in the four cheeses in which *S. durans* was used as a supplemental starter is shown in Fig. 3. The pattern exhibited is nearly identical to that obtained with the control cheeses (Fig. 1).

TABLE 2. *Analysis of variance; proteolysis in enterococcus cheeses*

Source of variation	d.f.	M.S.	F values	Level of significance
Species (S)	1	6524.82	1.12	
Concentration (CN)	1	3319.09	0.57	
S × CN	1	2343.24	0.40	
Error	4	5827.35		
Cooling procedure (C)	1	208.30	2.62	
Curing temperature (T)	1	10766.31	135.56	1%
C × T	1	26.41	0.33	
S × C	1	5.17	0.07	
C × CN	1	12.11	0.15	
S × T	1	138.21	1.74	
CN × T	1	14.39	0.18	
Days of curing (D)	6	27219.09	342.72	1%
S × D	6	1667.26	20.99	1%
CN × D	6	265.56	3.34	1%
C × D	6	10.16	0.13	
T × D	6	1287.20	16.21	1%
Pool error	179	79.42		

The analysis of variance presented in Table 2 confirms statistically that the effect of curing temperature, both alone and when interacting with days of cure, created a significant difference in proteolytic activity. The statistical analysis also indicates that the supplemental enterococcus species used and amount (number of cans) of concentrate significantly affect the degree of proteolytic activity when these two effects interact with days of cure. The lesser amount of concentrate, however, produced the greater degree of proteolysis over time.

TABLE 3. *Analysis of variance; differences in proteolysis between control and enterococcus cheeses*

Source of variation	d.f.	M.S.	F values	Level of significance
Species (S)	1	5663.58	4.81	10%
Concentration (CN)	1	1240.99	1.05	
S × CN	1	1285.64	1.09	
Error	4	1177.16		
Cooling procedure (C)	1	27.69	0.49	
Curing temperature (T)	1	26.26	0.47	
C × T	1	93.45	1.67	
S × C	1	2.61	0.05	
C × CN	1	11.67	0.21	
S × T	1	22.67	0.40	
CN × T	1	7.53	0.13	
Days of curing (D)	6	217.16	3.87	1%
S × D	6	465.91	8.31	1%
CN × D	6	32.60	0.58	
C × D	6	29.21	0.52	
T × D	6	66.55	1.19	
Pool error	179	56.07		

Data in Table 3 confirm that differences in noncasein tyrosine appearance between cheeses manufactured with *S. faecalis* and controls are significantly greater than

differences between the *S. durans* cheeses and their respective controls, as demonstrated by graphic comparison (Fig. 2 and 3).

Lactic acid

Figure 4 represents the averages of percentages of

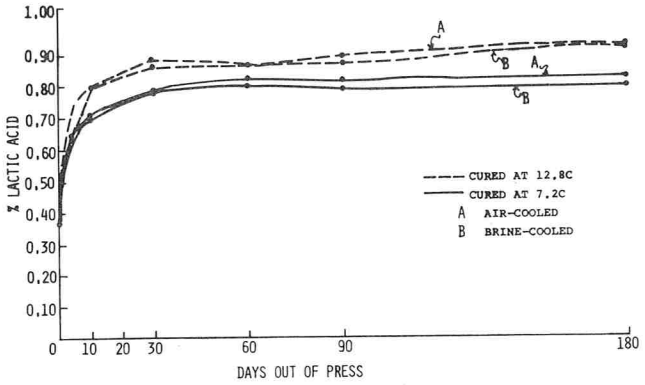

Figure 4. *Lactic acid content during curing of air- and brine-cooled control cheeses.*

lactic acid developed in the eight lots of control cheeses manufactured. Nearly all the lactic acid was developed in the first 10 days of curing. There was a small increase from 10 to 30 days, after which the lactic acid level stabilized. Cheeses cured at 7.2 C developed less lactic acid, with the difference being about 0.10% between those cured at 7.2 C and at 12.8 C at the end of 6 months. In those cheeses cured at 12.8 C, there was a slight increase in lactic acid from 30 days to 180 days, whereas in the cheeses cured at 7.2 C, there was no evident change in the same period. There was a small but consistent difference in lactic acid content between air- and brine-cooled cheeses cured at the same temperature,

TABLE 4. *Analysis of variance; lactic acid development in control cheeses*

Source of variation	d.f.	M.S.	F values	Level of significance
Lot	7	0.11164184	44.02	1%
Cooling procedure (C)	1	0.01577857	6.22	5%
Curing temperature (T)	1	0.27021607	106.54	1%
C × T	1	0.00064464	0.25	
Error	21	0.00253622		
Days of curing (D)	6	1.04340506	272.04	1%
C × D	6	0.00188690	0.49	
T × D	6	0.01220565	3.18	1%
C × T × D	6	0.00237381	0.62	
Error	168	0.00383554		

with brine-cooled cheeses exhibiting the smaller amount. These differences induced by coolant and curing temperatures are statistically significant, as shown on Table 4.

Figure 5 depicts the average percentage of lactic acid in the four lots of cheese made with *S. faecalis* as a supplemental starter. The over-all pattern of development is the same as seen in the control cheeses,

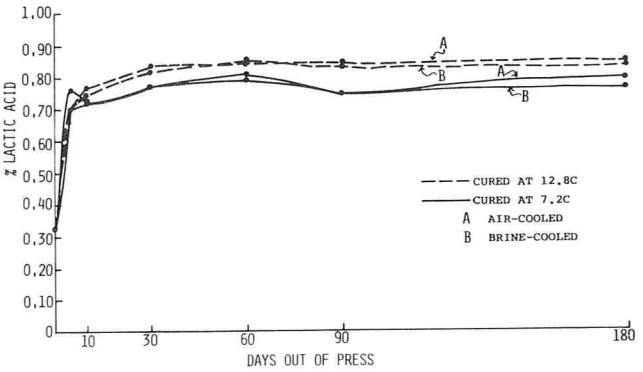

Figure 5. *Lactic acid content during curing of air- and brine-cooled experimental* (S. faecalis) *cheeses.*

but in the *S. faecalis* cheeses, the percentage of lactic acid increased by 0.40 to 0.45% in the first 5 days, whereas the amount in the control cheeses increased by 0.30 to 0.40% in the same period.

The average percentage of lactic acid for the four lots of cheese manufactured with *S. durans* is shown in Fig. 6.

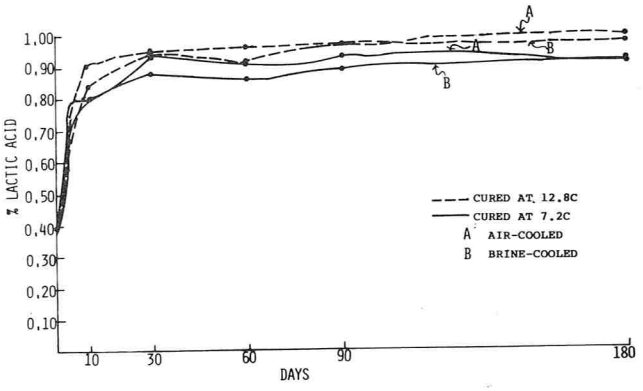

Figure 6. *Lactic acid content during curing of air- and brine-cooled experimental* (S. durans) *cheeses.*

Again, the trends for the four treatments are similar to the controls and *S. faecalis* cheeses, with more lactic acid in cheeses cured at 12.8 C and less in brine-cooled

TABLE 5. *Analysis of variance; lactic acid development in enterococcus cheeses*

Source of variation	d.f.	M.S.	F values	Level of significance
Species (S)	1	0.57410875	1.20	
Concentration (CN)	1	0.00642857	0.13	
S × CN	1	0.07651607	0.16	
Error	4	0.47791071		
Cooling procedure (C)	1	0.02614464	9.95	1%
Curing temperature (T)	1	0.08331429	31.70	1%
C × T	1	0.00008750	0.03	
S × C	1	0.00875000	3.33	
CN × C	1	0.00040179	0.15	
S × T	1	0.00030179	0.11	
CN × T	1	0.00825714	3.14	
Days of curing (D)	6	6.9603054	264.85	1%
S × D	6	0.07822500	29.76	1%
CN × D	6	0.05445893	20.72	1%
C × D	6	0.02143036	8.15	1%
T × D	6	0.05016071	19.09	1%
Pool error	179	0.00262800		

cheeses. There is, however, more lactic acid produced in the cheeses manufactured with *S. durans* than in the control cheeses and in those made with *S. faecalis.*

Data shown in Table 5 substantiate that significantly more lactic acid was present in air-cooled cheeses and cheeses cured at 12.8 C. Statistically significant interactions with the experimental cheese group also are evident when all variables (species used, concentration, curing temperature, and cooling treatment) are associated with the over-all days of cure.

The actual differences between enterococcus cheeses and their controls with respect to lactic acid development are given in Table 6. There is a significant difference in

TABLE 6. *Analysis of variance; differences in lactic acid development between control and enterococcus cheeses*

Source of variation	d.f.	M.S.	F values	Level of significance
Species (S)	1	0.03450179	0.59	
Concentration (CN)	1	0.00182857	0.03	
S × CN	1	0.00330179	0.05	
Error	4	0.05803214		
Cooling procedure (C)	1	0.00130179	0.40	
Curing temperature (T)	1	0.05344464	16.49	1%
C × T	1	0.00120714	0.37	
S × C	1	0.00002857	0.009	
CN × C	1	0.00171607	0.53	
S × T	1	0.00411429	1.27	
CN × T	1	0.00130179	0.40	
Days of curing (D)	6	0.06031250	18.61	1%
S × D	6	0.09797321	30.23	1%
CN × D	6	0.06709643	20.70	1%
C × D	6	0.006673214	2.06	
T × D	6	0.02348036	7.24	1%
Pool error	179	0.00324070		

the effects of curing temperature, with enterococcus cheeses yielding much more lactic acid at 12.8 C than their controls, but at 7.2 C, the over-all differences between experimental and control cheeses are minimal. Cheeses made with *S. durans* had significantly greater amounts of lactic acid as compared with their controls, yet *S. faecalis* cheeses and their controls produced comparable amounts of lactic acid.

The differences between experimental and control cheeses in the concentration × days of cure interaction is not immediately clear. When the mean differences of the two concentrations are examined graphically, however, there is a considerable difference between the two during early curing stages, with an equalizing effect at later stages.

DISCUSSION

Because hydrolysis of noncasein nitrogenous material in cheese is to a large extent bacterial in origin, it is paradoxical that, while the population declined rapidly at 7.2 C in the control cheeses (*7*), there was still a gradual increase in noncasein tyrosine. If most of the active protease present is from the original starter streptococci, it must have been exocellular in nature and secreted into the curd at an early stage. The continued activity of the enzyme would imply that it is active in the microenvironment of maturing cheese. Another possible explanation for continued proteolysis in cheese even after considerable decline in bacterial numbers is that the protease from starter streptococci is endocellular in nature and, as the cells die, they lyse and release the enzyme. Van der Zant and Nelson (*14*) characterized a proteolytic enzyme system from *Streptococcus lactis* and found that the pH optimum of this system occurred near neutrality; they suggested that the low level of proteolytic activity at pH 5.0 would limit the activity in most types of common cheese. Thus, it seems rather unlikely that the starter streptococci are responsible for the bulk of casein breakdown. Baribo and Foster (*1*) suggested that, since *S. lactis* and *Lactobacillus casei* are the predominant flora in ripening cheese, the characteristics of the proteolytic systems active in ripening cheese should be similar to those proteases possessed by the microorganisms. From these results, Baribo and Foster (*1*) concluded that these organisms possessed enzymes that only accounted for part of the active proteinase content of the cheese. It is most likely then that the gradual continuation of proteolysis in control cheeses cured at 7.2 C is due to adventitious flora and, possibly, residual rennet.

Since lactobacilli can initiate growth at pH 5.4 in milk (*2*), their protease systems must be capable to some extent of utilizing milk protein at this pH. Because lactobacilli have also been suggested as the primary organisms that proliferate extensively at 12.8 C (*5, 7, 8*), their activity is probably the major reason for the greater degree of proteolysis in cheeses cured at 12.8 C.

Statistical application has shown that early cooling treatment has no effect on the degree of proteolysis. This was substantiated by Miah (*10*) who found no significant differences in the degree of proteolysis between air- and brine-cooled cheeses.

Dovat (*4*) and Yates et al. (*16*) stated that *S. faecalis* cannot be described as actively proteolytic even when compared with relatively weakly proteolytic *S. lactis,* thus making increased proteolysis in cheeses manufactured with *S. faecalis* seem at first unusual. An explanation for this response would be that there are more viable organisms persisting and metabolizing because *S. faecalis* has been shown to survive in the cheese far more efficiently than the lactic streptococci (*7*). Even though *S. faecalis* may be much less proteolytic than *S. lactis,* the cumulative activity of the large populations may be significant in protein breakdown in cheese. It was expected that the noncasein tyrosine data from the *S. durans* cheeses would be more similar to those from the *S. faecalis* cheeses than to those from the controls since the survival of *S. durans* in the cheese was even greater than that of *S. faecalis* (*7*). Possibly *S. durans,* although highly capable of survival, produces so little proteolysis that any difference between *S. durans* cheeses and controls is unnoticeable. Wallace and Harmon (*15*) isolated an intracellular protease system from *S. durans,* the characterisitcs of which made it look promising in

Cheddar cheese ripening. Because its pH optima were 6.0 and 7.5, however, the lower pH of the cheese may have inhibited most of the activity. Also, because the system was endocellular, cell lysis would be necessary to release the protease, and in light of the essentially zero death rate of *S. durans* in the cheese, cell lysis probably did not occur to an appreciable extent.

There, too, is the possibility that *S. durans* survives so well simply because it is not proteolytic. Certain products of protein hydrolysis, such as amines and mercaptans, may be toxic to the very organism that produces the enzymes responsible for the proteolysis. The comparatively greater amount of protein hydrolysis by *S. faecalis* could be producing locally toxic conditions, being in part responsible for the death of the microorganism.

The effect of less proteolysis at a higher concentration of enterococcus starter, which shows as statistically significant (Table 2), is not clear at this time.

Dolby, McDowall, and Riddet (*3*) have stated that lactose disappears entirely from the cheese after 7 to 10 days of curing. The results of Miah (*10*) indicated that, even at 3 months, and average of 1.1 mM of lactose per 100 g cheese persisted. The initial rapid increase in lactic acid in the first 10 days reflects the utilization of lactose. The slight increase observed in cheese cured at 12.8 C between 30 and 180 days can probably be attributed to proliferation of large numbers of adventitious flora, most likely lactobacilli. Because there is a marked proliferation of these types (*7*), they must require some energy source and thus may be using secondary carbon sources in the curd, from which a small amount of additional lactic acid would likely be produced.

The more rapid development of lactic acid in the early curing stages in cheeses made with *S. faecalis* as compared with control cheeses is most likely due to their salt tolerance and resistance to the other envrionmental factors (*7*), resulting in higher numbers of enterococci. The rapid utilization of fermentable carbohydrates in cheeses made with *S. faecalis* may be of interest from the standpoint of uniformity and defect control in the finished product. The enterococci may help to overcome inconsistencies in acid production resulting from the varying extent of inhibition of the normal starter by the relatively high brine-salt concentration of the cheese. Furthermore, the more rapid depletion of the energy source will prevent growth of undesirable adventitious microorganisms and improve the chances for the pH to fall to the desired level of 5.1 to 5.3.

Miah (*10*) reported that there was significantly less lactic acid produced in brine-cooled cheeses. This is consistent with the data presented here and implies that nonuniform lactic acid development may be more likely than nonuniform protein breakdown when young, randomly stacked cheese is allowed to air cool. Early cooling treatment has no significant effect upon proteolysis.

The larger amounts of lactic acid in cheeses manufactured with *S. durans* are unusual since it is recognized that *S. faecalis* has more fermentative powers than *S. durans*. Although unable to ferment as broad a spectrum of carbohydrates as *S. faecalis, S. durans* may still ferment some component of the cheese more effectively. Because the development of lactic acid is complete in 30 days, it is unlikely that *S. durans* is utilizing residual lactose in the cheese. Although *S. faecalis* is considered to be more fermentative than *S. durans* under ideal conditions, *S. durans* seems to be more tolerant of the microenvironment of the cheese (*7*) and thus may be comparatively more fermentative under the circumstances.

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these strips to determine temperatures in heated water was tested against a mercury thermometer and a Mettler TM15 digital thermometer (Mettler Instrument Corp., Princeton, NJ).

In studying bacterial survival in relation to temperature at various positions within soup samples, temperature measurements as indicated by color change of the strips were recorded immediately after the exposure to microwaves. One-milliliter aliquots for determination of remaining viable cells were removed sequentially from bottom to top (unless otherwise noted) with a sterile pipette (samples in beakers), or were removed sequentially from top to bottom with a sterile 2-ml syringe fitted with a 21 gauge needle (samples in the modified graduated cylinder).

In all instances, samples for viable cell counts were removed from the cooked foods as quickly as possible to minimize the aftercooking heat effects on bacterial survival. Approximately 10-15 sec were required to remove samples from bottom, middle, and top regions of the soup in beakers, and 20-25 sec were required to remove the five samples from the modified graduated cylinder.

Modified graduated cylinder

The modified cylinder (Fig. 2) consisted of a 500-ml size graduated

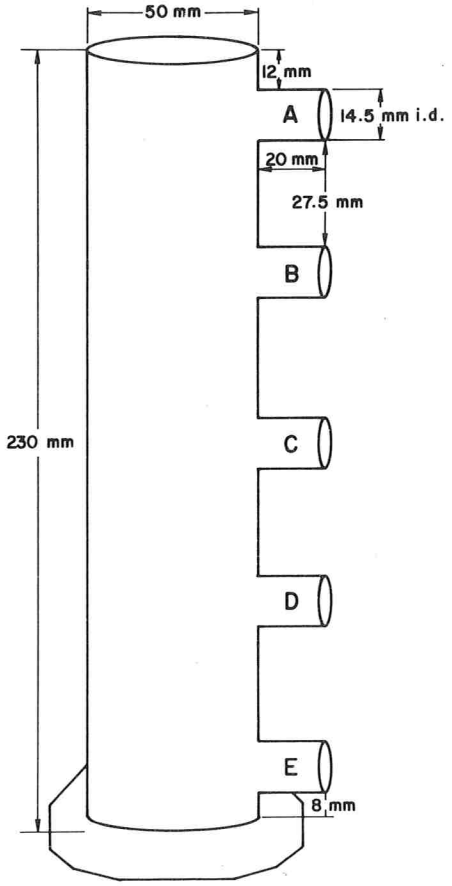

Figure 2. *The Modified Graduated Cylinder. Dimensions of the components are as labelled.*

cylinder with five short glass tubes (side-arms) attached to the cylinder at regular intervals. The open end of the side-arms were sealed with rubber serum caps. The use of the serum caps facilitated repeated samples withdrawal and at the same time minimized the chance of leakage. When filled to about 4 mm from the top, the cylinder accommodated 375 ml of liquid.

RESULTS

Survival patterns of E. coli and S. typhimurium in microwave cooked soups

For both *E. coli* and *S. typhimurium* results of three determinations of survival and temperature in bottom, middle, and top regions during timed microwave exposures of single serving portions (i.e., 200 ml) of soups were averaged. These values were then used to plot % survival versus exposure time as well as % survival versus temperature. Plots of temperature versus exposure time were also compiled for the three regions of the soup.

Tomato and vegetable soups. Results of experiments using tomato and vegetable soups were quite similar and comparable. Graphical data are presented only for tomato soup, but the findings, except was noted, pertain also to the data collected for vegetable soup.

The temperature profile for the three regions of tomato soup (Fig. 3) shows that, at any time, the middle

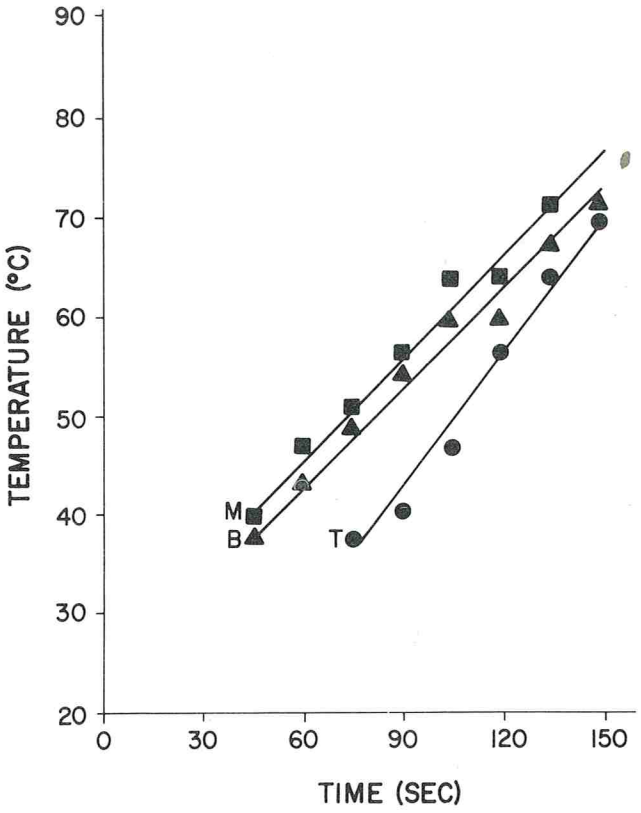

Figure 3. *Temperature profile of single serving of uninoculated tomato soup during microwave exposure. (Profiles obtained from inoculated soup showed consistent results.) Circles indicate Top (T), Squares indicate Middle (M), and Triangles indicated Bottom (B) regions of soup.*

region of this soup had the warmest temperature, while the top region exhibited the coolest temperature. Figure 4 and Figure 5 represent the decrease in survival with increasing exposure time for *E. coli* and *S. typhimurium* respectively in tomato soup. From these graphs it can be seen that, for any given time, organisms in the top region had the lowest percentage survival even though, according to data in Figure 3, this was the region of lowest temperature at that time. The organisms in the middle region (the warmest region from Fig. 3) had an intermediate level of survival and those in the bottom of the soup had the greatest survival over the entire heating period.

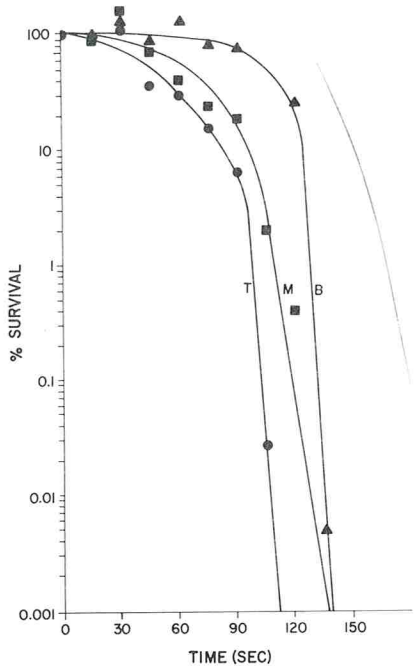

Figure 4. *Decrease in survival of* Escherichia coil *in single serving of tomato soup with respect to microwave exposure time. Circles indicate Top (T), Squares indicate Middle (M), and Triangles indicate Bottom (B) regions of soup.*

Further evidence that organisms in the top region were being inactivated at lower temperatures than organisms in the other regions of the soup is provided in Figures 6 and 7. These graphs present the decrease in survival of *E.*

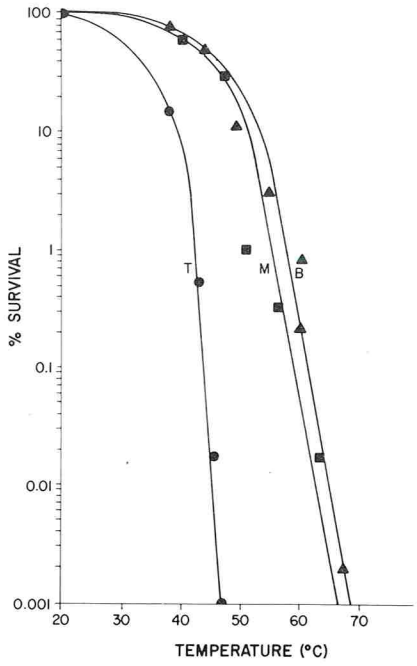

Figure 5. *Decrease in survival of* Salmonella typhimurium *in single serving of tomato soup with respect to microwave exposure time. Circles indicate top (T), Squares indicate Middle (M), and Triangles indicate Bottom (B) regions of soup.*

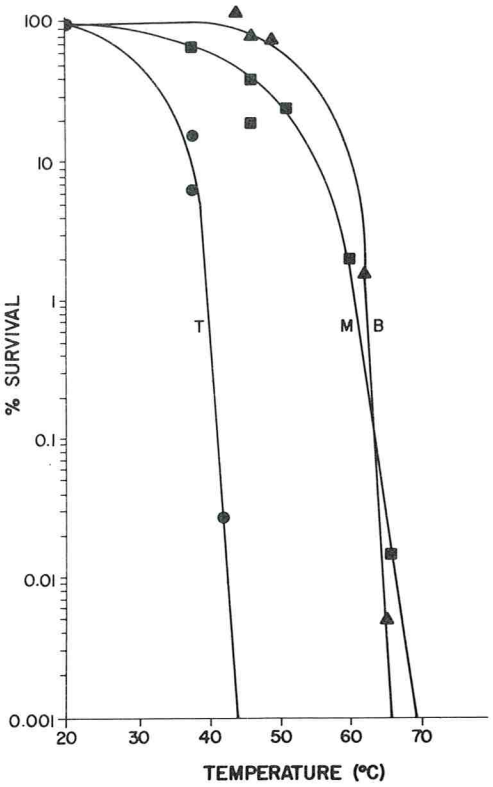

Figure 6. *Decrease in survival of* Escherichia coli *in single serving of tomato soup with respect to temperatures reached during microwave exposure. Circles indicate Top (T), Square indicate Middle (M), and Triangles indicate Bottom (B) regions of soup.*

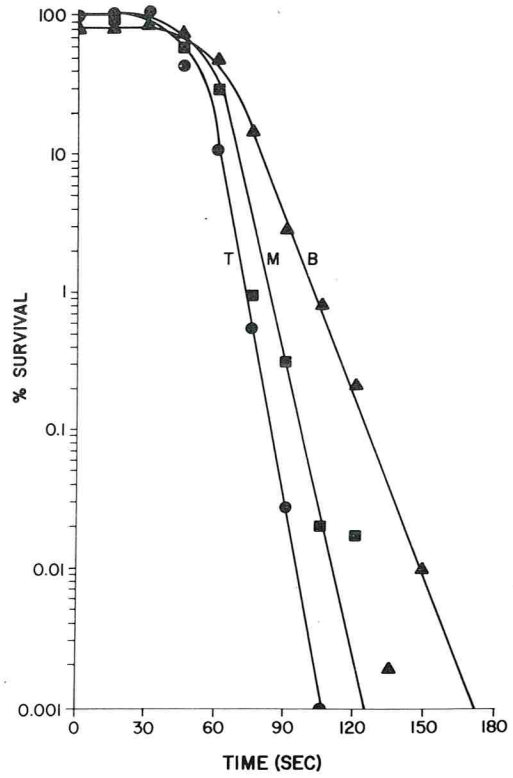

Figure 7. *Decrease in survival of* Salmonella typhimurium *in single serving of tomato soup with respect to temperatures reached during microwave exposure. Circles indicated Top (T), Squares indicate Middle (M), and Triangles indicate Bottom (B) regions of soup.*

coli and *S. typhimurium* respectively in terms of the temperatures achieved in the three tomato soup regions. From these graphs it can be noted that, for any given temperature, organisms at the top of the soup had decreased to the lowest level of survival. Organisms in the middle region generally had the intermediate survival, and organisms in the bottom of the liquid had the greatest percentage survival. Within the range of 30-60 C, the temperature at which a given percentage survival was reached in the top region was up to 22° (25° for vegetable soup) lower than the temperature at which that same percentage survival was reached in the bottom region.

Data collected for this experiment also indicate that viable *E. coli* were not detectable in the top tomato soup region when the temperature had reached ca. 45 C (55 C in vegetable soup). *S. typhimurium* was no longer detectable in the top region when the temperature had reached ca. 48 C (50 C in vegetable soup). In contrast, the temperature had reached 65-70 C before either organism was undetectable in the middle or bottom regions of either soup.

Beef broth. The temperature profile of beef broth (data not shown) indicates that the middle portion of the soup was the warmest region throughout the microwave heating period, as had been found in the other two soups. However, in beef broth, unlike the situation in the other soups, the bottom region was the coolest region during the exposure periods, and the top area had the intermediate temperature. Also, the temperature difference between the three regions at any given time was less in beef broth as compared to the other two soups (see Fig. 3).

The patterns of survival of both organisms in the three regions of beef broth in terms of exposure time or temperature were similar. At any given time organisms in the top region had the lowest survival, those in the middle had intermediate survival, and organisms in the bottom had the highest survival (data not shown). These results were similar to those found in tomato and vegetable soups (Fig. 4 and 5).

With respect to a given temperature, organisms in the top of the beef broth again had the lowest level of survival. Up to ca. 52 C, the middle was the region of intermediate survival and the bottom was the region of greatest survival with respect to temperature. In tomato and vegetable soups the difference in the temperatures which corresponded to given levels of survival in the top and bottom regions ranged up to 22-25°. In beef broth the temperature range was only 5-7°.

In the top region of beef broth, *E. coli* was non-detectable by the time the temperature had reached ca. 55 C; *S. typhimurium,* by the time the temperature had reached ca. 60 C. Organisms in the middle and bottom regions of this soup were non-detectable by the time the temperature had reached ca. 62-67 C.

Relationship of sampling procedures and soup volume to survival of E. coli in microwave cooked tomato soup

In the previous experiments, heated soups had been sampled sequentially from bottom to top (i.e., BMT). It was therefore necessary to determine whether the delay (10-15 sec) between removal of aliquots from the top and bottom regions influenced the pattern of survival of organisms in the three regions. In this experiment, samples from single servings of tomato soup inoculated with *E. coli* were removed sequentially from top to bottom (i.e., TMB) for viable counts of remaining cells. Duplicate determinations were made for each time period and results were averaged.

The results (data not shown) indicated that the sequence of removal of aliquots at the end of heating periods had no effect on the pattern of survival in the three soup regions. With respect to any given exposure time, organisms in the top region of the TMB-sequence soup still had declined to the lowest survival values, organisms in the middle region had intermediate levels of survival, and organisms in the bottom of the soup showed the greatest survival. In terms of any given temperature, it was once again evident that organisms in the top had the least survival, organisms in the middle had the intermediate survival levels, organisms in the bottom had the greatest survival. Also there was a difference of up to 23° between the temperatures in the top and bottom of the TMB-sequenced soup for which the same percentage survivals in these regions was achieved. This compares to a difference of 22° in the soup which was sampled in the reverse (i.e., BMT) sequence.

Although the sequence of sample removal had no effect on the pattern of survival of *E. coli* in tomato soup, increasing the volume of soup to 600 ml did show a difference in some of the results. The temperature profile for the larger soup volume was similar to that for the smaller volume in that the middle was the warmest region, the bottom had the intermediate temperatures, and the top was the coolest region throughout the microwave exposure. Also, in terms of exposure times, the pattern of survival of *E. coli* in the triple volume was similar to the pattern in the single serving volume. For almost all of the heating period, organisms at the top had the lowest survival, those in the middle had intermediate values, and organisms sampled from the bottom had the greatest survival. However, with respect to temperatures in the three regions, there was a difference in the survival pattern. Although for any given temperature, organisms in the top declined to the lowest levels, the relative levels of survival for the middle and bottom regions are reversed in comparison to the findings for the smaller soup volume. In the triple serving, it was the bottom which showed intermediate levels and the middle showed the greatest survival. The difference in the survival between middle and bottom regions in all experiments was relatively small compared to the difference between these regions and the top region.

Survival of E. coli in microwave cooked tomato soup contained in a modified graduated cylinder

The temperature profile (data not shown) of tomato soup inoculated with *E. coli* during the microwave exposures reveals that position D (located between the middle and bottom; see Fig. 2) and position C (the middle) had the warmest temperatures throughout the heating period. Position B (between the top and middle) was consistently the coolest region. Positions E (the bottom) and A (the top) were the intermediate temperature regions. This profile is similar to that for the single and triple servings of tomato soup in which the middle was the warmest region, the bottom was intermediate, and the top was coolest. In terms of exposure time, the pattern of survival in the modified cylinder was also consistent with previous findings. Figure 8 shows, for any given time, the closer the sampling level was to the top of the liquid the greater the decrease in survival of the organisms.

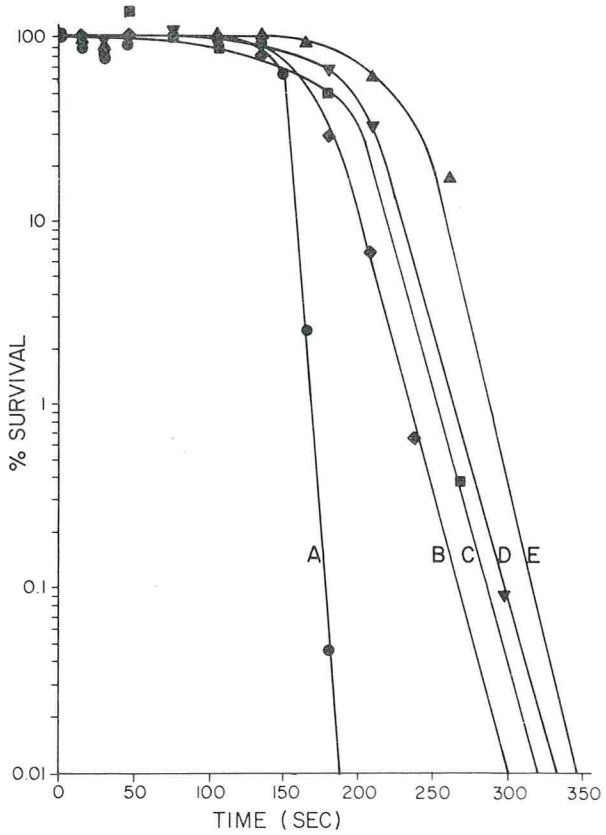

Figure 8. *Decrease in survival of* Escherichia coli *in tomato soup contained in the modified graduated cylinder with respect to microwave exposure time. Circles indicate Position A (Top), Diamonds indicate Postion B (Between Top and Middle), Squares indicate Position C (Middle), Inverted Triangles indicate Postion D (Between Middle and Bottom), and Triangles indicated Postion E (Bottom).*

In terms of the temperatures reached during the exposure, the survival pattern in the five regions in this experiment agree well with the previously found patterns for the single serving of tomato soup. Figure 9 indicates

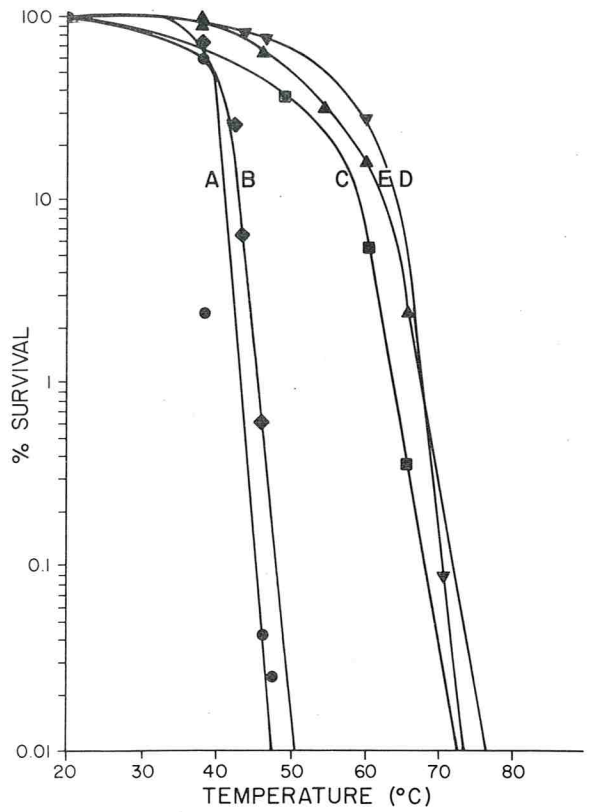

Figure 9. *Decrease in survival in* Escherichia coli *in tomato soup contained in the modified graduated cylinder with respect to temperatures reached during microwave exposure. Circles indicate Postion A (Top), Diamonds indicate Postion B (Between Top and Middle), Squares indicate Position C (Middle), Inverted Triangles indicate Position D (Between Middles and Bottom), and Triangles indicated Postion E (Bottom).*

that, for any given temperature, organisms in the two top regions (A and B) had declined to the lowest levels, the middle region (C) showed the intermediate level of survival, and organisms in the bottom regions (D and E) had the least decrease in survival. Also, there was again a difference of up to 23° in the temperature between the top and bottom regions for which a given value of survival was obtained. Organisms in the top two regions of the cylinder had declined to nondetectable levels by the time the temperature had reached ca. 55 C. In contrast, survivors were detectable in the middle and bottom regions until the temperatures had reached ca. 75-80 C.

DISCUSSION

Many of the literature reports concerning the biological effects of microwaves had attempted to ascertain whether the lethality of such radiation for microorganisms is solely due to the generated heat. In this study, experiments were done to investigate the relationship between temperatures generated in liquid foods and destruction of inoculated bacteria in these foods. To account for uneven energy distribution, temperatures achieved in several regions of the foods during the microwave exposure were measured and correlated with the percentage of surviving bacteria in

those regions. Copson (*4*) had determined that the temperature profile of agar cylinders heated with 915 MHz microwaves revealed that the interior or "core" section of the cylinders were heated to higher temperatures than the peripheral or surface regions. Heating of tomato, vegetable, and beef broth soups with 915 MHz microwaves in this work also revealed a similar profile in that the middle region of the soup was the warmest region throughout the exposrure period. In tomato and in vegetable soups, the finding that the top portion of the soup was the coolest region may have been a consequence of heat loss from the uncovered, non-insulated surface of these liquids to the cool, unheated oven air. However, in beef broth, the bottom region was the coolest portion of the soup. This different pattern of heating in beef broth may have been due to differences in the conduction and convection patterns that were induced in this soup as compared to the other two soups. Although conduction and convection are not responsible for the primary heating effect in microwave cooked foods, they do serve to redistribute the heat within the food once the primary heating has occurred (*4*).

If the lethal action of microwave energy on microorganisms were solely due to the heat generated by the waves, it would be expected that organisms sampled from the warmest region of the soups would have declined to the lowest survival values at any given time. Conversely, those sampled from the coolest region should show the greatest survival. However, results of microwave cooking of soups in this study indicate that a factor other than heat generated in the surroundings of the bacteria may be responsible for the inactivation of the inoculated organisms. Regardless of soup type or volume, sampling procedure, test organism, or relative temperatures of the sampled positions, the consistent finding was that, for any given exposure time, the closer the sampled region was to the top of the container, the greater the decrease in microbial survival. In terms of the temperatures reached in any of the given soup positions, it was also evident that organisms in the top regions of the liquids declined to given levels of survival at temperatures lower than those required to reduce organisms in the middle and bottom regions to the same given levels.

In contrast to the findings in this study, Goldblith and Wang (*9*) concluded that the lethal effects of 2450 MHz radiation for *E. coli* and *B. subtilis* spores were solely due to the heat generated by the microwaves. Lechowich et al. (*12*) also concluded that the inactivation of *S. faecalis* and *S. cerevisiae* by 2450 MHz microwaves could be explained solely in terms of the heat generated during the exposures.

Nevertheless, precedent does exist for the demonstration that temperature increase alone is insufficient in explaining the detrimental effects of microwaves for microorganisms and several mechanisms have been suggested to elucidate the nature of such apparently "non-thermal" effects. For instance, Olsen (*14*) postulated that microorganisms present a "preferential target" for the action of microwaves. That is, since microorganisms usually contain high intracellular concentrations of ionizable compounds, they heat extremely well and may reach higher temperatures than the surrounding matrix when placed in a microwave field of suitable strength. Carroll and Lopez (*3*) also stated that, depending on the relative chemical composition of microbial cells and their surrounding medium, the cells may be selectively heated by microwaves.

However, it has also been postulated that some of the apparent "non-thermal" effects of microwaves may actually be due to molecular level responses of the biological systems to the input of thermal energy. For instance, Vogelhut (*16*) has demonstrated how the input of microwave energy may effect structural changes in the bound-water layer surrounding biological macromolecules. Such changes can be expected to alter the stability and function of the macromolecules and, consequently, the biological processes in the cell itself. Carroll and Lopez (*3*) have proposed that microwave frequency radiation may be selectively absorbed by certain essential biochemical molecules. If such resonance frequencies exist, configurational changes may occur so that the molecule would be irreversibly denatured. Illinger (*11*) has considered some of the molecular mechanisms for microwave absorption by biological systems and has undertaken theoretical predictions, based on energy requirements and frequency dependencies, of which mechanisms could be significant.

In addition to inducing molecular level changes, it has also been suggested that microwaves may affect biological systems on a mechanical level. Copson (*4*) stated that it is to be expected that the net charge of bacterial cells will influence their behavior in an electromagnetic field. Carroll and Lopez (*3*) speculated that the presence of this charge may cause the cells to oscillate rapidly in a high frequency field. Mechanical disruption of cells would occur if the oscillations were rapid enough and of sufficient displacement to exceed the elastic limitations of the cell wall. Teixeira-Pinto et al. (*15*) demonstrated eventual rupturing of *Amoeba limax* with 27 MHz radiation as the field strength in their experiment was progressively increased.

It seems evident from the results in this study that two lethal effects of microwaves for bacteria were demonstrated. First of all, heat was generated which undoubtedly contributed much to inactivation of the inoculated organisms. In addition, the greater decrease in survival of organisms in the top regions of the liquids as compared to the decreases in survival in the middle and bottom regions indicates that the microwaves' irradiation also effected an inactivation of bacteria that could not be explained solely in terms of the relative temperatures generated in the soup regions. This greater decrease in survival in the top regions may be a reflection of the fact that the microwave field intensity was greater there than in the other soup regions. The waves entered from the top of the oven, and as they were absorbed by

successive regions in the liquid, the intensity would decrease. (It might be expected therefore that the top region would be the warmest region due to the greater intensity, but there is also much more heat dissipation from this region.) Whether the nature of the lethality of the microwave radiation for bacteria as noted in this study was molecular, mechanical, or a selective heating effect, it is likely that the effect would be greater for greater intensities. Results of this study do not indicate the exact nature of the lethal effects of microwaves for bacteria. However, they do cast doubt on the theory that heat alone as generated by microwaves in the surroundings of the bacteria is fully adequate to account for the destruction of the bacteria. In light of the fact that the exact nature of the biologic effects of microwave radiation for any living system are still not completely understood, it seems that continuing research in this area is definitely warranted.

ACKNOWLEDGMENTS

We thank M. Schmoyer for his technical assistance.

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(act of October 23, 1962; Section 4369, Title 39, United States Code)

Of The Journal of Milk and Food Technology, published monthly at 413 Kellogg, Ames, Iowa 50010 for December, 1974.

The names and addresses of the publisher, editor, and managing editor are:

Publisher, International Association of Milk, Food and Environmental Sanitarians, Inc., 413 Kellogg Avenue, Ames, Iowa 50010.

Editor, Dr. E. H. Marth, University of Wisconsin, Madison, Wisconsin.

Managing editor, Earl O. Wright, 413 Kellogg Avenue, Ames, Iowa 50010.

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Column one average No. copies each issue during preceeding 12 months—Column two single issue nearest to filing date.

A. Total No. Copies Printed (Net Press Run)	4200	3900
B. Paid Circulation		
1. To term subscribers by mail, carrier delivery or by other means	3900	3585
2. Sales through agents, new dealers or otherwise	45	42
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J. Milk Food Technol. Vol. 38, No. 1, Pages 16-19 (January, 1975)

Effect of the Postmilking Teat Dip "Bovadine" on the Incidence of *Staphylococcus aureus* and Mastitis[1]

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(Received for publication September 17, 1973)

ABSTRACT

The University milking dairy herd was divided into two groups according to breed. Diagonally opposite teats of each cow were dipped with "Bovadine" after each milking. The remaining two teats served as controls. The Wisconsin Catalase Test, Wisconsin Mastitis Test, and Direct Microscopic Somatic Cell Count were used to detect incidence of mastitis in composite samples from dipped and control teats. Samples of milk were plated on Baird-Parker Agar to detect staphlococci. The postmilking teat dip caused a 31.4% reduction in new cases of mastitis and 42.3% reduction in staphylococcal infections during the 19 months of the study. The teat dip caused a reduction in new cases of staphylococcal infections and in number of quarters infected with mastitis; however, these reductions were non-significant. The WMT had the highest correlation when compared with all the other tests used to detect mastitis. Results indicated that the WMT would be the best single test to use to detect cows with mastitis.

Complete eradication of bovine mastitis appears unlikely since it may be caused by both microbial and non-microbial factors. Control of this costly disease may be achieved by use of a proper milking hygiene program. Inclusion of a postmilking teat dip in such a program appears to be useful in controlling udder infections by preventing the spread of pathogens from cow to cow during milking (*5, 7, 8, 10*). As a result, microbially related mastitis should be reduced.

The use of a teat dip to reduce the incidence of microbially related mastitis was recommended as early as 1916 (*4*); however, it has been only during the last decade that serious consideration has been given to the use of this procedure as a means for controlling mastitis.

This study was designed to determine the effect that the teat dip "Bovadine" would have on the incidence of *Staphylococcus aureus* in the dairy herd at the University of Kentucky. The effect of the teat dip on the incidence of mastitis also was determined. In addition, interrelationships were determined among three tests used to indicate cows with mastitis, and among tests used to indicate pathogenic strains of staphylococci.

MATERIAL AND METHODS

Diagonally opposite teats of each milking cow were dipped in "Bovadine" (West-Agro Chemical, Co.), a commercial iodine (10,000 ppm) teat dip preparation, after each milking. The right front and left rear teats of each Holstein and the left front and right rear teats of each Jersey were dipped immediately after each milking. The remaining two teats of each cow were not dipped and served as controls.

A total of 183 cows were on experiment for at least one month of the 19-month study. There were 87 Jerseys and 96 Holsteins.

Before sampling, each udder was washed with a 20% (by weight) quaternary ammonium sanitizer and dried with a paper towel. Then each teat was wiped with an individual piece of cotton soaked with 70% alcohol and allowed to air-dry. Two composite foremilk samples were obtained from each cow. One sample consisted of approximately 40 ml of foremilk from each of the two dipped teats, while the second sample contained foremilk from the two non-dipped teats. Samples were collected in sterile plastic bags and placed in ice for transportation to the laboratory where they were then stored at 2 C until analyzed. Samples were analyzed within 12 h of being collected

Before the start of the study, 176 two-quarter composite samples were obtained from the milking herd which consisted of 43 Holsteins and 45 Jerseys. The composite samples were obtained from the two diagonal teats selected to be dipped and from those teats selected to serve as controls. These samples were used to obtain information as to number of composite quarters containing detectable levels of staphylococci and from cows with mastitis.

A total of 978 two-quarter composite foremilk samples were collected from 183 cows six times during the 19 months of the study. Samples were obtained after 4, 8, 14, 18, and 19 months of dipping the teats.

Surface-inoculated Baird-Parker Agar (BBL) plates were used for selective isolation of staphylococci. Plates were inoculated by spreading 0.1 ml of sample on the surface with sterile glass "hockey" rods. Plates were incubated for 48 h at 37 C. Two colonies showing morphological characteristics of *S. aureus,* shinny black colonies with halos, were picked into separate tubes of Trypticase Soy Broth (BBL). Tubes of broth were incubated for 18 h at 37 C, transferred to Trypticase Soy Agar (BBL) slants, incubated overnight at 37 C and then stored at 2 C.

Active cultures for use in identification of an isolate as *S. aureus* were obtained by transferring several times in TSB before doing the following tests: gram strain (*2*), coagulase (*1,2*) catalase (*1,2*)deoxyribonuclease activity (*1,2*), phosphatase activity (*1,2*), and oxidative-fermentative utilization of glucose and mannitol (*3*). Isolates were identified as *S. aureus* using the criteria of Baird-Parker (*1*).

The following screening and confirmatory tests (*6*) for detection of mastitis were done on each sample: Wisconsin Mastitis Test (WMT), modified Wisconsin Catalase test (WCT), and the field counting procedure for determining the direct microscopic somatic cell count (DMSCC). The WCT was modified so that calibrated 15-ml screw-capped centrifuge tubes and 9 ml of milk were used in the procedure.

The following criteria were used to identify a cow with mastitis: WCT greater than 29% oxygen, WMT greater than 29 mm and DMSCC greater than 1.5 million cells/ml. For this study, a cow was considered to have mastitis if values greater than those given above for the three tests were obtained by any one of the tests on a composite sample.

A cow was considered to have a new staphylococcal infection when staphylococci were not detected in a composite sample during one sampling period but were detected during the next sampling period.

Analysis of variance was used to determine the effects of breed, treatment, sampling period, breed × treatment, breed × sampling period and treatment × sampling period. Correlations were made among DMSCC, WMT, WCT and staphylococcus-positive samples and among the test used to identify an isolate as *S. aureus.*

[1]*Published with the approval of the Director of the Kentucky Agricultural Experiment Station as journal article no. 73-5-103*

The percent reduction in new cases of mastitis and in staphylococcal infections due to the teat dip was determined using the equation:

$$\%\,\text{Reduction} = \frac{\text{Number of positive samples from controls} - \text{Number of positive samples from treated teats}}{\text{Number of positive samples from controls}}$$

RESULTS AND DISCUSSION

The isolates obtained from Baird-Parker Agar plates all were catalase-positive, gram-positive cocci. All except 17 isolates were coagulase positive. Further testing of the coagulase-negative isolates, identified them as having characteristics similar to *Streptococcus faecalis* (*9*).

During preliminary examination of the herd, 36.9% of the composite samples were identified as being from cows with mastitis and 47.2% were found to contain staphylococci. More composite samples obtained from diagonal teats selected to serve as controls indicated mastitis (39.8%) than did the teats selected to be dipped (34.1%). The reverse was observed for staphylococci, where 50.0% of the composite samples from teats to be dipped contained staphylococci compared with 44.3% from the controls. More composite samples which indicated mastitis (38.9%) and also contained staphylococci were obtained from Jerseys (51.5%) than were obtained from Holsteins (34.9% and 44.3%).

The results obtained during the preliminary period and the five sampling periods are shown in Table 1 and Table 2.

As expected from previous observations (*10*), more two-quarter composite samples from control teats (29.7%) indicated cows with mastitis than were obtained from dipped teats. More staphylococci also were detected (32.7%) in the samples from the control teats.

The percentage of samples from control and dipped teats found to contain detectable numbers of staphylococci and from cows with quarters indicating mastitis generally decreased with an increase in the length of the study. The lowest percentage of samples

TABLE 1. *Percentage of composite samples from dipped and non-dipped teats of Holsteins and Jerseys found to be positive for mastitis by screening tests*

Sampling period	Number composite samples		Mastitis positive[a] Breed			
			Holstein		Jersey	
	Holstein	Jersey	Dipped	Control	Dipped	Control
Prelim[c]	86	90	32.6	37.2	35.6	42.2
1	104	96	37.6	36.5	37.5	39.6
2	96	78	20.8	27.1	25.6	30.8
3	104	94	17.1	36.5	31.9	19.1
4	120	96	26.7	35.0	35.4	29.2
5	110	80	14.5	23.6	20.0	15.0
Total periods 1-5	534	444	22.8	31.8	30.6	27.0

[a]Sample considered to be from cow with mastitis if one test exceeded: WCT 29% 0_2; WMT 29 mm; DMSCC 1,499,999 cells/ml.
[b]Half of the samples were from dipped and half from non-dipped teats.
[c]Preliminary period, samples were obtained the milking prior to start of dipping.

TABLE 2. *Percentage of composite samples from dipped and non-dipped teats of Holsteins and Jerseys found to contain staphylococci*

Sampling period	Number of composite samples[b]		Staphylococcus-positive[a] Breed			
			Holstein		Jersey	
	Holstein	Jersey	Dipped teats	Control teats	Dipped teats	Control teats
Prelim[c]	86	90	46.5	39.5	53.3	48.9
1	104	96	38.5	34.6	47.9	39.6
2	96	78	29.5	45.8	28.2	20.5
3	104	94	15.4	38.5	27.7	25.5
4	120	96	15.0	41.7	27.1	16.7
5	110	80	18.2	34.5	20.0	20.0
Total periods 1-5	534	444	22.8	39.0	30.6	24.8

[a]Sample considered staphylococci positive if black colonies appeared on Baird-Parker Agar within 48 h at 37 C.
[b]Half of the samples were from dipped and half from non-dipped teats.
[c]Preliminary period, samples were obtained the milking prior to start of dipping.

positive for staphylococci or indicating mastitis was obtained during sampling period 5, which corresponded to the end of the study.

Differences were found in the percentage of composite samples from cows indicating mastitis and those which contained detectable staphylococci between the two breeds, between the preliminary and test periods and between dipped and control teats of each breed. While a definite decrease in percentage differences was observed, analysis of variance indicated that the differences between breeds, treatments, sampling periods and their interaction were not significant ($P < 0.05$).

The purpose of a postmilking teat dip is to prevent new infections of the mammary gland by preventing cross-infection from interior or exterior to exterior of teats due to the spread of microorganisms capable of causing mastitis. The effectiveness of a teat dip can be determined by comparing the number of incidences or new cases of mastitis or staphylococcal infections occurring in samples from controls with those from dipped teats (*10*).

During the study 86 new cases of mastitis were identified by at least one of the tests used to detect mastitis, with 51 cases being from controls and 35 from dipped teats. This indicates that dipping the teats resulted in a reduction of 31.4% in cases of mastitis.

$$\%\,\text{Reduction} = \frac{51 - 35}{51} \times 100 = 31.4\%$$

A total of 82 new staphylococcal infections occurred during the study. A new infection was considered to have occurred when staphylococci were not detected in a sample during one sampling period, but were detected during the next sampling period. Control teats had 52 new infections compared with 30 from dipped teats. This was a reduction of 42.3% in staphylococcal infections due to the teat dip.

$$\%\,\text{Reduction} = \frac{52 - 30}{52} \times 100 = 42.3\%$$

The teat dip appeared more effective in reducing staphylococcal infections in Holsteins than in Jerseys. A 48.6% reduction in new staphylococcal infections was observed for Holsteins compared with 31% for Jerseys. Wesen and Schultz (*10*) reported an overall reduction of 53.2% in new infections when the teats on the right side were dipped in "Bovadine" and individual quarter samples analyzed.

A comparison was made between new staphylococcal infections and new cases of mastitis. The comparison showed that 48 of the 82 new staphylococcal infections (58.5%) came from samples which also indicated they were from quarters with mastitis. A higher percentage of composite samples (58.3%) from non-dipped teats were positive for staphylococcal infections than were samples from dipped teats (41.7%). This indicates that a majority of the samples positive for staphylococci also came from quarters infected with mastitis.

Correlations were determined among the various tests used to detect mastitis as well as between the various tests used to identify the black colonies isolated from Baird-Parker Agar. (Table 3).

TABLE 3. *Correlation coefficients between isolates from Baird-Parker Agar and tests used to characterize the isolates*[a]

	PHOS[b]	DNA[b]	CAT[b]	MAN[b]	COAG[b]
BP	o.7286**	0.7294**	0.7299**	0.8357**	0.7699**
COAG	0.8829**	0.8826**	0.8810**	0.7655**	
MAN	0.8587**	0.8582**	0.8580**		
CAT	0.9937**	0.9973**			
DNA	0.9954**				

[a]Data represents 1154 samples.
[b]COAG – Coagulase Test; PHOS – Phosphatase Test; DNA – Desoxyribonuclease activity; CAT - Catalase Test; MAN - Mannitol Fermentation; BP - Black colonies isolated from Baird-Parker Agar.
** $P < 0.1$

The three tests used in this study to indicate mastitis had differences which were all highly significant ($P < 0.01$) when compared with each other. Highest correlation was obtained between WMT and WCT ($r = 0.7801$). The WMT had the highest correlation when compared with the other tests used to detect mastitis and would be the best single test to use to indicate cows with mastitis.

Correlations between the black colonies isolated from Baird-Parker Agar and tests used to characterize them as *S. aureus* were highly significant ($P < 0.01$) and are shown in Table 3. Highest correlations were between CAT and PHOS and DNA, and DNA and PHOS. All isolates obtained from Baird-Parker Agar were cocci and were considered to be staphylococci due to the selectivity of this medium. Characterization of these isolates has been done and reported (*9*). Results obtained were similar to those reported by Baird-Parker (*1*) and White et al. (*11*).

Use of a postmilking teat dip proved effective in reducing the number of new incidences of mastitis and new staphylococcal infections. Results obtained in this study are in agreement with those obtained by other investigators in establishing the value of postmilking teat dips (*5, 7, 8, 10*). The use of a postmilking teat dip caused up to a 50% reduction in new cases of staphylococcal infections and quarters infected with mastitis. These results appear to be practical and of economic importance to the dairy farmer, however, statistical treatment of the data showed these reductions to be non-significant. In addition, the value of this teat dip in reducing staphylococcal infections was establised in this study. Since staphylococci are presently considered a major cause of mastitis, the use of a teat dip appears to be a valuable part of a program to control mastitis.

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UW Scientists Nearing Better Food Poisoning Test

Food scientists at the University of Wisconsin-Madison are working on a test which offers some promise of becoming a practical method for determining if foods contain toxins which may cause food poisoning.

The test uses reverse passive hemoagglutination, according to M.S. Bergdoll, head of the Food Research Institute (FRI) staphylococci testing group. In conducting the test, staph enterotoxin antibodies are coupled to sheep red blood cells and mixed with food extract. If the food extract contains enterotoxin, the red blood cells agglutinate or clump together.

Staph enterotoxins, proteins produced by growing bacteria, are responsible for almost half of the food poisoning incidents in the United States. Public health and regulatory agencies and food processors need a simple, sensitive test to determine if enterotoxins are present in foods to make sure that contaminated foods don't reach the consumer.

The technique now in use was developed as a result of FRI research, but the physical limits of the microslide procedure prevent the necessary sensitivity, Bergdoll says. "We must be able to detect less enterotoxin than makes a sensitive person ill," Bergdoll says, "and that amount may be less than regulatory agencies can detect in their labs at present."

The stumbling block in the microslide technique is that food extract must be highly concentrated for the test. The reverse passive hemoagglutination method favored by Bergdoll is a simple, sensitive procedure that requires less food extract concentration and purification.

The test still has some problems, however. FRI researchers are having trouble getting antibodies from one enterotoxin to stick to the red blood cells. Another problem is that the test has sometimes given false positive reactions caused by the buffer solution and some food extracts. "Making the buffer solution fresh each day and some simple treatments of the food extracts have eliminated the false positive reactions," Bergdoll says, "but we won't recommend this method until we are sure the results cannot be misinterpreted."

A problem common to all enterotoxin detection methods is that the technician must test for five different known enterotixins. Some enterotoxins haven't been identified by their antibodies, but are known to exist from monkey feeding tests. "Until we have antibodies for them we cannot use the reverse passive hemoagglutination test," Bergdoll says. "The Food Research Institute is tackling this problem by trying to develop one reagent that will react with all the enterotoxins including the unknowns."

J. Milk Food Technol. Vol. 38, No. 1, Pages 20-24 (January, 1975)

Distillate From Milk Can Affect Growth and Activity of *Streptococcus lactis*[1]

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ABSTRACT

Raw whole milk was processed under vacuum at 60, 68.3, or 76.6 C and distillate (5 ml/100 ml of milk) was collected in cold traps. Unprocessed and processed samples of milk were autoclaved, steamed, or heated in a waterbath at 60, 68.3, or 76.6 C. Each heated processed milk was subdivided into two portions; distillate (5:95) was added to one portion and distilled (in glass) demineralized water (5:95) was added to the other. All samples were inoculated with *Streptococcus lactis* 4175 and incubated at 22 C. Growth and activity of *S. lactis* were monitored by determining the number of organisms, pH, and titratable acidity after 0, 4, 7, 10, and 13 h of incubation.

Distillate collected at 60 C slightly retarded growth of *S. lactis* in steamed milk. Both growth and activity of this organism were reduced when distillate collected at 68.3 C was added to autoclaved and waterbath-treated (68.3 C) milk; this was not true for steamed milk. When milk was processed at 76.6 C, the resultant distillate had only a very slight effect on behavior of *S. lactis* in autoclaved milk. In steamed milk, although its generation time was unaffected, acid production by *S. lactis* was enhanced when the distillate was added. When processed milk was heated at 76.6 C, distillate collected at 76.6 C caused a slight increase in the generation time of *S. lactis*.

Heating induces certain changes in milk and often makes it more suitable for growth of starter cultures. Speck (*20*) has reviewed the effects of different heat treatments on starter growth in milk and hence only a limited discussion of the subject is given in this paper.

Pasteurization generally improves milk as a culture medium (*7*), possibly because it reduces the activity of inhibitory substances that are naturally present in milk (*17*). According to Olson (*15*) and Olson and Gilliland (*16*), most pure cultures of *Streptococcus lactis* and *Streptococcus cremoris* and also most commercial starter cultures developed acid more rapidly in milk pasteurized at 71 rather than 61 C, although activity of some cultures was greatest in milk heated at the lower temperature. High- rather than low-temperature pasteurization of milk for growth of starter cultures also was favored by Swartling and Mukherj; (*21*). Milk pasteurized at 72-75 C contained more free amino acids than did milk heated at lower temperatures (*14*). This was claimed as the reason for improved growth of starter cultures in milks given a high-heat pasteurization treatment (*14*).

Although gross overheating makes milk a poor medium for starter cultures (*6, 10*), autoclaving tends to improve milk as a substrate for such bacteria more than does pasteurization. This has been demonstrated for lactic streptococci (*7, 19*), *Streptococcus thermophilus* (*4, 5*), and *Lactobacillus casei* (*1, 3*).

Greene and Jezeski (*8-12*) observed that the amount of heat given to milk before it is used to grow lactic streptococci can induce a cycle of stimulation (62 C for 30 min to 72 C for 40 min), inhibition (72 C for 45 min to 82 C for 10-120 min or to 90 C for 1-45 min), stimulation (120 C for 15-30 min), and inhibition (120 C for more than 30 min). A cycle of inhibition and stimulation of starter culture growth in milks heated to various temperatures also was reported by other investigators (*2, 4, 21*).

Reasons given for improved growth of starter cultures in heated milk include: lowering of the oxidation-reduction potential (*8-12*), destruction of heat-labile inhibitors normally present in milk (*4, 8-12, 17, 18*), partial hydrolysis of milk proteins (*8-12, 14*), denaturing of serum proteins (*8-12*), removal of certain volatile sulfur compounds (*8-12*), and production of formic acid (*1, 3*).

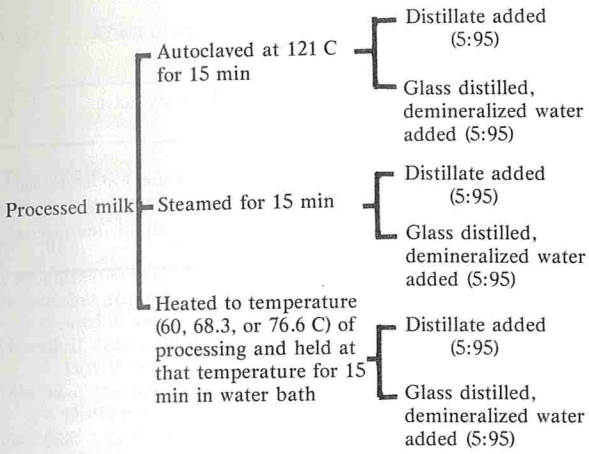
Except for sulfur compounds, none of the previous work took into account the fact that milk contains numerous volatile compounds and that removal of such materials during heat processing also might influence the suitability of milk as a substrate for certain starter bacteria. Consequently, experiments were done to determine if distillates obtained from milk heated at several temperatures could influence the growth and activity of *S. lactis*. Results of the tests are given in this paper.

MATERIALS AND METHODS

Raw whole milk was obtained from the University of Wisconsin dairy plant. Portions of this milk were processed at 60, 68.3, or 76.6 C in a "Precision" Laboratory Evaporator, supplied by Precision Scientific Company, Chicago, Illinois. Milk in the evaporator was brought to the processing temperature quickly and distillate was collected at the rate of about 5 ml/100 ml of milk. The distillate was condensed in a series of traps: (*a*) cold water, (*b*) dry ice and propylene glycol, (*c*) dry ice and propylene glycol, and (*d*) liquid nitrogen. Distillation was stopped when sufficient distillate was collected. Condensate from all traps was combined. Milk from which distillate was thus removed and unprocessed raw whole milk were treated as follows to obtain nine different samples of milk:

Raw milk —
- Autoclaved at 121 C for 15 min
- Steamed for 15 min
- Heated to temperature (60, 68.3, or 76.6 C) of processing and held at that temperature for 15 min in water bath

[1] *Research supported by the College of Agricultural and Life Sciences, University of Wisconsin-Madison.*



All samples were then inoculated with 0.7% of an active 12-16-h old culture of *S. lactis* 4175, obtained from the Marschall Division, Miles Laboratories, Inc., Madison, Wisconsin. To minimize loss of volatile compounds, epoxy-lined aerosol cans, supplied by Continental Can Co., Chicago, Ill., were used as test vessels. Cans were sealed soon after adding distillate or distilled water, using stainless steel caps without dip tubes. Five cans were used for each treatment of milk sample. Growth and activity of *S. lactis* during incubation at 22 C were monitored at 0, 4, 7, 10, and 13 h by determining (*a*) the number of organisms, using standard methods (*13*) but with APT agar (Difco) as the plating medium, (*b*) pH, and (*c*) titratable acidity (as percent lactic acid).

RESULTS AND DISCUSSION

Tables 1, 2, and 3 present data obtained when milk was processed at 60, 68.3, or 76.6 C. These data were used to calculate the generation time of this strain of *S. lactis,* the increase in titratable acidity (as % lactic acid), and the drop in pH.

Values thus obtained (Table 4) suggest that the generation time of *S. lactis* tended to be longer in processed than in unprocessed milk, but acid production was usually greater in processed than in unprocessed milks. When milk was autoclaved, neither processing nor adding distillate had any effect on generation time. Adding distillate reduced acid production in steamed processed milk but not in processed milks given other heat treatments. Generally, acid production was greater in processed autoclaved and steamed milks than in unprocessed milk that received the same heat treatments. When processed milk was heated at 60 C for 15 min, *S lactis* failed to produce much acid even though its generation time was similar to that obtained in processed milks given other heat treatments.

When milk was processed at 68.3 C, addition of distillate to both autoclaved and water bath-treated (68.3 C-15 min) milk retarded growth and acid production by *S. lactis.* Processed milk, when steamed, was more suitable for growth and acid production by *S. lactis* when distillate was added to it. Milk heated in a waterbath at 68.3 C, whether processed or not, generally, developed less acid than did autoclaved or steamed milk.

Unprocessed autoclaved and steamed milks were better for growth of *S. lactis* than were milks processed at 76.6 C. The reverse was true for milk heated in a water bath at 76.6 C. In this instance unprocessed milks were somewhat better suited for growth of *S. lactis* than were the processed milks. When processed milk was autoclaved, addition of distillate had no apparent effect on behavior of *S. lactis.* In steamed milks, although the distillate did not affect the generation time of *S. lactis,* it enhanced the capacity of this organism to produce acid. Addition of distillate to milk processed at 76.6 C and

TABLE 1. *Effect of different treatments after processing milk at 60 C on growth and activity of* Streptococcus lactis

Treatments	Log. of no./ml after h of incubation					T.A. (% lactic acid) after h of incubation					pH after h of incubation				
	0	4	7	10	13	0	4	7	10	13	0	4	7	10	13
Raw untreated milk (autoclaved)[1]	6.08	7.25	7.90	8.40	8.78	0.20	0.22	0.30	0.45	0.57	6.30	6.22	5.90	5.25	4.70
Raw untreated milk (steamed)[2]	5.93	7.06	7.71	8.25	8.61	0.16	0.21	0.26	0.35	0.51	6.55	6.35	6.10	5.70	5.10
Raw untreated milk (heated in water bath)[3]	5.87	6.95	7.69	8.23	8.57	0.17	0.18	0.21	0.23	0.25	6.60	6.50	6.30	6.20	6.10
Processed milk (autoclaved) + Dist. water[1,4]	6.16	7.15	7.85	8.35	8.69	0.20	0.25	0.37	0.58	0.74	6.40	6.10	5.55	4.80	4.55
Processed milk (autoclaved) + Distillate[1,5]	6.32	7.25	7.95	8.38	8.72	0.20	0.25	0.39	0.60	0.73	6.40	6.15	5.55	4.85	4.55
Processed milk (steamed) + Dist. water[2,4]	6.25	7.20	7.79	8.27	8.73	0.16	0.22	0.33	0.57	0.66	6.60	6.25	5.90	5.25	4.60
Processed milk (steamed) + Distillate[2,5]	6.26	7.05	7.75	8.24	8.74	0.16	0.21	0.28	0.39	0.52	6.60	6.30	5.95	5.55	4.75
Processed milk (heated in water bath) + Dist. water[3,4]	6.65	7.22	7.66	8.10	8.77	0.16	0.19	0.22	0.23	0.26	6.60	6.45	6.30	6.20	6.00
Processed milk (treated in water bath) + Distillate[3,5]	6.63	7.22	7.74	8.20	8.85	0.16	0.18	0.22	0.23	0.26	6.60	6.45	6.35	6.15	6.05

[1]Autoclaved at 121 C for 15 min.
[2]Steamed for 15 min.
[3]Heated in water bath to 60 C and maintained for 15 min.
[4]Milk processed in evaporator to remove distillate with added distilled water.
[5]Milk processed in evaporator to remove distillate with added distillate.

TABLE 2. *Effect of different treatments after processing milk at 68.3 C on growth and activity of* Streptococcus lactis

Treatments	Log. of no./ml after h of incubation					T.A. (% lactic acid) after h of incubation					pH after h of incubation				
	0	4	7	10	13	0	4	7	10	13	0	4	7	10	13
Raw untreated milk (autoclaved)[1]	6.50	7.27	7.75	8.29	8.71	0.21	0.22	0.31	0.52	0.68	6.30	6.25	5.80	5.30	4.85
Raw untreated milk (steamed)[2]	6.66	7.35	7.80	8.31	8.72	0.16	0.22	0.29	0.44	0.65	6.65	6.30	6.10	5.40	4.85
Raw untreated milk (heated in water bath)[3]	6.60	7.15	7.65	8.00	8.06	0.16	0.18	0.20	0.21	0.25	6.65	6.55	6.45	6.30	6.15
Processed milk (autoclaved) + Dist. water[1,4]	6.69	7.29	7.80	8.26	8.77	0.19	0.23	0.31	0.44	0.63	6.40	6.15	5.75	5.30	4.80
Processed milk (autoclaved) + Distillate[1,5]	6.71	7.23	7.75	8.23	8.72	0.19	0.23	0.25	0.42	0.60	6.40	6.20	5.90	5.40	4.90
Processed milk (steamed) + Dist. water[2,4]	6.59	7.25	7.79	8.28	8.67	0.15	0.20	0.28	0.37	0.58	6.60	6.35	6.05	5.60	5.00
Processed milk (steamed) + Distillate[2,5]	6.62	7.10	7.65	8.26	8.67	0.15	0.19	0.30	0.44	0.64	6.70	6.40	5.95	5.40	4.75
Processed milk (heated in water bath) + Dist. water[3,4]	6.53	7.26	7.80	8.28	8.53	0.10	0.18	0.27	0.36	0.44	6.75	6.50	6.15	6.15	5.35
Processed milk (heated in water bath) + Distillate[3,5]	6.49	7.21	7.68	8.24	8.53	0.15	0.18	0.26	0.33	0.39	6.70	6.50	6.20	6.20	5.55

[1]Autoclaved at 121 C for 15 min.
[2]Steamed for 15 min.
[3]Heated in water bath to 68.3 C and maintained for 15 min.
[4]Milk processed in evaporator to remove distillate with added distilled water.
[5]Milk processed in evaporator to remove distillate with added distillate.

TABLE 3. *Effect of different treatments after processing milk at 76.6 C on growth and activity of* Streptococcus lactis

Treatments	Log. of no./ml after h of incubation					T. A. (% lactic acid) after h of incubation					pH after h of incubation				
	0	4	7	10	13	0	4	7	10	13	0	4	7	10	13
Raw untreated milk (autoclaved)[1]	6.35	7.15	7.69	8.13	8.29	0.22	0.25	0.28	0.44	0.62	6.30	6.15	5.80	5.35	5.00
Raw untreated milk (steamed)[2]	6.34	7.25	7.77	8.26	8.37	0.18	0.24	0.25	0.37	0.50	6.60	6.25	6.15	5.70	5.30
Raw untreated milk (heated in water bath)[3]	6.38	7.16	7.75	8.18	8.26	0.17	0.21	0.22	0.33	0.45	6.70	6.55	6.35	5.85	5.55
Processed milk (autoclaved) + Dist. water[1,4]	6.36	7.14	7.69	8.24	8.38	0.20	0.26	0.27	0.48	0.59	6.40	6.15	6.05	5.25	4.90
Processed milk (autoclaved) + Distillate[1,5]	6.34	7.14	7.74	8.29	8.38	0.20	0.25	0.26	0.46	0.59	6.45	6.20	6.10	5.35	5.00
Processed milk (steamed) + Dist. water[2,4]	6.33	7.10	7.69	8.26	8.37	0.17	0.21	0.25	0.37	0.50	6.65	6.35	6.20	5.65	5.30
Processed milk (steamed) + Distillate[2,5]	6.30	7.00	7.49	8.12	8.36	0.18	0.24	0.24	0.40	0.54	6.60	6.30	6.25	5.65	5.10
Processed milk (heated in water bath) + Dist. water[3,4]	6.30	7.00	7.66	8.23	8.36	0.17	0.21	0.25	0.40	0.51	6.65	6.50	6.25	5.65	5.30
Processed milk (heated in water bath) + Distillate[3,5]	6.34	7.17	7.66	8.22	8.35	0.17	0.21	0.25	0.36	0.50	6.70	6.50	6.25	5.70	5.40

[1]Autoclaved at 121 C for 15 min.
[2]Steamed for 15 min.
[3]Heated in water bath to 76.6 C and maintained for 15 min.
[4]Milk processed in evaporator to remove distillate with added distilled water.
[5]Milk processed in evaporator to remove distillate with added distillate.

TABLE 4. *Effect of different treatments after processing milk at different temperatures on generation time and activity of* Streptococcus lactis

Treatments	Gen. time (h) at different temp. (C)			Increase in T. A. (% lactic acid) at different temp. (C)			Decrease in pH at different temp. (C)		
	60	68.3	76.6	60	68.3	76.6	60	68.3	76.6
Raw untreated milk (autoclaved)[1]	1.7	1.8	1.5	0.37	0.47	0.40	1.60	1.45	1.30
Raw untreated milk (steamed)[2]	1.3	1.9	1.5	0.35	0.49	0.32	1.45	1.80	1.30
Raw untreated milk (heated in water bath)[3]	1.1	1.9	1.5	0.08	0.09	0.28	0.50	0.50	1.15
Processed milk (autoclaved) + Dist. water[1,4]	1.7	1.7	1.6	0.54	0.44	0.39	1.85	1.60	1.50
Processed milk (autoclaved) + Distillate[1,5]	1.7	1.8	1.6	0.53	0.41	0.39	1.85	1.50	1.45
Processed milk (steamed) + Dist. water[2,4]	1.7	1.7	1.5	0.50	0.43	0.33	2.00	1.60	1.35
Processed milk (steamed) + Distillate[2,5]	1.7	1.6	1.5	0.36	0.49	0.36	1.85	1.95	1.50
Processed milk (heated in water bath) + Dist. water[3,4]	2.0	1.7	1.4	0.10	0.34	0.34	0.60	1.40	1.35
Processed milk (heated in water bath) + Distillate[3,5]	1.8	1.8	1.5	0.10	0.24	0.33	0.55	1.15	1.30

[1]Autoclaved at 121 C for 15 min.
[2]Steamed for 15 min.
[3]Heated in water bath to 60, 68.3, or 76.6 C
[4]Milk processed in evaporator to remove distillate with added distilled water.
[5]Milk processed in evaporator to remove distillate with added distillate.

then heated in a waterbath at the same temperature was somewhat unfavorable for growth of *S. lactis*.

Data obtained in these experiments suggest that different heat treatments and distillates obtained from milk at three temperatures exerted a variable influence on the suitability of milk as substrate for growth and activity of *S. lactis*. Thus suitability of milk for use to manufacture fermented dairy products is dependent on heat treatment given the milk before it is used. The lowest processing temperature we used was slightly below the temperature of pasteurization by the holding method and 76.6 C was somewhat above the temperature for the high-temperature short-time pasteurization process (in practice milk often is pasteurized at 76.6 C or at even higher temperatures), 68.3 C was selected as intermediate between the two.

It has been reported by other workers (*6, 8, 9, 10, 11*) that cycles of stimulation and inhibition occurred when different heat treatments were given to milk. According to findings of Greene and Jezeski (*12*) heat treatments at both 60 and 68.3 C were within the first stimulatory phase and treatment at 76.6 C was in the first phase of inhibition.

It is evident that distillates collected in our experiments sometimes stimulated and sometimes inhibited growth and activity of *S. lactis* in milk. This suggests that the distillates and/or milks were variable in their composition. Evaluating the effect of distillates was complicated by the heat treatments-autoclaving, steaming, and heating in a water bath-given to processed (at three temperatures) or unprocessed milks. It is probable that heat treatments given milk before inoculation with *S. lactis* caused changes which made it more suitable for growth of this organism and that the distillate when added inhibited the bacterium. Furthermore, it also is possible that the heat treatment sometimes made milk unsuitable for growth of *S. lactis* and the distillate then improved the medium and thus enhanced growth of the bacterium. Finally, it is possible that the heat treatment and added distillate had the same effect (either positive or negative) on subsequent growth of *S. lactis*. Hence results we obtained are the final outcome of the synergistic or antagonistic effects of both distillate and heat treatment.

It was mentioned earlier in this paper that other investigators provided some explanations for changes in growth of starter cultures when milk receives certain heat treatments. Whatever causes stimulation or inhibition of *S. lactis* (or starter cultures in general) in milk given the different heat treatments, results of these experiments indicate that at least some compounds are removed from milk by a vacuum treatment and this may affect growth of the bacteria. Furthermore, the compounds that are removed may have different effects on growth of bacteria, depending on the heat treatment given to milk. Hence, it is necessary to know the heat treatment given milk if one wishes to predict the behavior of starter cultures in milk. Vacuum treatment of milk also is indicated to obtain steady (and probably faster) growth of and acid production by starter cultures. This would result in more uniform and higher quality fermented dairy products.

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8th International TNO Conference Rotterdam, 27 and 28 February 1975

The Netherlands Organization for Applied Scientific Research—TNO is organizing its 8th International Conference, which will be held in the Hilton Hotel at Rotterdam on 27 and 28 February, 1975.

The TNO Conferences aim at providing a forum for R&D representatives on the one hand and senior commercial representatives of industry in the broadest sense of the word on the other hand to analyse and discuss problems re the communication between these two groups. At the same time these representatives are invited to discuss from their own standpoint problems that are facing the industry as a whole. Against this background the following theme has been chosen for 1975 Conference: EFFECTS ON INDUSTRY OF TRENDS IN FOOD PRODUCTION AND CONSUMPTION.

The following speakers will deliver lectures: Dr. H. A. B. Parpia, Senior Officer, Food and Agricultural Industries Service of the Agriculture Department of FAO, Rome, Italy; Professor Dr. Fredrick J. Stare, Chairman of the Department of Nutrition in cooperation with Dr. James Austin, Assistant-Professor in the Department of Agri-business of the Harvard Business School, Boston, USA; Mr. T. L. V. Ulbricht, Head of the Planning Section of the Agricultural Research Council, London, Great-Britain; Mr. Mogens Jul, Danish Meat Products Laboratory of the Royal Veterinary and Agricultural College, Copenhagen, Denmark; Mr. C. A. Shacklady, Group Nutrition Manager of British Petroleum Proteins Limited, London, Great-Britain; and Professor Dr. J. Boldingh, Director of the Unilever Research Laboratory, Vlaardingen, the Netherlands. Moreover, Dr. Ir. F. D. Tollenaar, Research Co-ordinator of the Organization for Nutrition and Food Research TNO, will speak on "Qualitative factors in Food Production."

Additional information on the Conference can be obtained from the Secretariat, c/o Holland Organizing Centre, 16 Lange Voorhout, The Hague, the Netherlands.

J. Milk Food Technol. Vol. 38, No. 1, Pages 25-28 (January, 1975)

Betalaines as Colorants in Dairy Products[1]

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(Received for publication July 5, 1974)

ABSTRACT

Color in foods is one of the most important attributes and is subject to great changes that are not easily controlled. Therefore, to control appearance of food, artificial dyes often need to be added. In recent years the safety of artificial dyes has been questioned and, as a result, their use in the future will be limited if not eliminated. It is for this reason that interest has developed in obtaining pigments from natural sources. The red beet is a rich source of pigments, which warrant investigation in dairy products. In addition, beet powder is permitted as a colorant under the 1960 Color Additive Amendment. The term betalaine refers to the class of pigments found in the beet which contains both betacyanines (red) and betaxanthines (yellow). The betanine content (major red pigment) in beets is in excess of 100 mg/100 g fresh weight and juice concentrates and powders available range in dye content between 0.2 and 1%. Use of betalaines as colorant and color stability of betalaines in yogurt, ice cream, and sherbet has been evaluated. Subjective and objective color values were measured and, as an example, yogurt colored with 45 ppm pigment, calculated as betanine, was judged to compare favorably with a black cherry shade.

Color of foods is an important factor in consumer acceptance. Some foods are naturally colored, but frequently manufactured foods require color addition. The number of artificial dyes available for use is small, and recent investigations into the safety of such dyes have further limited their availability. Among the red dyes permitted under the 1960 Color Additive Amendment to the Food Drug and Cosmetic Act of 1938 are FD&C Red No. 2, 3, 4, and 40. Use of FD&C Red No. 2 and 4 is severely restricted. FD&C Red No. 4 can only be used to produce maraschino cherries and the amount cannot exceed 150 ppm, while no food may contain more than 30 ppm FD&C Red No. 2 (*3*). FD&C Red No. 3 and Red No. 40 are routinely used but their physical and chemical properties restrict their application in some foods. For example, FD&C Red No. 3 is light sensitive and insoluble at low pH values, whereas FD&C Red No. 40 has an orange hue, thus making it difficult to obtain some red shades. The dairy industry uses approximately 30,000 lb of red dyes annually (*1*). With the restriction of FD&C Red No. 2, the need for additional red color clearly exists.

The red table beet (*Beta vulgarus*) is a rich source of red pigments termed betacyanines. Together with a small amount of yellow pigments, betaxanthines, these pigments comprise a class of compounds called betalaines (*5*). Of the betacyanines, betanine is the principle component, accounting for 75% to 95% of the total betacyanine content of the beet (*11*). Betanine displays a spectral maximum (A_{max}) at 537-538 nm over the pH range 4.0 to 7.0, with no color change. Below pH 4.0, i.e. pH 2.0, a decrease in intensity occurs accompanied by a shift to an A_{max} of 535 nm and an increase in absorbance at 575 to 650 nm. Above pH 7.0, i.e. pH 9.0, the A_{max} shifts to 544 nm along with a decrease in intensity. Additionally, an increase in absorbance in the regions of 575-650 nm and 400-450 nm and a marked change in color from red to violet occurs (*6,10*).

Studies have shown that in the range of pH 3.0 to 7.0 stability of betanine is greatest at about pH 5.0 and the pigment degrades following first-order kinetics (*10*). The half-life at 25 C for betanine in a model system at pH 5.0 has been calculated as 1100 ± 100 min. Betanine, like other natural pigments, is subject to degradation by air and/or light and exclusion of oxygen from the system will significantly increase the measured half-life (*10*). In model systems (glycerol-water) of low water activity, the rate of heat degradation decreases. For example, in systems with a water activity of 0.8 the rate constant decreases by approximately one-half compared to a system with a water activity of 1.0 (*7*).

Properties of foods, such as a minimal heat treatment during manufacturing, a short shelf-life, a favorable pH range (4.0-5.5), and/or a reduced water activity would facilitate using betalaines as colorants (*8,9*). Since dairy products possess one or more of these conditions, the purpose of this study is to show the applicability of betalaines as a food colorant in sherbet, ice cream, and yogurt.

MATERIAL AND METHODS

Experimental samples

Samples containing betalaines as well as commercial samples were used in all evaluations. Commercial samples were purchased at local supermarkets. To prepare experimental samples, uncolored yogurt, sherbet and ice cream mixes were obtained from the University of Wisconsin Dairy Plant. Beet juice concentrate (B-20, Chr. Hansen's Lab Inc., Milwaukee, Wis.) was added to each of the mixes. The betanine content in B-20 colorant was determined to be 0.20% by the method of von Elbe et al. (*11*). Different red shades were obtained with the addition of water-soluble annatto color containing 1.4% bixin (Chr. Hansen's Lab, Inc., Milwaukee, Wis.). The various amounts of betanine and bixin used in dairy products are listed in Table 1.

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TABLE 1. *Levels of Colorants Added to Dairy Products*

Sample	Betanine (ppm)[a]	Bixin (ppm)
Sherbet	12	—
	26	—
	20	1.3
Ice cream	26	—
	17	5.1
	26	5.1
Yogurt	36	—
	43	—

[a]Betanine and bixin added as B-20 and annatto colorants, respectively, Chr. Hansen's Lab, Inc.

After addition of the colorants, yogurt was allowed to gel; sherbet and ice cream mixes were frozen in a laboratory scale ice cream freezer (Emery Thompson Machine and Supply Co., N.Y.) with overruns of 60 and 80%, respectively. Samples were then stored; yogurt at 4 C, and ice cream and sherbet at − 20 C, until subsequent analysis.

Color Measurements

Objective evaluation. Color measurements of all products were made with a Hunter Color and Color Difference Meter (Model D25, Hunter Lab. Assoc., Fairfax, Va.) and results recorded as Hunter color reflectance values L, a_L, and b_L. The instrument was standardized with standard plate No. D25-1302 (L =74.5, a_L =12.6, b_L =7.3). The quantities $\tan^{-1}(a/b)$, $(a_L^2 + b_L^2)^{1/2}$, and $\Delta E = [(L_1 - L_2)^2 + (a^{L1} - a_{L2})^2 + (b_{L1} - b_{L2})^2]^{1/2}$ were calculated. Standard viewing conditions were used. Color measurements were made for yogurt at 0 days and after storage for 21 days, measurements for ice cream and sherbet were made at 0 days and after storage for 60 days.

Subjective evaluation. Subjective evaluations were done by the University of Wisconsin Sensory Evaluation Laboratory. Each visual panel consisted of both commercial samples and samples that contained betalaine. All samples were viewed under a MacBeth daylight-type lamp system (MacBeth Daylight Corp., Newburgh, N.Y.). Untrained panelists were asked to rate each sample on a 1 to 7 hedonic scale (1 = least desirable, 7 =most desirable). A minimum of 50 panelists participated. Mean scores, analysis of variance, and least significant differences were determined using a UNIVAC 1108 computer (2).

RESULTS AND DISCUSSION

Hunter color reflectance values and mean visual panel scores for sherbet, ice cream, and yogurt are given in Table 2. Color values for commercial samples illustrate the greatest difference within three sherbet samples, three ice cream samples, and four yogurt samples. The magnitude of these ranges indicates the wide variation of colors among commercial products.

Of the commercial samples, raspberry sherbet had the greatest color range. Total color difference (ΔE) between samples was calculated as 22.2. This difference can be attributed to differences in lightness (L) and redness (a_L). The hue $[\tan^{-1}(a_L/b_L)]$ for these samples was the same but the saturation value $(a_L^2 + b_L^2)^{1/2}$, because of the difference in a_L, was different. The more saturated $[(a_L^2 + b_L^2)^{1/2} = 43.6]$ and darker (L = 35.4) sample was preferred by panel (visual score = 4.89). These results indicate that the consumer might prefer a more highly pigmented product.

The total color difference between commercial strawberry ice cream samples was 10.0. The greatest contribution to the observed difference was the large yellow component (b_L). The presence of yellow color ($b_L = 7.3$) in one sample and the absence of yellow ($b_L = -0.3$) in the other sample resulted in distinct differences in the hue $[\tan^{-1}(a_L/b_L) = 90.7$ and 70.3, respectively]. As with sherbet, the visual panel results for

TABLE 2. *Hunter Color Reflectance Values and Mean Visual Panel Scores of Dairy Products*

	L	a_L	b_L	$\tan^{-1}(a_L/b_L)$	$(a_L^2 + b_L^2)^{1/2}$	ΔE^2	Mean scores[3] visual panel
			Sherbet (Raspberry)				
Commercial	35.4	43.5	—2.8	93.7	43.6	—	4.89[a]
Commercial	55.3	33.0	—2.6	94.5	33.1	22.2	3.73[b]
12 ppm betanine[1]	61.3	27.9	—3.0	96.1	28.1	30.0	4.22[ab]
26 ppm betanine	54.3	37.2	—4.8	97.3	37.5	20.1	3.57[b]
20 ppm betanine + 1.3 ppm bixin	57.5	33.0	—3.0	95.1	33.6	24.2	4.43[ab]
			Ice Cream (Strawberry)				
Commercial	70.1	24.4	—0.3	90.7	24.4	—	5.40[a]
Commercial	65.1	20.4	7.3	70.3	21.7	10.0	2.98[c]
26 ppm betanine	61.1	30.4	—3.9	97.3	30.6	11.2	3.04[c]
17 ppm betanine + 5.1 ppm bixin	66.0	24.3	6.0	76.1	25.0	7.5	3.74[bc]
26 ppm betanine + 5.1 ppm bixin	61.0	28.2	3.9	82.1	28.5	10.6	4.02[b]
			Yogurt (Black cherry)				
Commercial	57.9	19.9	—4.9	103.8	20.5	—	4.34[a]
Commercial	47.9	19.8	—2.2	96.3	19.9	10.3	—
36 ppm betanine	60.6	36.4	—7.2	101.2	37.1	16.6	4.43[a]
43 ppm betanine	58.0	38.6	—7.3	100.7	39.3	18.6	4.77[a]

[1]Betanine and bixin added as B-20 and annatto colorants, respectively, Chr. Hansen's Lab, Inc.
[2]ΔE = total color difference compared to sample with highest mean visual score.
[3]1 = least desirable, 7 = most desirable; mean scores followed by the same letter are not significantly different at the 1% level.

strawberry ice cream showed that the sample with the greater saturation value (24.4) was preferred.

The total color difference between commercial black cherry yogurt samples was 10.3. The observed total color difference, as well as difference in hue, can be attributed, at least in part, to a difference in the $-b_L$ value (blueness). Like other products, samples with the greatest saturation value received the highest visual panel score. Similar results were obtained with strawberry and raspberry yogurts.

Samples containing betalaines had mean visual panel scores not significantly different from commercial samples. Scores for sherbet that contained betalaine were lower but not significantly different from the most preferred commercial sample, whereas scores for yogurt with betalaine, although not significant, were greater than scores for commercial samples.

Table 4 suggests amounts of colorants to be added to sherbet, ice cream, and yogurt to obtain various shades. These concentrations are relatively low when considering that similar shades produced with artificial dyes (FD&C Red No. 2 or 40) would require a concentration ranging from 60 to 120 ppm. The absorptivities ($A^{1\%}_{1cm}$) of FD&C Red No. 2 and Red No. 40 are 460 and 580, respectively (*4*), while the absorptivity of betanine is 1120 (*12*). This means that the tinctorial strength of pure betanine is approximately twice that of artificial dyes and explains the differences in concentration.

Color reflectance values and visual preference scores of samples containing betalaines as the colorant indicate that betalaines can be used in dairy products to produce the desired red shades. In all products tested, samples contining betalaines alone had a a $-b_L$ value indicating the typical violet hue characteristic of beet pigment. As a result, to obtain colors with little or no blue hue, addition of a yellow pigment such as annatto color is needed. Presently the production of beet color is limited and therefore cost comparison with artificial dyes is difficult to assess. With increased use and the greater color strength of betanine this colorant could become competitive.

TABLE 3. *Hunter Color Reflectance Values of Dairy Products After Storage*

Sample	Storage time (days)	L	a_L	b_L	$\tan^{-1}(a/b)$	$(a_L^2 + b_L^2)^{1/2}$	ΔE
SHERBET							
commercial	0	35.4	43.5	—2.8	93.7	43.6	—
	60	36.7	48.9	—2.3	92.7	48.4	5.6
20 ppm betanine[a] + 1.3 ppm bixin	0	57.5	33.0	—3.0	95.1	33.6	—
	60	60.7	31.1	—3.6	96.6	31.3	3.8
ICE CREAM							
commercial	0	70.1	24.4	—0.3	90.7	24.4	—
	60	76.0	21.2	—0.2	90.5	21.2	6.7
26 ppm betanine + 5.1 ppm bixin	0	61.0	28.2	3.9	82.1	28.5	—
	60	65.6	26.1	2.5	84.5	26.2	5.2
YOGURT							
commercial	0	66.7	22.2	—1.0	92.6	22.2	—
	21	66.2	38.0	—0.9	92.2	23.0	0.9
43 ppm betanine	0	58.0	38.6	—7.3	100.7	39.3	—
	21	60.0	33.1	—3.8	96.5	33.3	6.8

[a] Betanine and bixin added as B-20 and annatto colorants, respectively, Chr. Hansen's Lab, Inc.

Table 3 lists Hunter color reflectance values of sherbet, ice cream, and yogurt after storage. In all instances, changes in color values were similar for commercial samples and those that contained betalaine. During storage L values increased while a_L values decreased indicating a loss in pigment. Yogurt had a slightly greater total color difference (ΔE) after storage than did the frozen products. This might be attributed to a less favorable pH and storage at refrigerated rather than freezing temperatures.

TABLE 4. *Suggested Betalaine Levels in Dairy Products*

Product	Shade	Betanine (ppm)[a]	Bixin (ppm)
Sherbet	Raspberry	20	1.5
	Cherry	12	
Ice Cream	Strawberry	26	5.0
Yogurt	Strawberry	30	5.0
	Raspberry	30	
	Black Cherry	45	

[a]Betanine and bixin added as B-20 and annatto colorants, repectively, Chr. Hansen's Lab, Inc.

ACKNOWLEDGMENTS

The authors wish to acknowledge the assistance of the University of Wisconsin Sensory Evaluation Laboratory.

Research supported by the College of Agricultural and Life Sciences, University of Wisconsin-Madison, and Chr. Hansen's Lab, Inc., Milwaukee.

Presented at the 61st Annual Meeting of the International Association of Milk, Food, and Environmental Sanitarians, St. Petersburg, Florida, August 12-14, 1974.

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Lexington-Fayette County, Kentucky, Health Department Wins Consumer Protection Award

The Health Department of Lexington-Fayette County, Kentucky, today was announced as the winner of the 1974 Samuel J. Crumbine Consumer Protection Award for outstanding achievement in public food and beverage sanitation.

The Award is presented annually by the Single Service Institute to a local health authority picked by a jury of environmental health professionals as having done the most to protect consumers in public eating and drinking establishments.

The Crumbine Award is named for the public health pioneer who, as the Kansas State Health Officer, was the first to ban common drinking cups from public facilities.

The 1974 Crumbine Award jury chose the Lexington-Fayette County Health Department as the health authority that best demonstrated outstanding growth and improvement in its food and beverage sanitation program over the past five years in accord with the recommendations made by the 1971 National Food Protection Conference.

The jury said that the Department distinguished itself as the first local health office in the United States to insitute a certification program for all foodservice workers. In the five years since the Department was reorganized to cope with increasing consumer protection concerns, more than 1,500 foodservice workers have studied for and passed the certification program test.

The jury also cited the Lexington-Fayette County Department's effective Consumer Advisory Council program.

Harry A. Marsh, Director of the Department's Division of Environmental Health, said the voluntary council is composed of concerned citizens who consult with the Department on public health needs and are active in persuading lawmaking bodies to financially support programs to meet those needs.

Marsh said the Department also meets regularly with the local restaurant association, and provides continuing education for its own professional staff.

Lexington-Fayette County also was acclaimed for its unusual restaurant grading system. All public eating places undergo at least four inspections a year, and some as many as 12 or 14, Marsh said. He explained that restaurants needing least inspection usually are "fast food" outlets where the use of disposable food service products reduces the risk of foodborne infection.

Of the County's 375 public eating and drinking places, Marsh said only about 100 meet the department's "Grade A" classification at any one time, and these are identified monthly in local news media.

The jury noted that Lexington-Fayette Country's programs are backed up by tough restaurant closure powers, but these punitive measures are only a part of the Department's progressive efforts to upgrade the sanitation standards of food and beverage establishments.

J. Milk Food Technol. Vol. 38, No. 1, Pages 29-30 (January, 1975)

A Research Note

A Sampling Device for Aseptic Portioning of Pasteurized Milk[1]

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ABSTRACT

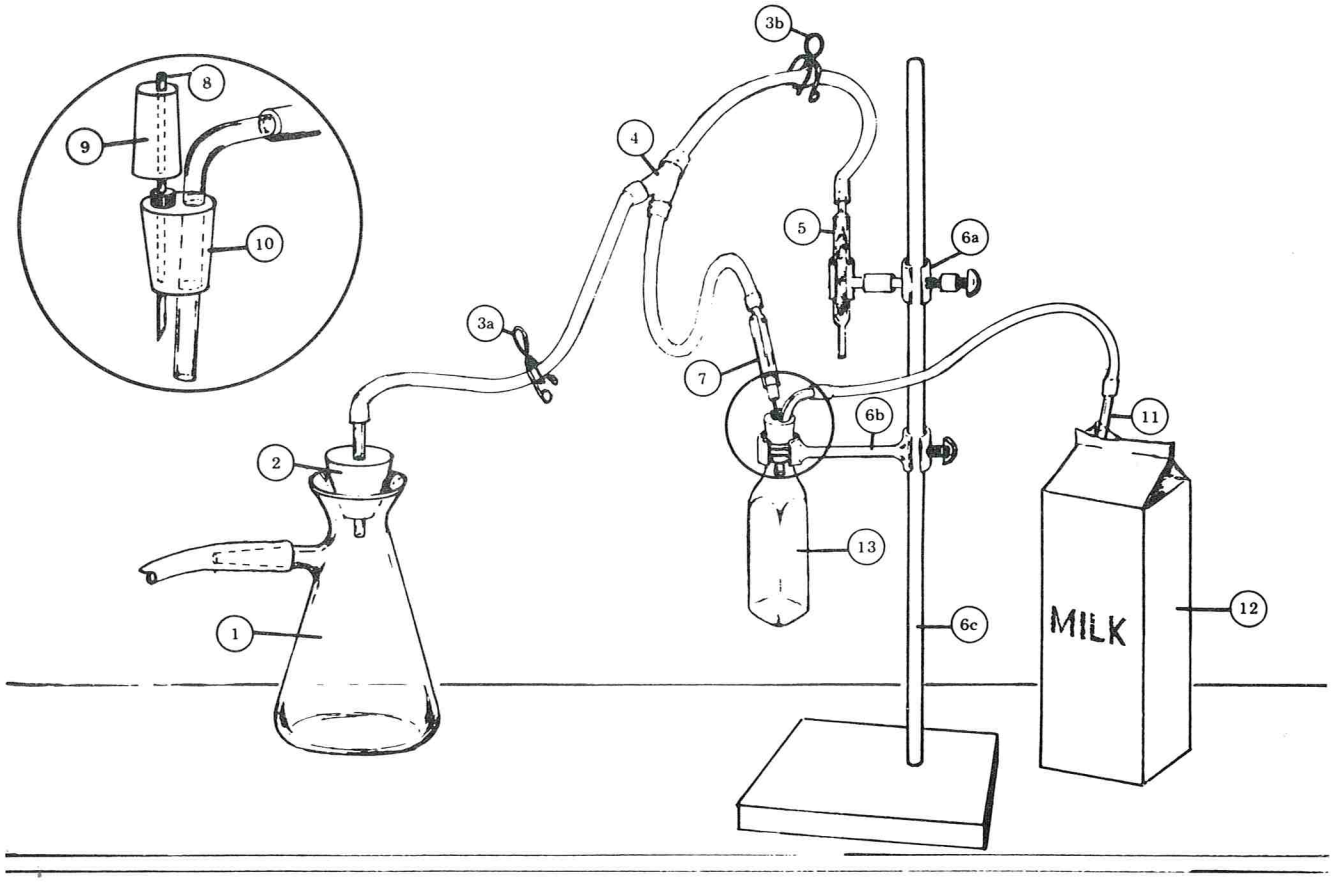
Pasteurized milk poured from its original container into smaller sterile containers showed evidence that contamination occurred during the pouring process. A simple vacuum sampling apparatus was devised to transfer, aseptically, milk from the original container to smaller containers. Uniform counts and shelf-life determinations were obtained from subsamples using the vacuum apparatus.

In early shelf-life studies, the pouring lip of a half-gallon carton of pasteurized fluid milk was sanitized with alcohol and subsamples of about 150 ml each were poured from the carton into prechilled milk dilution bottles. Erratic plate counts and flavor analyses on subsamples from the half-gallon sample indicated that contamination occurred during the portioning procedure. Use of the sampling device (Fig. 1) resulted in more uniform plate counts and shelf-life data, indicating that contamination during the sampling procedure was eliminated or at least greatly reduced.

Milk Sampling Device

The aseptic milk sampling device is shown in Fig. 1. A 1-liter filtering flask (1) closed by a size 7 rubber stopper (2) fitted with a glass tube was connected to a Y-connector (4) with surgical tubing. A pinchcock clamp (3a) was used to control the vacuum between the flask and the

[1] *Fla. Agr. Experiment Station Journal Series No. 5425.*



1 - Filtering Flask
2 - Rubber Stopper size 7
3a, 3b - Pinchcock Clamps
4 - Y-connector
5 - Cotton packed cream test pipette
6a, 6b - Burette Clamp
6c - Ringstand
7 - Pyrex adapter
8 - Bleeding needle
9 - Rubber Stopper size 00
10 - Rubber Stopper size 3
11 - Bacteriological pipette 1.1 ml
12 - Milk sample
13 - Milk dilution bottle

Figure 1. *Aseptic milk sampling device.*

Y-connector. Surgical tubing and a pinchcock clamp (3b) connected the Y-connector to a cotton-packed cream test pipette (5). An adapter (7) was made by drawing one end of a 10 cm long pyrex tube (15 mm OD) to about 7 mm. The reduced end was connected to the open arm of the Y-connector with surgical tubing. The 15 mm opening of the pyrex adapter was placed over an inverted size 00 rubber stopper (9) which had the blunt end of a California bleeding needle (76 mm × 15 gauge, Jen-Sal Labs. Kansas City Mo.) (8) forced through it. The tip end of the bleeding needle was inserted through a size 3 rubber stopper (10). The stopper contained a glass tube which was connected by surgical tubing to an inverted 1.1 ml bacteriological pipette (11). The size 3 rubber stopper (10) was tapered on the small end to make an air-tight seal with the milk dilution bottle (13). Before portioning a sample, the cream test pipette (5) and all of the apparatus between the filtering flask (1) and the inverted 00 stopper (9) was sterilized. Parts 8 through 11 were sterilized as a unit and a different unit was used for each sample portioned. All subsample bottles (13) were sterilized and chilled to 4.5 C before sampling.

Procedure

The following stepwise procedure for portioning a half-gallon sample was used:

1. Parts 1 through 7 were assembled with both clamps closed, and these parts remained in place until all of the samples were portioned.
2. After connecting the filter flask (1) to the water aspirator with heavy wall vacuum tube, the water valve controlling the aspirator was opened.
3. The sample container (12) was inverted 25 times and the pouring lip was sanitized with alcohol after the chilled milk dilution bottles were removed from 4.5 C storage.
4. The sterile unit, parts 8 through 11, was mounted on a ringstand and the pipette (11) was inserted into the milk sample (12). The adapter (7) was fitted to the rubber stopper (9).
5. The milk dilution bottle (13) was fitted to the rubber stopper (10).
6. Clamp 3a was opened and the bottle filled under vacuum.
7. Vacuum to the system was broken by closing clamp 3a and opening clamp 3b to allow air filtered through the cotton-packed cream pipette to enter the system.
8. Clamp 3b was closed.
9. Steps 5 through 8 were repeated for each subsample and the subsamples were immediately returned to refrigerated storage.
10. Before portioning the next sample, parts 8 through 11 were replaced with a sterile unit and the procedure was repeated.

Kentucky Educational Conference

The 1975 Educational Conference for Fieldmen and Sanitarians will be held February 25-26, 1975, at Stouffer's Inn, Louisville, Kentucky.

All county and state health department personnel (sanitarians, administrators and health officers) and milk and food industry fieldmen, plant managers and related service companies and university personnel are invited to attend.

The conference is sponsored by the Kentucky Association of Milk, Food and Environmental Sanitarians with assistance from the Kentucky State Department for Human Resources' Office of Consumer Health Protection, the Kentucky Dariy Products Association and the American Dairy Association of Kentucky.

The program will be broken into general sessions; food and environmental sanitarians section and milk sections.

Also, an awards luncheon will be held at the close of the meeting at which time awards will be presented to the Outstanding Sanitarians, Outstanding Fieldman and Outstanding Service Award.

Program topics to be included are as follows:
Water pollution Act—Admendments of 1972
Package Sewage Treatment Plants
Proposed Food Service Code
Air Pollution Program
Animal Disease Program
Aseptic Packaging of Milk & Milk Products
How Hot is Hot? (Cleaning Dairy Equipment)
Swimming Pool Design
Swimming Pool Operation & Safety
Kentucky's Milk Program
How to Stamp Out Culture Failures
Solid Waste Pollution
Urban Rate Control

J. Milk Food Technol. Vol. 38, No. 1, Pages 31-35 (January, 1975)

Split Defect of Swiss Cheese

II. Effect of Low Temperatures on the Metabolic Activity of *Propionibacterium*[1]

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ABSTRACT

In a preceding paper we reported that certain strains of *Propionibacterium* which grow at low temperatures are able to split Swiss cheese. The metabolic characteristics of these strains differ from those of strains unable to grow and produce CO_2 at low temperatures. The optimal pH for malate dehydrogenase activity of cell-free extracts of the low-temperature growing strains was 7.5, whereas it was 8.5 for strains lacking the ability to grow at low temperatures. Arrhenius plots of enzymic specific activity for lactate and malate dehydrogenases of cell-free extracts obtained from low-temperature growing strains showed greatest activities at temperatures below 10 C. At 15 C or greater, cell-free extracts of strains without low-temperature growth ability showed equal or greater lactate or malate dehydrogenase specific activities. Thus, enzymes of low-temperature growing strains showed greater capacities for activity at both lower temperatures and lower pH. These data support the hypothesis that such strains at low temperature are capable of CO_2 production which creates a predisposition for Swiss cheese to split when stored at temperatures of 10 C or lower.

Propionibacteria have been implicated in the development of the split defect of Swiss cheese (*7*). Certain strains are able to grow and produce large quantities of CO_2 at low temperatures. These strains seemingly have metabolic characteristics that differ subtly from non-split-forming strains.

Formation of propionate, acetate, and CO_2 by propionibacteria has been extensively investigated. The enzymes involved in the metabolic pathways have been purified and found to catalyze a wide variety of biochemical reactions (*4, 5, 6*).

To determine if identical enzymes from different strains would differ in activity at various temperatures, lactate, malate, and pyruvate dehydrogenases were studied. These enzymes play a significant role in the production of CO_2, acetate, and propionate by propionibacteria (*1, 2, 12*), and are among a large number of enzymes in the group of dehydrogenases linked to the coenzyme NAD. Lactate dehydrogenase specifically catalyzes the reversible reaction of pyruvate to lactate. Malate dehydrogenase catalyzes the oxidation of L-malate to oxaloacetic acid. The oxidation of pyruvate to acetyl-CoA and CO_2, catalyzed by the pyruvate dehydrogenase system, is indeed a very complex process and has been studied in detail (*1*). The objectives of this study were to elucidate certain differences in enzyme metabolism among strains of propionibacteria.

[1] *Journal Paper No. J-7907 of the Iowa Agriculture and Home Economics Experiment Station, Ames, Iowa 50010. Project No. 1839.*
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MATERIAL AND METHODS

Strains of Propionibacterium and cultural conditions

Strains of *Propionibacterium* were obtained from the culture collection of the Department of Food Technology, Iowa State University. All cultures were transferred daily on at least three consecutive days befor being used. The cultures were purified on sodium lactate agar and maintined in sodium lactate broth (*8*).

Growth and gas production at low temperatures

An estimate of the gas-producing abilities of various propionibacteria was determined by filling 50% cream-test Babcock bottles with sodium lactate broth to the base of the necks and inoculating at the rate of 0.2%. A paraffin and petroleum-jelly plug was placed over the broth, and the inoculated media were incubated at 3.8, 10, 21, and 32 C for 3 months, 3 months, 4 days, and 2 days, respectively. Broth turbidity was noted and the distances that the paraffin plugs moved up the necks of the bottles by the gas that was produced were recorded. Growth of the various strains of propionibacteria at low temperatures also was determined with the procedure of Park et al. (*13*).

Preparation of cell-free extracts of propionibacteria

After three successive daily transfers of a rapidly growing strain of *Propionibacterium* in glucose broth (glucose 10 g, Trypticase [BBL] 10 g, yeast extract [Difco] 10 g, dibasic potassium phosphate 0.25 g, and distilled water 1,000 ml), a 0.5% inoculum was transferred to 10 liters of gluscose broth and incubated for 24 h at 32 C. Cells were harvested by centrifugation, washed 3 times in 0.1 M, pH 8.0, potassium phosphate buffer, and resuspended in the buffer. Cell-free extracts (CFE) were prepared by disruption of the cells by repeated passage through a French press at 1266 kg/cm^2 and 5 min of maximum sonication in a 10 kc Raytheon sonic oscillator (Raytheon, Waltham, Massachusetts), followed by centrifugation of the homogenate at 37,000 × *g* at 4 C for 20 min. Protein in the CFE was determined by the method of Lowry et al. (*11*) with bovine serum albumin as the standard.

Enzyme activity

The activities of reduced nicotinamide adenine dinucleotide (NADH) oxidase and malate dehydrogenase were measured spectrophotometrically as described by Lowry (*10*) and Allen et al. (*1*).

Lactate dehydrogenase activity was determined by the formazan formation assay of Brodie (*3*) with the following modifications: (*a*) 4.8 ml of 60% sodium lactate with 95.2 ml of 0.1 M, pH 9.0, tris-HCl buffer were added to the reaction mixture; (*b*) the reaction mixtures were incubated at 5, 10, and 15 C; (*c*) the amount of formazan formed was measured by reading the absorption at 540 nm after 15 min; and (*d*) the blank was prepared by adding the acid to stop the reaction. A unit of lactate dehydrogenase activity is defined as that amount of enzyme yielding 1 μg formazan under assay conditions.

Methods used for determining pyruvate dehydrogenase activity in crude CFE were: (*a*) spectrophotometric decrease in absorption of NADH formation was determined as described by Allen et al. (*1*); (*b*) in the procedure employed to determine lactate dehydrogenase activity, pyruvate (0.5 M), coenzyme A (5 mg/ml), and TPP (0.02 M) were

substituted for sodium lactate as the substrate; (*c*) the dismutation assay described by Korkes (*9*) served to measure CO_2 liberation of 28 C in a Warburg respirameter (American Instrument Co., Silver Springs, Maryland); and (*d*) the dismutation assay described by Reed and Willms (*14*) was used to measure acetyl phosphate by the hydroxamic acid method. The mixture was transferred to optically matched cuvettes, and the color read at 540 nm (Bausch and Lomb Spectronic 88).

RESULTS AND DISCUSSION

To study the enzyme system of carbohydrate metabolism in propionibacteria, we selected a reaction that developed activity in crude CFE. This approach was followed by determination of the reacting enzyme's specific activity under various conditions of analysis. It was of primary importance to evaluate the reactions that produced CO_2 as an end product because of its primary role in this study.

An estimate of growth of *Propionibacterium* strains at various temperatures was determined as described by Park et al. (*13*). Strains of 9 of the 11 recognized species of *Propionibacterium* were randomly selected and tested for growth at 3.8, 6.8, 10, and 15 C. Of the species tested, *Propionibacterium shermanii* and *P. freudenreichii* exhibited the greatest ability to grow at low temperatures. Similar results were reported earlier by Park et al. (*13*).

An estimate is given in Table 1 of CO_2 production in sodium lactate broth at various incubation temperatures by strains of *Propionibacterium* selected for growth or lack of growth at low temperatures. Carbon dioxide was produced when growth occurred, and the quantity usually coincided with the rate of growth. Luxuriant growth and substantial amounts of CO_2 were produced by all strains at 32 C. When the temperature was decreased to 21 C, strains P-5, P-42, and P-129, selected for lack of growth at low temperatures, exhibited growth equivalent to, but lower amounts of CO_2, than that of strains able to grow at low temperatures. At 10 and 3.8 C, these same strains, P-5, P-42, and P-129 did not grow and produced no CO_2. *Propionibacterium shermanii* strains 47 and 83 separately produced greater amounts of CO_2 at 3.8 C than any strains tested.

Several techniques were investigated to determine the most efficient method for cell disruption. The greatest amount of protein was found in CFE disrupted by passage through a French pressure device and a sonic oscillation treatment. Lysozyme and sonic probe treatments proved unsatisfactory. The enzyme activity of several P-129 extracts decreased rapidly upon freezing. The pH of the suspending buffer was varied to determine its effect on the activity of P-129 CFE. A buffer pH of 8.0 was most suitable for maintaining malate dehydrogenase activity in P-129 CFE. Results in Figure 1 indicate that the *P. shermanii* 59 extract shows optimum activity at pH 7.5, whereas the *P. arabinosum* 129 extract has an optimum activity at pH 8.5. Because the activity of

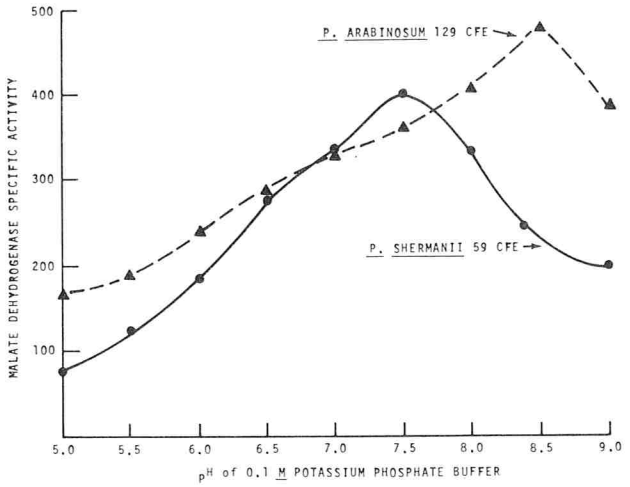

Figure 1. Effect of pH of 0.1 M potassium phosphate buffer on the specifice activity of malate dehydrogenase.

TABLE 1. *Growth and carbon dioxide production by strains of* Propionibacterium *at various temperatures of incubation.*

Species and strain no.[b]		Incubation time and temperature[a] 32 C, 2 days Growth ml CO_2[c]		21 C, 4 days Growth ml CO_2		10 C, 90 days Growth ml CO_2		3.8 C, 90 days Growth ml CO_2	
P. Shermanii	83	+[d]	2.0	+	1.0	+	1.8	+	0.8
P. shermanii	51	+	1.7	+	0.7	+	1.6	+	0.2
P. shermanii	109	+	2.0	+	0.6	+	1.0	+	0.1
P. shermanii	48	+	1.8	+	0.5	+	1.8	+	0.7
P. shermanii	33	+	1.5	+	0.6	+	1.5	+	0.2
P. freudenchii	39	+	1.4	+	0.3	+	1.1	+	0.5
P. shermanii	59	+	1.9	+	0.4	+	1.7	+	0.1
P. shermanii	47	+	1.7	+	1.0	+	1.9	+	0.9
P. pentosaceum	5	+	2.0	+	0.1	-	0	-	0
P. arabinosum	129	+	1.8	+	0.1	-	0	-	0
P. arabinosum	42	+	2.0	+	0.1	-	0	-	0

[a]Growth and CO_2 production determined in Sodium lactate broth.
[b]The first eight strains were selected on the basis of growth at low temperatures. The last three strains were selected on the basis of lack of growth at low temperatures.
[c]Volume of broth displaced on a calibrated column. All figures are averages of duplicate determinations.
[d]+ = definite growth.
- = No growth

malate dehydrogenase differs with respect to optimum pH in the two extracts, these data may indicate why one extract tends to be more stable in storage.

The presence of NADH oxidase in CFE will affect the results of other enzyme systems producing NADH. The NADH oxidase detected in all CFE tested was very low and did not significantly affect results. NADH oxidase activity was not considered to have a bearing on the results of the enzyme activities determined.

As lactic acid is oxidized to pyruvic acid, the coenzyme, NAD, is reduced. This reduction could be spectrophotometrically measured at 340 nm; the use of acetone-dried or whole cells, however, would interfere with the method. Therefore, the dye-reduction method was selected to improve the versatility of the method. The reduced coenzyme formed in the reaction will transfer the hydrogen ions and electrons to a tetrazolium dye via the action of a mediator, resulting in the reduction of the tetrazolium derivative to a red formazan.

TABLE 2. *Lactate Dehydrogenase Activity of Cell-Free Extracts of Propionibacteria at Low Temperature*

Species and strain No.		Units of activity[a] 5 C	10C	15 C
P. shermanii	59[b]	3640	7280	9440
P. shermanii	59	2000	4000	6000
P. shermanii	59	2810	5250	8380
P. shermanii	59	2960	6600	8150
P. shermanii	83	7190	10270	30000
P. arabinosum	42	3420	6000	9130
P. arabinosum	129[b]	2930	7270	10800
P. arabinosum	129	1040	1840	3440
P. arabinosum	129	840	1480	2680
P. arabinosum	129	1430	2400	3930
P. pentosaceum	5	1320	3860	4870
P. shermanii	51	1190	1710	3000

[a]Units of activity = ug formazan/mg protein
[b]Strains used to depict Arrhenius plot in Fig. 2

Table 2 shows the lactate dehydrogenase activity of CFE of selected propionibacteria at various low temperatures. Comparable values were obtained from most of the P-59 CFE. The CFE of P-83 repeatedly provided exceptionally high values of lactate dehydrogenase activity. The activity of CFE of several other strains of propionibacteria behaved much like the P-129 and P-59 extracts; i.e., low activity was obtained with P-5 and P-51 and relatively high activity from P-42 (P-129 and P-51 lacked the ability to grow at low temperatures, whereas P-42, P-59, and P-83 exhibited this ability). The P-59 CFE exhibited greater lactate dehydrogenase activity than the P-129 CFE at 5 C, whereas the reverse was true as the temperature increased to 15 C. These results were confirmed when several other CFE of similarly behaving strains were tested under similar conditions.

The effect of temperature on enzymic reactions is two-fold: (*a*) an increase in rate with temperature until maximal rate is achieved and, (*b*) a region at high temperatures in which the rate decreases with increase in temperature. Arrhenius postulated that not all the molecules in a system can react; only those molecules which have sufficient energy of activation are capable of reacting. Before a reaction may take place, a molecule of a substance must come into contact, or collide, with one of another substance. The rate of reaction will depend on the frequency of such collisions. Accordingly, the energy of activation could be obtained for rate processes in the same manner as ΔH for equilibrium processes. Since an increase in temperature will increase the rate of a chemical reaction, there must occur an increase in the rate of formation of active molecules in the equilibrium. Thus, the effect of temperature is on the equilibrium constant, where ΔH for this reaction is the heat of activation.

An Arrhenius plot of temperature dependence versus the log of enzyme activity of P-59 CFE and P-129 is depicted in Figure 2. The point at which the slope of the curves change is ambiguous because of an insufficient

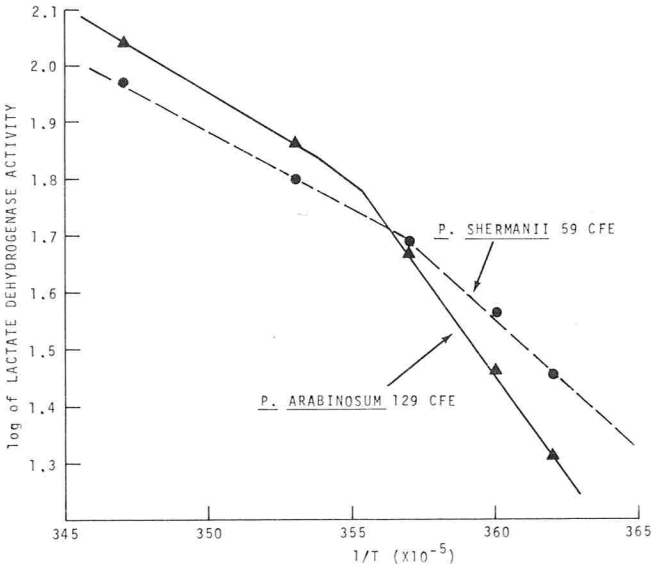

Figure 2. Arrhenius plot of temperature dependence vs. the log of specific activity of lactate dehydrogenase.

number of determinations. This change in slope may indicate that an alteration in the configuration of the enzyme occurs and influences its activity. This integer of change seems to be the rate-limiting point of the enzyme. The plot indicates that the heat of activation of the enzyme is greater at high than at low temperatures. Clearly, lactate dehydrogenase is a more effective catalyst for the reaction rate at low temperatures. The pertinent factor observed was that extracts obtained from propionibacteria that grew well at low temperatures have a greater enzyme activity when determined at low temperatures than do extracts obtained from strains that did not have this ability. Also, the results are not prejudiced in favor of a particular extract exhibiting overall greater activity. These results might be indicative of a greater ability of certain strains of *Propionibacterium* to produce CO_2 in Swiss cheese cured at low temperatures.

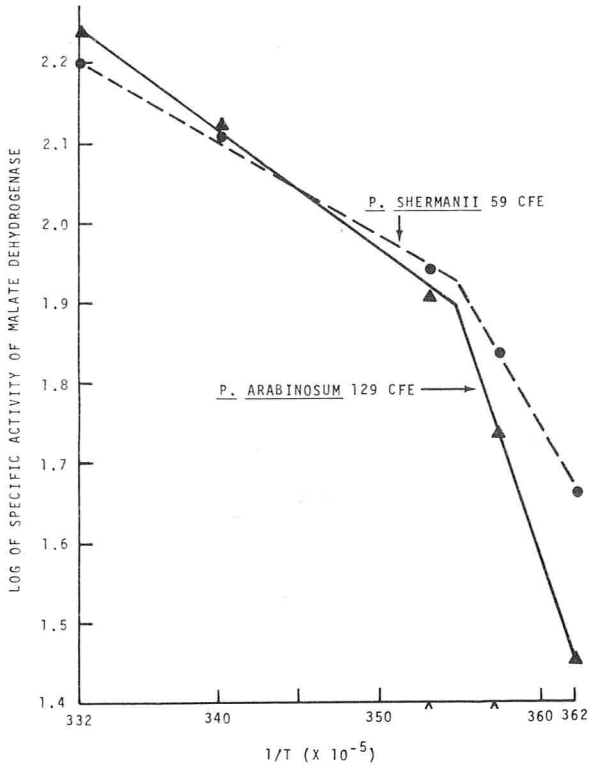

Figure 3. Arrhenius plot of temperature dependence vs. the log of specific activity of malate dehydrogenase.

Similar results were obtained when malate dehydrogenase activity was determined in extracts of P-59 and P-129 at various temperatures. Figure 3 shows an Arrhenius plot of temperature dependence versus the log of malate dehydrogenase activity of P-59 and P-129 CFE. Again, there is a possible change in the configuration of the enzyme at approximately 10 C, and the heat of activation also is greater at higher temperatures than at low temperature. Approximate calculations from the curve slopes indicate that the heat of activation at high temperature was 10,000 cal per mole and 4,500 cal per mole at low temperatures.

Pyruvate dehydrogenase is the enzyme responsible for production of CO_2 in propionibacteria. Study of this enzyme may provide insight as to the ability of certain strains of *Propionibacterium* to grow and metabolize at low temperatures.

The study of pyruvate dehydrogenase was initiated to establish activity of this enzyme or enzyme complex at various temperatures. Reed and Willms (*14*), in studying the pyruvate dehydrogenase complex in *Escherichia coli,* stated that a spectrophotometric assay, based on measurement of the rate of formation of NADH, can be applied after some purification of the enzyme complex. They found that the preparation had to be free of NADH oxidase and lactate dehydrogenase before activity could be measured spectrophotometrically. Because no activity was observed in *Propionibacterium* CFE, similar inhibitors may be present.

The dismutation assay for measurement of acetyl phosphate will indicate activity at all levels of purity in *E. coli* CFE (*14*). This method was attempted without added phosphotransacetylase; no activity was observed in *Propionibacterium* CFE. In an attempt to determine if an incorrect procedure was being followed and if the phosphotransacetylase addition affected the results, *E. coli* B, W, and K 12 CFE were prepared and tested by the method. *Escherichia coli* B and W CFE exhibited the presence of a slight amount of acetyl phosphate being produced. These results, though inconclusive, indicated that the procedure followed was correct, and that the addition of phosphotransacetylase was improtant to obtain greater activity in *E. coli* CFE. But Allen et al. (*1*) showed that phosphotransacetylase exists in CFE of *P. shermanii,* therefore, it should not be necessary to add this enzyme to our tests. This supposition, however, does not account for interference by inhibitors, inactivation by environmental conditions, feed-back inhibition by intermediates and products, and (or) the many other factors influencing activity.

Manometric determinations of CO_2 production by *Propionibacterium* CFE were negative. By the procedure employed, no CO_2 was liberated by highly active extracts of P-59, P-83, and P-129. Manometric measurements of CO_2 from *E. coli* B whole cells and CFE were negative, suggesting that the procedure employed in this laboratory was incorrect.

To determine if an alternative pathway for CO_2 formation existed differing from that pathway described by Allen et al. (*1*), several other substrates were substituted for pyruvate. When phosphoenolpyruvate and dihydroxyacetone phosphate were added to the test (or reaction) mixture for manometric measurement of CO_2 liberation and acetyl phosphate production, no activity was found.

The results on enzymic studies indicated that extracts from strains of *Propionibacterium,* considered as cheese splitters, had greater metabolic capabilities at lower pH and lower temperatures than did extracts of strains considered to be nonsplitters. These results support the hypothesis that certain propionibacteria are able to produce CO_2 at low temperatures and, thus, create the predisposition to split Swiss cheese.

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Ultrafiltration Concentrates Egg White

Better egg white concentrate for bakers and candy makers can be produced by using a process called ultrafiltration to remove as much water as possible before drying. Such processing is less expensive and requires less energy than other ways of doing the job, such as spray drying and freeze drying.

That's the conlcusion reached by three University of Wisconsin-Madison researchers who have recently studied the technical and economic aspects of the process. The researchers are R. Edward Payne and Charles G. Hill, Jr., of the UW College of Engineering, and Clyde H. Amundson of the College of Agricultural and Life Sciences.

Presently, egg white is usually concentrated by spray drying. This has two limitations—it costs more to remove water than it does by ultrafiltration, and the process causes some minor damage to the protein. For example, the spray-dried powders don't whip up very well when they're reconstituted. Freeze drying is even more expensive than spray drying.

Still, bakers and candy makers would like to use powdered egg white because it is easier to package, transport, and store than liquid egg white.

Research a few years ago showed that membrane separation techniques could produce a good egg white concentrate. With such techniques, the food being processed is pumped through a membrane which holds back some molecular species and lets other species pass through.

There are two types of membrane separation—reverse osmosis, in which the membrane lets through only the water, and ultrafiltration, which lets through some of the smaller dissolved species along with the water. This is the technique which looks most promising for egg white because it causes less damage to the protein and requires less pressure during processing.

The research provided several technical suggestions for operation of an ultrafiltration process for concentrating egg whites.

The scientists also made economic studies, based on a plant with 250,000 pound daily capacity. These showed that ultrafiltration would cost around 0.2 cents per pound of water removed, compared to a cost of nearly one cent per pound by spray drying. Freeze drying costs 7 to 15 cents per pound of liquid removed.

Thus, the researchers say that it's economically attractive to use ultrafiltration to obtain a product containing about 25 percent solids, then to follow up by spray drying or freeze drying it down to 3 percent moisture. Liquid egg white contains about 12 percent solids, so the ultrafiltration takes out about half the water.

Spray drying, even with high temperatures, doesn't injure the proteins in the egg white already concentrated by ultrafiltration.

Ultrafiltration can't do the whole drying job by itself because the material becomes harder to pump as it thickens, and the membranes don't work with viscous material.

J. Milk Food Technol. Vol. 38, No. 1, Pages 36-38 (January, 1975)

Ultra-Pasteurization of Whipping Cream

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(Received for publication June 19, 1974)

ABSTRACT

Ultra-pasteurization of whipping cream in the United States is now synonymous with commercial sterilization. Correct composition of whipping cream is essential to comply with the standard of identity and to produce a product with desirable qualities. Careful selection of the ultra-high-temperature (UHT) processing system is helpful to offset unwanted characteristics in the finished product. Functionality of various stabilizers and emulsifiers in the product is described. Homogenization of the product during UHT processing helps improve its physical stability. A long shelflife whipping cream with improved physico-chemical stability but containing no foreign additives can be produced commercially. Some of the physical conditions for whipping cream such as its cold storage, whipping time, and temperature are necessary to assure consumers of its peak performance.

Americans are known throughout the world for their pragmatism. When commercially sterilized dairy products made their debut in the American market, many regulatory agencies, dairy processors, and distributors began debating about the heat treatment, shelflife, labeling, etc. of these products. More than anything else labeling was subjected to nationwide controversy until recently when the FDA, after much deliberation, stipulated that all commercially sterilized milk products must be labeled as ultra-pasteurized.

Processors engaged in pasteurization of milk products claim that whipping cream is the most troublesome product. Regular pasteurized whipping cream is a product that turns over slowly, and this results in a consistently excessive amount of returns. In New York city, for example, pasteurized whipping cream is required to be sold within 66 h after its production.

The significance of the ultra-pasteurization process for dairy processors, distributors, and consumers has been reported elsewhere (*1*). Application of ultra-pasteurization in the dairy industry has, by and large, extended the shelflife of one of the most perishable milk products, i.e. whipping cream. Processing conditions for whipping cream with extended shelflife are somewhat different from those of its regular analogue. Aggarwal (*4*) has discussed, in general, the fundamental requisites for ultra-pasteurization of milk products. However, some of the factors involved in the commercial application of ultra-pasteurization to processing of whipping cream are outlined here.

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COMPOSITION OF THE PRODUCT

Initial composition of a dairy product is very important for its desirable performance in the consumer's hand.

Fat

It was experimently established (*2*) that cream containing as low as 18% butterfat could be made to whip. Nonetheless, the standard of identity requires a minimum of 30% milkfat in light whipping cream and 36% milkfat in heavy whipping cream. Many processors of whipping cream lable this product as "cream for whipping." But there is no standard of identity for a product named "cream for whipping." It could, therefore, imply that any cream with a milkfat content of 18% or more, and which is whippable, could be called cream for whipping!

Protein

A minimum of 2.30% protein was found (*2*) essential to achieve a good standability (stiffness in overrun) and performance in a whipping cream. If, for instance, a light cream is prepared from freshly separated 30% milkfat cream it contains 2.50% protein and needs no protein supplementation. On the other hand, if light or heavy cream is prepared from 36% or 40% milkfat cream the product has to be fortified with protein because there is only 2.0% protein in 40% milkfat cream and 2.20% in 36% milkfat cream. The protein content in whipping cream may be raised by addition of a calculated quantity of nonfat dry milk, condensed milk, or fresh skim milk. New York City, for one, permits standardization of cream by addition of only fresh skim or whole milk.

Solids-not-fat (SNF)

Protein content, however, is a part of the SNF but total SNF in whipping cream was found contributory to its final performance (*2*). A minimum SNF content of 6.25% is needed for optimum performance of whipping cream. Freshly separated 30% milkfat cream contains just enough SNF. Heavy cream made from 36% milkfat cream will need its SNF content raised by 0.50%. Cream with 40% milkfat contains 5.35% SNF. Therefore, the SNF content in a light whipping cream must be increased by 1.0%. The SNF content in whipping cream may be increased by addition of a calculated quantity of nonfat dry milk, condensed milk, or fresh skim milk. Again,

New York City is an example of a community which permits standardization of cream by addition of only fresh skim or whole milk. It may be pointed out here that the SNF in whipping cream should not be raised with commercial grade lactose because an excessive lactose addition could delay development of the peak overrun in the product.

EQUIPMENT

The type of ultra-high-temperature (UHT) processing equipment has much to do with the properties of finished milk products. Whipping cream that is ultra-pasteurized with a direct heat-exchange UHT system is more susceptible to "oiling off" than is its counterpart processed with an indirect heat-exchange UHT system. Hence, different types of equipment are designed for processing of different milk products. Since there are several suppliers of equipment in this country one frequently hears that a given UHT system is the best on the market. Some of the reasons given are: absence of scaling of heating surface, better regeneration, high product velocities, longer operation, etc. This is not always true because the scaling problem is common to all UHT systems. However, in light of this, design engineers must attempt, first, to minimize the deposit and second, to accommodate it within the system. Regenerative heating and cooling vary with the design of the UHT system as reported earlier (*3*). There is no scientific evidence that high velocities can avoid fouling—on the contrary very high product velocities provide conditions for physical removal of scale with subsequent risk of grittiness and sediment in the product. There is no large capacity commercial UHT system yet available which can operate efficiently and continuously for 12 h per day without an itermediate clean-up.

PROCESSING

The quality of raw ingredients determines the quality of finished ultra-pasteurized products.

Mixing of ingredients

A raw 40% milkfat cream, if used, should be stored below 40 F and must not be allowed to reach an acidity beyond 0.10% lactic acid. It must be agitated slowly for a few minutes before pumping or drawing out of a holding tank. If ingredients are mixed between 80 and 130 F, cream must be added last and a minimum holding time must be allowed. Longer holding of product mix at this temperature could induce lipolysis and also could encourage growth of heat resistant bacterial spores. Longer holding of product mix at an elevated temperature also can impair the whipping quality of the finished product. Stabilizers and emulsifiers must be added at appropriate temperatures so they can dissolve satisfactorily to have adequate affinity with fat and protein in the product.

Additives

The type and amount of additives for use in dairy products depend on the kind of product, its composition, its end usage, etc.

Emulsifiers

These are surface-active chemical compounds with a complex molecular structure. Their main function in an emulsion is to prevent two immiscible liquids from separating. They are used in food products as such or in combination with stailizer. Since emulsifiers are both lipophilic (fat-loving) and hydrophilic (water-loving) their selection for application in the food industry depends upon the water:fat ratio in products. Performance of emulsifiers is governed by water:fat solubility which is expressed by HLB (hydrophilic-lipophilic balance) numbers ranging from 1 to 20. Thus, in a whipping cream containing 30% milkfat the water:fat ratio is 70:30; when converted to the 20 point HLB scale this 14:6 ratio would be expressed as a HLB number of 14. Therefore, any emulsifier which is about 60 to 70% (HLB 12 to 14) soluble in water can bring maximum emulsion stability in whipping cream with either 30% or 36% Mono- and diglycerides are the most common emulsifiers used in whipping cream.

Stabilizers

These are used mostly to control the behavior of water in a variety of food products. Stabilizers are usually derivatives of natural products or products of animal origin. The hydrophilic and rheological properties of stabilizers are helpful in their selection for various dairy products. An emulsion stabilizer is necessary to improve viscosity, overrun, and degree of seepage, and to increase the stability of whipped cream. Acidity or pH value of the product is of significant importance in the functionability of stabilizers. Sodium alginate in conjunction with emulsifiers is the most common of all stabilizers used in whipping cream to provide long-term stability, fine particle size, and desirable rheology to the product. Sodium alginate is not easily soluble in the cold product, and so must be handled by trained plant personnel and added to the product at the right temperature. Gelatin is another stabilizer which sometimes replaces and other times is combined with sodium alginate for use in whipping cream. The Bloom value of gelatin has little or no effect on whipping properties of the finished product. One of the main features distinguishing sodium alginate from gelatin is that the latter drastically cuts down the amount of seepage in whipped cream during its subsequent storage. Gelatin may be added to the product at any temperature but must be allowed to imbibe some water before heating otherwise it will not dissolve satisfactorily.

Chemicals

The whippability of a whipping cream may be increased by hydrolyzing the raw cream from which it is made. Hydrolysis of raw cream is caused by addition of sodium or calcium hydroxide (food grade) so as to raise the pH of the cream to 9.5-10.0. At this pH value

hydrolysis proceeds so far that an albuminous product with a good whipping capacity is produced.

Because of recent advances in chemical technology an organic based chemical called triethanolamine has become available. It may be added instead of or together with alkaline compounds to produce dramatic improvement in whipping qualities of cream.

Addition of the chemicals just discussed to whipping cream is not widely practiced by the American dairy processors. Local regulatory agencies must be consulted before resorting to application of additives in dairy products.

Acidification

Acidity of cream may be raised quickly by addition of either commercial lactic acid or viscogen. In certain areas, New York, for example, no additive whatsoever is permitted in whipping cream. Therefore, these chemicals to raise acidity would be construed as foreign additives in the cream. However, acidity of raw cream may be increased by culturing it with lactic acid starter grown in fresh skim milk to 1.0% acidity. A calculated quantity of such a starter is added to raise the acidity in cream to at least 0.13% lactic acid just before ultra-pasteurization of the product. The time-temperature combination employed in commercial ultra-pasteurization of whipping cream readily destroys the starter organisms. This slight increase in cream acidity before ultra-pasteurization affects its casein and albumin. This chemical reaction enables these proteins to form a gelatinous consistency which is necessary to entrap air while the cream is being whipped. More than 0.16% lactic acid could be detrimental to the physical stability during subsequent longer cold storage of the product, and also could impart a "culture-like" flavor to the whipped product.

Homogenization

Whipping cream is a dairy product with a high fat content. An ultra-pasteurized whipping cream may be stored for weeks before it reaches consumers. Calcium-phosphocaseinate in the product could become unstable because of gravity after long storage and forms deposits at the bottom of a container which could cause product separation. Homogenization of whipping cream during its UHT processing seems necessary to preserve physical stability, and to reduce significantly product separation during long storage. Homogenization before final UHT processing requires a higher pressure to avoid product separation but higher pressure could affect whipping qualities. Better results are achieved by homogenizing after final UHT heat treatment. This requires an aseptic homogenizer or homogenizing valve to eliminate post ultra-pasteurization contamination. A homogenization pressure to 150 psig is recommended for whipping cream, and a pressure above 200 psig could produce undersirable properties in the finished product.

UHT heat treatment

Cream contains a somewhat larger number of bacteria than milk from which it is separated. Since whipping cream is one of the costlier milk products, a staggering loss could result if only slight carelessness is exercised in its processing. It is, therefore, advisable, just to be on the safe side, to ultra-pasteurize whipping cream at a slightly higher temperature than that used for other milk products. Many UHT systems have two operating capacities, i.e. a system can process either 350 or 700 gal per hour. Whipping cream should always be processed with equipment operating at full capacity. At less than full capacity product velocity is greatly reduced and its holding time at the final heating temperature is doubled. This slowdown of product in the UHT heating system could seriously affect product quality. The product must be cooled to below 40 F before packaging.

PERFORMANCE CONDITIONS FOR WHIPPING CREAM

There are many physical conditions which could cause an excellent whipping cream to fail in its expected peak performance if handled improperly by the customers. Whipping cream, immediately after ultra-pasteurization, possesses a slight cooked flavor and therefore, must be stored cold for 24 to 48 h to allow for the oxidation of sulfhydryl compounds that may have been produced during the heat treatment. Any rise in temperature above 40 F could not only cause churning of whipping cream but also protein dehydration which results in poor air entrapment during whipping of cream. To obtain optimal results a cold whipping cream, after some vigorous shaking, should be poured into a chilled bowl. It should then be whipped with an electric hand mixer at the "whip" setting. A good whipping cream should come to its maximum overrun within 1.5 min and if it fails to do so in 3 min it should be considered as unsatisfactory.

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J. Milk Food Technol. Vol. 38, No. 1, Pages 39-43 (January, 1975)

Impact of Vegetable Proteins on Dairy Products

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(Received for publication September 4, 1974)

ABSTRACT

The technological status of research trends directed toward creation of dairy product analogues based fully or partially on vegetable protein raw materials is reviewed. Particular emphasis is given to soya protein-based milk and cheese analogues. Nutritional and organoleptic properties of the analogues are critically discussed. Continued research activity is required by product developers to reach consumer satisfaction, nutritional adequacy, and legal approval for milk and cheese analogue products.

The food protein raw material supply and demand balance in the past year clearly indicated that neither we in the U.S., nor the rest of the world can any longer depend entirely on the traditional protein sources for human nutrition. The short supply of animal proteins has pointed toward the direction of more rational use of vegetable proteins as supplements or partial replacements of animal proteins in foods.

It is also expected that tastes of people for their protein-rich foods of animal origin is not going to change radically, the preference will likely be, as before, for meats, dairy products, eggs, etc. Therefore, if food demands are to be met by food producers, traditional products must be made available essentially in their commonly accepted form at a high level of nutritional quality and organoleptic appeal.

Current business trends show that red meats can be extended to consumer satisfaction with properly designed soy proteins. It is expected that with further research efforts extending dairy products and creating new vegetable protein-based dairy analogues seem to be rational objectives.

The purpose of this paper is to review the technological status of worldwide research trends directed toward creation of dairy product analogues based fully or partially on vegetable protein raw materials. Information to be reviewed is organized in two sections: vegetable milk analogues and cheese analogues. The "filled milk" product will not be considered in this paper. In the course of the review, step-by-step recognition of product properties and nutritional or organoleptic deficiencies of the respective dairy analogues will be critically discussed. Methods leading to product quality improvements are evaluated in the perspective of their organoleptic acceptability.

It is concluded that the major problem with the soya-based dairy analogues is off-flavor. Some of the flavor constituents can be removed or reduced in intensity, but there is still an undesirable flavor left. In these situations, flavor masking is often attempted. But this approach has serious inherent limitations which prohibits general applicability.

Continued research activity is required by product developers before consumer satisfaction can be anticipated at a level believed to be adequate for potential commercial success. Moreover, governmental laws or regulations may require that the vegetable analogues of the dairy products be positioned on the market with full disclosure of the nature of their origin, composition, and nutritional equivalency. This principle should be observed also when the vegetable protein sources are used only as extenders at a 10-30% addition level.

This review will consider as vegetable milk analogues, beverages made similar to bovine milk, using predominately plant raw materials such as soybeans, peanuts, or coconuts, for protein sources.

MILK ANALOGUES BASED ON SOY PROTEIN

The earliest attempts to produce a soya milk are reported in the Western literature in a German patent issued to Gossel and published in 1911; this was followed by other patent applications in Great Britain and in the U.S. Dehulled soybeans were soaked in water, mashed or milled, and the slurry filtered. Nutritional fortification of the soya milk analogue was considered and nonfat milk powder, calcium salts, and vitamins were suggested for use. Flavor improvements were thought to be necessary and proteolytic enzymes were suggested for this purpose in 1921. In the next decades, debittering trials were reported by Japanese and German sources.

The infant food industry moved in the direction of soya milks to supply infants allergic to cow's milk with nutritionally acceptable substitutes. Further work along the same lines, but directed toward mass feeding of infants by UNICEF in Indonesia, was done in the 1950's. Large scale production of liquid soya milk was carried out in the Far East in numerous locations in 1965. Dried soya milks were marketed in Japan (*40*).

In the U.S., Hand started soya milk research in the early 1960's (*25,26,27*). An acceptable bland soya milk analogue was produced by Wilkens during this period (*48*). Wilkens ground dehulled soybeans with water

between 80-100 C and maintained the beans at this temperature for 10 min to completely inactivate the lipoxidase enzyme. This development was adapted to the Philippine soya milk market by Steinkraus and co-workers from the New York State Agricultural Experiment Station in Geneva (*37*).

The Geneva group identified the chemical nature of the disturbing flavor by means of chromatographic methods. The status of soya milk technology, processing, economics, nutrition, and organoleptic properties are summarized by Bourne for a United Nations sponsored project (*11*). The mechanism of protein insolubilization in dried soya milk preparations was studied by Fukushima (*17*). This was followed by extensive process studies conducted by Khaleque et al. (*21*). Their findings indicated that it is possible to produce a sterilized fluid canned soya milk concentrate containing up to 15% solids (*21,45*). Removal of flatulence-causing oligosaccharides from soya milks was proposed by Sugimoto (*39*) who used α-galactosidase and invertase enzyme preparations obtained from *Aspergillus saitoi.* Chien treated soybeans with yeast to hydrolyze these oligosaccharides (*13*).

Growth and activity of lactic acid bacteria in soya milk was investigated very thoroughly by Angeles and Marth (*1,2,3*). If soya milk is to serve as a base for production of cheese-like products, these studies give valuable basic information not only on lactic acid formation, but lipolytic and proteolytic enzyme actions were also considered during these investigations.

The most significant soya milk commercial venture was the introduction of a heat sterilized, bottled soya milk under the trade name "Vitasoy" in Hong Kong by Lo. This project was the first successful commercial effort for promotion of soya milk. Results were satisfactory from both financial and nutritional points of view (*34*).

Several patents have been issued on the heels of the commercial successes of "Vitasoy." Muskatas proposed wet milling of full fat or defatted soya flours to 5-40μ size and homogenization of the slurry (*32*). A dry product is recovered by spray drying. It is claimed that by suitable formulation with fats and sweetners, highly nutritious drinks can be obtained having flavors which are acceptable to various cultural groups.

A patent by Arndt claims a nutritious substitute for dry skim milk as a food additive (*9*). The product is made by dry blending a vapor flash treated, deflavored soya protein with dried cheese whey solids. In a second patent, Arndt described preparation of a soya protein and vegetable fat containing whole milk analogue (*10*).

Wagner recommended a process for preparation of milk analogues by comminuting soya beans or other legumes in the presence of edible acids. Vapor phase chromatographical results are cited which indicate a reduction of off-flavor compounds (*41*).

A team headed by Steinberg made soya milks based on a new technology. In this process, undesirable flavor development is prevented by a rapid blanching operation (*47*). The authors have shown that the "beany" off-odors and -flavors are not inherent in the beans, but are induced by enzymes when ruptured cells are moistened. A simple heat treatment of beans before crushing was found to inactivate the enzymes, and, thereby, prevented formation of undesirable flavors. Steinberg's group is aiming for a dry milk analogue which would be easy to ship anywhere in the world where bovine milk is hard to come by.

Commercial steps were announced in 1973 for production of soya protein-based dry milk replacers for industrial use (*7*) and for production of "soya milk" (*8*). Nutritional properties of whey and soy protein combinations have been investigated and found to be supplementary, resulting in high protein efficiency blends (*14*).

The soya milk area was reviewed at the World Soy Protein Conference in Munich, Germany (*35*).

MILK ANALOGUES BASED ON PEANUT PROTEIN

Use of peanut proteins for preparation of milk analogues dates back to 1950. This early work is reviewed by Swaminathan (*40*). Large scale development work followed using recommendations of the Central Food Technological Research Institute, Mysore, India. This group saw in peanut milk an excellent extender for the fat-rich buffalo milk available in India.

"Miltone," a toned peanut protein milk product was placed in the highlights of the Institute of Food Technology Award in 1971 (*6*). The Central Food Technological Research Institute of Mysore, India was honored for developing a new dairy product that could double the supply of milk for the children of India. For this process, hydrogen peroxide treatment of the protein isolate was used to eliminate the aflatoxin that could be present in ground nut protein. "Miltone" has all the properties of milk and could be used in the same way as milk.

MILK ANALOGUES BASED ON COCONUT PROTEIN

Coconuts as raw materials for milk analogues have been brought into focus at the Tropical Products Institute by Dendy and Timmins (*15*). Traditionally, coconut is dried to produce copra and the oil is then obtained from copra by expression or solvent extraction methods. The residual product containing 18-25% protein is too fibrous for human food use; consequently it is sold as an animal food supplement.

With the increasing concern over the world food supply, considerable international attention has been directed towards the possibility of the utilization of coconut protein as a source of human food. The possible routes of utilization are cocnut milk, coconut cream, and a coconut protein isolate. However, to obtain these products a wet coconut milling process had to be developed which differs essentially from the copra route.

Steinkraus (*37*), under the sponsorship of U.S.AID, conducted development of flavored soya milks and

soya-coconut milks for the Philippine market. The paper reports of flavor studies, with taste panels composed of Filipino children; it also presents the best technology for making soya milks known at the time. It was found that unflavored soya milks were unacceptable in flavors to the majority of Filipino taste panelists.

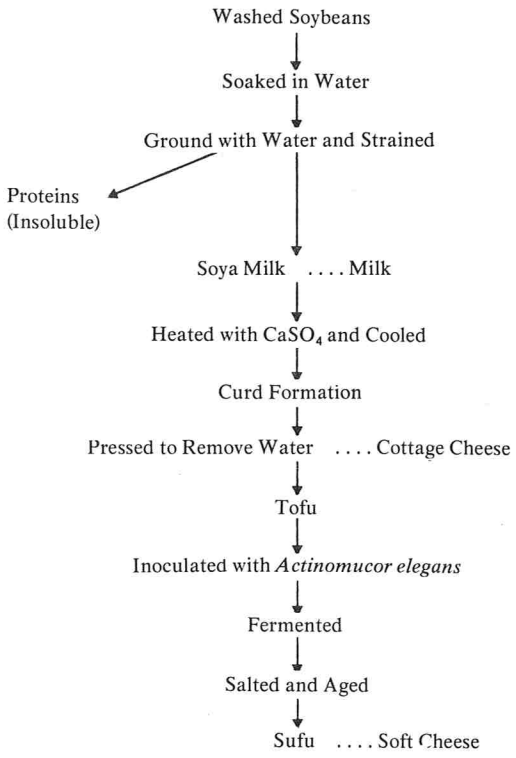
The children themselves suggested raising the sugar content. When the sugared soya milks were flavored with vanilla and chocolate syrup the acceptance was increased to 96% level. Addition of coconut milk also generally increased acceptability of the soya milks. The protein fraction of the coconut milk coagulates on heat exposure. This makes it impossible to produce a sterilized coconut milk, as the product is no longer fluid. However, when coconut milk was combined with soya milk, the product no longer showed any visible coagulation and the milk remained liquid. Hagenmaier published on the food value and functionality of dried coconut milk. A 12% solution of coconut skim milk is an acceptable beverage (*18*).

OBJECTIVE SENSORY METHODS

The potential for protein fortification of human diets is obviously an important public and technological issue. Milk-based products as protein supplements have proven value and acceptability. Recently, considerable attention was given to use of vegetable protein sources for competitive use with milk proteins. The major asset of milk protein materials has been their bland flavor, whereas, the major limitation with most non-milk protein supplements has been their unfamiliar or objectionable flavor characteristics. Objective chemical and sensory methods have been proposed by Maga and Lorenz for the study of this question to be able to standardize acceptability limits, or point out specific flavor differences (*30,31*).

CHEESE ANALOGUES BASED ON SOYA

The historical precursor of the vegetable-based cheese analogue is the Chinese food "sufu" which dates back to the era of Hang Dynasty (179-122 B.C.). Sufu is a soft cheese-like product made from cubes of soybean curd (tofu) by the action of molds. The analogous relationships between the Western dairy cheeses and the Far Eastern soy based sufu is best shown in a diagram (*20*).



In this diagram, we see that the soya beans when ground and cooked yield a soya milk. This milk can be coagulated to form a protein and fat containing curd in analogy to cottage cheese curd. The soft, bland, perishable curd will be transformed by mold fermentation (*Actinomucor elegans*) and brine soaking into the "sufu" which is a soft cheese analogue (*20*).

The process of making "sufu" in the Far East was considered a natural event to be carried out in the household, just like bread baking was in Western civilization. The technological inquisitiveness of the scientific mind did not touch this area for thousands of years. However, it was investigated during the period of 1929-1959. The investigations of Wai, from Taiwan, (*42*) described the sufu process in terms of chemistry and microbiology. Based on these fundamental findings, joint Far Eastern and Western efforts were started for creation of a soya based cheese analogue (*46*).

It is interesting to note that the temporary cottage cheese shortage during World War II resulted in the idea of substituting tofu for cottage cheese (*4*).

In Western food science, interest in making cheese analogues from vegetable sources, mainly from soybeans, gained some momentum during the middle 1960's (*19,19a*). However, in Japan, significant patent activity was recorded on the subject between 1938-1966. Use of *Neurospora* molds and enzymes derived from them were claimed in a Japanese patent (*38*).

Starting in 1967, using funds provided under Public Law 480, the U.S.D.A. supported Chinese and Japanese scientific groups engaged in soya cheese analogue research. Close cooperative activity was carried on between Hesseltine's group in the U.S., Wai in Taiwan, and Obara in Japan.

These efforts resulted in application of *A. elegans* and also a wide variety of proteolytic enzyme preparations in the soya cheesemaking process, in addition to use of lactic starter cultures (*33,43,44*).

At the University of Alberta, Schroder and Jackson continued the soy cheese analogue work both for texture and flavor improvement. However, beany and bitter flavor defects were observed in all the soy cheese analogues (*36*).

A U.S. patent issued to Lundstedt and Lo, reports on preparation of a soya curd blue cheese which was fortified with milkfat and nonfat milk solids. Inoculation was with *Penicillium roqueforti* and *Streptococcus diacetilactis.* After two weeks of curing, the cheese is claimed to possess the appearance, texture, and spreadability of blue cheese. A further patent by the same authors describes preparation of a heat-stable, meltable cheese analogue made of soya milk, milkfat, and nonfat milk solids (*28,29*).

Burkwall (*12*) claims a high protein simulated cheese product prepared from specified amounts of natural cheese, pregelatinized starch, a high protein binding agent (preferably soya protein), water, and sugars. The mixture is heated up to 195° F and extruded into the form of small strands. A similar product in semi-dry form was discussed by Dietz (*16*).

CHEESE ANALOGUES BASED ON OTHER VEGETABLE SOURCES

An Indian food research group associated with the Central Food Research Institute in Mysore did development work with peanut protein-based cheese spread analogues in 1968. As a bread spread, the product was quite acceptable, and is considered to be of value in those countries where peanut protein foods are commonly used.

This work was continued by the same authors up to 1971. The peanut protein-based cheese analogues, resulting from this extended research, was approaching cheese-like texture (*23,24*).

The 1967 Report of the Tropical Products Institute describes work on fermentation methods of cassava to improve its nutritional value. Cassava is widely cultivated in the tropics. It is high in starch, but has practically no protein. Tropical Products Institute (TPI) has examined microbiological methods for enrichment of proteins in the cassava cheese analogue. The TPI "vegetable cheese" product is a cake of cassava dough fermented with *Rhizopus stolonifer* (*5*).

CONCLUSIONS

"It is still hard to visualize"—writes prof. Kosikowski of Cornell University (*22*) "that in about 30 years enough inexpensive protein beverages can be produced to fill the protein needs of our expanding population, but one dreads the consequences if the goal is not reached. By the year 1990 we will need all the protein and calories that can be obtained through natural and unconventional food sources—whether plant food or animal foods are more desirable, or vice versa." Logically, in the face of foreseeable needs, we are going to need both the genuine and the analogue milks and cheeses. Their market positioning and their roles in the dietary patterns of various subcultures might be different. In any event, the analogues should possess adequate nutritive values commensurate with their dietary role, have satisfactory functionality when used in manufactured food formulations, and be aesthetically acceptable in the form offered for human consumption.

The big markets of the world are heavily interdependent today. The more vital roles the products play in human life, the more powerful are going to be the reactionary forces of adjustments if shortages, or inbalances are encountered. Dairy products are foods and foods are the most vital items for humanity. The future of the dairy industry is interconnected with the global food supply and demand situation. There is a great opportunity, as well as, obligation for the dairy research to contribute in a meaningful manner to the solution of the balanced food supply of the world population.

ACKNOWLEDGMENTS

This paper was presented in part at the Educational Conference for Fieldmen and Sanitarians, Louisville, Kentucky, February 26, 1974; and the 29th Annual Meeting of the American Dairy Science Association, University of Guelph, Guelph, Ontario, Canada, June 25, 1974.

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J. Milk Food Technol. Vol. 38, No. 1, Pages 44-51 (January, 1975)

Effect of Milking Machine Design and Function on New Intramammary Infection

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(Received for publication August 19, 1974)

ABSTRACT

Vacuum fluctuation at the teat end is a common cause of increased udder infection. Milking system design must incorporate large-bore milk and vacuum conveying systems that permit unobstructed air flow through an efficient vacuum pump. Effective control of static vacuum is essential.

In the past, milking machine (MM) design and function have been changed on an emperical basis (depending on experience or observation alone). Most of these changes have improved milk quality, increased milking rate, increased milk yield, resulted in more complete milking, and reduced labor costs. From the standpoint of labor economy, 15 inches (38.1 cm) Hg, 60 pulsation cycles/min, and a 75% (*24*) pulsation ratio are the most economical (*29*). However, a corresponding investigation regarding vacuum level, pulsation rate, and pulsation ratio and their effect on udder health has not been carried out.

Some aspects of MM design and function are apparently affecting the rate of intramammary infection (IMI). In this paper, suggestions will be made to decrease the high rate of IMI as related to the MM. However, I do not believe we need to completely justify our recommendations on the basis of improved udder health only.

The primary defense mechanism that prevents IMI is the teat canal (*9,14*). Abnormal function of the MM will probably have little adverse effect on the mammary glands if IMI is prevented, glands are properly evacuated, and injury to the end of the teat and teat canal is minimized. In addition, an efficient operator can overcome many deficiencies in milking equipment.

As a result of labor cost and shortage, we have overemphasized fast milking by each unit. If you want to milk more cows over a certain period, you should install more units rather than increase the rate of milking by each unit. However, more milking units per man may increase overmilking.

The main feature of this paper is to present the causes and effects of vacuum fluctuation on the incidence of new IMI. Several comprehensive reviews)*7,8,12,15,16, 17,21,28,29*) of the relationship between MM and mastitis should be referred to for details on milking machine function.

EFFECTS OF MACHINE MILKING

The primary function of the MM is to remove milk from the mammary gland by increasing the pressure differential across the teat canal. The increased pressure varies from 10 (25.0 cm) to more than 15 (38.1 cm) inches of Hg.

Greater labor efficiency is realized when the MM is used than when cows are hand-milked. One can hand-milk only 8 to 10 cows per hour, whereas one can milk 30 or more cows per hour with a machine. The amount of injury to the teat canal varies with the use of the MM (*29*). Stretching the teat canal and pinching the teat end can result in bacterial colonization of the injured areas and thus increase bacterial exposure to the teat canal. The best time to examine the teat ends for injury is immediately after removal of the MM. A high massage force of liner collapse can remove keratin from the teat canal and increase susceptibility to IMI.

The MM transfers bacteria from teat to teat within each cow and between cows (*17,29*). Bacteria are transferred within each udder when contaminated milk surges from the inside of one liner into another liner during milking (*14*). This mechanism results in contamination of the entire inner surfaces of the MM cluster. Bacteria are transferred between cows when the contaminated cluster is moved from cow to cow without thorough sanitation. Recent data (*1*) indicate that the MM increases contamination of the teat canal and, in some cows, the interior of the teat sinus. This action may be responsible for up to one-half of the IMI within a dairy herd.

POSSIBLE MECHANISMS THAT RESULT IN INTRAMAMMARY INFECTION

During nonmilking periods, the rate of IMI is high. Many infections occur before the time of first calving, during the dry period, and between milkings during lactation. Bacteria can colonize the teat canal and invade the teat sinus. This mechanism may be responsible for many IMI during the early dry period. There is no evidence that motile bacteria can migrate more readily through the teat canal compared to non-motile bacteria. Capillary action within the canal might carry bacteria into the mammary gland. Dilation of the proximal one-half of the teat canal between milkings due to

increased intramammary pressure may aid bacterial invasion (*17*). This mechanism becomes important, especially if the distal 4 mm of the teat canal is contaminated as a result of MM function.

In the average field herd, each cow contracts about two new IMI each lactation. Perhaps one-half of new IMI are specifically related to the function of the MM (*28*). During one lactation, each cow is milked 610 times, 2440 quarter milkings. If the milking time per cow is 5 min and the pulsation rate is 60, then 732,000 pulsation cycles are used to milk each cow for one lactation. We can assume that we are looking for mechanisms that result in IMI only about once in 732,000 times. However, any single mechanism may have an accumulative effect.

During machine milking, at least two mechanisms may cause IMI. First, the collapsing liner, when flooded during maximum milk flow, may force contaminated milk back through the teat canal. Second, near the end of milk flow, when the liner opens, increased vacuum levels within the body of the opening liner may cause a reversal of milk flow within the short milk tube (Fig. 1).

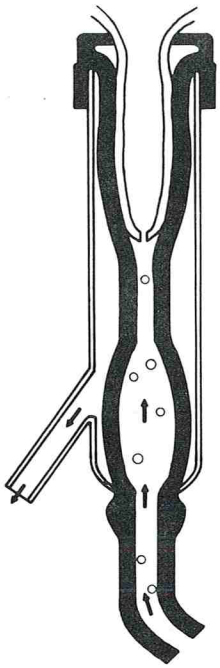

Figure 1. *Reversal of direction of flow in the short milk tube with opening of the liner.*

Under these conditions, droplets of contaminated milk may be thrown with considerable impact against the teat end and distal end of the teat canal. These events take place mainly near the end of milk flow in one quarter while milk is still flowing in one or more of the other quarters.

FUNCTIONAL COMPONENTS OF THE MILKING MACHINE

Air flow

Because a vacuum is created by removal of air within a system, air flow is the most logical starting place when discussing the MM. If air is continuously admitted to the system, e.g., through the vacuum regulator, increased air flow is required to maintain the static vacuum level.

The vacuum pump must be able to remove all air admitted into the system as a result of normal machine functioning. However, some air normally leaks into the system between the teat wall and the liner. Air is admitted through bleeder holes in the cluster. A large amount of air is admitted by the evacuation of the pulsator chambers during each pulsation cycle. The above air removal requirements can be measured and can be accurately planned for in any installation.

Vacuum reserve is the air admitted through the static vacuum regulator and includes all air flow above the actual requirements of the system. This reserve is necessary to maintain a stable vacuum when there is a sudden admission of air that can occur when clusters are changed or when a cluster drops off the udder. Numerous recommendations have been made concerning the amount of vacuum reserve that is necessary (*13*). Dynamic testing is the best method for accurate determination of vacuum reserve requirements. the static vacuum regulator must be able to function efficiently for maximum use of vacuum reserve (*19,24*).

Static vacuum

Vacuum within the MM system must be constant. The static vacuum level should be checked with a manometer. Vacuum stability at the teat end is one of the most important requirements of the functioning MM. The vacuum level used in the MM must be safe and result in minimal injury to the teat end (*11,22*). Vacuum levels over 13 inches (33.0 cm) of Hg result in excessive injury to the teat end and teat canal, result in more overmilking, and decrease vacuum reserve (*28*). If a system is functionally inadequate, increasing the static vacuum level further decreases the efficiency of the system. For efficient function of the system, vacuum in the milk and

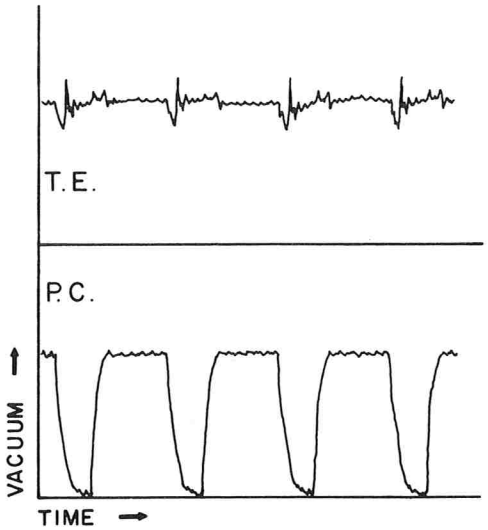

Figure 2. *Cyclic vacuum fluctuation at the teat end. T.E. = Teat end vacuum; P.C. = Pulsator chamber vacuum.*

other lines should not vary more than 1 inch (2.5 cm) of Hg (*2*).

Vacuum fluctuation (Instability)

Nearly all aspects of the design and operation of the system influence this component. Vacuum fluctuation is not detected during static testing. There are several types of vacuum fluctuation within the milking system.

Cyclic vacuum fluctuation

This is the change in vacuum level at the teat end with each pulsation cycle (Fig. 2). Factors that increase cyclic fluctuation (*2,3,25*) include elevation of milk, simultaneous (4 × 0) pulsation, decreased or lack of air admission, air admission remote from the teat end, wide ratio pulsation, rapid change of pressure in the pulsator chamber (snappy pulsation), larger bore liner, flooding of the liner, decreased capacity of the claw chamber, increased milk flow, and decreased diameter of the short or long milk tube (Fig. 3). High cyclic vacuum fluctuation

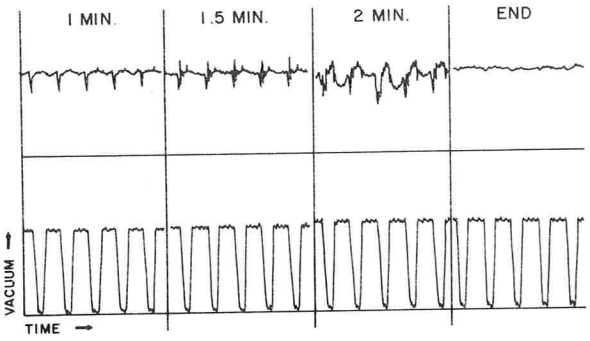

Figure 3. *Recordings during dynamic testing at four stages of milking.*

does not influence MM performance.

Irregular vacuum fluctuation (20)

This condition is a decline and subsequent recovery of the static vacuum level that persists over several pulsation cycles. Most irregular vacuum fluctuation is due to poor design or improper installation of the system and follows the sudden admission of large amounts of air. There are two types of irregular vacuum fluctuation: the first type takes place when there is an equal drop of vacuum at the teat end and in the pulsator chamber, and the second type occurs when milk is being lifted into a high pipeline and results in a drop in teat end vacuum without a concomitant drop in pulsator chamber vacuum (Fig. 4). When this second type occurs, liner ballooning, teat ballooning, and teat canal and teat injury result (*20,25*). Both types of fluctuation can occur when MM clusters are changed and excessive air is admitted or when a cluster drops off the udder (*20*). Irregular vacuum fluctuation may be localized or generalized throughout the entire system. Localized fluctuation is due to substantial obstruction of air flow in the vacuum system by air turbulence, physical blockage, or obstruction by milk in the milk collection system. Generalized

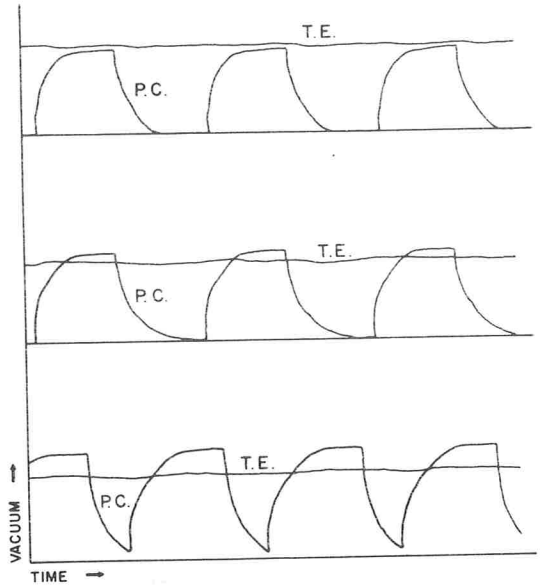

Figure 4. *Dynamic tests that show different maximum vacuum levels in the pulsator chamber (P.C.) compared with teat end vacuum (T.E.).*

fluctuation in the system is due to the inability of air to flow freely from the teat end through the exhaust of the vacuum pump. Factors that increase irregular vacuum fluctuation (*16,18*) include inadequate vacuum reserve (from inadequate vacuum pump capacity), malfunction or inefficient function of the vacuum regulator, poorly designed milk or vacuum lines, or both (restricted, undersized, or excessive length), milk entering the bottom half of the pipeline, excessive air admission (air leaks, inefficient changing of cluster by operator), improper slope and flooding of the milk pipeline, elevation of milk, milk filtration within the vacuum system, lack of air admission at or near the teat end, and rough-skin teats that do not fit tightly in the mouth of the liners.

The effects of irregular vacuum fluctuation include decreased maximum and average milk flow rates which result in slow milking (*18,19*). However, there may not be a great increse in machine-on-time (*20*). Excessive cluster drop-off may occur during maximum milk flow when the system is operating at full capacity.

There is good evidence that poor equipment design and inadequate vacuum reserve, which results in irregular vacuum fluctuation (*20*), is an important contributing cause of new IMI (*10,18,21*). Nyhan (*18*) has presented an excellent review of the effect of vacuum fluctuation on udder infection. In 1963, Nyhan and Cowhig were the first to show, under controlled experimental conditions, that irregular vacuum fluctuation was associated with an increase in the rate of IMI. This and other studies by Nyhan and Cowhig (*19*) are presented in

Tables 1 and 2. Typical vacuum line fluctuation is shown in Fig. 5.

TABLE 1. *Relationship Between Milking Machine Function and Intramammary Infection* (20)

Experiment no.	Year	No. cows	Length (Mo.)	No. infections	
				High reserve[1]	Low reserve[2]
1	1963	30	2.0	2	13[3]
2	1964	38	5.0	21	44
3	1966	46	6.0	24	52
				Stable vacuum[4]	Fluctuating vacuum[5]
4	1967	26	1.5	11	24
5	1967	24	0.5	10	23

[1]7.5 cfm free air/unit.
[2]1.5 cfm free air/unit.
[3]Vacuum line fluctuation from 8.5 to 15.0 inches (27.1 to 38.1 cm) of Hg.
[4]13.2 inches (33.5 cm) of Hg.
[5]Vacuum line fluctuation from 9.0 to 15.0 inches (22.9 to 38.1 cm) of Hg four times/min.

TABLE 2. *Relationship of Vacuum Reserve per Milking Unit to Intramammary Infection* (19)

Vacuum reserve/unit[1]	No. herds	No. cows	Infections (%)	
			Cows	Quarters
1.0-2.5	8	179	32	12
0.5-1	7	169	42	16
0.5	6	136	52	24

[1]Cfm free air

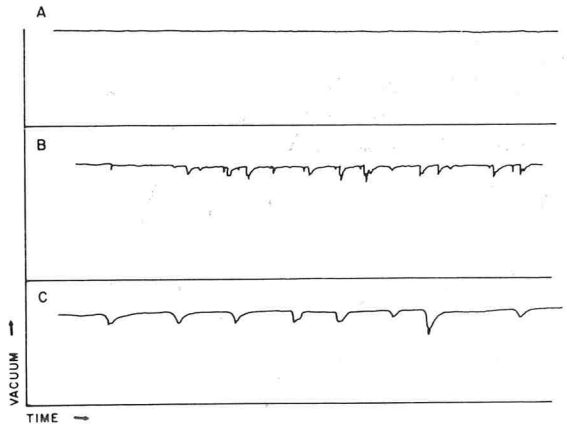

Figure 5. *Recordings taken within the vacuum line of low and high vacuum reserve systems. A = high vacuum reserve; B = low vacuum reserve, experiment 2; C = low vacuum reserve, experiment 3.*

Klastrup (*10*) and Wilson (*30*) have reported similar findings (Tables 3 and 4). In a field study involving two herds with high rates of IMI and two herds with low rates, Cousins (*4*) showed that irregular vacuum fluctuations were more frequent in the milking system used to milk the two herds with high rates of IMI. Cousins, et al. (*6*), Kingwill (*9*), and Thiel et. al. (*25,26*) have studied extensively the effects of vacuum fluctuation on udder infection. If a quarter MM with a separate milk collection system for each quarter was used, no effect on the rate of IMI was noted (*5*) (Table 5). During normal machine milking (Table 6), neither type of irregular vacuum fluctuation (as mentioned previously) alone increased IMI (*25*). Large cyclic fluctuation alone or with fast liner wall movement did not increase IMI (*25*). A combination of factors including irregular vacuum fluctuation at the teat end and in the pulsator chamber or at the teat end only, when combined with cyclic fluctuation and fast liner wall movement, were associated with a high rate of IMI (*25*). These are MM induced IMI.

When irregular and cyclic vacuum fluctuations along with fast liner wall movement were applied near the end of milk flow only (Table 7), the rate of IMI was similar to the rate when the predisposing conditions were applied

TABLE 3. *Vacuum Reserve and Intramammary Infections in 602 Cows in 47 Herds* (10)

Vacuum reserve/unit[1]	Infections (%)
0.0-1.0	49
1.0-2.3	52
1.8-2.7	50
2.7-3.6	38
3.6-5.4	34
5.4	16

[1]Cfm free air

TABLE 4. *Intramammary Infections in 12 Cows During a 14 Week Exposure Period* (30)

No. cows	Type vacuum	No. infections
6	Irregular & cyclic	23
6	Cyclic	1

TABLE 5. *Effects of Vacuum Fluctuation During Milking with a Machine Equipped with Quarter Milk Collection Systems* (5)

Animals: 20 cows
Methods: Length of experiment = 11 weeks
All teats dipped in bacterial cultures
Experimental design:

	Vacuum fluctuation	
Period	Irregular	Cyclic
1	X	X
2	X	—
3	—	X
4	—	—

Results: 14 new infections. No differences between treatments.

TABLE 6. *Relationship Between Milking Machine Function and Intramammary Infections* (25)

Experiment No.	Vacuum fluctuation		Varying pulsation rate	Fast liner wall movement	Infections	
	Irregular	Cyclic			No.	%
1	–	–	–	–	3[1]	7.4
2	+	+	+	+	23[2]	56
3	+	+	–	+	12	30
5	+[3]	–	–	–	1	2
6	–	+	–	+	4	10
7	+[3]	+	–	+	19	48

[1]Average of 5 trials.
[2]Average of 2 trials.
[3]Fluctuation in teat end vacuum only.

TABLE 7. *Time of Application of Milking Machine Conditions Predisposing to Intramammary Infection and the Number of Infections in 20 Cows* (6).

Experiment no.	Treatment during			No. infections
	Peak flow	Reduced flow	Stripping	
1,2,3	×	×	×	21[1]
4	×			8
5	×			6
6		×		12
7			×	19
8	×	×	—[2]	22

[1]Average.
[2]Unit removed before machine stripping.

throughout milking and stripping (*6*). Earlier, Thiel et al. (*26*) had shown that a high impact force at the teat end could force material reversibly through the teat canal into the teat sinus. These high impact forces are present with reverse flow in the short milk tube as the liner opens. Bacteria gaining access to the teat canal and teat sinus near the end of milk flow are less likely to be washed out by the small amount of milk remaining within the quarter (*6*). Subsequent research has shown that the high rate of IMI is associated with impacts on the end of the teat by milk droplets returning to the body of the opening liner (*25*).

Several methods are available to suppress milk droplets from impinging upon the teat end (*1*). Increasing the bore of the short milk tube and claw nipples to 7/16 to 1/2 inch (1.1 to 1.3 cm) in conjunction with a slow rate of liner opening will reduce cyclic fluctuations and impact forces. The short milk tube could be attached to the body of the liner at an angle that will prevent droplet impact on the teat end. A deflector or shield could be placed in or near the opening of the short milk tube into the body of the liner to intercept returning milk droplets (Table 8).

TABLE 8. *Effect of Shields Within the Short Milk Tubes on Intramammary Infection* (1)

No. quarters	Shields	No. infections
40	Present	1
40	Absent	26

Admission of air into each liner near the junction of the short milk tube and body of the liner will virtually eliminate reverse flow (*22,23*). Alternate pulsation and use of narrow bore liners decrease impact forces.

In 1969, Nyhan (*18*) stated that the evidence seems adequate, both from the point of view of udder disease and of milking efficiency, that machine standards should be adopted that will minimize irregular vacuum fluctuation at the teat end. Simple design changes can virtually eliminate cyclic vacuum fluctuations. With the additional research data since 1968, functional machine standards should be established that will minimize cyclic and irregular vacuum fluctuation.

With a low-level milk pipeline of adequate size, a system with adequate unrestricted air flow, and efficient static vacuum control, a pipeline system can provide vacuum stability that is similar to efficient bucket system. The system should maintain vacuum stability within ± o.r inch (1.3 cm) of Hg in the vacuum line, teat end and maximum pulsator chamber vacuum levels within ± o.5 inch (1.3 cm) of Hg of each other, vacuum no higher than 13 inches (33.0 cm) Hg at the teat end with 2 inches (5.1 cm) Hg or less fluctuation, and a pulsation ratio of 65% or less. Most of these functional conditions are not possible with a high milk pipeline installation.

Pulsation

Pulsation rates between 45 and 60 cycles/min have not been implicated in IMI (*28*). Air admission increases as the pulsator rate increases; therefore, high pulsation rates are associated with a decrease in the vacuum reserve (*19*).

No controlled experimental work concerning pulsation ratio and IMI has been carried out. Mean peak flow rate at several vacuum levels was at a maximum at a pulsation ratio of 65% and declined at higher and lower ratios. Field reports claim increased lesions on the teat end and greater mastitis problems when cows are milked with wide pulsation ratios.

CHANGES IN MILKING MACHINE DESIGN THAT SHOULD BE CONSIDERED

With further research data, a separate milk conveying system for each quarter may prove desirable. This would decrease the within cow transfer of bacteria and would decrease the MM related high rate of IMI. Washable pulsators and transparent clusters for each unit are desirable. No air injection into the milk collection system would appear desirable.

We need to increase the size and prevent flooding of the milk collection system. The bore of the short milk tubes and ferrules that leads into the claw should be at least 7/16 inch (1.1 cm). The capacity of the claw chamber should be sufficient and changed in design so that the milk inlets never flood. Milk should flow by gravity throughout the system. We need to stop lifting milk by installation of lower milk pipelines or receiver jars so that milk is lifted no more than 3 ft (91.4 cm) within the vacuum system.

Vacuum at the teat end should be 13 inches (33.0 cm) of Hg or less and never fluctuate more than 2 inches (5.1 cm) of Hg. Fluctuation in the vacuum supply and milk pipeline must be limited to no more than 0.5 inch (1.3 cm) of Hg even when changing clusters or when a cluster drops off. Teat end and maximum pulsator chamber vacuum should be within 0.5 inch (1.3 cm) of Hg of each other. All vacuum and milk pipelines should be looped and all dead ends eliminated. Vacuum regulators should be improved so that all air admission is stopped when static vacuum drops 0.5 inch (1.3 cm) of Hg. We need a monitor on static vacuum and vacuum reserve.

Because the liner is the only part of the MM that contacts the teat, its design and function are important. Teat massage force (the difference between the pressure differential required to collapse the liner when in the shell and teat end vacuum) should be between 6 and 10

inches (15.2 to 25.4 cm) of Hg. Stretched liners have lower massage force and minimize teat injury. A pulsation ratio of 65% or less is required to permit time for teat massage by the liner. Use of narrow bore liners decreases teat end injury. The body of the liner must be long enough to prevent the teat end from being forced into the short milk tube. Air should be admitted near the teat end.

From the standpoint of MM related IMI, labor efficiency, and equipment efficiency, the end of milking is a critical time. We need a monitoring system to indicate the end of milk flow. Overmilking should be prevented on a quarter basis. The teat cup should never be removed from the teat without relieving or shutting off the vacuum supply.

Recent date (*1*) indicate that we need to prevent milk droplets from impinging upon the teat end. Some mechanism such as a baffle or elbow in the short milk tube would be beneficial. Admission of air into the liner at the junction of the body and short milk tube and a decrease in the rate of opening of the liner will suppress impact forces.

TESTING THE MILKING MACHINE SYSTEM

Static testing is done when milk is not being transported within the system and cows are not being milked. In many herds that have serious mastitis problems, static tests indicate that the system is satisfactory (*7*). Designing a system with complete assurance that it will be functionally adequate will always be difficult. Upon completion, a system must be tested under full load to demonstrate to the dairyman that his system is adequate. We should establish functional performance standards for MM and then let the manufacturer design and install the equipment to meet these standards.

Dynamic testing is done with all units milking and accessories in use. Each cluster is evaluated while being used to milk a high producing, fast milking cow. A simultaneous recording of the teat end and pulsator chamber vacuum should be taken on each cluster (Figures 6-9). Also when alternate pulsation is used,

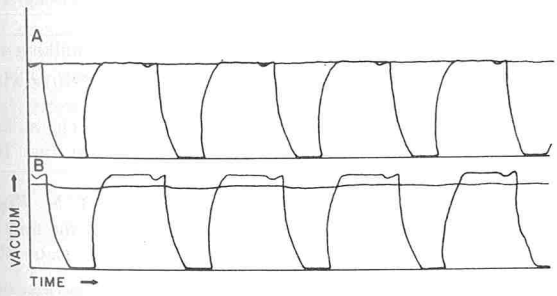

Figure 6. *Static and dynamic tests on the same system. A = static testing; B = dynamic testing.*

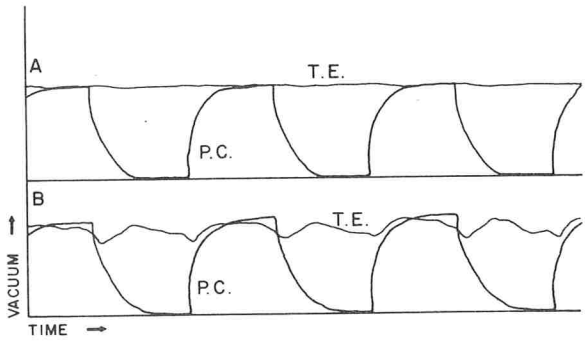

Figure 7. *Static and dynamic tests on the same system. A = static testing; B = dynamic testing; P.C. = pulsator chamber vacuum; T.E. = teat end vacuum.*

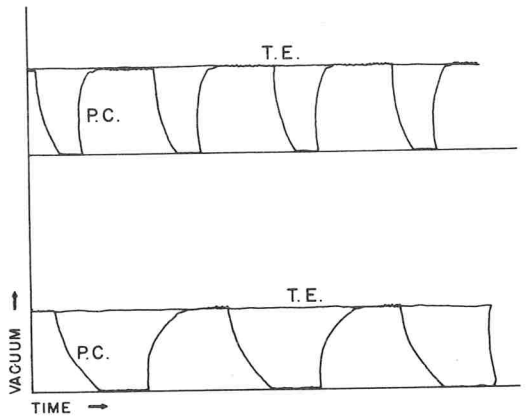

Figure 8. *Recordings during dynamic testing on two efficient systems. P.C. = pulsator chamber vacuum; T.E. = teat end vacuum.*

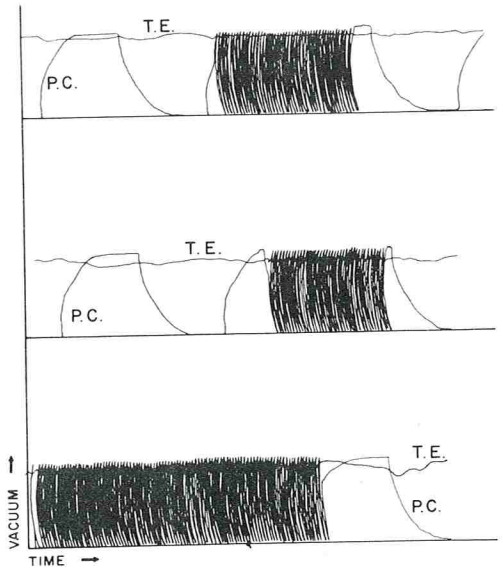

Figure 9. *Slow and high speed recording during dynamic testing of three systems. P.C. = pulsator chamber vacuum; T.E. = teat end vacuum.*

simultaneous recordings within alternating pulsator chambers should be taken. These recordings should be made with the liners that are normally used. However, use of transparent teat cup shell and liner will aid in determining whether liner flooding or ballooning is present. Functional vacuum reserve can be subjectively checked by listening for air admission into the vacuum

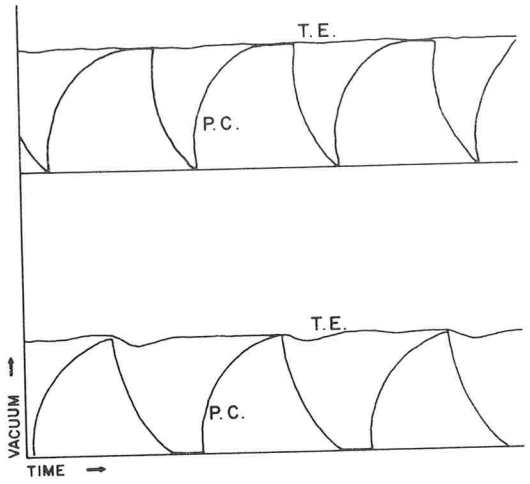

Figure 10. *Recordings during dynamic testing of two inefficient systems. P.C. = pulsator chamber vacuum; T.E. = teat end vacuum.*

reserve can be subjectively checked by listening for air admission into the vacuum regulator while the system is under full load. Check milk flow for slugs as it enters the receiver. Improper design and faulty functions as well as improper operation must be corrected. The major aspects to be noted in an analysis of a milking system can be recorded on the form in Table 9.

TABLE 9. *Milking System Analysis* (27)

Step I. Vacuum pump
1. Make _____ Model _____ RPM _____
2. _____ cfm[1] at _____ in. Hg.
3. Static vacuum _____ in. Hg.

Step II. System air flow capacity
1. Total _____ cfm[1] at static vacuum level.
2. Vacuum reserve _____ cfm[1].
3. Effective vacuum reserve _____ cfm[1]
4. Recovery after 5 in. Hg. drop _____ Seconds.

Step III. Vacuum line sizes
1. Pump to distribution tank _____ in.
2. Tank to moisture trap _____ in.
3. Moisture trap to receiver jar _____ in.
4. Pulsator line _____ in.
5. Stall cock line _____ in.
6. Milk pipeline _____ in.

Step IV. Milking units
1. Number _____
2. Type of shells _____
3. Type of liners _____
4. Air bleeder holes open _____
5. Diameter of long milk tube _____ in.
6. Diameter of short milk tube _____ in.

Step V. Pulsation
1. Rate _____ Action _____
2. Ratio _____
3. Check each pulsator with a recorder during maximum milk flow.

Step VI. Teat End Vacuum
Record on a fast milking, high producing cow with all units milking and all accessories functioning.
1. Maximum teat end _____ in. Hg
2. Minimum teat end _____ in. Hg
3. Fluctuation _____ in. Hg
4. Average _____ in. Hg
5. Difference between maximum pulsator chamber and teat end end _____ in. Hg
6. Milk: Rest Ratio _____

[1]Free air (ASME)

CONCLUSIONS

The characteristics of functionally efficient MM systems are as follows: (*a*) total pump capacity of 8 to 12 cfm free air/unit; (*b*) vacuum reserve of 3 to 5 cfm/unit; (*c*) alternate pulsation; (*d*) pulsation ratio between 50 and 60%; (*e*) vacuum at the teat end near 12 inches (30.5 cm) of Hg; (*f*) not more than 2 inches (5.1 cm) of Hg. vacuum fluctuation at the teat ends; and (*g*) virtually no drop in line vacuum when clusters are transferred. We must emphasize capacity, simplicity, and compactness in the design and installation of a MM system.

Responsibility of the MM operator is concerned with conservation of vacuum reserve. He must minimize unnecessary air admission by preventing cluster drop off, keeping short milk tubes bent over claw ferrules when putting on or removing cluster, shutting off vacuum before cluster removal and when recorder jar is evacuated, and not allowing units to run idle.

I predict that we will see more, rather than less, mastitis problems directly related to the MM. In newly installed systems, more complex equipment design and automation will be the basic reason for these increases in mastitis problems.

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A Note to Readers of the Journal . . . .

The December, 1974 issue of the *Journal* was the last one to be printed by Roeder's Franklin Printing Service, Inc., of Franklin, Indiana. This firm printed the *Journal* for about 18 years. Excellent cooperation and excellent workmanship by the folks at the Franklin Printing Service is gratefully acknowledged.

When the headquarters of IAMFES was moved to Ames, Iowa it became evident that printing of the *Journal* should be done at the same location. Consequently, a contract to print the *Journal* was negotiated with Heuss Printing of Ames, Iowa. The January, 1975 issue of the Journal is the first one to be printed by Heuss Printing.

This change in printer allowed some changes to be made in appearance of the *Journal.* The major changes include: (*a*) modifications in the design of the cover, (*b*) a redesigned "table of contents" page, (*c*) use of a new typeface throughout the *Journal,* (*d*) more liberal use of boldface type for headings in articles, (*e*) complete citation of each article just above the title, and (*f*) use of upper- and lower-case letters for the title of each article. Many of these changes are possible because composition of the *Journal* is now being done by a cold-type computer process. It is hoped that the new format will make the *Journal* more appealing to both readers and authors.

E. H. MARTH

Editor
Journal of Milk and Food Technology

J. Milk Food Technol. Vol. 38, No. 1, Pages 52-53 (January, 1975)

Instructions to Contributors

Journal of Milk and Food Technology

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The *Journal of Milk and Food Technology* is intended for publication of papers dealing with: (*a*) food, dairy, and environmental sanitation and hygiene; (*b*) foodborne disease hazards (microbiological, chemical, etc.); (*c*) food and dairy microbiology, including methodology; (*d*) food and dairy chemistry, including methodology; (*e*) food and dairy engineering; (*f*) food and dairy technology (processing, packing, etc.); (*g*) food additives (intentional and unintentional additives); (*h*) food service and food administration, (*i*) quality control and assurance in the dairy and food industry; (*l*) food and dairy regulatory programs; and (*m*) agricultural sciences (animal, dairy, and poultry science; entomology; agronomy; horticulture; soil science; etc.) as they relate to food production, quality, safety, and processing and to environmental control.

Papers concerned with other subjects in the areas of food and dairy science, environmental control and health, and sanitation also are suitable for publication in the *Journal*. Authors who may have a question about the suitability of their manuscript for publication are invited to request an opinion from the Editor.

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The author is notified when a manuscript is received and also when it is submitted to the printer for preparation of proofs. An author must return proofs promptly or publication of his paper may be delayed.

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Upon recommendation of the Journal Management Committee, it was voted by the Executive Board of IAMFES to institute a page charge of $25.00 per printed page for publication of all *research* papers received after January 1, 1969. This charge is necessitated by increases in costs and also will serve to permit expansion of the *Journal* so that a greater number of research papers can be published promptly.

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The *Journal* enjoys a wide readership in the United States and in foreign countries. Readers include persons at various levels in industrial, regulatory, and academic organizations. As a consequence, the *Journal* attempts to publish a variety of papers so that it is of maximum benefit to its readers. The following types of papers are acceptable for publication in the *Journal.*

Research paper

The research paper reports results of original research which has not been published elsewhere. It usually consists of 8 to 12 double-spaced typewritten pages plus appropriate tables and figures. A research paper deals in some depth with its subject.

Research note

A research note is a short paper which describes observations made in a rather limited area of investigation. Negative results are sometimes best reported in the form of a research note. The research note should not be used as a vehicle for reporting inferior research. A research note generally consists of less than 5 double-spaced typewritten pages of text together with appropriate figures or tables. Organization of a research note is the same as that of a research paper. The designation, "A Research Note" will appear above the titles of these papers since the *Journal* does not devote a separate section to research notes.

The author should specify that his manuscript is a research note so that it can be properly evaluated during the reviewing process.

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Well written, thorough, well documented review papers on subjects of concern to the readers of the *Journal* are encouraged and will be

published promptly. If a review paper substantially exceeds approximately 25 double-spaced typewritten pages, it may be necessary to subdivide the manuscript so that it will appear in several issues of the *Journal.* Authors are invited to discuss their plans for review papers with the Editor.

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The *Journal* regularly publishes some nontechnical papers as a service to those readers who are not involved with the technical aspects of dairy and food science. These "grassroots" papers might deal with such topics as working with people, organization of a sanitation program, organization of a regulatory agency, organization of an educational program, use of visual aids, and similar subjects. *Papers of this type should be well written and properly organized with appropriate subheadings.* Often talks given at meetings can be modified sufficiently to make them appropriate for publication. Authors planning to prepare general interest nontechnical papers are invited to correspond with the Editor if they have questions about the suitability of their material.

Letter to the Editor

Readers are invited to submit letters to the editor to express their opinion on papers published in the *Journal* or on other matters which may be of concern to the entire readership. The letter to the editor also may be used to report limited observations made in the field or in the laboratory which cannot be published as a research note. This mechanism should be particularly valuable for the exchange of information by persons who are unable to attend annual meetings of IAMFES or by laboratory workers whose duties preclude publication of full-fledged research papers. A letter to the editor must be signed by its author(s).

Book reviews

Authors and publishers of books in the fields covered by this *Journal* (see earlier discussion of Subject Matter) are invited to submit their books to the Editor. Books will then be reviewed by a specialist in the field covered by the book and a review will be published in an early issue of the *Journal.*

PREPARATION OF MANUSCRIPTS

A. All manuscripts should be typed double-spaced on 8.5 by 11-inch bond paper. *Lines on each page should be numbered to facilitate review of pagers.* Use of paper with prenumbered lines is satisfactory. Side margins should be one inch wide and pages should not be stapled together.

B. The Editor assumes that the senior author has received proper clearance from his organization for publication of the paper. Authors should be aware of procedures for approval within their own organization.

C. A manuscript should be read critically by someone other than the author before it is submitted. This will help to eliminate errors and to clarify statements.

D. The third edition of *CBE Style Manual* (published by the American Institute of Biological Sciences, 3900 Wisconsin Ave. N.W., Washington, D. C. 20016) has been adopted by the *Journal* and should be consulted by authors for technical details of manuscript preparation. Abbreviations for journals and for botanical, chemical, physical, mathematical, and statistical terms should conform to the *Style Manual.*

E. *Organization of research papers and research notes*

1. The *title* should appear at the top of the first page. It should be as brief as possible, contain no abbreviations, and be truly indicative of the subject matter discussed in the paper. Care should be exercised by the author in preparing the title since it is often used in information retrieval systems. *Good information can be lost through a poor title!*
2. *Name(s) of author(s) and affiliation(s)* should follow under the title. If an author has changed location since the work was completed, his new address should be given in a footnote.
3. The *Abstract* appears at the beginning of the paper. It should be brief, factual, and not exceed 200 words. The abstract should be intelligible without reading the remainder of the paper. Generally, an abstract should not contain abbreviations. Abstracts of papers are reprinted by abstracting journals and so will be disseminated beyond the readership of the *Journal* to people who often do not have access to the entire paper. This suggests that abstracts should be prepared with great care.
4. The *text* should contain: (*a*) introductory statements, objectives or reasons for research, and related literature, (*b*) materials and methods (*c*) results, (*d*) discussion (may be combined with results), (*e*) conclusions (only if needed; should not repeat the abstract), (*f*) acknowledgements, and (*g*) references.
5. *Citation of references* must follow the style of the *CBE Style Manual.* Several examples of proper citations are given below.
   a. *Paper in a journal*
      Alderman, G. G., and E. H. Marth. 1974. Experimental production of aflatoxin in citrus juice and peel. J. Milk Food Technol. 37:308-313.
   b. *Paper in a book*
      Marth, E. H. 1974. Fermentations. pp. 771-882. In B. H. Webb, A. H. Johnson, and J. A. Alford (eds.) Fundamentals of dairy chemistry (2nd ed.), Avi Publishing Co., Westport, Conn.
   c. *Book*
      Fennema, O. R., W. D. Powrie, and E. H. Marth. 1973. Low-temperature preservation of foods and living matter. Marcel Dekker, Inc., New York. 598 p.
   d. *Patent*
      Hussong, R. V., E. H. Marth, and D. G. Vakaleris. 1964. Manufacture of cottage cheese. U.S. Pat. 3, 117, 870. Jan. 14.

   For citation of bulletins, annual reports, publications of federal agencies, etc., see *CBE Style Manual.* References should be listed in alphabetical order and numbered. Numbers in parentheses, independently or in conjunction with last names of authors, should be used in the text for designating references.

F. *Organization of review and general interest papers*
These papers must have a title, give name(s) of author(s) and affiliation(s), and the text must begin with an abstract. See items 1, 2, and 3 under E. The remainder of the text should begin with an introductory statement and then should be subdivided into appropriate sections each with a subheading which is decriptive of the subject matter in the section. Review papers, by their very nature, utilize numerous references. Citation of references in the text and listing of references at the end of the paper should be done as mentioned in section E-5 above.

G. *Preparation of figures*
Figures consisting of drawings, diagrams, charts, and similar material should be prepared in India ink on 8.5 by 11-inch tracing paper, white drawing paper, or blue linen. Do not use paper with green, red, or yellow lines since they cannot be removed and will appear in the final copy. A lettering guide must be used to prepare all letters which appear on figures. *Titles for all figures must be on separate sheets and not on the figures.* Use Arabic numbers for numbering of figures. Glossy prints of figures are suitable for use. They should be at least 4 by 5 inches in size. If photographs of equipment, etc. are submitted, the images should be sharp, there should be good contrast, and a minimum of distracting items should appear in the picture.

H. *Preparation of tables*
Each table should be typed on a separate sheet of 8.5 by 11-inch bond paper. *Tables should not be included in the text of the paper.* Use Arabic numbers for numbering of tables. *Titles should be as brief as possible but fully descriptive.* Headings and subheadings should be concise with columns or rows of data carefully centered below them. Use only horizontal lines to separate sections of tables. *Data in tables should not be repeated in figures.* When possible use figures instead of tables since the latter are more costly to prepare for publication.

News and Events

Design Innovation is Hallmark of Food and Dairy Expo '74

Computerizing of entire processing plants, innovative equipment design and advanced sanitary application mark the progress of the '70s at Food and Dairy Expo 74.

"Maximizing plant productivity results in improved cost control for both processor and the consumer," said Gordon Houran, president of Dairy and Food Industries Supply Association, sponsor of the show. "This Expo has shown that our industry is continuing to work for greater production efficiencies."

The exposition, held Oct. 20-24, 1974, at the Dallas Convention Hall, attracted 266 exhibitors serving the giant food processing industry. Exhibitors represented nine commodity grouping—processing equipment and components, ingredients, containers, general commodities, delivery, merchandising, chemicals and refrigerants and jobbers.

Exhibitors of packaging and container-forming equipment proved to be particularly innovative. Equipment and containers featured plastic for packaging, closures, bundling, bagging, cartoning, and casing. Plastic packaging included in-plant blow-molding equipment that forms the container for an integrated filling line. Integral packaging machines in which the paperboard or plastic container is formed, filled, and sealed in a continuous operation on the same machine were displayed.

Unique in the processing equipment area was the infra red pasteurizer from France, a "first" for a Food and Dairy Expo. The pasteurizer is being used on wines, fruit juices and other beverages in Europe. Another first, an infra red milk analyser measuring fat, lactose and protein in 40 seconds, was previewed at Expo. Analytical tools were also available for determining butterfat and other milk constituents, some of which can be used for automatic on-stream composition control.

Stainless steel equipment from the master craftsmen of sanitary design was evident in many forms. This year saw further integration of equipment into automated systems for both operation and clean-up. Particularly advanced demonstrations involved the application of computer technology to systems control and operations. This now makes it possible to program food processing operations to give simultaneous information on raw material inventory, product composition and product flow.

Instrumentation was exhibited which would sense and record liquid levels, pressure, temperature, weight, and gas analysis.

Strong emphasis continues on reverse osmosis and ultra filtration equipment with sanitary design. These new tools are invaluable for separation and concentration operations particularly applicable to whey handling.

Cheese making was represented in a new dimension. Automatic curd-making and handling, and mechanized cheddaring were novel approaches to the continuous manufacture of cheddar cheese.

For ingredients, new and extended applications were presented for flavors, stablizers, enmlsifiers, and specially modified milk solids. Demonstrating the growing consumer market, dietetic products available for franchise promotion and merchandising by dairy processors were displayed.

Precise overrun control for ice cream manufacturing, using metered flow of mix and air, was demonstrated.

Exhibitors were noticeably mindful of the needs of the diverse food processor industry. Pumps designed for movement of viscous food products and those with suspended materials were featured.

Filler and sealing equipment for application to salads and convenience foods were shown. Portion packaging and containers for use in institutional feeding were in frequent evidence.

Food processors could see meat equipment for extruding and forming meat products. There were also poultry chillers, and poultry cookers. Water removal equipment was displayed for spray drying, foam-mat drying, and agglomeration.

High speed fillers were demonstrated for paperboard containers of both metric and U.S. units in which volumetric fill is adjustable from the operating consol. This device enables the use of either quart or liter increments on the same machine through adjustment of fill at the control panel. Other fillers showed the use of three-quart paperboard containers. Flavors and spices were available for the broad spectrum food needs, including flavors for cultured products.

Plant construction, engineering, and architectural services were displayed, most of which were aimed at highly automated processing plants with maximum efficiency in materials handling.

Exhibitors demonstrated a willingness and ability to adapt to consumer oriented federal legislation. Impact of new laws in occupational health and safety were seen in equipment with protective devices for belts, shafts, excessive heat and noise. Labeling applications on filling and closing equipment for the industry's Universal Product Code were exhibited.

Association Affairs

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Letters To The Editor

Conclusions of the plate count are suspect?

DEAR SIR:

We have read with interest the recent article by Roughley, Johns, and Smith entitled "The Influence of Time and Temperature of Incubation on the Plate Count of Milk" (*J. Milk Food Technol.* 37:209-212, 1974). We feel that the conclusions resulting from the Duncan Multiple Range test are suspect and should be recalculated using analysis of variance after estimating the variance from the experimental data. We question why the authors elected to use an experimental error from previous research when a more appropriate and accurate error term for the immediate experiment could be normally calculated from their own data. The experiment as explained was a $2 \times 2 \times 2$ factorial experiment in which milk samples should be used as repetitions. The interaction of milk samples with the main effects and factorial interactions is an appropriate error for testing the factorial effects. The variance between plates, if more than single plates had been used, is an estimate of sampling variation and not of experimental error and therefore will generally be too small for a particular situation. Interactions of milk samples with other factors represents the failure of different milk samples to give the same results and as such is an approximate experimental error. We also feel that for statistical purposes all possible data should be used. *Standard Methods for the Examination of Dairy Products* recommends the use of plates containing 30-300 colonies. However, for statistical purposes the selection of plates for bacterial counts need not have this restriction. The statistical tests will overcome the errors in plating.

In addition, it is apparent that the percentages given in Table 1 could not have been based on 169 samples in each case, since $0.114 \times 169 = 19.226$ and $10/169 = 11.2$ not 11.4, etc. If all 8 combinations of the factorial were not evaluated for a sample, that sample should have been omitted from the data to retain comparability. Table 2 appears to be based on 74 samples in each case rather than the 76 mentioned in the text on page 210. The 2nd sentence in the 2nd full paragraph on page 210 does not appear to be borne out by the data.

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E. JAMES KOCH
Biometrical Staff
U.S. Department of Agriculture
Beltsville, Maryland 20705

Conclusions are valid say authors

DEAR SIR:

We appreciate the interest in our paper shown by Huhtanen and Koch. Their suggestion to use interaction to test incubation time and temperature effects on Standard Plate Counts of raw and pasteurized milk samples did not change the conclusions reported in the paper. The recalculated analysis of variance tables are as follows:

RAW MILK

Source	d.s.	m.s.
Samples (S)	130	1.086
Time (Ti)	1	1.879**
S × Ti	130	.016
Temperature (Te)	1	.595**
S × Te	130	.032
Ti × Te	1	.022
S × Ti × Te	130	.007

PASTEURIZED MILK

Source	d.s.	m.s.
Samples (S)	50	1.457
Time (Ti)	1	1.918**
S × Ti	50	.032
Temperature (Te)	1	.040
S × Te	50	.101
Ti × Te	1	.013
S × Ti × Te	50	.013

The target population for these comparative studies is the mid-range population. Very low counts are of little interest in regulatory work and very high counts will be consistently high under the methods used in this study. Excessively crowded plates also create bias in the data by yielding underestimates of the counts.

The title in Table 4 is correct and the total number of pasteurized samples throughout the text should be 74. The second sentence in the second full paragraph on page 210 should read "and 5.0% after PI" not "and 9.0% after PI."

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Research Branch
Canada Department of Agriculture
Ontario, Canada

K. L. SMITH
Department of Dairy Science
University of Florida
Gainesville, Florida 32611

Association Affairs

Planning the Program for the Sixty-Second Annual Meeting

The Sixty-Second Annual Meeting for IAMFES is to be held at the Royal York Hotel, Toronto, Canada on August 10-14, 1975.

Left to Right: Harold Thompson, Program Chairman; Parnell Skulborstad, President, IAMFES; William Kempa, Chairman of Local Arrangements.

The executive board, program planning committee and the local arrangements committee met on November 14 and 15 at the Royal York to plan the annual meeting. The local arrangements committee from the Ontario Milk and Food Sanitarians Association are making excellent plans for this meeting. Even though it has been twelve years since the last meeting was held in Canada many of our members will remember the great meeting of that year. Many outstanding speakers from Canada are being scheduled to appear along with U.S. speakers to make up an outstanding program.

The regional meeting of the National Mastitis Council will be held in conjunction with the annual meeting similar to last year's meeting in Florida.

Affiliate Membership Contest

The Executive Board of the IAMFES (International Association of Milk, Food and Environmental Sanitarians) has authorized the membership committee to conduct a contest to further our continuing effort to increase our IAMFES membership.

Each Affliliate Chairman will be encouraged to participate in this contest, not only to bolster their own membership, but also to earn a FREE, round trip airline ticket to Toronto, Canada, the sight of the 1975 Annual IAMFES Conference to be held in August 1975.

To qualify for this free ticket, the Affiliate Chairmen must increase their present membership by 25 members (or 25% of the present membership)-whichever is greater. This contest is effective beginning August 1, 1974 and ending July 1, 1975—at which time the local Affiliate Membership Chairman should notify the Executive Secretary to whom the ticket will be issued.

All applications and checks should be sent to Earl Wright, Executive Secretary, P.O. Box 701, Ames, Iowa 50010.

Local chairmen are encouraged to solicit their own team of membership promoters. It should be noted that membership goals should not be limited to the 25 (or 25%) membership increase—since special recognition will be given at the Annual International Conference for any and all outstanding performances in IAMFES membership recruitment.

Harold Y. Heiskell,
IAMFES Membership Chairman

Award to Florida Affiliate

Left to Right: Earl Wright presents a certificate to Dave Frye who is representing The Florida Affiliate.

The Florida Affiliate was awarded a certificate of appreciation for the way they conducted the Sixty-First Annual Meeting in St. Petersburg, Florida last year. This award was made availabe by Waleter Wilson of Los Angeles, California who was chairman of the Resolutions Committee.

Constitution Amendment

The Executive Board recommends that the Constitution be amended as follows:

ARTICLE IV — Section 1. Insert the words "and Secretary-Treasure" following the words "Second Vice-President" in line 4. Delete the "and" in lines 4 and 5. Add the words "and Second Vice-President" following the words "First Vice-President" in line 5. Delete the words "second Vice-President and" from lines 5 and 6.

ARTICLE VII—Section 1. Change the words "Secretary-Treasure" to the words "Executive Secretary."

By-Laws amended as follows:

ARTICLE II — Section B. Change the wording of "Secretary-Treasure" to "Executive-Secretary." Delete the word "an" and replace with the word "the." Change the wording of "Executive-Secretary" to "Secretary-Treasure."

This is being done to bring the Constitution and By-Laws up to date. This will also provide for the Secretary-Treasure to advance on the Executive Board as the other board members have in the past. If this amendment is voted favorable, elections will be changed to electing only a Secretary-Treasure each year instead of a Second Vice-President and Secretary-Treasure. This will be presented to the membership for action at the annual meeting in August at the Royal York Hotel, Toronto, Ontario, Canada.

Continuing Education Programs for the Food Industry

February 12-13, 1975—Ohio Dairy Industry Conference; Fawcett Center for Tomorrow, The Ohio State University, Columbus, Ohio. For further information contact: John Lindamood, Department of Food Science and Nutrition, 2121 Fyffe Road, The Ohio State University, Columbus, Ohio 43210.

March 24-28, 1975—Mid-West Workshop in Milk and Food Sanitation; Fawcett Center for Tomorrow, the Ohio State University, Columbus, Ohio. For further information contact: John Lindamood, Department of Food Science and Nutrition, 2121 Lyffe Road, The Ohio State University, Columbus, Ohio 43210.

Gouda Cheese from Holland Wins International Award

Mr. Rijkele Sijtsema, a cheesemaker from Holland with his award-winning Gouda.

A cheesemaker from Holland has proved that the best Gouda comes from the country where it all began! Rijkele Sijtsema shipped his 37-pound golden Gouda across the Atlantic to Green Bay, Wis., this month . . . and walked off with top honors for his class in the 10th Biennial World's Natural Cheese Championship Contest. Sponsored by the Wisconsin Cheesemakers Association, awards in six categories were presented at the close of the Association's 83rd annual meeting.

There were 173 entries, coming from Holland, France, Denmark, Finland, Canada, Switzerland, Italy, Sweden, Ireland, Australia and New Zealand. This marked the second consecutive World's Natural Cheese Championship Contest where a Dutch dairyman took top honors in his class.

The large Gouda entered by Mr. Sijtsema was made on May 31, 1974 in the cooperative at Opeinde, a village of 2,000 near Leeuwarden in The Netherlands (about 15 miles from where the champion cheesemaker was born).

Recognizing it to be "an ideal example of Gouda," Mr. Sijtsema entered it first in Dutch competition, where it got a 92 score. After reading about the Green Bay competition, he decided to enter . . . and shipped the cheese in early October. It had been kept at a constant 68 degree temperature to preserve its taste and texture.

American judges gave it an even higher score: 98.50 points.

This was the second highest score earned in the entire Competition, the grand champion scoring 99 points . . . a cheddar made in Blair, Wis. There were 72 cheddar entries; 18 in the Colby class; 20 in the Drum or Block Swiss section; 7 in the Brick cheese division; 14 in the Italian and 42 in Class 6 . . . all other types of natural cheese not covered by the other five sections.

Kenneth W. Hohe receives the American Intersociety Academy of Sanitarians Award

Mr. Kenneth W. Hohe, right, Chief of the Hospital and Institutions Section, Pennsylvania Department of Envrionmental Resources, is shown receiving the American Intersociety Academy of Sanitarian's Award from Dr. Sanford M. Brown, left, Associate Professor of Envrionmental Health, at East Tennessee State University, Johnson City, Tennessee.

The graduate faculty of the Department of Environmental Health has selected **Mr. Kenneth W.** Hohe to receive the AIACS Award for 1974. Mr. Hohe will graduate August 14, 1974.

Ken Hohe is a native of Allentown, Pennsylvania, and a graduate of Penn State University, with a B.S. in Dairy Science. His home address is 5029 Wynnewood Road, Harrisburg, Pennsylvania.

He has worked for the Pennsylvania Department of Health, now Environmental Resources, since 1960. He is now the Chief of the Hospital Facilities Section.

Acknowledgment of Assistance by Reviewers

Appreciation is expressed to all members of the Editorial Board who reviewed manuscripts during 1974. Dr. W. G. Walter, Montana State University, resigned from the Editorial Board because of additional duties that were assigned to him by the University. He has been replaced by Dr. H. W. Walker, Professor of Food Technology, Iowa State University. Thanks go to Dr. Walter for past service and to Dr. Walker for his willingness to serve in the future.

During 1974 manuscripts were reviewed by the following persons who are not regular members of the Editorial Board. Their help is acknowledged and appreciated.

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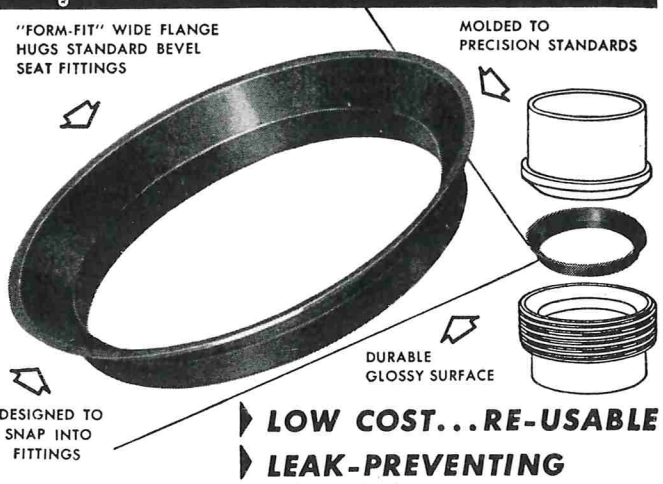

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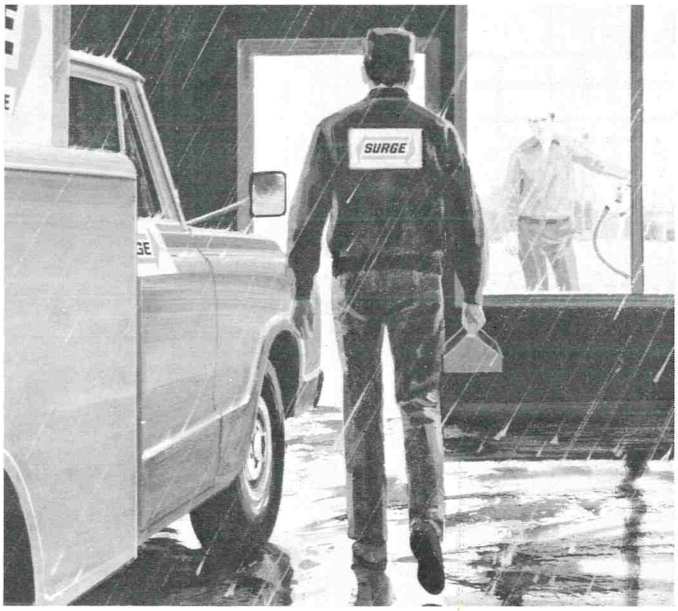

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