EFFECTS OF MEDIUM AND INCUBATION TEMPERATURE ON RECOVERY OF MICROORGANISMS FROM MANUFACTURING-GRADE, GRADE-A AND PASTEURIZED MILK[1]

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Abstract

Recovery of microorganisms by Standard Methods Agar and Eugonagar was compared at 7, 21, 28, and 32 C incubation temperatures for 10, 5, 4, and 2 days, respectively, from manufacturing-grade, grade-A, and pasteurized milk. The incubation temperature made a statistically significant difference in the mean logarithm of the count for all three grades of milk. The highest mean logarithm of the count with manufacturing-grade milk was obtained at 21 C for both agars. With grade-A raw milk, the greatest mean logarithm of the count was obtained at 21 C for Standard Methods Agar and at 28 C for Eugonagar. The highest mean logarithm of the count for pasteurized milk was obtained at 28 C with Standard Methods Agar and at 21 C with Eugonagar. There was a significant difference between means on Standard Methods Agar and Eugonagar on grade-A milk samples only; recovery was highest with Eugonagar.

Because of the presence of psychrophilic microorganisms, an incubation temperature lower than 32 C is needed for maximum recovery. Incubation at 28 C for 4 days was the optimum temperature-time combination in this study.

Since there is no recognized group of microorganisms that can be used as an index of production conditions on grade-A dairy farms, we must rely on recovering the maximum number of microorganisms from the milk as a partial measure of farm sanitation. The purpose of this work was to compare the use of different media and incubation temperatures to be sure the maximum number of microorganisms is recovered. The scope of the experiment was broadened to also include recovery from two other grades of milk. Two agars were incubated at each of four different temperatures with samples of manufacturing-grade, grade-A, and pasteurized milk.

Methods

Fifty-six manufacturing-grade, and 103 grade-A samples were aseptically collected from properly agitated bulk-tank milks and were placed in 6-oz. Whirl-Pak plastic bags for refrigerated transportation to the laboratory. The samples were collected in Iowa and Southern Minnesota. Most were collected by accompanying bulk-tank drivers on their routes; the remainder by driving to the farms. Samples were analyzed the evening of the collection day or on the following day. The grade-A producers' milk was collected every other day. Milk from 37 of the 50 manufacturing-grade farms was collected every third day or less frequently. Fifty pasteurized-milk samples were obtained from local supermarkets, the Iowa State University Dairy, and from products sent to the Iowa State University Food Products Analysis Laboratory for routine examination. Because pasteurized milk of different ages was plated, some counts were high. Several pasteurized samples were eliminated from the study because the Standard Plate Count (SPC) and the count on Eugonagar (EA) *(4)* at 32 C were both >100,000/ml.

Analysis of samples

Procedures listed in the 11th edition of *Standard Methods for the Examination of Dairy Products (2)* were followed in analyzing the samples unless another procedure is specified. Duplicate plates with Standard Methods Agar (SMA) *(2)* and EA *(4)* were incubated at each of the following temperature-time combinations: 32 C for 2 days, 28 C for 4 days, 21 C for 5 days, and 7 C for 10 days. The incubation temperatures were maintained within 1 C of the specified temperatures. Plates were counted within 3 hr of the specified time. Cover layers of the same plating medium were poured on plates incubated at 21, 28, and 32 C to minimize the problem with spreaders.

Counts of <1 were recorded as 0, and the data were transformed by taking the $\log_{10}$ (count + 1). A least-squares analysis was performed.

Results and Discussion

Mean logarithms of counts for each incubation temperature-time combination for the three grades of milk are presented in Table 1. The F tests of sample source, incubation temperature, and medium are presented in Table 2. The source of sample and the incubation temperature made a significant difference in the count of all three grades of milk. Both these sources of variation were expected to be statistically significant.

With grade-A milk samples, there was a significant difference in geometric means on the two agars (Table 2). Higher geometric means were obtained with EA, with the greatest increase occurring at 7 C (Table 1). On the other hand, there was no significant difference between the means obtained with the media on the other two grades of milk. Pelczar and Vera *(13)*, and Pessin and Black *(14)* obtained

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TABLE 1. MEANS OF TRANSFORMED[a] COUNTS OF MILK SAMPLES INCUBATED[b] AT FOUR TEMPERATURES WITH TWO AGARS

Agar	Incubation Temperature 7 C	21 C	28 C	32 C
	[Means of Log_{10} (Count + 1)]			
	56 Manufacturing-grade milks			
SMA[c]	5.849	6.233	6.196	6.072
EA[d]	5.823	6.213	6.178	6.056
	103 Grade-A milks			
SMA	3.359	4.174	4.155	4.074
EA	3.546	4.189	4.210	4.133
	50 Pasteurized milks			
SMA	1.885	3.180	3.203	3.075
EA	1.914	3.106	3.091	2.967

[a]Logarithm$_{10}$ (count + 1).

[b]With incubation temperatures of 7, 21, 28, and 32 C, the respective incubation times were 10 days, 5 days, 4 days, and 2 days.

[c]Standard Methods Agar.

[d]Eugonagar.

essentially the same results when comparing a medium with approximately the same composition as EA with tryptone-glucose-beef extract-milk agar (TGEM). The highest recovery of microorganisms from grade-A milk was obtained with EA at 28 C incubation. With SMA, the highest geometric mean was obtained at 21 C. With both agars, geometric means at 21 and 28 C were higher than those at 32 C. Further, there was a significant interaction between medium and incubation temperature because of the greater recovery on EA at 7 C. With improved cooling facilities and longer storage periods, psychrophiles are becoming more significant in raw milk. Because some psychrophiles do not grow at 32 C, lower incubation temperatures result in better recovery.

Research workers have recognized that incubation temperatures too high for many of the bacteria in milk have often been used. Bacteriological determinations on milk were first made at 37 C because that was the temperature laboratories were using for water analysis. Also, earlier investigators wanted to incubate at a temperature favorable for growth of pathogens. Pederson and Yale *(11)*, 1934, observed that 37 C was not the optimum temperature for bacterial counts of milk and that, at temperatures slightly above 37 C, the count was much lower. They said the optimum, which varied somewhat depending on the sample, was close to 32 C. In later studies, using an improved agar *(21)*, they also found 32 C better than 37 C. Other workers reported higher counts at 32 than at 37 C *(1, 5, 6, 8, 15, 16, 20)*. Pederson and Breed *(12)*, in 1940, recommended 32 instead of 37 C for determining milk counts. Thomas and Jenkins *(17)* obtained significantly higher counts at 30 C for 3 days, than at 37 C. Babel et al. *(3)*, 1955, reported that the logarithmic mean count at 32 C was generally higher than at 35 C. Mean counts at 26 and 32 C were essentially the same after 2 days, but after longer incubation, plates at 26 C gave the higher counts. They suggested that an intermediate temperature between 26 and 32 C possibly would give a higher count. In this experiment, 21 and 28 C gave higher geometric means than 32 C incubation. Although *Standard Methods for the Examination of Dairy Products (2)* recommends incubation at 32 C, this is not the optimum for many bacteria in milk.

With manufacturing-grade milk, there was no statistically significant difference in recovery with the two agars (Table 2); however, the higher geometric mean at all temperatures was with SMA (Table 1). No significant interaction indicates that relative recovery on the two agars was independent of the incubation temperature. The counts at 7 C were high. From samples collected in the same geographical area, LaGrange and Nelson *(9)*, 1958, reported that the psychrophilic count (PBC) on manufacturing-

TABLE 2. ANALYSIS OF VARIANCE OF BACTERIAL COUNTS[a] OF MILK SAMPLES

	Degrees of freedom	Mean squares	F values
	56 Manufacturing-grade raw bulk-tank milk samples		
Farms	55	8.3491	95.31**
Temperatures	3	3.4174	39.01**
Agars	1	0.0427	0.49
Temperature X agar	3	0.0005	0.006
Error	385	0.0876	
	103 Grade-A raw bulk-tank milk samples		
Farms	102	1.8323	41.95**
Temperatures	3	25.7618	589.78**
Agars	1	1.2897	29.53**
Temperature X agar	3	0.2858	6.54**
Error	714	0.0437	
	50 Pasteurized milk samples		
Samples	49	7.6217	29.14**
Temperatures	3	36.5944	139.91**
Agars	1	0.4332	1.66
Temperature X agar	3	0.1075	0.41
Error	343	0.2616	

[a]Counts were transformed by taking logarithm$_{10}$ (count + 1).

**P <0.01.

grade milk was nearly as high as the SPC and sometimes exceeded it greatly.

The smallest difference in count between agars and incubation temperatures was obtained with manufacturing-grade milk. This was probably because of the complex flora present, which included many psychrophiles. The highest geometric mean occurred at 21 C with both agars. The means at 28 C incubation were both higher than those obtained at 32 C. Although better recovery was obtained at temperatures lower than 32 C, this is of little or no significance when we consider the high bacterial population present.

With pasteurized milk, the incubation temperature had a significant effect on the count, but there was no statistically significant effect of agars. There was, however, more experimental variation with pasteurized milk (Table 2). With pasteurized milk, Pessin and Black *(14)* reported that counts were significantly lower on a medium with approximately the same composition as EA than on TGEM. With a 7 C incubation temperature, the higher geometric mean was obtained with EA. At the other three incubation temperatures, the highest mean count was obtained with SMA. The different recovery at 7 C was not large enough to cause significant interaction. The highest recovery was obtained with SMA at 28 C. With EA, the highest geometric mean was obtained at 21 C. The means for both agars were higher at 21 and 28 C than at 32 C.

Many workers have obtained greater increases in the count of pasteurized milk than in raw milk by lowering the incubation temperature from 37 to 32 C, which shows that the flora of pasteurized milk is more sensitive to the higher incubation temperature *(5, 7, 8, 11, 15, 16)*. Nelson and Baker *(10)* observed that incubation at 25 C for 3 days detected large numbers of bacteria in many market-milk products that did not develop colonies at 35 C. Babel et al. *(3)* reported higher counts from pasteurized milk samples by incubating at 26 C for 3 days than at 32 C for 2 days. Comparing 21, 28, 32, and 35 C incubation of plates of laboratory pasteurized milk, Thomas, Reinbold, and Nelson *(18)* found that 28 C incubation for 4 days was the temperature-time combination that produced the highest count. Studies with pure cultures indicated that thermoduric bacteria generally were more exacting in growth temperature requirements after they had been subjected to laboratory pasteurization than before. Also, they were more exacting in nutrient requirements *(19)*. Commercial market-milk samples, like those used in this experiment, will have a flora much different from laboratory pasteurized samples, however. The counts were quite high in the pasteurized milk used in this experiment.

Although *Standard Methods for the Examination of Dairy Products (2)* specifies a 32 C incubation temperature, results of this study show that higher counts were obtained at the lower temperatures. Because psychrophiles constitute an important part of the milk flora and since some psychrophiles may not grow at 32 C, a lower incubation temperature should be used for grade-A milk evaluation. Because incubation at 28 C requires 1 less day than incubation at 21 C, this would be the preferred incubation among those studied.

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OCEANS VAST RESERVOIRS OF FOOD

Oceans represent a vast potential of almost virgin territory as a reservoirs of food for people, according to Dr. Ernest R. Pariser of the Massachusetts Institute of Technology, Cambridge. Dr. Pariser told an audience of food processor executives, planners and researchers that in order to utilize this reservoir—whether plant, squid or fish—powerful prejudices and taboos against such products "must be overcome by processing, marketing and educational skills." This task, he emphasized, is the most difficult to accomplish. The scientist was addressing the Third Biennial Food Forum, a feature of the Food & Dairy Industries Expo, Oct. 13-17, at Chicago's International Amphitheatre. Both are sponsored by the Dairy and Food Industries Supply Association, a national trade group headquartered in Washington, D. C.

Dr. Pariser noted that while the total quantity of ocean plant biomass is "vastly superior to that of land plants," most marine plants are microscopic and difficult to spot and harvest. On the other hand, the larger marine plants being harvested and consumed are "entirely leafy vegetables having no roots, tubers, fruits, nuts or other food concentrating and storage members." Thus, these are only of "limited food value."

Although currently not used as human food, invertebrates account for more than 80% of the weight of marine animals, and, they represent an important protein reservoir that must be slowly tapped as other, more conventional supplies become insufficient to meet the world demand. Squids are being harvested in large quantities in some areas, but are used mainly for bait. These and their relatives could be used more extensively as human food, since they contain a high protein concentration, are perfectly safe and edible, widely distributed over the world's oceans, and easily harvested.

Of the vertebrates, fish represent the best known and widely used. However, only a handful of species of a total of 20 or 25,000 known species are consumed by man, and the annual world harvest is only 54 million metric tons compared to a potential annual harvest estimate of as many as 2 billion metric tons.

Considering the urgent need for food in general and for protein in particular, Dr. Pariser explained why more marine foods are not reaching hungry peoples of the world. "It's a complex question," he said, requiring "changes at different levels and directions—technological, economic, socio- psychological." First, the art of fishing—locating and surrounding a catch— is still almost prehistoric. New and more sophisticated methods must be developed. Second, marine organisms spoil more easily than most other foods, necessitating processing and preservation. Although freezing, freeze-drying, radiation preservation and canning are excellent procedures, he noted, they are expensive and for a long time will remain out of the reach of the poor. Less expensive methods are being developed, and new foods incorporating such preserved products will have to be formulated. Last, marine foods—especially fish—have been consumed and marketed in their recognizable forms for many years. Slowly and against much resistance, it's being established that marine proteins from one source or another can and should be used in a new form in which the original raw material loses its identity.