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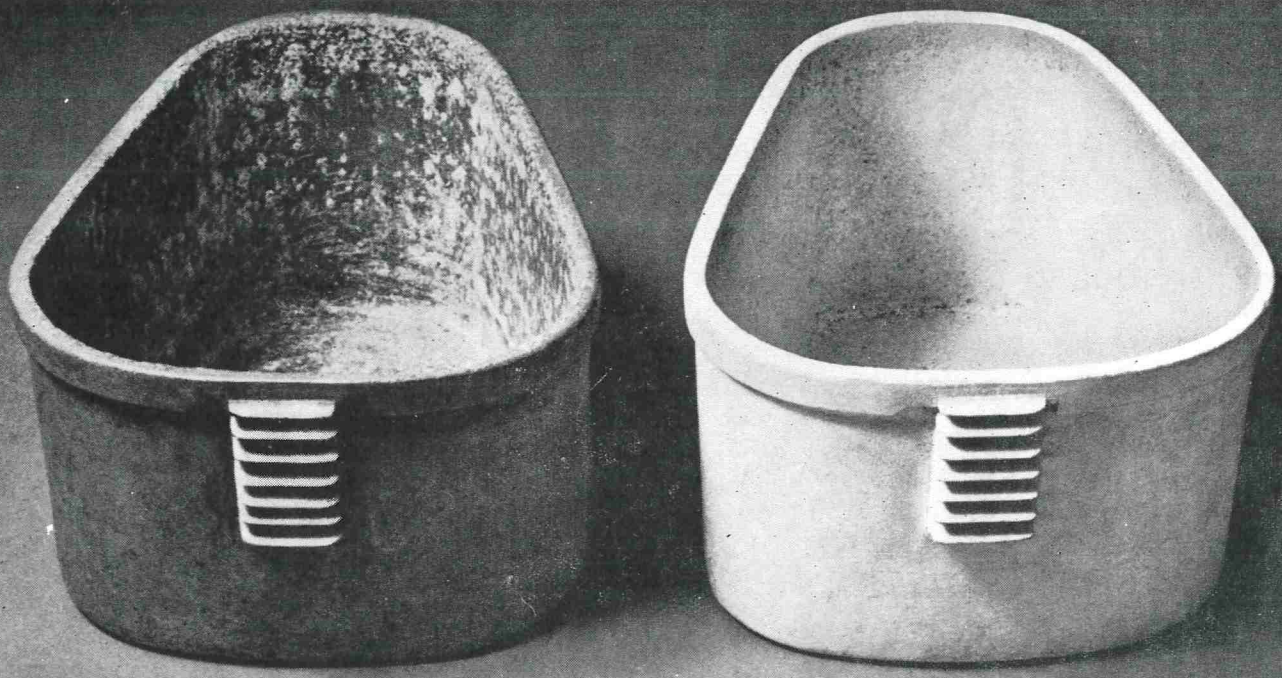

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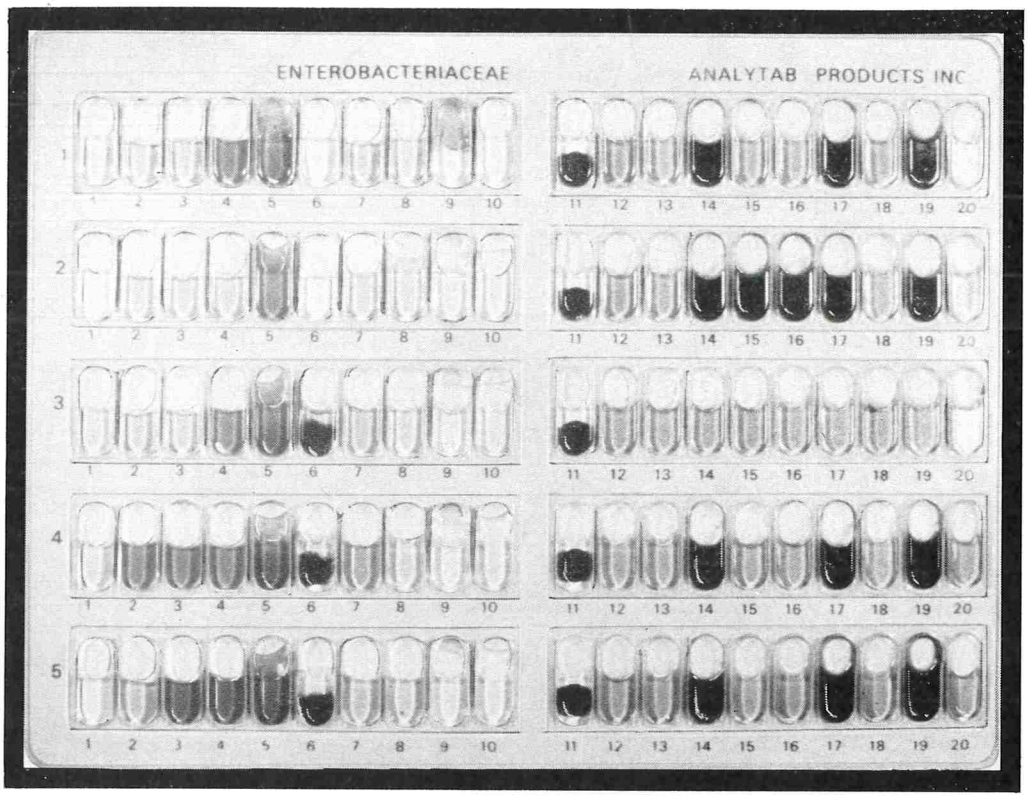

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BACTERIOLOGICAL TESTING OF MILK FOR REGULATORY PURPOSES—USEFULNESS OF CURRENT PROCEDURES AND RECOMMENDATIONS FOR CHANGE

V. PASTEURIZED MILK[1]

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(Received for publication October 22, 1970)

Abstract

Proper pasteurization and elimination of contamination subsequent to heat treatment suffice to ensure the consumer a milk supply that is free from pathogens and which has good shelf life. Bacterial plate count standards have remained practically unchanged during the past few decades in which many advances in processing and sanitary control have evolved. Standard Plate Counts and Coliform Counts on pasteurized milk are not reliable indices of either the safety or shelf life of the product. Current standards for these counts should be lowered in order to be commensurate with the counts obtained in well operated plants. However, low Standard Plate Counts and negative Coliform Counts are no assurance that post-pasteurization contamination has not occurred. The CVT Count and the 5-day—45 F Count are useful for detecting post-pasteurization contamination, with the latter count very useful for predicting shelf life. Satisfactory sanitary control of milk processing can be attained by application of these tests on a daily basis in a well equipped and staffed plant laboratory.

Since the first published reports of bacterial counts on milk in 1892, great changes have taken place in methods for production, processing, and distribution of milk as well as in methods for controlling the sanitary quality. With the prime objective of public supervision of milk supplies being that of protection of consumers, any discussion on this subject should maintain this objective as the central theme. We in the dairy industry can be proud of progress that has been made in improving the sanitary quality of our products but we should be conscious of even greater progress in the future.

Milk-borne epidemics were rather common 30 to 40 years ago but are now rather rare and are generally confined to raw products as the sources of infection *(14)*. From information we have there has been only one epidemic of disease traceable to pasteurized milk reported in the last 20 years or more *(13)*. This is evidence of what can be attained by the combined efforts of regulatory agencies and dairy plants.

We are all aware of the great changes that have occurred in methods of producing, processing, and merchandising of milk during the past several years. Some of these changes have compelled us to change our methods of testing for adequate sanitary control. While protection of the consumer from milk-borne diseases is foremost in any sanitary control program, the problem of attaining good shelf life has been one of great concern in recent years. Changes that have occurred in our industry have largely eliminated some old problems but, on the other hand, have created new ones or amplified others.

The change from the low temperature holding method of vat pasteurization to high temperature — short time processing has largely eliminated the problem of thermophiles and "pin point" colonies. Likewise, adoption of single service containers has largely eliminated a source of contamination which sometimes proved very troublesome. The increasing use of automated in-place cleaning has resulted in uniformly good cleaning and sanitizing procedures by removing variations in effectiveness in manual cleaning caused by differences in training, diligence, efficiency, and integrity of the personnel involved. Also, research has developed cleaners and sanitizers that are more effective than those used several years ago. The change to fewer and larger milk plants has resulted in elimination of many small plants that did not have facilities and personnel necessary for proper processing, sanitary control, and handling of milk.

The milk marketed currently must be of much better sanitary quality than formerly because of the stress to which it may be exposed in passing from the processing plant to the consumer's table. Much of the milk is marketed through stores and supermarkets which are often several hundred miles from the source. The long hauls, infrequent deliveries necessitating storage, and unsatisfactory refrigeration conditions during transportation and storage at the retail outlet are often such that only milk with a long shelf life can withstand the rigors of such treatment. Furthermore, the increasing use of multiple quart containers together with purchases at stores and supermarkets adds up to less frequent purchases and lengthens the period between processing and eventual consumption. Thus, the milk we market today must be of such quality that it can withstand adverse conditions to which it may be exposed. A

[1]Presented at the Annual Meeting of the American Dairy Science Association, Gainesville, Florida, June 29, 1970.

bad carton of milk is not only a stigma for the plant whose name appears on the package but also mars the good image of the milk industry.

While we have been emphasizing the importance of long shelf life as an attribute of milk we must be conscious of the fact that any methods used for controlling contamination with spoilage organisms also serve to eliminate the sources of pathogens that could possibly contaminate the milk.

Before proceeding further, we would like to inject the following quotation:

> "*Pasteurized milk.*—Milk of this class shall come from cows free from disease as determined by physical examinations by a qualified veterinarian and shall be produced and handled under sanitary conditions such that the bacterial count at no time exceeds 200,000 per cubic centimetre. All milk of this class shall be pasteurized under offical supervision, and bacterial count shall not exceed 10,000 per cubic centimetre at the time of delivery to the consumer."

The regulatory standards given for bacterial counts are not greatly different from those we have today. It is interesting to note that the above quotation appeared in *Public Health Reports* in 1913 *(7)*.

In discussing current procedures for controlling the sanitary quality of milk we rely mostly on our experiences in this field. Our main concern in working with various milk plants is to attain good product shelf life as well as to satisfy requirements of regulatory agencies in regard to Standard Plate Counts (SPC) and Coliform Counts.

Standard Plate Counts

Several investigators *(1, 2, 4, 11, 12)* have attempted to demonstrate the correlation between SPC and shelf life but all concluded that no close correlation existed. The present standard for SPC is, in our opinion, very liberal. With the use of pasteurization exposures of 165 to 175 F for 16 sec, which is common in the plants in our area, we have observed that most SPC's were $<$1,000/ml *(10)*. We believe lowering the standard count for pasteurized milk would be realistic and would not be detrimental to any plant. On the other hand, lowering the standard for the count on pasteurized milk would be no insurance of a better and safer milk because it is types rather than numbers of bacteria that determine whether the milk is free of pathogens and has good shelf life. Our attitude on Standard Plate Counts is to be certain that we comply with the regulatory standard and not to use them to evaluate the overall sanitary quality.

Coliform Counts

Coliform counts have been valuable in the past for detecting contamination or deficiencies in pasteurization but currently we do not consider them sensitive enough to be used as the sole measure of contamination since the medium employed is selective for this group of organisms and does not detect many of the types that cause spoilage in milk at refrigeration temperatures. Furthermore, we believe that a standard of $<$10 coliforms/ml in the freshly pasteurized milk is entirely too liberal. Since coliforms are killed by proper pasteurization *(3)*, presence of any coliforms would indicate contamination subsequent to the heating process. In plants we assist in controlling the sanitary quality we strive for negative coliform tests and we find that this goal is nearly always attainable.

Although coliforms indicate contamination *(6)*, there is no close correlation between coliforms and the shelf life of milk *(8)* and we believe that these counts are inadequate for satisfactory sanitary control of milk quality. A negative coliform count and a low SPC is no assurance that there has been no post-pasteurization contamination and that the milk will have good shelf. Again, it is types rather than numbers that determine how well the milk will keep.

Crystal-Violet-Tetrazolium Counts

We have found that a medium which restricts growth of organisms normally surviving pasteurization while permitting growth of gram-negative types which are common causes of spoilage is more useful than coliform counts for detecting post-pasteurization contamination *(9)*. This medium (CVT) contains crystal violet to inhibit the thermoduric organisms and 2, 3, 5-triphenyl tetrazolium chloride to add a distinctive color to colonies of gram-negative organisms. Since this medium detects gram-negative organisms in addition to the coliforms, it is much more useful than the coliform count. Here again, since it is types rather than numbers that determines the rate at which milk will spoil under refrigerated storage, a negative CVT test is no assurance of good shelf life, nor is a relatively high CVT count indicative of poor shelf life, although our investigations have shown a fairly good correlation between these counts and the keeping quality of refrigerated milk *(12)*. The CVT count was developed to detect contamination and we use it primarily for this purpose. We like to determine the CVT count on the first carton from each filling machine as a measure of the overall efficiency of cleaning and sanitizing the equipment.

Five Day — 45 F Counts

The 5 day — 45 F count *(5)* involves plating of milk initially and replating after holding the milk for five days at 45 F. This method detects organisms likely

to cause spoilage but which may have been present in numbers too low to be detected by counts on the fresh milk. We believe that this is the best method for determining the overall sanitary quality of milk and also that this is the best method of predicting shelf life. In different trials we have found correlation coefficients of 0.80 to 0.95 between these counts and shelf life *(12)*.

DAIRY PLANT LABORATORIES

It is our belief that all milk plants should maintain control laboratories with sufficient equipment and trained personnel to maintain strict sanitary control over their products. We have found it essential to conduct counts every day on every product in order to prevent laxity on the part of plant personnel. We believe that sampling only at intervals is insufficient for adequate supervision. Since it is impractical for regulatory officials to maintain a daily watch over every plant, it does not seem unreasonable to require the plants to make daily tests and then make their records available for inspection at all times. Regulatory officials can be very helpful to the plants in maintaining a satisfactory sanitary control program.

RECOMMENDED CHANGES

On the basis of our experience with the sanitary control of milk, we believe that the following suggested changes should be considered:

(*a*) Standard Plate Counts—Lowering the regulatory standard to a more realistic level on the basis of counts prevailing in well operated plants.

(*b*) Coliform Counts—Lowering allowable count to a considerably lower level. A negative coliform count in 3 out of 4 samples is not unreasonable.

(*c*) Requiring all plants to maintain a laboratory staffed with trained personnel and requiring them to make their records of counts available for inspection by regulatory officials.

In the future, more sensitive tests for detection of contamination and measurement of overall cleaning and sanitizing procedures may be developed. We should take note of these and change our methods and standards in line with these developments.

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BACTERIAL CONCENTRATIONS IN RAW MILK, IMMEDIATELY AFTER LABORATORY PASTEURIZATION AND FOLLOWING 10 DAYS STORAGE AT 7.2 C[1]

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(Received for publication September 21, 1970)

Abstract

No relationship was found among bacterial counts on raw milk samples, the thermoduric counts following laboratory pasteurization, or subsequent growth in pasteurized portions held at 7.2 C for 10 days. Considerable variation in numbers of bacteria in raw milk samples from farms supplying various plants was found. Also, thermoduric count ranges varied greatly among various plant supplies. No psychrophilic organisms were recovered from 1 ml portions of freshly pasteurized samples. After 10 days storage at 7.2 C, 10.4% of the samples showed viable psychrophilic organisms. This agrees with findings by others and suggests that temperatures below 7.2 C should be recommended for storage of pasteurized milk. The number of organisms surviving pasteurization and subsequently growing in refrigerated samples varied widely among the eight plant supplies. This suggests that quality programs advocated by the various plants may have a selective action in bacterial destruction and survival of organisms able to grow at a low temperature.

Current consumer oriented publicity on freshness of foods available for sale in stores has created the general impression that age *per se* of a food is closly related to acceptability. So far as fluid milk is concerned, the question of dating of retail packages, long a relatively dormant subject, has been reopened.

Witter's *(11)* review of psychrophilic bacteria shows either laboratory or commercial pasteurization effective in destroying them in the concentrations usually found in milk. Dabbah et al. *(4)* found a psychrophilic organism that was inactivated when heated at 55 C for 30 min, but growth was initiated when this organism was held at 20 C for 48 to 72 hr in a selective medium. It is significant that Dabbah et al. were unable to find surviving organisms when the test culture was heated at 60 C for 30 min.

Grosskopf and Harper *(7)* found that fluid pasteurized milk aseptically packaged and stored at 4.4 C had a shelf life of 4 weeks. Subsequent loss of quality was attributed to growth of a spore-forming microorganism. They isolated other psychrophilic spore-formers able to survive even ultra high temperature pasteurization from about 25% of producers' milk supplies. This suggests that these organisms are associated with ultimate spoilage of pasteurized milk even when milk is protected from post-pasteurization contamination.

Many studies *(2, 3, 5, 6, 8, 9, 12)* have shown that as the storage temperature of pasteurized milk increases the bacterial population also increases, and have indicated that pasteurized milk should be held at 5 C or below to achieve maximum shelf life.

Materials and Methods

Milk samples representing all of the farm supplies from eight Pennsylvania processors were collected. Milk producers per processor varied from 17 to 162. Except for Plant F, which had can shippers, samples were collected from farm tanks by truck drivers. Samples from Plant F were collected from the weigh vat. Sterile paper sampling pipettes and sterile screw capped tubes were furnished for sample collection. Immediately after collection samples were cooled in ice and delivered to the Pennsylvania State University Creamery Laboratory.

Standard Plate Counts were immediately made on the raw samples. Thermoduric and psychrophilic counts were made after laboratory pasteurization at 62.7 C for 30 min and following 10 days storage at 7.2 C. Prior to pasteurization, the original samples were subdivided for each bacterial determination to prevent contamination and subsequent growth of stored samples. Portions to be laboratory-pasteurized were completely submerged in a thermostatically controlled water bath to insure heating of all milk contact surfaces.

All bacterial determinations were made in conformity with procedures outlined in *Standard Methods for the Examination of Dairy Products (1)*. A wide range of dilutions was used to attempt to have plates with 30-300 colonies, but the many low counts, especially on pasteurized milks, made it necessary in some instances to use data from plates with less than 30 colonies.

Results and Discussion

Bacterial counts on the raw samples are shown

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TABLE 1. PERCENTAGE DISTRIBUTION OF STANDARD PLATE COUNTS ON 500 RAW MILK SAMPLES FROM EIGHT DAIRY PLANTS[1]

Count range	Plant								% of Total
	A	B	C	D	E	F	G	H	
Up to 1,000	4.9	0	0	6.7	0	0	0	1.3	1.8
1,001 - 10,000	62.7	41.4	42.6	26.7	7.7	5.9	34.6	50.0	46.0
10,001 - 100,000	28.2	54.3	40.4	53.3	69.2	23.5	57.7	39.7	42.8
>100,000	4.2	4.3	17.0	13.3	23.1	70.6	7.7	9.0	9.4
Total %	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0

[1]No. of samples from each plant: A, 142; B, 162; C, 47; D, 15; E, 13; F, 17; G, 26; and H, 78. Total 500.

TABLE 2. PERCENTAGE DISTRIBUTION OF THERMODURIC COUNTS ON 500 MILK SAMPLES FROM EIGHT DAIRY PLANTS

Count range	Plant								% of Total
	A	B	C	D	E	F	G	H	
To 100									
1.[a]	58.4	45.7	57.5	46.7	38.4	5.9	73.1	65.4	53.4
2.[b]	36.6	38.3	48.9	53.3	30.8	5.9	57.7	51.3	41.0
101 - 1,000									
1.	29.6	32.7	25.5	33.3	30.8	11.8	15.4	24.3	28.2
2.	28.9	26.5	31.9	26.7	30.8	17.6	30.8	35.9	29.2
1,001 - 10,000									
1.	11.3	17.3	10.6	20.0	30.8	23.5	7.7	7.7	13.6
2.	17.6	20.4	12.8	20.0	38.4	29.4	7.7	11.5	17.6
>10,000									
1.	0.7	4.3	6.4	0	0	58.8	3.8	2.6	4.8
2.	16.9	14.8	6.4	0	0	47.1	3.8	1.3	12.2

[a]1. On day of pasteurization.
[b]2. After 10 days storage at 7.2 C.

in Table 1, as well as the number of samples from each plant. Data are presented in per cent of samples from each plant falling within the various count ranges.

Considerable variability is evident in the bacterial concentrations in milk samples from the eight plants, probably reflecting the quality control programs in effect. While 90.6% of the samples had Standard Plate Counts of <100,000 per ml, in a few instances excessive counts were apparent. This is especially true with Plant F. Since the U.S.P.-H.S. Grade A *(10)* bacterial standard for raw milk at the farm level is not >100,000 per ml, it is evident that several of the plants had non-complying supplies.

Standard Plate Counts on the laboratory pasteurized samples on the day of pasteurization and following 10 days storage at 7.2 C are shown in Table 2. As with the plate counts on the raw supplies, considerable variability was evident among the thermoduric concentrations on shipper's supplies of the various plants.

The most interesting fact evident from Table 2 is that increases in counts on laboratory pasteurized milks varied so greatly among the various plant supplies. Plant A had the highest percentage of raw milk supplies with counts of <10,000/ml, with only 0.7% of the plate counts on freshly laboratory pasteurized portions above 10,000/ml. However, after 10 days storage at 7.2 C, 16.9% of the Plant A supplies exceeded this level. Plant B yielded roughly similar data. Plant F, with the highest percentage of high count raw milk samples also had the highest per cent with thermoduric counts >10,000 per ml, but after 10 days storage at 7.2 C the percentage had dropped. In total, counts >10,000/ml on the pasteurized samples increased from 4.8 to 12.2%, with supplies from Plants A and B being

TABLE 3. PERCENTAGE DISTRIBUTION OF BACTERIAL COUNTS ON 500 MILK SAMPLES FROM EIGHT DAIRY PLANTS

Count range	SPC per ml			Pasteurized Psychrophilic Count per ml	
	Raw	Pasteurized			
	0 days	0 days	10 days[a]	0 days	10 days[a]
<1	—	—	—	100	89.6
1 - 100	0.2	53.4	41.0	0	0.8
101 - 1,000	1.6	28.2	29.2	0	2.6
1001 - 10,000	46.0	13.6	17.6	0	2.0
10,001 - 100,000	42.8	4.8	12.2	0	5.0
>100,000	9.4	—	—	—	—
Total per cent	100.0	100.0	100.0	100.0	100.0

[a]After 1 days storage at 7.2 C.

entirely responsible for the change.

As shown in Table 3, no recoverable psychrophiles were found in 1 ml portions of any of the freshly laboratory-pasteurized samples. After 10 days storage at 7.2 C, 89.6% of the samples still had <1/ml, and in no instance did the concentration of psychrophiles exceed 100,000/ml. Perhaps the most important observation is that 95% of the samples had psychrophilic counts of 10,000/ml or less after 10 days storage at 7.2 C.

The data from this study confirm the observations of many that freshly pasteurized milk contains few if any psychrophilic organisms surviving pasteurization that are recoverable using methods as advocated by Standard Methods. Some organisms surviving pasteurization and capable of low temperature growth over extended times have been observed by other workers. The data in the study supports these findings, with 10.4% of the samples showing microorganisms recoverable after 10 days storage at 7.2 C.

Previous work *(2, 3, 5, 6, 8, 12)* suggested that 7.2 C is less satisfactory than lower temperatures if milk is to be held for extended periods. Rather obviously, the higher the storage temperature the greater likelihood for bacterial multiplication. The finding that milk from shippers supplying certain plants appeared to harbor organisms capable of surviving pasteurziation and subsequently growing at low temperatures suggests that quality programs advocated by these plants may have resulted in selective destruction of microorganisms. The data also suggest that no relationship existed between high bacterial levels of raw milk and subsequent growth in laboratory pasteurized portions held under the conditions indicated.

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COLLABORATIVE STUDY OF CONFIRMATORY TESTING PROCEDURES FOR SOMATIC CELLS IN MILK

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Abstract

Collaborative studies were done to establish the experimental error (replicate variance) and the among-analyst variance of three modifications of the Direct Microscopic Somatic Cell Count and the Electronic Somatic Cell Count when used in laboratories that test milk routinely. The lowest total variance of logarithms of 0.00639 was obtained with the electronic procedure, followed by 0.00964 for the field counting modification of the Direct Microscopic Somatic Cell Count. The strip-reticle modification of the latter procedure had a total variance of 0.01251, and the Direct Microscopic Somatic Cell Count modification that involves counting all somatic cells on one strip without the use of a reticle had a variance of 0.03041. The coefficients of variation for the experimental error for the Electronic Somatic Cell Count and for the field counting, strip count with reticle, and strip count without reticle modifications of the Direct Microscopic Somatic Cell Count were 8, 17, 14, and 17%, respectively.

The abnormal milk program promulgated by the Food and Drug Administration requires that milk which exceeds certain screening test scores be tested by a confirmatory procedure to establish the somatic cell count. At present, there are two approved methods for confirmatory testing; namely, the Direct Microscopic Somatic Cell Count and the Electronic Somatic Cell Count *(6)*. The microscopic procedure has three alternate techniques for counting somatic cells and these are *(a)* counting the cells in a given number of microscopic fields *(2)*, *(b)* counting the cells in one field-wide strip across the diameter of a 1-cm² area circular film *(2)*, or *(c)* counting the number of cells that appear between the lines of a reticle when the horizontal and verticle diameters of two replicate milk films are traversed *(3)*. We have received many requests for an evaluation of the relative precision of these techniques when used in routine testing laboratories. This paper reports the results of collaborative studies on the precision of the direct microscopic and electronic somatic cell counting procedures when done in routine testing laboratories.

Materials and Methods

Direct Microscopic Somatic Cell Count

States were selected by use of a random number table. The state-designated Milk Laboratory Survey Officer(s) was asked to name analysts in milk testing laboratories who were qualified to count milk somatic cells microscopically, and each analyst was asked to participate in the collaborative study. Fifty-four agreed to participate.

Ten samples of 48-hr old bulk tank milk were obtained from farm tanks and were used to make milk films in both known and blind duplicates for a total of 40 films to be counted by each analyst by each of three counting procedures. Milk films were prepared by use of a metal syringe in accordance with the procedure described in *Standard Methods for the Examination of Dairy Products (1)*. The slides were packaged and sent to each analyst together with instructions for counting the films. Each analyst stained his own slides using the Levowitz-Weber modification of the Newman-Lampert stain *(1)*. Complete sets of data were returned by 26 analysts. All strip-reticle counts were made with the 4-mm reticle.

The objective of the statistical analysis of results from this study was to obtain estimates of the two variance components, the experimental error (error on blind replicates by one analyst), and the variation between the analysts in the study. These components are similar to those proposed by Youden *(7)* for the AOAC; he refers to these as random and systematic error, respectively.

Electronic Somatic Cell Count

The technique developed in our laboratories for using a Coulter[1] Counter to count somatic cells *(6)* was evaluated for precision in milk testing laboratories by selecting laboratories in the United States that indicated a desire to use this procedure for routine testing of milk samples for somatic cells, and which agreed to participate in a collaborative study of the precision of the method. These laboratories composed the total known population of laboratories using the Electronic Somatic Cell Count procedure.

A series of four sets of 20 samples each was sent to all participating laboratories, and the results were obtained. The Coulter Counters were calibrated either by a representative of Couler Electronics or by the laboratory using the counter. An indication of the somatic cell count was included with the initial set of 10 samples so that the participating laboratories could check the calibration of their instruments. These data were not used in the calculation of the results from the study inasmuch as count information was given the participants. Following this, a series of four sets of samples, each con-

[1]Mention of commercial products implies neither endorsement nor criticism by the Food and Drug Administration.

TABLE 1. COMPONENTS OF VARIANCE OF THREE MODIFICATIONS OF THE DIRECT MICROSCOPIC SOMATIC CELL COUNT

Component of variance	Modification of DMSCC		
	Field count	Strip count	Strip-reticle count
	(Variance)		
Experimental error	0.00527 (250)[a]	0.00567 (250)	0.00357 (250)
Among-analysts	0.00437	0.02474	0.00894
Total variance	0.00964	0.03041	0.01251
	(Coefficient of variation) (%)		
Experimental error	17	17	14

[a]Degrees of freedom.

TABLE 2. EFFECT OF COUNTING ONE, TWO, AND FOUR STRIPS ON THE EXPERIMENTAL ERROR OF THE STRIP RETICLE MODIFICATION OF THE DMSCC

Strips counted	Experimental error		DMSCC/ml	
	Variance	Coeffcient of variation	Geometric mean	Range
1	0.00684 (250)[a]	19	1,400,000	630,000 – 1,900,000
2	0.00437 (250)	15	1,400,000	690,000 – 1,800,000
4	0.00357 (250)	14	1,400,000	730,000 – 1,800,000

[a]Degrees of freedom.

TABLE 3. TIME REQUIRED TO COUNT THE SOMATIC CELLS IN ONE SAMPLE BY EACH OF THREE COUNTING MODIFICATIONS OF THE DIRECT MICROSCOPIC SOMATIC CELL COUNT

	Modification of the DMSCC		
	Field count	Strip count	Strip-reticle count
Average counting time (min)	4.7	6.2	8.3
Range of counting times (min)	2-10	3-20	3-18

taining 10 samples in blind duplicate, was sent to each laboratory over a period of approximately 1 year.

The statistical analysis of the data was primarily concerned with the same parameters as those obtained from the study of the microscopic counting procedure; namely, the experimental error and the variation between analysts. All counts were transformed to $\log_{10}$ and it was assumed that these transformed values were normally distributed. Variances are reported in log units.

RESULTS AND DISCUSSION

Data from 26 analysts were used for the initial calculation of means, variance among analysts, and the experimental error of the three modifications of the Direct Microscopic Somatic Cell Count. After these calculations were performed, one analyst was found to have extremely large experimental error values on all three modifications, and his data were deleted as outliers. All variance values were recalculated using the data from 25 analysts (Table 1). The total variance of the strip-count modification without reticle was large primarily because of an exceptionally high variance among analysts. This was caused by the low counts recorded by four analysts for all samples examined by this modification. The among-analyst variance was about one, four, and two times as large as the experimental error for the field, strip, and strip-reticle modifications, respectively. This component has generally been the major one for the studies we have done on the Standard Plate Count (*4*, *5*) and on confirmatory tests for somatic cell count. The experimental error or the variation in results each analyst obtained between blind duplicates that he tested was slightly less for the strip-reticle modification than for the other two methods of counting (coefficient of variation of 14% opposed to 17%).

Since the strip-reticle method involves counting four strips on two films, data were obtained regarding the effect on experimental error of counting a horizontal and a verticle diameter-wide strip on one film as well as, more simply, counting a single horizontal strip on one film (Table 2). Counting a single strip was inferior in precision to a count involving four strips as measured by experimental error. There was little difference between the experimental errors obtained for a count involving two as opposed to four strips.

The time required to count one sample (no consideration was given to slide preparation time) by each of the modifications was obtained from the analysts (Table 3). A wide range of counting times was reported; the field counting modification gave the shortest average counting time for the 25 analysts.

Experimental error was calculated for each laboratory participating in the collaborative study of the Electronic Somatic Cell Count for each set of split samples (Table 4). In general, the experimental error was low for all laboratories with an occasional exception. The experimental error expressed as variance was 0.00133, or the coefficient of variation was 8%.

Since the major component of variance has been the among-analyst component for most techniques we have collaboratively tested, we calculated this value for the Electronic Somatic Cell Count and found it to be 0.00506 or almost four times the experimental error component. The total variance for

TABLE 4. EXPERIMENTAL ERROR FOR FOUR SETS OF SAMPLES SENT TO EIGHT LABORATORIES FOR ELECTRONIC SOMATIC CELL COUNT

Laboratory	Experimental error[a]					Coefficient of variation on pooled results (%)
	Samples					
	Set 1	Set 2	Set 3	Set 4	Pooled	
1	0.00129(10)[b]	—	0.00118	0.00026	0.00091(30)	7
2	0.00098	0.00023	—	0.00050	0.00051(30)	5
3	0.00079	0.00079	0.00059	0.00012	0.00057(40)	5
4	0.00232	0.00169	0.00023	0.00100	0.00131(40)	8
5	0.00083	0.00089	—	0.00425	0.00199(30)	10
6	0.00084	0.00035	0.00533	0.00028	0.00170(40)	9
7	0.00530	0.00123	0.00070	0.00092	0.00204(40)	11
8	—	0.00023	0.00377	0.00062	0.00153(30)	9
Pooled	0.00176(70)	0.00077(70)	0.00196(60)	0.00099(80)	—	—

[a]The overall error was 0.00133 with 280 degrees of freedom and an 8% coefficient of variation.

[b]Degrees of freedom.

the Electronic Somatic Cell Count was the sum of the two components, or 0.00639. As was true in the collaborative study of the Direct Microscopic Count, all data were from laboratories engaged in the routine testing of milk. Data from our laboratory were not included since we are not engaged in routine analyses.

When the data on precision of the microscopic and electronic testing procedures for milk somatic cells are compared, the total variance of 0.00506 for the electronic count is substantially lower than that for any of the microscopic procedures. We believe this is the result of the larger sample counted in the electronic procedure and the objectivity of an electronic count. Undoubtedly, lack of fatigue is a factor in the electronic procedure and enables a lower total variance.

An evaluation of the relative precision of the three modifications of the Direct Somatic Cell Count together with a consideration of the time spent in performing the counting phase of the procedure indicate there is little difference with the exception of the among-analyst variance in the strip-counting modification without reticle. This lack of difference is further substantiated by the observation that the among-analyst component of variance for the field counting modification is lower in this study than one would anticipate from other unpublished data we have on the precision of this modification when used in routine testing laboratories.

To put the absolute value of the variance in perspective, variance results from split samples of milk sent to routine testing laboratories for testing by Standard Plate Count typically give an experimental error of about 0.00500 expressed as variance of $\log_{10}$ count per milliliter and an among-analyst variance of 0.00700 for a total variance of 0.01200 *(4, 5)*. Thus, the electronic procedure had about one-half the total variance of the Standard Plate Count when done on milk products. The field counting modification of the Direct Microscopic Somatic Cell Count had about three-fourths the total variance of the Standard Plate Count, and the strip-reticle modification gave a total variance about the same as that of the plating procedure.

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REPORT OF THE COMMITTEE ON FOOD EQUIPMENT SANITARY STANDARDS, 1969-1970

The IAMFES Committee on Food Equipment Sanitary Standards, known hereafter as the Committee, is charged with the responsibility of cooperating with other interested health organizations and related industries in the formulation of sanitary standards and educational materials for the fabrication, installation, and operation of food equipment and to present to the membership those standards and educational materials which the Committee recommends be endorsed by the Association.

The purpose of this cooperative program is to aid industry in improving the design, construction, and installation of equipment so that it will lead to easy cleaning and proper functioning when placed into service in food establishments. It is the Committee's further purpose to cooperate with industry in the preparation of standards or guidelines which public health agencies will accept, thereby securing uniformity in the manufacture and nationwide acceptance of such equipment.

The following report will outline the Committee's activities during the past year in working with two health and industry organizations (National Sanitation Foundation's Joint Committee on Food Equipment Standards and the National Automatic Merchandising Association's Automatic Merchandising Health-Industry Council) and progress in meeting its purposes and objectives. It is expected these organizations will be the two groups that the Committee will work with during the coming year.

NATIONAL SANITATION FOUNDATION (NSF)

The Committee was represented at the 1970 meeting of the National Sanitation Foundation's Joint Committee on Food Equipment Standards, where action was taken on several proposals; and prior to the meeting, the Committee reviewed and submitted comments on each draft of these proposals. Since the meeting, the Committee has also reviewed and submitted comments on proposed changes to standards.

Basic criteria for special equipment and/or devices

At the request of the Joint Committee, the NSF staff reviewed the definitions now contained in the various NSF Food Equipment Standards and Criteria as well as those contained in the NAMA, BISSC and 3A Standards for the purpose of promoting a higher degree of uniformity of interpretations and applications. The proposed basic definitions for the terms accessible, cleanable, food, food contact surfaces, non-food contact surfaces, readily accessible, readily removable, sanitizing, sealed, smooth, splash zone, and toxic with some modifications were recommended for approval by the public health representatives. These basic definitions eventually will be incorporated in each of the NSF Standards, and hopefully will be incorporated in other National Food Equipment Standards.

Standards for soda fountain and food service equipment

At the 1968 Joint Committee meeting, the public health representatives recommended that all wheeled utensil storage equipment be tightly enclosed to a height of 18 inches from the floor. However, during the past two years, industry reviewed this proposal and experiencing some difficulty to comply therewith received approval by a majority of the public health representatives at the 1970 Joint Committee meeting to recind the previous recommendation. The action taken previously probably should have provided greater safeguards, as the PHS Code is not only concerned with protection of clean utensils from splash but also from all other forms of contamination.

Standard for cooking and warming equipment

The NSF staff's survey of industry and users of equipment as to the need and feasibility of thermometers in cooking and warming equipment was reported to the public health representatives. Because of solid opposition from both the manufacturers and users of cooking and warming equipment to this proposal at the present time, no action was taken to amend Standard No. 4. However, the Joint Committee requested the Foundation to continue exploring ways and means of sensing and indicating food temperatures, with particular attention to hot food storage cabinets. It is believed that all food temperature control equipment (hot and cold) should be equipped with appropriately located accurate temperature indicating equipment, and the appropriate standards will be so amended in the near future to accomplish this public health objective.

(Continued on Page 293)

THE MICROBIAL CONTENT OF SOME SALADS AND SANDWICHES AT RETAIL OUTLETS[1, 3]

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Abstract

Samples of commercially prepared salads and sandwiches were examined for microbial content. Total counts varied considerably among the samples of different products and between samples of the same type product. Pimento cheese spread had the highest median count (5.4×10^6/g) and cole slaw had the lowest median count (3.6×10^3) for the salad products. Median counts for the different type sandwiches ranged between 1×10^5 and 2.7×10^5/g. Approximately 40% of the samples contained <10 coliforms/g. The median yeast and mold counts for these products generally were $<1 \times 10^4$/g. Approximately 39% of the salads and 60% of the sandwiches contained coagulase positive staphylococci at levels which exceeded 1×10^5/g for some salads and 1×10^6/g in one sandwich. Possible *Salmonella* isolates were obtained from one salad sample and two sandwiches. Two salad samples and one sandwich were positive for *Clostridium perfringens*.

Commercially prepared salads and sandwiches are included in a large group of products known as convenience foods. Many of these, including the salads and sandwiches, are not heated or otherwise processed to ensure the absence of pathogenic and spoilage organisms in the finished product. Thus, the microbiological quality of these foods depends on the quality of the raw materials, sanitation practices during production, and the amount of microbial growth in the finished product.

Little information is available concerning the microbial content of commercially prepared salads and sandwiches. Prewrapped sandwiches (108 samples) purchased in the Cincinnati, Ohio area were included in a study of different market foods *(6)*. Total counts for these sandwiches ranged from less than 10 to 3×10^7/g. Coliform counts ranged from less than 10 to 4.6×10^4/g with a mean of 4.3×10^3/g and a median of 9/g. No salmonellae or shigellae were detected. These sandwiches had a 16.7% incidence of coagulase-positive staphylococci and 9.2% contained *Clostridium perfringens*.

The purpose of the present study was to determine the microbial content of some locally produced salads and sandwiches as they appear on the retail market in an attempt to gain some insight into potential microbiological problems associated with these products.

Materials and Methods

Collection of samples

Samples were purchased from retail outlets in the Raleigh, North Carolina area. The non-sandwich products were sold from refrigerated display cases. Most sandwiches were displayed and sold at ambient temperature. All samples were transported to the laboratory packed in ice and then held at 4 C for up to 24 hr before microbiological assays were made.

Preparation of samples for analysis.

One hundred grams of salad was aseptically weighed into a sterile, chilled Waring Blendor bowl containing 300 ml of sterile distilled water. Following 2 min of high speed blending, the pH of the sample was measured and then adjusted to 7.0 with sterile 2 N NaOH. Sandwich samples were prepared similarly to the salads, except that the entire sandwich was weighed and blended with sufficient sterile distilled water to make a 1:4 dilution.

After adjusting the pH, blended samples of salads and sandwiches were diluted as required for microbiological analyses in sodium phosphate buffer. Appropriate dilutions were plated in duplicate for all plate counts.

Microbiological analyses and media

Total counts were determined on pour plates of Plate Count Agar (Difco). Dilutions of 10^{-1} to 10^{-7} of sample were plated and plates incubated at 32 C for 48 hr. Violet Red Bile agar (Difco and BBL) was used for enumerating coliforms. Plates were inoculated with 10^{-1} through 10^{-5} dilutions of the food material and counted after 18-24 hr of incubation at 35 C. Yeasts and molds were enumerated by plating 10^{-2} through 10^{-6} dilutions of sample on Potato Dextrose Agar (PDA, Difco) acidified to pH 3.5 with sterile 10% tartaric acid and Cooke Rose Bengal Agar (Difco) containing 35 μg chlortetracycline/ml. The plates were incubated at ambient temperature (ca. 23 C) for 5 days. Samples were diluted from 10^{-2} to 10^{-5} and plated on spread plates of Tellurite Polymyxin Egg Yolk Agar (TPEY, BBL) for enumeration of staphylococci. Typical staphylococcal colonies were counted after 24 and 48 hr incubation at 35 C. These colonies or a representative number were transferred into Brain Heart Infusion Broth (BHI, BBL) and incubated at 35 C for 24 hr. Tube coagulase tests were done on the broth cultures using rabbit coagulase plasma (BBL). The

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[3]The use of trade names in this publication does not imply endorsement by the North Carolina Agricultural Experiment Station of the products named, nor criticism of similar ones not mentioned.

TABLE 1. THE pH OF PREPARED SALADS

Product	No. of samples	pH range	Median pH
Pimento cheese	15	4.9-5.7	5.4
Ham salad	8	4.7-5.0	4.8
Chicken salad	10	4.5-5.9	5.2
Bar-B-Q pork	19	4.5-5.8	5.0
Potato salad	11	4.2-5.2	4.7
Cole slaw	5	4.0-4.5	4.2

number of coagulase-positive staphylococci was determined by multiplying the staphylococcal colony count on TPEY by the fraction of coagulase-positive isolates for each sample. For detection of *Salmonella* a 100-ml aliquot of the blended pH-adjusted sample (25 g of food material) was transferred to 400 ml of Lactose Broth (Difco) prepared at 1.25 × the concentration recommended by the manufacturer. Following incubation, 1 ml of the lactose broth culture was transferred to 9 ml of Selenite Cystine Broth (BBL). Streak plates on Brilliant Green Agar, Bismuth Sulfite Agar, and Salmonella Shigella Agar, all Difco products, were prepared from Selenite Cystine tubes showing growth. Suspect colonies from the selective media plates were transferred to Triple Sugar Iron Agar (TSI, Difco) slants. Suspect isolates from the slants were tested for urease and lysine decarboxylase activity by inoculation into Urease Test Broth (BBL) and Lysine Iron Agar (Difco and BBL), respectively, and for their ability to ferment lactose and dulcitol in fermentation tubes of Purple Broth Base (BBL) containing 1% carbohydrate. All of the above tests were incubated at 35 C for 24 hr. Suspect isolates also were tested for agglutination with *Salmonella* 0 polyvalent antiserum (BBL). A direct plating and an enrichment technique were used to detect *Clostridium perfringens*. For direct plating, pour plates were made on Sulfite-Polymyxin-Sulfadiazine (SPS) agar (2) prepared from the individual ingredients. Enrichment cultures were prepared by transferring 8 ml of blended sample into tubes containing 8 ml double strength Fluid Thioglycollate Broth (Difco). The tubes were incubated at 35 C and observed daily for 7 days for gas production. Streak plates were prepared from the gassing tubes on SPS agar. All plates were overlaid with SPS agar and incubated in Gaspak jars (BBL) at 35 C. Plates were checked after 24 hr, those with no growth were incubated an additional 24 hr in recharged jars. Representative isolates from both types of plates were tested for motility, and ability to reduce nitrate, liquify gelatin, and from spores.

RESULTS

pH

The pH of the blended salads ranged from 4.0 to 5.9 (Table 1). Approximately 58% of the samples had values of pH 5.0 or less. Only 15% of the samples had a pH >5.5. Some products (chicken salad, Bar-B-Q pork, and potato salad) exhibited ranges of 1 pH unit or more.

The pH values for sandwich samples are not given. Because of the heterogeneous nature of the products, the pH of the blended samples would not be meaningful.

Total counts

Total counts ranged from a few hundred to nearly a billion organisms per gram (Table 2). Approximately 36% (19 of 53) of the salad samples and 16% (10 of 62) of the sandwiches had counts greater than a million per gram. Only seven of the salads and no sandwiches had <1,000 organisms per gram.

Coliform counts

Approximately 57% of the samples contained <100 coliforms/g and 40% contained <10/g. Only 12% had counts $>1 \times 10^3$ coliforms/g.

Yeast and mold counts

Counts on acidified PDA and Cooke Rose Bengal Agar did not differ significantly. The data shown in Table 4 were obtained on PDA. The yeast and mold counts ranged from <100 to 3.8×10^7/g. Only six samples (one of pimento cheese, one Bar-B-Q and four ham sandwiches) had counts >1 million per gram. Ten samples contained <100 yeasts and molds per gram.

TABLE 2. TOTAL COUNTS IN SOME PREPARED SALADS AND SANDWICHES

Product	No. of samples	Range of count/g	Median count/g
	Salads		
Pimento cheese	9	1.4×10^3-2.9×10^7	5.4×10^6
Ham salad	7	8.0×10^2-8.7×10^6	1.5×10^6
Chicken salad	8	1.2×10^4-1.4×10^7	6.7×10^5
Bar-B-Q pork	15	1.0×10^2-8.0×10^7	3.0×10^5
Potato salad	9	4.0×10^2-1.2×10^5	1.5×10^4
Cole slaw	5	5.0×10^2-3.4×10^4	3.6×10^3
	Sandwiches		
Chicken salad	15	6.6×10^3-2.4×10^6	2.7×10^5
Ham and chopped ham	20	2.6×10^4-7.0×10^8	1.3×10^5
Ham salad	7	1.5×10^4-1.8×10^5	1.1×10^5
Miscellaneous[a]	20	1.3×10^3-5.3×10^7	1.0×10^5

[a]The miscellaneous sandwiches included roast beef, egg salad, bologna, sliced turkey, cheeseburger, and smoked sausage sandwiches.

TABLE 3. COLIFORM COUNTS IN PREPARED SALADS AND SANDWICHES

Product	No. of samples with detectable coliforms/total	Range of counts/g	Median counts/g
	Salads		
Pimento cheese	5/9	<10-3.5×10^3	2.0×10^1
Ham salad	6/8	<10-1.5×10^4	3.0×10^2
Chicken salad	6/8	<10-2.5×10^4	1.9×10^3
Bar-B-Q pork	10/15	<10-1.6×10^4	2.0×10^1
Potato salad	1/9	<10-2.0×10^1	<10
Cole slaw	0/5	—	<10
	Sandwiches		
Chicken salad	14/15	<10-5.7×10^3	3.3×10^2
Ham and chopped ham	11/20	<10-5.6×10^2	1.5×10^1
Ham salad	6/7	<10-5.0×10^2	1.6×10^2
Miscellaneous[a]	11/20	<10-1.2×10^5	4.0×10^1

[a]The miscellaneous sandwiches included roast beef, egg salad, bologna, sliced turkey, cheeseburger, and smoked sausage sandwiches.

TABLE 4. YEAST AND MOLD COUNTS IN PREPARED SALADS AND SANDWICHES

Products	No. of samples with counts/total	Range of counts/g	Median count/g
	Salads		
Pimento cheese	8/9	<100-3.4×10^6	3.6×10^3
Ham salad	8/8	4.0×10^2-1.6×10^5	1.2×10^5
Chicken salad	8/9	<100-1.1×10^5	1.0×10^4
Bar-B-Q pork	13/15	<100-9.1×10^6	2.0×10^2
Cole slaw	2/5	<100-2.0×10^2	<100
	Sandwiches		
Chicken salad	15/15	4.0×10^2-1.7×10^5	8.2×10^3
Ham and chopped ham	20/20	2.0×10^2-3.8×10^7	1.1×10^4
Ham salad	7/7	3.9×10^3-1.4×10^5	9.9×10^3
Miscellaneous[a]	14/18	<100-5.6×10^6	8.7×10^2

[a]The miscellaneous sandwiches included roast beef, egg salad, bologna, sliced turkey, cheeseburger, and smoked sausage sandwiches.

Coagulase positive staphylococci

Approximately 76% of the samples contained organisms which developed as typical staphylococcal colonies on TPEY agar. However, coagulase positive isolates were obtained from only 50% of the total samples, i.e., approximately 39% of the salads and 60% of the sandwiches. For the salads, the incidence of these organisms was highest for chicken salad with 6 of 10 samples containing the organisms and lowest for cole slaw, 1 of 5 samples (Table 5). For the sandwiches, coagulase positive staphylococci were isolated from 5 of 7 ham salad sandwiches (the highest incidence) and 10 of 19 sliced and chopped ham sandwiches (the lowest incidence). The detectable counts of these organisms in the salads ranged from 1×10^2 (the minimum detection level) in one sample of potato salad to 2×10^5/g in a sample of pimento cheese spread and a sample of chicken salad. Counts for sandwiches ranged from 1×10^2/g in one ham salad sandwich to 3×10^6/g in a ham sandwich.

Preliminary experiments have indicated that staphylococci may not be able to grow in the salads. Commercial samples of chicken salad (pH 5.2), pimento cheese (pH 5.4) spread, and ham salad (pH 4.8) inoculated with approximately 10^6 coagulase positive staphylococci/g were incubated at 4 and 37 C. At 37 C, daily counts on TPEY revealed a steady decrease in the number of staphylococci in each of the three products. In chicken and ham salad, the staphylococcal count decreased to <100/g in 4 days. In pimento cheese, staphylococci could be detected after 7 but not after 8 days of incubation. The rate of decline was much slower at 4 C than at 37 C. After 15 days of storage at 4 C, the staphylococcal count was 5.4×10^4/g in chicken salad, 9.0×10^4 in ham salad, and 5.0×10^5 in pimento cheese spread.

Total counts were determined for samples held at 37 C. The count in chicken salad increased from an initial level of 1.1×10^7 to a maximum of 5.3×10^8/g after 2 days. In 3 days the count in ham

TABLE 5. COAGULASE POSITIVE STAPHYLOCOCCI COUNTS IN PREPARED SALADS AND SANDWICHES.

Product	No of samples with counts/total	Range of counts/g in samples containing the organism
Salads		
Pimento cheese	6/13	2×10^3-2×10^5
Ham salad	2/8	1×10^4-2×10^4
Chicken salad	6/10	3×10^3-3×10^4
Bar-B-Q pork	5/19	3×10^3-2×10^5
Potato salad	5/11	1×10^2-8×10^4
Cole slaw	1/5	1×10^3
Sandwiches		
Chicken salad	9/15	2×10^2-2×10^4
Ham and chopped ham	10/19	4×10^2-3×10^6
Ham salad	5/7	1×10^2-2×10^4
Miscellaneous[a]	14/21	1×10^3-2×10^5

[a]The miscellaneous sandwiches included roast beef, egg salad, bologna, sliced turkey, cheeseburger, and smoked sausage sandwiches.

salad increased from 5.0×10^6 to 8.1×10^7/g and in pimento cheese from 5.1×10^6 to 3.0×10^8.

Salmonella

Organisms tentatively classified as *Salmonella* were isolated from only three samples, one sample of Bar-B-Q, one roast beef sandwich, and one cheeseburger. These isolates produced acid butts and alkaline slants, but were H_2S negative on TSI agar; were urease negative and lysine decarboxylase positive; did not ferment lactose; were plus-minus in terms of dulcitol fermentation; and agglutinated poly-o-somatic antiserum.

Clostridium perfringens

Clostridium perfringens was isolated from three products, two samples of Bar-B-Q and one smoked sausage sandwich. For the two Bar-B-Q samples, *C. perfringens* was detected by the direct plating technique at concentrations of 12 and 60/g. No *C. perfringens* isolates were obtained from the enrichment cultures made from these two samples. For the smoked sausage sandwich, the organism was detectable by enrichment culture but not by direct plating. The lowest dilution of sample plated was 1:4.

DISCUSSION

Refrigeration storage and the generally low pH of the salads would inhibit growth of most microorganisms. Lactic acid bacteria, some of which are both psychrophilic and acid tolerant, might grow and contribute to the total count. Yeasts and molds also might be able to grow in these products. However, except for high counts in a few samples, the yeast and mold counts in most of the salads did not indicate extensive growth of these organisms. Improper refrigeration would enhance the possibility for growth particularly in samples with the higher pH values.

Ingredients such as natural Cheddar cheese used in pimento cheese spread by some producers and raw vegetables used in many of the salads and sandwiches may have contributed to the high total counts in some of these products. These ingredients often have high microbial populations *(6)*.

There is the possibility that initial contamination levels of salmonellae and coliforms were higher than those found in samples obtained at the retail level. Acetic acid (vinegar) is added to all the salads. Apart from lowering the pH, acetic acid has an inhibitory or even lethal effect on some coliforms and salmonellae *(3, 4, 5)*.

Microbial growth in the sandwiches is possible since the sandwiches may be held at room temperature for several hours before sale. Although parts of the sandwich would have conditions similar to those in the salads (i.e. low pH and the presence of acetic acid), the heterogeneous nature of the sandwich seemingly would provide areas where these factors would have little effect on growth. Hall et al. *(6)* found that the mean total count of sandwiches increased from an initial level of 4.2×10^6/ml to 2×10^8 after 48 hr storage at room temperature. Although this storage time may have been excessive from a practical viewpoint, the results do show that growth can occur in the sandwiches.

The true incidence of coagulase-positive staphylococci in these foods no doubt is higher than the stated value since staphylococci present at levels of less than 1×10^2/g would not have been detected by the methods used. Also, because of the tendency for staphylococci to be killed when inoculated into some of the salads, the levels of these organisms found in retail samples may be lower than the levels initially contaminating the products.

Staphylococci probably would be unable to grow in properly refrigerated salads. Also, as suggested by preliminary trials, growth is unlikely even in salads held at 37 C. Thus, the levels of coagulase-positive staphylococci found in the salads appear to originate in raw materials and/or from contamination during production, giving cause to question the quality of the raw materials and the sanitation practices. Growth of staphylococci in raw materials could be accompanied by production of enterotoxin which would be introduced into the final product.

Comments concerning sources of staphylococcus contamination of salads also would apply to sandwiches. In addition, given that the sandwiches may be held at room temperature for several hours there is the potential danger of growth and enterotoxin

production in these products. Hall et al. *(6)* demonstrated that staphylococci could grow in sandwiches held at room temperature for 48 hr, although the populations did not reach hazardous levels. Sandwiches in this experiment were contaminated with coagulase-positive staphylococci at levels detectable by enrichment culture only. Following incubation, counts had increased but the highest count was only 5.8×10^3 staphylococci/g. Perhaps the staphylococci were inhibited or outgrown by other organisms in the sandwiches. The poor competitive abilities of staphylococci are well known. As indicated above, staphylococci were present in very low numbers, whereas the initial mean total count of these sandwiches was 4.2×10^6/g. In the present study the staphylococcal contamination levels were much higher than in the above study. The higher populations would not only enable the staphylococci to compete more effectively, but also would increase the likelihood of the organisms being located in an area of the sandwich where pH or other factors would not affect growth.

ACKNOWLEDGEMENT

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REPORT OF COMMITTEE ON FOOD EQUIPMENT SANITARY STANDARDS 1969-1970

(Continued from Page 288)

Standard for (soft ice cream) dispensing freezers

The Joint Committee in 1967 initiated a comprehensive review of Standard No. 6 because of the numerous changes in the ice cream field within the past 5 years. This review has been completed, and numerous revisions, including those mandating facilities to assure continuous storage of the ice cream mix (whether integrally or remotely located) at safe temperatures, have been approved by the Joint Committee. These revisions which have been under consideration for several years by both public health and industry members were made for the purpose of updating the Standard and making it more applicable to public health, industry, and consumer needs.

During the final review of the proposed revisions to Standard No. 6, the public health representatives expressed a need for cautioning users of this equipment that use of the freezers for other than dairy products, particularly for acid type foods, might result in food poisoning caused by dissolution of the heavy metals found in such equipment. Furthermore, they requested the Foundation to review this matter with the manufacturers of the freezers to determine the feasibility of requiring a cautionary statement in their instruction manual and on the cleaning instruction label. Should this not suffice to prevent a food-borne incident from the heavy metals, it would probably necessitate a change in the Standard requiring use of other materials.

Standard for walk-in and reach-in refrigerators and freezers

After several years of discussions and studies by both public and industry representatives and failure by many members of the industry to comply with the requirement, effective July 1, 1969, that all joints and seams in the food zones (the food zone is defined by Standard No. 7 to include the entire interior of a reach-in refrigerator or freezer) should be filled to conform with Item 3.01 has been rescinded by action of a majority of the public health representatives. Furthermore, they approved an amendment to Item 4.052 stating that "Open joints and seams in the walls and ceilings need not be filled and finished to conform to Item 3.01". This latter statement would contradict the following first sentence in Item 4.052: "Joints and seams in the food zone shall be closed and sealed."

This Committee objected to rescinding the statement requiring joints and seams to comply with Item 3.01 and to the above amendment to Item 4.052 especially without further amending the Standard to delete the concept that the entire interior of a reach-in refrigerator or freezer is considered a food zone.

New standards

The proposed Standards for Dinnerware; Air Curtains for Entrance Ways to Food Service Establishments; Laminated Plastics for Surfacing Food Service Equipment; and Pots, Pans, and Utensil Spray-Type Washing Machines were thoroughly reviewed, appropriately amended, and finally recommended for adoption by the Joint Committee. The manufacturers and users of such equipment and/or utensils and public health representatives have long recognized the need for properly designed and constructed food service facilities of the nature. However, the application of the Standard for Laminated Plastics will be limited to use in equipment evaluated under Standards Nos. 1 and 2; and the

(Continued on Page 299

USE OF YEAST BETA-GALACTOSIDASE IN MILK AND MILK PRODUCTS[1]

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Abstract

Experiments were carried out to study factors affecting the enzyme activity of β-galactosidase of *Saccharomyces fragilis* NRRL Y-1109 in milk products. Both the type of lactose-containing substrates and their method of preparation greatly affected β-galactosidase activity. Lactose in an aqueous solution was hydrolyzed more easily than it was in milk products. Of milk products tested, whey was the best substrate for the enzyme. Milk solids, other than lactose, exhibited some inhibitory effect on hydrolysis of lactose by the *S. fragilis* β-galactosidase. The maximum rate of hydrolysis in milk products was obtained when milk or whey was fortified with 0.1 M potassium sulfate and 10^{-4} M manganese chloride.

Nonfat dry milk and whey powders, in which portions of the lactose were hydrolyzed to simple sugars, were prepared. These products were of good flavor, appearance, and stability.

Use of β-galactosidase in dairy products is one of the most promising applications of enzymes in food processing. Hydrolysis of lactose by β-galactosidase (commonly called "lactase") will alleviate the problems of low solubility and lack of sweetness which are often experienced in concentrated milk products and ice cream. Pomeranz *(6)* has reviewed the possible ways in which this enzyme might be utilized in the food industries.

In earlier studies of lactose hydrolysis in dairy products, the β-galactosidases from lactose-fermenting yeasts were generally used *(7, 9, 12, 13, 15)*. Some factors affecting hydrolysis of lactose by these enzymes have been reported. However, the specific strains of yeast from which these β-galactosidases were obtained were never indicated.

Previous studies have shown that β-galactosidases from various lactose-fermenting yeasts vary in some of their chemical and physical properties *(3, 18)*. This investigation was made primarily to study the factors affecting β-galactosidase activity of *Saccharomyces fragilis* Y-1109 in milk and milk products. In addition, studies were also made on properties of dried milk products in which a portion of lactose had been hydrolyzed by β-galactosidase.

[1]Research supported by the College of Agricultural and Life Sciences, University of Wisconsin, Madison and by the Cooperative State Research Service, United States Department of Agriculture.

Materials and Methods

Organism

Saccharomyces fragilis NRRL Y-1109 was obtained from USDA Northern Utilization Research and Development Division Laboratories. The yeast was maintained by occasional transfers on slants of agar medium containing 20 g lactose, 5 g yeast extract, and 15 g agar per liter.

Growth

Studies of the enzyme from *S. fragilis* Y-1109 were made with enzyme extracted from large quantities of dried yeast produced in the University of Wisconsin pilot plant. *Saccharomyces fragilis* Y-1109 was grown on a medium containing 100 g lactose, 5 g yeast extract, 3 g K_2HPO_4, and 1 g ammonium sulfate per liter. The pH of the medium was adjusted to 4.5 with phosphoric acid. A 750 gal stainless steel fermentor with mechanical agitation was used to grow 400 gal quantities of yeast culture. The medium was inoculated with 45 l of *S. fragilis* culture, which had been actively growing in a sterile medium for 12 hr. The temperature of the fermentation was maintained at 28 C, the pH maintained at 4.5 by addition of ammonia, and the aeration rate held at 40 ft^3 per min. When maximum growth was reached, yeast cells were harvested and washed in a DeLaval PX-309 sludge separator. The yeast cream obtained was spray-dried according to the method of Stimpson *(14)* on the University of Wisconsin tower dryer *(2)* to obtain a β-galactosidase-active, zymase-inactive, preparation.

Cell-free extracts

All extractions were carried out in a cold room at 5 C. Potassium phosphate buffer (0.1 M at pH 7.0) containing 10^{-4} M $MnCl_2$ was used routinely in extraction of the enzyme. The procedure for extracting the enzyme from the dried yeast cells consisted of mechanically agitating a 10% (w/v) yeast slurry in the pH 7.0 buffer for 16 hr. Approximately 5% (w/v) sterile sea sand was added to aid in disruption of the yeast cells. Cell-free extracts were obtained by centrifuging the treated cell preparation at 1000 × g for 15 min.

Enzyme and lactose assays

Enzymatic activity was determined by the amount of glucose released from hydrolysis of lactose. The procedure involved two steps: hydrolysis of lactose in dairy products by the enzyme preparation, and microdetermination of glucose in the hydrolysis products.

Hydrolysis of lactose was performed in test tubes in a water bath at 40 C. Two and one-half milliliters of milk or whey, fortified with 10^{-4} M $MnCl_2$, were placed in the test tubes and equilibrated in the bath. All milk products were adjusted to pH 6.5 with either phosphoric acid or potassium hydroxide. The quantity of enzyme extract added to the milk product was the extract obtained from a measured

amount of dried yeast cells equivalent to 2% of the lactose content of the product. After a 1 hr incubation period, the activity was stopped by heating the tubes in a boiling water bath for 5 min. Proteins in the hydrolyzed milk and whey samples were removed by the method of Somogyi *(11)*. The diluted supernatants were analyzed for glucose.

For the determination of glucose, 1.0 ml aliquots of the diluted supernatants were placed in test tubes. A 4.0 ml aliquot of Glucostat reagent (Worthington Biochemical Corp.) was delivered into each tube, and the tubes were incubated at 25 C for 10 min. One drop of 4 N HCl was added to stop the reaction, and the optical density was recorded at 400 mμ. A standard curve was prepared with glucose. All values reported were measured with Mn++ added to the milk product and were corrected for the dilution effect from adding the enzyme solution to the milk and whey samples.

The concentration of lactose in the dairy products varied considerably in the various studies but the enzyme to lactose ratio was always constant. Thus, to obtain true comparisons enzyme activities were reported as per cent of lactose hydrolyzed.

Lactose in the milk products was determined by the method of Lawrence *(4)*.

Production of dried, enzyme-treated milk products

Raw skimmilk and whey were obtained from the University of Wisconsin dairy plant and vat-pasteurized at 74 C for 30 min. The pasteurized skimmilk was divided in equal parts of which one was concentrated to contain 15% lactose. The other was cooled and stored at 5 C until ready for treatment with enzyme. The skimmilk, whey, and skimmilk concentrates were adjusted to pH 6.5 with phosphoric acid or potassium hydroxide, and 10^{-4} M $MnCl_2$ then was added to each sample. The three samples were brought to 40-45 C and cell-free extract of S. *fragilis* was added. The quantity of the enzyme extract added was the extract obtained from a measured amount of dried yeast cells equivalent to 2% of the lactose content of the product. When the desired amounts of lactose hydrolysis were achieved, samples of the product were removed from the incubating vat and enzyme activity was arrested by heating them at 74 C for 10 min. The samples were then concentrated to 30% solids. The various hydrolyzed skimmilk concentrates were dried in a Necro-Niro portable spray drying unit. The inlet temperature was 170-180 C and the outlet temperature was 75-80 C. The atomizer was rotated at 50,000 rev/min. Samples were stored at room temperature.

Hydrolyzed whey was spray dried in the experimental tower dryer of the University of Wisconsin *(2)*. The product was atomized through a Spraying Systems 62-21 pressure nozzle at either 1500 or 3500 psi (1500 psi with high pressure pump and 2000 psi with compressed air from a Corblin A2CV250 two stage diaphragm type compressor). Inlet air temperatures were maintained at 150-155 C. Outlet air temperatures (at the point of powder and air separation) were maintained at 98-100 C. Powders were stored in polyethylene-lined barrels at room temperature.

Analysis of powders

Bulk density was determined as the weight per milliliter of a lightly tapped 50 g sample. Moisture in the powders was determined by the toluol distillation method *(5)*. Solubility index was measured by the method prescribed by the American Dry Milk Institute *(1)*. Glucose in the powders was determined with Glucostat reagent. The flavor of these dried hydrolyzed milk products was evaluated by a trained taste panel. Powders were reconstituted with deionized water to make solutions containing 10% total solids.

Reagents

Glucostat reagent was obtained from Worthington Biochemical Corp. (Freehold N. J.). All other reagents used in this investigation were analytical reagent grade. The various milk products used in this study were obtained from the Department of Food Science, University of Wisconsin, and were of the highest quality. Deionized distilled water was used for all experiments.

RESULTS

Lactose hydrolysis in milk products

The type of lactose-containing food products, used as the substrate for S. *fragilis* β-galactosidase, greatly influenced the extent of lactose hydrolysis (Table 1). Enzyme activity was determined both with and without 10^{-4} M $MnCl_2$ added to the milk products. The lactose content of all products was adjusted to 5% by dilution of the products with deionized water. The pH of milk products was adjusted to 6.5 by addition of either phosphoric acid or potassium hydroxide. Enzyme activity with Mn++ was greater than in its absence. The extent of lactose hydrolysis was greatest in the lactose buffer solution and least in the whole milk.

In previous studies *(18)*, it was found that β-galactosidase from S. *fragilis* Y-1109 required Mn++ as a cofactor to produce a stable enzyme assay when using lactose solutions. Studies of the enzyme-time-product relationship were made to determine if Mn++ also was necessary for a stable enzyme system in milk. The amount of glucose liberated from lac-

TABLE 1. THE INFLUENCE OF VARIOUS LACTOSE-CONTAINING SUBSTRATES ON THE RATE OF HYDROLYSIS OF LACTOSE.

Substrate[a]	Lactose hydrolyzed (%)	
	Without Mn++	With Mn++
Lactose solution	—	13.5
Whey	7.8	9.8
Skimmilk	7.2	8.2
Whole milk	6.3	6.8

[a]Lactose content adjusted to 5% in all samples

TABLE 2. ENZYME-TIME RELATIONSHIP WITH SKIMMILK AS SUBSTRATE.

Quantity of enzyme (ml)	Time of hydrolysis (min)	Lactose hydrolyzed (%)	
		Skimmilk	Skimmilk + 10^{-4} M Mn++
0.500	7.5	3.6	6.1
0.250	15.0	3.4	6.1
0.125	30.0	2.1	6.0

tose in skimmilk was measured both with and without addition of 10^{-4} M $MnCl_2$ to the skimmilk. Hydrolysis was conducted for 1 hr at 40 C. Table 2 shows that Mn^{++} was necessary to produce a relatively stable enzyme system and a constant value for the enzyme-time relationship in milk.

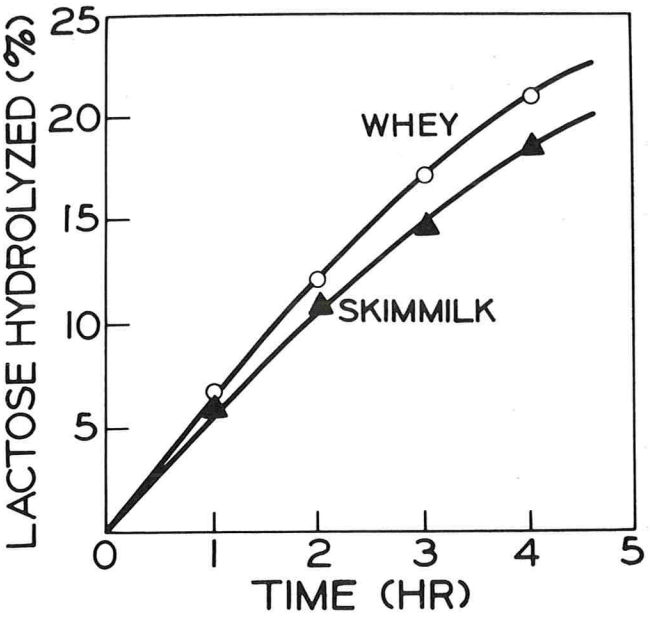

Figure 1. Hydrolysis of lactose in skimmilk and whey.

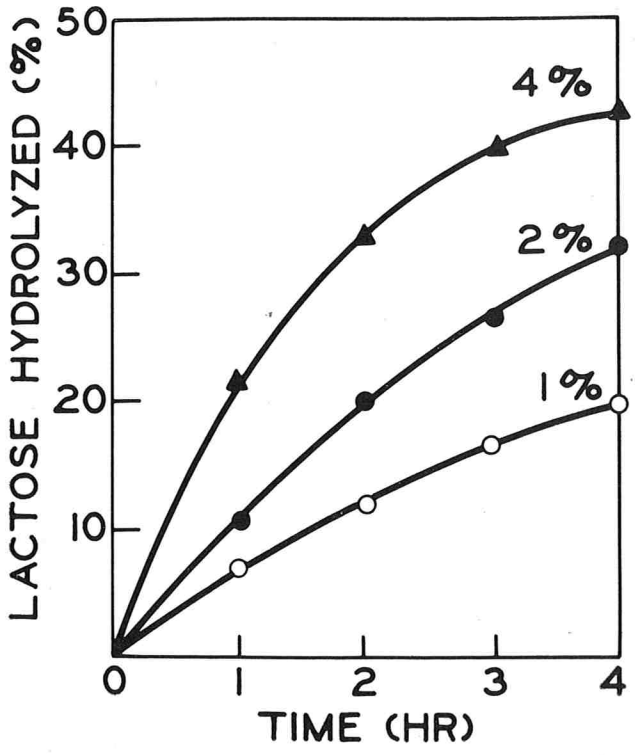

Figure 2. Effect of enzyme concentration on the rate of lactose hydrolysis in skimmilk.

In another study *(19)*, the effect of heat treatment of milk on rate of lactose hydrolysis by β-galactosidase was determined by an analysis of the enzyme activity in lots of skimmilk subjected to heat treatments (30 min) ranging from 62.8-85.0 C. In raw skimmilk 8.6% of the lactose was hydrolyzed. The maximum enzyme activity, 9.9% lactose hydrolyzed, was obtained with a heat treatment of 74 C for 30 min. Temperatures >74 C had no additional effects on hydrolysis of lactose in skimmilk.

The rate of lactose hydrolysis by *S. fragilis* β-galactosidase in both skimmilk and whey was determined hourly over a 4 hr period. Both the skimmilk and whey solutions had a pH of 6.5, contained 5% lactose and 10^{-4} M $MnCl_2$, and were given a heat treatment of 74 C for 30 min. Figure 1 shows that the enzymatic hydrolysis of lactose in skimmilk and whey did not follow zero-order kinetics as it did in a lactose solution *(18)*.

To determine the effect of concentration of milk solids on the activity of β-galactosidase in milk and whey, dried whey and high-heat nonfat dry milk (NDM) were reconstituted with deionized water containing 10^{-4} M $MnCl_2$ to various concentrations of total solids. To each of the samples, enzyme was added at a concentration of 2% of the lactose content and enzyme activities were determined. Table 3 shows that maximum hydrolysis of lactose was obtained in reconstituted whey which contained 20% total solids and in reconstituted NDM which contained 20-30% total solids. These concentrations of milk and whey solids were equivalent to approximately 10-15% lactose in solution.

To determine the effect of milk solids other than lactose on lactose hydrolysis by the *S. fragilis* enzyme, dried whey, low-heat NDM, and high-heat NDM were reconstituted with deionized water containg 10^{-4} M $MnCl_2$ to various concentrations of

TABLE 3. EFFECT OF TOTAL SOLIDS CONTENT OF RECONSTITUTED NDM AND WHEY ON THE RATE OF LACTOSE HYDROLYSIS.

Product	Total solids in product (%)	Lactose hydrolyzed (%)
Reconstituted whey	5	9.5
	10	9.8
	20	10.4
	30	9.6
	40	9.4
Reconstituted NDM (high heat)	5	9.4
	10	9.7
	20	10.2
	30	10.3
	40	9.8

TABLE 4. EFFECT OF MILK SOLIDS OTHER THAN LACTOSE ON THE RATE OF LACTOSE HYDROLYSIS.

Product[a]	Milk solids other than lactose in product (%)	Lactose hydrolyzed (%)
Reconstituted whey	3.6	10.3
	2.7	10.5
	1.8	11.1
	0.9	12.9
Reconstituted NDM (low heat)	10.0	7.3
	7.5	8.6
	5.0	10.1
	2.5	11.9
Reconstituted NDM (high heat)	10.0	9.5
	7.5	9.9
	5.0	10.6
	2.5	11.5

[a]Lactose content adjusted to 10% in all samples

TABLE 5. EFFECT OF ADDITION OF POTASSIUM TO MILK OF THE RATE OF LACTOSE HYDROLYSIS.

Concentration of K+ added (moles/l)	Lactose hydrolzed (%)
None	8.0
0.05	8.4
0.10	8.9
0.20	8.8
0.50	7.9

solids. These concentrations were equivalent to 10, 7.5, 5, and 2.5% lactose in solution. Lactose was then added to the samples to adjust the lactose content of all samples to 10%. Table 4 shows that as the milk solids other than lactose were decreased, the rate of hydrolysis of lactose in the milk products increased. Enzyme activity when using low-heat NDM was generally less than when using high-heat NDM or whey.

Addition of lactose activators *(16)*, such as sodium sulfite and sodium bisulfite, had no effect on enzyme activity when 10^{-4} M $MnCl_2$ was added to the milk products. Potassium metabisulfite slightly increased β-galactosidase activity, but it was later found that this increase resulted from addition of potassium ions.

Additional studies on the effect of potassium ions on β-galactosidase activity in milk were conducted. To heat-treated skimmilk containing 5% lactose and 10^{-4} M $MnCl_2$, various amounts of K^+ were added in the form of potassium sulfate. The addition of 0.1 M K^+ gave the maximum rate of lactose hydrolysis in skimmilk (Table 5).

The rate of lactose hydrolysis in heat-treated skimmilk containing 10^{-4} M Mn^{++} and 10^{-1} M K^+ was determined by adding β-galactosidase in increments of 1, 2, and 4% of the lactose content. Samples were incubated at 40 C and aliquots were removed for glucose analysis hourly over a 4 hr period. Figure 2 shows that, under optimum conditions, the *S. fragilis* β-galactosidase in skimmilk did not follow the zero-order kinetics observed in lactose solutions. Rather, mixed-order kinetics was observed. Inhibition of the β-galactosidase by the hydrolysis products was evident above 40% hydrolysis.

Dried hydrolyzed milk products

Nonfat dry milk (NDM) powders were prepared in which 0, 15, 30, or 45% of the lactose in the product was hydrolyzed to the corresponding monosaccharides. The resulting milk powders were of good appearance and stability. Dried whey powders also were prepared in which 50% of the lactose was hydrolyzed. The powders had a satisfactory light color but were rather hygroscopic. The general characteristics of these dried modified milk products are shown in Table 6. In general, as the percentage of lactose hydrolyzed in the product was increased, the moisture content of the powder increased and the bulk density decreased.

Flavors of the resulting NDM and whey powders were generally good. The powders did not have the "yeasty" or "malty" flavor sometimes associated with hydrolyzed milk concentrates. However, as the percentage of lactose hydrolyzed in the NDM powders increased, the sweetness increased.

TABLE 6. CHARACTERISTICS OF DRIED MODIFIED MILK PRODUCTS.

Sample	Lactose hydrolyzed (%)	Bulk density (g/ml)	Moisture (%)	Solubility index (ml)
Nonfat dry milk[a]				
1	0	0.58	2.9	0.04
2	15	0.56	3.4	0.10
3	30	0.52	3.7	0.08
4	45	0.50	3.8	0.07
Nonfat dry milk[b]				
1	0	0.58	2.9	0.04
2	15	0.51	3.0	0.06
3	30	0.50	3.1	0.07
4	45	0.50	3.6	0.08
Dried whey				
1[c]	50	0.46	3.6	2.00
2[d]	50	0.22	4.0	1.80

[a]Hydrolyzed before concentration.
[b]Hydrolyzed after concentration.
[c]Spray-dried with no injected gas.
[d]Spray-dried with injected gas.

Discussion

Beta-galactosidase from lactose-fermenting yeasts has been extensively studied for possible use in the food industry. Several workers *(7, 9, 13, 17, 19)* have reported various factors affecting lactose hydrolysis in milk products. Studies were conducted to determine if all of these factors affected the enzyme activity of S. *fragilis* Y-1109. The type of lactose containing substrate greatly influenced the rate of lactose hydrolysis by the S. *fragilis* enzyme. Lactose in aqueous solution was hydrolyzed more easily than it was in milk products. Of the milk products tested, whey was the best substrate for the yeast enzyme. This agreed with the effect of substrates reported by Reed *(8)*. Manganese and potassium had to be added to the milk products to provide maximum hydrolysis and a stable enzyme system. Lactase activators, such as sulfites and bisulfites, had no effect on enzyme activity when Mn^{++} was added to heat-treated milk. Reed *(8)* reported that patent literature showed activation only in unpasteurized milk or whey when reducing compounds were added.

Sfortunato and Conners *(10)* reported that unpasteurized milk products contained some material which suppressed enzyme activity. In the present work, this suppressor of enzyme activity in milk products also was observed. This enzyme suppressor was inactivated with a heat treatment of 74 C for 30 min.

Very little previous work has been reported on the effect of concentration of milk solids on lactose hydrolysis by the yeast enzyme. Maximum hydrolysis of lactose by the S. *fragilis* enzyme was obtained when milk products were concentrated to contain 15% lactose. However, milk solids other than lactose were found to either inhibit or suppress β-galactosidase activity in milk products. One would expect that maximum rates of lactose hydrolysis would then be obtained by hydrolyzing whey or milk containing added increments of lactose. This general procedure was used by Sfortunato and Connors *(10)* as the basis for a patent on lactose hydrolysis in milk products.

Beta-galactosidase from S. *fragilis* Y-1109 has been shown to actively hydrolyze lactose in various milk products. The enzyme was most efficient at hydrolysis levels below 40%. Above 40%, the rate of hydrolysis of lactose by the yeast enzyme proceeded more slowly.

The potential use of β-galactosidase for production of dried hydrolyzed milk products has been reported by several workers *(7, 9, 13)*. Potter and Webb *(7)* reported that powder prepared from hydrolyzed skimmilk was sweeter, more hygroscopic, and had more tendency to darken than powder prepared from normal skimmilk. Sampey and Neubeck *(9)*, however, obtained milk powders of good appearance and stability. In the present study, we found that powders having up to 50% of the lactose hydrolyzed were of the same appearance as normal NDM. As the percentage of lactose hydrolyzed in the products increased, the products became more hygroscopic. There was little evidence of caking or clumping in the modified NDM powders. However, there was some clumping evident in the dried hydrolyzed whey.

Results of this study indicated that portions of lactose in milk and whey can be hydrolyzed to provide dried milk products with increased sweetness plus good flavor and stability. These sweeter dried products have a great potential use in ice cream, baked goods, candies, and any other food products in which milk or whey solids and sugar are incorporated. With addition of these dried modified products, less sugar would have to be added, thus offering substantial savings in ingredient costs.

Acknowledgments

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REPORT OF COMMITTEE ON FOOD EQUIPMENT SANITARY STANDARDS 1969-1970

(Continued from Page 293)

air curtains are intended to prevent flying insects from passing through an opening and are not intended to exclude cats, dogs, or other animals nor to serve as a replacement for a security door.

The proposed Standard for Commercial Cooking Equipment Exhaust Systems which was recommended for adoption by the Joint Committee at its 1969 meeting was reviewed once again by the Committee. One of the major changes made in this final review was in the method used in calculating the quantity of air to be exhausted from a canopy type hood. This method led to the adoption of the following terminology and three formulas:

"Q"—Shall mean the total quantity of air required to be exhausted by the ventilating system in cubic feet per minute.

"A"—Shall mean the cross section of the plane extending over the canopy hood opening in square feet.

"P"—Shall mean the perimeter of the open sides of the canopy hood.

"D"—Shall mean the distance between the cooking surface and the face of the hood.

$Q = 150\ A$ (Open four sides)

$Q = 100\ A$ (Open three sides or less)

$Q = 50\ PD$

It was also agreed that the NSF Testing Laboratory in evaluating other than canopy type hoods would require the manufacturer to indicate on his equipment the basis for comparative testing, i.e., 150 A, 100 A, or 50 PD. Further, the Testing Laboratory Listing would indicate the basis of qualification.

Policies and procedures for the NSF Joint Committee

The NSF Joint Committee on Food Equipment Standards believing it would function better and more efficiently under a set of written policies and procedures developed such and recommended to the National Sanitation Foundation that such written procedural guidelines be adopted. With some minor modification, the Foundation adopted the proposed set of written policies and procedures for the Joint Committee in 1966.

Since the Foundation's adoption of these written policies and procedures, periodically, a question is raised at NSF Joint Committee meetings as to who is permitted to vote on approving or amending a standard or criterion. According to the NSF Manual on Organization-Structure-Policies for Joint Committees: NSF Standards are public health sanitation standards and as such must be acceptable to the professional public health representatives on Committees. Therefore, the professional representatives of public health are asked to vote on the public adequacy of a proposed standard. The industry and other liaison members of the Committee are invited to present their recommendations and freely advise on the portions of the Standard in which they have an interest and on which they have competent technical background. However, all members of the Joint Committee (Industry and Public Health) have an equal right to object to the adoption of a standard by the Board of Trustees of the National Sanitation Foundation.

A review of the aforementioned material taken from the NSF Policy and Procedural Manual for Joint Committees served to clarify this matter and should prevent need for further discussion at future Joint Committee meetings.

NATIONAL AUTOMATIC MERCHANDISING ASSOCIATION (NAMA)

The National Automatic Merchandising Association's Automatic Merchandising Health-Industry Council (AMHIC) held its 14th annual meeting during October 1969, and this Association and other public health organizations and the affected industries were represented and participated in AMHIC's discussions.

The afternoon of the first day was reserved solely for a meeting of the public health representatives and was used by them to discuss and clarify their view on public health objectives and policies to be followed in their work with the entire membership of the AMHIC. The Chairman of the IAMFES Food Equipment Committee was re-elected Chairman of the Public Health Group and also served as Co-Chairman of AMHIC during 1969-1970.

Evaluation manual and checklist

The members of AMHIC received a report that no requests for changes in the Evaluation Manual or Checklist had been received during the year, but that changes would probably be forthcoming in the areas of cut-off controls location and testing and in ice-maker design and construction.

However, during the Council meeting tentative approval was given to amending Section 505.2 of the Manual to read as follows: "If a cup storage turret or magazine is so located that splash or spillage may contaminate the stored cups, such turret or magazine shall be equipped with a fitted, overlapped lid or cover." Tentative approval was also given to amending Section 603.1 of the Manual to read as follows: "In those vending machines in which failure of the check valves upstream from the carbonator would result in the backflow of carbon dioxide or carbonated water into potentially-toxic tubing or components inside the machine or outside, an air gap, atmospherically-vented valve or other venting device shall be installed between the check valves and any potentially-toxic water contact surfaces upstream. NOTE: A brass pump housing shall not be considered to be "potentially-toxic" in a system which is otherwise free from copper or other materials listed in 602.3." The latter proposal if not in exact wording but at least in principle will be approved by the NAMA and NSF. However, representatives of the NSF Joint Committee and AMHIC have questioned the advisability of the former (505.2) amendment.

(Continued on Page 306)

CHARACTERIZATION OF BETA-GALACTOSIDASE FROM *SACCHAROMYCES FRAGILIS*[1]

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Abstract

Studies were made on physical and chemical characteristics of β-galactosidase in cell-free extracts of *Saccharomyces fragilis* Y-1109. The enzyme was stable at pH 6.0-7.0. When frozen in buffer solution, it was stable for more than 3 months but at 51 C, it lost 96% of the activity in 10 min. The optimum pH for lactose hydrolysis at 37 C was 6.5.

The enzyme was activated by K+ while Mn++ served as the cofactor for the enzyme. Manganese appeared to be important in maintaining the integrity of the secondary and tertiary structure of the enzyme molecule. Inactivation of the enzyme by urea indicated the importance of secondary and tertiary structure in the enzymatic function of the yeast β-galactosidase.

Beta-galactosidase appeared to be a sulfhydryl enzyme since heavy metals, *p*-chloromercuribenzoate, and iodoacetate inhibited the enzyme. Cysteine and galactose were competitive inhibitors of the enzyme, whereas glucose and various amines were non-competitive inhibitors.

Lactose-fermenting yeasts are excellent sources of β-galactosidase (E. C. 3.2.1.23 β-D-galactoside galactohydrolase) and have commonly been used for its preparation *(2, 3, 11, 12, 16,17)*. A survey of eight strains of lactose-fermenting yeasts obtained from the USDA Northern Regional Research and Utilization Laboratory has shown that *Saccharomyces fragilis* Y-1109 produces large quantities of enzymes *(19, 20)*.

The present study began as an attempt to isolate and purify the β-galactosidase from the yeast. Our attempts were complicated by the lability of the enzyme to many treatments involving precipitation of the enzyme or reaction of the enzyme with ion-exchange resins. Therefore, studies of the physical and chemical characteristics of β-galactosidase were conducted on cell-free extracts of *S. fragilis* Y-1109. This report presents some of the physical and chemical properties of the β-galactosidase from this *S. fragilis* strain.

Materials and Methods

Organism

Saccharomyces fragilis NRRL Y-1109 was obtained from USDA Northern Regional Research Laboratories. The yeast was maintained by occasional transfers on slants of agar medium containing the following ingredients per liter: lactose, 20 g; yeast extract, 5 g; and agar, 15 g.

Growth

Studies to characterize the enzyme from *S. fragilis* Y-1109 were made with enzyme extracted from large quantities of dried yeast produced in the University of Wisconsin biochemical pilot plant. *Saccharomyces fragilis* Y-1109 was grown on a medium containing the following ingredients per liter: lactose, 100 g; yeast extract, 5 g; K_2HPO_4, 3 g; and ammonium sulfate, 1 g. The pH of the medium was adjusted to 4.5 with phosphoric acid. A 750 gal stainless steel fermentor with mechanical agitation was used to grow 400 gal quantities of yeast culture. The medium was inoculated with 45 l of *S. fragilis* culture which had been actively growing in a sterile medium for 12 hr. The temperature during fermentation was maintained at 28 C, the pH was maintained at 4.5 by addition of ammonia, and the aeration rate was held at 40 cfm. When maximum growth was reached, yeast cells were harvested and washed in a De Laval, PX-309, sludge separator. The yeast cream obtained was spray-dried according to the method of Stimpson *(15)* on the University of Wisconsin tower dryer *(1)* to obtain a β-galactosidase-active, zymase-inactive, dried preparation.

Buffer solutions

Potassium phosphate buffer (0.1 *M* at pH 7.0) was used routinely to extract the enzyme, whereas potassium phosphate buffer (0.1 *M* at pH 6.5) was used in the enzyme assay substrate solution. Manganese chloride (10^{-4} *M*) was used in the buffer solution in the experiments indicated.

Cell-free extracts

All extractions were carried out in a cold room at 5 C. The procedure for extracting the enzyme from the dried yeast cells consisted of mechanically agitating a 10% (w/v) yeast slurry in a pH 7.0 buffer for 16 hr. Approximately 5% (w/v) sterile sea sand was added to help disrupt the yeast cells. Cell-free extracts were obtained by centrifuging the broken cells at 1000 × *g* for 15 min.

Enzyme and protein assays

Enzymatic activity was determined by the amount of glucose released from the hydrolysis of lactose. The procedure involved two steps: hydrolysis of pure lactose by the test preparation, and microdetermination of glucose in the hydrolysis products.

The procedure for hydrolysis of lactose was a modification of the method of Jasewicz and Wasserman *(8)*. Hydrolysis was performed in test tubes in a water bath at 37 C. One milliliter of a 5% (0.139 *M*) lactose solution in 0.1 *M* potassium phosphate buffer of pH 6.5 and 0.8 ml of the buffer solution were mixed and equilibrated in the bath. The total test volume was brought to 2.0 ml by addition of 0.2 ml of a buffer-diluted cell-free extract containing the equivalent of 10 mg of dried yeast cells per milliliter. After a 15 min

[1]Research supported by the College of Agricultural and Life Sciences, University of Wisconsin, Madison and by the Cooperative State Research Service, United States Department of Agriculture.

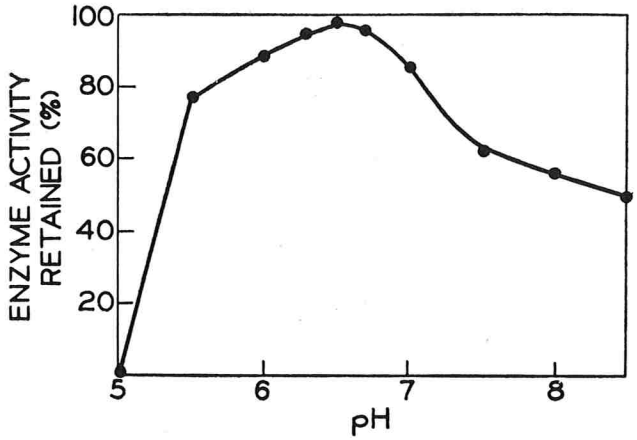

Figure 1. Effect of pH on stability of S. *fragilis* enzyme; 0.1 *M* potassium phosphate solution.

incubation period, the activity was stopped by heating the tubes in a boiling water bath for 5 min. The denatured protein residue was removed by centrifugation, and the supernatant was analyzed for glucose. All values reported were measured with Mn^{++} present in the buffer, unless stated otherwise.

To determine glucose, 1.0 ml aliquots of a 1:20 dilution of the supernatants were placed in test tubes. A 4.0 ml aliquot of Glucostat reagent (Worthington Biochemical Corp.) was delivered into each tube, and the tubes were incubated at 25 C for 10 min. One drop of 4 N HCl was added to stop the reaction, and the optical density was recorded at 400mμ. A standard curve was prepared with glucose. A unit of β-galactosidase, as defined by Jasewicz and Wasserman *(8)*, is the amount of enzyme which will liberate 1 μm of glucose from lactose in 15 min at 37 C. In characterization studies on the enzyme, enzyme activity was reported only in μmoles of glucose liberated per ml of reaction per 15 min.

With *o*-nitrophenyl-β-D-galactoside (ONPG) as substrate, the *o*-nitrophenol released was determined spectrophotometrically by a modification *(14)* of the procedure described by Lederberg *(9)*. The reaction was carried out in a volume of 3.0 ml with a final concentration of 0.001 *M* ONPG and 0.05 *M* phosphate buffer (pH 7.0). After incubation at 37 C for an appropriate period of time, (5-10 min) depending upon the activity of the extracts, 2.0 ml of 1 *M* K_2CO_3 were added to stop the reaction and to develop the color of the *o*-nitrophenol released. The amount of *o*-nitrophenol released was measured at 420 mμ with a Beckman model DU spectrophotometer. The values obtained were expressed as μmoles of *o*-nitrophenol released.

The Lowry Folin-phenol method *(10)* was employed to determine total protein. Standard curves were established with crystallized bovine serum albumin.

Reagents

Glucostat reagent was obtained from Worthington Biochemical Corp. (Freehold, N. J.); *o*-nitrophenyl-β-D-galactopyranoside (ONPG) was obtained from Mann Research Laboratories, Inc. (New York, N. Y.); and crystallized bovine serum albumin was acquired from Nutritional Biochemical Corp. (Cleveland, Ohio). All other reagents used in this investigation were analytical reagent grade. Deionized distilled water was used for all experiments.

RESULTS

Stability of enzyme

Beta-galactosidase in cell-free extracts of S. *fragilis* was stable for at least 3 months when stored at −20 C, whereas, it was stable only 2-3 weeks at 5 C. The effect of pH on stability was examined by incubation of the enzyme in the absence of lactose for 30 min at 25 C at various pH values. After the incubation period, the pH was readjusted to 6.5. Then the amount of enzyme activity retained was determined under standard conditions. The enzyme was relatively stable from pH 6.0 to 7.0 (Fig. 1). At pH 5.0, the enzyme was precipitated as a white flocculent material and enzyme activity was completely lost. When this material was resolubilized by addition of alkali, enzyme activity was not restored.

Stability of yeast β-galactosidase toward heat was measured at 51 C without Mn^{++} added and at 51 and 56 C with Mn^{++} added to the buffer. Samples of cell-free extract in phosphate buffer, with and without Mn^{++}, were held at 51 or 56 C and aliquots of the extracts were removed at various time intervals and assayed by the standard procedure. Figure 2 shows that the enzyme is relatively heat-labile at 51 C. Addition of Mn^{++} to the cell-free extract at 51 C provided some protection against heat inactivation of the enzyme.

Table 1 shows the effect of dialysis on enzyme activity of cell-free extracts. Ten-milliliter aliquots of cell-free extract were dialyzed over a 24-hr period against three 1000-ml changes of either water or various buffer solutions at 5 C. Specific activities were measured both with and without 10^{-4} *M* Mn^{++} in the assay buffer. The S. *fragilis* enzyme was irreversibly inactivated by prolonged dialysis against distilled water. Mercaptoethanol and Mn^{++} gave some protection against the dialysis effect, whereas Mg^{++} and buffer gave little protection when assayed in the absence of Mn^{++}. When Mn^{++} was added to the assay buffer, extracts dialyzed against the various buffers showed no appreciable difference in activities.

The results from the studies on stability of S.

TABLE 1. THE EFFECT OF DIALYSIS ON THE β-GALACTOSIDASE ACTIVITY OF S. FRAGILI- EXTRACTS.

Dialysis for 24 hr against	Specific activity (units/mg) Without Mn^{++}	With Mn^{++}
Control (not dialyzed)	49.5	71.5
Distilled water	0	2.2
Phosphate buffer, pH 7.0	11.5	65.9
Buffer + 10^{-2} *M* mercaptoethanol	36.1	65.1
Buffer + 10^{-3} *M* $MgCl_2$	13.9	63.5
Buffer + 10^{-3} *M* $MnCl_2$	38.6	63.6

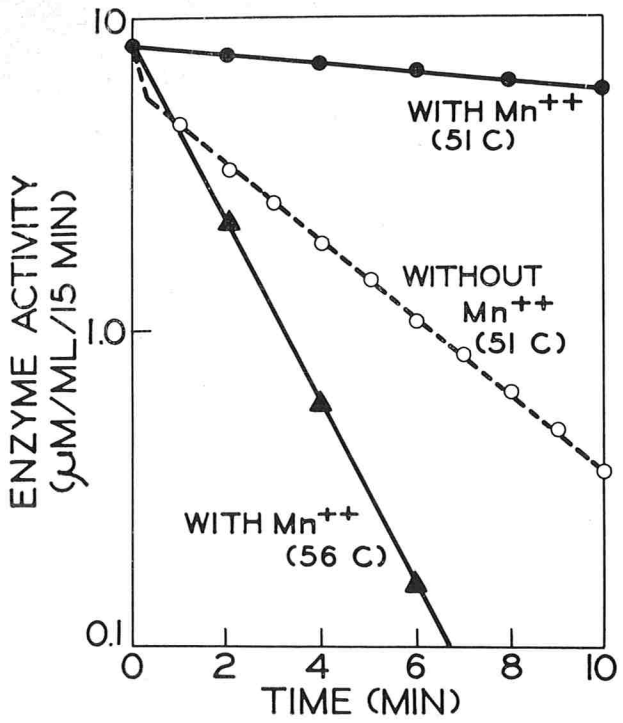

Figure 2. Thermal inactivation of S. *fragilis* enzyme.

fragilis β-galactosidase indicated that the enzyme in phosphate buffer without Mn^{++} added was extremely labile, but with Mn^{++} added the enzyme was relatively stable to extreme conditions. These observations led to investigation of the enzyme-time-product relationship because of the possibility that Mn^{++} served as a cofactor for the enzyme. Table 2 shows that the enzyme, when assayed in phosphate buffer alone, did not yield a constant value for the enzyme-time relationship. With 10^{-4} *M* Mn^{++} added, however, a constant relationship was obtained. Addition of 10^{-4} *M* Mg^{++} did not produce the same effects as Mn^{++}, which indicated a unique specificity for the manganese ion.

With the addition of Mn^{++} as cofactor, the curve for the hydrolysis of lactose by S. *fragilis* β-galactosidase appeared to obey zero-order kinetics up to about 40% hydrolysis (Fig. 3). The velocity of the reaction was directly proportional to enzyme concentration, indicating the stability of the enzyme assay system when Mn^{++} was included in the buffer.

TABLE 2. RELATION BETWEEN QUANTITY OF ENZYME AND TIME OF HYDROLYSIS FOR S. *fragilis* ENZYME.

Quantity of enzyme (ml)	Time of hydrolysis (min)	Glucose liberated (μmoles)		
		Buffer	Mg^{++}	Mn^{++}
0.40	7.5	5.0	5.4	6.5
0.20	15.0	3.2	4.4	6.5
0.10	30.0	0.6	1.5	6.6
0.05	60.0	0.1	0.3	6.3

Effect of pH

The effect of pH on the rate of lactose hydrolysis was studied by using 0.1 *M* potassium phosphate solutions containing 10^{-4} *M* $MnCl_2$. Maximum enzyme activity was obtained between pH 6.3-6.5 (Fig. 4). At pH 5.0, the yeast enzyme was irreversibly inactivated.

Effect of temperature

The effect of temperature on the rate of lactose hydrolysis was examined over the range of 5-45 C. Results, plotted in the conventional Arrhenius man-

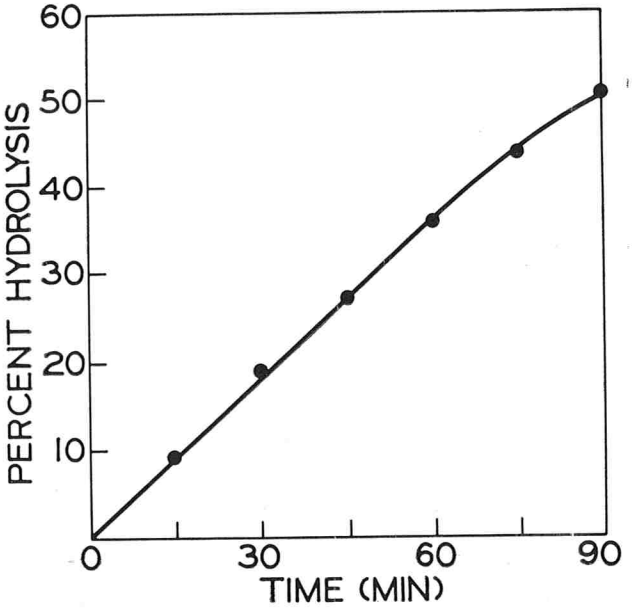

Figure 3. Enzymatic hydrolysis of lactose by S. *fragilis* enzyme: initial substrate concentration, 0.07 *M;* enzyme concentration, 1 mg per ml; pH 6.5; $[K^+] = 0.1$ *M* (Phosphate); $[Mn^{++}] = 10^{-4}$ *M*.

ner (log *k* versus 1/T; *k* = zero-order velocity constant, T = absolute temperature), are shown in Fig. 5. A deviation from linearity was observed above 37 C. According to these data, the average energy of activation *(13)* over the range of 5-37 C was 12,-900 cal per mole.

Activation by ions

Beta-galactosidase of S. *fragilis* was inactive when assayed in deionized water containing lactose. The enzyme was strongly activated by potassium and to a lesser extent by ammonium. Optimum activity was achieved with 0.1 *M* potassium in the form of potassium phosphate. Manganese and magnesium both gave further increases in hydrolysis rates, even in the presence of 0.1 *M* K^+. The concentration of Mn^{++} used in the assay buffer was limited to 10^{-4} *M* because of the low solubility of manganese phosphate. Various anions studied in this investigation appeared to have no effect on enzyme activity.

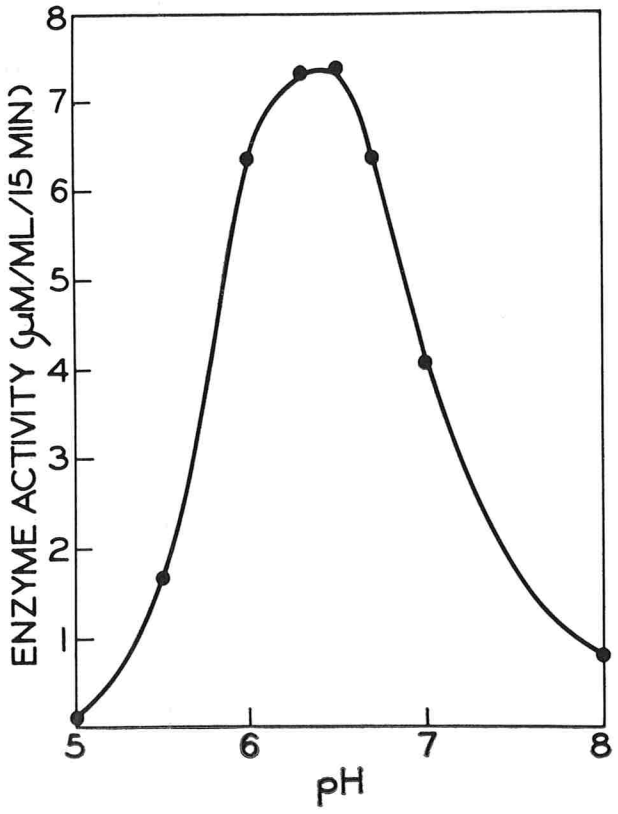

Figure 4. Effect of pH on enzyme activity.

Inactivation with urea

Enzyme activity of β-galactosidase was determined when varying concentrations of urea were added to the assay solution. The rate of lactose hydrolysis, when assayed in the presence of urea, progressively decreased as the urea concentration increased (Table 3.) Inactivation by urea could not be reversed when the assay solution was diluted with phosphate buffer containing 10^{-4} *M* Mn^{++}.

Inhibition

The β-galactosidase of S. *fragilis* Y-1109 was inhibited by several different types of compounds. The effect on enzyme activity of various metal ions in the presence of 0.1 *M* potassium ion is shown in Table 4. In general, the heavy metal ions inhibited the enzyme completely, whereas the monovalent cations inhibited the enzyme to a lesser degree. Ammonium ion, which by itself activated enzyme activity, inhibited β-galactosidase activity, when adequate K^+ was present.

In preliminary studies on purification of the S. *fragilis* β-galactosidase, it was noted that whenever cysteine was added to the buffer systems, enzyme activity decreased slightly. This effect of cysteine on enzyme activity was investigated by assaying the enzyme with various concentrations of cysteine added to the solution. The pH of the solutions was readjusted to pH 6.5 with KOH before hydrolysis. At 10^{-3} *M* cysteine, 83% of the enzyme activity was left and at 10^{-2} *M* cysteine, only 55% of the activity remained. As shown in Fig. 6, the cysteine-inhibited system followed the kinetics of reversible (competitive) inhibition.

The instability of the enzyme in the presence of heavy metals led to investigation of the effect of sulfhydryl inhibitors on enzyme activity. Various concentrations of iodoacetate or *p*-chloromercuribenzoate (PCMB) were added to the enzyme assay solution and the hydrolysis reaction was begun by addition of lactose. As shown in Table 5, PCMB, at a concentration of 10^{-4} *M*, greatly inhibited enzyme activity but iodoacetate inhibited it to a lesser degree. Figure 7 shows that inhibition of the enzyme by PCMB was reversible with excess substrate, indicating a competition between the substrate and sulfhydryl reagent for the catalytic site of the enzyme.

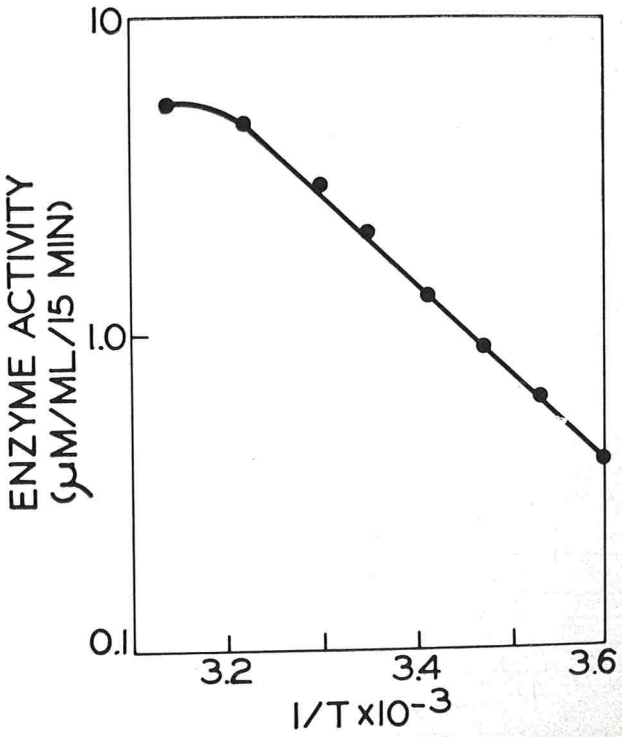

Figure 5. Arrhenius plot for lactose hydrolysis.

TABLE 3. INACTIVATION OF S. *fragilis* ENZYME WITH UREA.

Molarity of urea	% of the original activity
0	100
1	81
2	48
3	2
4	1

TABLE 4. INHIBITION OF POTASSIUM-ACTIVATED S. *fragilis* ENZYME BY OTHER CATIONS.

Cation	Enzyme activity (units/ml/15 min)	Inhibition of K+ activated enzyme (%)
None	11.1	—
Na+, 10^{-1} *M*	4.7	58
NH_4+, 10^{-1} *M*	6.1	45
Li+, 10^{-1} *M*	6.7	39
Zn++, 10^{-1} *M*	11.2	0
Ca++, 10^{-1} *M*	3.3	70
Ca++, 10^{-2} *M*	0	100
Ag+, 10^{-2} *M*	0	100
Hg++, 10^{-2} *M*	0	100

TABLE 5. INHIBITION OF S. *fragilis* ENZYME BY SULFHYDRYL AGENTS.

Inhibitor	Concentration (moles/1)	Inhibition (%)
None		0
PCMB	1×10^{-4}	86
	1×10^{-5}	13
	1×10^{-6}	11
Iodoacetate	1×10^{-3}	9
	1×10^{-4}	9
	1×10^{-5}	8
	1×10^{-6}	1

TABLE 6. INHIBITION OF S. *fragilis* ENZYME BY AMINES.

pH	Amine	Concentration (Moles/1)	Inhibition (%)a
7.4	Tris	0.01	42
7.0	Tris	0.01	17
6.5	Tris	0.01	8
7.4	Histidine	0.1	26
7.0	Histidine	0.1	14
6.5	Histidine	0.1	9

[a]Values are based on controls at each respective pH level.

TABLE 7. ENZYME-TIME RELATIONSHIP WITH ONPG AS SUBSTRATE.

Quantity of enzyme (ml)	Time of hydrolysis (min)	o-Nitrophenol liberated (μmoles)	
		Buffer	Mn++
0.60	5	8.2	11.3
0.30	10	3.6	11.4
0.15	20	1.1	11.4

The hydrolysis of lactose by S. *fragilis* was found to be inhibited by histidine and tris (hydroxymethyl) aminomethane (Tris) (Table 6.) The inhibition by these amines was unique in that they inhibited more strongly at alkaline than at acid pH. In further investigations, it was found that the inhibition of enzyme activity by these amines was of the non-competitive type and could not be reversed by excess substrate.

The β-galactosidase from S. *fragilis* was inhibited by high concentrations of both glucose and galactose. The enzyme activity of yeast β-galactosidase showed 7% inhibition when 0.01 *M* galactose was in the assay solution and 60% inhibition when 0.01 *M* glucose was in the assay solution. Glucose was found to be a non-competitive inhibitor of the enzyme. In the presence of galactose, a competitive inhibition of the hydrolysis of lactose with the β-galactosidase of S. *fragilis* Y-1109 was observed.

Substrate specificity

When lactose was used as the substrate, a typical Michaelis-Menten relationship was observed between the substrate concentration and the initial velocity (zero-order kinetics) of the reaction. The Michaelis constant (K_M) at pH 6.5 was estimated from the Lineweaver-Burk plot (Fig. 8) to be approximately 2.4×10^{-2} *M* and the maximum velocity of reaction

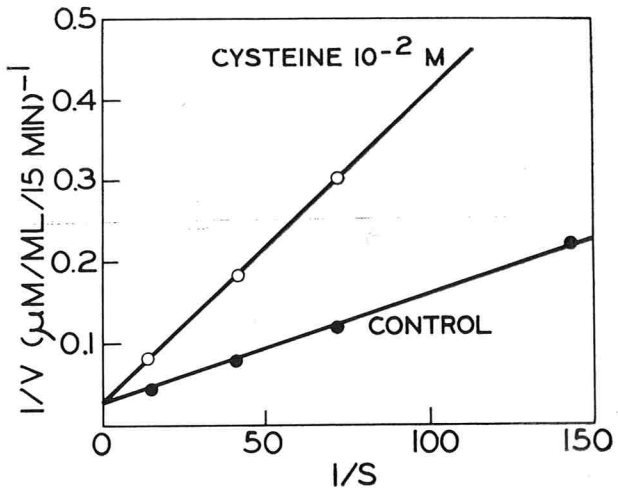

Figure 6. Inhibition of S. *fragilis* enzyme by cysteine.

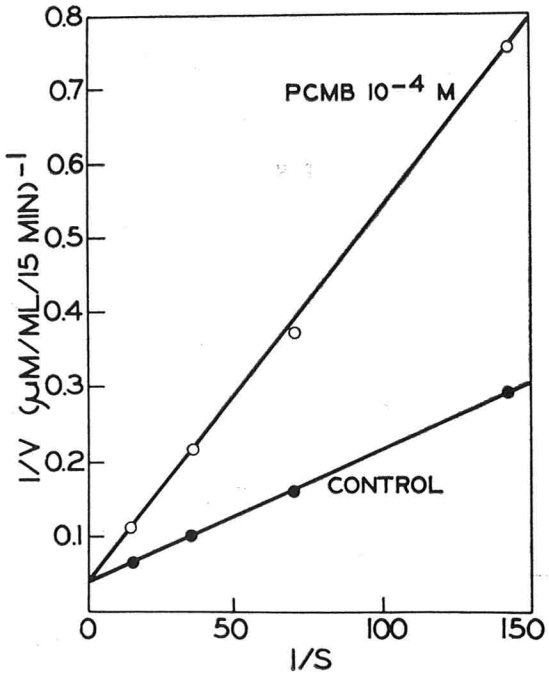

Figure 7. Inhibition of S. *fragilis* enzyme by PCMB.

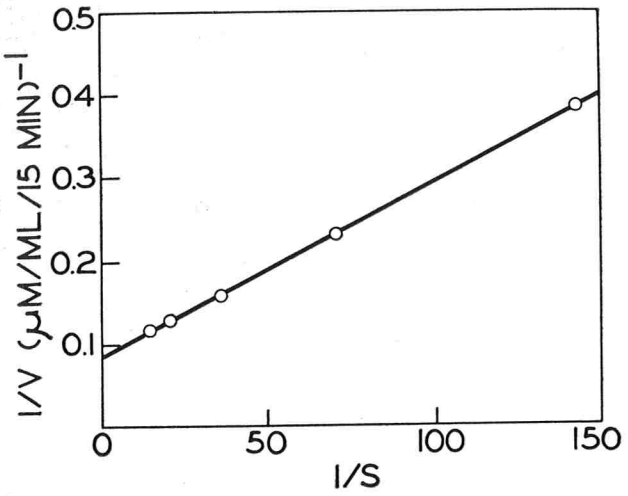

Figure 8. Lineweaver-Burk plot for lactose hydrolysis.

(V_{max}) was found to be 11.6 μmoles 1 ml/15 min.

A study was conducted to determine if Mn^{++} was required by the enzyme for stable activity when ONPG was used as a substrate. The enzyme-time relationship was compared both with and without Mn^{++} present in th assay solution. When Mn^{++} was not added to the phosphate buffer, the activity decreased with decreasing enzyme concentrations (Table 7). However, when Mn^{++} was present in the buffer, a constant activity was obtained indicating the requirement of Mn^{++} for a stable enzyme system. When ONPG was the substrate, the Michaelis constant (K_M) for the *S. fragilis* β-galactosidase was 2.5×10^{-3} *M* and V_{max} was 0.66 μmoles of *o*-nitrophenol liberated per minute.

Discussion

Properties of β-galactosidase from different strains of lactose-fermenting yeasts vary considerably. The β-galactosidases of *S. fragilis* Y-1109, *Saccharomyces lactis* Y-1140, and *S. lactis* Y-1193 required Mn^{++} to produce a stable enzyme system, whereas the enzyme from *Candida pseudotropicalis* Y-83 was stable without addition of any metal ions *(19, 20)*. An absolute requirement for magnesium ion for maximum hydrolysis of ONPG by a *S. fragilis* β- galactosidase was reported by Gilmour (M. H. N. Gilmour, Diss. Abstr., 17: 946, 1957), while Szabo and Davies *(16)* reported that manganous ion was essential for maximum hydrolysis by the *S. fragilis* (Jorgensen) enzyme. All of the *S. fragilis* β-galactosidases, however, were activated by potassium ion when lactose was the substrate.

The optimum pH and temperature for lactose hydrolysis by the enzyme of *S. fragilis* Y-1109 was within the range of other values reported for *S. fragilis* β-galactosidases. The activation energy required by the yeast β-galactosidase was slightly less than values reported for yeast α- and β-glucosidases *(5, 6, 7)*.

Although various mechanisms have been proposed for the hydrolysis of β-D-galactosides by both bacterial and calf-intestinal enzymes *(18)*, there is little information available on the nature of the functional groups involved in the enzyme activity of yeast β-galactosidase. The inactivation of *S. fragilis* Y-1109 β-galactosidase by heavy metals, which are sulfhydryl binding agents, indicated the presence of sulfhydryl groups on the enzyme molecule. The possible involvement of some of these sulfhydryl groups in the catalysis of lactose hydrolysis was indicated by the competitive inhibition of enzyme activity by PCMB. Inactivation of the enzyme by urea suggested that the secondary and tertiary structure of the enzyme molecule must remain intact to insure the hydrolysis of lactose by the yeast enzyme.

The β-galactosidase of *S. fragilis* Y-1109 also was competitively inhibited by cysteine. Wallenfels and Malhotra *(18)* felt that inhibition of enzymes by cysteine could result from the presence of an enzyme-bound, heavy metal activator such as manganese or iron. In the present study, manganese served as an essential cofactor for *S. fragilis* β-galactosidase and possibly aided in maintaining the intactness of the secondary and tertiary structure of the enzyme molecule. Whether or not Mn^{++} forms an integral part of the native β-galactosidase molecule can only be decided by examination of the pure enzyme of *S. fragilis* Y-1109.

The β-galactosidase from *S. fragilis* Y-1109 showed a greater rate of hydrolysis of lactose than of ONPG. However, ONPG had a greater affinity than lactose for the enzyme. Michaelis constants (K_M) calculated for the enzyme from *S. fragilis* Y-1109 were slightly higher than those reported by De La Fuente and Sol *(4)* for *S. fragilis* 189-KS.

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REPORT OF COMMITTEE ON FOOD EQUIPMENT SANITARY STANDARDS 1969-1970

(Continued from Page 299)

Both the NAMA and NSF are to be commended for working together on these two proposed changes in the evaluation programs as a continuing joint effort to develop uniform evaluation guidelines.

Ice-maker studies

It has been recognized for several years that in order to dispense quality cold drinks from vending machines ice-making and dispensing equipment must be properly protected from contamination and be of cleanable construction. Studies to aid in accomplishing these features are being carried out by a member of this Committee and other members of AMHIC to determine the bacteriological design and construction aspects of current ice-making equipment being used in vending machines. The first draft of a report on the bacteriological study of three different ice-makers has revealed the following tentative conclusions: *(a)* that there is no single cleaning method that can be applied to all ice-makers, and that total "in-place" cleaning is not presently feasible; *(b)* that access to certain components for cleaning and examination is not presently satisfactory; *(c)* that meltwater "refreezing" properly controlled does not appear to be a problem; *(d)* that a further look at other ice-maker types would be beneficial; and *(e)* that operators should completely clean and sanitize new machines before placing them in operation.

These studies, which have been underway for about 3 years, are being continued; but findings and recommendations to construct and maintain an even easier to clean ice-maker should soon be available to this Committee for consideration and review.

Cut-off controls for machines vending potentially hazardous foods

It was emphasized during the Council meeting that effective and functionable cut-off controls and a rapid field test to determine the reliability of such controls are needed so that the public health officials and vending machine operator could determine whether a machine complied with the Federal Code in preventing the vending of any potentially hazardous foods which had been stored in the machine at unsafe temperatures.

The USPHS Vending Code has provided the Cut-Off Control Requirement to safeguard the food in two respects: *(a)* to interrupt vending of food if the machine has not recovered safe temperatures within a specified period after loading or serving; and *(b)* to discontinue vending after temperature stablizations if, for any reason, the air temperature has risen above safe limits in the storage compartment. The first function is to guard against delivery of foods to the machine at illegal or dangerous temperatures. The second guards against power interruptions, system failure, high thermostat settings, etc.

There have been several problems associated with these controls since 1957, earlier reported as mostly due to the operating personnel but more recently reported as due to failure of the controls involving both the manufacture and the operator. These problems are listed and identified as follows: *(a)* wide tolerances and controls overlap (problems of the manufacturers of the controls); *(b)* premature cut-off controls and discontinuation of vending due to a forgetful routeman (problems of the operators); and *(c)* by-passing of controls, failure to provide access of controls for field testing (problems for the public health personnel).

(Continued on Page 311)

EFFECT OF POTASSIUM SORBATE ON YOGURT CULTURES[1]

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Abstract

The effect of 0.05 and 0.10% potassium sorbate on acid and acetaldehyde production and growth of three commercial yogurt cultures was studied. Either amount of potassium sorbate retarded growth of both *Lactobacillus bulgaricus* and *Streptococcus thermophilus* strains in the commercial cultures and also decreased the rate of acid production of cultures incubated at 45 C. Acetaldehyde in these cultures decreased during storage and decreased more in samples containing potassium sorbate. In all trials 0.1% potassium sorbate had a greater effect than 0.05% on the above characteristics of the yogurt cultures.

Sorbic acid and its salts have been found to inhibit growth of several different kinds of bacteria and fungi in foods *(2, 7, 12)*. Moustafa and Collins *(11)* reported that potassium sorbate inhibited growth of *Pseudomonas fragi* in broth, skim milk, and half-and-half. They also found *(4)* the addition of 0.05 to 0.10% potassium sorbate extended shelf life of cottage cheese.

The effect of adding potassium sorbate to yogurt has not been reported. However, a patent was issued to Chenier *(3)* for use of fungicidal and/or fungistatic agents such as sorbic acid in diluted fermented milk drinks made from yogurt; another patent was issued to Corn Products Company *(5)* who claimed that addition of 0.03 to 0.2% by weight of sorbic acid, or its salts, to cultured milk products promoted growth of desirable bacteria and inhibited the spoilage bacteria.

This work was done to study the effect of potassium sorbate on production of lactic acid and acetaldehyde by yogurt cultures as well as on growth of the organisms used to make up yogurt cultures.

Experimental Procedures

Cultures

Three combined commercial yogurt cultures were used. Culture R1 was obtained from Chr. Hansen's Laboratory[1], and cultures 403 and 405 came from Moseley Laboratory[2].

All cultures were maintained in fresh milk with 3% added instant, non-fat dry milk (NFDM). The medium was prepared by dissolving the NFDM in whole milk and autoclaving at 116 C for 10 min at 15 psi. The cultures were transferred daily, using 3% inoculum, and incubated at 45 C.

Preparation of yogurt

For each phase of the study 1.5 l of fresh homogenized milk were placed in screw-capped, 2-l Erlenmeyer flasks, fortified with 3% of instant NFDM, and autoclaved for 10 min at 15 psi, then cooled to 45 C, and inoculated with 3% culture. The inoculated milk was thoroughly mixed and 150 ml were poured into 8 oz polyethylene cups and incubated at 45 C. Incubation was carried out until the pH of yogurt reached 4.6 in the control milk and then all cups were placed in a refrigerator at 4 C. This procedure provided an undisturbed cup of yogurt for examination at each observation time. The cups were held at 45 C for 8 hr in those trials carried out to determine the effect of potassium sorbate on pH changes during incubation.

Potassium sorbate was added at a level of 0.05 or 0.10% to the milk immediately before inoculation with the yogurt culture used. Control flasks of milk, with no added potassium sorbate, were inoculated to serve as controls.

Analytical determinations

The changes in pH and bacterial population were determined during incubation and storage of the yogurt cultures. The acetaldehyde concentration during storage also was determined.

Measurements of pH were made in duplicate with a Corning Model 12 pH meter, utilizing the expanded scale feature of this instrument. Bacterial numbers were determined by plating dilutions of the yogurt sample on lactic agar as described by Elliker et al. *(6)* for enumerating *Lactobacillus bulgaricus* and on trypticase soy agar *(1)* to enumerate *Streptococcus thermophilus*. All plates were incubated at 37 C and colonies were counted after 72 hr. Acetaldehyde concentration was determined by the 3-methyl-2-benzothiazolone hydrazone hydrochloride test, as modified by Lindsay and Day *(10)*. It was necessary, because of high concentration of acetaldehyde in yogurt samples, to reduce sample weight of ripened yogurt to less than 1 g. It also was necessary to prepare a reagent blank at each test period because of the color change which occurred in the reagent blank held for more than 0.5 hr.

The yogurt was evaluated organoleptically by a panel of 4 to 6 judges. The judges, in making their evaluation, classified the flavor quality of the yogurt as: excellent, good, fair, or poor. Criticisms of flavor also were noted. The yogurt samples were coded to prevent identification of the treatment by the judges. Since the number of samplings made was comparatively small, no statistical analyses of the evaluation were made; however, the judges' opinions were summarized.

Results and Discussion

Table 1 shows the effect of two levels, 0.05 and 0.1% of potassium sorbate on the acid production of three yogurt cultures, 403, R1, and 405, during 8 hr of incubation at 45 C. Samples treated with

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[1]Published with approval of the Director, Wyoming Agricultural Experiment Station, as Journal Paper 459.

[1]Milwaukee, Wisconsin

[2]Indianapolis, Indiana

TABLE 1. EFFECT OF POTASSIUM SORBATE ON pH VALUES OF THREE YOGURT CULTURES DURING INCUBATION AT 45 C.

Time of incubation	Culture R1			Culture 403			Culture 405		
	A[1]	B[2]	C[3]	A	B	C	A	B	C
(hr)	(pH)								
0	6.30	6.30	6.30	6.20	6.20	6.20	6.30	6.30	6.30
1	5.50	5.65	5.75	5.55	5.75	5.85	5.80	5.95	6.00
2	4.75	5.00	5.10	4.75	4.90	5.15	4.85	5.05	5.25
3	4.35	4.60	4.70	4.40	4.50	4.60	4.45	4.65	4.75
4	4.10	4.40	4.50	4.20	4.30	4.40	4.20	4.40	4.50
5	3.95	4.30	4.40	4.00	4.10	4.20	4.05	4.25	4.35
6	3.95	4.20	4.25	4.00	4.10	4.10	3.95	4.10	4.20
7	3.90	4.10	4.20	3.95	4.00	4.10	3.95	4.10	4.15
8	3.85	4.05	4.15	3.90	4.00	4.05	3.90	4.05	4.10

[1]Control, no potassium sorbate added
[2]0.05% by weight added potassium sorbate
[3]0.10% by weight added potassium sorbate

TABLE 2. EFFECT OF POTASSIUM SORBATE ON pH OF THREE YOGURT CULTURES DURING STORAGE AT 4 C.

Storage	Culture R1			Culture 403			Culture 405		
	A[1]	B[2]	C[3]	A	B	C	A	B	C
(days)	(pH)								
0	4.60	4.70	4.75	4.65	4.75	4.75	4.60	4.60	4.70
1	4.35	4.55	4.50	4.35	4.50	4.70	4.30	4.45	4.60
3	4.20	4.40	4.45	4.20	4.35	4.45	4.20	4.25	4.35
6	4.10	4.30	4.40	4.10	4.25	4.40	4.10	4.20	4.30
9	4.10	4.25	4.30	4.00	4.20	4.35	4.05	4.15	4.15
14	3.90	4.10	4.15	4.00	4.20	4.35	4.00	4.10	4.15
21	3.90	4.10	4.20	3.90	4.20	4.35	3.95	4.10	4.15
28	3.90	4.10	4.20	3.90	4.20	4.30	3.95	4.10	4.15
32	3.85	4.05	4.10	3.85	4.00	4.20	3.95	3.95	4.15

[1]Control, no potassium sorbate added
[2]0.07% by weight added potassium sorbate
[3]0.10% by weight added potassium sorbate

either level of potassium sorbate had higher pH values than samples without potassium sorbate, regardless of the culture used. Addition of 0.1% potassium sorbate retarded acid production more than did 0.05%. The effect of potassium sorbate on acid production was somewhat greater during the first 4 to 5 hr of incubation than during the remainder of the incubation period. A representative example for this effect is shown in Fig. 1.

Table 2 shows the effect of potassium sorbate on acid production during storage at 4 C for 32 days. The initial pH of the yogurt containing added potassium sorbate generally was higher than that of the control because of the slight decrease in acid production in the sorbate-treated milk during the incubation period prior to storage. All three cultures showed retarded acid production during storage when treated with potassium sorbate. The pH of all controls dropped to below 4.0 after the second week of storage, whereas the pH of all samples con-

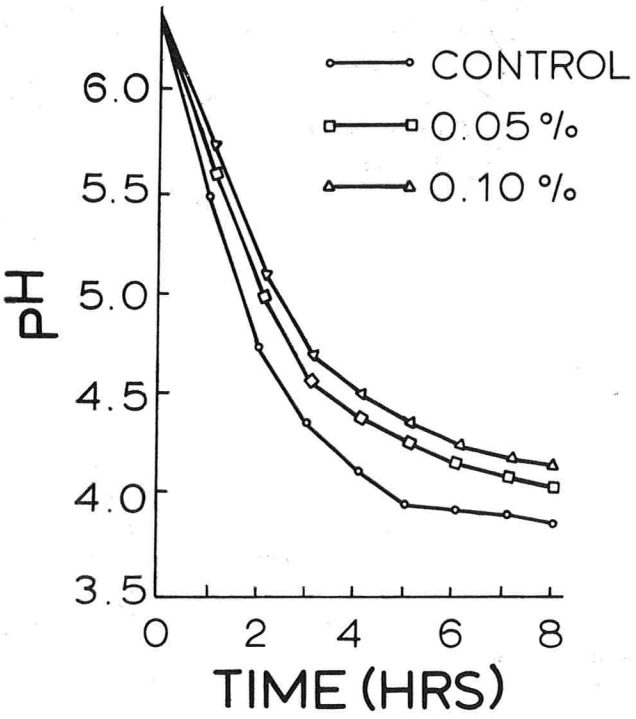

Figure 1. Effect of added potassium sorbate on pH of culture R1 during incubation at 45 C.

taining potassium sorbate remained about 4.0 even after 4 weeks of storage. If the pH of yogurt is to be used as an index of shelf life, these data indicate that the shelf life can be increased by the addition of potassium sorbate.

The effect of added potassium sorbate on flavor evaluation during refrigerated storage of plain yogurt made with culture R1 is shown in Table 3. The flavor evaluations of the yogurt at zero days storage were inconsistent. The judges' principal criticism was that of "low acidity" or "lacking yogurt flavor". The pH of the yogurt, as shown in Table 1, ranged from 4.60 to 4.75 depending upon the culture used and amount of potassium sorbate added. The flavor of the samples containing potassium sorbate was preferred over that of the control samples as the storage period lengthened. The most common flavor criticism, particularly with the control samples, after 2 weeks of storage was "high acidity." The yogurt containing potassium sorbate had a more mild acid flavor than the control samples, and was, in fact, criticized as slightly lacking in acid flavor the first 3 days of storage. An off-flavor characterized as "astringent" or "slight chemical flavor" was noted by one of the judges in some yogurt samples containing 0.10% potassium sorbate and stored 14 days or more. This flavor was not observed in the yogurt to which 0.05% potassium sorbate had been added. The other cultures used responded in much the same way and developed a milder acid flavor following addition of potassium sorbate.

TABLE 3. EFFECT OF POTASSIUM SORBATE ON FLAVOR OF YOGURT MADE WITH CULTURE R1 AND STORED THREE WEEKS AT 4 C.

Storage	Potassium sorbate	Flavor preference			pH
		Good	Fair	Poor	
(days)		(Per cent)			
3	0.00	75.00	00.00	25.00	4.20
	0.05	00.00	25.00	75.00	4.40
	0.10	25.00	25.00	50.00	4.45
6	0.00	33.30	66.70	00.00	4.10
	0.05	16.65	66.70	16.65	4.30
	0.10	50.00	50.00	00.00	4.40
14	0.00	00.00	100.00	00.00	3.90
	0.05	50.00	50.00	00.00	4.10
	0.10	50.00	50.00	00.00	4.15
21	0.00	40.00	40.00	20.00	3.90
	0.05	80.00	20.00	00.00	4.10
	0.10	80.00	20.00	00.00	4.20

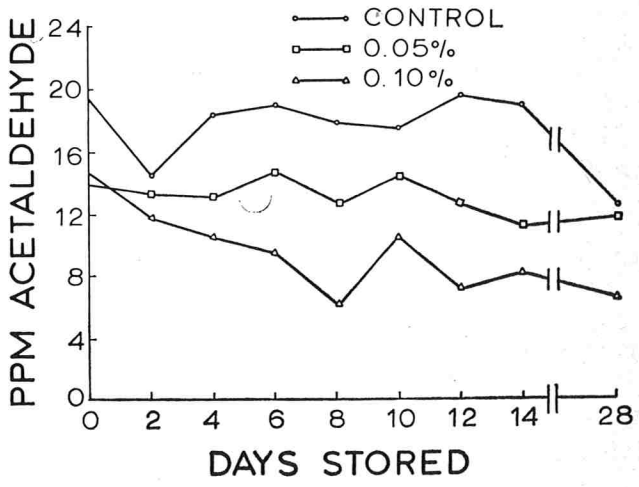

Figure 2. Effect of potassium sorbate on acetaldehyde concentration of culture R1 during storage at 4 C.

Figure 2 shows the effect of potassium sorbate on the concentration of acetaldehyde in yogurt culture R1 during 4 weeks of storage at 4 C. The acetaldehyde concentration was reduced in all three cultures containing added potassium sorbate. Since acetaldehyde has been recognized as an important flavor component of good quality yogurt *(8)*, the reduction in acetaldehyde concentration following addition of potassium sorbate may be considered by some to be a reason for not adding potassium sorbate to yogurt even though the rate of acid production during storage was slowed thus increasing the

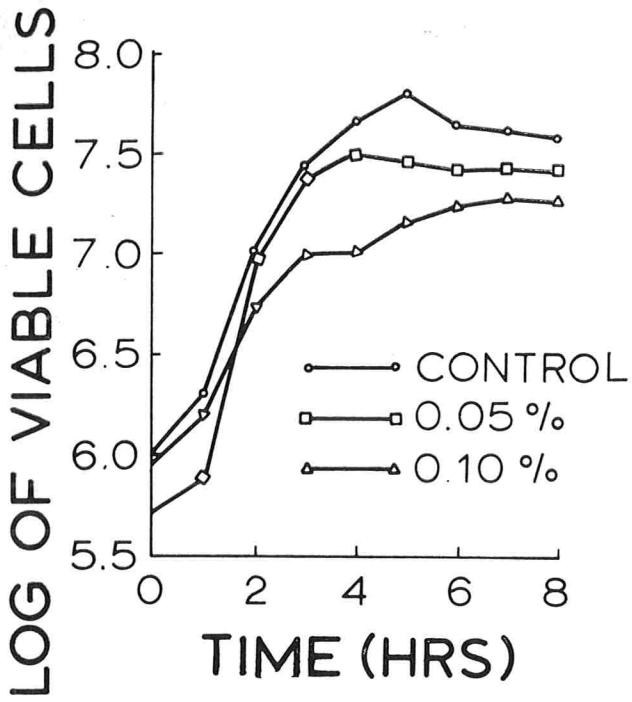

Figure 3. Effect of potassium sorbate on the growth of *L. bulgaricus* in culture 403.

time required for the development of an undesirable, pronounced, acid flavor. The members of the judging panel, however, did not consistently identify or indicate a preference for those yogurt samples with the higher levels of acetaldehyde.

Potassium sorbate did retard the increase in numbers of both *L. bulgaricus* and *S. thermophilus* during an 8 hr incubation period at 45 C. This effect was noted with the strains of these organisms present in all three commercial cultures. Representative growth curves for the *L. bulgaricus* and *S. thermophilus* strains present in culture 403 are shown in Fig. 3 and 4, respectively. These show that both levels of potassium sorbate reduced the maximum number of viable organisms attained by either organism. The effect was less evident during the first 2 hr of incubation. The greatest differences in cell count were found during the period the highest cell counts were obtained. The reduction in cell count of *S. thermophilus* in yogurt containing potassium sorbate was greatest when the pH reached 4.6 to 4.1. The maximum reduction in numbers of *L. bulgaricus* occurred when the pH of the yogurt ranged from 4.4 to 4.1. Maximum growth for *S. thermophilus* with or without added potassium sorbate was reached between the second and fourth hour of incubation, whereas the maximum growth for *L. bulgaricus* was reached at the fourth or fifth hour of incubation. A concentration of 0.05 or 0.10% of potassium sorbate decreased the numbers of both organisms present throughout the incubation period. This reduction in numbers of the culture organisms by potassium sorbate may explain the reduction of lactic acid and acetaldehyde production as shown in the previous experiments. In support of this contention, Keenan et al. *(9)* reported that acetaldehyde production by several single-strain lactic organisms appeared to parallel the increase in microbial population.

It was evident from this study that growth and acid production of the lactic cultures used were inhibited by potassium sorbate. Thus, the decrease in pH that normally occurs during storage of yogurt was slowed, allowing a longer storage period before the flavor became more acid than desired. It also was found, however, that the concentration of acetaldehyde, a compound associated with the desired flavor in yogurt, decreased during storage of the product. Addition of potassium sorbate lowered the acetaldehyde content. The relative importance of these two effects would have to be assessed in making a decision as to whether or not potassium sorbate should be used in making yogurt.

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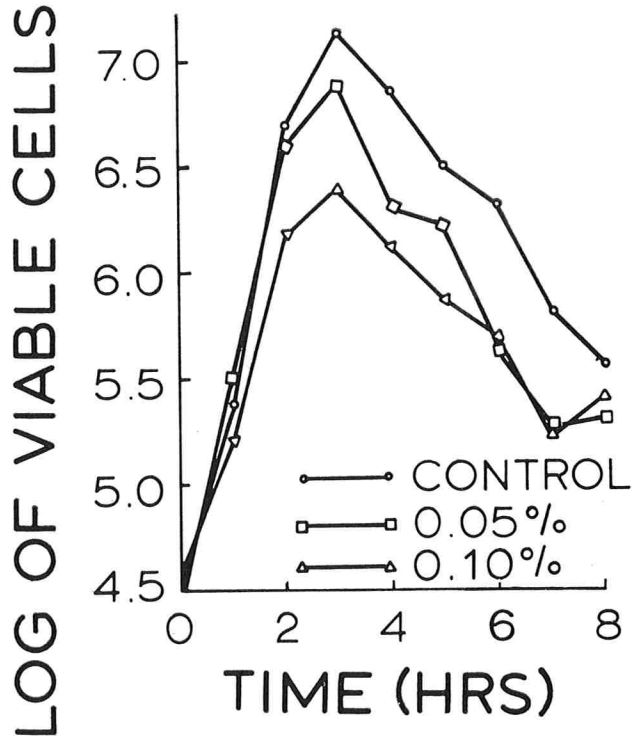

Figure 4. Effect of potassium sorbate on the growth of *S. thermophilus* in culture 403.

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REPORT OF COMMITTEE ON FOOD EQUIPMENT SANITARY STANDARDS 1969-1970

(Continued from Page 306)

In order to obtain solutions to these cut-off control problems, the members of the Council recommended the following course of action: *(a)* the controls manufacturer will be asked to perform time-temperature tests on controls set at different cut-off points from 45 F to 80 F, or thereabout; *(b)* a rapid field test using tap water will be studied and reported by the manufacturers (recent research studies by the manufacturer of the controls have indicated that additional studies will be needed to develop a practical field test); *(c)* NAMA will develop an instructional booklet for each model setting forth the method of determining when the model has become "inoperative," as required by the Code and Manual; *(d)* the Committee will have a report submitted to the Manual and Checklist Revision Committee far enough in advance of the 1970 AMHIC meeting to allow amendment of the Manual, if necessary, or a recommendation for a Code change; and *(e)* the machine manufacturers, generally, agree that factory test procedure to carry out their responsibilities in this area of cut-off controls can be met, but first it must be evaluated against various models during the next few months.

Remanufactured vending machine evaluation program

The Remanufactured Vending Machine Evaluation Program formerly has been known as the Other-Than-New Vending Machine Evaluation Program. This Program of completely rebuilding used vending machines and renovating them so as to not only meet the current requirements of the NAMA Vending Machine Evaluation Manual but also to make some significant innovations in design has received favorable response from public health officials and vending machine operators. To date, machines of two large companies have been evaluated under this program; and several other companies have indicated a keen interest in this relatively new program.

Labeling guide

As indicated in the 1969 Committee Report, the advent of the Fair Packaging and Labeling Act, the Wholesome Meat Act, and the Wholesome Poultry Products Act has extended uniform Federal labeling requirements to a vast majority of foods prepared and packaged by vending operators—even in intrastate commerce. AMHIC has already taken steps to develop a uniform labeling guide in cooperation with the FDA and USDA for the purpose of aiding industry in complying with these Federal Acts and also to aid in promoting uniformity in their interpretation and application. Principles developed and applied with these two agencies in developing guidelines to comply with the Federal labeling requirements may be used with possibly some minor modifications with foods vended only in intrastate commerce.

The first draft of a proposed NAMA Food Product Labeling Guide for Vending Operators has been presented to this Committee and to the FDA and USDA for review, comment, and approval. This Guide when eventually approved by AMHIC and the above Federal agencies should minimize interpretation problems for the vending machine operator and governmental officials and promote voluntary compliance with the appropriate laws and regulations.

Commissary construction guide

The first draft of a Commissary Construction Guide which is intended to advise vending machine commissary operators of basic commissary layout and construction requirements in their respective states and at the Federal level has been reviewed by this Committee and is currently being reviewed by NAMA, FDA, USDA, and USPHS. The restaurant type requirements in this first draft have been taken from the USPHS 1962 Food Service Sanitation Manual, and the agriculture type requirements are those recommended by USDA in various documents and advisory letters to NAMA. Standards of both Federal agencies are included in the proposed Guide; but it is hoped before the final draft is completed that the USDA, FDA, USPHS, and NAMA can agree on single requirement statements in each paragraph of the Guide which treats a specific subjct.

Other educational materials

An informative and interesting piece of literature developed by NAMA-AMHIC in cooperation with this Committee should prove of interest to the general public and to members of industry and public health. This publication, *Copper Poisoning Prevention—A Guide for Post-Mix Soft Drink Equipment Operators,* is intended to aid the vending industry in preventing a food-borne illness caused by copper poisoning. The publication on copper poisoning applies to both manual and

(Continued on Page 325)

E-3-A SANITARY STANDARDS FOR INLET AND OUTLET LEAK PROTECTOR PLUG VALVES FOR BATCH PASTEURIZERS

Serial #E-1400

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

It is the purpose of the IAMFES, USPHS, USDA, IAPI, and DFISA in connection with the development of the E-3-A Sanitary Standards program, to allow and encourage full freedom for inventive genius or new developments. Leak protector plug valve specifications which are developed and which so differ in design, material, construction or otherwise, so as not to conform with the following standards, but which in the opinion of the manufacturer or fabricator are equivalent or better, may be submitted at any time for the consideration of IAMFES, USPHS, USDA, IAPI, and DFISA.

MATERIAL:

1. These valves shall be constructed of stainless steel, optional metal or equally corrosion-resistant metal that is non-toxic and non-absorbent. (See Appendix for the composition of an acceptable optional metal alloy.)

DESIGN:

1. The design of leak protector valves shall conform to the recommendations set forth in Section 7, item 16p (A) 3 and 4 of the Grade "A" Pasteurized Milk Ordinance, 1965 Recommendations of the United States Public Health Service.
   An abstract of these requirements follows and drawings of acceptable valves are appended to this standard.

   (a) All leak protector valves shall be provided with leak diverting grooves which when the valve is in a closed position, will prevent leakage past the valve either by way of the valve seat or the plug channel. A closed position shall mean any position of the valve seat which stops the flow of product into or out of the holder. It includes the "just-closed" position, which means that closed position of the plug in which the flow into or out of the holder is barely stopped or any closed position within 5/64-inch thereof as measured along the maximum circumference of the valve seat. It also includes the "fully-closed" position, which means the closed position of the valve seat which requires the maximum movement of the valve to reach the fully-open position, and all positions between the "just-closed" and "fully-closed" position. Leak-protector grooves must begin to function within 5/64-inch after the "just-closed" position is passed in closing.

   (b) All leak-protector grooves shall be at least 3/16-inch wide and at least 3/32-inch deep at the center. Mating grooves shall provide these dimensions throughout their combined length whenever the valve is in, or approximately in, the "fully-closed" position. All single-leak grooves, and all mating leak grooves when mated, shall extend throughout the entire depth of the seat, so as to divert leakage occurring at all points throughout the depth of the seat, and so as to prevent air bindings. Washers or other parts shall not obstruct leak-protector grooves.

   (c) All leak-protector valves shall be provided with a stop to guide the operator in closing the valve so that unpasteurized product may not inadvertently be permitted to enter the outlet line or the holder. The stop shall be so designed that the plug will be irreversible when the plug is provided with any grooves or their equivalent, unless duplicate, diametrically opposite grooves are also provided. In the case of 2-way, plug-type valves (i.e. those having only one inlet and one outlet), a 180° stop, or any combination of stops permitting two fully-closed positions, may be substituted for a 90° stop, provided that there are no air-relief grooves in the plug and that all leak grooves are located symmetrically with respect to the valve inlet. Stops shall be so designed that the operator cannot turn the valve beyond the stop position, either by raising the plug or by any other means.

A valve stop means a guide which permits turning the valve plug to, but not beyond, the fully-closed position. A 90° stop means a stop so designed as to prevent turning the plug more than 90°. A 180° stop means a stop which prevents turning the plug more than 180°. A valve with an irreversible plug shall mean one in which the plug cannot be reversed in the shell.

(d) Leak-protector inlet valves shall be provided with grooves to provide air-relief to the inlet line to the holder when the valve is in any closed position.

(e) Leak-protector outlet valves shall be so designed as to prevent the accumulation of unpasteurized product in the product passages of the valve when the valve is in any closed position.

(f) Leak-protector outlet valves shall be so designed that the combined length of the inlet passage in the shell and of any passage of corresponding diameter in the pasteurizer does not exceed the diameter of the passage in the valve.

CONSTRUCTION:

1. All product contact surfaces shall be finished to an equivalent of not less than 120 grit properly applied.
2. All parts having product contact surfaces shall be readily removable or shall be accessible for cleaning and inspection. All exterior surfaces shall be smooth, easily cleanable, and self-draining.
3. The diameter of the product passage and the dimensions of the sanitary thread and of the fitting seal shall conform with the standards established for the similar counterpart in the "3-A Sanitary Standards for Fittings Used on Milk and Milk Products Equipment and Used on Sanitary Lines Conducting Milk and Milk Products, Serial #0809," as amended.
4. The stop may be cut or cast in the body and/or plug. If a stop pin is used, it shall have:
   (a) A minimum shear strength of 30,000 P.S.I.
   (b) The pin shall be attached with a thread having a Class 3 fit and a minimum length of 1/2 inch.
   (c) The pin shall have a minimum diameter of not less than 5/16-inch O.D. throughout its entire length except at root of thread at threaded end.
   (d) The pin shall be made of material that will withstand the repeated impact that occurs in continued operation without breaking or becoming sufficiently distorted to cause improper register of the leak protector parts.
5. The handle of the valve shall be at right angles to the direction of flow when in fully-closed position. If valve handle is so positioned as not to be at 90° when fully closed, the valve shall be marked to show the fully-closed position.

Appendix

OPTIONAL METAL ALLOY

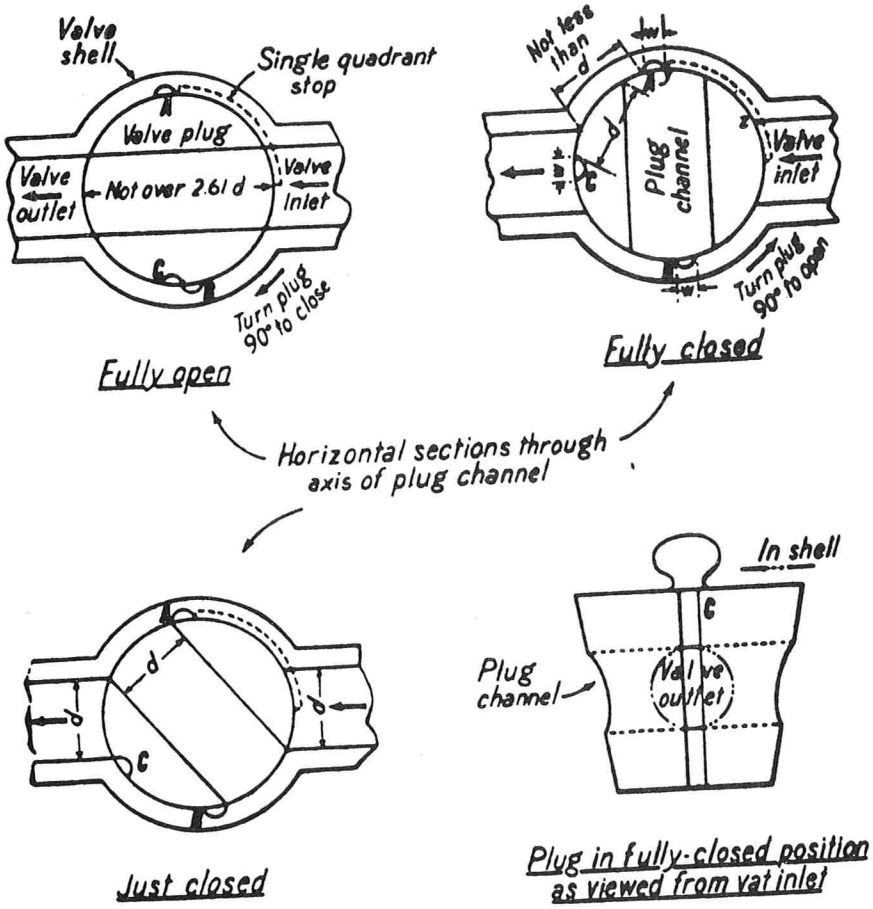
An optional metal alloy having the following minimum and maximum composition is deemed to be in compliance with the MATERIAL section:

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

An alloy of the composition given above is properly designated "nickel silver" or, according to ASTM[1] Specification #B 149-52, may be entitled, "leaded nickel bronze."

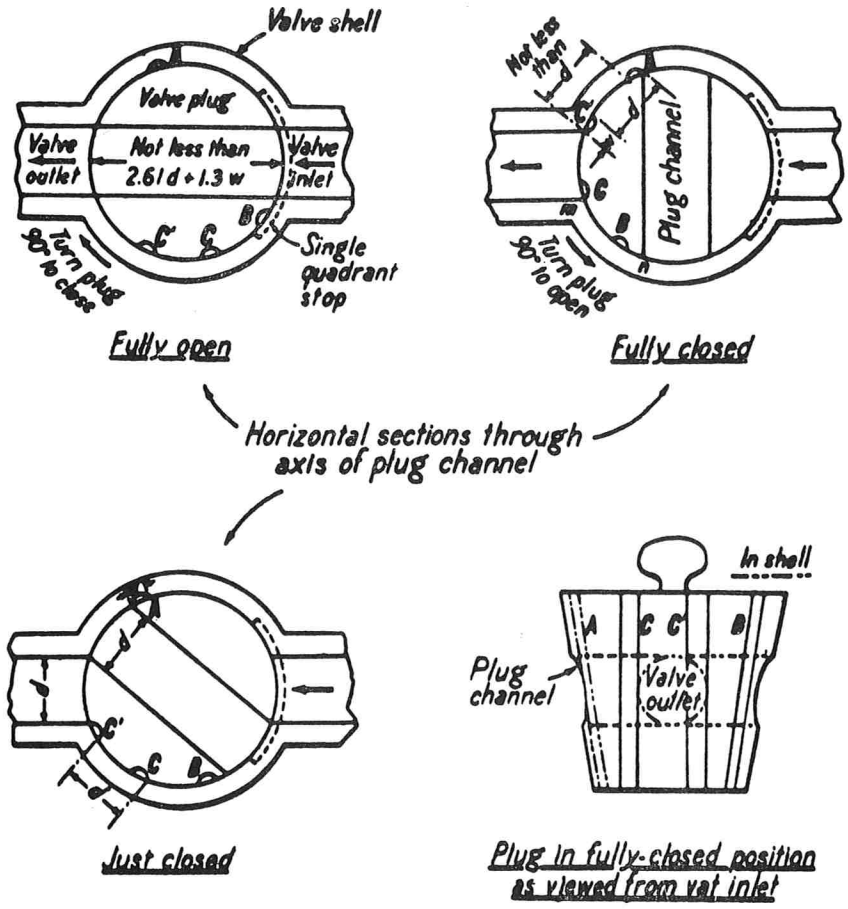
[1]Available from American Society for Testing and Materials 1916 Race St., Philadelphia, Pa. 19103.

This standard is effective Aug. 28, 1971.



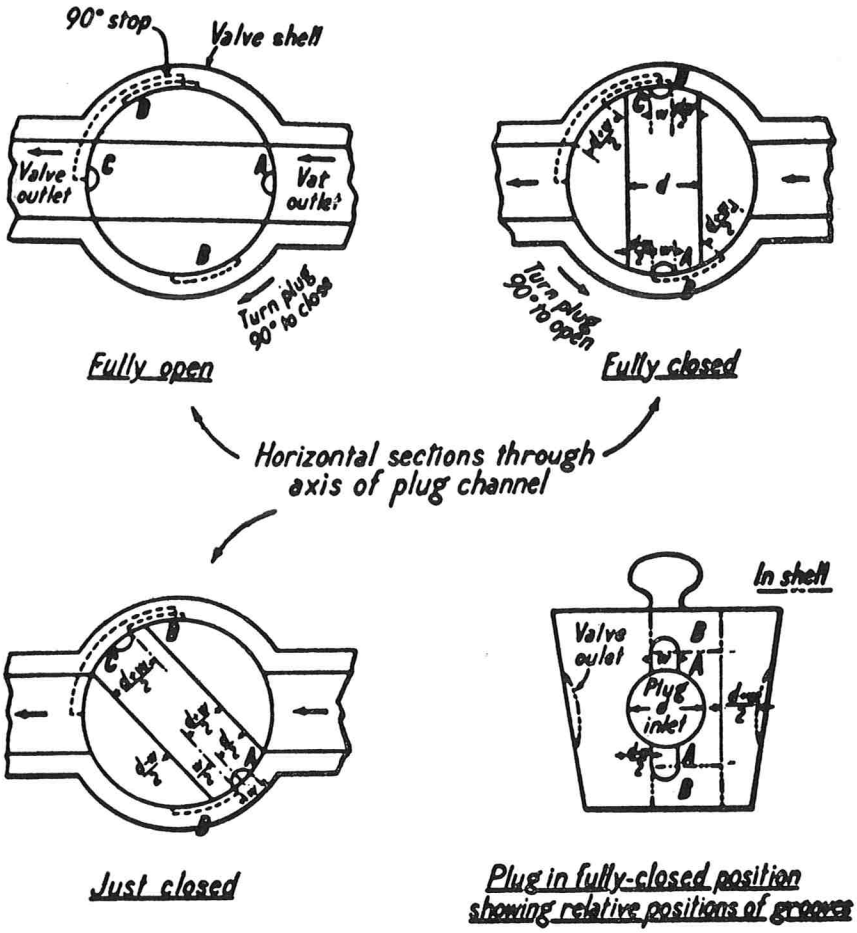
Notes: A and B are leak-protector grooves. C is an air-relief groove. All grooves are full length, but upper or lower half of C may be omitted.
W = not less than 3/16″

TWO-WAY HORIZONTAL PLUG-TYPE INLET VALVE (DESIGN - A)



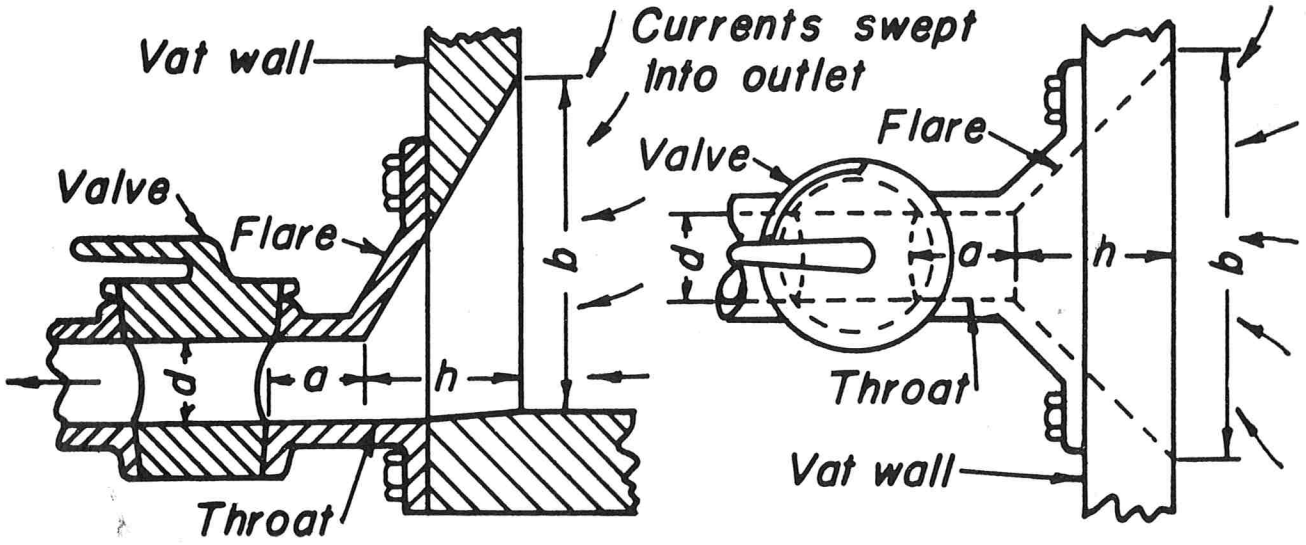
Notes: *A and B are leak-protector grooves. C and C¹ are air-relief grooves. All grooves are full length, but upper or lower halves of C and C¹ may be omitted. W = not less than 3/16″*

TWO-WAY HORIZONTAL PLUG-TYPE INLET VALVE (DESIGN - B)



Notes: A, B, C, and D are leak-protector grooves. A extends part way in the plug and mates with B (in upper and lower shell) in all closed positions. Grooves C and D are diagonally opposite A and B.
W = not less than 3/16″

PLUG-TYPE SIDE-OUTLET VALVE (DESIGN-E)

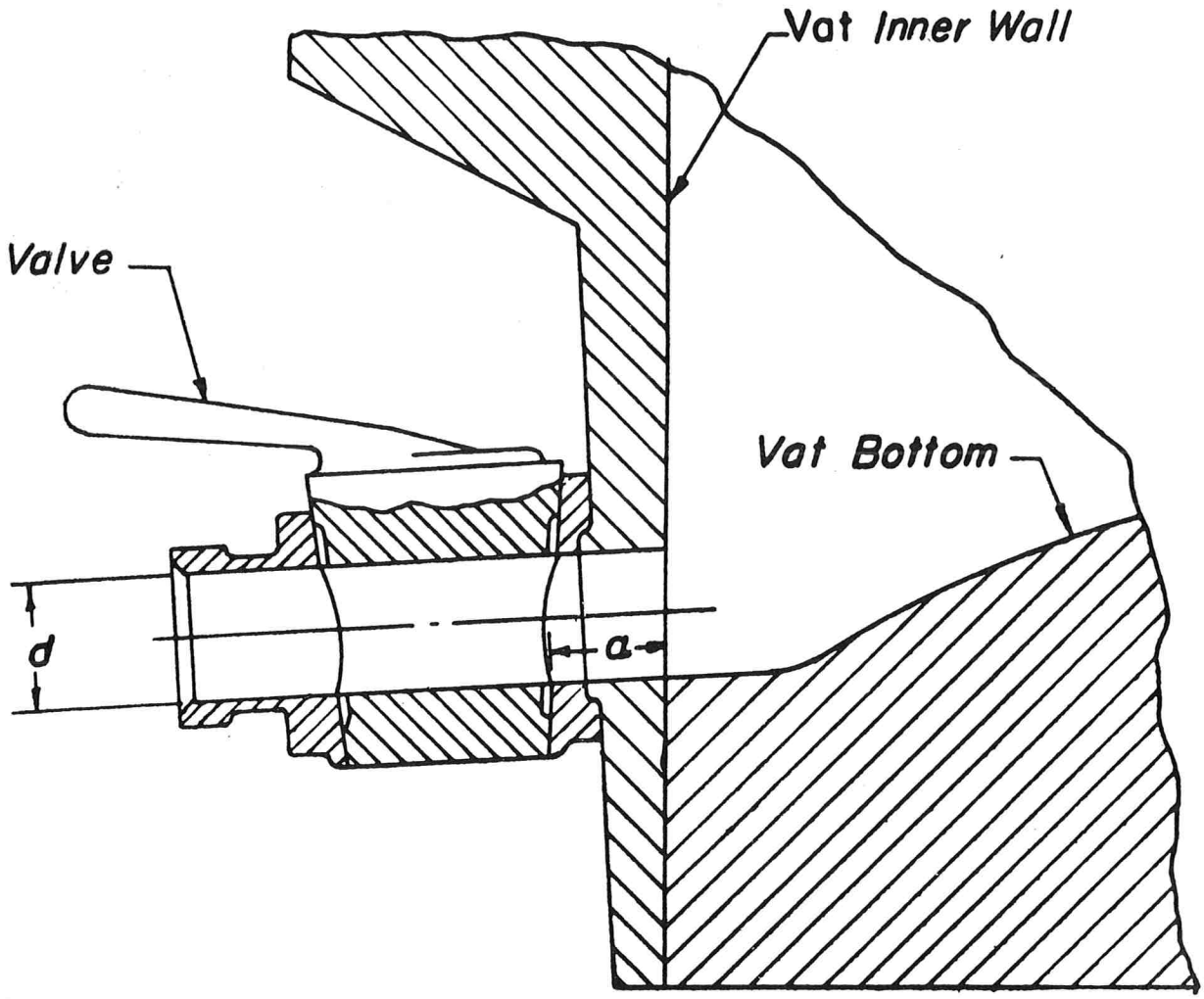


VERTICAL SECTION PLAN

Note: Close-coupled side-outlet valve connected to holder, showing design requirements. d = diameter of outlet. h = depth of flare. a = greatest distance from valve seat to small end of flare (shall be not more than d.) b = smallest diameter at large end of flare (shall be not less than h + d.)

CLOSE-COUPLED SIDE-OUTLET VALVE (DESIGN-D)

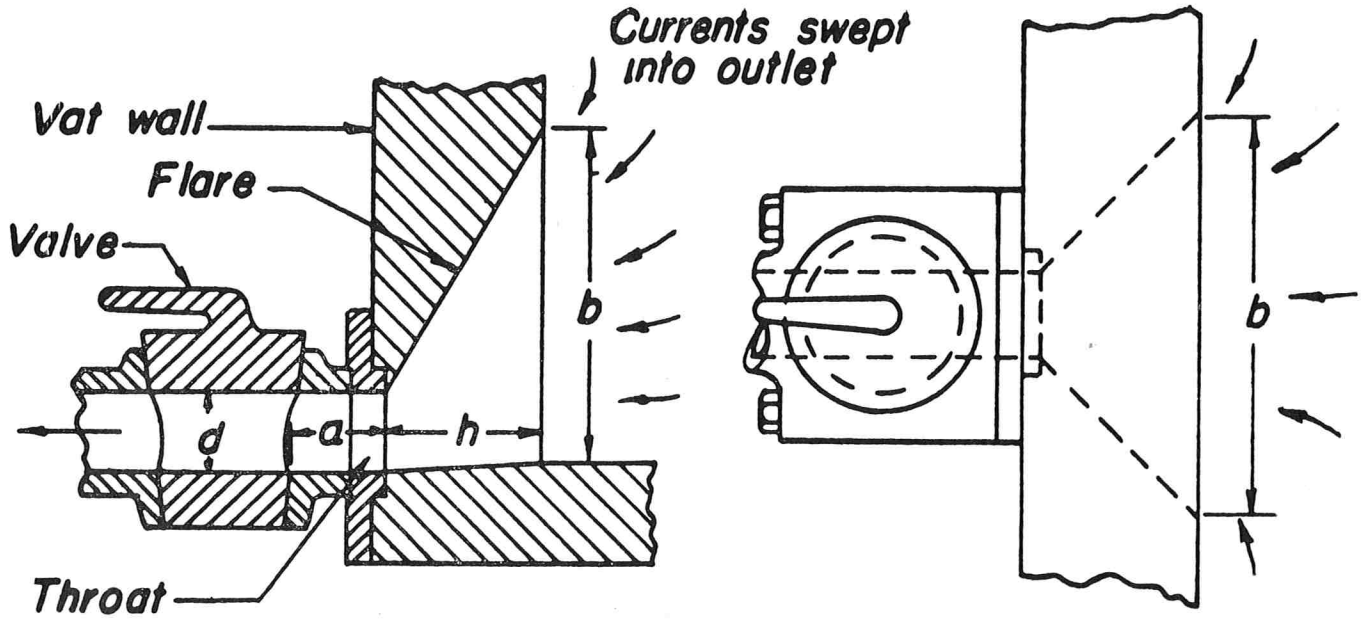
SANITARY FITTINGS
3-A STANDARD
3-A—100—19



Note: Close-coupled side-outlet valve connected to holder, showing design requirements. d = inside diameter of outlet valve. a = greatest distance from valve seat to vertical side of inner wall. (In no case shall it exceed d.) No flare required for reason that a is less than d.

CLOSE-COUPLED SIDE-OUTLET VALVE
(DESIGN-D ALTERNATE)

SANITARY FITTINGS
3-A STANDARD
3-A—100—20



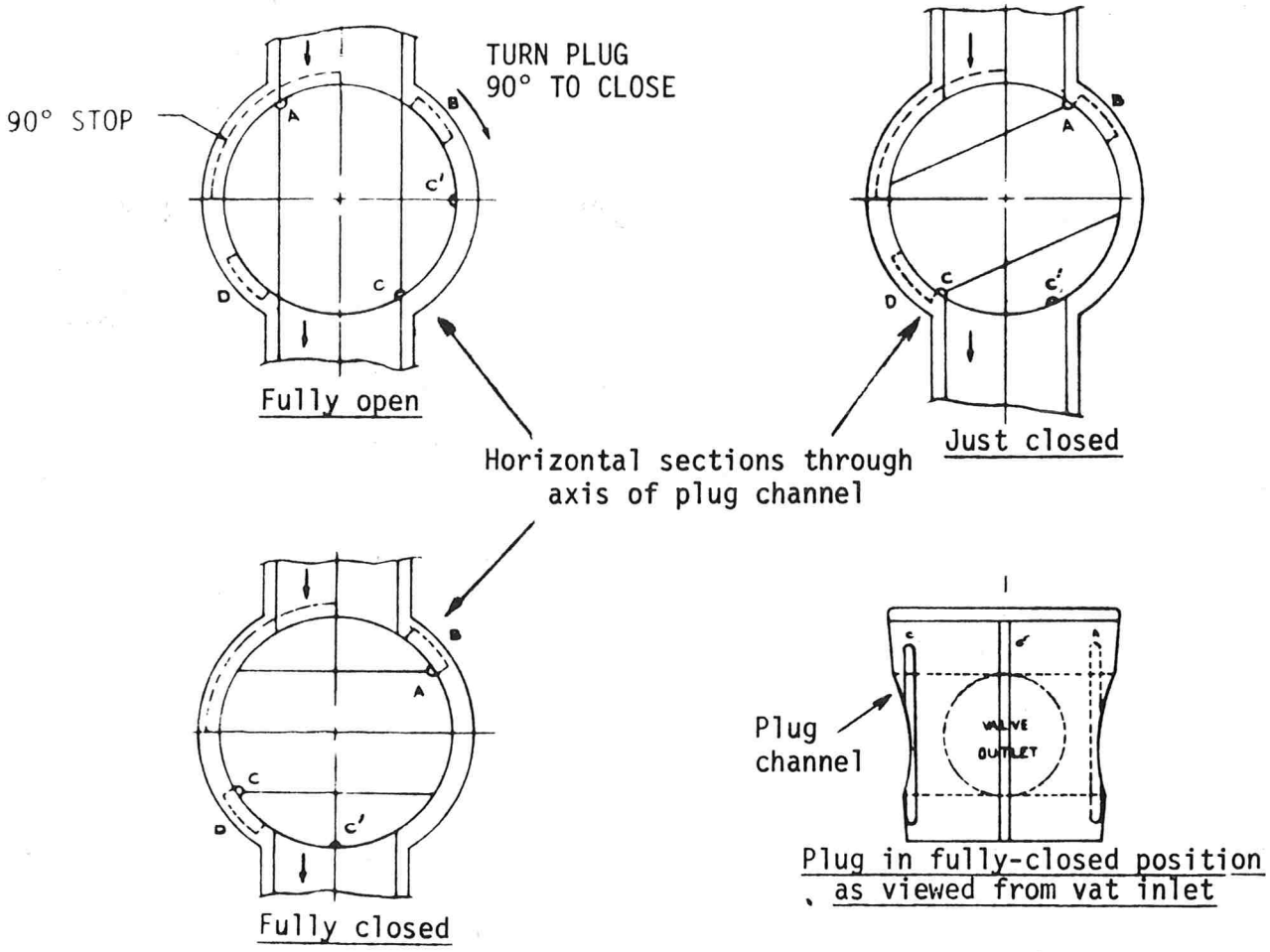
VERTICAL SECTION

PLAN

Note: Close-coupled Side-outlet valve connected to holder, showing design requirements. d = inside diameter of outlet. h = depth of flare. a = greatest distance from valve seat to small end of flare. (Shall not be more than d.) b = smallest diameter at large end of flare (shall not be less than h plus d)

CLOSE-COUPLED SIDE-OUTLET VALVE (DESIGN-D ALTERNATE)

SANITARY FITTINGS
3-A STANDARD
3-A—100—21



TWO-WAY VERTICAL PLUG-TYPE INLET VALVE

NOTES: A, B, C and D are leak-protector grooves. A extends part way in the plug and mates with B (in upper and lower shell) in all closed positions. Grooves C and D are diagonally opposite A and B.

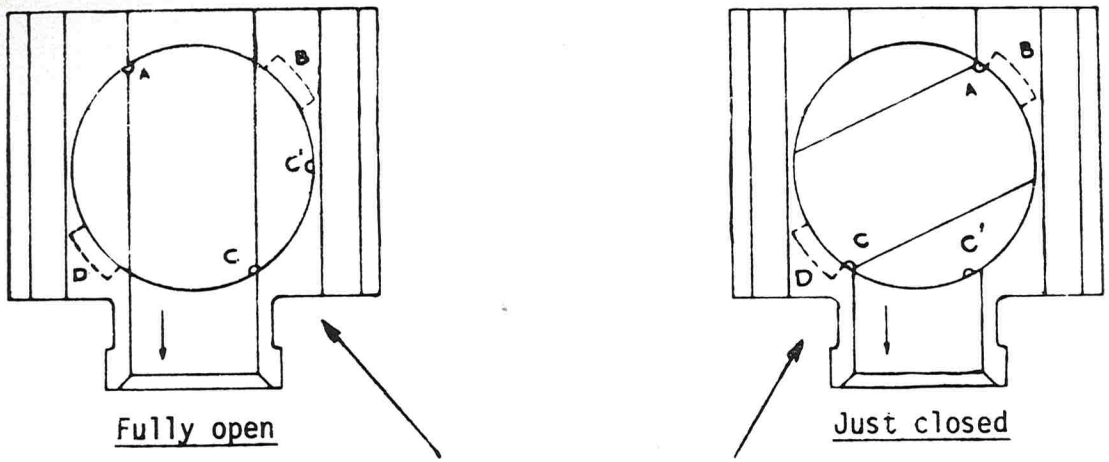
C^1 is an air-relief groove for the outlet. Upper or lower half of C^1 may be omitted.

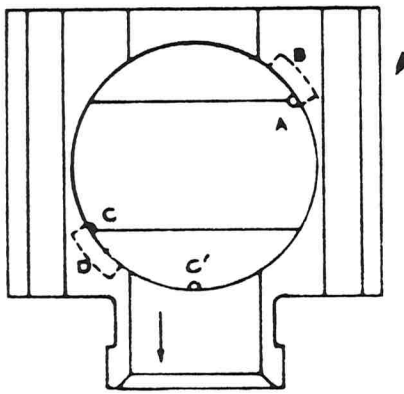
Leak-protector grooves must begin to function within 5/64 of an inch after the "just closed position" is passed in closing.

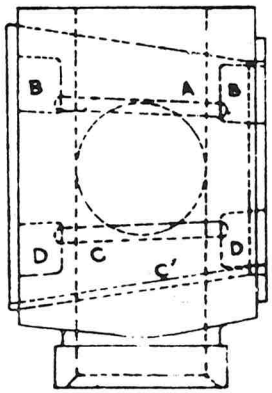
The configuration of leak-protector grooves D (the drain) shall be such that there will be no pocket to trap product and all product will be drained.

A 90° stop shall be provided. The positioning of the stop shall be such that the product in the passage in the plug will drain completely.

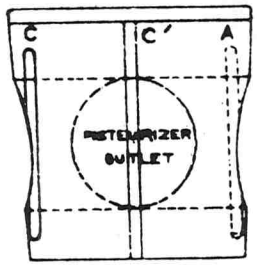
SANITARY FITTINGS
3-A STANDARD
3A-100-27







Side view of valve showing position of leak protector grooves and air relief grooves when plug is in fully closed position

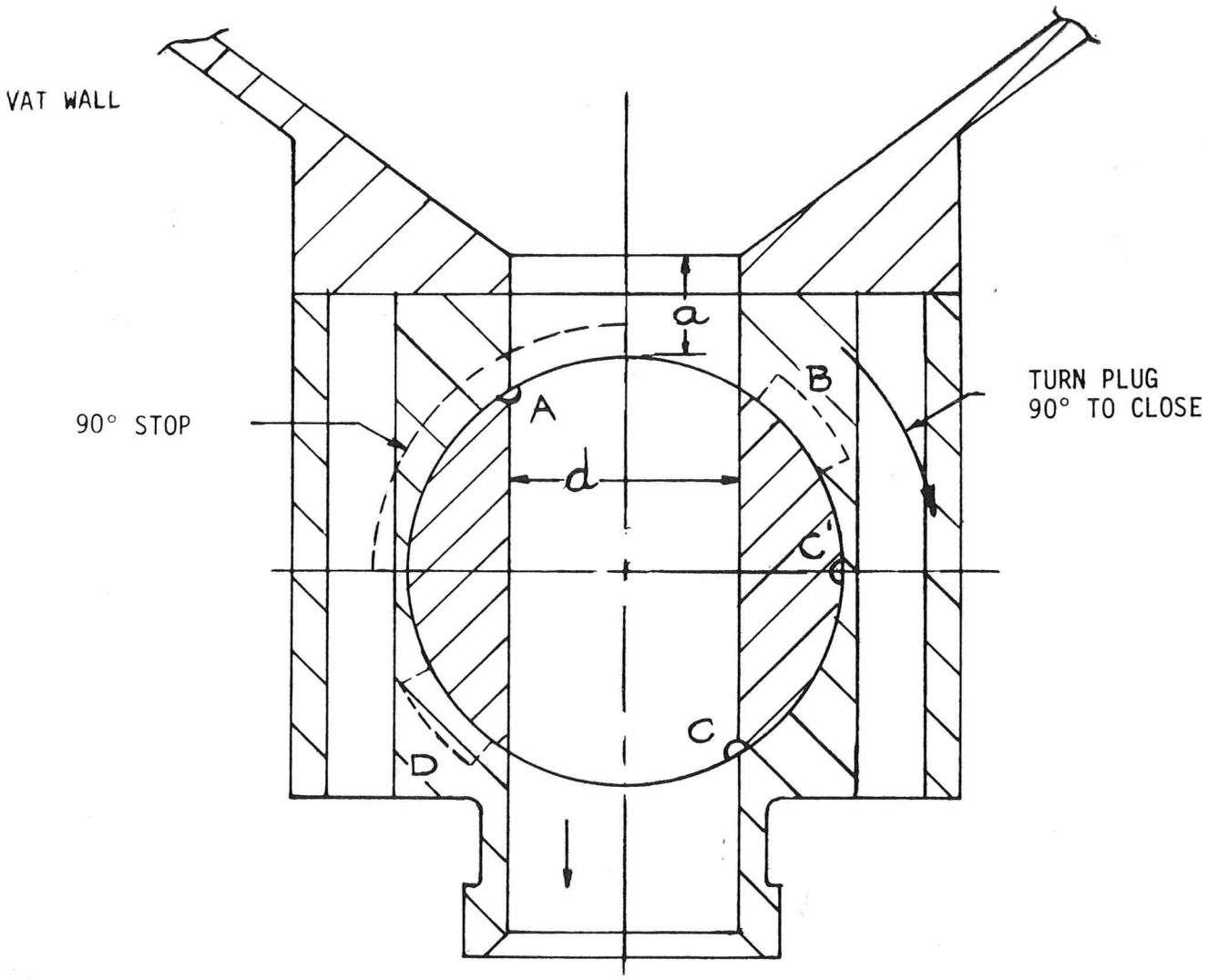


Plug in fully-closed position
showing relative positions of grooves

PLUG-TYPE BOTTOM-OUTLET VALVE

Notes: A, B, C and D are leak-protector grooves. A extends part way in the plug and mates with B (in upper and lower shell) in all closed positions. Grooves C and D are diagonally opposite A and B.
C¹ is an air-relief groove for the outlet. Upper or lower half of C¹ may be omitted.
Leak-protector grooves must begin to function within 5/64 of an inch after the "just closed position" is passed in closing.
The configuration of leak-protector groove D (the drain) shall be such that there will be no pocket to trap product and all product will be drained.
A 90° stop shall be provided. The positioning of the stop shall be such that the product in the passage in the plug will drain completely.

SANITARY FITTINGS
3-A STANDARD
3A-100-28



VERTICAL SECTION

NOTE: Close coupled bottom outlet valve connected to holder showing design requirements.

d = inside diameter of outlet valve.

a = greatest distance from valve seat to end of holder portion of outlet having same diameter as the passage in the valve.

a shall not be more than d.

CLOSE-COUPLED BOTTOM-OUTLET VALVE

SANITARY FITTINGS

3-A STANDARD

3-A-100-29

3-A SANITARY STANDARDS FOR HOMOGENIZERS AND PUMPS OF THE PLUNGER TYPE

Serial #0403

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

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

A.

SCOPE

A.1

These standards cover the sanitary aspects of homogenizers and pumps of the plunger type for milk and liquid milk products.

A.2

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

B.

DEFINITIONS

B.1

Product: Shall mean milk and liquid milk products.

B.2

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

B.3

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

B.4

Surfaces:

B.4.1

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

B.4.2

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

C.

MATERIALS

C.1

Product Contact Surfaces:

C.1.1

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

C.1.2

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

C.1.3

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

C.1.4

Plastic materials may be used for gaskets, seals, and parts used in similar applications. These materials shall comply with the applicable provisions of the "3-A Sanitary Standards for Multiple-Use Plastic Materials Used as Product Contact Sur-

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

[2]Alloy Casting Institute, 300 Madison Ave., New York, N. Y. 10017.

faces for Dairy Equipment, Serial #2000", as amended.

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

C.2
Non-Product Contact Surfaces:

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

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

D.
FABRICATION

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

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

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

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

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

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

D.7
Inlet and outlet connections shall conform with the applicable provisions of the "3-A Sanitary Standards for Fittings Used on Milk and Milk Products Equipment and Used on Sanitary Lines Conducting Milk and Milk Products, Serial #0809", as amended.

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

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

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

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

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

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

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

D.12
The space between the cylinder(s) and the drive shall be readily accessible for cleaning, self-draining and protected so that liquids will not enter the drive. This space shall be provided with a cover or shield. The cover may be designed to permit observation without removing it from the homogenizer or pump.

D.13

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

APPENDIX

E.

STAINLESS STEEL MATERIALS

Stainless steel conforming to the applicable composition ranges established by AISI for wrought products, or by ACI for cast products, should be considered in compliance with the requirements of Section C.1.1 herein. Where welding is involved the carbon content of the stainless steel should not exceed 0.08%. The first reference cited in C.1.1 sets forth the chemical ranges and limits of acceptable stainless steels of the 300 series. Cast grades of stainless steel equivalent to types 303, 304, and 316 are designated CF-16F, CF-8, and CF-8M, respectively. These cast grades are covered by ASTM[3] specifications A296-67 and A351-65.

F.

PRODUCT CONTACT SURFACE FINISH

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

G.

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

These standards shall become effective September 15, 1971 at which time the 3-A "Standards Covering Homogenizers and High Pressure Pumps of the Plunger Type, As of June 23, 1947" and amendments thereto, are rescinded and become null and void.

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

REPORT OF COMMITTEE ON FOOD EQUIPMENT SANITARY STANDARDS 1969-1970

(Continued from Page 311)

automatic post-mix soft drink equipment and covers in easy to understand terminology the following points: What is copper poisoning? How can it happen in post-mix devices? What carbonated water systems are approved? What systems are "fail-safe?" What have manufacturers done? What new standards are in effect? What should the vending machine operator do?

RECOMMENDATIONS

1. That the Association reaffirm its support of the National Sanitation Foundation and the National Automatic Merchandising Association and continue to work with these two organizations in developing acceptable standards and educational materials for the food industry and public health.

2. That the Association urge all sanitarians to obtain a complete set of the National Sanitation Foundation's Food Equipment Standards and Criteria and a copy of the National Automatic Merchandising Association—Automatic Merchandising Health-Industry Council's Vending Machine Evaluation Manual; to evaluate each piece of food equipment and vending machine in the field to determine compliance with the applicable sanitation guidelines; and to let this Committee and the appropriate evaluation agency know of any manufacturer, installer, or operator failing to comply with these guidelines.

3. That the Association urge all sanitarians and regulatory agencies to support the work of the Association's Committee, and subscribe, by law or administrative policy, to the principles represented by the Standards, Criteria, and Evaluation Manual for Food Equipment and Vending Machines.

Karl K. Jones, *Chairman*, Purdue University, West Lafayette, Indiana.

Irving L. Bell, State Department of Health, Frankfort, Kentucky.

Glenn Brauner, National Canners Association, Washington, D.C.

Carl Henderson, New Mexico Department of Public Health, Santa Fe, New Mexico.

Lloyd W. Regier, Fisheries Research Board, Halifax, Nova Scotia, Canada.

Jerome Schoenberger, City Department of Health, New York, New York.

Harold Wainess, Harold Wainess and Associates, Chicago, Illinois.

ASSOCIATION AFFAIRS

PROGRAM NATIONAL MASTITIS COUNCIL SUMMER MEETING AUGUST 16, 1971

Sheraton Motor Inn—Harbor Island
San Diego, California

The program chairman is NMC's Vice President, Dr. D. E. Jasper, School of Veterinary Medicine, University of California, at Davis. Local arrangements for hosting the meeting is chaired by Harold Y. Heiskell, Babson Bros., Company, Sacremento, California.

The program listed below will present needed information and the speakers selected are national authorities in their fields:

The National Mastitis Council is Helping You. W. L. Arledge, Pres. N.M.C.—Dairymen, Inc., Louisville, Kentucky.

California Milk Quality Program Progress. George DeMedeiros, Dairyman's Cooperative Creamery, Assoc., Tulare, California.

A Dairyman's Experience With Mastitis Control. Tom Sawyer, Calif. Holstein-Fresian Assoc., Waterford, California.

A Veterinarian's Experience With Mastitis Control. Jack W. Morse, Calif. Veterinary Medical Assoc., Trulock, California.

Some Factors that Affect the Incidence of New Udder Infection. John S. McDonald, National Animal Disease Laboratories, Ames, Iowa.

Udder Health Management—Part I. John W. Woods, Dairy Practitioner, Mesa, Arizona.

The Leucocyte and Udder Health. O. W. Schalm, School of Veterinary Medicine, U. of Calif., at Davis, California.

Relationship of Milking Machine Design and Function to Udder Disease. J. S. McDonald, National Animal Disease Laboratories, Ames, Iowa.

A Rationale for Milking System Design. F. F. Smith, Farm Advisor, Los Angeles, California.

Udder Health Management—Part II. John M. Woods, Dairy Practitioner, Mesa, Arizona.

Herd Evaluation Using Bulk Tank Milk. D. E. Jasper, R. B. Bushnell, J. D. Dellinger, U. of Calif., at Davis, California.

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30TH ANNUAL MEETING OF THE IOWA ASSOCIATION OF MILK, FOOD AND ENVIRONMENTAL SANITARIANS, INC.

Charles Yeager, Jr., receives Dr. M. P. Baker award, (left to right) Alvin Gray, First Vice-President; Farris Biggart, Pres.-Elect; Mr. Yeager; Don Jaeger, President.

The 30th annual meeting of the Iowa Association of Milk, Food and Environmental Sanitarians, Inc. was held at the Ramada Inn, Ames, Iowa on March 23, 1971. The meeting was well attended and many favorable comments were received on the program.

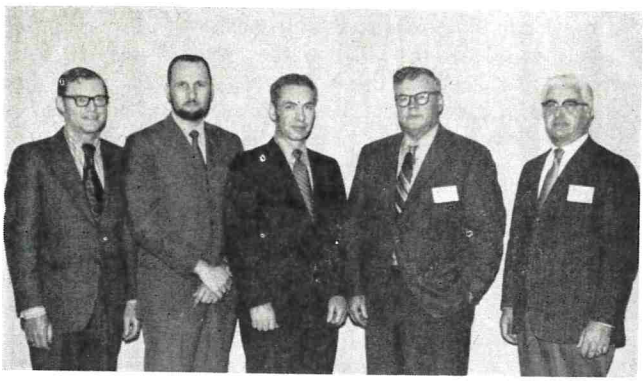
The annual banquet was held at the Ramada Inn with 92 attending. At this time the Dr. Merle P. Baker Award for meritorious contribution in the field of milk and food sanitation was presented to Charles Yeager, Jr.

The recipient of this Award shall have made a meritorious contribution in the field of milk, food or environmental sanitation to the Public Health and Welfare of a municipality or county within the State of Iowa, or to the State of Iowa. A $50 savings bond accompanies the Award.

Mr. Yeager was cited for his leadership in the field of sanitation in the State of Iowa. He is a past president of this Association and has contributed a great deal of time and effort in making the organization a success.

He received a B. S. degree in Dairy Industry from Iowa State University in 1947, and went to work as a milk sanitarian for the City of Sioux City. In February 1948, he was employed by the Anderson-Erickson Dairy Company of Des Moines, Iowa, where he remained until 1969 when he accepted a position with Beatrice Food Company, Chicago, Illinois.

The social hour at the annual banquet was again sponsored by Klenzade Products, Dairy Equipment Company, Sepko Chemicals Company, and The Schleuter Company.



Officers of the Missouri Association of Milk and Food Sanitarians, left to right, Erwin P. Gadd, Secretary-Treasurer; Harold Bengsch, Second Vice-President; Robert Gillilan, President; William McCowan, Immediate Past President; Charles Van Landuyt, First Vice-President; Auditors, James Ragan and Ronald Sibley absent.

William McCowan, President of Missouri Association of Milk and Food Sanitarians, presents Sanitarians Award to C. W. Dromgold (left).

SEVEN PROJECTS COMPLETED AT 3-A MILWAUKEE MEETING

Seven major projects, including significant Accepted Practices for Instantizing Systems for Dry Milk and Dry Milk Products, were completed by the 3-A Sanitary Standards Committees at their April 20-22 meeting. The Dry Milk document, providing sanitary criteria and guidelines for instantizing systems, is particularly timely, following by several months a new Dryer Practice. Both practices will appear in the *Journal of Milk and Food Technology*, with an effective date of March 14, 1972, for the Dryer Practices; effective date for the Instantizer Practices has not yet been established.

A completely new 3-A Standard for Air Eliminators for milk and Liquid Products, complementing Meter Standard #2800 published in February, was adopted. Publication of this document will be later this year. It will become effective about June, 1972. Revisions for Plate Type and Tubular Heat Exchangers, first published in 1952, were completed and soon will be signed and prepared for publication. A 1964 published Air Under Pressure Practice was also revised. Two Amendments—one for the HTST Pasteurization Practices to provide for handling of concentrated skim milk for drying, and the other adding polysulfone resins as a new generic class of plastics to the 3-A Plastic Standard.

1971 DAIRY FIELDMEN'S CONFERENCE

The 29th Annual Dairy Fieldmen's Conference was held at The Pennsylvania State University on June 8 and 9, 1971. All meetings were held in the J. O. Keller Conference Center. Highlighting this year's Conference were discussions from industry, regulatory, and university personnel on current problems in milk handling, especially those problems related to sampling, milk procurement, and metering of farm milk. Several speakers discussed operational problems of CIP equipment on farm handling equipment. Installation criteria for bulk headed farm tanks and precooling of bulk tank milk were discussed. Panels discussed current sanitation problems at the farm level and possible solutions to high somatic levels.

Features of the Conference was a discussion of research and teaching activities related to Pennsylvania agriculture by Dean R. E. Larson and a banquet speech by the new University President, Dr. John Oswald.

WARD DUEL APPOINTED ASSISTANT DIRECTOR ENVIRONMENTAL HEALTH AMA

Ward Duel has been employed by the American Medical Association as Assistant Director in the Department of Environmental, Public, and Occupational Health. The central thrust of this Department is to maintain liason with other national organizations in the above fields in order to develop, collect, evaluate, and disseminate technical information to the medical profession, state and local medical associations and others concerned with the influence of the environment on health.

It is expected that medical societies and physicians indivdually will be taking a larger part in problem solving and assuming environmental health leadership roles at the community level. To do this, AMA Headquarters will back them up with techni-

cal support.

His address is AMA Headquarters, 535 North Dearborn, Chicago, Illinois 60610. The phone number is (312) 527-1500 Ext. 578.

BOOK REVIEW

DISINFECTION. *Melvin A. Benarde (editor).* Marcel Dekker, Inc., New York (1970), 466 pages.

Control of microorganisms is of major importance in enterprises such as the food industry and medical practice. Principles of microbial control are always the same but the job can be done in different ways, depending on limitations imposed by a particular environment. Approximately 350 pages of this book are devoted to "doing the job" on spores and viruses, with gases, in hospitals, and in the dairy, seafood, food processing, and beverage industries.

As is common practice today, the eleven chapters in the book were written by different authors who, presumably, were selected because of their expertise in the areas which are covered by their contributions. Preparation of a book by a collection of authors has certain advantages but also often leads to problems; this book suffers from several. For example, approximately 12% of the pages in the book are devoted to an in-depth (and excellent) discussion of gaseous disinfection, a process of substantially less importance than disinfection by heat or by certain other chemicals, at least in hospitals and the food industry—the principal potential beneficiaries of this book. Neither the use of heat nor the use of the other chemicals is considered in detail anywhere in the book. Instead, both topics are discussed superficially and repeatedly throughout the book. Use of iodine compounds is mentioned in chapters 4, 5, 7A, 7B, 8, 9, 10, and 11 but the topic never does receive a thorough treatment. Some repetition, perhaps, is needed since the person who reads the chapter on disinfection in the seafood industry might not read other parts of the book. If this logic would prevail, however, then the first four chapters of the book would not be needed.

The first two chapters (nutrition and metabolism of microorganisms and mechanisms of disinfection) are intended to set the stage for what is to follow. Unfortunately, much of what they have to say seems to miss the mark for this particular goal. When these chapters discuss inactivation of microorganisms they deal with antibiotics, sulfa drugs, dyes, nitrofurans, and similar compounds. These chemicals may have interesting effects on microorganisms but they find little application in disinfection as practiced in hospitals (except for treating patients) or the food industry. Chapters on hospital and food plant disinfection are useful but suffer from variation in depth of coverage and from careless proofreading. Whittlestone is spelled as just indicated on page 373, but appears as Wittleson on page 329, and as Whittleston on page 324. This reviewer is puzzled by inclusion of a chapter on disinfection in Russian hospitals. If this was deemed necessary, what about disinfection in German, Greek, or Nigerian hospitals? Surely different practices might exist in these and other countries.

The book appears to be well manufactured. It is very readable and chapters are outfitted with appropriate subheadings. Photographs and line drawings are generally well done and often aid in understanding the topic. Two indexes are provided; the traditional subject index (5.5 pages) and a listing of all authors cited in the text (11 pages). This reviewer would prefer a more detailed subject index and elimination of the author index. An author index in a book such as this seems to be most useful to those persons who wish to conveniently learn if one of their papers was cited by the authors.

In spite of its limitations, many of which are organizational in nature, the book contains much useful information and should be, not only on their bookshelves, but actually used by those persons in hospitals and the food industry who are concerned with the control of microorganisms. This really includes nearly all persons who are associated with either enterprise.

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

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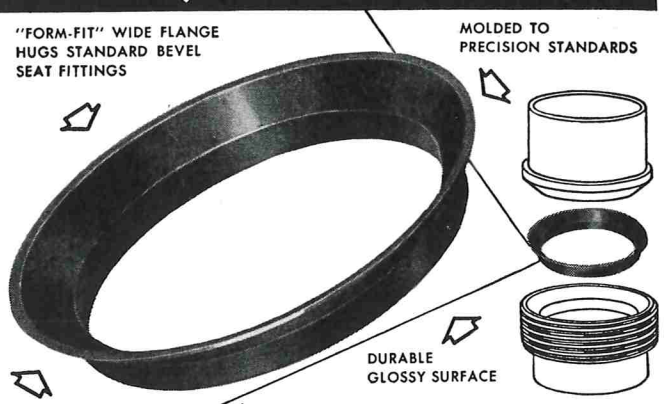

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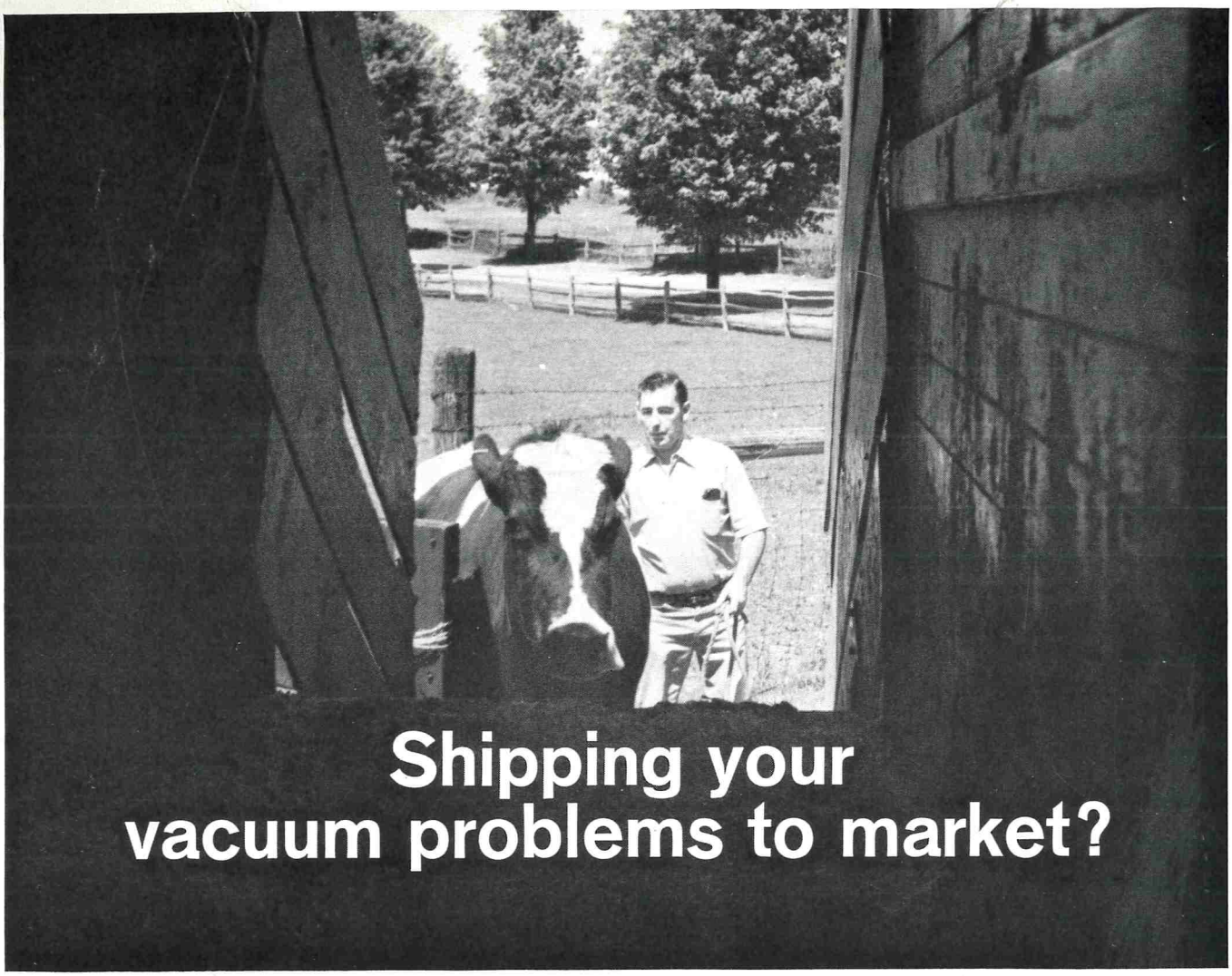

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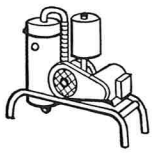

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