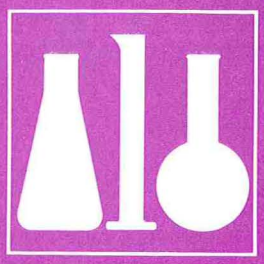

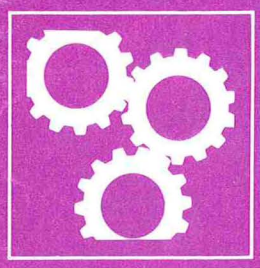

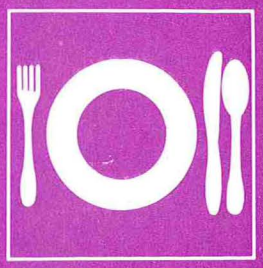

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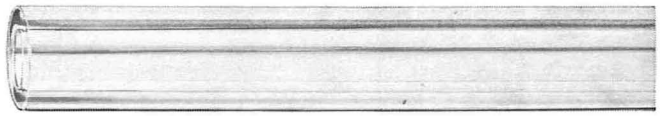

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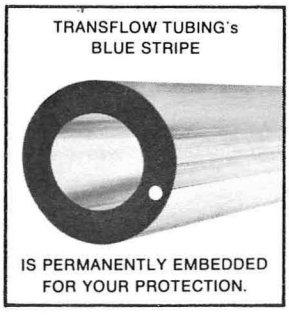

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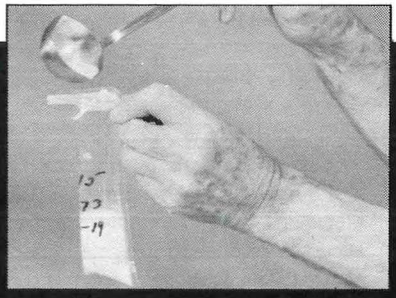

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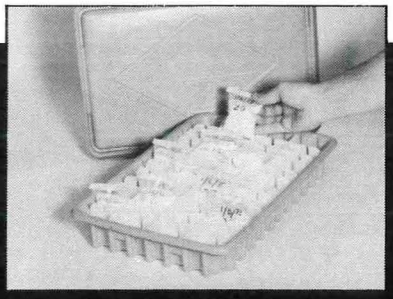

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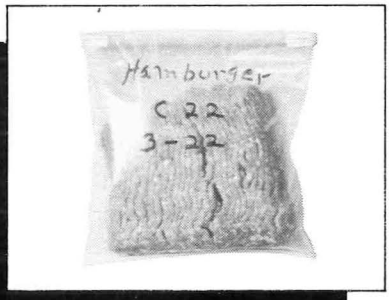

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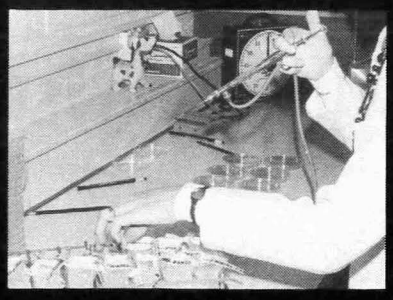

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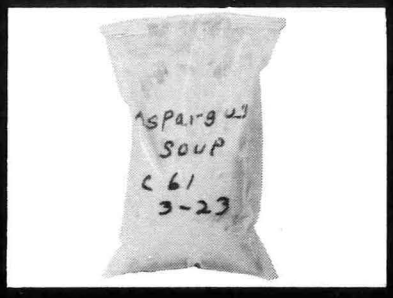

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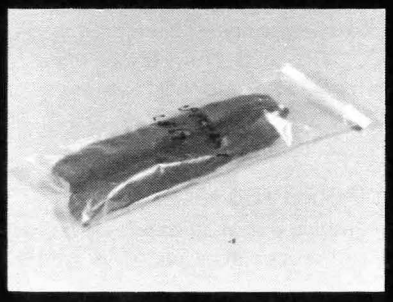

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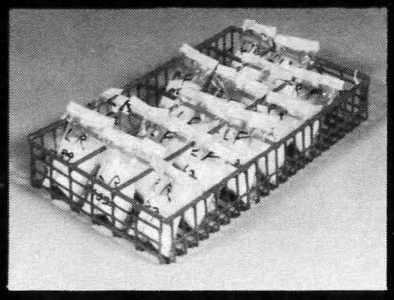

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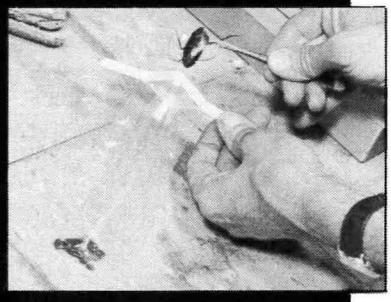

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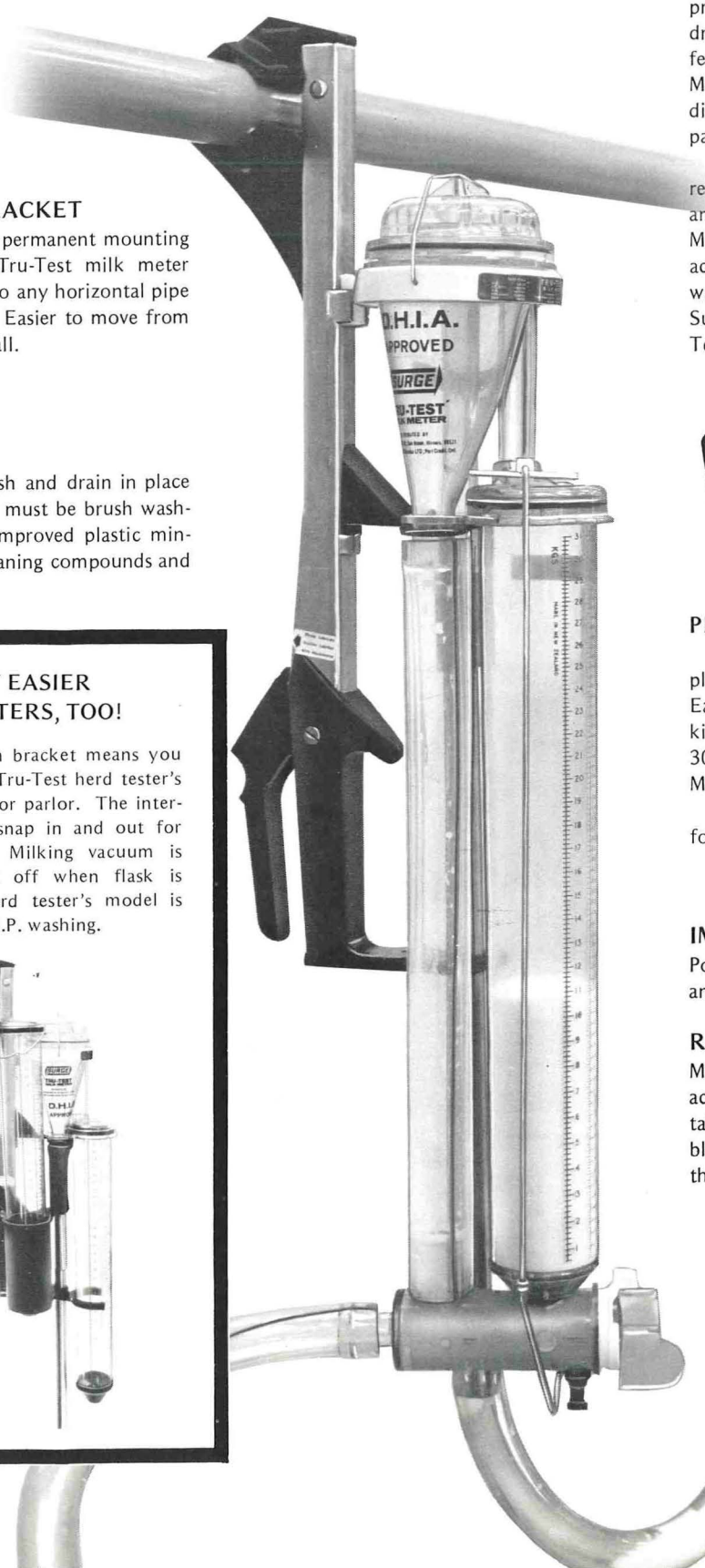

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Journal of Food Protection Vol. 40, No. 7, Pages 442-444 (July, 1977)

Thermal Destruction of Microorganisms in Meat by Microwave and Conventional Cooking[1]

F. LEON CRESPO and H. W. OCKERMAN

The Ohio State University, Columbus, Ohio 43210 and The Ohio Agricultural Research and Development Center, Wooster, Ohio 44691

(Received for publication November 8, 1976)

ABSTRACT

When heating ground beef to internal temperatures of 34, 61, and 75 C, high temperature (232 ± 6 C) oven cooking was more effective for bacterial destruction than low temperature (149 ± 6 C) oven cooking. Low temperature oven cooking was more effective than microwave cooking. These differences in microbial destruction rates became significant (P<05) when the meat reached the 75-C internal temperature level.

One of the primary objectives of the cooking process is thermal destruction of microorganisms, thus foods are usually heated in such a manner that they can be consumed with a high degree of safety. It has been reported (*7*) that conventional cooking reduces the usual microbiological contamination in meat to levels that represent little food poisoning danger to the consumer.

New methods of meat cookery include low temperature-long time roasting and microwave cooking. Low temperature roasting has been shown (*2*) to improve the palatability characteristics of roast beef. Microwave cooking is gaining acceptance because it is fast and convenient and also saves energy (*10*).

Since thermal destruction of microorganisms depends on a time-temperature relationship in each food item, these new cooking techniques should be evaluated for microbiological survival. Low temperature-long time oven cooking should be evaluated due to the low temperature involved. Microwave cooking is open to suspicion due to the short time involved.

The objective of this research was to compare (*a*) conventional high temperature oven cooking, (*b*) low temperature oven cooking, and (*c*) microwave cooking, with respect to the effect on the microbiological population usually present in meat tissue.

MATERIALS AND METHODS

Samples of ground meat (approximately 3.5 kg) were obtained from The Ohio State University Meat Laboratory, inoculated with microorganisms by successive passes over a meat cutting table, mixed, and stored under refrigeration (5 ± 3 C) for 24 h to simulate poor commercial conditions. From each batch, 10 samples (approximately 250 g) were prepared and randomly assigned to the treatment cells. One sample was used as a control, three were heated in a microwave oven (Hobart Model 125R) for 30, 60, and 90 sec, three were heated in a conventional electric oven (Toastmaster Model H54O-R) set at a high temperature of 232 ± 6 C, and three were heated in the same oven set at a temperature of 149 ± 6 C, (low temperature). The cooking time in the conventional electric oven was monitored and regulated to produce final internal temperatures (34, 61, and 75 C) as close as possible to the ones achieved by the microwave oven. This experimental design was replicated six times.

After heat treatment the internal temperature of each sample was recorded and 50-g core samples were taken for microbiological evaluation (*1*). Total bacterial flora were enumerated using standard plating techniques for aerobic bacteria and Tryptone Glucose Agar (Difco). Plates were incubated in a 25-C oven for 4 days.

RESULTS AND DISCUSSION

The uncooked beef samples used in this study had a bacterial range from 24×10^4 to 80×10^6/g (Table 1) which is slightly higher than the normal contamination found in commercial ground beef (*9*). The variability among replications in initial microbial counts and the slight differences encountered in the final internal temperatures complicated the analysis of these data (to remove the unavoidable bias, the analysis was done in the following manner). To remove the inital microbiological count variation the logarithmic reduction produced by heating rather than total count was analyzed. It is generally accepted that the bacterial reduction by heat follows a logarithmic pattern and the proportion of the population destroyed by the time-temperature treatment is not dependent upon initial numbers (*3,6*). However, since all cooking treatments were represented in each replication (same initial count) even a non-adjusted calculation should not have been biased for cooking

TABLE 1. *Initial microbiological contamination of ground beef samples*

Trial	Total count/g
1	80×10^6
2	13×10^6
3	50×10^6
4	65×10^6
5	24×10^4
6	30×10^4
$\bar{X}$	26×10^6
σ	32×10^6

[1]*Approved as Journal Article No. 107-76 by the Ohio Agricultural Research and Development Center, Wooster.*

treatment.

The logarithmic reduction was also individually adjusted at each of the three internal temperature locations (reference temperatures) by regression analysis to the meat temperature for all treatments (34, 61, 75 C) at this location. Final temperature ranges for each cooking method in all instances bracketed the mean reference temperatures of 34, 61, and 75 C. These adjusted values were then analyzed by analysis of variance and individual means were evaluated by the t-test.

When the internal temperature of reference was 34 C the bacterial survival was very high (Fig. 1). There was almost no change in the samples heated by microwave

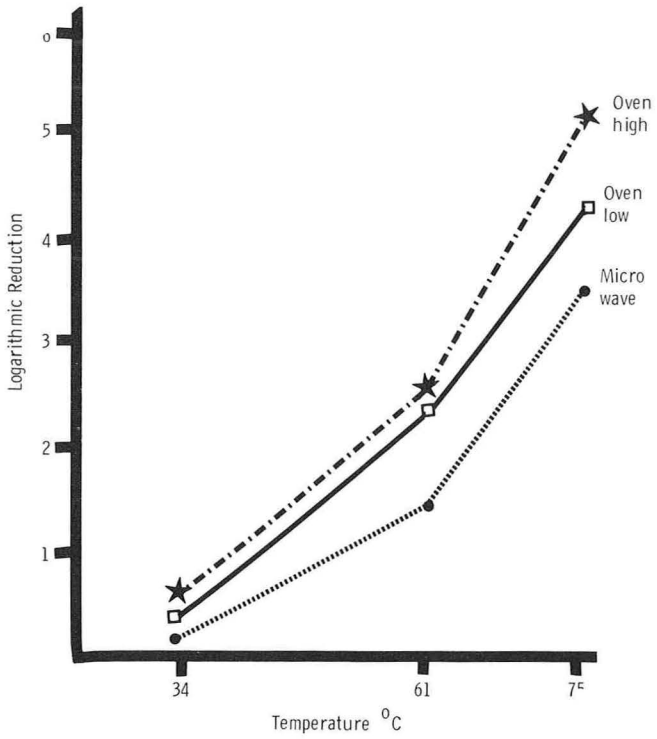

Figure 1. *Comparison of the logarithmic reduction in microorganism to final internal cooking temperature.*

(only 0.18 logarithmic reduction), slightly higher lethality (0.39 logarithmic reduction) in samples given a low temperature oven treatment and the greatest reduction (0.54 logarithmic reduction) was obtained with the high temperature oven treatment. These cooking procedure differences were not large enough at the 34-C internal temperature point to be significant ($P<05$).

When the internal temperature of reference was 61 C, there was a significant ($P<05$) difference between the logarithmic destruction produced by microwave cooking (1.48 logarithmic reduction) and that produced by low temperature oven cooking (2.39 logarithmic reduction) and a highly significant ($P<01$) difference between microwave cooking and high temperature oven cooking (2.79 logarithmic reduction). Although the lethality produced by cooking at high temperature exceeded that produced by low temperature cooking this difference between treatments was not large enough to be significant.

The reference temperature of 61 C is considered in the top range for beef cooked to the rare state (*8*). These data agree with those of Patterson and Gibbs (*7*) in that a considerable quantity of microorganisms in meat can survive this cooking temperature.

When the internal temperature of reference was 75 C there were clear differences in the destruction produced by the three treatments. The treatment producing the greatest destruction at this temperature was cooking in a high temperature oven (5.24 logarithmic reduction). This treatment effect can be explained by the thermal gradient inside the meat. In the high temperature oven sample there was a large portion of the sample, particularly in the surface regions, at a considerably higher temperature than the center where the internal temperature was measured. With this method of cooking one-half of the samples (three of six replications) yielded no microbial growth on the media employed.

When the low temperature oven samples were evaluated (4.32 logarithmic reduction), there was a significantly ($P < .05$) lower destruction than in the high temperature oven samples. Only one sample out of the six replicates had no bacterial recovery from this treatment. This temperature is in the top range for "medium" cooked beef (*8*). Low temperature oven cooking resulted in less bacterial destruction than conventional high temperature oven cooking.

The microwave cooking technique was the least effective from a bacterial destruction standpoint at the 75 C reference temperature (3.61 logarithmic reduction). The microwave destruction was significantly ($P<05$) lower than the low temperature oven and highly significantly ($P < .01$) lower than the high temperature oven treatments.

These results agree with Hone (*4*) who reported a greater bacterial survival with microwave cooking than with conventional oven cooking of microbiologically inoculated pork tissue. Also, Lacey et al. (*5*) observed that microwave cooking is not very effective for bacterial destruction. The low thermal destruction with microwave cooking can be explained by the quick rise in temperature in this process and implies that the microorganisms are exposed to the lethal temperature for a shorter time. Also, temperature is more uniformly distributed in this type of cooking than in oven cooking and therefore the surface temperature does not exceed the internal temperature as much in these samples as in conventional oven-cooked samples. To achieve the same microbiological safety as with traditionally cooked samples, it will be necessary to increase the final internal cooking temperature or maintain the product at the final temperature for a longer time.

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Evaluation of the Stomacher for Preparation of Food Homogenates

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(Received for publication December 6, 1976)

ABSTRACT

Two methods to prepare food homogenates, the Stomacher and blender, were evaluated by comparison of aerobic plate counts on replicate portions of mixed samples of ground beef, celery, and wieners. Low and high speeds on the blender with 1:10 dilutions were compared against dilutions of 1:2 and 1:10 on the Stomacher. No significant difference in aerobic plate counts was observed among the four methods on three types of foods.

The usual method to prepare food homogenates for bacteriological examination is by mechanical blending of a portion of the food with sterile diluent at a weight: volume ratio of 1:9 (*5, 7*). In 1972 Sharpe and Jackson (*4*) described a new method for preparing bacterial suspensions from foods called "stomaching" after the type of action employed by the "Stomacher" device, which utilizes a set of paddles for alternately compressing the food held in a plastic bag. In 556 comparisons of various types of foods, Sharpe and Jackson found the Stomacher and Ato-Mix blender equivalent except for high fat (95%) beef cuts, shortcrust pastry and dairy cream, where the Stomacher counts were lower. Tuttlebee (*6*) further compared the Stomacher against various other homogenization methods, observing that counts on prawns and cooked foods were significantly higher than by the Ato-Mix blender. The ratios of Stomacher to blender counts obtained by Tuttlebee for individual samples varied greatly with the type of food, ranging from 0.01 for Pate de Marcassin to 250 for Puporc sausage; but median ratios for classes of foods showed less variation with a range from 1.0 for cheeses and powders to 3.5 for cooked foods.

Sharpe and Harshman (*3*) recently reported a comparison of the Stomacher with the Osterizer blender (Sunbeam Corp. of Canada, Ltd.) for recovery of *Clostridium perfringens, Staphylococcus aureus,* and molds from foods, concluding that while counts were equivalent for most foods, the Stomacher gave a slightly lower recovery for foods with high fat, a decrease which was recoverable by addition of Tween 80. The Stomacher was considered less lethal than the blender for *S. aureus, Streptococcus faecalis,* and *Escherichia coli.*

This report concerns an evaluation of the Stomacher used according to the method of Tuttlebee (*6*) with 100 g of food and 100 ml of diluent (1:2 dilution), and the more common procedure using 25 g of food with 225 ml of diluent (1:10 dilution). Recoveries measured by the aerobic plate count were compared against those obtained when the blender operated at low and high speeds. Three foods representing different textures and composition were chosen for study, ground beef, celery, and wieners. To reduce the influence of non-uniform distribution of bacteria in foods, replicate trials were completed on most of the food samples after pre-mixing.

MATERIALS AND METHODS

Food samples

To reduce as much as possible the variability in bacterial densities that occur between separate portions of heterogeneous food samples, three foods, ground beef, celery, and wieners, were chosen as representative of different food texture and composition and from which reasonably uniform samples could be prepared for analysis. All samples were purchased from retail stores and held under refrigeration until examination no more than 2 days later. Samples of sufficient size of ground beef to provide for all trials by each method were first mixed in a beaker (2 liter), then small portions from different areas were removed to make up the weighed sample. The leafy upper end and base of celery stalks were removed, and the middle portions were cut into approximately 1/4-inch pieces, which were placed in a plastic bag for thorough shaking before weighing out individual sample portions. Wieners were similarly cut into pieces and shaken in a bag before weighing individual samples.

To further reduce the influence of sample variations on the comparisons, duplicate or triplicate trials by each method were completed on most of the food samples.

Homogenates

Four sample portions were weighed out for each comparison: (*a*) Two 25-g portions into separate blender cups, (*b*) one 100-g portion into a plastic bag (Fisher No. 1812-120, 12" × 8", 0.004" thick) for stomaching, and (*c*) one 25-g portion into a second Stomacher bag. Although these bags were not sterile, rinse tests on selected samples found the aerobic counts always less than 10 per bag, presenting no significant contribution to plate counts after dilutions.

Weighed samples were held in the refrigerator until ready for testing, but brought to room temperature before homogenization by blender (Waring Model 702B, two speed) or Stomacher (Stomacher 400, Model

BA6021, A. J. Seward & Co., Ltd., London). Phosphate buffer (pH 7.2) was used for all homogenates and dilutions, with 225 ml added for preparing 1:10 dilutions of the sample, and 100 ml for an initial 1:2 dilution. No surfactant was used with ground beef or wiener samples. Homogenization times were exactly 2 min.

The blender was compared on low and high speeds, which represented, according to a Strobscope, 11,200 and >18,000 rpm, respectively.

A 1:10 dilution was prepared from the 1:2 dilution with the Stomacher by pouring off 20 g of homogenate and adding 80 ml of diluent. This mixture was mixed well with a spatula in a beaker, and the fluid pipetted for preparation of further dilutions.

Plate counts

Sample dilutions were poured with Standard Methods agar (BBL), and plates were incubated at 35 C for 44-48 h. Dilutions producing 30 to 300 colonies were selected for colony counts used to calculate the Aerobic Plate Count (APC) per gram of food. Reported counts represented the arithmetic mean of five replicate plates.

Plate counts for ground beef and celery samples were completed by one laboratory worker. To reduce the possibility of a counting bias on the conclusions, plate counts for wiener samples were based on the arithmetic mean of counts by three laboratory workers.

Statistical tests

Mean counts for each trial were used to calculate arithmetic means, standard deviations, and coefficients of variation, and for calculation of recovery ratios between methods for each type of food. The results for each food were further subjected to analysis of variance (ANOVA) for determining the statistical significance of observed differences.

RESULTS

The results of comparisons on ground beef, celery and wieners are presented in Tables 1, 2 and 3, respectively. Only the results with ground beef suggest any difference

TABLE 1. *Comparison of Stomacher and blender for preparation of homogenates of ground beef*

Sample No.	Trial	APC/g (X 10^4)[a]				Count ratios		
		Blender		Stomacher				
		L	H	A	B	L:H	A:B	B:L
1	1	159	203	296	732	0.78	0.40	4.60
2	1	47.0	33.8	102	53.0	1.39	1.92	1.13
3	1	8.62	14.5	6.78	5.36	0.59	1.26	0.62
4	1	163	168	145	169	0.97	0.86	1.04
	2	157	205	219	290	0.77	0.76	1.85
5	1	4.74	12.4	3.98	6.34	0.38	0.63	1.34
	2	1.84	1.54	4.86	3.02	1.19	1.61	1.64
6	1	432	664	654	574	0.65	1.14	1.33
	2	282	460	458	788	0.61	0.58	2.79
	3	516	1160	596	698	0.44	0.85	1.35
7	1	315	208	127	78.8	1.51	1.61	0.25
8	1	1420	1170	578	828	1.21	0.70	0.58
	2	1380	1100	726	1710	1.25	0.42	1.24
9	1	2360	1550	1520	1540	1.52	0.99	0.65
10	1	778	1196	770	1340	0.65	0.57	1.72
	2	1200	1210	1310	952	0.99	1.38	0.79
11	1	147	167	84.3	138	0.88	0.61	0.94
	2	235	87.2	129	162	2.69	0.80	0.69
	3	221	110	106	166	2.01	0.64	0.75
Mean		517	512	412	539	1.08	0.93	1.33
SD		636.3	532.5	438.4	544.6			
CV		123.0	104.1	106.3	101.1			

[a]Arithmetic mean of five replicate plates. L = low speed. H = high speed. A = 100 g sample + 100 ml diluent. B = 25 g sample + 225 ml diluent. SD = standard deviation. CV = coefficient of variation.

TABLE 2. *Comparison of Stomacher and blender for preparation of homogenates of celery*

Sample No.	Trial	APC/g (X 10^5)[a]				Count ratios		
		Blender		Stomacher				
		L	H	A	B	L:H	A:B	B:L
1	1	28.4	9.80	12.5	13.5	2.90	0.93	0.48
	2	15.9	24.0	17.2	10.5	0.66	1.64	0.66
2	1	4.76	9.10	6.48	4.10	0.52	1.58	0.86
	2	5.14	5.58	4.76	6.94	0.92	0.69	1.35
	3	8.38	6.08	3.50	3.40	1.38	1.03	0.41
3	1	15.0	15.9	13.1	11.7	0.94	1.12	0.78
	2	9.76	23.6	17.2	17.5	0.41	0.98	1.79
4	1	16.6	9.46	9.50	9.46	1.75	1.00	0.57
	2	15.0	21.7	10.6	18.1	0.69	0.59	1.21
	3	11.0	11.0	15.4	9.26	1.00	1.66	0.84
5	1	9.90	5.24	8.12	6.56	1.89	1.24	0.66
	2	4.30	4.86	6.38	4.04	0.88	1.58	0.94
	3	4.48	6.22	8.10	4.98	0.72	1.63	1.11
Mean		11.4	11.7	10.2	9.00	1.07	1.21	0.87
SD		6.819	7.153	4.574	5.124			
CV		59.7	61.0	44.8	56.9			

[a]Arithmetic mean of five replicate plates. L = low speed. H = high speed. A = 100 g sample + 100 ml diluent. B = 25 g sample + 225 ml diluent. SD = standard deviation. CV = coefficient of variation.

between methods, where Method A (100 g sample + 100 ml of diluent) with the Stomacher has a seemingly lower mean recovery (4.12×10^6) compared to the blender on high speed (5.12×10^6), the next lowest recovery. In addition, the mean ratio of recoveries by Method B (25 g sample + 225 ml of diluent) with the Stomacher to the blender on low speed (1.33) suggests a 33% greater recovery with the Stomacher. However, the results on two samples (number 1 and trial 2 of number 6) are so removed from the others that they might be considered outliers, and when these two results are deleted the comparison ratio becomes 1.05. Furthermore, a statistical analysis on the entire data (Table 4) concludes that these differences are not significant.

The results from comparisons with celery and wieners (Tables 2 and 3) show less varibility in the data than with ground beef. The agreement between trials on the same food samples was best with wieners. A statistical analysis (Table 4) concludes that there is no significant difference between any of the four methods for preparation of homogenates from celery and wieners for aerobic plate counts.

DISCUSSION

One of the major problems associated with evaluation of food homogenization methods is the heterogeneous distribution of the bacterial flora in foods. This could account for the large variation in recovery ratios reported by Tuttlebee (*6*) between samples of the same type of food, and for the occasional lack of agreement between the Stomacher and blender on relatively homogeneous foods where such variation is not expected, such as dairy cream (*4*), powdered egg (*6*), and potato salad (*3*). Oblinger and Kennedy (*2*) and Goepfert (*1*) have observed this variability between weighed potions from a single food sample. Goepfert found, for example, a 30-fold variation in *E. coli* MPN values between 50-g portions from one sample of ground beef.

This study attempted to reduce the effect of non-uniform bacterial distribution by selecting three foods from which reasonably uniform mixtures could be prepared, and by using replicate comparison trials on the same sample. The greater varibility in results with ground beef, a coarsely comminuted meat, compared to wieners, a finely comminuted meat product, further suggests the influence of uniformity in the sample on counts. With ground beef, there may be the additional influence of fats on both distribution and removal of bacteria by an aqueous diluent, which was used in this study without further addition of a surfactant. As emphasized by Goepfert (*1*), this lack of homogeneity is an important consideration in establishing bacterial limits for foods and sampling and examination schemes for determining compliance.

TABLE 3. *Comparison of Stomacher and blender for preparation of homogenates of wieners*

Sample No.	Trial	APC/g (X 10^6)[a]				Count ratios		
		Blender		Stomacher				
		L	H	A	B	L:H	A:B	B:L
1	1	18.7	20.1	17.3	19.3	0.93	0.90	1.03
	2	17.0	15.8	13.1	19.8	1.08	0.66	1.16
2	1	25.3	12.8	39.0	46.0	1.98	0.85	1.82
	2	23.5	25.0	34.5	29.5	0.94	1.17	1.26
3	1	6.39	11.5	5.40	10.0	0.56	0.54	1.56
	2	6.21	6.82	4.88	6.95	0.91	0.70	1.12
	3	5.45	3.78	5.07	4.93	1.44	1.03	0.90
4	1	12.7	10.5	8.71	15.9	1.21	0.55	1.25
	2	12.9	10.7	10.7	15.7	1.21	0.68	1.22
	3		6.57	10.3	8.93	2.05	1.15	0.66
5	1	15.8	14.0	13.6	14.6	1.13	0.93	0.92
	2	14.8	17.4	12.9	13.4	0.85	0.96	0.91
	3	15.7	16.1	16.4	16.3	0.98	1.01	1.04
6	1	55.9	46.4	72.8	72.1	1.19	1.01	1.31
	2	54.6	71.3	67.9	68.8	0.77	0.99	1.26
	3	59.6	60.8	75.6	62.3	0.98	1.21	1.05
Mean		22.3	21.8	25.5	26.5	1.14	0.90	1.15
SD		17.80	19.93	25.02	22.67			
CV		79.7	91.2	98.1	117.0			

[a]Arithmetic mean of five replicate plates. L = low speed. H = high speed. A = 100 g sample + 100 ml diluent. B = 25 g sample + 225 ml diluent. SD = standard deviation. CV = coefficient of variation.

TABLE 4. *Analysis of variance table for aerobic plate counts on three types of foods by four methods*

Type of food		SS	df	MS	F[a]
Ground beef	Within	21,192,993	72	294,347	
	Between	180,327	3	60,109	0.204
Celery	Within	1,738.1	48	36.21	
	Between	60.76	3	20.25	0.559
Wieners	Within	27,810	60	463.5	
	Between	258.0	3	86.00	0.186

[a]$F_{3,60;0.95} = 2.76$. $F_{3,40;0.95} = 2.84$.

This study found no difference in recoveries when using either low (11,200 rpm) or high (> 18,000 rpm) speed for 2 min on the blender. High speed blending has been considered deleterious to bacterial recovery because of excessive heating. One method described by Thatcher and Clark (*5*) specifies a blending sufficient to give a total of 15,000 to 20,000 revolutions. High speed on our blender was in excess of 18,000 rpm, which would provide with 2 min mixing more than 36,000 revolutions with no apparent decrease in recovery.

Two methods for preparing homogenates with the Stomacher were compared, one using a 1:1 ratio of food to diluent as described by Tuttlebee (*6*), and the other a 1:9 ratio. No differences in bacterial recoveries were found between the two approaches. The 1:2 dilution provides a slurry too thick for pipetting, therefore use of the 1:10 dilution is a more convenient method.

This study concludes that the Stomacher is an alternative method with many advantages over the blendor for preparing food homogenates. It is also a convenient procedure for preparing *Salmonella* enrichments by weighing the sample directly into the bag, which is held upright in a beaker during incubation. The method has additionally been very satisfactory for preparing dilutions of fecal specimens for enumeration of spores of *C. perfringens*.

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Concentration of Egg White by Ultrafiltration [1]

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ABSTRACT

Unpasteurized egg white was concentrated by ultrafiltration to about one half of its original volume. Twenty four batches, each started with 5,000 pounds of fresh egg white, were carried out in a 12-month period with a SANOVO Unit in an egg products plant. The optimum operating pressure was found to be about 415 psi. Higher pressure did not benefit the overall efficiency; on the contrary, it increased the chances of fouling and possibly compaction of the membrane and shortened the membrane life. As no refrigeration was provided in the system, egg white temperature rose linearly in all trials. The aerobic plate count of microorganisms was found to be doubled within 1-3 h when the ending temperature was above 20 C and over 10 h when the ending temperature was less than 16 C. The logarithm of permeation rate (R, lb./min) was linearly proportional to operating time (t, min) and the percent solids content of egg white (S). The solids content at a given time can be predicted by the equation:

$$S = 11.3 + 0.025\ t$$

The estimated cost of concentrating egg white from 11.5 to 23% solid and at a volume of 10,000 lb. per day or 2,500,000 lb. per year, was $0.0295 per pound of concentrate. The accountable net savings for liquid egg white which would be shipped in tankers from the west coast to the midwest, and subsequently spray dried, was about $0.0117 per pound of concentrate and $0.0095 for frozen products. The return on capital investment would be approximately 35-40%.

Egg white has been extensively used as an ingredient in the baking, confectionary, and cake mix industries. Approximately 200 million lb. of liquid, frozen, and dried products were produced each year during the last decade (*1*). The relationship among the three types of products varies slightly each year. In 1972, for example, about 50% of the total production was consumed in liquid form and 25% in frozen. The amount of dried egg white produced from either liquid or frozen white was 20.4 million lb.

Fresh egg white contains approximately 88.5% water. The solids are predominantly proteins with minor amounts of free carbohydrates and inorganic elements (*17*). The proteins are sensitive to heat (*3*) and shearing force (*12*) and susceptible to microbiological spoilage. Therefore, fresh egg white needs to be refrigerated or frozen in stroage.

The economic advantages of removing a portion of the water from egg white to save the cost of processing, packaging, storage refrigeration, transportation, and drying have long been recognized. However, as far as we know, no liquid or frozen egg white is commercially concentrated in the US.

Reverse osmosis and ultrafiltration are separation processes that use pressure to force water and selected solutes through a structurally supported semipermeable membrane. Since these methods concentrate aqueous products without phase change and the application of heat, they offer a potential method for concentrating the heat labile egg white.

The application of reverse osmosis to concentration of egg white was first investigated by Lowe et al. (*11*). They devised a unit of alternating layers of membrane and porous matrix sheet in a sandwich-type construction (*10*). The possible damage of product quality by shearing force was minimized by using a specially designed device to withdraw the concentrate gradually from the high pressure zone. They reported that at 600-1200 psi and 18-20 C, with a given membrane, the permeation rate was dependent on neither pressure nor velocity. However, recycling of product, thin membrane spacing, and continuous agitation would increase the unit efficiency.

Payne et al. (*15*) investigated the concentration of egg white with two tubular membrane systems. They found that the permeation rates increased with increasing operating pressures or temperatures, but the rate became less dependent on the operating pressure as it approached 400 psi. They postulated that at high operating pressure the accumulated egg white solid at the membrane surface, a "cake" layer, became the limiting factor to the permeation rate.

These workers demonstrated the technical feasibility of concentrating egg white with a membrane process. As

[1] *Reference to a company or product name does not imply approval or recommendation of the product by the U.S. Department of Agriculture to the exclusion of others which may also be suitable.*
[2] *U.S. Department of Agriculture.*
[3] *Nulaid Foods, Inc.*

their investigations were carried out under laboratory conditions, and considered neither the microbiological property of egg white nor the sanitary requirements of a processing facility, it has been difficult to evaluate the adaptability of the membrane process from the practical standpoint.

The present study was initiated to investigate the concentration of egg white with a membrane process in a commercial production environment. A ROVOSAN ultrafiltration unit was used to determine the effect of operating temperature on bacterial growth, the effect of pressure and solids content on process efficiency, and the economics of the operation. Data on the effects of ultrafiltration, pasteurization, dehydration, and chemical additives on the quality of egg white concentrate will be presented in subsequent articles.

MATERIALS AND METHODS

Ultrafiltration of egg white

ROVOSAN ultrafiltration unit, SANOVO, Food and Engineering Ltd. A/S, Denamrk, Fig. 1, was installed in an egg product plant in Central California, USA. The unit consists of a column (rear right), a control panel (front left), and a feeding pump (under the panel), all mounted on a stainless steel frame. The column consists of a stack of three identical modules, which were piped in parallel to enlarge the total capacity. Each module included a stack of 48 sets of membranes and porous matrix sandwich-combination, similar to the simple WURSTACK unit (*10*). Four types of cellulose acetate membranes (#900, 975, 985, 990 SANOVO DANMARK) were used and the total effective surface was 301 ft². The panel consists of indicators of feed and concentrate temperatures, operating pressure and motor speed, pH recorder, and the various controls for the system.

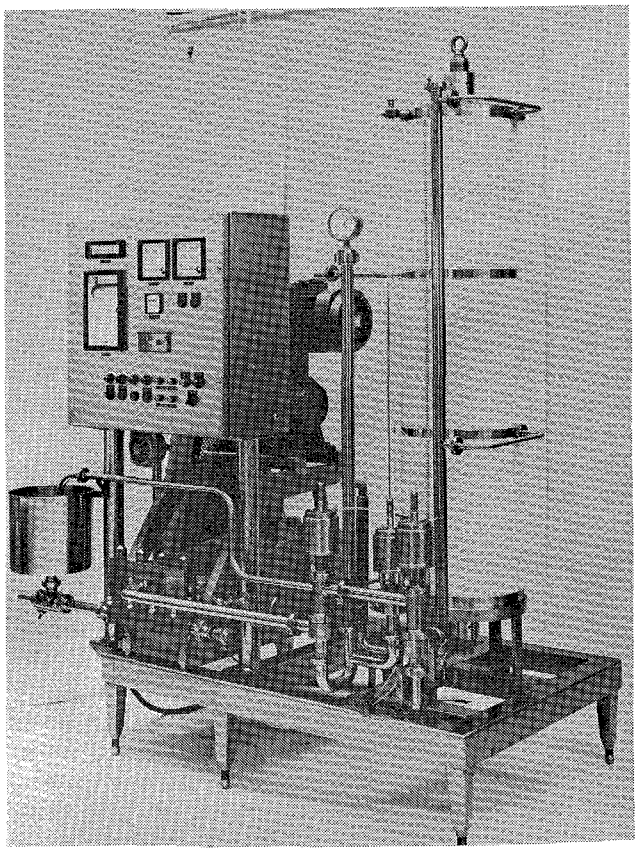

Figure 1. *ROVOSAN ultrafiltration unit. It consists of a control panel (front left), a feeding pump (under the panel), a column (rear right) and piping, all mounted on a stainless steel frame. The panel consists of indicators of feed temperature, operating pressure and motor speed, pH recorder, and the various controls for the system.*

[4] *A proteolytic enzyme preparation supplied by SANOVO.*

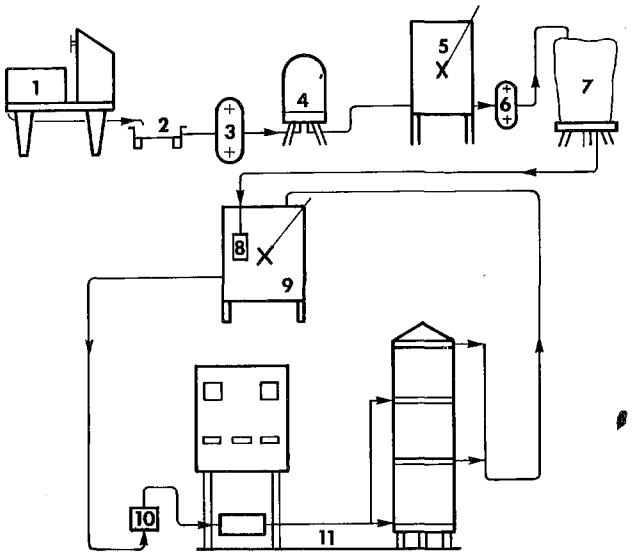

Figure 2. *Schematic flow diagram of the ultrafiltration process. 1. Egg breaking/separating, 2. Collection pot, 3. Pump, 4. Filter, 5. Tank for storage and pH adjustment, 6. Pump, 7. Bag filter, 8. Inline filter, 9. Feed tank, 10. Double inline filter, 11. ROVOSAN ultrafiltration unit.*

The flow diagram of the process is shown in Fig. 2. Egg white from the breaking machine was strained and acidified with acetic acid (10%) to pH 7 in the storage tank. After the egg white was allowed to settle for an hour it was filtered again through a bag filter (SANOVO) and was ready for ultrafiltration.

Egg white accumulated in the feeding tank was pumped through an inline filter into the column and the concentrate was recycled back. Continuous agitation in the tank was provided with a sweep-type agitator to prevent formation of a concentration gradient. The permeate from the column (not shown on the flow diagram) was collected periodically and weighed to determine the permeation rate. Operating pressure and the temperature of the feed were read from the indicators on the panel. The pH of the egg white before entering the column was recorded continuously by the recorder. The process was terminated when the solids content of the whites in the tank reached the specific level. Then, the column was immediately rinsed with water and followed with circulating Lactonaise HB 103[4] (0.9%) solution at pH 7.5-8.0 for 1 h and then in reverse flow for another hour. It was then rinsed with water and followed with nitric acid solution (pH 3) for about 15 min, water for 5 min, and finally preserved in 1.75% iodine solution or formaldehyde solution if held for longer than 1 week.

Analytical methods

Total solids content of egg white was determined by the oven method described in the USDA *Laboratory Methods for Egg Products* (*18*). Total microbial counts were determined by the method for egg products recommended by the Association of Official Analytical Chemists (*14*).

RESULTS AND DISCUSSION

About 40 trials were made from October, 1973 to September, 1974 (Table 1). Various batch sizes up to 5,000 lb. were tested in the preliminary investigation. Only those started with 5,000 lb. are reported as that was

the most economical volume the facility could accommodate in an 8-h shift.

The process was terminated when egg white was concentrated to one-half of its original volume. Further concentration was found impractical due to the rapid decrease of permeation rate.

The average solids content of egg white used was 11.3%, but varied from 10.4 to 12.0% (Table 1). Addition of acetic acid before concentrating did not significantly change the solids. The low solids content suggested that the egg whites were produced mostly from eggs laid by older hens (*2*); such eggs are generally larger and have higher magma to shell ratio than those laid by younger hens (*2,8*). The egg product industry uses extensively old hens' eggs because of their higher yield.

Temperature

The effects of operating temperature on bacterial growth are summarized in Table 2. The data are listed in descending order of ending egg white temperatures. As

TABLE 1. *Water removal and average permeation rates of the ultrafiltration of egg whites in 5,000-pound batches*

Trial	Date	Initial solids, %	Water[a] removal, %	Average Permeation rate lb./h	Temperature, C	
					Starting	Ending
4	10/30/73	10.4	50	500	11	20
5	10/30	10.4	54	540	14	24.5
6	11/19	10.9	57	407	11	20
7	11/26	11.5	53	379	11	17
14	12/10	11.3	55	392	5	18
15	12/11	10.9	59	328	24	28
16	12/12	11.3	56	400	20	28
17	12/13	11.0	57	317	12	23
21	1/8/74	10.9	52	329	16	17
22	1/10	11.1	51	283	7	19
23	2/6	11.1	51	255	7	17
24	2/7	11.3	49	245	10	17
25	2/12	11.1	52	325	9	18
26	2/13	11.2	50	278	10	19
27	2/26	11.3	50	294	10	18
28	2/27	11.2	50	270	7	19
29	6/4	11.5	49	377	9	17
31	7/31	11.6	49	306	8.5	15.5
32	8/2	12.0	46	219	6	16
33	8/5	12.0	48	282	5.5	13
34	8/8	11.9	48	267	6.5	14.5
36	8/27	11.6	49	272	8.5	19
37	8/28	11.7	49	306	10	16
39	9/10	11.2	52	305	10.5	18

[a] % water removed from original egg white.

TABLE 2. *Effect of temperature on microbial growth in egg white*

Trial	Temperature, C		APC[a] X 10^3/g		Generation[b] time, h
	Beginning	Ending	Beginning	Ending	
15	24	28	13	8,000	1.2
16	20	18	670	19,000	1.9
5	14	24.5	830	16,000	2.0
17	12	23	690	12,000	3.0
6	11	20	17	320	2.2
4	11	19	280	960	6.3
26	10	19	120	670	6.3
36	8.5	19	66	260	10.0
22	7	19	160	790	7.1
28	7	19	26	150	6.7
39	10.5	18	76	190	6.7
27	10	18	39	180	7.1
25	9	18	48	300	5.0
14	5	18	390	1,100	20.0
7	11	17	160	960	5.0
24	10	17	460	2,700	6.7
29	9	17	28	40	—[c]
23	7	17	250	1,700	5.9
21	6	17	130	400	16.7
37	10	16	28	64	50.0
32	6	16	82	170	250.0
31	8.5	15.5	77	260	11.1
34	6.5	14.5	44	170	10.0
33	5.5	13	95	260	23.1

[a] Aerobic Plate Count.

[b] Generation time: time required to double the microbial counts under the specified condition.

[c] It was impossible to calculate the generation time, since the net bacteria growth was less than doubled during the experiment.

no refrigeration was provided in the system, egg white temperature rose linearly in all trials. The final temperature depended on the initial and ambient temperatures, as well as the duration of the experiment.

The microbial count of the beginning egg white varied from 13×10^3 to 830×10^3 per gram. There was no apparent correlation between counts and temperature, nor was there any correlation with season. The 60-fold variation in microbial count perhaps merely reflected the difference in shell egg qualities and the sanitary condition of the facility.

The microbial growth in albumem was expressed in generation time — the time (hour) required to increase by 100% the total count (APC). When the ending temperatures were above 20 C, the generation times were in the range of 1 to 3 h. When the ending temperatures were less than 16 C (5 trials), the generation times were all over 10 h. In other words, microbial count will be less than doubled in 10 h of operation. For example, an initial count 1×10^5 per gram of albumen may increase to a maximum of 2×10^5 after 10 h, but the actual bacterial count of the concentrate will be 4×10^5 as bacteria are also concentrated because they are excluded by the membrane.

Obviously, operating temperature should be kept to a minimum to ensure the albumen's microbiological quality; but, lowering temperature has an adverse effect on membrane permeability (*15*) and increases operating cost. Therefore, once the desired final solid concentration (which determines the operating time) is known, the operation temperature could be selected based on the initial counts and the maximum tolerable counts of the concentrates.

In the United States, the Egg and Egg Product Inspection Law (*4*) requires that unpasteurized egg white which will be held in excess of 8 h to be cooled to below 7.2 C (45 F) within 2 h from the time of breaking and separating. Egg whites which will be pasteurized within 8 h are required to be held below 12.8 C (55 F).The maximum holding temperature for egg white which will be stabilized by desugaring (*7*) and subsequently dried is 12.8 C (55 F) for those held in excess of 8 h, and 21.1 C (70 F) for those held less than 8 h. Therefore, to operate at about 12.8 C the total operation time is confined by present regulation to a maximum of 8 h which limits the concentration of the finished product to under 23% solids, as will be shown in the following section. However, if egg white is to be stabilized and dried, the operating time may exceed 8 h at 12.8 C.

Egg white contains 0.38% of free glucose (*11*). The interaction of glucose and the amine group of proteins, followed by other changes (*9*), results in formation of a brownish color and loss of whipping properties. This browning reaction is known to be the primary cause of quality deterioration of dried egg whites, and several methods have been developed to retard this deterioration (*7*). The most prevalent process for stabilizing egg white is to reduce the free glucose to a minimum level through controlled bacterial or yeast fermentation. The process is commonly carried out by inoculating with the desired culture and incubating at pH 7.0 and about 30 C. Pasteurized egg whites are used to minimize the growth of undesirable organisms. The other popular desugaring method, although not extensively used in egg white because of its higher cost, is the enzyme method. It uses glucose oxidase to catalyze oxidation of glucose to gluconic acid in the presence of molecular oxygen. Its optimum pH is also at about 7.0, but it can be carried out at a lower temperature than bacterial fermentation (*6*). Since the requirements of physical condition for ultrafiltration and desugaring are similar, they may be done concurrently to save processing time, handling, equipment and waste, and to lessen the chance of product abuse. A study to investigate the feasibility of combined ultrafiltration and desugaring of egg white has been initiated.

Pressure

Figure 3 is a typical example of operating pressure conditions. The figure includes the four trials (31,33,34, 37) carried out at temperatures ranging from 6 to 16 C (Table 1). The operating pressure rose rapidly to 410 psi as egg white was pumped into the membrane column, remained at this pressure until the solids content of the feed reached 20%, and then, a gradual increase of pressure was required to compensate for the sharp decrease in permeation rate. However, a further increase in pressure above 500 psi did not benefit the overall efficiency. On the contrary, a "cake" layer of egg solids

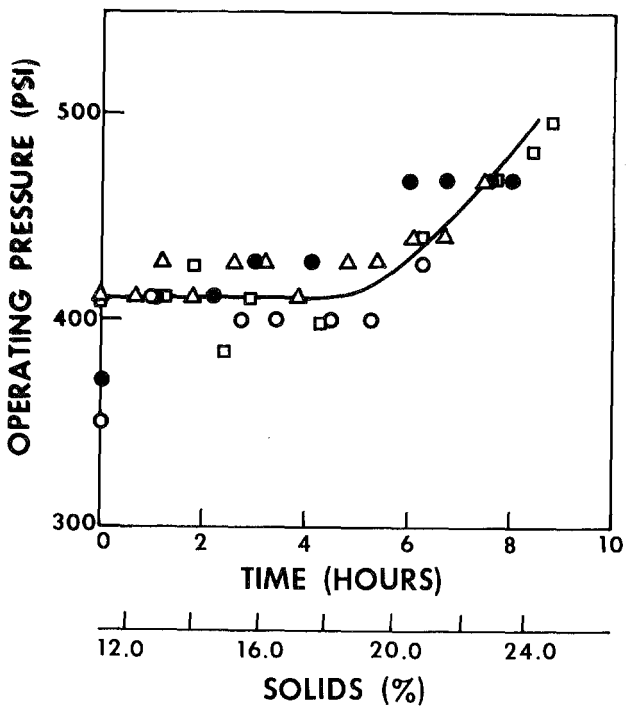

Figure 3. *Typical operating pressure at the inlet of the ultrafiltration column. Trials 31* (O), *33* (△), *34* (•) *and 37*(□) *of ending temperature below 16 C are included.*

was observed to deposit on the membrane. The tightly packed deposit could not be completely removed with the available cleaning methods, and caused irreversible damage to the membrane.

Permeation rate

The permeation rates of those trials (31,33,34,37) carried out under 16 C were plotted against operating time (Fig. 4) and solid content (Fig. 5). The logarithm of

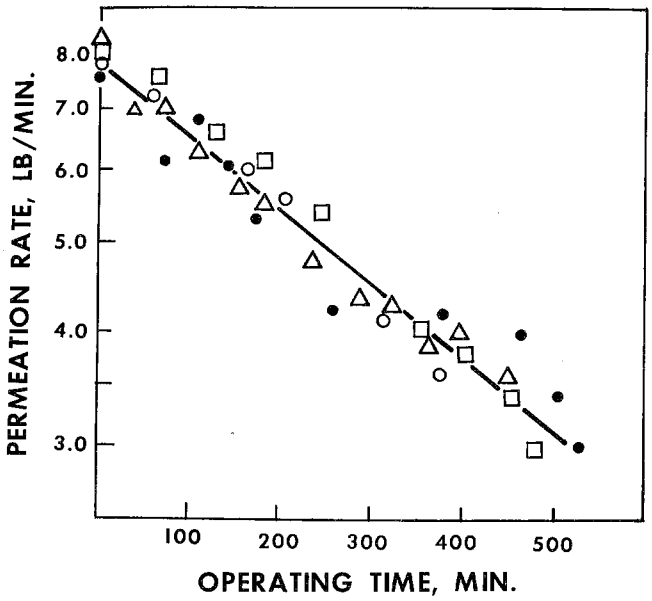

Figure 4. *Relationship between permeation rate and operating time. Trials 31* (O), *33* (△), *34* (•) *and 37* (□) *of ending temperatures below 16 C are included.*

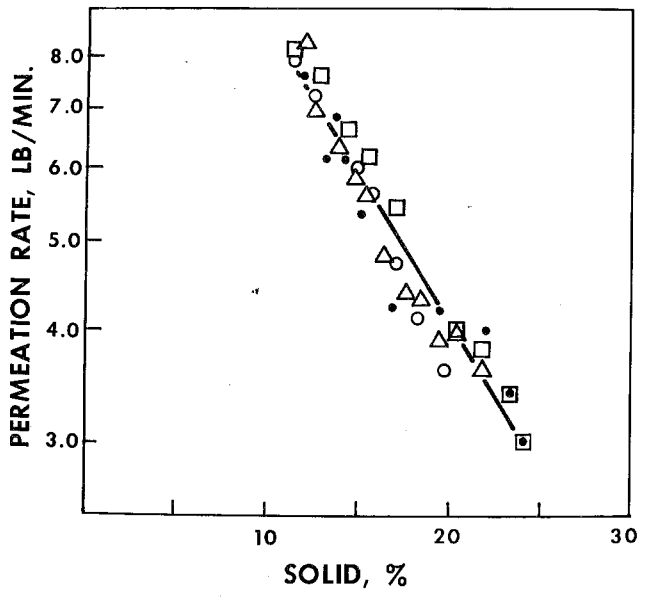

Figure 5. *Relationship between permeation rate and percent solids content of the feed. Trials 31* (O), *33* (△), *34* (•) *and 37* (□) *of ending temperatures below 16 C are included.*

permeation rate (R) was found to decrease linearly with time (t).

$$\log R = 0.89 - 0.00078t$$

A similar relationship between the logarithm of permeation rate and percent solid (S) in egg white was also obtained.

$$\log R = 1.26 - 0.032S$$

By the combination of equation I and II, the solids content of the concentrate was found to be linearly proportional to operation time.

$$S = 11.3 + 0.025\ t$$

This equation was used to predict the time required to concentrate egg white to the desired solids content.

All four trials analyzed were in good general agreement. The coefficients of determination for permeation rate vs time, and vs solids content, were 0.952 and 0.934, respectively.

The permeation rate at 12% solids was 7.4 lb./min, equivalent to 4.3 gal/ft²-day, and at 23% solids was 3.3 lb./min or 1.9 gal/ft²-day. Lowe et al. (*11*) using the WURSTACK apparatus at 900 psi and 18-20 C observed similar permeation rates (4.5 and 1.3 gal/ft²-day for feeds of 12 and 23% solids). The permeation rate of feed at 12% solids, calculated from the data reported by Payne et al. (*15*) using an Abcor ultrafiltration module at 32.8 C (91 F), 25 psi, and 15 gpm of feed flow rate, was 4.5 gal/ft²-day. Despite the wide variations in equipment design and operating conditions among these three studies, the similarity in permeation rates suggests that membrane type, temperature, and pressure, are not the primary determining factors of the process. The efficiency of ultrafiltration is, rather, governed predominantly by the content of egg white solids, which seems to further substantiate the hypothesis that, in egg white concentration, polarization is the primary source of resistance, as described by Payne et al. (*15*). Similar phenomena were also observed in whey concentration by Marshall et al. (*13*), and in other food systems by Ginnette and Merson (*5*).

One of the major design differences between the apparatus used in the current study, and that used by Lowe et al. (*11*), was that the concentrate was discharged to atmospheric pressure in the former, whereas in the Lowe system a special pressure release device was added to maintain high pressure differences across the membrane and to insure gradual withdrawl of concentrate from such high pressure. Lowe et al. (*11*) stated that such a device was essential to prevent functional damage of egg white by shearing. By operating at lower pressures than those used by Lowe et al. and elimination of the pressure release device, the equipment design and operation in the current study was greatly simplified. The efficiency of the two processes, as judged by the comparable permeation rates found in the two studies, were comparable and no functional damage to concentrated egg white was observed.

Membrane deterioration

Although egg white was concentrated to one-half of its volume, there was a wide variation of total processing time. The average permeation rate, which is calculated by dividing the the total amount of water removed by processing time, decreased from 500 lb./h to 300 lb./h during the initial 15 trials, and remained at the 300 lb./h throughout the rest of the study (Table 1). Obviously, temperature and pressure are not the cause of these differences, as comparable conditions were employed during both periods.

The irreverisble decrease in permeation rate may be attributed to compaction and/or fouling of the membranes. Compaction, as it is described by Podall (*16*), has been generally considered to be the result of the plastic creep of the membrane structure, which reduces the porosity, or increases the effective thickness of the permeation barrier. As might be expected, this effect increases with increasing pressure. Membrane fouling, on the other hand, depends on the tightness of the membrane and the solutes in the feed. Egg white proteins could deposit on and "plug" the more "open" pores. They could also interact with the membrane until a dynamic equilibrium is reached. To minimize membrane deterioration by "plugging," egg white was filtered in four stages (see Fig. 2) to remove suspended particles. Most of the fibrous chalaza was removed by the first perforated stainless steel filter. The bag filter located after the acidification tank removed the denatured proteins resulting from acidification, mechanical agitation, or pumping. The in-line filter located before the feeding pump provided further protection from suspended solids entering the column. However, it is doubtful that these precautions could prevent the entering of very fine particles in the feed, which inevitably causes a certain amount of membrane fouling. Because the effects of both compaction and deposition on membrane increase with increasing pressure, it would be advantageous to adopt the lowest possible pressure so as to extend membrane life.

Economics

The expenditures which will contribute to the cost of the ultrafiltration process are: (*a*) equipment amortization, (*b*) equipment replacement, (*c*) chemicals, (*d*) labor, and (*e*) utilities. The cost of such a process on a unit basis depends on the volume of product produced and also the solids content of the finished product.

Table 3 shows an example of the itemized unit cost of concentrating egg white from 11.5 to 23% solids with the SANOVO unit. The estimation was based on the assumption that 2 × 5,000-lb. batches of egg whites were concentrated each day to 5,000 pounds of concentrate (23% solids). The operating time for each batch was 8 h, but an additional 4 h were allowed for preparation, and equipment cleaning and sanitizing. The annual production based on 250 working days per year was 1,250,000 lb. of concentrate. Capital investment was amortized in 10 years with 10% annual interest rate. The replacement cost included the cost of two sets of new membranes and labor for replacing them. The total cost of concentrating was $0.0295 per pound concentrate, in which labor accounts for 71%. If equipment is installed in an egg products plant, cost of labor could be reduced significantly as operation and cleaning require little attention, and can be shared at least partially by existing operators. The total cost could vary from $0.0086 to $0.0296 per pound, depending on the labor situation.

TABLE 3. *Cost of egg white concentration from 11.5% to 23% solids*[a] *by ultrafiltration*[b]

Item	Cost per lb. of concentrate	%
Equipment amortization	$0.0030	10
Equipment replacement	0.0018	6
Chemical supplies		11
for processing	0.0002	
for cleaning	0.0030	
Labor		71
for processing[c]	0.0126	
for cleaning[d]	0.0084	
Utilities	0.0006	2
	$0.0296	100

[a]50% of the original water was removed.
[b]Based on concentrating 2 × 5,000 lb batches per day and 2,500,000 lb. per year.
[c]6 man-hour at $5.25 per hour, including 28% fringe factor.
[d]4 man-hour at $5.25 per hour, including 28% fringe factor.

Egg whites from a breaking plant are marketed in bulk liquid form delivered in tankers or other reusable containers, and also in frozen form in 30-lb. cans. The economic advantages of concentrating egg white differ according to geographic and other factors including intended use of the product. The major accountable savings in packing concentrates rather than regular egg white are illustrated in Table 4. It was assumed that the breaking plant was located in one of the western states of the United States where a drying facility is not available. In the case of selling liquid egg white to drying plants located in midwest states, the major accountable savings are in transportation and drying cost which,

TABLE 4. *Major accountable savings by concentrating egg whites from 11.5 to 23% solids in an egg product plant*[a]

Item	Cost per lb.-Egg white		Saving per lb. concentrate
	Unconcentrated	Concentrated	
Liquid products to driers			
Transportation to midwest	$0.0300	$0.0300	$0.0300
Drying to egg solid 8% moisture[b]	0.0199	0.0085	0.0113
		Total	$0.0413
Frozen products			
30-lb. can at .90 each	0.0300	0.0300	0.0300
Freezing and storage[c]	0.0095	0.0095	0.0095
		Total	$0.0395

[a]50% of the original water was removed.
[b]Calculated from the data reported in Marketing Research Report #917, ERS, USDA, 1971, page 18.
[c]Includes frozen storage for 1 month.

together, amounted to $0.0413 per pound of concentrate. The net saving would be $0.0117 per pound, which amounts to approximately 40% return on the capital investment.

For frozen product the anticipated savings are from containers (30-lb. can at $0.90 each) and freezing and storing cost (at $0.0095 per pound of product). The overall savings were $0.0345 per pound of concentrate. The net savings were $0.0095 and the return on capital investment would be approximately 35%. In addition, the concentrated egg white had many other advantages that are more difficult to quantify. It increases user's holding capacity, especially by reducing refrigeration requirements, as well as reducing handling time, transportation costs, etc. To those who are seeking new ingredients in food formulation, concentrates can offer a wide range of protein contents in a unique product of unmatched whipping characteristics. It may broaden the usage of egg white in products where it was not accepted previously due to its high moisture content.

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Collaborative Study of the Coulter Counter-Chemical Method for Counting Somatic Cells in Raw Milk[1]

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ABSTRACT

Variation between laboratories for Electronic Somatic Cell Counting by the chemical method (ESCC) was evaluated by a collaborative study. Eight laboratories counted somatic cells in 12 milk samples (six replicated samples) by the ESCC method. The somatic cell count for the same milk samples was also evaluated by the Direct Microscopic Somatic Cell Counting procedure (DMSCC) as a comparison for the level of error. The standard deviation of the variation of logarithms of ESCC counts between laboratories was 0.04368. The standard deviation for the variation of logarithms of DMSCC counts between technicians was 0.08617. The corresponding value for the DMSCC analysis of the last set of federal split milk samples was 0.141. An earlier study of electronic counting by the centrifuge method showed a standard deviation of 0.0711.

The Electronic Somatic Cell Count by the Chemical Method (ESCC) is the official test of milk quality in Canada and several European countries. The method has not been approved for this purpose in the United States, primarily due to lack of collaborative studies. A recent report compared ESCC results with those of the Direct Microscopic Somatic Cell Count (DMSCC) with a coefficient of correlation of 0.96 (*14*). The same authors reported coefficents of variation for the DMSCC, Wisconsin Mastitis Test (WMT), and ESCC to be 15.6, 6.3 and 4.2% respectively. This work and that of other investigators (*2,3,6-9,12*) suggests that the ESCC method could be used in routine somatic cell counting in the Abnormal Milk Control Program in the United States, as recommended by the Interstate Milk Shippers Conference.

This collaborative study was initiated to determine the variation in ESCC results between laboratories. This variation was compared with the variation in DMSCC results.

MATERIALS AND METHODS

Eight laboratories geographically distributed in the United States and South Central Canada were selected to participate in this research effort. Each laboratory was given a complete outline of the ESCC procedure to be followed. This technique was modified by Macauley and coworkers (5). All collaborating laboratories had 1 month to learn the procedure and to clarify specific details. All of the laboratories had previous experience with electronic counters, but not all had used the specified ESCC prodcedure.

A trail test was done before the actual experiment to identify and correct problems experienced in shipping and analyzing milk samples. This was an important aspect of the study. The quality of results depends on the familiarity of technicians with the procedure. In this investigation the quality of data in the final experiment was considerably better than that obtained during the trial test. We believe that further tests would have shown improved results.

The electronic counting equipment (Coulter Counters, models FN, ZB, ZBI, and ZF) was calibrated before the inital test by representatives of Coulter Electronics. All equipment was standardized on 3.40 -μm latex particles (Coulter Electronics Inc., Hialeah, Florida) from the same production batch. Each laboratory was provided with a precalibrated automatic diluter for making reagent/milk dilutions and accuvettes for handling diluted samples. Critical details of methodology were discussed with personnel in each laboratory before the initial test and uniform forms for results were distributed to all laboratories. The procedure for the initial test and the experiment were identical except as noted in the following paragraphs.

Raw milk samples were collected from six dairy herds on the first morning of the experiment. The farms were selected on the basis of general herd health and ESCC counts taken the previous day. The criterion of selection was to obtain samples with somatic cell counts representing the entire range normally encountered in a laboratory. We attempted to select herds that would provide two samples with somatic cell counts below 500,000, two samples with counts between 500,000 and 1,000,000, and two samples with counts in excess of 1,000,000/ml.

All milk samples were initially delivered to the Dairy Quality Control Laboratory, Inc., in plastic containers immersed in a crushed ice/water mixture in insulated coolers. After mixing, aliquots were taken from

[1]*This study was sponsored by the subcommittee on laboratory methods for the examination of milk and milk products, I.A.M.F.F.S. [R. E. Ginn and V. S. Packard, Jr., Co.-chairmen). The collaborating laboratories in alphabetical order are: Associated Milk Producers, Inc., Arlington, Texas, Gerald Hein; Cornell University, Ithaca, New York, Roger Natzke; Coulter Electronics, Hialeah, Florida, Shepard Kinsman; Dairy Quality Control Institute, Inc., St. Paul, Roy Ginn; Dawson Research Corporation, Orlando, Florida, Patsy Rambo; Iowa Department of Agriculture Dairy Laboratory, Des Moines, Iowa, B. J. Reed; Manitoba Department of Agriculture, Dairy Section, Manitoba Canada, D. M. Macauley; and North Carolina State University, Raleigh, North Carolina, R. D. Mochrie.*
[2]*Dairy Quality Control Institute, Inc.*
[3]*Department of Agricultural Engineering.*
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each sample for a preliminary ESCC analysis. This analysis served only to assure a distribution of somatic cell counts. The bulk samples were then split into six smaller sample units containing approximately 200 ml. Both the bulk samples and the smaller aliquots were held in the refrigerator at 3 C. Before all sampling or splitting of samples, milk was mixed by gentle rotation through an arc of 180° at least six times in the partially filled containers.

Each 200-ml samples was divided among several 50-ml glass tubes with screw-on plastic caps for shipping to the collaborators' laboratories. A total of 12 samples, six of which were duplicates, were prepared for each laboratory. Samples were randomized by a computer procedure that provided independent randomization for each set of six samples. After randomization samples were renumbered 1 through 12 and the laboratories were told only that they were receiving 12 milk samples for analysis. None of the laboratories were given any information on the randomization procedure used on their samples.

The 12 vials of milk for each laboratory were packed in insulated picnic-type coolers for transporting to the collaborating laboratories. During the initial test a protective plastic bubble overwrap was placed around the vials and held in position with a rubber band. Frozen ice packs were used as refrigerant inside the insulated cooler. Several laboratories observed that this did not provide a uniform temperature control for all milk samples. Better temperature control was achieved by eliminating the overwrap and packing crushed ice around the milk samples shipped for the actual experiment. No temperature control problems were observed with these samples.

All samples were shipped to the participating laboratories by special flight delivery. This provided delivery to the collaborating laboratories on the same day as the herd samples were collected. One set of samples was shipped to Des Moines, Iowa and returned to the Dairy Quality Control Institute, Inc., by special flight service to compare with a set of samples which were held under refrigeration in the laboratory. Each laboratory analyzed the samples they had received at a specified time on the following day.

Each of the collaborating laboratories prepared one aliquot from each of the 12 samples of milk. The 12 aliquots were all counted twice on the electronic counters, and both results were reported.

Four additional sets of milk samples (48 vials) were prepared and held at the Dairy Quality Control Institute, Inc., for DMSCC analysis on the following day. Each set of 12 samples was randomized by the same procedure used with the ESCC test. A film was made for each of these milk samples. The direct microscopic counts of cells on these films were made by technicians in the Dairy Quality Control Institute, Inc., and the official Minnesota State Laboratory. Each set of 12 films was counted once in the official laboratory and once by a technician at Dairy Quality Control Institute. One set of films was counted a second time by a fifth technician at the Institute. The single strip method of counting was outline in *Standard Methods for the Examination of Dairy Products* (*4*) was used for these counts.

RESULTS AND DISCUSSION

Somatic cell counts for milk samples shipped to Des Moines and back were not significantly different from those counts for samples which had remained in the laboratory. From this we concluded that shipping of milk samples did not significantly affect the results. Samples subjected to the temperature distribution experienced during the initial test (partially frozen to near room temperature) also showed no significant variation in cell count by the electronic method from samples that had been held under refrigerated storage. Others (*11*) have noted a reduction in count when milk samples were frozen. In this study, though, samples were only partially frozen, and under relatively mild freezing conditions.

The ESCC data were initially analyzed with laboratories, milk samples, and duplicate electronic counts, each considered as an independent source of error. The analysis of variance showed that duplicate counts on the instrument and all interactions between them and the laboratories and samples contributed insignificant levels of error. None of the error terms involved in the duplicate counts was greater than 25% of the pure error. From this it was concluded that duplicate counting of cells added no additional information beyond that obtained by a single counting.

The ESCC results were reanalyzed using a single electronic count for each milk samples. Components of the analysis of variance are in Table 1. Each source of error (laboratories, milk samples, and the interaction term) is significant at the 5% level. Logarithm transformations of counts were used in this statistical analysis to properly account for the change in variability with the number of cells counted (*1*).

TABLE 1. *Analysis of variance of the logarithm transformation of the ESCC results*

Source of error	Degrees of freedom	Sum of squares	Mean squares	Calculated F
Laboratories	7	0.181	0.02586	8.74
Milk samples	5	12.083	2.4166	816.
Laboratory × sample	35	0.289	0.00826	2.79
Within replicates	48	0.142	0.00296	

The standard deviation was calculated as an estimate of the expected variation in counts between laboratories. For this calculation the mean square for the laboratories was assumed to contain the sum of the mean square for the pure error and the variance between laboratories multiplied by the number of samples.

$$MS_L = MS_{PE} + 12s^2_L$$

Where

MS_L is the mean square for laboratories

MS_{PE} is the mean square within replicates

s^2_L is the estimated variance between laboratories.

or

$$s_L = \sqrt{\frac{MS_L - MS_{PE}}{12}}$$

$$s_L = 0.04368$$

A standard deviation was also calculated for the variation between results of replicated samples. Each laboratory received six different milk samples in duplicate, for a total of 12 samples. The standard deviation for these replicated samples is the square root of the within replicates - mean square, 0.0544.

For DMSCC results, standard deviations between technicians and between replicated samples were 0.0862 and 0.1109, respectively. These numbers are based on the analysis of variance, Table 2, of the logarithms of the DMSCC results.

Another measure of the variation in DMSCC results is available from the federal split milk samples program.

TABLE 2. *Analysis of variance of the logarithm transformation of the DMSCC results*

Source of error	Degrees of freedom	Sum of squares	Mean squares	Calculated F
Technicians	8	0.811	0.1014	8.24
Milk samples	5	21.950	4.3900	357
Technician × sample	40	0.039	0.0010	0.0813
Within replicates	54	0.666	0.0123	

The standard deviation of the logarithm of counts from the last set of split samples (September, 1975) was 0.141 (*13*). This represents a between-laboratory variation like the corresponding value for the ESCC method in this study. The DMSCC results for the study came from several technicians, but only two laboratories. Therefore, the DMSCC results do not generate a measure of between-laboratory variation, but a between-technician value.

In an earlier study of the Coulter Counter-Centrifuge Method (*10*) the variance among analysis was reported as 0.00506. This corresponds to a standard deviation of 0.0711, which indicates somewhat greater variability than was observed in this study of the chemical method.

The substantially smaller variation in ESCC results between laboratories than either the DMSCC between-technicians or the DMSCC federal split milk samples is shown in Table 3. In fact, the ESCC variation is only one-third to one-half of the DMSCC variation. Figure 1 shows that these ratios are constant for any level of count above 300,000.

TABLE 3. *The Limits for 95% confidence on a count of 500,000 somatic cells*

Method	Log mean limits	Lower limit	Upper limit
DMSCC-			
Federal split sample	± 0.282	260,000	965,000
DMSCC-			
Between technicians	± 0.172	336,000	744,000
ESCC-			
Between laboratories	± 0.087	409,000	611,000

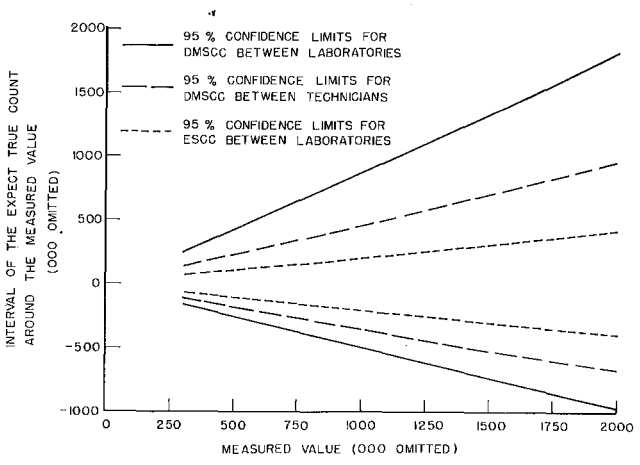

Figure 1. *Expected 95% confidence intervals for ESCC and DMSCC.*

CONCLUSIONS

Results of this study indicate that use of repeated counts on electronic instruments when making ESCC measurements is not necessary. Variation between counts was much less than the variation between replicated samples.

Reproducibility of ESCC results was found to be substantially better than for the single strip DMSCC method. The standard deviation of the variation within replicates for the ESCC method was approximately one-half of the same measure for the DMSCC method. The variation in ESCC results between laboratories was also one-half of the variation between technicians for the DMSCC results and only one-third of the variation between laboratories for the DMSCC results of the last federal split milk samples.

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Effect of the Base Layer in the Cylinder-Plate Method for Analysis of Penicillin

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ABSTRACT

Using the *Sarcina lutea* protocol for detection of penicillin, experiments comparing zones of inhibition in the presence and absence of the base layer indicated that omission of the uninoculated base layer enhanced detection of smaller concentrations of the inhibitory agent. It is, therefore, suggested that the requirement for the base layer be removed from the official, approved procedure.

The cylinder plate method for the assay of substances which diffuse through an agar medium was introduced by Abraham et al. in 1941 (*1*). In that procedure, an unseeded base layer was used, presumably to provide a flat working surface in the concave glass plates then in use. After the base layer solidified, agar which had been seeded with an indicator organism was added to each plate, and when that, in turn, solidified, the cylinders which served as reservoirs for the inhibitory materials were deposited on it.

Although a number of changes in the original procedure have been introduced since that time (the nature of the cylinder, the organism used, the components of the medium, etc.), use of an unseeded base layer has been retained and incorporated into official procedures (*2,3*). In an early evaluation of the cylinder plate procedure, Schmidt and Moyer (*6*) observed that as the agar in the plate became deeper, the diameter of the zone of inhibition decreased. This observation, however, was not accompanied by substantiating data. In any event, the use of a 25-ml base layer was recommended in that report because, when using *Staphylococcus aureus* as the indicator organism, a sharp zone delineation was obtained; reduction of the base layer to 15 ml rendered the zone edge indistinct. The current protocol advocated by the Association of Offical Analytical Chemists (*3*) and the Food and Drug Administration (*2*) mandates use of *Sarcina lutea* and a 10-ml base layer in a 90-mm diameter petri dish.

In a recent memorandum from the FDA (*4*) to the Health Officers, Agriculture Commissioners, Dairy Officials, and Laboratory Directors of each State, the cylinder plate method was adopted as the official assay for detection of penicillin in milk and milk products. In that memorandum, the opinion was advanced that "...failure to employ the more sensitive Cylinder Plate Method would present a significant public health problem to individuals highly sensitive to penicillin."

It follows from this opinion that any modification in the procedure which would enhance sensitivity for detection of reduced quantities of the antibiotic would be desirable. Logically, this could be accomplished by reducing the thickness of the uninoculated base layer, provided such reduction in the thickness did not introduce any undesirable characteristics into the assay. Accordingly, experiments were done to document the observations of Schmidt and Moyer by comparing sizes of zones produced by penicillin on the plates with and without the 10-ml base layer.

MATERIALS AND METHODS

The organism used in this protocol was *S. lutea* (ATCC 9341). Base layer agar was Antibiotic Agar No. 1 (Difco) and the overlayer agar was Antibiotic Agar No. 4 (Difco). To ensure that all plates contained an equivalent number of bacteria, molten agar tempered at 49 C was inoculated with *S. lutea* and the inoculated agar dispensed in 4-ml volumes either onto the surface of plates with the 10-ml base layers or into empty plates. The possibility that uneven distribution of the inoculated agar would occur in the empty plates was minimized by heating each plate to 50 C before addition of agar. During the addition, each plate was agitated to spread the agar over the surface of the plate. The plate was then removed to room temperature and the agar allowed to solidify. Six cylinders were placed on each plate and penicillin at a concentration of 0.05 unit/ml was added to three alternating cylinders. Penicillin was added to the remaining cylinders on each plate at one of the following concentrations: 0.00625 u/ml, 0.0125 u/ml, 0.025 u/ml, 0.1 u/ml, or 0.2 u/ml. All penicillin solutions were prepared in phosphate buffer. pH 6.0. Three plates were prepared for each penicillin concentration other than 0.05 u/ml. Plates were incubated at 32 C for 16-18 h. Zones of inhibition were measured to the nearest 0.5 mm.

RESULTS AND DISCUSSION

A graphic representation of standard curves obtained with the standard procedure and with the base layer free technique is shown in Fig. 1. The points were plotted according to the recommended FDA procedures (*2*). For each base layer, 15 plates were used, three plates for each

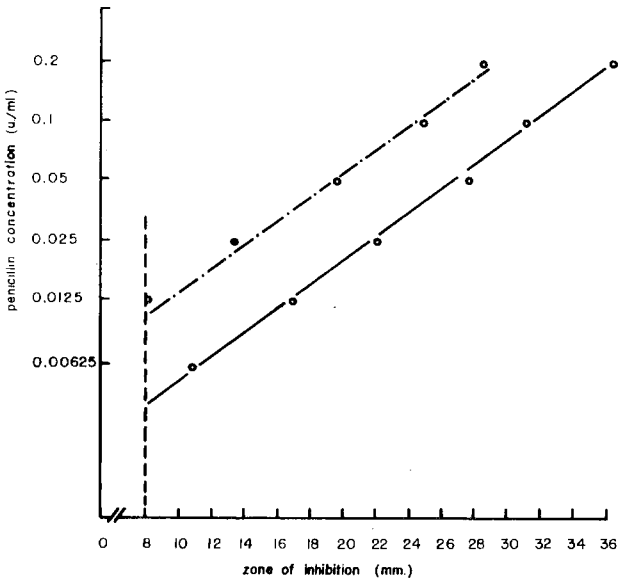

Figure 1. *The effect of an underlayer of uninoculated agar on zone sizes produced by various concentrations of penicillin. The upper curve (broken line) represents the response when 10 ml of agar was used as a base layer; the lower curve (solid line) is the response when no base layer was used.*

concentration of penicillin except 0.05 u/ml. Thus, 45 values were obtained for 0.05 u/ml and nine values for each of the other concentrations. The mean values obtained for each of the penicillin concentrations were corrected for individual plate variations according to the FDA protocol. When no base layer was used, the zone size at each penicillin concentration was increased. Further, the use of a single layer procedure rendered the method more sensitive by enabling detection of lower levels of penicillin. This was indicated by the intercepts with the 8-mm line, which represented the smallest diffusion zone possible when using cylinders with that diameter. It would be expected that use of uninoculated agar base layers of less than 10 ml would results in curves intermediate between the two shown in this figure. We confirmed this in our study.

The zone sizes plotted for each penicillin concentration shown in Fig. 1 were subject to variation. Zone size may be affected by age of the culture used, temperature of incubation, time of storage of antibiotic, etc., and was especially sensitive to inoculum size. Larger inocula displaced both lines of Fig. 1 upwards and smaller inocula generated the reverse effect. However, the qualitative relationship of the two curves which is generated by the thickness of the agar base layer was unaffected by variations in the inoculum size, provided that the inoculum in both sets of plates remained the same. Table 1 indicates values obtained during two seperate experiments; in each, the omission of the base layer provided larger zones of inhibition.

Schmidt and Moyer (*6*) asserted that reduction of the depth of the agar rendered the zone edge indistinct. In our experiments, no difficulty was experienced in measuring the zone sizes, even when the base layer was omitted. As shown in Fig. 2, edges of zones on base layer plates and those devoid of the base layer appeared to have similar sharpness.

TABLE 1. *Mean values* $(\bar{x})$ *and standard deviations (S.D.) of diameters of zones of inhibition in the presence and absence of a base layer*

Penicillin concentration, unit/ml.	Inhibition zone diameter, $\bar{x}$, S.D. in mm. With base layer: Expt. 1 n = 9, except for 0.05, where n = 45	With base layer: Expt. 2 n = 5	Without base layer: Expt. 1 n = 9, except for 0.05, where n = 45	Without base layer: Expt. 2 n = 5
0.2	29.0 ± 1.0	28.6 ± 0.814	36.8 ± 2.56	35.0 ± 0.067
0.1	25.05 ± 0.082	23.4 ± 0.894	32.0 ± 0.0	31.3 ± 1.25
0.05	19.75 ± 0.803	19.0 ± 1.0	27.7 ± 1.08	26.1 ± 1.73
0.025	12.66 ± 0.707	16.2 ± 3.11	22.1 ± 0.60	21.2 ± 1.32
0.0125	—	8.2 ± 0.44	16.4 ± 0.72	15.4 ± 1.95
0.00625	—	—	10.2 ± 0.83	8.5 ± 1.22

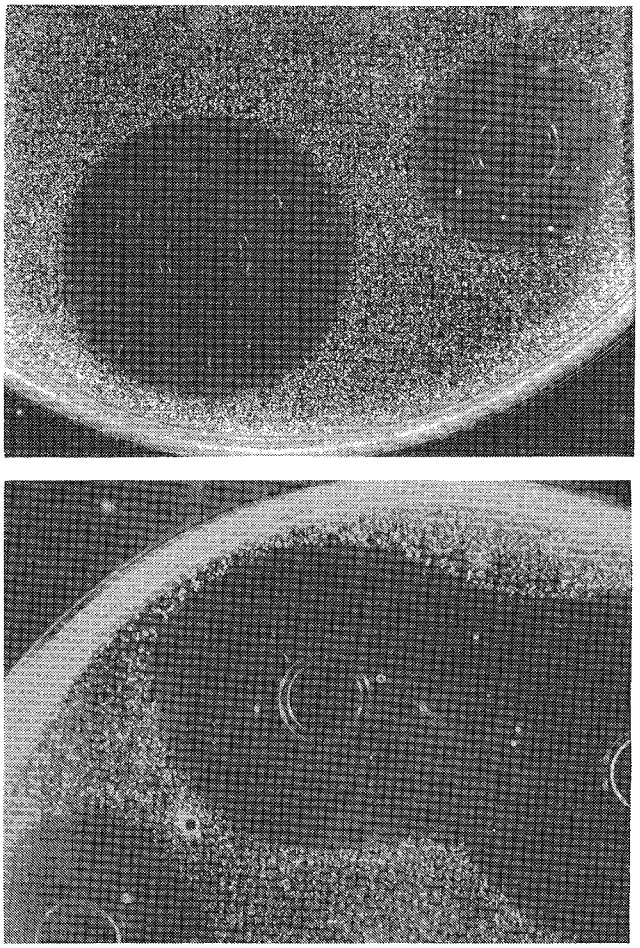

Figure 2. *Zones of penicillin inhibition in plates with (a) and without (b) a base layer, enabling a comparison of the sharpness of zone edges.*

According to the Food and Drug Administration, the permissible level of penicillin in dairy products is zero (*5*). Practically speaking, it is unlikely that any analytical procedure will ever be so exquisitely sensitive. On the other hand, the currently mandated, offical procedures

of the FDA and the AOAC appear to unnecessarily restrict the sensitivity of the bioassay by demanding the inclusion of the base layer. The results provided here suggest that it would be appropriate to reconsider the current procedures and to revise them by omitting the base layer in order that the sensitivity of the assay be enhanced.

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A Collaborative Study of the Spiral Plate Method for Examining Milk Samples

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ABSTRACT

The spiral plating procedure is a rapid method for determining bacteriological counts. Results from a collaborative study indicate that the procedure should be useful in milk analysis. Typical milk samples (homogenized milk, raw milk, chocolate drink, 2% milk, and 20% cream) were sent to six analysts to be examined by standard plate count (SPC) and spiral plate count (SPLPC). Analysis of duplicate samples shows that the SPC and SPLPC values did not differ at the $a = 0.01$ level. Components of variance for replicate determinations among laboratories and laboratory-sample interaction were computed. The standard deviation was 0.109 compared to the 0.110 estimate reported for SPC in state laboratories. Results from the SPLPC method compared favorably to the results of conventional (SPC) pour procedure.

State central laboratories participate in an annual program to check analysts' techniques (*6*) for determining the bacterial count of milk by examining common samples that are split among the 180-200 participating analysts. The total bacteria count is reported as standard plate count (SPC) (*1*). During the last survey, 14 typical samples were prepared for the study, with 12 of the samples consisting of duplicate portions of six products. These products were homogenized milk, chocolate drink, 20% cream, and raw milk (three samples). Two single samples of 20% cream and 2% milk completed the 14 units. As an adjunct to this survey, a collaborative study was designed to test a new method using these samples.

The spiral plate method (SPLPC) (*4*) was designed to serve as a general microbiological method. Its mechanical features reduce the time, equipment, and space required to do analyses. Only one plate is needed in the 500 to 300,000/ml count range. The method has been employed to count pure cultures and enumerate organisms in milk and foods within one laboratory. At present, six analysts in six laboratories have the spiral plating machines. Thus six additional sets of samples were prepared for the state laboratory survey to collaboratively test the SPLPC procedure on typical milk samples. The six analysts did both the SPC (*1*) and the SPLPC (*3,4*) on the 14 sample units.

MATERIALS AND METHODS

Samples

Samples were fluid milk that had not been heated in the laboratory. Raw milk was added to homogenized milk to provide phosphatase-positive samples, and mesophilic bacteria were added to produce a desired count range. Fourteen samples were sent out in the 1975 survey — two homogenized, two chocolate drink, one 2% milk, three 20% cream, and three sets of raw milk duplicates. The preparation and shipment of samples were essentially as described in *Evaluation of Milk Laboratories* (*6*). Samples were received and analyzed within 2 days after they were shipped. A single plate of each sample was used to determine the bacterial concentration.

SPLPC method

The spiral plating machine, its operation, and its application for counting bacteria in milk have been described (*3,4*).

SPC method

A description of the SPC procedures is found in *Standard Methods for the Examination of Dairy Products* (*1*).

Agar

Standard Methods agar was used in these studies. The spiral plates (150 × 15 mm) were prepoured, and the agar was allowed to harden and dry before they were inoculated (*3*). The pour plates (100 × 15 mm) were inoculated and the agar poured according to Standard Methods.

Statistical methods

The counts by SPLPC and SPC were converted to $\log_{10}$ count and assumed to be normally distributed with homogeneous variance. The assumptions and computations for analysis of variance were given by Ostle (*5*). Presentation of components of variance was demonstrated by Youden and Steiner (*7*) for collaborative studies. All tests of significance were done at the $a = 0.01$ level.

RESULTS AND DISCUSSION

Table 1 shows the geometric mean for the SPC results obtained in the state milk laboratories and in the laboratories with spiral plating machines. Most of the SPC values (pour plates) from the SPLPC laboratories were close (within 15% of the state milk laboratory values), with the exception of sample 6. This SPC value was 28.6% higher than those obtained by the state milk laboratories on the 2% milk sample. Values observed for SPLPC ranged from -18.2% lower to 14.3% higher than the geometric means from the state milk laboratories.

TABLE 1. *Geometric means per ml of split milk samples obtained by state milk laboratories and results from six analysts in spiral plate laboratories*

Sample	State milk laboratories	Spiral plate laboratories		% Difference of geometric mean per ml compared to state milk laboratories	
	SPC/ml	SPC/ml	SPLPC/ml	SPC	SPLPC
1 Homogenized milk	8,500	9,700	7,300	14.1	−14.1
	(192)[a]	(6)	(6)	—	—
2 Chocolate drink	110,000	110,000	96,000	0.0	−12.7
	(192)	(6)	(6)	—	—
3 20% cream	10,000	9,000	9,600	−10.0	− 4.0
	(192)	(5)	(5)	—	—
4 Duplicate of 2	110,000	110,000	90,000	0.0	−18.2
	(192)	(6)	(6)	—	—
5 20% Cream	17,000	17,000	15,000	0.0	−11.8
	(192)	(6)	(6)		
6 2% milk	14,000	18,000	16,000	28.6	14.3
	(192)	(5)	(5)		
7 Duplicate of 3	10,000	9,900	11,000	− 1.0	10.0
	(192)	(6)	(5)	—	—
8 Duplicate of 1	9,000	8,800	7,900	− 2.2	−12.2
	(192)	(6)	(6)	—	—
9 Raw milk	230,000	230,000	230,000	0.0	0.0
	(184)	(6)	(6)	—	—
10 Duplicate of 9	230,000	220,000	190,000	− 4.3	−17.4
	(184)	(6)	(6)	—	—
11 Raw milk	180,000	160,000	150,000	−11.1	−16.7
	(184)	(6)	(6)	—	—
12 Duplicate of 11	180,000	160,000	150,000	−11.1	−16.7
	(184)	(6)	(6)	—	—
13 Raw milk	200,000	210,000	220,000	5.0	10.0
	(184)	(6)	(5)	—	—
14 Duplicate of 13	200,000	190,000	200,000	—	0.0
	(184)	(6)	(6)	—	—

[a]Figures in parentheses are the number of observations.

An analysis of variance was done on the $\log_{10}$ SPLPC as shown in Ostle (5). This analysis, presented in Table 2, further assumes that analyst effect is random since the total population of spiral plating analysis in the USA is included. The sample effect is fixed because of the deliberate choice of products (raw milk, chocolate drink, etc.). This analysis was done on five samples in blind duplicate. Since samples 5 and 6 (Table 1) were not duplicates and some participants did not do analyses of 3 and 7, these samples were not included in the analysis of variance.

The sums of squares due to the analysts, samples, interaction of analysts and samples, and replicate error are presented in Table 2. The F ratios (5) indicate that there is a significant component of variance due to analysts and interaction. Estimates of these components are listed, and the reproducibility standard deviation is computed as suggested in the statistical manual of the AOAC (7).

A standard value of reproducibility variance used in the split milk samples (2) is 0.012 (S = 0.110). The variance estimated for the sprial plating machine is $\sigma_X = 0.01181$ with 35 degrees of fredom. Degrees of freedom are based on the linear combination of components of variance. This estimation procedure for $\hat{\sigma}_X$ is recommended as a way of presenting the error term in the statistical manual of the AOAC (7). In these calculations, blind duplicate samples were required. Five of the six available pairs were employed in the analysis. An F ratio of 0.01181 to the 0.012 standard is 0.98 and is nonsignificant at the $a = 0.01$ level. Thus the spiral plating components of variance do not differ significantly from the standard.

An estimate of the overall mean ($\log_{10}$ counts) for the five samples (units 1-8, 2-4, 9-10, 11-12, and 13-14) from the state data is 4.97204 (geometric mean, 94,000/ml). The mean and variance components for state laboratories used in this comparison were obtained from a random sample of 20 state laboratories. The overall mean ($\log_{10}$ counts) for the spiral data is 4.93623 (geometric mean,

TABLE 2. *Analysis of variance for spiral plating machine milk sample results and variance components*[a]

Source[b]	Sum of squares	Degrees of freedom	Mean square	F ratio
Analysts	0.11548	5	0.02310	4.57[c]
Samples	17.64771	4	4.41193	295.11[c]
Analysts and Samples	0.29907	20	0.01495	2.96[c]
Error	0.15158	30	0.00505	—
Total	18.21384	59	—	—

[a]Estimates of components of variance, where A = analysts, and AB = interaction.

Variation among replicate determinations $\hat{\sigma}^2 = 0.00505$

Variation of laboratory-sample interaction $\hat{\sigma}^2_{AB} = 0.00495$

Variation among laboratories $\hat{\sigma}^2_A = 0.00181$

Reproducibility standard deviation

$$\hat{\sigma}_X = \sqrt{\hat{\sigma}^2 + \hat{\sigma}^2_{AB} + \hat{\sigma}^2_A} = 0.10867$$

[b]Analysts factor is random and the samples factor is assumed fixed.

[c]Significant at $a = 0.01$ level.

86,000/ml). These two values were compared using a t test to examine the null hypothesis that the two population means (state SPC and SPLPC) were equal. A linear combination of variance components was used as an error term. The t = -0.68, which is not significant at the $a = 0.01$ level. Thus, the SPLPC method yielded similar components of variance to the standard SPC method, and the mean of $\log_{10}$ counts for samples did not differ significantly compared to the estimate of reproducibility. An automatic (laser) colony counter can also be used with this machine. However, the laser counter was not considered in the present study.

Table 2 shows the individual elements of the components of variance. The variance among replicate determinations is $\hat{\sigma}^2 = 0.00505$, the variation of laboratory-sample interaction is $\hat{\sigma}^2_{AB} = 0.00495$, and the variation amoung laboratories is $\hat{\sigma}^2 = 0.00181$. Values of $\hat{\sigma}^2 = 0.005$ and $\hat{\sigma}^2_A = 0.007$ have been reported (2) in milk analysis. However, there is no standard for $\hat{\sigma}^2_{AB}$. Thus, the sample-analyst gemoetric means/ml are given in Table 3 to illustrate the extent of the interaction. As an example of interaction, analyst 3 (A_3) has the second highest overall mean, but the lowest value on sample 1,8. These deviations do not seem to be excessive.

The duplicate samples (3 and 7) deleted from the analysis were 20% cream in composition. Estimates of the geometric means in Table 1 indicate that the reported values are close (within 15%) to the one computed value for state laboratory data. The SPLPC method therefore yields adequate results compared to SPC results on typical milk samples.

The SPLPC procedure is a rapid test to do microbiological analysis. At the time of the milk study, six laboratories had the machine for experimental testing. Results from the collaborative study on milk samples indicate that the procedure should be useful in the analysis of milk. This conclusion is based on the estimate of variance components and comparison of mean results on samples.

TABLE 3. *Spiral plate count geometric mean/ml for six analysts and five samples*

Sample	Analysts (geometric mean/ml)						Sample means
	A_1	A_2	A_3	A_4	A_5	A_6	
1, 8	9,600	8,200	6,400	7,000	6,700	8,300	7,600
2, 4	100,000	52,000	120,000	88,000	97,000	110,000	93,000
9, 10	200,000	160,000	210,000	200,000	230,000	250,000	210,000
11, 12	150,000	120,000	180,000	120,000	190,000	160,000	150,000
13, 14	190,000	270,000	200,000	200,000	170,000	260,000	210,000
Analyst means[a]	91,000	74,000	91,000	79,000	86,000	100,000	—

[a]Overall mean is 86,000.

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Destruction of *Salmonella* and *Staphylococcus* During Processing of a Nonfermented Snack Sausage

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ABSTRACT

Survival of *Salmonella dublin, Salmonella senftenberg* 775W, *Staphylococcus aureus* 196E, and *S. aureus* 184 was studied during processing of an inoculated beef, nonfermented snack sausage. No viable staphylococci or salmonellae were detected in sausages that had been heated at an internal temperature of 53.9-55.0 C or 57.8-58.9 C for 3.5 h followed by drying at 21 C and 50-55% relative humidity for 4 days. Heating at an internal temperature of 51.1-52.2 C for 3.5 h followed by drying did not produce a salmonellae- or staphylococci-free sausage.

In the 10-year period from 1965 to 1975, snack food sales in the United States increased from 2.6 to 6.4 billion dollars. Snack meat products commanded about 2% of the 1975 snack food market with a value of 143 million dollars (*2*). Among snack meats, snack sausages (both fermented and unfermented), beef jerky, and pork skins were the biggest sellers (*2*).

Information concerning formulation and preparation of meat snacks is not readily available. A procedure for production of a hot bar sausage (*6*) and a nontechnical description of the preparation of beef jerky (*5*) have been published. Meat chips, a potential meat snack, have been described by Fox and Ackerman (*4*).

We found no published data on the microbiology of snack sausages. Further, the fate of food-borne pathogens, such as *Salmonella* and *Staphylococcus*, during processing of snack sausages has not been considered. Therefore, survival of *Salmonella* and *Staphylococcus* species during processing of a nonfermented all-beef snack sausage was investigated.

MATERIALS AND METHODS

Preparation of snack sausages

Either fresh or frozen whole beef chuck was used. Frozen beef (previously ground through a 3/4-inch plate, packed in 5-10-kg amounts into Cry-O-Vac[2] bags and frozen at -27 C) was thawed overnight at 10 C. The thawed or fresh beef was ground through a 3/16-inch plate and mixed with spices, sugars, salt, nitrate, and nitrite according to the formulation shown in Table 1. The mixture was stuffed into 13-mm diameter collagen casings (#130-712-0, Devro, Somerville, New Jersey). Sausages were placed in an air-conditioned smokehouse with the dry bulb and wet bulb set at temperatures needed to maintain the desired internal temperature of the product for 3.5 h. The time necessary to reach the desired internal temperature for sausages was 25 ± 5 min. The internal temperature was monitored by a thermocouple probe inserted into the center of the sausage. Heavy smoke was maintained during the heating step. After heating, the smoked product was placed in a drying room maintained at 21 C and 50-55% relative humidity (RH) for 4 days.

TABLE 1. *Ingredients for experimental sausage*[a]

Ingredient	g/kg Beef
NaCl	20.0
$NaNO_3$	1.9
$NaNO_2$	0.123
Sucrose	5.0
Glucose	5.0
White pepper	4.0
Allspice	1.0

[a]The sausages used for data in Tables 2-5 had a pH range of 5.7 ± 0.1 at zero time (immediately after stuffing) and a pH range of 5.6 ± 0.2 at the end of the heating period.

Experiments with salmonellae and staphylococci

Difco Tryptic Soy Broth (TSB) cultures of *Salmonella dublin, Salmonella senftenberg* 775 W (the most heat resistant strain of salmonella known), *Staphylococcus aureus* 196E, and *S. aureus* 184 were grown for 24 h at 37 C. The cultures were diluted with sterile 0.1% Difco Peptone water to give the appropriate concentration of cells. Suspensions of the pathogens were added to the meat during the formulation step. The viable count of the *Salmonella* strains were determined by the method of Smith et al. (*7*).

The following 3-tube MPN procedure was used to isolate, quantitate, and identify coagulase positive staphylococci: 50 g of sausage were weighed aseptically into sterile blender jars, and 200 ml of sterile 0.1% peptone water was added. The material was blended at high speed for 1 min. Appropriate dilutions of the blended material were placed into tubes of Difco Brain Heart broth (BHI). The tubes were incubated at 37 C for 48 h. Growth from each BHI tube was steaked onto Vogel-Johnson agar plates (950 ml of Difco Vogel-Johnson agar plus 50 ml Difco EY Tellurite Enrichment) and then incubated 48 h at 37 C. Representative typical black colonies were selected for catalase, gram stain, and coagulase tests. For the coagulase reaction, 0.2 ml of TSB, in 12 × 100 mm tubes, was inoculated and incubated at 37 C for 24 h.

[1]*Agricultural Research Service, U.S. Department of Agriculture.*

[2]*Reference to brand or firm name does not constitute endorsement by the U.S. Department of Agriculture over others of a similar nature not mentioned.*

TABLE 2. *Effect of heating at an internal temperature of 57.8-58.9 C on the survival of salmonellae and staphylococci during processing of a snack sausage*[a]

Organism	Sample number	MPN[b] of cells/g sausage at zero time	MPN cells/g after heating	MPN cells/g after drying
Salmonella dublin	1	6.9×10^1	<0.03	<0.03
Salmonella dublin	2	6.4×10^3	<0.03	<0.03
Salmonella senftenberg 775W	3	6.9×10^1	<0.03	<0.03
Salmonella senftenberg 775W	4	3.6×10^3	<0.03	<0.03
Staphylococcus aureus 196E	5	9.3×10^2	<0.3	<0.3
Staphylococcus aureus 196E	6	2.4×10^6	0.3	<0.3
Staphylococcus aureus 184	7	2.1×10^2	<0.3	<0.3
Staphylococcus aureus 184	8	2.4×10^6	<0.3	<0.3

[a]The smokehouse was initially set at a dry bulb of 65.5 C, wet bulb set at 57.2 C; the settings were adjusted as needed to maintain the internal temperature of the sausages at 57.8-58.9 C for 3.5 h, the sausages were dried at 21 C and 50-55% RH for 4 days.
[b]The three tube MPN method was used.

Then 0.2 ml of Difco Coagulase Plasma was added, tubes were incubated at 37 C for 4-5 h, and the coagulase reaction was observed. Micrococci that were positive for catalase, gram stain, and coagulase tests were considered to be *S. aureus*. The MPN/g was calculated by the use of a table (*9*).

RESULTS

Preliminary experiments indicated that a satisfactory snack sausage could be prepared by heating and smoking the product at an internal temperature of approximately 58 C for a least 3.5 h followed by drying for 4 days. Viable cells of *S. senftenberg* 775W or *S. dublin* were not detected (< 0.03 cells/g) after heating snack sausages at an internal temperature of 57.8-58.9 C for 3.5 h nor after 4 days of drying (Table 2). When *S. aureus* 196E was present initially in the snack sausages at more than 10^6/g, an estimated MPN of 0.3 viable cell/g was found at the end of the heating period but none after drying. With low numbers of *S. aureus* 196E and both high and low numbers of *S. aureus* 184, no viable cells (< 0.3/g) were found at the end of the heating or drying periods (Table 2).

Efforts were then directed towards determining the minimum internal temperature necessary to heat snack sausages to destroy salmonellae or staphylococci. When the internal processing temperature of the sausages was lowered to 53.9-55.0 C and maintained for 3.5 h, viable cells of *S. senftenberg* were not detected (< 0.03/g); however, *S. aureus* 196E was still present in low numbers after the heating step but not after drying (Table 3). When the internal processing temperature was reduced to 51.5-52.5 C, neither salmonellae nor staphylococci were eliminated from the snack sausages during the heating step and viable cells of both pathogens were detected at the end of the drying step (TAble 4).

TABLE 3. *Effect of heating at an internal temperature of 53.9-55.0 C on the survival of salmonellae and staphylococci during processing of snack sausage*[a]

Time	MPN[b] cells/g sausage: *Salmonella senftenberg* 775W	MPN[b] cells/g sausage: *Staphylococcus aureus* 196E
Zero time	5.6×10^3	1.1×10^4
Heated 3.5 h	<0.03	0.3
After drying	<0.03	<0.3

[a]The smokehouse was initially set at a dry bulb of 61.1 C, wet bulb at 51.7 C, and the settings adjusted as needed to maintain the internal temperature of the sausages at 53.9-55.0 C for 3.5 h; the sausages were dried at 21 C and 50-55% RH for 4 days.
[b]The three tube MPN method was used.

TABLE 4. *Effect of heating at an internal temperature of 51.1-52.2 C on the survival of salmonellae and staphylococci during processing of a snack sausage*[a]

Time	MPN[b] cells/g sausage: *Salmonella senftenberg* 775W	MPN[b] cells/g sausage: *Staphylococcus aureus* 196E
Zero time	1.9×10^3	4.3×10^1
Heated 2 h	1.1×10^2	1.1×10^1
Heated 3.5 h	1.1×10^1	4.3×10^1
After drying	0.073	2.1

[a]The smokehouse was initially set at a dry bulb of 60 C, wet bulb at 46.1 C, and the settings were adjusted as needed to maintain the internal temperature of the sausages at 51.1-52.2 C for 3.5 h; the sausages were dried at 21 C and 50-55% RH for 4 days.
[b]The three tube MPN method was used.

Use of the lower temperature resulted in sausages that did not have a cooked appearance and that did not dry to form the typical snack sausage.

A commercial snack sausage meat mixture containing 39.4% fat and an experimental mix containing 7.3% fat were contaminated with either salmonellae or staphylococci and then processed into snack sausages. The data in Table 5 indicate that the amount of fat in sausages did not influence thermal destruction of either *S. senftenberg* 775W or *S. aureus* 196E.

DISCUSSION

The survivor data obtained and presented in this study are the result of end point determinations, i.e., the number of pathogens (salmonellae or staphylococci) which were detectable at the end of the selected heating conditions given the snack sausage. The data indicate that heating snack sausages at an internal temperature of > 54 C for 3.5 h followed by 4 days of drying at 21 C and 50-55% RH led to the complete destruction of salmonellae and staphylococci. In custard and chicken á la king, Angelotti et al. (*1*) showed that temperatures which killed *S. senftenberg* 775W, the most heat resistant strain of *Salmonella* known, were satisfactory for thermal destruction of other salmonellae as well as strains of *S. aureus*. In general, data obtained with *S. senftenberg* 775W in this study support the conclusions of

TABLE 5. *Effect of fat content on the destruction of* Salmonella senftenberg *and* Staphylococcus aureus *during processing of snack sausages*[a]

Formula	% Fat[c]	MPN[b] cells/g sausage					
		Salmonella senftenberg 775W			*Staphylococcus aureus* 196E		
		Zero time	After heating	After drying	Zero time	After heating	After drying
Commercial formula	39.4	9.0×10^1	<0.03	<0.03	4.6×10^3	<0.3	<0.3
Experimental formula	7.3	9.0×10^1	<0.03	<0.03	2.4×10^3	<0.3	<0.3

[a]Sausages were heated at an internal temperature of 57.8-58.9 C for 3.5 h, then dried at 21 C and 50-55% RH for 4 days.
[b]The three tube MPN method was used.
[c]Fat content was determined on the unprocesed sausages by the standard AOAC method (3).

Angelotti et al.

Many food technologists believe that microorganisms are more resistant to thermal inactivation in the presence of large amounts of fat even though real proof is lacking. Data in Table 5 indicate that snack sausages contaminated with salmonellae or staphylococci heated at 57.8-58.9 C for 3.5 h contained no survivors of either bacterial type regardless of the fat level of the sausage. Recently Smith et al. (8) utilizing frankfurters and coarsely comminuted sausages, have shown that there was little difference in the thermal destruction of pseudomonads or micrococci when the fat level of sausages varied from approximately 10-40%.

In conclusion, heating snack sausages at an internal temperature of 53.9-55.0 C or 57.8-58.9 C for 3.5 h followed by drying for 4 days gave salmonellae- or staphylococci-free products. However, heating at an internal temperature of 51.1-52.5 C for 3.5 h did not eliminate the food-poisoning bacteria; drying reduced the bacterial population but did not completely eliminate it.

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Bacteriological Survey of Chopped Liver Produced at Establishments Under Federal Inspection

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ABSTRACT

At the time of manufacture, 74% of 27 sets of chopped liver (2 to 10 finished product units/set) collected from eight firms had aerobic plate counts (arithmetic averages) of fewer than 50,000/g, and 52% had 10,000 or fewer/g. Of the total of 209 finished product units, 57.4% were coliform-positive, but only 8.6% were *Escherichia coli*-positive and only one unit was *Staphylococcus aureus*-positive. All units were salmonellae-negative.

A survey was conducted to determine the bacterial levels of Kosher-style chopped chicken liver and Kosher chopped beef liver during preparation and as packaged for shipment from establishments under federal inspection in the United States.

In descending order of prominence, the product is a finely ground mixture of cooked livers, cooked onions, cooked eggs, cracker meal, vegetable oil or chicken fat, salt, and pepper. Two establishments (both producing chopped beef liver) did not add eggs to the product, two establishments added sugar (0.3% by weight), and one establishment did not add cracker meal. The Kosher chopped beef liver was prepared under rabbinical supervision. The product was either ladled manually or dispensed mechanically by a filling machine into 1- to 5-lb. plastic-lined cardboard containers, or into 4- to 8-oz. plastic containers.

Conditions of sanitation in the firms looked very good. All food contact surfaces were treated with a sanitizing agent after being cleaned, and hand-sanitizing solutions were used by employees. The cooked ingredients and finished product were chilled promptly. The firms seemed acutely aware that the product is very perishable.

MATERIALS AND METHODS

Sampling

From September 1974 to March 1976, samples were collected in eight establishments producing chopped liver. Four of the firms produced chopped chicken liver, three produced chopped beef liver, and one firm produced both products. The firms are located in the vicinity of New York City and represent most of the firms producing this product under federal inspection. Three of the firms froze the product and five refrigerated the product for shipment to outlets within 24 h.

A total of 211 production line samples and 209 containers of the finished product (units) were collected and analyzed. Each collection included samples of the ingredients, a set of two to 10 units (containers of the finished product) related to the production line samples, and when available, a set of two to 10 units produced the day before the plant visit. Groups of samples were collected from five of the firms on more than one date. The samples were frozen promptly and shipped under dry ice to the laboratory for analyses 3 to 4 weeks after collection.

Laboratory methods

Methods for aerobic plate counts (APC), coliforms, *Escherichia coli, Staphylococcus aureus,* and salmonellae have been described (*3*).

RESULTS AND DISCUSSION

Table 1 presents results of bacteriological examination of the finished chopped liver. Being a homogeneous product, the arithmetic average and geometric mean of the APC's of the units within a set were nearly the same.

A total of 95 samples of freshly cooked ingredients were collected in the eight firms. As expected, these samples were negative for coliforms and *S. aureus,* and had very low APCs. However, as seen in Table 1, the process of combining and packaging the cooked ingredients as a finished product usually resulted in some contamination despite the observed good sanitary conditions.

In Firms A and C, the cooked, chilled, ground ingredients increased in bacterial content after being combined as the finished product in a 200-lb. capacity horizontal tilt-type mixer. In both firms, the mixers had been washed and rinsed with a detergent and hot water, rinsed with a hypochlorite solution of more than 100 ppm Cl_2, then rinsed with potable water. However, because this type of mixer is not designed for routine disassembly, crevices at the junctions of the horizontal rotating shaft and the sides of the mixer, and at the junctions of the shaft and mixing blades, are not accessible for thorough cleaning. Firm C was one of only two firms where some *E. coli*-positive units were found and was the only firm that produced chopped liver with APC's greater than 100,000/g (Table 1).

A brief test was conducted at Firm C. Just before use,

the mixer was operated about 2 min while partially filled with potable water. Samples of the water before and after contact with the mixer were collected in sterile jars containing a few crystals of sodium thiosulfate to neutralize residual Cl_2. The samples were immersed in crushed ice and delivered to the laboratory for examination within five hours. The potable water was sterile in 0.1-ml portions, but the water from the mixer contained 1,000 coliforms/ml and had an APC of 200,000/ml. Because of these findings, the firm was advised to operate the cleaned mixer filled with a sanitizing agent for at least 5 min just before final rinsing and use. Unfortunately, samples to measure the effect of this treatment could not be collected because the firm discontinued manufacture of the product.

In Firm B, the cooked chilled ingredients increased in bacterial content after being ground and mixed in a chopper ("silent cutter"). Before use, the cleaned chopper bowl was filled with an I_2 solution of more than 100 ppm for 0.5 h, then operated while draining the I_2 solution and rinsed with potable water. It seems that the cutting blades of a chopper must be removed and disassembled for thorough cleaning and sanitization before using this equipment for a cooked product.

Firm D produced both chopped chicken liver and chopped beef liver. The chicken liver ingredients were cooked together and, while hot, ground and mixed in a chopper along with cooked, chilled onions and dry ice pellets (for rapid chilling). The beef liver ingredients were cooked together and, while hot, passed through a grinder directly into a horizontal tilt-type mixer for blending with cooked, chilled onions and dry ice pellets. The product increased in bacterial content after contact with the chopper or the mixer. Just before use, the cleaned equipment was rinsed with an I_2 solution of more than 100 ppm.

In Firm E, the freshly cooked ingredients, while hot, were mixed and ground and pumped directly through a column-type heat exchanger for rapid chilling. When contamination occurred, it was noted in the samples collected at the discharge of the heat exchanger. Firm E was the second of two firms where some *E. coli*-positive finished chopped liver units were found (Table 1). The heat exchanger was cleaned-in-place with recirculated detergent solution, rinsed, and partially disassembled for treatment with a solution of 100 ppm of a quaternary ammonium compound. However, the heat exchanger, with numerous interior scraper-blades, is difficult to clean and santize unless disassembled completely.

In Firm F, the freshly cooked ingredients, while hot, were ground with cooked, chilled onions; then mixed in a free-standing dough mixer. Before use, the cleaned, detached, one piece vertical mixing arm and smooth-walled portable mixing bowl were treated with a hypochlorite solution of more than 100 ppm Cl_2. The warm product was spread on chilled, shallow pans; covered with clean white paper; and placed in a freezer for rapid chilling before being packaged about 3 h later. Occasionally, slight contamination of the product resulted from passage through a mechanical cup-filling

TABLE 1. *Results of analyses of finished chopped liver units*

Firm	Liver	No. of units/set[a]	No. of units with[b]			Aerobic plate counts/g	
			Coliforms	*E. coli*	*S. aureus*	Arith. av.	Geom. mean
A	Chicken	10	10	0	0	90,000	87,000
		5	4	0	0	7,000	6,600
B	Chicken	5	5	0	0	4,000	4,000
		5	5	0	0	45,000	44,000
C	Chicken	10	10	7	0	820,000	820,000
		2	2	1	0	700,000	700,000
		10	3	0	0	350,000	350,000
		10	10	0	0	500,000	500,000
		2	2	0	0	65,000	65,000
D	Chicken	10	6	0	0	3,200	3,100
		5	2	0	0	2,400	2,200
		10	10	0	0	65,000	64,000
		10	10	0	0	14,000	10,000
G	Chicken	10	0	0	0	3,000	3,000
E	Beef	10	6	0	0	2,200	1,900
		4	0	0	0	1,700	1,600
		10	10	10	0	17,000	16,000
		10	0	0	0	11,000	11,000
		5	1	0	0	26,000	26,000
F	Beef	10	0	0	1	300	300
		2	0	0	0	23,000	23,000
		10	5	0	0	2,000	2,000
		10	4	0	0	4,000	4,000
D	Beef	10	1	0	0	3,300	3,200
		4	2	0	0	40,000	30,000
		10	10	0	0	10,000	9,000
H	Beef	10	2	0	0	2,000	2,000

[a]Each set represents a different day of production
[b]Every unit was salmonellae-negative in 25-g portions

machine.

In Firms G and H, the cooked chilled ingredients were ground and then mixed in a free-standing dough mixer. Before use, the cleaned detached mixing arm and portable mixing bowl were treated with solutions of either 100 ppm I_2 or 100 ppm of a quaternary ammonium compound. Very little contamination of the product was noted in these firms.

Table 2 shows the effect of processing equipment on the coliform content of chopped liver. Most of the coliform contamination resulted from the use of equipment hard to clean and sanitize. The samples of chopped liver prepared in the dough mixer (equipment easier to clean and sanitize) had a lower incidence and fewer numbers of coliforms.

TABLE 2. *Effect of processing equipment on the coliform content of finished chopped liver units*

Equipment	No. of units	No. of units with coliforms/g at 10^1	10^2	10^3	% of Units colif.-pos.
Chopper	45	16	13	9	84
Tilt-mixer	73	41	12	1	74
Column-exchanger	39	12	5	0	43
Dough-mixer	52	11	0	0	21

On three occasions, sets of chopped liver units delivered under crushed ice for examination within 24 h were reexamined after 3 weeks of frozen storage at -23 C. The results are presented in Table 3. In all, there appeared to be some reduction in viable coliforms during frozen storage but no significant differences in APCs.

On four occasions, sets of chopped liver units (two of chicken and one each of beef produced with and without eggs) were delivered under crushed ice on the day of manufacture and stored in the laboratory refrigerator at 2 C. At intervals, portions were examined bacteriologically and organoleptically. Figure 1 shows the rate of bacterial growth (APCs) in these products during refrigerated storage. The chopped chicken liver was the most perishable and developed an off-odor after 17 days of refrigerated storage. As expected, the chopped beef liver formulated without eggs was the least perishable, but developed a slightly sour odor after 21 days. There was little, if any, growth of coliforms during refrigerated storage, and all samples were negative for *S. aureus* and salmonellae at every examination. The dominant microorganisms isolated from the organoleptically unacceptable chopped liver were identified as pseu-

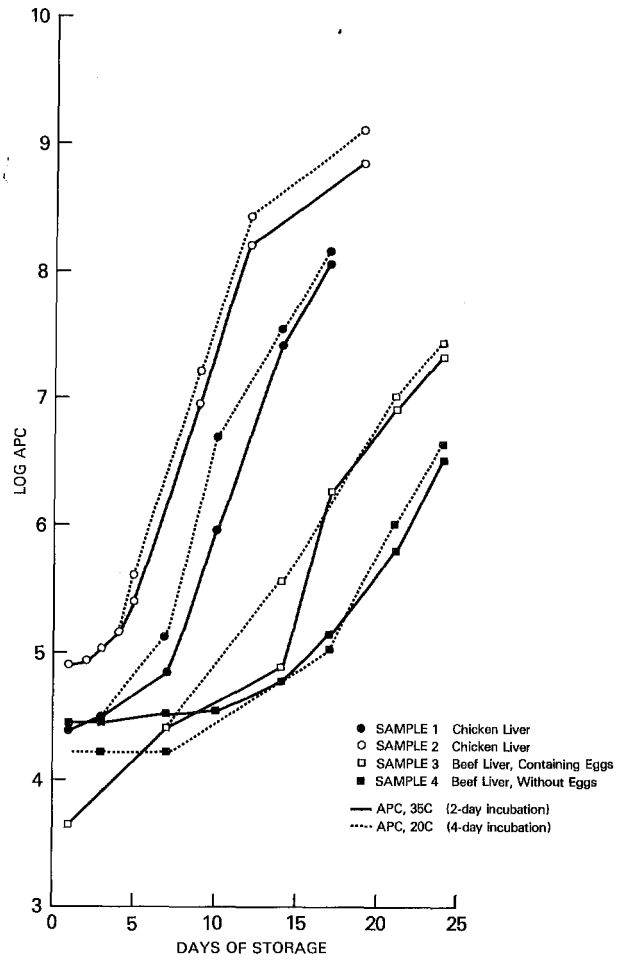

Figure 1. *Aerobic plate counts/g of finished chopped liver units during storage at 2 C.*

TABLE 3. *Results of analyses of finished chopped liver units before and after 3 weeks storage at -23 C*

Liver species	No. of units[a]		No. units with coliforms/g at $< 10^1$	10^1	10^2	10^3	Arith. av. of APCs/g
Chicken	5	Before freezing	0	0	3	2	45,000
		After storage	3	2	0	0	39,000
Chicken	5	Before freezing	0	0	2	3	3,900
		After storage	0	1	4	0	2,500
Beef	5	Before freezing	0	3	2	0	14,000
		After storage	0	4	1	0	13,000

[a]All units were negative for *E. coli, S. aureus,* and salmonellae at every examination.

domonads and enterococci.

This survey shows that at the time of manufacture and by the laboratory methods employed, 20 (74.1%) of the 27 sets of finished chopped liver units (2 to 10 units/set) had APCs (arithmetic average) of fewer than 50,000/g; and 14 sets (51.9%) had APCs of 10,000 or fewer/g. Of the 27 sets, 22 (81.5%) contained coliform-positive units. Of the 209 units, 120 (57.4%) were coliform-positive but only 18 (8.6%) were *E. coli*-positive and only one (0.5%) was *S. aureus* positive. All units were salmonellae-negative in 25-g portions.

We found only two articles in the literature referring to this type of product. Pace (*2*), during a surveillance of delicatessen foods, collected 12 samples of "liver spread" at a central production kitchen and found that the aerobic plate counts ranged from 100 to 100,000/g and found that four of the 12 samples contained 10 to 100 coliforms/g.

In 1968, consumption of chopped chicken liver packed in a glass jar and produced in a non-federally inspected plant resulted in a case of botulism (*1*). However, U.S.D.A. investigation at that time revealed evidence that the jar of chopped liver, though labeled "keep refrigerated," had been stored at room temperature by both the retailer and the purchaser. As a result of this case, the U.S.D.A. does not permit perishable pasteurized products to be packaged in hermatically sealed rigid containers (glass jars or cans) unless the product is pH 4.5 or lower.

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Production of *Bacillus cereus* Enterotoxin in Defined Media in Fermenter-Grown Cultures

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ABSTRACT

Enterotoxin, measured in capillary permeability factor (CPF) units, was produced by cultures of *Bacillus cereus* growing in defined media containing glucose, basal salts, and amino acids under controlled conditions in a small fermenter.

Bacillus cereus produces an enterotoxin whose biological activity may be measrued in rabbit ileal loop (*6*) and vascular permeability (*4*) assays, and in a guinea pig dermal necrotic assay (*2*). Brain heart infusion broth has been the growth medium of choice for production of large amounts of entertoxin by shake flask cultures, but we have recently reported production of high enterotoxin levels by cultures growing in shake flasks and under defined conditions in a fermenter, in a Casamino Acids-yeast extract medium (*3*). Formulation of defined growth media that support entertoxin synthesis by *B. cereus* would be very beneficial, both in determining the nutritional requirements for enterotoxin production, and in facilitating enterotoxin purification from the spent growth medium, This paper presents the compositions of such defined media.

MATERIALS AND METHODS

Bacterial strain

Strain B-4ac-L, a variant of our standard enterotoxin producing strain B-4ac, was used. Stock cultures were maintained on nutrient agar slants at room temperature. Working cultures were steaked weekly from stock cultures onto nutrient agar plates, incubated overnight at 32 C, and stored at 4 C.

Growth media

The semidefined medium, designated CAD, which supports enterotoxin synthesis, was adapted from the medium of Evans et al. (*1*) and was formulated as described previously (*3*). It contained (g/1): Vitamin-Free Casamino Acids (Difco), 20; yeast extract (Difco), 6; glucose, 10; NaCl, 2.5; K_2HPO_4, 8.71;$MgSO_4 \cdot 7H_2O$, 0.05; $MnCl_2$, 0.005; $FeCl_3 \cdot 6H_2O$, 0.005. The pH was adjusted to 8.0 before autoclaving.

All defined media (pH 7.0) contained glucose (10 g/1) and a basal salts mixture (BS) which was composed of (g/1): $(NH_4)_2SO_4$,2;K_2HPO_4, 14; KH_2PO_4, 6; sodium citrate $\cdot 2H_2O$, 1; $MgSO_4 \cdot 7H_2O$, 2. Amino acids and vitamins were added to defined media in the same concentrations as present in CAD medium (Table 1). These concentrations were determined using an amino acid analysis of Vitamin-Free Casamino Acids (*5*) and a partial analysis of yeast extract supplied by Difco. Amino acids (all L-forms), purines and pyrimidines, and phosphorocholine were obtained from Nutritional Biochemicals Corporation. Vitamins were obtained from Sigma Chemical Company. Inositol was a gift from Dr. J. C. Garver.

All components were sterilized by autoclaving at 121 C for 15 min except for tryptophan, vitamin B-12, folic acid, pyridoxine, inositol, phosphorocholine, and $FeCl_3 \cdot 6H_2O$, which were filter-sterilized. Defined media were prepared by aseptically mixing the appropriate sterile ingredients and were prewarmed overnight at 32 C before use. All solutions were prepared in glass-distilled water.

Growth conditions

Inocula were prepared by inoculating from a single colony from a working culture into 25 ml of the desired medium in a 125-ml Erlenmeyer flask and incubating overnight (10-15 h). For studies to determine nutritional requirements for growth, the overnight inoculum was washed once, resuspended, and if necessary, diluted in BS so that between 5×10^5 and 5×10^6 cells were inoculated per flask. Inocula for fermenter studies were grown by making a 1% transfer from the overnight inoculum into a second flask and incubating 2 h before inoculating the fermenter medium. All shake flask cultures were incubated at 32 C on a reciprocal shaker.

Fermentation control equipment used was described previously (*3*). Standard cultural conditions employed were temperature, 32 C; agitation rate, 400 rpm; air flow rate, 50 ml/min; culture volume, 300 ml; pH controlled at 7.0; inoculation level, 1-5%.

Enterotoxin assay

Harvesting of culture supernatant fluid and testing of the fluid for enterotoxin activity by the vascular permeability assay were done as previously described (*3*).

RESULTS

Since entertoxin production in complex media is associated with vigorously growing cultures (*4*), it was appropriate first to establish the minimum nutritional requirements for rapid growth (defined as the ability of a shake flask culture to increase from an OD_{560} of 0.01 to 1.0 within 6 h). The experiments to establish the influence of defined medium composition on culture growth rate were done using shake flask cultures.

Before examining the organic growth requirements,

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TABLE 1. *Amino acid and vitamin composition of CAD and defined media*

Nutrient	Concentration[1]	Present in CAD[2]	MIN[3]	MOD[4]	MAX[5]
	(*mg/l*)				
alanine	400	+			+
arginine	460	+	+	+	+
asparagine	500[6]				+
aspartic acid	910	+		+	+
cysteine	40	+	+	+	+
glutamic acid	2,000	+	+	+	+
glutamine	500[6]				+
glycine	390	+		+	+
histidine	360	+	+	+	+
isoleucine	700	+	+	+	+
leucine	1,370	+	+	+	+
lysine	1,180	+		+	+
methionine	400	+	+	+	+
phenylalanine	280	+	+	+	+
proline	1,420	+			+
serine	660	+	+	+	+
threonine	710	+	+	+	+
tryptophan	500[6]				+
tyrosine	42	+		+	+
valine	910	+	+	+	+
vitamin B-12	0.025	+			
biotin	0.024	+			
folic acid	0.12	+			
inositol	1.0[6]				
lipoic acid	0.25[6]				
nicotinic acid	2.4	+			
PABA	0.144	+			
Ca-pantothenate	0.6	+			
phosphorocholine	1.2	+			
pyridoxine	0.18	+			
riboflavin	0.3	+			
thiamine	0.6	+			

[1]Concentrations determined from the composition of Vitamin-Free Casamino Acids and of yeast extract.
[2]Semidefined medium which supports good growth (generation time $\leq$ 45 min) and good enterotoxin production (maximum activity obtained $\geq$ 400 capillary permeability factor units/ml (CPF/ml).
[3]Defined medium which supports good growth but poor enterotoxin production (maximum activity obtained = 20 CPF/ml).
[4]Defined medium which supports good growth and moderate enterotoxin production (maximum activity obtained = 140 CPF/ml).
[5]Defined medium which supports good growth and the highest enterotoxin production attained in defined media (maximum activity obtained = 400 CPF/ml).
[6]Not listed in analyses for casamino acids or yeast extract. Chosen concentration arbitrary.

we first substituted BS for the inorganic salts of CAD medium. This new formulation was equivalent to the old in the ability to support rapid growth and was used for defined media in all subsequent studies.

The amino acid composition of CAD medium was duplicated in a defined medium using a mixture of amino acids. This medium supported rapid growth equally as well as did CAD. Omission of any of the following 11 amino acids reduced the cell yield attained in 6 h: arginine, cysteine, glutamic acid, histidine, isoleucine, leucine, methionine, phenylalanine, serine, threonine, and valine. A defined medium containing glucose, BS, and these 11 amino acids was formulated and designated MIN (Table 1), the minimal medium to support rapid growth. Addition of vitamins did not improve either culture growth rate or cell yield.

No detectable enterotoxin was produced in shake flask cultures grown either in MIN or in defined medium containing all the amino acids (MAX; see Table 1) with or without vitamins. However, when cultures were grown in defined media under controlled conditions in a fermenter, detectable enterotoxin was synthesized in amounts dependent on the amino acid composition of the medium. About 20 times as much enterotoxin activity was detected in MAX-grown cultures as in MIN-grown cultures. The only amino acid additions to MIN capable of boosting enterotoxin synthesis were the combination of aspartic acid, glycine lysine and tryosine. Cultures grown in the medium with these added amino acids (MOD; see Table 1) produced six times more enterotoxin than did cultures grown in MIN. The vitamins listed in Table 1 and the purines and pyrimidines (adenine, cytosine, guanine, thymine, uracil, each at 10 mg/l) also were included in some defined media preparations but had no effect on enterotoxin synthesis.

Although the enterotoxin activity measured in cultures grown in MAX was in the same range as the acitivity measured in CAD-grown cultures, it was not difficult to obtain two to three times higher levels in the richer medium. Addition of the trace salts used in CAD, increase of the sodium chloride concentration to the level found in CAD, and control of culture pH at different values did not further increase enterotoxin synthesis in defined media.

DISCUSSION

We have obtained enterotoxin synthesis by *B. cereus* in defined media under controlled cultural conditions in a fermenter. The observation that higher levels of enterotoxin activity were detected as more amino acids were added to the growth medium may have several explanations. Presence of added nutrients may relieve cells of the need to synthesize essential constituents and thus free the cell's protein synthesizing apparatus to make secondary metabolites not essential for normal cell growth, e. g. enterotoxin. Increased nutrient concentration might relieve the toxicity of medium components; for example, by complexing heavy metal ions. Alternatively, high concentrations of organic compounds in the growth medium may help to stabilize toxic activity, as was found by Wu and Bergdoll with staphylococcal enterotoxin B (*7*). In the present case, organic nutrients might protect the toxin against action of concurrently secreted proteases. The inter-action of two or more organic constituents in CAD medium, or presence of an unidentified ingredients of yeast extract could also be significant.

The fact that enterotoxin was produced in defined media containing few ingredients beyond those required for rapid growth indicates that the nutritional requirements for enterotoxin synthesis are relatively simple. Probably *B. cereus* could produce enterotoxin in any food product in which it could grow well, if possible unknown toxin synthesis inhibitors were absent.

The value of the ability to control cultural conditions in the fermenter is underscored by the fact that cultures growing in defined media could produce enterotoxin only in the fermenter and not in shake flasks. It is not unreasonable to assume that optimization of fermenter conditions will greatly increase enterotoxin yield in defined media.

Production of enterotoxin in media containing no complex organic constituents not only has defined the minimum nutritional requirements for toxin synthesis but also has simplified efforts to purify the enterotoxin molecule.

ACKNOWLEDGMENTS

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Lactic Acid Production by *Streptococcus thermophilus* and *Lactobacillus bulgaricus* in Milk Precultured with Psychrotrophic Bacteria

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ABSTRACT

Raw milk was incubated at 7 C for 5 days after it was inoculated with psychrotophic bacteria previously isolated from other raw milks. Then a portion of each sample of milk was pasteurized; the remainder was autoclaved. *Streptococcus thermophilus* and *Lactobacillus bulgaricus* were individually inoculated into all pasteurized and autoclaved milks which were then incubated at 37 C and titratable acidity was measured at 2-h intervals. Pasteurized milks precultured with psychrotrophic bacteria supported more acid production than did pasteurized control milks. *S. thermophilus* and especially *L. bulgaricus* produced more acid in autoclaved than in pasteurized milks. Cell-free filtrates from cultures of psychrotrophic bacteria were added to raw, pasteurized, and autoclaved milks before they were inoculated with the lactic acid bacteria. Results were similar to those obtained when psychrotrophic bacteria were added to milk. When the cell-free filtrate was added to milks simultaneously with starter cultures, less than 10 h were required to produce conditions in milk favorable for enhanced lactic acid production.

Previously Cousin and Marth (*1*) reported that acid production by *Streptococcus lactis* and *Streptococcus cremoris* was enhanced in milk that had supported growth of psychrotrophic bacteria or that contained their metabolic products. This paper considers acid production by *Streptococcus thermophilus* (2 strains) and *Lactobacillus bulgaricus* in milk precultured with psychrotrophic bacteria or in milk that contained their exocellular metabolic products.

MATERIALS AND METHODS

Cultures

Psychrotrophs used in this study were isolated and characterized as reported previously (*1*). The psychrotrophs selected for the research were: *Flavobacterium* sp. (No. 26), *Lactobacillus* spp. (No. 29 and 34), *Micrococcus* sp. (No. 32), and *Pseudomonas* spp. (No. 1, 10, 13, 31, 36). *S. thermophilus* (ST and ST4) and *L. bulgaricus* (LB2) were obtained from Marschall Division of Miles Laboratories, Madison, Wisconsin.

Milk samples and cell-free filtrates

Methodology used in these experiments was the same as previously reported (*1*) except that milks were incubated at 37 C with acid production measured at 2-h intervals until milks were coagulated.

RESULTS

Plate counts

The initial psychrotrophic counts for inoculated milks for use with *S. thermophilus* (ST and ST4) ranged from 3.4×10^3 to 6.4×10^6/ml; those for use with *L. bulgaricus* ranged from 1.0×10^4 to 6.3×10^6/ml. After 5 days at 7 C counts ranged from 5.5×10^6 to 9.5×10^7/ml for milks to be inoculated with *S. thermophilus* (ST and ST4) and from 5.0×10^6 to 2.5×10^8/ml for those to be inoculated with *L. bulgaricus*. Uninoculated milks had initial counts in the range of 7.5×10^2 to 2.9×10^4/ml which increased to 1.1×10^6/ to 1.9×10^8/ml after 5 days incubation at 7 C. Plate counts were of the same magnitude as psychrotroph counts (data not given).

Experiments with psychrotrophic bacteria

Milks which were precultured with psychrotrophs (Fig. 1 and 2) were markedly superior to control milks as substrates for acid production by *S. thermophilus* (ST). All psychrotrophs studied gave results similar to those observed for control and inoculated milks when *Lactobacillus* sp. (No. 34) was evaluated (Fig. 2) except *Pseudomonas* sp. (No. 10) which acted as did *Flavobacterium* sp. (No. 26) (Fig. 1). All values in Fig. 1 and 2 and in other figures are averages of four replications.

Pasteurized milks which previously had supported growth of the normal flora and those which supported simultaneous growth of *S. thermophilus* (ST) plus the psychrotrophs gave results similar to those of pasteurized control milks (Fig. 1 and 2). In only one instance (Fig. 2) did pasteurized milk which supported growth of the normal flora have more acid produced by *S. thermophilus* (ST) than did milks in which the psychrotrophs and starter culture grew concurrently. Autoclaved milks were always most favorable for acid production by *S. thermophilus*.

Results obtained with *S. thermophilus* (ST4) in milks precultured with psychrotrophs were similar to those observed with strain ST (Fig. 3). In this instance acid production in milks with the normal flora was similar

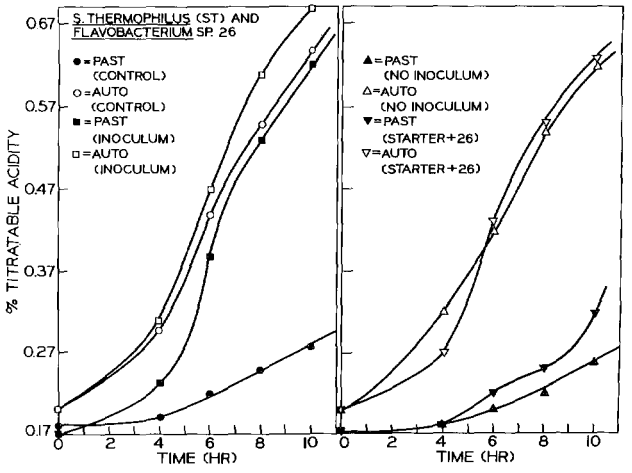

Figure 1. *Lactic acid production by* S. thermophilus *(ST) in milks precultured with* Flavobacterium *sp. 26. All samples were from the same lot of milk. Split samples were used for autoclaved and pasteurized milks. Key: Past (control): Raw milk was pasteurized, held at 7 C for 5 days, and inoculated with lactic culture; Auto (control): Raw milk was autoclaved, held at 7 C for 5 days, and inoculated with lactic culture; Past (Inoculum): Raw milk was inoculated with psychrotroph, held at 7 C for 5 days, pasteurized, and inoculated with lactic culture; Auto (Inoculum): Raw milk was inoculated with psychrotroph, held at 7 C for 5 days, autoclaved, and inoculated with lactic culture; Past (No Inoculum): Raw milk was held at 7 C for 5 days, pasteurized, and inoculated with lactic culture; Auto (No Inoculum): Raw milk was held at 7 C for 5 days, autoclaved, and inoculated with lactic culture; Past (Starter + 26): Raw milk was pasteurized, held at 7 C for 5 days, and inoculated simultaneously with psychrotroph and lactic culture; Auto (Starter + 26): Raw milk was autoclaved, held at 7 C for 5 days, and inoculated simultaneously with psychrotroph and lactic culture.*

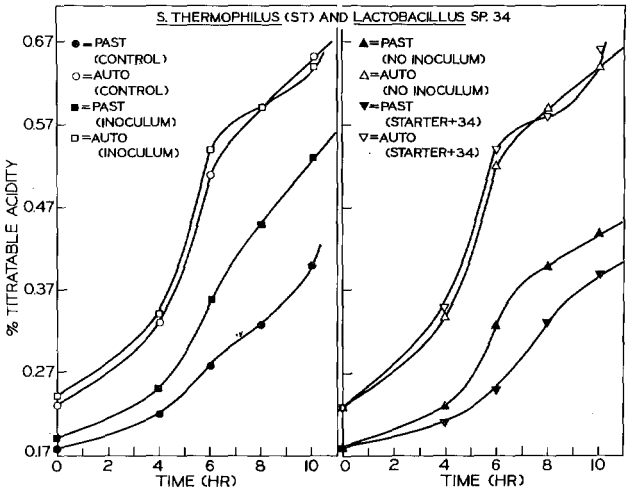

Figure 2. *Lactic acid production by* S. thermophilus *(ST) in milks precultured with* Lactobacillus *sp. 34. See Fig. 1 for key to treatments.*

to that obtained in milks precultured with psychrotrophs. *Micrococcus* sp. (No. 32) and *Pseudomonas* sp. (No. 36) acted as did *Flavobacterium* sp. (No. 26) in that the pasteurized inoculated milk yielded more acid than did the autoclaved control milk (Fig. 3); all other psychrotrophs studied acted as did *Pseudomonas* sp. (No. 13) where autoclaved milks developed more acid than did pasteurized milks (data not given).

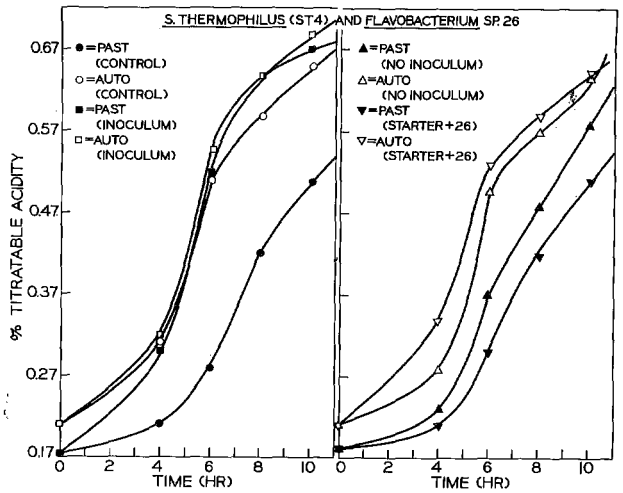

Figure 3. *Lactice acid production by* S. thermophilus *(ST4) in milks precultured with* Flavobacterium *sp. 26. See Fig. 1 for key to treatments.*

Autoclaved milks were superior to pasteurized milks as media for lactic acid production by *L. bulgaricus* (LB2). Pasteurized milks which had been inoculated with a psychrotroph or where the normal flora grew (Fig. 4) yielded more titratable acid than did controls. Milk which supported concurrent growth of *L. bulgaricus* and psychrotrophs generally was simialr to control milk in lactic acid production (Fig. 4 and 5).

To further demonstrate that differences existed in results obtained when milks were or were not precultured with psychrotrophic bacteria, statistical analysis using Duncan's New Multiple Range Test (*9*) (P at 0.05) was done on data obtained when milks inoculated with the starter cultures were incubated more than 8 h. Significant differences occurred when *S. thermophilus* (ST and ST4) grew in milk that had supported growth of *Flavobacterium* sp. No. 26 (Fig. 1 and 3) and when *L. bulgaricus* grew in milk that had supported growth of *Pseudomonas* sp. No. 10 (Fig. 4).

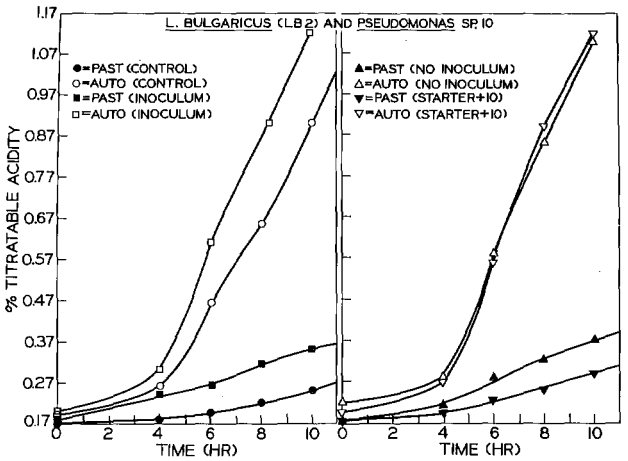

Figure 4. *Lactic acid production by* L. bulgaricus *in milks precultured with* Pseudomonas *sp. 10. See Fig. 1 for key to treatments.*

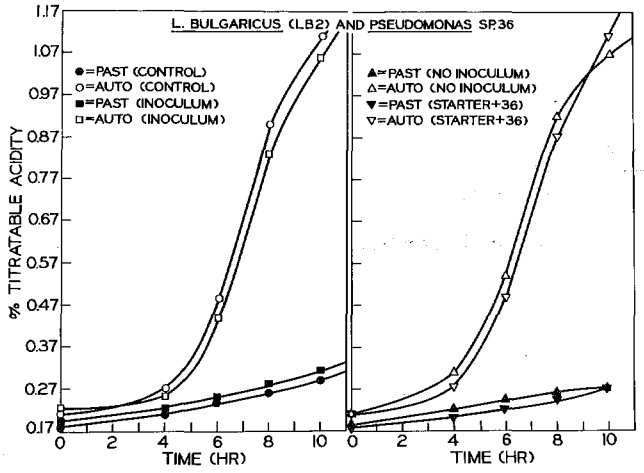

Figure 5. *Lactic acid production by* L. bulgaricus *in milks precultured with* Pseudomonas *sp. 36. See Fig. 1 for key to treatments.*

Experiments with cell-free filtrates

As reported previously (1), the adding to milk of cell-free filtrates from cultures of psychrotrophic bacteria allowed for acid production by *S. lactis* and *S. cremoris* which was similar to that obtained when the psychrotrophic bacteria themselves were added. When similar cell-free filtrates were added to milk together with *S. thermophilus* or *L. bulgaricus*, acid production was the same as in milks which had the cell-free filtrate added five days earlier (Tables 1,2,3). All treated pasteurized samples gave results that were significantly different from those obtained with pasteurized control milks after 8 h of incubation as determined by Duncan's New Multiple Range Test (9).

DISCUSSION

Milks precultured with psychrotrophic bacteria were better substrates than uncultured milks for lactic acid production by *S. thermophilus* and *L. bulgaricus* probably because the psychrotrophs through proteolysis increased amounts of usable nitrogenous compounds available to the lacic acid bacteria. Knaut (*7*) reported that some *Pseudomonas* spp. increased the nonprotein nitrogen (NPN) content of milk 15 times over that present in uninoculated milk. Other investigators (*2,6*) examined casein after psychrotrophic growth occurred in milk and found a decrease in β-and a_S-casein fractions and an increase in fractions of smaller-sized proteinaceous materials.

Autoclaved milks were good growth media for lactic acid bacteria used in these tests. There are reports (*3,4,5*) that lactic acid bacteria grew better in autoclaved than in

TABLE 1. *Acid production by* Streptococcus thermophilus *(ST4) in milks precultured with cell-free filtrates*

Treatment of milk	Titratable acid (%) after incubation				
	0 h	4 h	6 h	8 h	10 h
Pasteurized					
Control	0.17	0.19	0.23	0.27	0.33
Filtrate 26[1]	0.18	0.36	0.60	0.67	0.75
Filtrate 26 + *S. thermophilus*[3]	0.18	0.21	0.46	0.64	0.77
Filtrate 34[2]	0.18	0.30	0.57	0.68	0.74
Filtrate 34 + *S. thermophilus*[3]	0.18	0.28	0.52	0.66	0.73
Autoclaved					
Control	0.18	0.28	0.41	0.50	0.61
Filtrate 26[1]	0.20	0.33	0.58	0.70	0.80
Filtrate 26 + *S. thermophilus*[3]	0.19	0.30	0.58	0.73	0.78
Filtrate 34[2]	0.21	0.33	0.61	0.77	0.79
Filtrate 34 + *S. thermophilus*[3]	0.19	0.32	0.60	0.73	0.76

[1]*Flavobacterium* sp. (No. 26)
[2]*Lactobacillus* sp. (No. 34)
[3]Simultaneous inoculation

TABLE 2. *Acid production by* Streptococcus thermophilus *(ST4) in milks precultured with cell-free filtrates*

Treatment of milk	Titratable acid (%) after incubation				
	0 h	4 h	6 h	8 h	10 h
Pasteurized					
Control	0.16	0.20	0.25	0.34	0.41
Filtrate 13[1]	0.16	0.20	0.45	0.71	0.86
Filtrate 13 + *S. thermophilus*[3]	0.15	0.20	0.46	0.67	0.74
Filtrate 26[2]	0.17	0.25	0.59	0.74	0.76
Filtrate 26 + *S. thermophilus*	0.17	0.24	0.56	0.70	0.77
Autoclaved					
Control	0.17	0.28	0.44	0.72	0.85
Filtrate 13[1]	0.18	0.25	0.55	0.71	0.77
Filtrate 13 + *S. thermophilus*[3]	0.17	0.25	0.53	0.69	0.77
Filtrate 26[2]	0.19	0.26	0.54	0.72	0.73
Filtrate 26 + *S. thermophilus*[3]	0.19	0.28	0.61	0.73	0.77

[1]*Pseudomonas* sp. (No. 13)
[2]*Flavobacterium* sp. (No. 26)
[3]Simultaneous inoculation

TABLE 3. *Acid production by* Lactobacillus bulgaricus *(LB2) in milks precultured with cell-free filtrates*

Treatment of milk	Titratable acid (%) after incubation				
	0 h	4 h	6 h	8 h	10 h
Pasteurized					
Control	0.15	0.18	0.20	0.22	0.28
Filtrate 10[1]	0.15	0.19	0.25	0.32	0.41
Filtrate 10 + *L. bulgaricus*[3]	0.15	0.19	0.22	0.30	0.36
Filtrate 36[2]	0.16	0.19	0.27	0.36	0.43
Filtrate 36 + *L. bulgaricus*[3]	0.16	0.19	0.26	0.30	0.38
Autoclaved					
Control	0.17	0.24	0.47	0.84	1.03
Filtrate 10[1]	0.18	0.25	0.65	0.98	1.21
Filtrate 10 + *L. bulgaricus*[3]	0.17	0.24	0.57	0.93	1.09
Filtrate 36[2]	0.18	0.24	0.56	0.95	1.20
Filtrate 36 + *L. bulgaricus*[3]	0.18	0.24	0.55	0.91	1.09

[1]*Pseudomonas* sp. (No. 10)
[2]*Pseudomonas* sp. (No. 36)
[3]Simultaneous inoculation

unheated milks probably because the heat treatment caused an increase in the supply of readily available nitrogenous compounds. Thadon and Ganguli (*10*) noted that sterilization of milk at 121 C for 15 min caused an increase in the NPN fraction.

Nearly always when *S. thermophilus* (ST) and *L. bulgaricus* (LB2) were studied, milk with growth of the normal flora allowed acid production similar to that of the control. This suggests that the normal flora either produced substances that were somewhat inhibitory to subsequent acid production by these lactic bacteria or that the flora was not sufficiently proteolytic to provide favorable conditions for increased acid production by the lactic cultures. When *S. thermophilus* (ST4) was evaluated, acid production in milks where the normal flora grew was similar to that observed in precultured milk (Fig. 3). This could have been caused by either strain variation or possible proteolytic action of psychrotrophs which constituted the normal flora.

Milks in which the starter culture plus psychrotrophs grew concurrently gave results similar to those of controls undoubtedly because most of these psychrotrophs did not grow well at 37 C. Those that did grow at 37 C did not grow very rapidly since this temperature is beyond the optimum for their growth. Optimum temperatures for growth of most psychrotrophs are 20 to 30 C, with some able to grow between 30 and 40 C, and only a few below 15 C (*11*).

Since experiments with cell-free filtrates gave results similar to those in which psychrotrophic bacteria themselves were used, it seems likely that proteolytic enzymes were produced by the psychrotrophs and excreted into the medium. These enzymes probably degraded the casein so that nitrogenous compounds became available for use by the lactic starter cultures. When psychrotrophs and lactic cultures were simultaneously grown in milk at 37 C, acid production was similar to that in controls. But when the cell-free filtrate was added simultaneously with the lactic culture and milk was incubated at 37 C, acid production was similar to that in milk pretreated with the cell-free filtrate for 5 days. This suggests that even though the bacteria may not grow, enzymes can act at this high temperature. Peterson and Gunderson (*8*) reported that acitvity of both endo- and exocellular enzymes increased when the temperature was increased beyond that at which psychrotrophic *Pseudomonas fluorescens* could grow. The greatest proteolytic activity was attained within 8 h at 37 C.

These data suggest that milks precultured with psychrotrophic bacteria or pretreated with their cell-free filtrate were better media for acid production by *S. thermophilus* (ST and ST4) and *L. bulgaricus* (LB2) than were control milks. Autoclaved milks were superior to pasteurized milks for lactic acid production by these cultures. The amount of acid produced depended on the kind of psychrotrophic bacteria used, heat treatment given milk, and lactic culture inoculated into milk.

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Influence of Pasteurization Before and After Separation of Cream on the Oxidative Stability of Ripened Cream Butter

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ABSTRACT

Ripened cream butter manufactured from cream separated from pasteurized milk (80 C for 15 sec) was more susceptible to oxidative changes than the corresponding butter manufactured from cream which was separated from milk at 50 C and the cream subsequently pasteurised (80 C for 15 sec). The propensity to oxidation is related to changes in copper distribution between the serum and fat globule phases and also to the relative ratio of serum to fat at the time of pasteurization.

The thermal treatment of cream for buttermaking differs in different regions of the world. On the European continent, milk is preheated for separation and the cream is then pasteurized and ripened for manufacture of butter. Most manufacturers of sweet cream butter in Ireland pasteurize milk at 80 C for 15 sec and then cool by regeneration for separation. In some instances cream is repasteurized at temperatures ranging from 80 to 95 C. In recent years the manufacture in Ireland of ripened cream butter for export has increased. The susceptibility of ripened cream butter to oxidative deterioration is well established.

In view of the studies by Samuelsson (*12*) and the more recent studies in this laboratory (*3*) on the changes in copper distribution between the serum and cream phases during heat treatment and the influence of the latter on oxidative changes in cream, it was decided to study the influence on lipid oxidation of pasteurization pre and post separation of cream for the manufacture of ripened cream butter.

MATERIALS AND METHODS

Cream treatment

Creams containing 35% fat for manufacture into ripened cream butter were obtained from bulk milk. The following treatments, designated A and B, were applied to the milk.

A. Part of the milk was pasteurized at 80 C for 15 sec in a plate heat exchanger and separated after regenerative cooling to 50 C. The cream was then cooled to 10 C in the cooling section of the plant.

B. The remainder of the milk was heated to 50 C for separation. The cream was pasteurized at 80 C for 15 sec and then cooled to 10 C in the cooling section of the plant.

Butter samples

Both creams were ripened with a lactic culture to a pH of 4.6 and were not neutralized before churning. After ripening they were cooled to 10 C, held overnight, and individually churned. The moisture and salt contents were controlled to 16.0 and 0.8%, respectively. After working, butters were printed into 1-lb rectilinear prints, wrapped in parchment, and placed in a store at −20 C. Three replications of the experiment were made.

Peroxide values of the samples were determined at 1 and 8 weeks after 0, 12, 24, and 36 h in a display cabinet at 5 C at a light intensity of 50 lumens/ft^2. Peroxide analyses were also made after 4, 6, 8, and 9 months storage at −20 C and after holding these samples for one week at 10 C.

Peroxide test

Peroxide analyses were made by the ferric thiocyanate method of Loftus Hills and Thiel (*10*) as modified by Holloway (*5*). Quantities of 0.5 ml or 0.1 ml of fat were used depending on the expected magnitude of the peroxide values which are expressed as m equiv of oxygen per kg of fat.

Determination of copper

A modification of the method of Mertens et al. (*11*) was used. A 50-g portion of butter was melted at 43-45 C in a 250-ml beaker. After gravity separation, the fat layer was decanted. Traces of curd remaining in the fat were washed free of fat with petroleum ether and the curd added to the serum. The serum was dried in an oven at 102 C and the residue ashed in a muffle furnace at 550 to 600 C for 10 to 12 h until a white colored ash was obtained. When cooled, the ash was dissolved in 15 ml of a nitric acid and 10 ml of perchloric acid. The acids were then boiled off for 20 min (using a glass reflux) until about 1 to 2 ml remained. After cooling, distilled water was added to give a final volume of 20 ml.

The copper concentration of this solution was determined with a Perkin-Elmer Atomic Absorption Spectrometer using the following instrument settings: wavelength, 3257A°; oxidant, air at 45 psig pressure and a flow rate of 26.5 1/min; fuel, acetylene gas at 8 psig pressure and a flow rate of 3.5 1/min; lamp current, 8 ma. Readings were made against a reagent blank of nitric acid and perchloric acid carried through the same procedure i.e. evaporating 1-2 ml by boiling and subsequent dilution with distilled water.

Glassware

All glassware, after washing with hot detergent solution, was steeped for 24 h in dilute nitric acid and then thoroughly rinsed with deionized water and dried.

RESULTS

When milk was heated to 50 C for spearation and the cream subsequently pasteurized at 80 C × 15 sec the cream had considerably lower peroxide values after holding under light than cream separated at 50 C from milk which had been previously pasteurized at 80 C (Fig. 1).

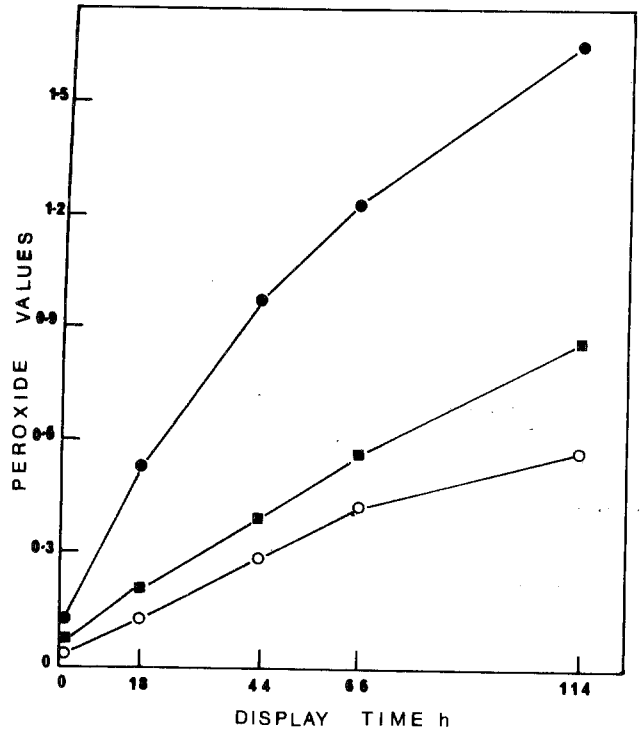

Figure 1. *The effect of pasteurization before and after separation on the peroxide values of cream before and after display of the cream under fluorescent light.* O—O *Control; milk preheated to 50 C, separated cream cooled to 5 C.* •—• *Milk pasteurized at 80 C for 15 sec, separated at 50 C, cream cooled at 5 C.* ■—■ *Milk preheated to 50 C, separated, cream pasteurized at 80 C for 15 sec, cooled to 5 C.*

An unpasteurized control sample separated from milk at 50 C gave lower peroxide values than either of the heat treated samples. The control sample, rather than becoming oxidized tended towards hydrolytic rancidity.

Figure 2 shows that butter B made from the cream which had received treatment B had lower peroxide values after 1 week at -20 C and after display under fluorescent light for up to 36 h than butter A made from the cream subjected to treatment A.

After 2 months at -20 C the peroxide values had increased considerably. (Fig. 3).

Butter B continued to have lower peroxide values before and after exposure to fluorescent light than butter A. At this stage, butter A had a peroxide value over 1 before display which is about the value at which an oxidized flavor becomes noticeable in sweet cream butter (*4*). The same definite trend was evident in the two other replications of the experiment.

Table 1 shows the peroxide values of butters A and B

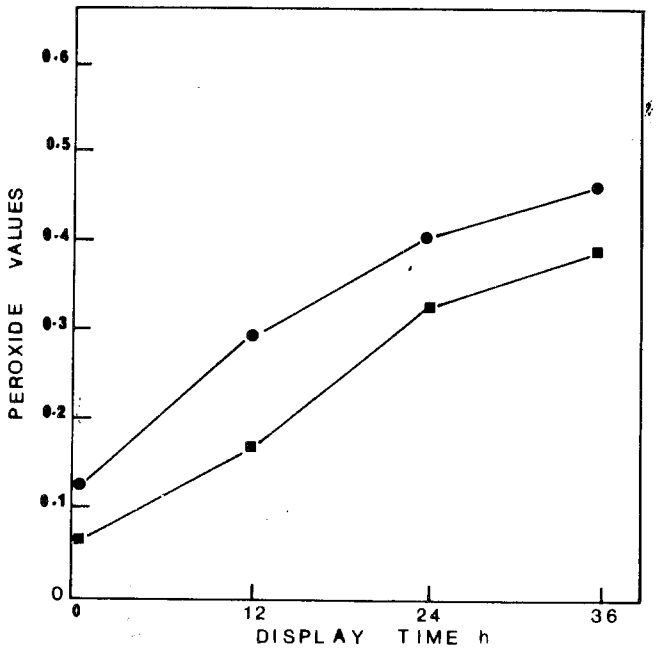

Figure 2. *The effect of pasteurization before and after separation on the peroxide values of ripened cream butter after 1 week of -20 C and after display under fluorescent light for up to 36 h.* •—• *Butter A-milk pasteurized at 80 C for 15 sec, separated at 50 C, cream ripened, cooled, and churned.* ■—■ *Butter B-milk preheated to 50 C, separated, cream pasteurized at 80 C x 15 sec, ripened, cooled, and churned.*

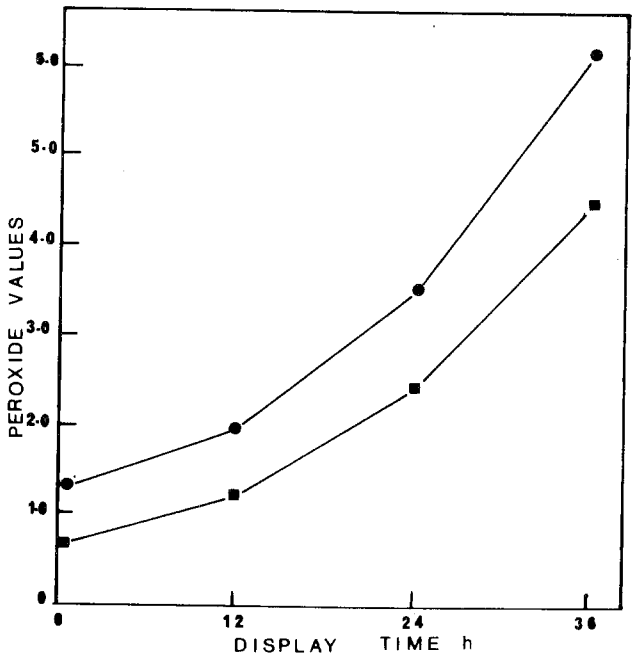

Figure 3. *The effect of pasteurization before and after separation on theperoxide values of ripened cream butter after 2 months at -20 C before and after display under fluorescent light for up to 36 h.* •—• *Butter A-Milk pasteurized at 80 C for 15 sec, separated at 50 C, cream ripened, cooled, and churned.* ■—■ *Butter B-Milk preheated to 50 C, separated, cream pasteurized at 80 C for 15 sec ripened, cooled, and churned.*

after cold storage at -20 C for 4,6,8 and 9 months and after holding these samples for 14 days at 10 C. The greater pro-oxidant tendencies of butter A is evident in all comparisons.

TABLE 1. *Peroxide values of butters A and B after extended storage at -20 C and after holding the stored butters at 10 C for 2 weeks*

Storage time (months)	Held at -20 C		Held at -20 C and 2 weeks at 10 C	
	(A)	(B)	(A)	(B)
4	1.04	0.70	1.37	1.13
6	1.20	0.94	1.41.	1.12
8	3.37	1.05	3.52	1.95
9	4.80	2.00	6.92	3.62

Table 2 gives the copper concentrations of butters A and B for the three replications of the experiment.

TABLE 2. *Copper concentrations (ppm) of ripened butters manufactured from differently treated creams (A and B)*

Trial	Copper concentration (ppm)	
	Butter A	Butter B
1	0.068	0.035
2	0.063	0.047
3	0.060	0.048

Concentrations of copper in series B are distinctly lower than those in series A. Evidently, the different cream treatments influenced the copper concentrations of the respective butters which in turn influenced their oxidative stability.

DISCUSSION

Ripened cream butter is manufactured in Ireland mainly for export to Germany and to certain areas of the United Kingdom where there is a preference for the ripened cream variety. It is more susceptible to flavor deterioration during storage than unripened butter. The level of copper is a critical factor in oxidative degradation of butterfat and the catalytic effect of copper is far greater in ripened than is sweet cream. Koops (*8*) showed that by lowering the pH of cream to 4.6 about 30 to 40% of added copper migrated from plasma proteins to the fat globule membrane.

He also showed that the phospholipid fraction, cephalin in particular, oxidized more rapidly when the pH was lowered. Oxidation of fatty acids occurred as oxidation proceeded (*9*). The serum of ripened cream butter, which is not washed during manufacture, is rich in phospholipids.

Factors other than processing may influence the level of copper in cream. Thus the copper content of milk varies with the stage of lactation (*6*). Cooling fresh milk to 6 C for 2 h reduces the copper content of cream (*2*). The milk used for this study was in the main held in refrigerated stainless steel bulk tanks on farms before delivery to the creamery and was unlikely to contain much contaminating copper. Both natural and contaminating copper have a catalytic effect on off-flavor development during cold storage of butter (*8*).

Foley et al. (*3*) noted that the oxidative stability of cream decreased progressively as the temperature treatment was increased from 60 to 95 C. They showed that the pro-oxidant influence was associated with migration of copper from the cream serum to the fat globule phase when cream was heated.

According to King et al. (*7*), Samuelsson (*12*), and Aulakh and Stine (*1*), the greater portion of copper in milk is in the serum phase and associated with the milk protein fraction. It is likely therefore that the ratio of serum to fat in a system would influence the extent of copper migration during heat treatment. Samuelsson (*12*) when studying the effect of heat treatment on distribution of added labelled copper, found that the serum to fat ratio influenced the amount of copper which migrated from the serum to the fat globules. Van Duin and Brons (*13*) showed that pasteurization of milk before centrifugation gave a relatively high copper content in the resultant cream.

When the ratio of serum to fat is high, as in milk, more copper associates with the fat during the heat treatment than happens when the ratio is lower in a product like cream. Thus the concentration of copper in butter A obtained from pasteurized milk (80 C) is higher than in butter B, the cream for which was pasteurized (80 C) post separation, when the serum to fat ratio was reduced from about 24:1 to approximately 2:1. The increased susceptibility of cream A to photocatalyzed oxidation is almost certainly due to its higher copper content (*3*). Likewise, butters made after treatment A had higher peroxide values after holding at -20 C and after exposure to light than butters from cream subjected to treatment B. The higher levels of copper in butters A explains their lower oxidative stability.

The study clearly shows that the practice of pasteurizing milk, before separation of cream, is not conducive to oxidative stability of ripened cream butter. Separation of milk preheated to 40 to 50 C and subsequent pasteurization of cream gives better results.

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A Research Note

Organisms From Positive MPN Tubes Inoculated With Samples That Yielded No Growth on Pour Plates

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ABSTRACT

Twenty samples of cold ready-to-eat cereal were analyzed for total organisms using the pour plate and Most Probable Number technique (MPN). Nine samples enhibited growth in MPN tubes but not on pour plates. Of the 28 isolates obtained from the MPN tubes, 16 were members of the genus *Bacillus* and the remainder were predominately other gram-positive organisms. The data support the working hypothesis that aside from the mathematical bias associated with the MPN procedure, microbial recovery is more favorable in a liquid environment than in an agar medium.

In previous reports (*5,6*) when comparing the MPN method to the pour plate method for recovery of total organisms it had been observed that some samples exhibited growth in MPN tubes but not on pour plates. This fact was observed a sufficient number of times in our laboratory, particularly with dry cereals, to warrant further investigation. It was hoped that identification of the organisms would lead to a possible explanation of this phenomenon.

MATERIALS AND METHODS

Twenty samples of ready-to-eat cold cereal were obtained from retail stores in the Gainsville, Florida area. Plate Count agar and Plate Count broth were made from the separate ingredients and sterilized.

Serial 1:10 dilutions of the samples were made and duplicate pour plates of each dilution were prepared (*1*). A three-tube MPN series was also prepared at each dilution (*7*) and 1-ml aliquots of each dilution were used to inoculate both plates and MPN tubes. Incubation was at 20 C for 5 days.

Those samples that did not exhibit growth in any of the pour plates, but did show growth in broth were selected for further study. The growth-positive tubes were streaked onto duplicate plates of Plate Count agar, with one plate incubated aerobically and the other anaerobically in a GasPak® jar.

Representative colonies from the aerobic plates were transferred to Plate Count agar slants, whereas colonies from the anerobic plates were picked into thioglycollate broth.

Identification of the isolates was made according to the descriptions in *Identification Methods for Microbiologists* (*4*) and the 8th edition of *Bergey's Manual of Determinative Bacteriology* (*2*).

[1] *Florida Agricultural Experiment Stations Journal Series No. 266.*

RESULTS AND DISCUSSION

Of the 20 samples analyzed, nine samples showed growth in the broth but not on the pour plates (Table 1).

Recognizing the positive bias associated with the MPN procedure due to sample size and probability, one might expect no growth on plates but growth within tubes approximately 40% of the time. These factors might explain the tabular values of 3.6 but not the higher estimates obtained with some samples. However, our purpose was not to question the statistical determinations but rather to explain the differences in recovery from a practical standpoint. Two samples exhibited growth only under anaerobic conditions (C-4 and I-5) with only one of the two isolates, a strict anaerobe. Of the 28 isolates, 16 were members of the genus *Bacillus* with the remainder also being predominately gram-positive organisms. *Acinetobacter calcoaceticus* was isolated from one sample and it was recovered from three of the five growth-positive tubes for that sample, indicating a level of about 15 organisms per gram. Yeasts were isolated from two samples (A-2 and G-10) and were the only fungi found.

That the majority of the organisms were gram-positive is not surprising, in that they are known to be more resistant to adverse conditions such as heat and desiccation. It might be assumed that conditions for outgrowth were more favorable in a liquid environment than in a gel. This may be related to medium components, oxygen tension, formation of toxic substances between the agar and other medium constituents, etc. Additionally, conditions in a liquid environment may possibly be more favorable for germination due to the constant bathing of the spore in fresh medium during the early phases of outgrowth.

While it is not possible to establish exactly why recovery by the MPN procedure was better as opposed to direct plating, the data do point out the need for a better understanding of the recovery of microorganisms from foods and the particular needs of such organisms during

TABLE 1. *Identification of organisms recovered from growth-positive MPN tubes*

Sample	Dilution of sample 10^{-1}	10^{-2}	10^{-3}	MPN/g	Isolates
A-2	0[1]	2	0	6.2	Yeast, *Nocardia convoluta*
A-3	3	2	1	150	*Bacillus megaterium, B. subtilis*
C-4	1	0	0	3.6	*Clostridium fallax*
D-7	1	0	0	3.6	*Micrococcus luteus*
E-8	3	2	0	93	*B. subtilis, B. megaterium, B. cereus B. firmus, B. licheniformis*
F-9	3	2	0	93	*Corynebacterium poinsettiae, Acinetobacter calcoaceticus, B. lentus, B. licheniformis*
G-10	1	0	0	3.6	Yeast, *B. licheniformis*
H-1	1	0	0	3.6	*M. luteus*
I-5	1	0	0	3.6	*Corynebacterium* spp.

[1]Number of positive tubes of three tubes inoculated

periods of resuscitation (*3*). Our work does, however, support the working hypothesis of many microbiologists that, at least with some foods, recovery of certain organisms appears to be better in liquid rather than solid medium and that when low levels of organisms are to be recovered, use of a procedure employing a liquid environment should be considered.

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An Assessment of *Yersinia enterocolitica* and Its Presence in Foods

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ABSTRACT

Yersinia enterocolitica is one of the few human pathogens that grow at refrigeration temperature for foods, 0-5 C. Typical strains of *Y. enterocolitica* do not ferment rhamnose. These have been recovered from human infections, various animals, and pig's feces, but only rarely from foods. The atypical strains tend to utilize rhamnose, raffinose, esculin, salicin, α-methyl glucoside, or Simmon's citrate. These atypical strains have been recovered from fish, meat, oysters, and water as well as human patients. Inoculated food studies indicate that present recovery methods for *Y. enterocolitica* need improvement, but its identification is uncomplicated provided that the typical and atypical strains are taken into consideration. The public health implication of its presence in foods cannot be assessed until the incidence and virulence of the food isolates are determined.

Yersinia enterocolitica, Yersinia pestis (the plague bacterium formerly named *Pasteurella pestis*), and *Yersinia pseudotuberculosis* are now placed in the same genus in the family *Enterobacteriaceae* (*30*). Until recently *Y. enterocolitica* infections did not receive much attention in the United States, even though sporadic cases and outbreaks were reported as early as 1939 (*43*). Most of the recent microbiological and clinical studies of *Y. enterocolitica* were conducted by several European investigators who provided us with much of the present knowledge of this bacterium (*27,32,51*).

The clinical aspects of *Y. enterocolitica* infections have been reviewed in considerable detail (*5,27,55*). Its symptoms are different for various age groups (*55*). The predominant symptoms in infants are fever and diarrhea, but older children have mesenteric lymphadenitis and ileitis which mimic symptons of acute appendicitis (*55*). Adults may experience abdominal pains, acute enteritis, arthritis, and erythema nodosum (*55*). Septicemia may affect the aged and immune-deficient or immune-suppressed patients (*55*). Like *Salmonella* infections, *Y. enterocolitica* can be systemic and invade tissues outside of the digestive tract; it has been recovered form various abcesses, lesions, and eye infections (*1,35*).

Most of the *Y. enterocolitica* infections have involved individual cases or small clusters of people. Three large outbreaks of *Y. enterocolitica* infections in Japan were reported but sources of infections could not be identified (*6,57*). In these outbreaks, 931 persons (mostly school children) were infected out of a total population of 2520 students in three elementary schools. Two large outbreaks of *Y. enterocolitica* infections involving 137 children and one adult out of 831 persons who had gone on outings were reported in Quebec, Canada. Raw milk was implicated as the source of both outbeaks (*4*). Recently, an outbreak of *Y. enterocolitica* infections affecting at least 30 persons (mostly children) in the village of Holland Patent, New York, with a population of 600 was reported; chocolate milk was implicated as the source of infection (Morbidity and Mortality Weekly Report 26:53-54. 1977). The clinical picture of *Y. enterocolitica* infections in the U.S. is still not complete because it is not a notifiable disease and the bacterium is recovered infrequently by clinical laboratories. The reported recovery rates for *Y. enterocolitica* worldwide range from almost 0 to 1-2% of the fecal specimens; three Belgian hospitals consistently had the highest recovery rates (*28, 39*). The discrepancy of recovery rates must be partly due to different recovery methods employed. It is interesting to note that two Canadian and Belgian Hosptials have reported that the recovery rates for *Y. enterocolitica* were higher than the rates for *Shigella* (*14,39*). For reasons yet unclear, most of the clinical isolates of *Y. enterocolitica* from Canada (*47*), Japan (*56*), and Europe (*50*) belong to the Nilehn's biotype 4 serotype 0:3 strain, whereas most of the U. S. clinical strains belong to Nilehn's biotypes 2 and 3 with many different 0 serotypes (*35,53*). Some U. S. clinical strains are *Y. enterocolitica*-related bacteria which utilize Simmon's citrate, rhamnose, melibiose, and raffinose (*1,8*).

Recent DNA homology studies have shown that the four biotypes of *Y. enterocolitica* are a closely related group with 85-100% homology among various strains and thus should be considered a homogenous species (*10,31*). On the other hand, biochemical characteristics and DNA homology of *Y. enterocolitica*-related bacteria are different from those of the four biotypes of *Y. enterocolitica* and in fact consist of three distinct groups of bacteria as determined by DNA homology (*10*). The DNA of *Y. enterocolitica* and *Y. enterocolitica*-related

strains have about 60% homology (*10*). By comparison, *Salmonella* and *Escherichia coli* DNA have 40-60% homology (*9*).

Y. enterocolitica-related bacteria were also isolated from human patients with conjunctivitis and urinary tract infections, etc. by Bottone et al. (*1,8*). Thus, it is possible that *Y. enterocolitica*-related bacteria may cause disease in people with different foci of infection and symptoms than is observed in the typical *Y. enterocolitica* infections. However, this as well as the public health significance of these microorganisms remain to be documented.

OCCURRENCE

Y. enterocolitica and related bacteria have been commonly isolated from a variety of animals, foods, and water sources, but the biotypes were not identified in some of the reports. Among animals, pigs were found to harbor *Y. enterocolitica* with the same biotypes (*4*) and serotype (0:3) as the most common clinical strain isolated from humans in Canada, Japan, and Europe (*15,48,49, 50,58*). For this reason Mollaret (*27*) considered pigs an important zoonotic source of *Y. enterocolitica* infections. The bacterium has also been isolated from sick cats (*38,46*), cattle (*20,25*), chickens (*25*), deer and monkeys (*33*), a beaver, a Canadian goose, a raccoon and a Pekin robin (*18*), hares (*38*), and chinchillas (*46,50*). Recently one case of *Y. enterocolitica* infection in the U.S. was traced directly to sick puppies (*54*), and similarly, puppies were implicated in another outbreak which infected 16 of 21 people, with two fatalities (*17*). The evidence just presented suggests that zoonosis may be an important mode of transmission of *Y. enterocolitica* and that the reservoir of infection may reside in diseased animals. Presence of *Y. enterocolitica* in animals suggests, therefore, that it may be present in meats.

In the extensive survey conducted by Leistner et al. (*25*), feces and meat of chicken, cattle, and pigs were found contaminated with *Y. enterocolitica*. They recovered 35 *Y. enterocolitica* and 56 related bacteria from 121 samples of chicken meat, 10 *Y. enterocolitica* and 10 related bacteria from 29 samples of pork, and four *Y. enterocolitica* and six related bacteria from 37 samples of beef (*25*). *Y. enterocolitica* was isolated from 15 of 61 samples of beef in Japan (*20*). In the U.S., *Y. enterocolitica* and related bacteria were recovered from 10 of 98 samples of vacuum-packed beef and two of 18 samples of vacuum-packed lamb meat which had been stored at 1-3 C for 21-35 days (*19*). In the course of testing various enrichment procedures, four native *Y. enterocolitica* and three related bacteria were isolated from eight pork samples; also, two native *Y. enterocolitica* and five related bacteria were isolated from 10 samples of oysters (unpublished results). Toma (*46*) in Canada isolated biotype 1 *Y. enterocolitica* from four of 17 oyster samples. *Y. enterocolitica* was recovered from mussels (*44*), milk (*4,42*), ice cream (*29*), banana (*29*), and fish (*21,38*).

Both *Y. enterocolitica* and related bacteria have been isolated from drinking water (3,22,23; Saari, T. N., and T. J. Quan, 1976, Abstr. Am. Soc. Microbiol. C119). The source of infection in one case of *Y. enterocolitica* septicemia was traced to drinking water obtained from a mountain stream in upper New York State (*22*). From these data, it can be inferred that certain biotypes of *Y. enterocolitica* are fairly common in meat, oysters, and water. The public health significance of *Y. enterocolitica* in foods and water sources must await assessment by epidemiological and virulence studies.

ISOLATION PROCEDURES

Isolation methods for enrichment of *Y. enterocolitica* were developed primarily for clinical and fecal specimens (*46,52*), and initially some of these methods were also used for testing of food samples (*20,25*). For instance, cold enrichment with pH 7.6, 0.067 M sodium phosphate buffered saline (PBS) was originally developed for isolation of *Y. pseudotuberculosis* from clinical material (*34*), but now is used widely for isolation of *Y. enterocolitica* from foods (*16,20,25,58*). In one study with beef, cold enrichment with PBS was superior to enrichment with selenite-F broth plus 40 mg of novobiocin/liter (*20*). In another study, PBS cold enrichment was used with good results to examine 215 meat samples (*25*), and cold enrichment has been recommended for the isolation of *Y. enterocolitica* from foods (*16*). Preliminary study with inoculated foods indicated that PBS cold enrichment could not recover the two clinical strains of *Y. enterocolitica* inoculated into meat and oysters, but that the modified $MgCl_2$ enrichment broth of Wauters (*52*) was fairly effective for recovery of the inoculated biotype 4 serotype 0:3 strain (*24*). Because many *Y. enterocolitica* strains are sensitive to $MgCl_2$, Wauter's broth (*52*) failed to recover an inoculated biotype 2 serotype 0:8 strain as well as the native *Y. enterocolitica* in meat and oysters which were recoverable by other enrichment methods (*24*). These results indicate that the current enrichment methods may not be adequate for recovery of some clinically important biotypes present in foods and thus must be improved.

Various enteric plating media such as deoxycholate citrate, eosin methylene blue, lysine sucrose urea (*32*), MacConkey, *Shigella Salmonella* (SS) and YL agars (T. Saari, personal communication) have been used for isolation of *Y. enterocolitica* in clinical work. Some of these media are inhibitory to the more sensitive strains of *Y. enterocolitica* (*8, 13*). However, Mac Conkey and SS agars adjusted to pH 7.4 gradually have gained acceptance (*14,16,25*). One of the problems encountered with SS, YL, or MacConkey agars in examining foods is that it is difficult to distinguish the lactose-negative *Y. enterocolitica* colonies from many other food-borne lactose-negative bacteria on these agars. Toma (*46*) and Wauters (*52*) reported that *Y. enterocolitica* colonies could be recognized on SS agar when the colonies were

examined under a dissecting microscope with oblique illumination. Recently, Vanderzant (personal communication) found that *Y. enterocolitica* formed typical black colonies on bismuth sulfite (BS) agar after incubation for 2-3 days at 25 C, and that BS agar was useful for the isolation of *Y. enterocolitica* from meats. Random selection of colonies reduces the sensitivity of the isolation procedure and also makes the procedure tedious. Part of this problem can be resolved by adding a fermentable sugar like sucrose to the DHL agar (Eiken, Japan) (*25*) or mannitol to YM agar (T. Saari, personal communication). Some strains of *Y. enterocolitica* have lipase and lecithinase activity but lack nuclease activity (*13, 51*). These properties were utilized to aid in the recognition of *Y. enterocolitica* colonies by addition of Tween 80 to MacConkey agar, and sorbitol and Tween 80 to "DNAse" agar (*24*). *Y. enterocolitica* forms typical colonies on the above agars after 48 h of incubation at 25 C and generally can be distinguished from other bacterial colonies with some practice (*24*). Identification of *Y. enterocolitica* is not difficult provided that allowance is made for the five biotypes and the various related bacteria. These characteristics have been described (*1,8,13,32*). The biotypes and biochemical reactions of *Y. enterocolitica* isolates should continue to be reported until the taxonomy of this species becomes clear.

VIRULENCE TESTS

Unlike the other two *Yersinia* species, *Y. enterocolitica* was not considered to be virulent to laboratory animals (*2*) until recently, when two stains of *Y. enterocolitica* isolated from humans were found to be pathogenic to mice (*12,35*). Alonso et al. (*2*) found that only five, possibly six, of 4,500 "old" strain of *Y. enterocolitica* in the Pasteur Institute collection were pathogenic to normal mice, but three of the "non-virulent" strains could infect immuno-deficient athymic nude mice. Rabson et al. (*36*) found that mice treated with ferric ammonium citrate were also susceptible to *Y. enterocolitica* infections. The virulence of *Y. enterocolitica* has been tested with germ-free mice (*7*), HeLa tissue culture (*40*), gerbils (*35*), monkeys (*26*), rabbits (*37,40*), rabbit ileal loop test (*41*), and the Sereny test using guinea pigs (*45*). However, the adequacy of the above tests is not yet assured. A reliable procedure must be sought for determining the virulence of *Y. enterocolitica* because it is essential for investigating the pathogenic potential of food and water isolates.

SURVIVAL

Resistance of *Y. enterocolitica* to heating, drying, NaCl, or fresh and salt water has not been determined. All three species of *Yersinia* grow at about 0-4 C (*11,25,34*) and the number of *Y. enterocolitica* in chicken broilers decreased only slightly after freezing for 90 days at -18 C (*25*).

Without question, *Y. enterocolitica* grows at recommended food refrigeration temperatures below 5 C, but it is not known whether it can compete with other bacteria that also grow well at low temperatures. In one study large numbers of *Y. enterocolitica* were isolated from vacuum-packed meats where such packaging would be expected to suppress the outgrowth of common spoilage bacteria (*19*). However, the true incidence of this microorganism in refrigerated foods and its role as a food-borne pathogen must await further investigation.

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Use of the Fossomatic Somatic Cell Counts in a Mastitis Control Program[1]

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ABSTRACT

The Fossomatic determination of somatic cells present in individual cow milk samples was instituted in the Virginia Dairy Herd Improvement testing program in June, 1976. During the first 6 months the average count was 449,060 cells/ml for 113,735 observations. The average percentage of cows according to ranges in cell counts was: under 150,000, 47.9%; 150-400,000, 25.8%; 400-800,000, 12.5%; 800-1,500,000, 7.2%; and over 1,500,000, 6.6%. The percentage of cows with less than 150,000 cells/ml was reduced for September, 1976. On a herd basis, 66% averaged less than 500,000 cells/ml, 30% between 500 to 1,000,000, and 4% exceeded 1,000,000. The percentage of herds with less than 500,000 cells/ml decreased from June to September, 1976 but increased in October. Quarters infected with primary pathogens were estimated by Westgarth's equation to be 10.4% resulting in a calculated 5% reduction in total herd milk production. Procedures are described which would assist dairymen in the interpretation of somatic cell counts.

Evaluation of the mastitis status in individual cows has been conducted in many states by use of the California Mastitis Test (CMT). Although the CMT is an inexpensive cow side test, it provides only a subjective assessment of the degree of inflammation. Previous studies have shown that the correlation between the CMT score and the direct microscopic somatic cell count (DMSCC) was 0.76 (*3*).

Electronic somatic cell counting recently has been implemented in mastitis control programs, with the introduction of the Fossomatic[1] in Europe (*4*), and more recently in North America (*3*). The Coulter Counter and the filter-DNA method (*12*) have also been used. The correlation between the Fossomatic and DMSCC is 0.96-0.99 (*3,4*). Heald et al. (*3*) have indicated that there was considerable discrepancy between somatic cell counts when the Fossomatic count was compared to the CMT score as determined by Dairy Herd Improvement (DHI) supervisors.

In June, 1976, the Virginia Federation of DHI's commenced screening of somatic cells in preserved samples of milk from individual cows. The results for the first six months are in Table 1. The 6-month average somatic cell count was 449,060 cells/ml.

In equating the Fossomatic count with the CMT scores for reporting on DHI records, we have assumed the following relatiosnhips as proposed by Schalm et al. (*11*).

Fossomatic count (× 10^3)	CMT score
Under 150	Negative
151-400	Trace
401-800	1
801-1,500	2
Over 1,500	3

The actual somatic cell counts for each cow are returned immediately to dairymen. Any count which exceeds 400,000 is encircled. Renner (*10*) has suggested a

[1]*Approved for publication by the Director, Division of Animal and Veterinary Sciences.*
[1]*Fossomatic, Foss Electric, Hillerød, Denmark.*

TABLE 1. *Summary of the first 6 months of somatic cell screening with the Fossomatic in the Virginia DHI program*

	June	July	August	September	October	November	6-mo. ave.
Number of herds	181	213	240	242	242	272	
Number of cows	14,055	16,799	19,742	19,387	20,843	22,909	113,735
Average somatic cell count	433,409	458,934	468,183	478,562	392,619	458,839	449,060
% of cows							
Under 150,000	52.6	50.8	50.6	44.6	47.8	43.2	47.9
150-400,000	23.4	22.9	23.1	26.2	28.4	29.0	25.8
400-800,000	10.8	11.7	12.1	13.1	11.6	14.7	12.5
800-1,500,000	6.7	7.1	7.1	8.4	6.3	7.5	7.2
Over 1,500,000	6.5	7.5	7.1	7.7	5.9	5.7	6.6
% of herds							
Under 500,000	70.6	63.0	66.0	56.8	71.5	68.7	66.0
500,000-1 million	22.6	33.2	28.9	38.0	26.0	28.7	29.8
Over 1 million	6.8	3.8	5.1	5.2	2.5	2.6	4.2

threshold value of 400,000 cells/ml for detection of subclinical mastitis. It was pointed out that at this threshold value, 21% of the samples would be false-negative (abnormal milk but < 400,000 somatic cells/ml), while the false-positive would approximate 15% (normal secretion but > 400,000 somatic cells/ml). From 24 to 29% of the Virginia cows screened were found to exceed the 400,000 cell threshold.

INTERPRETATION OF INDIVIDUAL COW SOMATIC CELL COUNTS

Since this screening program for mastitis is new, many dairymen are not familiar with interpretation of the counts. We encourage dairymen to record a 12-month history of monthly somatic cell counts and milk yields for individual cows (Table 2). This record provides an easy check on the status of any cow during the lactation. This procedure is more convenient than checking each DHI sheet over several months.

Dairymen are encouraged to establish a mastitis treatment program in consultation with their veterinarian. Treatment during lactation should not be based solely on results from the Fossomatic. The CMT should be conducted on milk from cows testing over 400,000 cells/ml to determine which individual quarters have an inflammation. Milk should be obtained from treatment-resistant or chronic quarters and cultured for identification of specific pathogens and their sensitivity established for antibiotic treatments. The strip cup should be used to determine the presence of any abnormal secretion.

Cows in early lactation or whose production exceeds 23 kg daily have > 400,000 cells/ml should be brought to the attention of the herd veterinarian. Many veterinarians recommend treatment of these cows if an infection is diagnosed. Philpot (*9*) has indicated that approximately one-third of the subclinical infections present at parturition will become clinical during the ensuing lactation. A subclinical infection for the entire lactation could reduce milk production by more than 700 kg (*7*).

Cows in mid to late lactation whose somatic cell count exceeds 400,000 cells/ml should not be treated unless abnormal milk is detected. Dry cow therapy is recommended for these cows.

The somatic cell count should continue to rise or remain high for cows prone to chronic "flare-ups" of mastitis. The CMT would indicate the number of abnormal quarters. Deep udder palpations are indicated to determine the extent of udder tissue damage. These cows may be candidates for culling.

INTERPRETATION OF HERD MASTITIS STATUS

Dairymen should determine the monthly herd average somatic cell count. Monthly comparisons of these herd averages allow dairymen to measure the progress of their mastitis prevention and control program. Also it makes them aware of the extent of subclinical mastitis present and its economic impact.

Herds whose average somatic cell count exceeds 500,000 have been defined as "Mastitis Affected Herds" (*8*). Approximately 34% of the Virginia dairy herds enrolled in the Fossomatic program would satisfy this definition (Table 1). At least 4% of these herds would have severe mastitis problems. Using the filter-DNA method, Wisconsin (*1*) screened the somatic cell counts of milk from cows in 134 herds. Herd percentages were 61.6% below 500,000 cells/ml, 20.5% between 500,000 and 1 million, and 17.9% above 1 million. Cell counts were highest in July and August. There was a close similarity between actual bulk tank cell numbers and bulk tank count estimated from individual cows in the herd.

The proportion of Virginia herds averaging less than 500,000 cells decreased by 20% from June through September, 1976 (see Table 1). This was accompanied by a 68% increase in herds between 500,000 and 1 million cells/ml, with the greatest change occurring during September. The number of herds enrolled in the somatic cell count program was similar for August and September. The percentage of herds exceeding 1 million cells and the average somatic cell count declined during October.

At the average somatic cell count in these Virginia herds, over 10% of the quarters would be infected with primary pathogens, using the equation of Westgarth (*13*). A lactation reduction of 700 kg of milk per infected quarter (*7*) suggests that the average cow's production is

TABLE 2. *Monthly somatic cell count and milk production history*

Cow		Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep
Lilly	—FOM[a]	—	—	—	120	558	773[b]	621	435	139[b]	2,311	1,768	982
	—Milk	—	—	—	73	57	68	58	60	48	52	56	44
Daydron	—FOM	58	29	77	—	—	125	129	143	619	1,387[b]	1,901	1,508
	—Milk	51	42	33	—	—	97	92	89	80	70	72	64
Franny	—FOM	—	—	792[b]	128	94	147	233	177	139	255	490	841
	—Milk	—	—	76	78	77	57	62	62	56	50	43	34
June	—FOM	62	98	—	—	—	78	95	139	141	567[b]	142	935	1,672
	—Milk	41	36	—	—	—	80	74	68	60	53	50	48	42
Amelia	FOM	113	—	—	—	138	125	141	1,730[b]	859	129	88	73
	—Milk	9	—	—	—	94	103	93	76	82	80	77	62

[a]FOM = Fossomatic somatic cell count (ooo's)
[b]Treated for mastitis

reduced by 371 kg, which, at $22/100 kg, represents an annual loss of $82 per cow. Janzen (*6*) suggested that reduced milk production accounts for 80% of the cost of mastitis. Thus, annual mastitis losses could average $102 per Virginia cow. After 3 years of teat dipping and dry cow therapy in 27 dairy herds, the incidence of infected quarters was reduced to 7% (*7*). This would be comparable to a herd average of 385,000 cells/ml and could reduce the average cost of mastitis to approximately $52/cow.

The average somatic cell count in 73 dairy herds and the proportion of cows in each CMT score were subjected to multiple regression analysis in an attempt to develop equations for dairymen to use for determination of herd average.

(*a*) Herd average = 412.2 − 4.54 (% negative) + 37.36 (%3's)
count (000) $R^2 = 0.92$; SD = ± 195

(*b*) Herd average = 133.7 + 44.71 (%3's)
count (000) $R^2 = 0.89$; SD = ± 226

Equation (*b*) suggests that herds with more than 30% of the cows exceeding 1,500,000 cells/ml would be in danger of violating state regulations (Table 3). For a herd to average less than 400,000 cells/ml, no more than 6% of the cows may exceed 1,500,000 cells/ml.

TABLE 3. *Estimated relationship between herd somatic cell count, proportion of cows in the herd with above 1,500,000 cells/ml, reduction in milk production, and proportion of quarters infected with primary pathogens*

Herd count (X 10^3 cells/ml)	% Cows over 1.5 X 10^6	% Lost milk	% Infected quarters[a]
50	—	—	—
100	—	—	—
200	1.5[b]	—	—
300	3.7	1.4	3.0
400	6.0	3.6	7.7
500	8.2	5.9	12.4
600	10.4	8.1	17.1
700	12.7	10.3	21.8
800	14.9	12.6	26.5
1,000	19.4	17.0	35.9
1,500	30.6	28.1	59.3

[a]Westgarth (*13*)
[b]Equation (*b*)

Dry cow therapy is recommended for all cows in the herd when the herd average count exceeds 400,000 cells/ml. Herds with less than 400,000 cells/ml, may choose to treat only cows whose count exceeded 400,000 cells/ml or cows which had clinical mastitis at some time during the lactation. However, this latter recommendation is subject to question. For the first 6 months of the Fossomatic screening program, 73.7% of the cows produced milk with less than 400,000 cells/ml. Heald et al. (*3*) have shown that cows with less than 300,000 cells/ml were infected by *Streptococcus agalactiae*, other *streptococci*, and *Staphylococcus aureus* in 9% of the quarters. Thus, it would be possible that 26 infected quarters could exist in these cows below 400,000 cells. At 1.1 infected quarters/cow (*2*), 33% of the cows which would not be treated at drying-off could be infected. At two infected quarters/cow, 13% of the infected cows in a herd would not be treated if selective dry therapy was used in a herd.

EFFORTS TO IMPROVE THE HERD MASTITIS STATUS

At the end of every month, the average somatic cell counts are reviewed for each herd. Any herd whose count exceeds 800,000 cells/ml is contacted by mail. The herd count and the estimated lost milk production are described. Dairymen are encouraged to consult their veterinarian and have milk samples from at leat 25% of the cows in the milking herd cultured in the state diagnostic laboratory. We recommend that they review their milking practices, have the milking system analyzed, use an effective teat dip, and implement a dry cow management program in consultation with their veterinarian. Also dairymen are encouraged to seek the assistance of their dairy fieldman, dairy inspector, or Extension Agent.

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Foodborne Disease in Canada - 1974 Annual Summary

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ABSTRACT

Data on foodborne disease in Canada in 1974 were compared with data for 1973. A total of 442 incidents, comprising 387 outbreaks and 55 single cases, and involving 4,338 ill persons was recorded for 1974. The number of outbreaks increased by 17% and single cases by 57% over that reported for 1973. As in 1973, *Staphylococcus aureus* was responsible for more incidents (*34*) than any other agent. Other incidents were caused by *Salmonella* (*24*), *Bacillus cereus* (*5*), *Clostridium botulinum* (*4*), *Clostridium perfringens* (*3*) and *Trichinella spiralis* (*6*). Paralytic shellfish poison caused illness in 43 persons. Similar to the previous year, non-microbiological agents accounted for less than a quarter of the incidents of known etiology. Nine persons were reported to have died through foodborne disease. About 36% of incidents and 31% of cases were associated with meat and poultry. Vegetables, fruits, sandwiches, and bakery products also played a significant role. Mishandling of food took place primarily in food service establishments (29% of incidents) or homes (14%). More than 50% of foodborne illnesses occurred in Ontario and Quebec; the number of incidents per 100,000 population, however, was highest in the Northwest Territories (5.3) and British Columbia (4.8).

The rationale for establishing a national system for collecting and disseminating data on foodborne disease in Canada was presented previously (*3*). In October, 1976, a detailed summary report of the 1973 data (*2*) was published in English and French and distributed to health agencies and interested persons in Canada and other countries; copies are available from the author on request. A similar detailed summary is also planned for the 1974 data.

The following definitions, used in this summary, are identical to those previously published (*3*).

Food: Any substance for human consumption, excluding drinking water.

Outbreak: An incident in which two or more persons experience a similar illness after ingestion of a common vehicle (food), and epidemiological evidence implicates the vehicle as the source of the illness.

Case: A person who has been ill as a result of consuming food shown to be contaminated on the basis of epidemiological evidence or laboratory analysis.

Single case: One case, as far as can be ascertained, unrelated to other cases in respect to vehicle consumed.

Incident: An outbreak or single case.

Laboratory confirmed incident: Where the specific etiological agent is determined by laboratory analysis.

THE DATA

The present report is a summary of foodborne illnesses occurring during 1974 reported by the Health Protection Branch, provincial epidemiologists, provincial laboratories, and municipal and regional health authorities. Data were collated, tabulated, and returned to the originators for verification. From the information listed under each incident the following tables were prepared.

Table 1 shows that 442 foodborne incidents, comprising 387 outbreaks and 55 single cases, occurred in 1974, and involved 4,338 cases of illness. Compared with figures for 1973, outbreaks increased by about 17%, single cases by 57%, and total cases by 26%. The percentage of incidents in the different etiological categories, however, remained essentially the same for both years; only about 23% were laboratory-confirmed, and of these microbiological agents were the most frequent cause of illness (18-19%). The percentage of laboratory-confirmed cases, however, dropped from 48.2% in 1973 to 39.9% in 1974, mainly owing to the incomplete identification of microbiological agents.

Table 2 lists incidents caused by specific agents,

TABLE 1. *Number of foodborne incidents and cases in 1974 and 1973*

	Outbreaks		Single cases		Total incidents		Percentage incidents		Total cases		Percentage cases	
Etiology	1974	1973	1974	1973	1974	1973	1974	1973	1974	1973	1974	1973
Microbiological	67	57	17	12	84	69	19.0	18.3	1569	1516	36.2	45.3
Parasitic	4	3	2	2	6	5	1.4	1.3	50	65	1.2	1.9
Plant	1	2	—	—	1	2	0.2	0.5	43	4	1.0	0.1
Chemical	9	6	1	7	10	13	2.3	3.4	64	29	1.5	0.9
total known	81	68	20	21	101	89	22.9	23.5	1726	1614	39.9	48.2
Unknown	306	275	35	14	341	289	77.1	76.5	2612	1733	60.2	51.8
Total	387	343	55	35	442	378	100.0	100.0	4338	3347	100.1	100.0

including the range and median number of cases per outbreak. The agent responsible for most outbreaks (29) and single cases (5) was *Staphylococcus aureus* with a median of 6 cases per outbreak. Although *Salmonella* ranked second in the number of outbreaks (22) and single cases (2), more persons were ill with salmonellosis (622) than with staphylococcal enterotoxemias (611). *Bacillus cereus* was third in order of importance; although the number of incidents (5) and cases (87) was much lower than for *Salmonella*, the median number of cases per outbreak for both agents was similar. Considerably, fewer outbreaks and cases of *Clostridium perfringens* occurred in 1974 than during the previous year, but the median number of cases per outbreak was very high (90). Generally the median for all agents was higher in 1974 than in 1973. Incidents were caused by four agents unreported in 1973 — *Shigella, Escherichia coli, Vibrio parahaemolyticus,* and *Brucella abortus.* Six incidents of trichinosis occurred through consumption of improperly cooked meat. Although no illness was reported from vascular plants[1] in 1974, an outbreak of paralytic shellfish poison occurred through consumption of shellfish contaminated with the alga, *Gonyaulax tamarensis.* The presence of iron and tin in canned foods, caustic wash and sulfuric acid in bottled drinks, and gasoline in canned sunflower seed oil led to a number of incidents.

Nine deaths were associated with foodborne disease in 1974. Two Eskimos died in separate outbreaks after eating game meat contaminated with *Clostridium botulinum* type E toxin. Two west coast Indians were also victims of the same toxin present in fermented salmon eggs. Two Indian children succumbed to trichinosis after consuming uncooked or partially cooked bear meat. One elderly person was hospitalized and subsequently died after eating egg salad contaminated with *S. aureus.* Finally, paralytic shellfish poison claimed the lives of two persons eating contaminated shellfish gathered from the St. Lawrence estuary.

Foods associated with illnesses are shown in Table 3. In the 1973 summary (*3*), foods which were the probable vehicles of infection were included under the appropriate headings. This practice was discontinued in 1974 and all 'probable' categories of foods have been relegated to the 'unknown' food categories. It follows that the 1973 figures listed in Table 3 are, therefore, somewhat different from those in the corresponding table in the 1973 summary (*3*). Table 3 also lists all incidents whether associated with a specific agent or not; therefore, etiological information pertaining to many of these incidents is incomplete. About 36% of incidents and about 31% of cases were associated with meat and poultry. Vegetables and fruit, especially canned and bottled low acid products, sandwiches, and bakery products also played a significant role.

Table 4 shows that mishandling in foodservice establishments was responsible for 29.0% of all incidents and 63.2% of all cases. Mishandling in the home (14.0% of incidents), food-processing establishments (8.8%), and retail food establishments (3.4%) was less significant. The trend was the same for both 1973 and 1974. Again, the figures for 1973 (Table 4) differ from the figures in the 1973 summary (*3*), because the 'probables' have been taken from the specific categories and put into the 'unknown' category.

[1]*See Addendum at end of paper*

TABLE 2. *Number of persons ill in foodborne incidents by specific cause in 1974 and 1973*

Etiology	Number of outbreaks		Number of cases in outbreaks		Number of cases/Outbreak: Range		Number of cases/Outbreak: Median		Single cases	
	1974	1973	1974	1973	1974	1973	1974	1973	1974	1973
MICROBIOLOGICAL										
Staphylococcus aureus	29	27	606	600	2-110	2-131	6	4	5	6
Salmonella sp.	22	13	620	334	2-126	2-150	13	10	2	1
Bacillus sp.	4	3	86	19	2-40	2-15	18	2	1	—
Clostridium botulinum	4	2	10	5	2-3	2-3	2.5	2.5	—	3
Clostridium perfringens	3	7	200	535	10-100	2-325	90	23	—	—
Shigella flexneri	1	—	12	—	NA[a]	NA	NA	NA	—	—
Escherichia coli	1	—	12	—	NA	NA	NA	NA	—	—
Streptococcus sp.	1	2	2	4	NA	2	NA	2	—	—
Vibrio parahaemolyticus	1	—	2	—	NA	NA	NA	NA	—	—
Brucella abortus	—	—	—	—	NA	NA	NA	NA	1	—
Mold and yeast	1	3	2	7	NA	2-3	NA	2	8	2
total microbiological	67	57	1552	1504	2-126	2-325	10	4	17	12
PARASITIC										
Trichinella spiralis	4	3	48	63	3-29	2-53	8	4	2	2
PLANT										
Alkaloid	—	1	—	2	NA	NA	NA	NA	—	—
Mushroom toxin	—	1	—	2	NA	NA	NA	NA	—	—
Gonyaulax tamarensis	1	—	43	—	NA	NA	NA	NA	—	—
CHEMICAL										
Metals	2	4	4	14	2	2-5	2	3.5	—	—
Cleaning and disinfecting solutions	1	—	7	—	NA	NA	NA	NA	—	6
Other chemicals	6	2	52	8	2-25	3-5	4	4	1	1
UNKNOWN	306	275	2577	1719	2-350	2-175	3	3	35	14
TOTAL	387	343	4283	3312	2-350	2-325	3	3	55	35

[a]NA = not applicable

The incidents caused by mishandling of foods in food-processing establishments are summarized in Table 5. Although these represent only 8.8% of the incidents, some of these were widespread in nature. The processor was responsible for 39 incidents and 256 cases. Eight (20.5%) of these incidents, responsible for 163 (63.7%) cases, involved microbiological agents, whereas 5 (12.8%) incidents, responsible for 31 (12.1%) cases, were associated with chemical agents. Two of the outbreaks, *Salmonella eastbourne* in chocolate and *Salmonella weltevreden* in pepper, had begun in 1973; the first lasted from August till April, the second from December to April. Sixty-two persons suffered enterotoxemia when they had eaten iced cakes; the icing had been made from salt-free butter contaminated with *S. aureus*. Tin at a level of 360-370 ppm in canned vegetable soup caused 2 cases of poisoning. As in 1973, caustic wash was left in bottles of soft drinks and 7 persons suffered from burning throats.

The main peak of food-borne disease in 1974 took place in October, whereas, in 1973, peaks occurred in June and November. Thus, there does not appear to be a distinct seasonal pattern. There is, however, some pattern in the regional distribution of incidents (Table 6).

TABLE 3. *Foods associated with foodborne incidents and cases*

	Incidents				Cases			
	Number		Percentage		Number		Percentage	
Food	1974	1973	1974	1973	1974	1973	1974	1973
Meat	117	117	26.5	30.9	1021	1281	23.5	37.1
beef	17	23	3.9	6.1	389	373	9.0	10.8
ham and pork	25	19	5.7	5.0	297	391	6.8	11.3
cooked hamburger	20	21	4.5	5.5	64	94	1.5	2.7
sausages	31	26	7.0	6.9	108	66	2.5	1.9
other or unspecified	24	28	5.4	7.4	163	357	3.7	10.4
Fish	18	10	4.1	2.6	57	27	1.3	0.8
Shellfish	8	12	1.8	3.2	68	129	1.6	3.8
Poultry	42	37	9.5	9.8	321	583	7.4	16.9
chicken	23	25	5.2	6.6	103	105	2.4	3.0
turkey	18	7	4.1	1.9	214	368	4.9	10.7
other or unspecified	1	5	0.2	1.3	4	110	0.1	3.2
Eggs	2	1	0.4	0.3	4	1	0.1	0.0
Dairy foods	17	9	3.9	2.4	95	99	2.2	2.9
ice cream	6	2	1.4	0.5	12	74	0.3	0.2
cheese	5	2	1.1	0.5	12	13	0.3	0.4
milk and other products	6	5	1.4	1.3	71	12	1.6	0.3
Bakery products	24	29	5.4	7.7	92	131	2.1	3.8
cakes and bread	9	11	2.0	2.9	39	57	0.9	1.6
pizzas	7	6	1.6	1.6	21	18	0.5	0.5
pies, puddings and pasta products	5	6	1.1	1.6	26	40	0.6	1.2
other baked products	3	6	0.7	1.6	6	16	0.1	0.5
Confectionery	11	3	2.5	0.8	121	27	2.8	0.8
Vegetables and fruits	51	33	11.6	8.7	281	114	6.5	3.3
raw vegetables and fruits	5	6	1.1	1.6	37	11	0.9	0.3
processed low acid vegetables	18	17	4.1	4.5	56	44	1.3	1.3
processed acid vegetables and fruits	11	5	2.5	1.3	67	44	1.5	1.3
other vegetables and fruits	17	5	3.9	1.3	121	15	2.8	0.4
Chinese foods	19	23	4.3	6.1	62	104	1.4	3.0
Salads	13	9	2.9	2.4	111	31	2.6	0.9
Sandwiches	25	18	5.7	4.7	370	173	8.5	5.0
Beverages	9	13	2.0	3.4	25	27	0.6	0.8
Multiple vehicles	11	1	2.5	0.3	196	6	4.5	0.2
Other foods	10	8	2.3	2.2	208	37	4.8	1.1
Unknown	65	55	14.7	14.6	1306	577	30.1	19.6
TOTAL	442	378	100.1	100.1	4338	3347	100.0	100.0

TABLE 4. *Places where food was mishandled in foodborne incidents in 1974 and 1973*

	Number of incidents		Percentage of incidents		Number of cases		Percentage of cases	
Establishment	1974	1973	1974	1973	1974	1973	1974	1973
Foodservice establishments								
hotels, restaurants	64	82	14.5	21.7	625	587	14.4	17.5
catering companies	21	7	4.8	1.8	1243	516	28.7	15.4
others	43	42	9.7	11.1	873	1171	20.1	35.0
Homes	62	63	14.0	16.7	338	351	7.8	10.5
Food-processing establishments	39	41	8.8	10.8	256	135	5.9	4.0
Retail food establishments	15	30	3.4	7.9	61	123	1.4	3.7
Other	7	4	1.6	1.1	74	10	1.7	0.3
Unknown	191	109	43.2	28.8	868	454	20.0	13.6
TOTAL	442	378	100.0	99.9	4338	3347	100.0	100.0

TABLE 5. *Incidents of foodborne illness caused by mishandling of food in food-processing establishments in 1974*

Etiology	Vehicle	Number of Incidents	Number of Cases
Salmonella eastbourne[a]	chocolate	1	78
Salmonella typhimurium and *anatum*	salami	1	2
Salmonella weltevreden[a]	pepper	1	14
Staphylococcus aureus	butter	1	62
Staphylococcus aureus	omelette seasoning mix	1	3
Staphylococcus aureus	Camembert cheese	1	2
Mold	canned creamed mushrooms	1	1
Mold and yeast	beer	1	1
Tin	canned vegetable soup	1	2
Caustic wash	bottled soft drink	1	7
Gasoline	canned sunflower seed oil	1	19
Can sealing compound	canned soft drink	1	1
Sand-like material	bottled soft drink	1	2
Probably gasoline	canned soy bean oil	1	2
Unknown	canned low acid foods	7	9
Unknown	canned acid foods	4	13
Unknown	bottled and canned soft drink	3	9
Unknown	wieners	2	6
Unknown	ice cream	2	6
Unknown	chocolate bars	1	5
Unknown	doughnuts	1	3
Unknown	cheddar cheese	1	3
Unknown	powdered infant formula	1	2
Unknown	gefilte fish	1	2
Unknown	frozen pre-cooked shrimp	1	1
Unknown	turkey	1	1
TOTAL		39	256

[a]Other cases of this outbreak also occurred in 1973.

As in 1973, most incidents occurred in central and western Canada, with Ontario and Quebec accounting for more than 50% of incidents. The Northwest Territories and British Columbia ranked first and second, respectively, in the number of incidents per 100,000 population. The number of incidents occurring in Ontario decreased with respect to 1973 data, but those from British Columbia, Alberta, Nova Scotia, Prince Edward Island, and Newfoundland increased considerably. One outbreak occurred outside the country when personnel in a Canadian Forces plane en route between Pakistan and Tanzania fell ill with shigellosis.

EXAMPLES OF OUTBREAKS

The following are examples of unpublished outbreaks which occurred in 1974 in Canada. Not all of the examples chosen were necessarily the best documented. Some of them illustrate incomplete epidemiological or laboratory analysis and indicate why so few incidents reported are of known etiology.

E. coli enteritis after a wedding dinner

Caterers in Burnaby, British Columbia, provided a wedding dinner in a community hall on June 22. The foods, including ham, turkey, sausage rolls, meat loaf, salads, and cheese, were prepared on June 21 and stored under refrigeration until served at 5 p.m. the next evening. Since this was a buffet style dinner, guests ate from 6 p.m. until about 11 p.m. Between 5 and 15½ h after eating, 12 of the 44 guests became ill with nausea, vomiting, cramps, diarrhea, and fever (the median incubation period was 13 h). Three guests, including the bride, were taken to a hospital and one required overnight treatment. Epidemiological investigation showed that turkey, ham, chicken, and vegetable salad were the foods eaten by the majority of ill persons. Samples of meat loaf, sliced ham, chicken, turkey, potato salad, and vegetable salad were examined microbiologically. No *Salmonella, S. aureus, C. perfringens* or *B. cereus* were found. The vegetable salad contained several million *a*-haemolytic streptococci/g, and the meat loaf and ham had aerobic colony counts of $> 10^9$/g and enterococcal counts of 10^4-10^5/g. Fecal coliforms were also found in the meat loaf (4.3×10^3/g) and ham (9.3×10^6/g), but only the ham yielded enteropathogenic *E. coli* [2 of 15 isolates positive by rabbit ileal loop test

TABLE 6. *Regional distribution of foodborne incidents in 1974 and 1973*

Province or Territory	Number of incidents		Percentage of incidents		Number per 100,000 population[a]	
	1974	1973	1974	1973	1974	1973
British Columbia	115	73	26.0	19.3	4.8	3.2
Alberta	18	7	4.1	1.9	1.1	0.4
Saskatchewan	19	13	4.3	3.4	2.1	1.4
Manitoba	16	12	3.6	3.2	1.6	1.2
Ontario	188	198	42.5	52.4	2.3	2.5
Quebec	49	56	11.1	14.8	0.8	0.9
New Brunswick	10	9	2.3	2.4	1.5	1.9
Nova Scotia	11	2	2.5	0.5	1.4	0.3
Prince Edward Island	5	—	1.1	0.0	4.3	0.0
Newfoundland	6	2	1.4	0.5	1.1	0.4
Northwest Territories	2	3	0.5	0.8	5.3	7.9
Yukon	—	—	0.0	0.0	0.0	0.0
More than one province or territory	2	3	0.5	0.8	NA[b]	NA
Other[c]	1	—	0.2	0.0	NA	NA
CANADA	442	378	100.1	100.0	2.0	1.7

[a]Based on 1973 and 1974 estimates by the Census Division, Statistics Canada
[b]NA: not applicable
[c]Other: Canadian Forces plane abroad

(*1*)]. The enterococcal isolates failed to cause distension of the rabbit loops, and were considered non-enterotoxigenic. Ham, therefore, appears to have been the vehicle of infection with *E. coli* as the etiological agent. The symptoms and incubation period are compatible with illnesses caused by this organism. Unfortunately, no data are available from analysis of stool specimens. This incident resulted from bacterial growth that occurred during the 5 h of buffet-style service.

Staphylococcal enterotoxemias in a home for the aged

Some members of a home for the aged in Nova Scotia were preparing for a party held on October 17. On October 15, ham and chicken were cooked and chopped into small pieces by one member and refrigerated. On October 16, someone else boiled eggs, chopped them into small pieces and added mayonnaise and refrigerated them. During the morning of the party, a number of ladies prepared egg salad, ham salad, and chicken sandwiches and put them into bags. Two ladies ate some egg salad for lunch; within 4 h both were ill. Unfortunately, no one connected the illnesses of these two persons with the food they had eaten, and the party went on as scheduled. Between 7 and 8 p.m. that evening about 40 persons ate the sandwiches; the first case of illness occurred at 11:00 p.m. A total of 17 cases was recorded with symptoms of nausea, vomiting, and diarrhea with incubation periods between 3 and 6 h. The egg salad eaten by the two ladies in the afternoon and the egg salad and chicken salad sandwiches eaten in the evening had very high aerobic colony counts (4×10^7-5×10^8/g) and *Staphylococcus aureus* counts (2.5×10^6-3.5×10^7/g). Although no nasal or skin swabs of the food handlers were taken, it would appear that one of the food handlers or a utensil was the source of *S. aureus.* The organism grew to large numbers during the time of exposure to room temperature (4 h total) and dissemination of the organisms was possible because of the continuous handling and mixing of the ingredients.

Trichinosis from pork sausage

In October, suspected cases of trichinosis were reported to an Ontario health unit. Ill people gave a history of eating smoked sausage bought at a corner store, and two meat markets in three separate towns, and also raw sausage obtained from a meat packer in another town. Eventually, it was determined that 12 persons from 6 families were ill, 11 confirmed with cysts of *Trichinella spiralis,* and one suspect case showing typical symptoms. The meat packer was found to be the origin of all the infected meat, and pork from a sow slaughtered between September 10 and 17 was the probable source of infection. Laboratory examination of raw pork sausage, produced later than that eaten, failed to show *Trichinella* parasites, and, although the farm source of every sow being processed was known, no infected live animal was found. Six of 7 hospitalized cases had eaten raw smoked sausages purchased at the corner store. This store had processed its own sausage, which had been lightly smoked for an hour to impart a smoked flavor; however, it was not intended to be eaten raw. The situation had at once been corrected by using labels to warn consumers that cooking of these sausages is required. The other 6 cases ate small amounts of raw sausage either when preparing cooked meals from products bought at the meat markets, or when nibbling during the processing at the meat packer's. This incident underlines the dangers of eating even small portions of raw pork meat.

Caustic wash in bottled soft drinks

On May 25 in Quebec a mother and grandmother drank bottled soft drinks and almost immediately felt a burning sensation in their throats. The grandmother had to be taken to the hospital where chemical damage of the esophagus was diagnosed. The incident was brought to the attention of the Health Protection Branch a few days later, after the family had the contents of the suspect bottle analysed privately; the pH of the contents was 14. Inspectors from the Branch took samples from the remaining bottles but all were found to be satisfactory. Further examination of the suspect bottle indicated that the pH of the contents was 13.3 with evidence that sodium hydroxide was present. Two additional complaints to the company led to a voluntary recall of 7 oz. and 28 oz. bottles on June 1. This action, unfortunately, was not sufficient to prevent illness in another family. Three children became ill on June 1 and the father on June 2 after drinking the same product. Symptoms included vomiting and headaches, as well as a sore throat, On June 4 a general recall, announced in the press, was initiated and no more illnesses were known to have occurred. Sodium hydroxide is used for cleaning glass bottles, which are normally rinsed before filling with the beverage. Improper rinsing presumably led to the presence of caustic in the bottles. Similar incidents, though unproven through lack of chemical analyses, were reported in 1974; one of these was associated with a defective bottle washing system. In 1973, 6 separate incidents involved either caustic soda or chlorine; one plant was responsible for two of these.

Illness from vegetable oil contaminated with gasoline

In February, two consumer complaints were received by the Health Protection Branch concerning the off-odor and off-flavor of canned sunflower seed oil in Sault Ste. Marie, Ontario. At the same time, retail chain stores of one company were recalling this product sold in the city. The Health Protection Branch visited the oil canning company in Toronto and found the firm, which had received 17 consumer complaints, was initiating a recall from the stores from where consumer complaints had originated. Further investigation showed that a number of the complainants claimed to have been ill. A few days later the company agreed to a voluntary recall of all canned sunflower seed oil produced after mid-January. The problem was further complicated when it was found that a food color was being illegally used in the oil; the company agreed to remove the color from existing

supplies.

The nature of the off-odor was not determined until mid-March when 50-100 ppm of gasoline was detected in the oil. There was also a possibility of other hydrocarbons being in the oil, although these were not identified. The can manufacturing company did not prove to be a likely source of solvents, but there were indications that one tank car containing 150,000 lb. of oil had been contaminated. However, the production manager of the sunflower oil refinery felt that the plant operations could not have lead to gasoline contamination. When tanks arrive at the canning company, the product is packed into cans without the addition of other compounds; therefore, if a large tank car were contaminated, there should have been more than 17 complaints.

In May, another product, soy bean oil, was recalled by the same company when a petroleum-like smell was again detected. Only two persons reported ill and the number of complaints was fewer, probably because the problem was swiftly dealt with by the company. Sabotage was considered a strong possibility in this second contamination, and may also have been the reason for the first. Although the source of contaminating chemicals was not determined, the net result of the two incidents was that 21 persons in different parts of Ontario complained of illness with a variety of symptoms—nausea, vomiting, cramps, diarrhea, headaches, and dizziness, and the cost and inconvenience of the recalls to the company were high. One other interesting feature is that investigation of one problem revealed another totally unrelated one.

ADDENDUM

Since this manuscript was submitted, a single case attributed to consumption of the roots of Western Water Hemlock (*Cicuta douglasii*) in British Columbia in April 1974 came to our attention.

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I acknowledge the following agencies for allowing me to summarize unpublished data as expamples of outbreaks: (*a*) British Columbia—Burnaby Health Unit, City of Vancouver Health Department, Provincial laboratories; (*b*) Ontario—Bruce County Health Unit; (*c*) Nova Scotia—Atlantic Health Unit; (*d*) Federal—Food Directorate and Field Operations Directorate (Ontario region, Québec region), Health Protection Branch.

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Evaluation of State Foodservice Sanitation Programs

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ABSTRACT

Outlined are procedures for evaluating foodservice sanitation programs with State Foodservice Sanitation Rating Officers certified by the Food and Drug Administration. Modifications are outlined to provide more continuity between the sanitary status of the establishments within the jurisdiction being evaluated and enforcement program being carried out by the regulatory agency. A greater degree of program uniformity is needed as the present trend of interjurisdictional movement of prepared foods for direct service to the consumer continues and increases. The increasing number of chain foodservice establishments also increases the need for standardized and certified foodservice programs and personnel.

In Kentucky the Foodservice Establishment, Retail Food Market and Vending Inspection and Enforcement Programs are carried out through district or county health departments. Therefore, comments in this paper will be directed largely toward evaluation of District and County Foodservice inspection programs. I feel regulatory agencies as a whole do not place enough emphasis on retail food service.

The importance of retail food service was borne out by a paper, "The Federal Food Service Program." by Bowers and Davis (*1*). The authors indicate that the foodservice industry is the fourth largest business in the United States. This industry alone has an estimated gross sales of $62 billion annually. The National Restaurant Association projections through 1977 anticipate approximately $80 million in sales.

The *Kentucky Restaurateur* (*2*), a publication of the Kentucky Restaurant Association, indicates that according to a 1974 survey conducted by the Department of Labor, approximately 27% of the food dollar is spent on food prepared outside the home. Since 1974 this percentage has increased to over 33%.

No wonder supermarkets are beginning to consider the restaurant as their prime competitior. I feel this has brought about the current trend of many retail food markets going into the foodservice business. In Kentucky this includes not only the sandwich business, but also plate lunches and all the trimmings. The bicentennial celebrations throughout the United States have brought into existence more mobile foodservice and temporary food service units this year than have ever proviously existed at one time.

Increased interstate movement, not only of people, but also of food, food ingredients, and food additives has, in my opinion, increased the need for uniformity and standarization by regulatory officials on the Federal, state and local levels.

FDA is presently making strides in this direction. Revision of the 1962 Food Sanitation Ordinance and the 1965 Vending Machine Ordinance are under way. Also, a new model Ordinance for Retail Food Markets is nearing completion. Hopefully these revision will be available for state adoption in 1977.

How ever great the words standarization, uniformity, revised model food ordinances, etc. sound to us in the regularly field, I cannot, in good faith believe any or all of these things will greatly improve the overall condition of foodservice in the United States.

I feel in most areas, the problems with substandard foodservice establishments can largely be traced to the lack of industry training and the failure of regulatory agencies to take a positive attitude toward their foodservice inspection and enforcement programs.

The function of the Kentucky Food Branch is primarily to provide adminstrative and technical support to local health units in the areas of: program planning, monitoring, and integrating. This is done through: (*a*) standard setting, (*b*) technical consultation, (*c*) training, (*d*) program standardization, (*e*) certification, (*f*) permit issuance, and (*g*) construction plan approval. The Branch also provides direct services in food manufacturing and storage facilities, state-owned institutions and interstate carrier facilities. Much of this work is carried out under a contract with the Food and Drug Administration. The areas I'll attempt to deal mainly with here today are program standarization and certification.

THE KENTUCKY PROGRAM

Kentucky presently has four FDA Certified Food Service Sanitation Rating Officers. The State is divided into four areas. The Survey Officers' time is spent in the following areas: (*a*) foodservice Establishment Surveys, 30% of time; (*b*) retail food market surveys, 30% of time; (*c*) environmentalist and industry training, 15% of time; (*d*) interstate travel sanitation program (under FDA contract), 10% of time; and (*e*) special problems and technical assistance to local health units, 15% of time.

In Kentucky, regulations are adopted by the Department for Human Resources and are effective in all areas of the State. Although local health boards have the authority to adopt more stringent regulations, this is rarely done. Some counties do require annual health examinations or tuberculosis tests for foodservice employees, and Lexington-Fayette County has a grading system for their foodservice establishments.

Surveys are conducted according to requirements of the Kentucky Food Service Code. The present Code was revised in late 1974, and became effective April 4, 1975.

Kentucky has not adopted the Federal Ordinance; however, we have patterned our regulation very closely after the recommended Ordinance. In fact, our present regulation was patterned closely after early draft of revision being done by the FDA with the 1962 Ordinance. Whenever the final FDA Ordinance revision is completed for state adoption, we will revise our regulation accordingly.

We are presently attempting to survey the food service and retail food market programs for each local unit a minimum of biennially. More will be said about this later.

Procedures for Evaluating Foodservice Sanitation Programs as recommended by the FDA are followed very closely as regards foodservice establishment data.

In 1974 we attempted to review the foodservice establishment. Surveys conducted over the previous 2 years. Although we are basically satisfied with the establishment data collected, we found what we considered a weakness in the administrative or enforcement data collected. In most instances the enforcement rating was not compatible with the establishment rating.

In too many instances the establishment rating was below the acceptable level; however, at the same time the admininstrative or enforcement rating was rather high. This meant one of two things: (*a*) enforcement porcedures were not sufficient, or (*b*) we weren't using the right evaluation methods.

In reviewing closely the items considered as administrative data, under the FDA recommended procedures, we found many of these items to have no direct bearing on the inspection and/or enforcement program. These items were eliminated from the evaluation sheet.

Examples of these items are: (*a*) Does the inspection staff equal a minimum of one inspector/15,000 population of 300 establishments? (*b*) Are pre-assembled sample kits available for investigation of foodborne disease outbreaks? (*c*) Is an approved laboratory available to assist in foodborne outbreaks? (*d*) Are the local media utilized for dissemination of program information? (*e*) Are standards equivalent to State and FDA standards? (*g*) Are report forms equivalent to State and FDA recommendations? (*h*) Are construction plans for new establishments reviewed and approved? (*i*) Do inspectors attend workshops and seminars on a regular basis? (*j*) Is at least one trade of professional journal subscribed to?

Although credit is not presently given for these items, they are documented and spoken to in the survey narrative report. At best most of these items have only an indirect effect on program operations. If, in the opinion of the Survey Officer, they are not being carried out and they have a bearing on the overall program, recommendations are made along these lines.

We then took a look at what was actually required, under the Code, to carry out the inspection and enforcement requirements, and a new Enforcement Evaluation Sheet was developed accordingly.

Basically, when we reviewed the Kentucky Code, there were nine areas which required the regulatory agency to carry out some type of enforcement action. Therefore, the "Food Service Establishment Survey Report" (Fig. 1) was developed accordingly. We feel this enforcement evaluation approach is much simpler and actually places emphasis where it is needed.

Those readers having knowledge of Interstate Milk Shippers Program, may recognize a similarity between the form shown in Figure 1 and the one the Milk Sanitation Rating Officer uses in conducting milk surveys under the cooperative State-Public Health Service Program for Certification of Interstate Milk Shippers.

We have been using this system for about a year and find the establishment and enforcement rating to be much more compatible. We feel it given us a much better picture of the enforcement being carried out in the program.

Once the Survey Officer completes the survey, and it is sent to the State Office for typing and review, copies of the completed summary sheets, enforcement sheet, and narrative report are forwarded to: (*a*) district/county administrator or health officer, (*b*) sanitarian(s) responsible for the program, (*c*) community health services, (*d*) FDA District office, and (*e*) survey officer.

As Manager of the Food Branch, I also submit a cover letter with each survey report, emphasizing weaknesses and areas which need immediate action and offering technical and administrative assistance if needed. If the survey report indicates the local program plan, which was approved and funded, is not being carried out and the objectives not being met, this also receives attention.

USE OF REPORTS

The survey reports are used by the program

Consumer Health
Protection - 46
11-75

COMMONWEALTH OF KENTUCKY
DEPARTMENT FOR HUMAN RESOURCES
BUREAU FOR HEALTH SERVICES

FOOD SERVICE ESTABLISHMENT SURVEY REPORT

OF _____ DATE _____

Health Officer:	Environmentalist:		Supervisor:	
Survey Officer:	Date Certified:	County:	Population:	Code in effect:

SUMMARY OF SURVEY RESULTS

Total No. of Establishments _____ Sanitation Compliance Rating _____

No. Inspected on Survey _____ Enforcement Rating _____

ENFORCEMENT EVALUATION

Number	ITEMS	Number Inspected	Number Complying	Percent Complying	Weight	Credit	Number	ITEMS	Number Inspected	Number Complying	Percent Complying	Weight	Credit
1.	All establishments hold valid permits				15		6.	Est. below 70 issued suspension notice				10	
2.	Permits posted				5		7.	Requirements Interp. according to Reg.				15	
3.	All Establishments Inspected 1/6 months				15		8.	Enforcement Procedures carried out				10	
4.	Est. scoring 70-84 Inspected in 30 days				10		9.	Records, systematically filed, complete, current				10	
5.	Est. with 4 or 5 point Insp. in 10 days				10			ENFORCEMENT RATING					

Remarks:

Figure 1. *Foodservice establishment survey report form used in Kentucky.*

administrator in developing program plans in the future. They may be used locally to request budgeting support for additional staff, staff training, or administrative support.

The Office of Community Health Services, which is responsible for appointing state funds to local health units, may use the survey report to determine if program plans which were approved and budgeted with State funds are being carried out effectively and efficiently. State funds may be withheld in certain program areas based on the failure of a program plan being administered. However, this is a last resort action and rarely done.

More usually in problems areas, we attempt to work more closely with the sanitarian and provide him with additional training. In areas where program problems

result from poor administrative support from the health officer or administrator, problems are sometimes brought before the local board of health.

Based on surveys we have conducted over past 1.5 years under the present Code, the Statewide average for Kentucky Foodservice Establishments Rating is 82.6% with an average Enforcement Rating of 84.3%.

PROPOSED ADDITIONS

Two program additions which we are presently considering putting into effect are: (*a*) A certification program for all district/county inspectors carrying out the foodservice inspection and enforcement program. (*b*) Annual spot checks of all district/county programs.

Under this program, complete surveys would be made approximately once each 2-3 years or more often upon request and in problem areas. Under the annual spot check system, each district/county unit would be required to keep an up-to-date record, of their rating, based on their last inspection.

On the spot check, the survey officer would: (*a*) Make a complete record review. (*b*) Select at random somewhere between 8-15 establishments for inspection. This will vary based on the size of the program being surveyed. We realize these few establishments would not be a statistically sound number, however, we do feel they would give us an indication of the program operational status. The ratings of the establishments selected would be compared with the district/county average as compiled by the local unit. If the rating we complied was within + or -5% of the local established rating, we would accpet the present program rating as being accurate. (*c*) Spot checks falling over 5% lower than the local rating would require us to complete a full survey.

This would free our Survey Officers to spend more time in problem areas and would strengthen the overall state program. We will also be adding one survey officer to our staff as soon as he receives some extra training and is certified by FDA.

IN CONCLUSION

In closing, I would say that as Program Managers and Survey Officers we need to maintain a positive attitude, and constantly look for new ways to strengthen the foodservice program. For too long, too many of us, have taken the attitude, that little can be done in foodservice programs. There is no reason why through a positive enforcement and training programs much needed improvement cannot be achieved. We don't have to wait for a national disaster to get concerned. To do less than our best in the field of foodservice would be unexcusable.

ACKNOWLEDGMENT

Presented at the FDA Food Survey Officer Seminar, Jackson, Mississippi, October 21, 1976.

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National Sanitation Foundation Holds Election

At the Annual meeting of the board of trustees of the National Sanitation Foundation, two Michigan women were elected officers of NSF. Carole VanLuven was named treasurer and Janice Gaertner was named secretary of the environmental organization which is headquartered in Ann Arbor.

Mrs. VanLuven, 347 Willis Road, Saline joined the staff of NSF in 1969 after completing courses in accounting at Cleary College. She began as an accounting clerk and progressed to the position of personnel director and assistant treasurer.

Mrs. Gaertner of 173 Center Street, Petersburg joined NSF in 1970 as secretary to the vice president. She later became a data processing programmer/analyst and then data processing manager.

Also at the meeting of the board, two new trustees were chosen and the resignation of another was accepted. Elected was Arthur C. Avery, professor and director of research, hotel and institutional management, Purdue University. Also elected was Bruce Benner, president and chief executive officer of Ann Arbor Bank and Trust Company. Retiring from the NSF board is Joseph B. Foster, chairman of Ann Arbor Bank and its holding company, American Bankcorp.

Two members of the NSF board were presented with the Spes Hominum (hope of mankind) Award for outstanding contributions to environmental quality. Honored was Charles A. Farish of Ann Arbor, retired executive director of NSF, and Blucher A. Poole of Indianapolis, former assistant commissioner of the Indiana state board of health.

The National Sanitation Foundation is a non profit, non governmental agency which assists public health officials, manufacturers and consumer representatives in establishing standards for environmentally related products.

New Product Saves Energy Two Ways on Dairy Farms

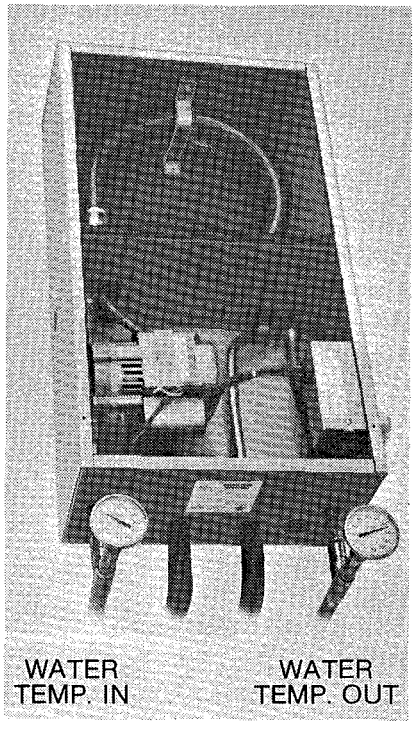
A new energy converter cuts energy costs two ways by relieving milk cooling tank condensing units while converting their rejected heat into usable FREE heated water, according to Babson Bros. Co., manufacturers of Surge dairy farm equipment.

As much as 90% can be saved on the cost of heating water, and less electricity is required to run the condensing unit when it is attached to a new Surge ARC Energy Converter. By decreasing energy requirements, the Surge ARC Energy Converter could pay for itself in 6 to 18 months.

The energy converter adapts to virtually all existing and new installations regardless of the type of refrigerant used or make of tank or condensing unit.

Installation can be accomplished in only a few hours, thanks to a clever design permitting maximum use of existing plumbing equipment.

The low cost and ease of installation makes this a practical, high return on investment item for almost any dairyman who is cooling milk.



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OUR GOAL SINCE 1920

Melding marketing know how with member energy, quality control, capital and land resources to keep consumers supplied with highly nutritious dairy products.

Maryland and Virginia Milk Producers Association, Inc.
1530 Wilson Boulevard
Arlington, Virginia 22209

and subsidiary

Southside Milk Producers
Marshall Building
Blackstone, Virginia 23824

Walter A. Martz
President

C. Thomas Sollenberger
First Vice President

David Weitzer
Second Vice President

James E. Click
Secretary-Treasurer and General Manager

"Whitehead Receives Recognition"

Dick B. Whitehead, right, Chairman of the Committee on Sanitary Procedures of The International Milk, Food and Environmental Sanitarians, was recognized by 3-A for distinguished service to the program. Whitehead retired in 1976 from the Mississippi Health Department. Presentation was made by Fred J. Greiner, Executive Vice-President of Dairy and Food Industries Supply Association on behalf of the Dairy Industry Committee.

Entirely New Plants May Be Created in the Laboratory

A new method of plant improvement, developed by scientists at Texas A&M University, has the potential to literally create new plants.

All present plant improvement, however successful, has come up against certain genetic barriers. These limitations have meant, for example, that no matter how you improved cotton or soybeans, they could never be as efficient in photosynthesis as corn or sugar cane.

Now, by a technique developed by Dr. Robert Halliwell, a scientist with the Texas Agricultural Experiment Station (TAES), there is hope that these genetic barriers can be breached and plants such as cotton or soybeans made much more efficient.

The technique developed by Halliwell is called single cell microinjection. A geneticist with TAES and Texas A&M, Dr. James Smith, will work with Halliwell on experiments that will transform albino tobacco cells into normal green tobacco cells.

The purpose of this is to refine techniques of mechanical transfer of genetic material. If this reseach is successful, scientists may no longer be restricted to plant improvement within the limits of genetic compatibility but can transfer desired characteristics between *unrelated species.*

In addition to the already mentioned increase in photosynthetic efficiency, another possibility would be transfer of nitrogen fixation capability.

This could reduce need for artificial nitrogen fertilizer by species that presently require it. Our nitrogen fertilizer manufacturing is a heavy user of energy.

Halliwell and Smith will be aided by another Smith, Dr. Roberta Smith, a TAES plant physiologist, who will supervise tissue culture.

These three scientists stress that the work they are doing is expected to take a number of years but they are convinced of its feasibility. If they succeed in transferring genetic material between unlike species of plants, they may well unlock the gates that presently bar agriculture from undreamed of yields of food and fiber production.

Corning Establishes Marketing Group For Industrial Biological Systems

To expand its immobilized enzyme technology from the laboratory into industrial applications, Corning Glass Works has established an industrial biological systems group within its Science Products Division.

Enzymes have long been used in the manufacture of cheeses, wines, beer, pharmaceutical products and a host of other items, Corning has developed technology for binding such biologically active materials—catalysts—to inert, insoluble supports such as glasses or ceramics. This makes these agents usable in continuous flow-through processes at considerable economic savings. Added benefits are stabilization of these processes and, in most cases, increased usable life for the immobilized biological agents.

The first industrial commercialization of this technology was in the conversion of cornstarch into a high-fructose corn syrup sweetner. Corning licensed CPC International (Corn Products Corp.) to its technology for immobilizing the enzyme dextrose isomerase onto porous support materials. Thus immobilized, this catalyst is being used in several CPCI plants in the U.S. and abroad. Corning continues to supply CPCI with the porous carrier materials used in the process.

Other areas of interest, Corning reports, are the conversion of cheese whey—normally a waste product—into the nutritive sweetner glucose, and the conversion of wastes into usable or less noxious materials. Test installations already exist in the U.S. and in Europe for cheese whey conversion, Corning reports.

Named to the new post of manager-industrial biological systems is Gerald A. Levine. R. Dale Sweigart and John T. Smolick have been named sales manager and market analyst, respectively.

American Cultured Dairy Products Institute 1977 Conference Set

The Annual Conference of the American Cultured Dairy Products Institute will be held September 15-16, 1977, at the Colony Motor Hotel, Clayton, Missouri.

This year's Conference will include general sessions as well as specific panel presentations relative to quality assurance and manufacturing procedures for cultured dairy products. Additionally, international speakers will discuss various aspects of manufacturing cultured dairy products in their particular country.

Attendance is open to representatives of all cultured dairy products companies—this is the only dairy meeting devoted entirely to cultured dairy products.

The final program will be announced this summer and nonmembers of the ACDPI should write the Institute office for further meeting information. The address is American Cultured Dairy Products Institute, 910 17th St., NW, Washington, D.C. 20006.

NRA Vows to Work For Adoption of 1976 FDA Model Ordinance

Noting that the new 1976 U.S. Food and Drug Administration Model Ordinance sets a "consensus" sanitation standard for foodservice operations, the National Restaurant Association has vowed to seek its adoption by all public health authorities around the country.

NRA expects to mobilize its Action Network, a body of over 1,000 foodservice operators nationwide, to push for implementation of the model sanitation code by state and municipal governments. Additionally, NRA with the cooperation of FDA has produced a 40-minute color filmstrip entitled "Protecting the Public Health." The film, which details all aspects of the new model ordinance, will be available to state restaurant associations and public health officials. NRA also has distributed copies of the film to FDA for use in the Government's regional office instruction programs.

Endorsement of the model ordinance came during the NRA's Spring board meeting. In a policy statement issued by the board, the association said: "The adoption of the 1976 ordinance by local public health authorities will benefit the consumer and the foodservice operator by assuring effective, equitable and uniform regulations regardless of the geographic location.

"Uniformity of FDA ordinance requirements will especially enhance the efficiency and effectiveness of sanitation and food protection programs of the growing number of enterprises operating in several public health jurisdictions."

We Can Grow Fuel for Vehicles

The feasibility of supplementing our fuel supplies with alcohol is being investigated by scientists with the Texas Agricultural Experiment State (TAES), in cooperation with Louisiana, Florida and USDA.

An automobile engine can be adjusted to run on fuel that's partly alcohol. And alcohol can be made from many of the crops we raise as food or feed.

Already many countries are having to cope with shortages and fuel prices that make our situation in the U.S. look desirable.

Brazil, for example, has gasoline prices three times as high as ours, in the range of $1.80 to $2.00 per gallon.

At present, Brazil is using an estimated 500 million liters of alcohol per year in their gasoline (a 5 percent alcohol-gasoline mixture). Plans call for an increase of alcohol production to 3,000 million liters per year by 1980 to allow for a 20 percent alcohol-gasoline mixture.

Sugar cane and cassava are used in Brazil for manufacturing alcohol, according to Dr. Fred Miller, an Experiment Station grain sorghum breeder.

Miller recently returned from Brazil where he conferred with agricultural officials. Acting on his recommendation that they consider TAES improved sweet sorghums as an additional resource, Brazilians have ordered tons of seed from here.

Estimates are that Brazil's present production of about 66 million metric tons of sugar cane will have to be increased to 100 million metric tons to provide the additional alcohol.

Though no more sugar will be produced, Brazil will still benefit in two ways: 1) it will reduce the drain of money for imported fuel, and 2) it will increase employment in sugar agriculture.

In the cooperative research being conducted by Texas Experiment Station scientists and those in Louisiana, Florida and USDA, "total biomass" are key words. This means all of the plant material that can be produced per acre, according to Dr. Sim Reeves, a TAES agronomist specializing in sugar crops. Reeves explains that the total plant, not just the sugar, can be converted to fuel production.

Reeves is located at the TAES Weslaco Center and is conducting research on the use of sweet sorghum for alcohol production. Scientists in Florida and Louisiana are working on sugar cane for the same purpose.

In recent years, TAES scientists have greatly improved sweet sorghum varieties to extend both milling season of Texas sugar mills and the area of production. Sorghum can be produced in areas not suited to sugar cane.

According to Miller and Reeves, the improved Rio variety can be grown to harvest in 135 days and in South Texas this permits two crops per year. This gives yields per acre of about 3½ tons of sugar per year. Another variety, Roma, is expected to give even higher yields.

Alcohol production from this sugar is about 250 gallons per acre per year. Even the leaves and stalks can be converted to another fuel, methanol, or can be used to fuel the sugar mills.

National Restaurant Association Recognizes Promotional Ideas

The Frankenmuth Bavarian Inn, Frankenmuth Mich., has been chosen the Grand Award winner in the National Restaurant Association's (NRA) 1st Annual Operators' Promotion Idea Exchange (OPIE). Six Gold and seven Silver Award winners were also chosen in the contest, which offers the nation's restaurateurs the opportunity to "blow their own horns" and share their most successful promotions with fellow operators.

The Bavarian Inn centered its successful promotion around a multi-purpose 10-oz. clear glass boot. The boot was adapted from a 1-liter German drinking boot and embossed with the restaurant's name and logo of a dancing German couple.

William A. Zehnder, assistant manager of the Inn, reports that the boot was designed to promote sales of beer, wine and cocktails, as well as candy and mixed nuts in the Inn's Gourmet Shop. Boots were even filled with dried flowers and used as centerpieces on the Inn's tables.

"We initiated our promotion during Frankenmuth's Bavarian Festival in 1975," Zehnder states. "During the first week, we sold more than 13,600! To date, more than 150,000 of the boots have been sold.

"These items have become unique advertising tools. Customers take the souvenir boots into their homes where they become constant reminders of pleasant times in Frankenmuth," adds Zehnder.

Frankenmuth Bavarian Inn's promotion along with other winning OPIE entries will be part of a special lunch hour program, "Merchandising Techniques That Work!" at the 58th Annual NRA Restaurant Hotel/Motel Show, May 22-25 at Chicago's McCormick Place.

The following foodservice establishments are recipients of Gold OPIE Awards: *Wine*—Pinehurst Country Club, Littleton, Colo.; *Food Item*—Saga Corporation, Menlo Park, Calif.; *Charity*—Friendly Ice Cream Corporation, Wilbraham, Mass.; *Increased Sales Overall*—The Original Ice Cream Saloon Inc., Beaverton, Ore.; *Increased Sales for a Day/Period*—Sheraton-Ritz Hotel, Minneapolis, Minn.; and *Other*—Ecobelli's Inc., Ballston Spa, N.Y.

Recipients of Silver OPIE Awards: *Wine*—Harris O. Machus Enterprises, Inc., Birmingham, Miss., and The Frank Unkle Restaurant, Toledo, Ohio; *Food Item*—Victorian House, Inc., Chicago, Ill.; *Charity*—International Dairy Queen, Inc., Minneapolis, Minn.; *Increased Sales Overall*—Trukadero Coffee Shop, Sacramento, Calif.; *Increased Sales for a Day/Period*—Tito & Pat Enterprises, Bloomington, Minn.; and *Other*—YWCA of Greater Milwaukee, Milwaukee, Wisc.

Sustaining Member

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Egg Substitutes Show Mixed Cooking and Eating Qualities

A number of "egg substitute" products have been nationally marketed during the past several years and advertised for use in low cholesterol diets as substitutes for egg products.

Most of these substitutes are egg albumen combinations with food additives designed to replace normal yolks according to research by two Texas Agricultural Experiment Station scientists.

The relative functional efficiency of egg substitutes and whole eggs were compared in experiments by Dr. Fred A. Gardner and M. L. Beck of the Poultry Science Department at Texas A&M University.

Whole eggs were compared to four brands of egg substitutes. Two frozen substitutes, a liquid refrigerated substitute and a dehydrated substitute were evaluated.

The two frozen substitutes contained egg white (about 80 to 85 percent) and a number of food additives to replace the color, function and natural characteristics of egg yolk. Both products were intended for use as packaged after thawing.

The liquid refrigerated product, like the frozen products, contained about 85 percent egg white and a number of food additives to replace the yolk characteristics. The product is reported to have a shelf-life of about 30 days and is intended for use as packaged.

"In all of these products, the yolk had been completely eliminated and the cholesterol content therefore had been reduced to 0 milligrams per egg equivalent. However, since the egg yolk normally provides emulsification and thickening properties, chemical stabilizers were used to produce thickening properties," Gardener said.

"The dehydrated product contained egg white and about 20 percent of the normal content of egg yolk. Cholesterol content therefore was reduced to less than 60 milligrams per egg equivalent. This product must be reconstituted with water prior to use," Gardener added.

In addition, all substitute products evaluated contained a substantial quantity of nutritional additives which were intended to replace nutrients normally found in the yolk. These include not only protein and fat soluble vitamins but also a rather large number of minerals.

The egg products were prepared and either presented to a taste panel or evaluated by laboratory tests.

"Scrambled eggs prepared from whole eggs were judged superior to these prepared from egg substitutes in tenderness, moisture, flavor and overall acceptability.

"Sponge cakes made from the egg substitute products were larger in volume but lacked the fine structure and tenderness associated with those prepared from whole eggs.

"In addition, baked custards from the egg substitutes were less susceptible to spread and sag but lacked the tenderness and flavor characteristics of whole egg custards.

"Our results indicate that the flavor of egg substitutes, when used alone or in a bland mixture, are generally inferior to that of whole egg products.

"However, when used in products which contain other flavor components, the flavor deficiencies of egg substitutes are somewhat masked.

"In addition, the incorporation of thickners and stabilizers in egg substitutes yielded products which lacked the tenderness and delicate structure normally associated with whole egg products," Gardener concluded.

The results of this study indicate that the cooking and organoleptic properties (flavor, taste, appearance) of products prepared from egg substitutes differ from those prepared from whole egg. However, it should be recognized that the egg substitute products satisfy a rather specific need of our egg marketing system. Since these products are either very low in cholesterol or are cholesterol free, they do offer a way of incorporating egg characteristics into the meals of individuals who on the advice of a physician are modifying their total dietary intake of cholesterol.

IAMFES Sustaining Members

Babson Bros. Co.
2100 S. York Road
Oak Brook, Illinois 60101

Darigold, Inc.
635 Elliott Ave. W.
Seattle, Washington 98119

Knudsen Corp.
231 East 23rd St.
Los Angeles, California 90011

Maddelena's Inc.
139 Lakeville St.
Petaluma, California 94952

Maryland & Virginia Milk Producers Assn., Inc.
P.O. Box 9154 Rosslyn Station
Arlington, Virginia 22209

Nasco, Inc.
901 Janesville Ave.
Fort Atkinson, Wisconsin 53538

Norton Co.
P.O. Box 350
Akron, Ohio 44309

Polson Co.
625 S. Lander St.
Seattle, Washington 98124

Seiberling Associates, Inc.
417 Eastern Ave.
So. Beloit, Illinois 61080

Southside Milk Producers
Marshall Building
Blackstone, Virginia 23824

Valley of Virginia Co-operative Milk Producers Assn.
41 W. Washington St.
Harrisonburg, Virginia 22801

West Agro-Chemical, Inc.
P.O. Box 1386
Shawnee Mission, Kansas 66222

Association Affairs

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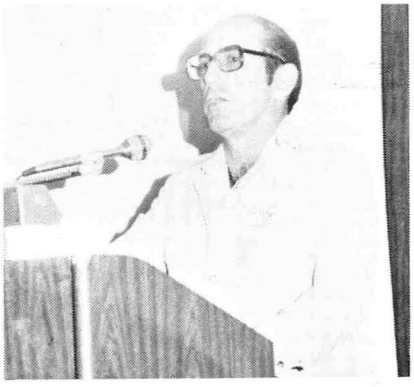
FAMFES officers and Board of Directors (Left to Right): Jay Boosinger Past-President, Mary Ann Ash, Margaret Reis, Doris Marchetti, Joe Hayes President Elect, Cecil Hickox, Carl Kroppman, Ron Richter Sec-Treas., Tom Hart President.

"Doc" L. A. Scribner receiving award for Outstanding Contributions to the FAMFES from Ron Richter, chairman of awards committee.



Dave Fry, President-Elect IAMFES up-dating FAMFES members on developments and activities of the International association.

News from Florida

The Florida affiliate held its annual meeting at the Kayhler Plaza in Orlando, Florida on March 15-17, 1977. More than 120 people attended this annual educational program again this year. Program chairman Tom Hart organized another outstanding educational program for the association. The one and one-half day program had many informative presentations and featured a one-half day sessions on the sterilization and aseptic packaging of milk.

Awards were presented to "Doc" L. A. Scribner for his outstanding contributions to the association and to Harvey Jordan for his many years of faithful participation in the association.

Joe Hayes was elected President-Elect and Ron Richter appointed as Secretary-Treasurer with Tom Hart moving into the role of President. New Board of Director members elected were Carl Kroppman, Mary Ann Ash, Doris Marchetti, Margaret Reis, and Cecil Hickox. Jay Boosinger serves on the board as Past-President.

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1977 ANNUAL MEETING I.A.M.F.E.S.

Advance Registration Form
Sioux City, Iowa

64th Annual Meeting
August 14-18, 1977

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Make checks payable to IAMFES — 1977 Meeting Fund and mail to Mr. Carl Webster

Please indicate your Professional Field

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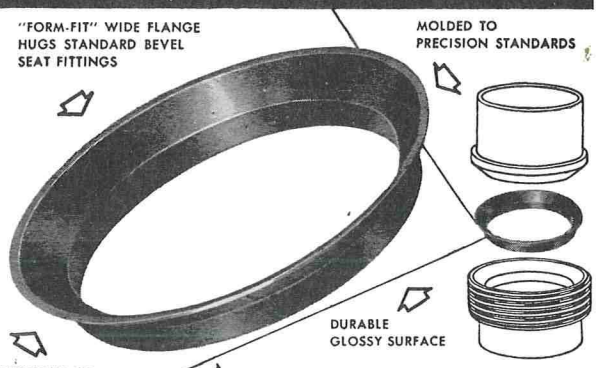

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Two Easy Steps to More Milk and Healthy Udders

By Dr. L.J. Bush
Professor, Department of Animal Sciences and Industry
Oklahoma State University—Stillwater

Significant advances have been made in recent years in the design and development of milking equipment. Now the challenge is for the dairyman to take full advantage of what they can do for him. By using this new equipment in accordance with suggested milking procedures, a dairyman can help keep his cows healthy and increase profits from his herd. Two important steps in getting the most from equipment are proper adjustment of milking units and prompt removal of the units as milking is completed.

Proper Adjustment Avoids Injury and Improves Milk Flow

To do the best job of milking cows by machine, it is important that the teat cups remain in the correct position on the teats. If the teat cups are allowed to creep up on the teats as milking progresses, the passageway for milk is partially blocked due to compression of the tissue by the teat cup liners. Injury to the tissue at the base of the udder may occur. This can be prevented by providing a slight downward pull on the teat cups as the cow is milked out. With "claw" type units this usually can be accomplished only by holding down on the unit as milking nears completion, whereas suspended units can be positioned to provide the tension needed. The mere fact that the milking unit is suspended does not insure proper downward tension on the teat cups, however. It must be adjusted properly to get the job done right. Higher milk yields will result due to more complete milking, and less machine stripping time is required.

Remove Teat Cups When Milk Flow Stops

Another important step in milking is to remove the milking unit as soon as the flow of milk has stopped. Removing teat cups too early will rob you of milk. On the other hand, there is evidence that overmilking causes severe injury to teats of some cows. Hemorrhage and inflammation of the membrane lining the teat cavity often occurs, and, in some cases, the streak canal is injured. These injuries lower resistance to bacterial invasion and mastitis may result.

Cows vary in milk yield and rate of milk flow. Individual quarters of the same cow vary in the same way. Therefore, the ideal situation is for individual teat cups to be removed from the quarters as each one is milked out. Milking units designed for automatic removal of individual teat cups accomplish this with precision, if serviced regularly and used in the recommended way. It should be noted though that the same results can be achieved with conventional milking units by careful attention to good milking procedures.