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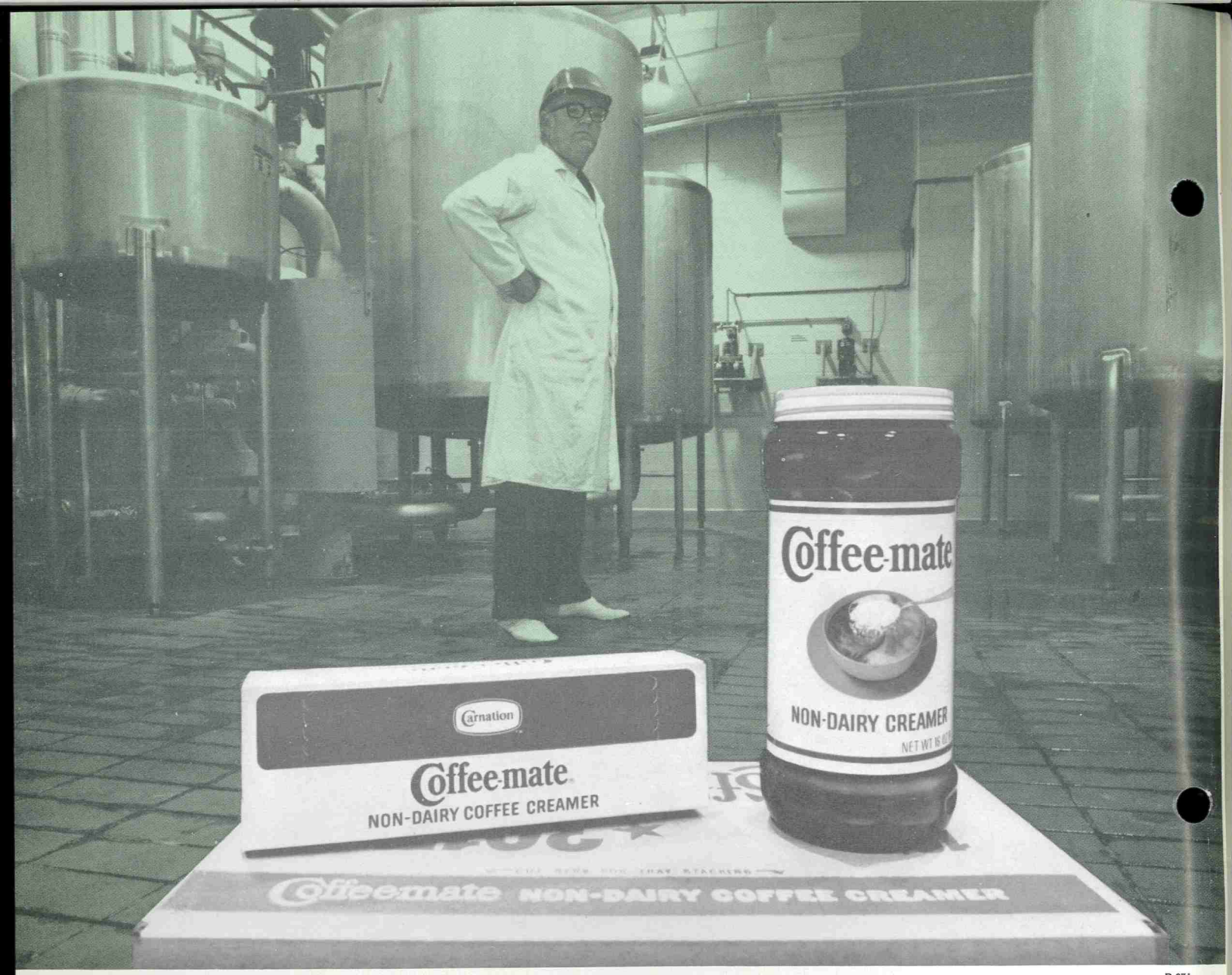

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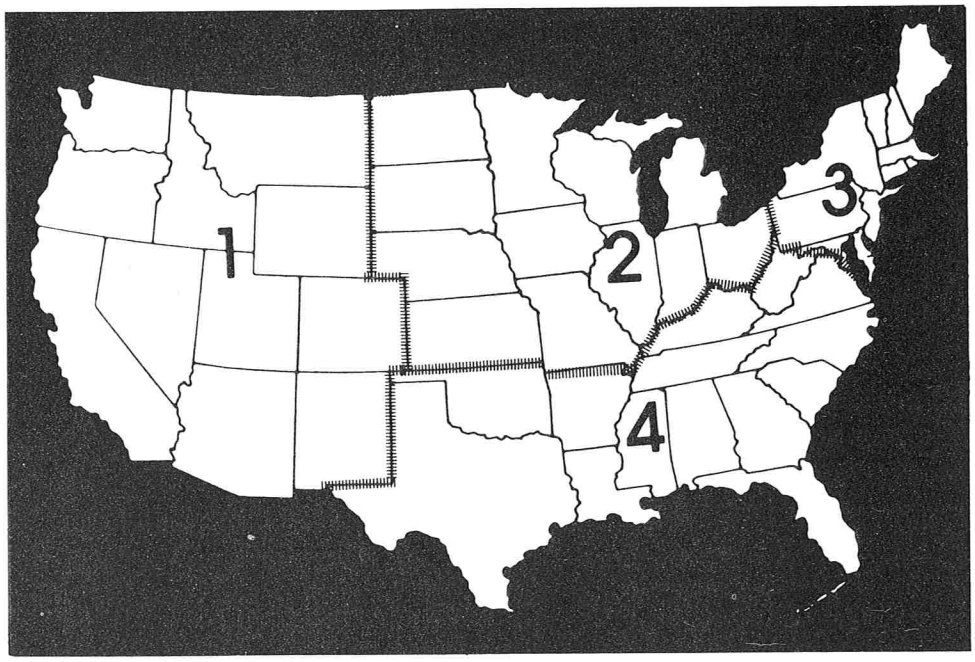

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J. Milk Food Technol. Vol. 38, No. 9, Page 504 (September, 1975)

A Research Note

A Cellulose Sponge Sampling Technique for Surfaces

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(Received for publication March 13, 1975)

ABSTRACT

An improved surface sampling technique using cellulose sponges is described. The technique has been found particularly effective for detection of *Salmonella* on surfaces but can also be used in place of the contact swab method for detection of other microorganisms on surfaces.

The classical approach to microbiological sampling of surfaces is the swab contact method in which moist, sterile swabs are rubbed over a measured area (*1*). On heavily soiled surfaces the sample obtained with this technique may be superficial and not reflect the true microbial load. Further, only a relatively small area may be sampled with each swab and the method permits the technician to apply a limited amount of pressure to the handle.

To overcome the limitations of the swab contact method we have made use of cellulose sponges for surface sampling. The sponges are cut into a convenient size of approximately 5 cm^3 and placed in individual small kraft paper bags and steam sterilized.

The sampler prepares for sponge swabbing by washing his hands thoroughly with soap and water, followed with sanitization in a solution of 200 ppm available chlorine. This preparation step is convenient when sampling for *Salmonella.* Alternatively, the hands may be covered with sterile or sanitized plastic or rubber gloves. The latter step is preferred when sampling for quantitative microbiological analysis.

The sterile sponge is removed aseptically from the bag and moistened with a 1% solution of Tergitol Anionic-7 (Union Carbide, Chicago). The surface is sampled by vigorously rubbing the sponge over the designated area until the soil is removed. An area of several square meters may be efficiently sampled. If the surface is flat, the Tergitol solution may be applied directly and then taken up into the sponge by the rubbing action. After sampling, the sponge is introduced into a sterile plastic bag and transported to the laboratory under refrigeration.

We have found the cellulose sponge technique particularly efficient in detecting *Salmonella* on food plant equipment and in the environment. In at least one instance, a plant was known to be contaminated because of *Salmonella*-positive results on a variety of finished product and environmental samples. However, the conventional swab contact method failed to detect *Salmonella* on equipment. We then applied the cellulose sponge technique to the equipment and environment and found a high percentage of the samples positive for *Salmonella.* For *Salmonella* analysis, the sponge is introduced directly into lactose broth which is then analyzed by conventional techniques (*2*).

However, the sponge-sample may be subjected to a variety of analytical procedures; for example, total plate count, coliforms, yeasts and molds, staphylococci, etc. It may be desirable, depending on the organism being sought, to replace the Tergitol solution with another sampling fluid, such as 0.1% peptone water, phosphate buffered distilled water, etc., because of the possible deleterious effects of Tergitol on the Gram-positive bacteria. When performing quantitative tests on the sponge-sample, the entrapped microorganisms are removed by adding 99 ml of diluent to the bag containing the sponge and then repeatedly compressing the sponge for 30 sec or more. Appropiate dilutions are made and then handled according to the analytical method desired. In quantitative determinations we have found this technique to produce consistent and useful information for evaluating cleaning and sanitizing procedures. The cellulose sponge technique has been used to sample equipment surfaces, floors, walls, and carcasses.

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J. Milk Food Technol. Vol. 38, No. 9, Pages 505-508 (September, 1975)

Survival of *Clostridium perfringens* During Preparation of Precooked Chicken Parts

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ABSTRACT

Vegetative cells of *Clostridium perfringens* were completely destroyed and the number of viable spores was reduced by procedures used to process precooked chicken thighs and breasts. A mixed inoculum of vegetative cells of *C. perfringens* CDC strains 7947 and 7948 was killed in thighs and breasts cooked in water at 82 C for 20 min and at 93 C for 15 min. Heat-sensitive spores of *C. perfringens* strain 7947 were reduced to low levels after 43 min at 82 C and completely eliminated after 38 min at 93 C. Heat-resistant spores of *C. perfringens* strain 7948 were not reduced when water-cooked at 82 C for 50 min nor completely destroyed after 45 min at 93 C. *C. perfringens* 7948 spores survived during frozen storage of inoculated thighs which were cooked at 82 C for 20 min (40-50%) and at 93 C for 45 min (23-30%). Numbers of spores were further reduced after reheating frozen pieces at 192 C for 30 min. Thighs were coated with inoculated batter and breading and flash fried at 192 C for 30, 45, or 60 sec resulting in destruction of more than 99% of vegetative cells and more than 90% of heat-resistant spores.

Poultry products are often the vehicles in food poisoning outbreaks caused by *Clostridium perfringens* (*3*). Carcasses have been shown to be contaminated with *C. perfringens* at various stages of processing (*4, 5, 9*). Roberts (*11*) reported that 63% of frozen chicken carcasses (containing giblets) sampled from processing plants carried *C. perfringens.* Lillard (*5*) isolated *C. perfringens* by enrichment techniques from 2.6% of chicken cooked in processing plants.

Use of commercially-processed chicken is increasing in chain food-service establishments, restaurants, schools, and hospitals. Water cooking is widely used by processors in preparing a variety of precooked chicken products. Since some spores of *C. perfringens* are heat-resistant, they may survive cooking procedures, and grow readily at appropriate temperatures in cooked chicken (*8, 11*) creating a potential health hazard.

The purpose of this study was to determine the effect of water-cooking, flash-frying, frozen storage, and reheating on survival of *C. perfringens* in precooked chicken parts.

EXPERIMENTAL PROCEDURE

Vegetative cell and spore suspensions of *C. perfringens* CDC strains 7947 and 7948 were prepared according to the procedure of Craven and Lillard (*2*).

Raw chicken thighs and breasts packed in ice were purchased from a local processor and within 2 h removed from ice, placed in freezer bags and stored at − 34 C. Before cooking experiments, portions were thawed 2-4 h at room temperature and then overnight at 4 C. Only those thighs from 80 to 120 g and breasts from 120 to 160 g were used.

For each trial, each of two breasts and two thighs was inoculated with a total of 1.0 ml of an inoculum by injecting 0.2 ml of either vegetative cell or spore suspension at five points (approximately equidistant from each other) at a 15-mm depth with a syringe and 25-gauge needle. The average maximum thickness of breasts was 30 mm and average maximum of thighs was 36 mm. Thighs and breasts were inoculated with a mixture of *C. perfringens* strains 7947 and 7948 vegetative cells (1.1×10^8 cells per piece), 7947 spores (7.0×10^5 spores per piece) or 7948 spores (4.8×10^6 spores per piece). Although surface contamination of chicken parts would be more likely in commercial practice, there is some evidence that internal tissues may become contaminated during processing (*6, 7*). Thus internal rather than surface inoculation was used. In addition, reduction in numbers of cells of spores by washing off if surface inoculation had been used was avoided. Preliminary experiments showed that there was no loss of cells or spores by washing out or leaking of the internal inoculum. The inoculated breasts and thighs held at 10 - 13 C were immersed in a pre-heated, 82 C ± 1, water bath, for 20, 28, 35, 43, or 50 min or in a preheated, 93 C ± 1, water bath for 15, 23, 30, 38, or 45 min. Two breasts and two thighs were cooked at each time-temperature relationship. Water temperatures and times were selected to represent a range that would encompass commercial water-cooking conditions for broiler parts. Copper-constantan thermocouple probes attached to a Honeywell potentiometer (Electronik 16) were inserted into each inoculated breast and thigh at a depth of 15 mm to follow internal temperatures. After cooking for specific time and temperature, pieces were removed and placed for 20 min in tap water at room temperature. Inoculated raw breasts and thighs held at 10-13 C during the cooking procedure served as controls. Pieces were sampled for survival of *C. perfringens.*

To determine the effect of processing steps subsequent to water cooking and of frozen storage, raw thighs were inoculated as above with *C. perfringens* 7948 spores and water-cooked at the lower and upper treatment extremes (82 C for 20 min or 93 C for 45 min). After cooling in running tap water (23 C) thighs were dipped in flour-based batter (Newly Weds Cracker Co. #234) followed by breading (Newly Weds Cracker Co. #497) and flash-fried at 192 C for 45 sec in frying fat (Frymax). The fully-processed thighs were placed in small sterile plastic bags and stored in a blast freezer (− 23 C) for 1 or 4 weeks. After frozen storage 50% of the thighs were placed in a rotary dry-heat oven (Despatch) and cooked at 192 C for 30 min (temperature and time recommended by processor to reheat frozen precooked chicken).

In a separate trial, inoculated thighs were water-cooked at 82 C for 20 min or 93 C for 45 min, frozen at − 23 C for 1 week, and reheated in the dry-heat oven (192 C) to an internal temperature of 71 C. After removal from the oven, maximum internal temperatures were recorded. The reheated thighs were sampled for *C. perfirngens.*

To determine the effect of flash-frying on *C. perfringens* in batter and breading, a group of thighs were water-cooked at 93 C for 23 min,

allowed to cool to room temperature, battered and breaded. A 1.0-ml inoculum of a mixture of *C. perfringens* 7947 and 7948 vegetative cells or a 1.0-ml inoculum of 7948 spores was placed over the surface of the breading with a syringe and allowed to absorb. The inoculated thighs were flash-fried at 192 C for 30, 45, or 60 sec. Pieces were sampled for *C. perfringens* immediately after frying. Other pieces were sampled after frozen storage (– 23 C) and after reheating at 192 C for 30 min.

In all trials numbers of *C. perfringens* surviving were determined by aseptically separating all meat of each piece from the bone and placing it in a sterile blender jar. A sufficient volume of 0.1% peptone, 0.1% sodium thioglycollate solution was added to yield a 1/5 dilution. The mixture was blended for 1 min at high speed in an Oster blender and serial dilutions were prepared. Pour plates were prepared using sulfite-polymyxin B-sulfadiazine (SPS) agar. Plates were incubated at 35 C for 24 h in an atmosphere of 80% nitrogen, 10% hydrogen, 10% carbon dioxide. Enrichment techniques using cooked meat medium were also used (*2*).

Results were reported as percent survival,

$$\text{i.e. \% survival} = \frac{\text{colony forming units/cooked piece}}{\text{colony forming units of uncooked control}} \times 100$$

RESULTS

Cooking chicken thighs and breasts in water at 82 C for 20-50 min and 93 C for 15-45 min resulted in internal temperatures of 77 C or higher (Table 1). Both internal temperature attained and time that internal temperature was above 77 C were related to temperature-time treatment at the 1% level of significance. No statistical difference was noted between breasts and thighs when internal temperatures or times above 77 C were compared.

TABLE 1. *Mean internal temperature (C) and minutes above 77 C of chicken breasts (120-160 g) and thighs (80-120 g) after water cooking*

Water cooking Temperature-time (C-min)	Final internal temperature		Time above 77 C	
	Breasts (C)	Thighs (C)	Breasts (min)	Thighs (min)
82-20[1]	79^{a}	77^{a}	4^{a}	0^{a}
82-28[2]	$80^{a,b}$	80^{b}	9^{b}	8^{b}
82-35[1]	$82^{b,c}$	82^{b}	17^{c}	16^{c}
82-43[2]	$82^{b,c}$	82^{b}	31^{d}	28^{d}
82-50[2]	$82^{b,c}$	82^{b}	29^{d}	30^{d}
93-15[1]	84^{a}	85^{a}	4^{a}	6^{a}
93-23[2]	91^{b}	89^{b}	13^{b}	10^{a}
93-30[1]	92^{b}	$91^{b,c}$	19^{c}	17^{b}
93-38[2]	92^{b}	92^{c}	24^{d}	25^{c}
93-45[2]	92^{b}	92^{c}	33^{e}	30^{d}

[1]Values represent mean of 8 pieces.
[2]Values represent mean of 4 pieces.
For each temperature treatment, values within a column followed by the same letter are not significantly different.

When thighs and breasts inoculated with *C. perfringens* 7947 spores (heat-sensitive) were cooked at 82 C, survival decreased with increasing cooking times resulting in low levels of cells (breasts, 0.3%; thighs, 5.5%) after 43 min (Table 2). When breasts and thighs were cooked at 93 C for 15 to 30 min, low levels of heat-sensitive spores were detected. No viable 7947 spores were isolated from chicken cooked at 93 C for 38 min.

Levels of *C. perfringens* 7948 spores (heat-resistant) were not reduced in thighs and breasts cooked in water at

TABLE 2. *Percent survival of* Clostridium perfringens, *CDC strains 7947 and 7948 vegetative cells and spores in chicken breasts and thighs after water cooking*

Water Cooking Temperature-time (C-min)	Vegetative cells[1]		CDC strain 7947 spores[2]		CDC strain 7948 spores[3]	
	Breasts (%)	Thighs (%)	Breasts (%)	Thighs (%)	Breasts (%)	Thighs (%)
82-20	0[4]	0	$13.8^{5,a}$	44.1^{a}	$132.3^{5,a}$	99.2^{a}
82-28			5.4^{b}	24.7^{b}		
82-35			0.8^{b}	11.8^{b}	119.2^{a}	87.0^{a}
82-43			0.3^{b}	5.5^{c}		
82.50					95.6^{a}	112.2^{a}
93-15	0	0	5.2^{a}	21.9^{a}	101.5^{a}	176.4^{a}
93-23			0.05^{a}	0.8^{b}		
93-30			0.04^{a}	0.2^{b}	44.4^{b}	61.9^{b}
93-38			0^{a}	0^{b}		
93-45					42.8^{b}	38.5^{c}

[1]Mixed inoculum of cells from CDC strains 7947 and 7948-1.1×10^{8} cells/piece.
[2]Initial population (inoculum)-7.0×10^{5} spores/piece.
[3]Initial population (inoculum) - 4.8×10^{6} spores/piece.
[4]Each value for vegetative cells represents average of 8 samples. "0" denotes no recovery of cells by direct plating or enrichment techniques.
[5]Each value for spores represents average of 4 samples.
For each temperature treatment, values within a column followed by the same letter are not significantly different.

TABLE 3. *Percent survival of* Clostridium perfringens *CDC strain 7948 spores[1] in thighs after water cooking + flash frying + freezing and after reheating of frozen thighs*

Treatment	Average internal temperature after reheating (C)	Frozen storage[2] 1 week (%)	Frozen storage[2] 4 weeks (%)
Water cooked 82 C - 20 min + flash frying[3] + frozen storage		54.0[4]	54.8[4]
Water cooked 82 C - 20 min + flash frying + frozen storage + reheating[5]	67	10.9[4]	16.8[4]
Water cooked 93 C - 45 min + flash frying + frozen storage		30.3[6]	23.1[6]
Water cooked 93 C - 45 min + flash frying + frozen storage + reheating[5]	79	0.6[6]	2.6[6]

[1]Initial population (inoculum) - 4.8×10^{6} spores/piece
[2]-23 C
[3]191 C for 45 sec
[4]mean of 6 samples
[5]191 C for 30 min
[6]Mean of 9 samples

82 C for 50 min or 93 C for 15 min (Table 2). In some treatments spores were apparently stimulated and germinated to give higher counts than the initial inoculum. Spore counts were reduced by about 40% to 60% after 30 to 45 min of water cooking at 93 C.

Analysis of variance showed that survival of heat-sensitive *C. perfringens* 7947 spores was higher in thighs than breasts, and dependent on cooking temperature and time ($p = .01$). Survival of heat-resistant *C. perfringens* 7948 spores at 93 C depended on cooking time alone, while at 82 C survival was not decreased by time of heating ($p = .01$).

Frozen storage (–23 C), for 1 and 4 weeks, of thighs previously inoculated with heat-resistant (7948) spores and cooked in water at 82 C for 20 min or 93 C for 45 min (Table 3), resulted in a greater reduction ($p = .01$) of viable spores as compared to pieces water-cooked only (Table 2). Reheating (192 C for 30 min) the frozen thighs cooked in water at 82 C for 20 min resulted in 11% to 17% survival ($p = .01$) of *C. perfringens* 7948 spores (Table 3). Reheating of thighs cooked in water at 93 C for 45 min resulted in significantly lower (0.6% to 2.6%) survival ($p = .01$) of these spores. No statistical difference in survival was noted between thighs stored for 1 week and 4 weeks.

TABLE 4. *Percent survival of* Clostridium perfringens *7948 spores in thighs after water cooking and flash frying and freezing and reheating to an internal temperature of 71 C*

Original water-cooking time-temperature combination	%Survival after flash-frying frozen storage (1 week), and reheating[1] to 71 C
82 C for 20 min	4.1[2]
93 C for 45 min	2.7[2]

[1]Reheating times varied from 27 to 35 min.
[2]Mean of 10 samples.

Table 4 shows the extent of survival of heat-resistant 7948 spores in frozen (1 week), cooked thighs when reheated to an internal endpoint temperature of 71 C-72 C. The average percent survival of spores after reheating was 4.1 for thighs cooked at 82 C for 20 min and 2.7 for those cooked at 93 C for 45 min. This difference was significant at the 5% but not at the 1% level.

Table 5 shows the results after flash-frying, freezing, and reheating of breaded and battered thighs that had been surface inoculated with *C. perfringens* vegetative cells or spores. After thighs were flash-fried, some vegetative cells (0.1-1.0%) and heat-resistant spores (1.6-8.1%) survived. After frozen storage of thighs for 1 to 4 weeks, vegetative cells were detected in low numbers (0.04-0.1%) and spore levels were reduced to 0.5-5.5%. After reheating (192 C for 30 min) frozen thighs, which resulted in an average internal end point temperature of 77 C, vegetative cells could be detected only by enrichment in 2 of 18 samples. Spores were reduced to a low levels (0.02-1.0%) after reheating.

Frying time (30, 45, or 60 sec) did not significantly affect survival of vegetative cells after initial flash frying or after frozen storage and reheating. However,

TABLE 5. *Percent survival of* Clostridium perfringens *CDC strains 7947 and 7948 vegetative cells[1] and CDC strain 7948 spores[2] in batter and breading of thighs after flash frying, frozen storage and reheating*

Treatment	Initial frying		1 week		Frozen storage 4 weeks	
	Veg. Cells (%)	Spores (%)	Veg. Cells (%)	Spores (%)	Veg. Cells (%)	Spores (%)
Flash frying[3]						
30 sec	1.0[a]	8.1[a]				
45 sec	0.4[a]	2.6[b]				
60 sec	0.1[a]	1.6[c]				
Flash frying						
30 sec			0.1[a]	5.5[a]	0.1[a]	4.8[a]
45 sec			0.1[a]	2.1[b]	0.1[a]	1.5[b]
60 sec			0.04[a]	0.6[c]	0.1[a]	0.5[c]
+ frozen storage[4]						
Flash frying						
30 sec			0[6,a]	1.0[a]	ND[7]	0.2[a]
45 sec			0[8,a]	0.3[a]	ND	0.1[a]
60 sec			0[9,a]	0.1[a]	ND	0.02[a]
+ frozen storage						
+ reheating[5]						

[1]1.1×10^8 cells/piece
[2]4.8×10^6spores/piece
[3]191 C
[4]–23 C
[5]191 C/30 min
[6]0 of 5 + by enrichment
[7]not determined
[8]1 of 6 + by enrichment
[9]1 of 7 + by enrichment
For each treatment, values within a column followed by the same letter are not significantly different.

differences in frying time did significantly affect survival of spores after initial flash-fry and after frozen storage, but not after reheating. Both freezing and reheating significantly reduced survival. Survival of vegetative cells or spores after 4 weeks of frozen storage was not significantly different from that after 1 week of frozen storage.

Survival of *C. perfringens* 7948 spores was higher at all time-temperature combinations of water cooking (Table 2) as compared to flash-frying (Table 5). However, vegetative cells did not survive water cooking but a few did survive flash-frying.

DISCUSSION

Precooked chicken, as prepared by the poultry processor, is used in a variety of convenient foods that are widely distributed. Chicken pieces (thighs, breasts, drumsticks, and wings) are cooked in water, steam, oil, or by microwaves. Cooking in water is one of the most widely used methods particularly in the preparation of frozen pre-fried chicken. Pieces are cooked in water for a given time, cooled in tap water, covered with commercial batter and breading, browned by flash-frying, and frozen. The product is reheated from the frozen state (usually by dry-heat oven or microwave oven) immediately before serving.

As shown in Table 2, vegetative cells of *C. perfringens* are easily destroyed by cooking thighs and breasts in water at any of the temperature-time combinations which results in a minimum final internal temperature of

77 C. Heat-sensitive spores of *C. perfringens,* however, can be significantly reduced only by internal temperatures of 82 C. Heat-resistant spores survived all temperature-time combinations at high levels indicating that subsequent processing and handling would determine the relative safety of chicken contaminated with such spores.

Strong and Canada (*12*) reported that *C perfringens* vegetative cells are greatly reduced in chicken gravy when frozen. Spores survive at high levels after such treatment. After heating in beef (*1*) and in meat pies (*10*) spores lost most of their heat resistance and decreased to low levels after further heating. In our studies frozen storage (Table 3) reduced survival of spores in thighs previously exposed to cooking in water and flash-frying in fat. Since vegetative cells are sensitive to freezing temperatures, the reduction indicates that many spores germinated during water-cooking or flash-frying and were subsequently destroyed. The further reduction to low levels after reheating the thighs also suggests that spores had germinated and lost their heat resistance. Table 3 shows that when thighs previously inoculated with spores, cooked in water at 93 C for 45 min and frozen were reheated at 191 C for 30 min, levels of survival (0.6-2.6%) were lower than in thighs treated in like manner but originally cooked in water at 82 C for 20 min (10.9-16.8%). These results could be due to greater germination and destruction of spores in thighs cooked at 93 C for 45 min rendering surviving spores more sensitive to reheating. But the fact that thighs originally water-cooked at 93 C for 45 min reached an internal temperature of 79 C when reheated while thighs water-cooked at 82 C for 20 min reached an internal temperature of 67 C when reheated (Table 3) could also explain the difference in survival of spores from the two treatments after reheating. When in a separate experiment all thighs were reheated for various times to an internal temperature of 71 C (Table 4), those previously inoculated and water-cooked at 93 C for 45 min showed spore survival at the 2.7% level and those cooked at 82 C for 20 min showed spore survival at the 4.1% level. Since this difference is significant at the 5% level but not 1% level, the difference (Table 3) between survival of spores after reheating of thighs originally cooked at 82 C for 20 min and 93 C for 45 min was the most part due to different internal temperatures after reheating.

C. perfringens may contaminate the surface of chicken after water-cooking. The bacterium has been detected in 9.5% of batter and 29.4% of flour for processing (*5*). Flash-frying, freezing, and reheating thighs reduce *C. perfringens* vegetative cells and spores which may contaminate batter and breading (Table 5), but a small percentage of vegetative cells and spores may still survive.

Evidently, the combination of water-cooking at certain temperature-time combinations (Table 3) and flash frying reduces survival of spores and causes others to germinate as shown by less growth after reheating. However, in both heat treatments, some spores retain their heat-resistant properties and survive even reheating temperatures. Since spores were not completely eliminated by any individual processing step (water cooking, flash frying, freezing, or reheating), or even by any combination of steps, subsequent mishandling of precooked chicken could provide temperatures which would allow germination of spores and subsequent multiplication of vegetative cells. Although levels of spores used in this study would rarely be encountered in commercially prepared chicken, precooked chicken parts contaminated with *C. perfringens* spores, even at low levels, could conceivably present a food poisoning hazard. Since it would be impractical to cook chicken at a temperature-time combination sufficient to insure destruction of all spores, elimination of food poisoning hazard from precooked chicken products must be attained by proper handling during distribution and marketing and during preparation for serving.

ACKNOWLEDGMENTS

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Evaluation of Household Dishwashing Machines for use in Small Institutions[1]

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ABSTRACT

Performance of three kinds of household dishwashing machines was evaluated with respect to certain criteria for performance of commercial spray-type machines. These criteria concern removal of soil from tableware, achievement of a specified "cumulative heat factor," and reduction of bacteria on tableware below a maximum permissible count. Machines of one group had a sanitizing wash or rinse in their cycles. This step ended only after temperature of the sanitizing wash or rinse water reached a minimum of 150 F. These machines met the criteria regardless of the temperature of their inlet water. The second type of machine had no sanitizing wash or rinse in its cycle. The third type had a sanitizing step, but it ended after 15 min regardless of the temperature of the water; this type also had a forced hot-air drying feature. Machines of the second and third groups met the criteria only when the temperatures of their inlet water was 155 F or higher.

It is seldom economical or practical to use either commercial spray-type (institutional) dishwashing machines or manual dishwashing for food service operations in day care centers, nurseries, kindergartens, nursing homes, half-way houses, and other small establishments that serve food once a day or that serve three meals a day to only a few people. Household dishwashing machines could be an answer, however. But does their capability meet the standards established for tableware washed in commercial spray-type (institutional) dishwashing machines?

Standards for construction and performance of commercial spray-type dishwashing machines have been set. Mallmann et al. (*6*), Mallmann and Kahler (*5*), and the National Sanitation Foundation Testing Laboratory (*7*) conducted extensive studies on physical and bacteriological cleanliness of tableware. Thermal (*2*) and bacteriological (*10, 11*) standards were established for tableware washed in commercial establishments. A Joint Committee on Food-Equipment Standards (*3*) sponsored by the National Sanitation Foundation set construction and operational standards for commercial spray-type dishwashing machines. These standards have rarely been applied either to the installation or operation of household dishwashing machines or to the evaluation of the cleanliness of tableware washed by such machines. This study evaluates household dishwashing machines on the basis of these standards.

MATERIALS AND METHODS

Dishwashing machines were tested in day care centers, kindergartens, nursing homes, private homes, and equipment manufacturers' and distributors' showrooms. Most machines had been in use for sometime, in some instances for years. Only a few new or demonstrator models were evaluated. Machines were operated with the recommended amount of a commercial dishwashing machine detergent and available or selected-temperature hot water (of from 100 F to 161 F) at full and at sanitizing (if available on the machine) cycles. Machines were operated with full or nearly full loads depending upon the amount of soiled tableware available for washing (except some trials of machine G). Tableware was arranged in manufacturers' recommended positions or as routinely placed in the machine by the operator. Eleven dishwashing machines, representing a total of seven brands, were evaluated. (In fact, this evaluation represented many more brands because a few manufacturers make several brands.) Usually, more than one test was done on each machine at different cycles and water temperatures.

Three types of household dishwashing machines were evaluated. (*a*) Dishwashers that lacked or that were operated without sanitizing wash or rinse cycles (identified as machines that lacked sanitizing cycles). (*b*) Dishwashers that had sanitizing wash or rinse cycles and a thermostat that had to sense a temperature of 150 F or higher before the machine would advance to the next step in its cycle (identified as machines that have temperature-activated sanitizing cycles). (*c*) Dishwashers that have sanitizing rinse cycles and have a thermostat as above, which advance to the next step in its cycle after 15 min if the temperature required to operate the thermostat is not reached. These machines were also equipped with a fan to blow heated air over tableware during the drying (identified as machines with time-controlled sanitizing rinse cycles and forced air-flow drying).

Tableware[4] temperature evaluations

Temperatures of tableware were taken by affixing premium grade, type T[5], open-ended thermocouples to tableware in three ways: (*a*) taped to the surface so that the thermocouple sensor was in contact with the tableware surface but still exposed to the water and air; (*b*) taped to the surface so that the thermocouple was completely covered by the tape; and (*c*) inserted into small diameter glass tubing closed at one

[1] *Use of trade names is for identification purposes only and does not constitute endorsement by the U.S. Department of Health, Education, and Welfare or the Georgia Department of Human Resources.*
[2] *Center for Disease Control*
[3] *Georgia Department of Human Resources*
[4] *Tableware refers to a china or plastic plate, bowl, or cup; glass; or stainless-steel knife, fork or spoon.*
[5] *Type T refers to copper-constantan junction.*
[6] *Temperature were recorded on Chart 5270 (type T), 0 to 500 F range in an Electronik 16 multipoint Recorder, Honeywell, Fort Washington, Pa.*

end, and taped to the tableware surface. Because no significant difference was observed in temperature recordings by the three procedures, later recordings were made by the second method only. Scotch pressure-sensitive, high-temperature, tape No. 236 or No. 238 was used to affix thermocouple or tubing to the tableware. Air or spray-water temperatures were taken with the same type thermocouples. The leads of which were wrapped around racks or taped in such a way that temperatures were registered within an inch of tableware, in the bottom drain area, or close to fans. Data were recorded on a chart in a potentiometer[6].

The cumulative heat factor—cumulative heat exposure in seconds at or above 140 F on tableware surface during the entire dishwashing period (or a complete phase of its cycle) equivalent to the cumulative time-temperature exposure required for pasteurization of milk—was calculated from recorded data. Heat units were computed by dividing 1800 by the seconds required for pasteurization, based upon a line drawn through former milk pasteurization standards of 1800 sec at 143 F and 15 sec at 161 F when these data were plotted on semi-log paper (*2, 7*). For instance, one heat unit is equivalent to 1 sec of 143 F. Calculations were based on a line drawn through a point of 1 heat unit equivalent at 143 F and a point of 120 heat unit equivalents at 161 F when these data were plotted on semi-log paper (z-value = 8.7 F). Heat units were calculated by selecting the unit equivalent to the nearest degree of temperature at which tableware was entirely above for 1 min. Each minute period was multiplied by 60. The total time of exposure was added to derive the cumulative heat factor.

Physical cleanliness evaluations

A test soil, as described by Mallmann et al. (*6*), was applied to tableware with a paintbrush and allowed to air dry approximately 30 min before the tableware was washed. The washed tableware was then evaluated by sight and touch.

Bacteriological evaulations.

Two milliliters of a 24-h old broth culture of *Serratia marcescens* was added to 100 ml of the test soil. This organism, although relatively heat-sensitive, was used to ensure the presence of bacteria on dishes but yet not introduce a foodborne disease into kitchen environments. The inoculated soil was applied to selected tableware previously contaminated by use or, in some instances, through long-time storage and allowed to air dry. Thus, organisms other than those in the test soil were present on dishes before washing. Half of the contaminated tableware was swabbed before washing. The other half of the tableware—of the same kinds as had been swabbed before washing—was marked and also swabbed after washing. Tableware was swabbed and samples were analyzed according to the methods recommended by the Subcommittee of Food Utensil Sanitation (*10*). Organisms recovered from washed dishes were not identified.

TABLE 1. *Temperature of tableware surfaces during each cycle of washing in household dishwashing machines*

Brand	Machine	Tap water temperature (F)	Highest surface temperature of tableware during prewash cycle (F)	Highest surface temperature of tableware during wash cycle (F)	Highest surface temperature of tableware during rinse cycle (F)	Highest surface temperature of tableware during sanitizing cycle (F)	Highest surface temperature of tableware during drying cycle (F)
A	1	138	107	126	133	NA[1]	137 (p)[2]
	1	149	118	134	142	NA	144 (p,u)
B	1	154	145	150	149	NA	155 (g)
							151 (p)
							150 (u)
	1	158	140	148	152	NA	156 (g,u)
	2	152	135	150	149	—[3]	182 (p)
							147 (c)
							144 (u)
							146 (p)
	2	160	138	152	151	175	173 (p,c,u)
C	1[4,5]	142	134	148	147	144	187 (p)
							160 (u)
D	1[5]	140	NA	146	146	150	149 (p,u)
E	1	154	134	140	148	—[3]	212 (p)
							164 (c)
							152 (u)
	1	154	112	153	151	148	203 (p)
							196 (c)
							152 (u)
F	1[4]	126	108	115	119	176	175 (p)
							164 (c,u)
G	1	126/110[6]	113	118	118	140	140 (p)
G	2[4]	134/100[6]	122	130	130	140	139 (p)
	2	140	123	134	138	150	148 (u)
							145 (p)
	2	146	130	142	143	150	147 (p)
							145 (u)
	2	150	135	144	147	154	151 (u)
							148 (p)
Gm[7]	1	158	145	155	158	[8]	157 (p)
Gm	1	161	150	160	157	—[8]	157 (p)
							154 (u)
							144 (b)
Gm	2	153	133	141	144	160	157 (p,g)
Gm	2	155	132	140	142	159	157 (p)

[1]Na = Cycle not available on machine. [2](p) indicates temperature of plate, (u) stainless steel utensil, (g) glass, (c) cup, (b) bowl.
[3]Cycle available on machine but not used.
[4]New machine.
[5]Sanitizing wash cycle as well as rinse cycle.
[6]Drop in water temperature from prewash cycle to rinse cycle.
[7]Machine modified by replacing factory thermostat with a 160 F thermostat for sanitizing cycle control.
[8]Cycle skipped because of water temperature.

TABLE 2. *Heat unit equivalents on tableware surfaces during each cycle of washing in household dishwashing machines*

Brand	Machine	Tap water temperature (F)	Heat factor accumulated during each cycle[1]					Cumulative heat factor total of all cycles[1]	Cumulative heat factor including drying in machine after washing[1]
			Prewash	Wash	Rinse	Sanitizing	Drying		
A	1	*138*	0	0	0	NA[2]	0	0	0 (p)[3]
	1	149	0	0	120	NA	1,100	1,200	1,200 (p,u)
B	1	154	360	1,300	2,000	NA	7,000	11,000	15,000 (g)
							2,700	6,400	14,000 (p)
							2,400	6,100	7,300 (u)
	1	158	60	700	3,500	NA	8,100	12,000	21,000 (g)
							6,300	11,000	20,000 (u)
							3,900,000	3,900,000	10,000,000 (p)
	2	152	0	700	1,400	—[4]	300	2,400	2,400 (c)
							170	2,300	2,300 (u)
							100	2,200	2,200 (p)
	2	160	0	1,800	1,200	630,000	95,000	730,000	740,000 (p)
							440,000	1,100,000	1,100,000 (c)
							94,000	730,000	730,000 (u)
C	1[5,6]	142	0	600	260	380	6,500,000	6,500,000	70,000,000 (p)
							17,000	18,000	29,000 (u)
D	1[6]	140	—	460	570	540	1,700	3,300	3,700 (p,u)
E	1	154	0	270	2,000	—[4]	9,100,000,000	9,100,000,000	13,000,000,000 (p)
							72,000	74,000	136,000 (c)
							6,900	9,200	11,000 (u)
	1	154	0	9,500	1,800	1,200	850,000	860,000	840,000,000 (p)
							5,200,000	5,200,000	170,000,000 (c)
							8,000	21,000	21,000 (u)
F	1[5]	126	0	0	0	2,500,000	330,000	2,800,000	4,600,000 (p)
							280,000	2,800,000	2,900,000 (c)
							260,000	2,800,000	2,800,000 (u)
G	1	126/110[7]	0	0	0	52	0	52	52 (p)
G	2[5]	134/100[7]	0	0	0	0	0	0	0 (p)
	2	140	0	0	0	210	120	330	330 (u)
							27	240	240 (p)
	2	146	0	62	410	410	46	930	930 (p)
							27	910	910 (u)
	2	150	0	310	1,100	770	840	3,000	3,000 (u)
							250	2,500	2,500 (p)
Gm[8]	1	158	110	6,000	24,000	—[9]	6,100	36,000	36,000 (p)
Gm	1	161	9,300	26,000	5,000	—[9]	640	42,000	43,000 (p)
							320	42,000	42,000 (u)
							200	42,000	42,000 (b)
Gm	2	153	0	130	330	9,000	8,200	17,000	18,000 (p)
							8,000	17,000	17,000 (p,g)
Gm	2	155	0	60	330	3,600	5,900	10,000	10,000 (p)

[1]Based on time-temperature standards for pasteurization of milk; to first two significant numbers.
[2]NA = Cycle not available on machine.
[3](p) indicates temperature of plate, (u) stainless steel utensil, (g) glass, (c) cup, (b) bowl.
[4]Cycle available on machine but not used.
[5]New machine.
[6]Sanitizing wash cycle as well as rinse cycle.
[7]Drop in water temperature from prewash cycle to rinse cycle.
[8]Machine modified by replacing factory thermostat with a 160 F thermostat for sanitizing cycle control.
[9]Cycle skipped because of water temperature.

RESULTS

The highest temperatures on tableware surfaces and the cumulative heat factors at each phase of the dishwashing cycle of each dishwashing machine tested are shown in Tables 1 and 2, respectively. Typical sanitizing rinse cycles), 3, 4, 5, and 6 (machines with time-temperature recordings for dishwashers are illustrated in Fig. 1 and 2 (machines that lacked temperature-activated sanitizing cycles), and 7 and 8 (machines with time-controlled sanitizing-rinse cycles with forced air-flow drying).

Heat treatment of tableware varied with different cycles. Pre-wash cycles usually did not offer any appreciable heat treatment. Measurable heat was generated on tableware during this cycle in only machines B1 and G1. Both were operated with water temperatures above 150 F. When inlet water temperatures, as measured at a nearby water faucet, were above 150 F, wash and rinse cycles usually generated considerable heat on tableware surfaces. Half the machines that had sanitizing cycles generated enough heat during this cycle to satisfy requirements for commercial spray-type dishwashing machines. All machines, except machines G and Gm which had forced air-flow drying, generated most heat during the drying cycle. This was in part due to the effect of the heat previously generated during the earlier cycles and, thus, the temperature of the tableware just before drying. Tableware washed in machines (G, Gm) with forced air-flow drying showed an initial drop in surface temperatures during the drying cycle; temperature did not recover until late in the drying cycle, if at all. Only a little heat was generated on tableware surfaces during

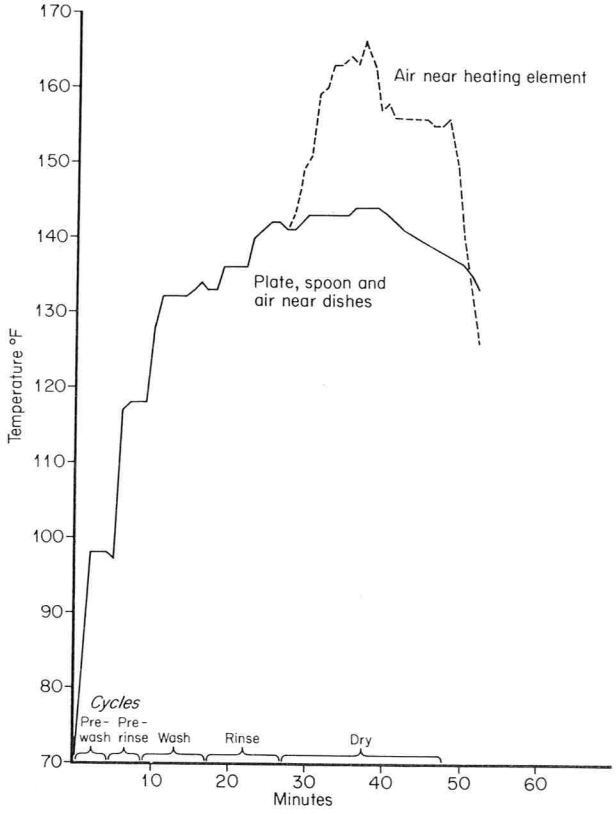

Figure 1. *Temperatures of tableware and water in household dishwashing machine without sanitizing cycle operated with water at 149 F.*

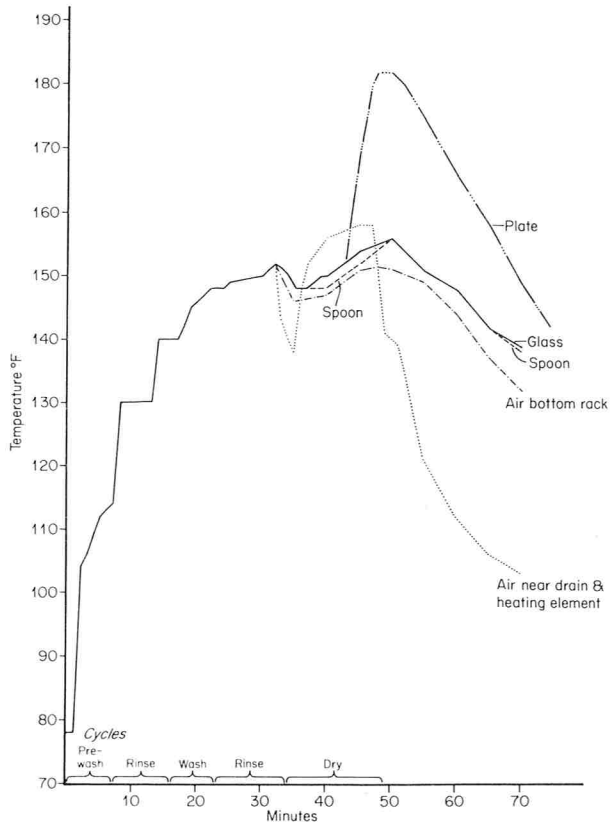

Figure 2. *Temperatures of tableware and water in household dishwashing machine without sanitizing cycle operated with water at 158 F.*

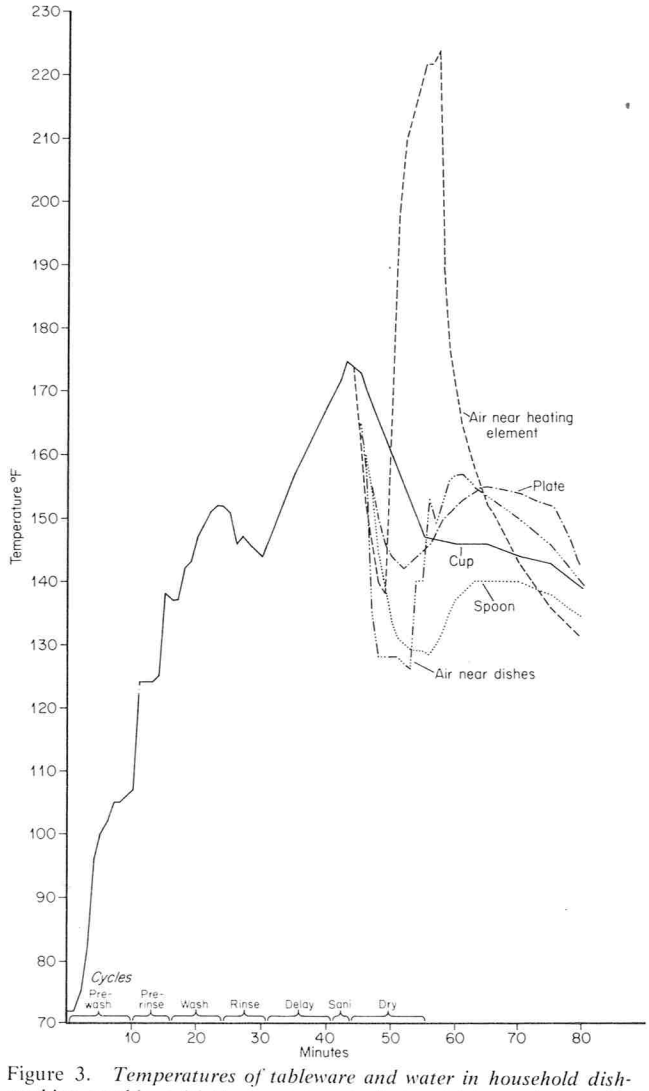

Figure 3. *Temperatures of tableware and water in household dishwashing machine with thermostat-controlled sanitizing cycle operated with water at 160 F.*

the forced air-flow drying cycle. Additional heat effects occurred on tableware after the drying cycle was completed if tableware was left in a machine with the door closed.

With the exception of plates washed with 126 to 100 F water in machine G4, tableware was clean to sight and touch after washing. Numbers of bacterial colonies of 10^3 to 10^8 per piece of soiled and contaminated tableware were reduced to low or nondetectable levels on washed tableware. On only two occasions did individual pieces of tableware or sets of four pieces have counts higher than 100 per piece. Median bacterial colony counts of washed tableware were 16 or less, frequently zero. A comparison of bacterial colony counts on tableware washed in different machines is shown in Table 3.

DISCUSSION

According to Fuchs (*2*), time-temperature exposure on the surface of tableware should be equivalent to time-temperature exposure of milk during pasteurization—30 min, or 1800 sec, at 143 F. To meet standards

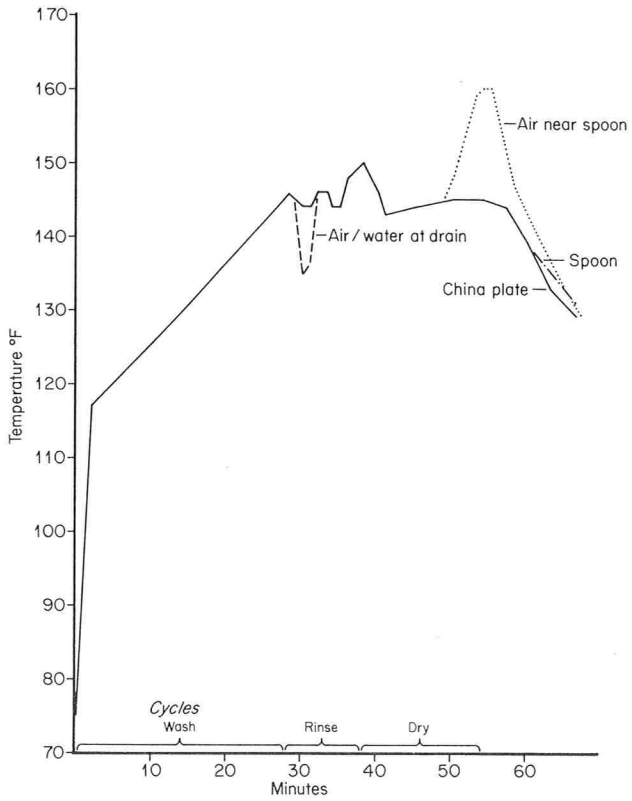

Figure 4. *Temperatures of tableware and water in portable household dishwashing machine with sanitizing cycle operated at water temperature of 140 F.*

for commercial spray-type dishwashing machines, the National Sanitation Foundation (*3*) requires machines to have water temperatures and time cycles that produce a cumulative heat factor that is twice as great as the time-temperature standard for pasteurization of milk. This requirement gives a considerable margin of safety

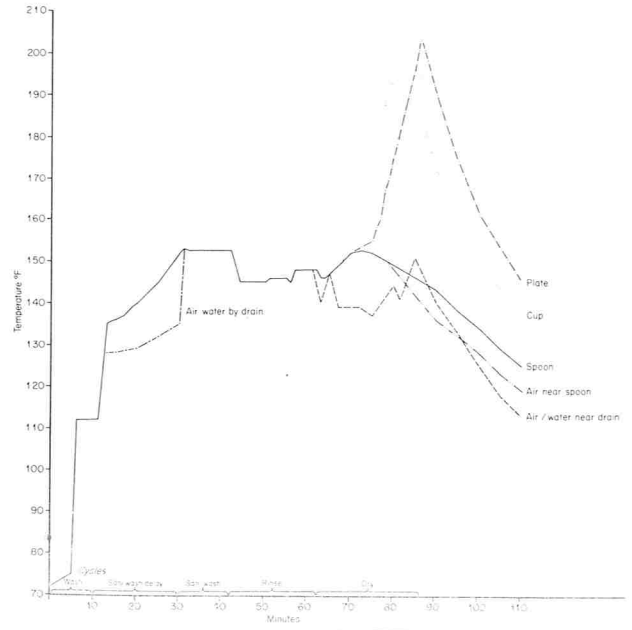

Figure 5. *Temperatures of tableware and water in household dishwashing machine with long cycle and thermostat-controlled sanitizing cycle operated at water temperature of 154 F.*

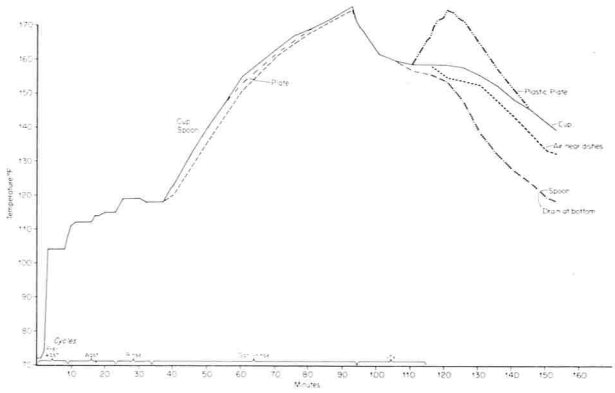

Figure 6. *Temperatures of tableware and water in household dishwashing machine with thermostat-controlled sanitizing cycle operated at water temperature of 126 F.*

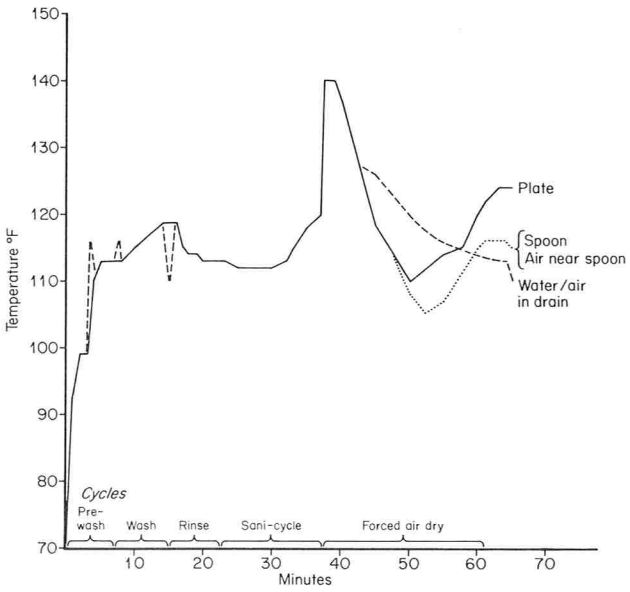

Figure 7. *Temperatures of tableware and water in household dishwashing machine with time-controlled sanitizing cycle and forced air drying operated at water temperature of 126 to 110 F.*

for the following reasons: (*a*) It is twice the heat exposure required for pasteurization of milk. (*b*) Bacteria in water are killed by a lower cumulative heat factor than is required to kill bacteria in milk because water is less viscous than milk. After initial flushing and washing of tableware, the medium on tableware surfaces in spray-type dishwashing machines would be more similar to water than to milk. (*c*) The standard for pasteurization of milk, provides a considerable margin of safety. North and Park (*8*) reported a safety factor for milk pasteurized at 142 F for 30 min to be 20 min and 6 F. During pasteurization of milk, a part of the lethal effect attributed to the holding time at a specific temperature is caused by the heat coming-up period. Cooling-down periods have a similar effect on remaining bacteria (*1*). Thus, the rate of rise and decline of temperature as well as holding time at a standard temperature affect survival of bacteria. According to thermal death time curves constructed by Kells and Lear (*4*), the former pasteurization standard of 30 min at 143 F provides a

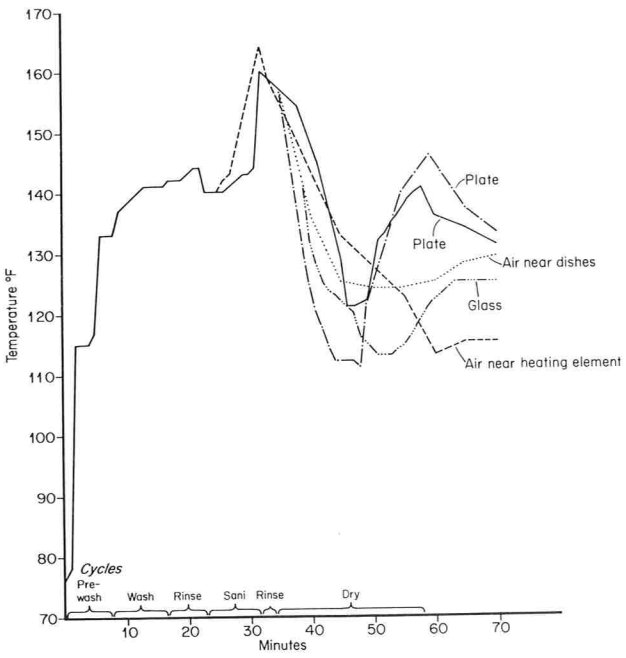

Figure 8. *Temperatures of tableware and water in household dishwashing machine with time-controlled sanitizing cycle and forced air drying-operated at water temperature of 153 F.*

margin of safety of approximately 28-½ min and the standard of 15 sec at 161 F provides a margin of safety of approximately 14 sec for destruction of *Mycobacterium bovis.* Thus, 10^6 *M. bovis*/ml heated at 143 F would be killed in 90 sec. (*d*) Pasteurization standards are based on destruction of large numbers (10^6) of *M. tuberculosis,* but tableware, which may have had very high numbers of bacteria on their surfaces before washing, would be expected to have relatively low numbers of bacteria on their surfaces after prewashing, washing, and rinsing (the cycles generally completed before most of the heat units are applied). On the otherhand, enterococci and *Coxiella burnetii* have greater heat resistance than *M. tuberculosis* or *M. bovis;* nevertheless, the other reasons are still applicable.

The criteria upon which the cumulative heat factors were established may be high—particularly in an energy-conservative era. Nevertheless, these are the criteria used to evaluate tableware washed in household dishwashing machines and as bases for recommendations for operations. They, therefore, conform to heat unit equivalent standards required for commercial, spray-type dishwashing machines.

Dishwashers that had temperature-activated sanitizing wash or rinse cycles, which operated as part of or after the full wash and rinse cycles were completed, generated enough heat on tableware surfaces to surpass requirements for commercial spray-type dishwashing machines approved by the National Sanitation Foundation, regardless of the inlet water temperature (Table 2, Fig. 3-6). This was caused by the heat generated during the delay between the full cycle and the sanitizing cycle and by spraying this sanitizing-cycle-heated water over tableware; thus, the tableware temperature was raised to a point at which the required cumulative heat factor was achieved, or tableware temperature was increased to a point that the subsequent drying cycle provided the required heat factor. The cumulative heat factors attained during dishwashing varied with make and model of machine, inlet water temperature, wattage of drying element, sanitizing-thermostat setting, and time of operation of each cycle.

Dishwashers that had time-controlled sanitizing-rinse cycles and forced air-flow drying failed to generate enough heat on tableware surfaces to meet required cumulative heat factors unless water temperature was at least 155 F or unless the machines were modified with a 160 F thermostat and water temperatures of 150 F or higher were used (Table 2, Fig. 7 and 8). This was caused by failure of these machines to raise tableware to a temperature of 150 F during wash or rinse cycles (unless water temperatures were 155 F or higher; 150 F if thermostat was replaced with a higher temperature one) and by lowering tableware temperature during initial forced-air drying and not providing ample recovery time. This occurred even though incoming air was reheated to 260 F. Cumulative heat factors on tableware surfaces were primarily a function of inlet water temperature. Sanitizing rinse water raised the temperature of tableware surfaces, but only high enough to satisfy heat unit equivalents when these surfaces were sufficiently hot from the effect of the heat generated during washing and rinsing.

TABLE 3. *Total aerobic colony count before and after washing tableware in household dishwashers at various cycles and temperatures*

		Temperature		Colony Counts per piece of tableware					
				Before wash			After wash		
Brand	Cycle[1]	Tap water	Highest on tableware	Number swabbed	Range	Median	Number swabbed	Range	Median
A	Full	138	137	10	$4.0 \times 10^3 - 7.6 \times 10^3$	4.8×10^3	10	0- 6	0.5
B	Full	154	155	6	$2.6 \times 10^7 - 1.1 \times 10^8$	6.4×10^7	6	0- 10	5
	Full	158	156	4	$5.0 \times 10^2 - 4.2 \times 10^4$	1.6×10^4	11	0- 50	0
	San 1	160	175	4	$3.0 \times 10^4 - 1.8 \times 10^6$	9.2×10^5	21	0- 0	0
C	San 1	142	187	18	$4.6 \times 10^4 - 4.8 \times 10^5$	6.7×10^4	18	0-110	16
D	San 1	140	150	16	$5.4 \times 10^4 - 1.5 \times 10^5$	6.6×10^4	16	0- 6	0
E	Full	154	212	15	$1.7 \times 10^5 - 4.4 \times 10^5$	1.7×10^5	17	0- 15	15
	San 1	154	203	17	$1.0 \times 10^5 - 1.5 \times 10^6$	1.5×10^6	17	0- 1	0
F	San 1	126	176	8	$5.6 \times 10^6 - 1.7 \times 10^7$	1.1×10^7	26	0- 0	0
Gm	San 2	158	158	12	$1.7 \times 10^4 - 1.0 \times 10^6$	1.7×10^4	29	0-200	15
G	San 2	126	140	16	$4.0 \times 10^5 - 5.2 \times 10^6$	1.2×10^6	16	0- 2	0

[1]Full refers to complete operation without sanitizing cycle; San 1 refers to machines that have temperature activated sanitizing cycles: San 2 refers to machines that have time-controlled sanitizing rinse cycles and forced-air drying.

Dishwashers that lacked sanitizing cycles also generated enough heat on tableware surfaces to equal or surpass required cumulative heat factors when inlet water temperatures were 155 F or higher (Table 2, Fig. 1 and 2), but this amount of heat was not achieved when inlet water was 152 F or below. Cumulative heat factors on tableware surfaces were also primarily a function of inlet water temperature. Drying helped add to the total but only did so when the tableware surfaces were high enough as a result of the heat generated during the wash and rinse cycles.

A temperature of 150 F on tableware surfaces will reach required (3600) heat unit equivalents in 10 min (2.5 min at 155, 40 sec at 160 F, 10 sec at 165 F, and less time at higher temperatures). So if 150 F is reached on tableware during washing or rinsing, it is probably that sufficient cumulative heat factors have been achieved when a relatively slow rate of rise and decline of temperature is considered. Inlet water temperatures of at least 155 F should cause this to happen in any type of machine operated at full or sanitizing cycles.

For practical purposes, temperatures of tableware and air (spray water) were the same during spraying operations, but during drying temperatures of different materials varied. The temperature registered in a glass encased thermometer or by a paper thermometer (*9*) after drying may be slightly different than the temperatures of individual pieces of tableware because of differences in heat absorption during drying. Thus, field-test checks should be made by reading thermometers secured to tableware, suspended near tableware, or put in racks before operation after all spraying cycles are completed, but before the drying cycle commences. A temperature of 150 F should be registered. Such testing however, will not provide assurance that the required cumulative heat factors were reached because time cannot be evaluated.

The capability of dishwashers without sanitizing cycles and those with time-controlled sanitizing rinse cycles and forced air-flow drying to achieve required cumulative heat factors can be field tested as above or simply by taking water temperature at a nearby faucet with a maximum-registering thermometer to see if it is 155 F or above. The same limitation applies as mentioned above.

The lower standard of 155 F inlet water household dishwashing machines as compared to 180 F water in the manifold of commercial spray-type dishwashing machines, the 150 F on tableware surfaces in household machines as compared to 160 F on tableware surfaces in commercial spray-type machines, can be approved because of the longer time tableware is subjected to lower but lethal temperatures in the household dishwashing machines. An analogy, for instance, is that in the approved procedures for pasteurization of milk, a temperature of 145 F for 30 min (in vat pasteurizers) is equivalent to a temperature of 161 F for 15 sec (in high-temperature, short-time pasteurizers).[7]

[7]*Calculations were based on former milk pasteurization standards because they were contemporary with the commercial dishwashing machine standards cited in this work.*

SUMMARY

Dishwashing machines (evaluated in this investigation) can, according to standards applied to commercial spray-type dishwashing machines, be used with assurance of effective bactericidal treatment of tableware if the following criteria are met: (*a*) dishwashers with properly-functioning temperature-activated sanitizing cycles can be operated at any inlet water temperature; and (*b*) dishwashers that either have no sanitizing cycle or have a time-controlled sanitizing-rinse cycle and forced air-flow drying should not be operated with inlet water temperature under 155 F.

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A Research Note

Predominant Psychrotrophic Bacteria on Fresh and Aged Ground Beef and Antelope

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ABSTRACT

Predominant psychrotrophic bacteria on fresh and aged ground beef and antelope were identified. In addition, psychrotrophic bacteria were enumerated. The predominant bacteria on ground beef and antelope were similar. A change in the predominant bacteria before and after aging was noted.

Surface bacteria normally encountered on meat include: *Pseudomonas* spp., *Achromobacter* spp., *Micrococcus* spp., *Streptococcus* spp., *Sarcina* spp., *Leuconostoc* spp., *Lactobacillus* spp., *Clostridium* spp., *Escherichia* spp., *Salmonella* spp., *Kurthia* spp., and *Streptomyces* spp. (*1, 5*). Flora shown to develop on beef under refrigeration temperatures include the following bacteria: *Pseudomonas* spp., *Achromobacter* spp., *Flavobacterium* spp., *Lactobacillus* spp., *Microbacterium* spp., *Micrococcus* spp., and *Proteus* spp. (*1, 6, 7*).

Game meat has some microbial characteristics which are similar to beef but total plate counts are often higher (*11, 12*). In addition, refrigerated poultry meat has been shown to develop microflora similar to other refrigerated meat (*3, 4, 8, 9*).

The purpose of the present study was to compare the predominant psychrotrophic bacteria on beef and antelope before and after storage at 5 C.

MATERIALS AND METHODS

During the 1973 antelope season in Wyoming two different samples of antelope meat trimmings (approximately 0.45 kg) from different carcasses were collected at each of three game processing plants. In addition, ground beef samples (approximately 0.45 kg) were collected from two of the three plants. All samples were from carcasses which had been held approximately 1 week at 5 C after slaughter.

Isolation and enumeration of bacteria were initiated on the day that the samples were collected. Antelope trimmings were ground and all samples were packaged in polyethylene coated freezer paper. They were maintained under aerobic conditions during 2 months of storage at 5 C.

Twenty-five grams of fresh or aged meat were placed in sterile Waring Blendor cups and 225 ml of sterile buffered water were added. The contents were blended at high speed for 3 min. Plate counts were performed on the blended material using plate count agar (Difco) with incubation at 5 C. After a 2-week incubation period, the plates were counted and a representative colony of each morphologically distinct colony type on the countable plates was picked for identification. Colonies were restreaked on plate count agar and purity was determined by means of a Gram stain. After purification of the isolates at 5 C, the incubation temperature for the remaining tests was changed to 20 C to obtain more rapid growth.

Identification was done using biochemical tests and several special staining procedures. Flagellation was determined by means of a flagella stain (*13*). The following biochemical tests were performed on each culture: sugar fermentation (dextrose, fructose, mannose and lactose), SIM (sulfide, indole and motility), litmus milk, catalase, oxidase, nitrate reduction, lipolysis, MR-VP (methyl red and Voges Proskauer), and gelatin and starch utilization. Cultures of gram positive rods were tested for the presence of spores by means of a spore stain and by heat shocking 2-week-old cultures at 80 C for 10 min followed by plating on plate count agar to determine survival. Cultures also were examined for pigment production, odor and morphological characteristics of the colonies. Fluorescence was determined by means of a Woods ultra violet lamp on litmus milk cultures. Identification of the isolates was made with the aid of *Bergey's Manual of Determinative Bacteriology* (*2*) and *Skerman's Guide to the Identification of the Genera of Bacteria* (*10*).

RESULTS AND DISCUSSION

Plate counts at 5 C on the fresh meat were quite variable between and within plants (Table 1). Plate counts on fresh antelope meat varied from 1.6×10^1 to 1.7×10^6/g while they were 2.2×10^4 and 4.4×10^6/g for the two fresh beef samples. After holding for 2 months at 5 C under aerobic conditions, meat samples were in the range of 10^9 bacterial cells/g.

TABLE 1. *Plate counts at 5 C per gram of fresh and spoiled antelope and beef at different processing plants*

Location	Type of Meat	Plate count	
		Fresh	Aged
Plant 1	Antelope	1.7×10^6	7.1×10^9
	Antelope	4.0×10^2	1.2×10^9
	Beef	4.4×10^6	3.0×10^9
Plant 2	Antelope	3.8×10^5	2.0×10^9
	Antelope	1.2×10^4	4.7×10^9
	Beef	2.2×10^4	2.4×10^9
Plant 3	Antelope	2.7×10^2	5.3×10^9
	Antelope	1.6×10^1	2.4×10^9

The genera and species of the predominant psychrotrophic bacteria found on the meat are presented in Table 2. A change in the types of predominant psychrotrophs occurred during aging. For example, the predominant psychrotrophs on fresh ground antelope

TABLE 2. *Genera and/or species isolated from fresh and aged meat*

Location	Type of meat	Genera and species isolated	
		Fresh	Aged
Plant 1	Antelope	*Alcaligenes* spp. *Microbacterium* spp. *Pseudomonas mephitica* *Pseudomonas* spp. (fluorescent)	*Aeromonas* spp. *Alcaligenes metalcaligenes* *Pseudomonas fragi* *Pseudomonas* spp. (flourescent)
	Beef	*Kurthia* spp. Unidentified gram negative cocci	*Aeromonas* spp. *Alcaligenes metalcaligenes*
Plant 2	Antelope	*Alcaligenes* spp. *Pseudomonas fragi* *Pseudomonas mephitica* *Psuedomonas* spp. (fluorescent) *Pseudomonas* spp. Unidentified gram negative cocci	*Aeromonas* spp. *Brevibacterium imperiale* *Flavobacterium aquatile* *Flavobacterium* spp. *Pseudomonas fragi*
	Beef	*Bacillus* spp. *Pseudomonas mephitica* *Pseudomonas* spp. (fluorescent) *Pseudomonas* spp.	*Alcaligenes metalcaligenes* *Microbacterium* spp. *Pseudomonas fragi* *Pseudomonas* spp. (fluorescent)
Plant 3	Antelope	*Alcaligenes* spp. *Kurthia* spp. *Micrococcus* spp. *Pseudomonas fragi* *Pseudomonas* spp. (fluorescent) Unidentified gram negative cocci	*Microbacterium* spp. *Pseudomonas fragi* *Pseudomonas* spp. (fluorescent)

from plant 1 were: *Alcaligenes* spp., *Microbacterium* spp., *Pseudomonas mephitica* and a fluorescent pseudomonad, whereas after aging for 2 months at 5 C the predominant psychrotrophs were *Aeromonas* spp., *Alcaligenes metalcaligenes, Pseudomonas fragi* and a fluorescent pseudomonad.

The widest variety of pseudomonads were isolated from fresh samples of antelope and beef from plant 2, thus indicating a possible inplant source of contamination. This suggests as would be expected that an important source of the microflora of the ground antelope is the processing plant. *Alcaligenes* spp. and fluorescent pseudomonads were common to all antelope samples which could mean that these bacteria were contaminants in all three processing plants. Alternatively, these bacteria could be part of the natural bacterial flora of the hoofs and hides of the antelope which could have contaminated the carcass at the time of evisceration or skinning.

The data presented here for ground antelope meat is similar to data which have been obtained for other meat (*1, 5, 6, 7*). Only the predominant psychrotrophic bacteria on fresh and aged meat exposed to aerobic conditions were studied. Other bacteria may have been present but not recovered by the procedures used in this study. Mesophilic bacteria were undoubtedly present but would not have been isolated due to the low temperature of incubation. Also had the meat been stored under anaerobic conditions it would have been expected that additional or different spoilage bacteria would have been isolated. Additional work is required to more completely identify all of the bacteria which may be associated with antelope meat.

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Organic Acid Profiles of Thermally Processed Carrot Puree[1,2]

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ABSTRACT

The aim of this investigation was to examine changes in organic acid concentration in carrot puree during processing at temperatures ranging from 240-300 F, with 20 F increments and an F_0 equal to 4.9. Two varieties of carrots from two different geographical regions were packed in Thermal Death Time (TDT) tubes, flushed with nitrogen, sealed, and processed. Following this, analyses were conducted to determine changes in organic acid content and pH. Significant increases in pyrrolidone carboxylic acid (PCA), citric acid, fumaric acid, acetic acid, and malic acid were noted between the fresh product and product processed at 240 F. The PCA concentration was significantly affected by changes in processing temperature, while pyruvic and fumaric acids exhibited possible variations. The greatest change in pH from the fresh (unheated) product occurred with the 240 F processed product in both Texas and California samples. As the processing temperature increased, with a constant F_0 value, the pH decreased. The pH readings for the carrot samples from the two different locations were similar.

Since the organic acids present in all plant cells are involved in metabolic pathways, their changes during growth, senescence, and storage have been of considerable interest. Weissberger et al. (*12*) examined the organic acid constituents present in cocoa beans because of the possible influence of variations in the acid content on the ultimate quality of the finished product.

A great deal of work has been done using tomatoes. Pyrrolidone carboxylic acid (PCA) was found to increase in stored processed tomato juice when compared to the fresh product (*9*). It was believed that this formation was influenced by the temperature of processing and storage conditions. El Miladi et al. (*2*) examined thermal processing effects on tomato juice and determined that acetic, lactic, PCA, malic, citric, and an unknown acid increased whereas succinic and alpha keto-glutaric acid decreased.

Spinach puree, processed for various times and temperatures and stored, was examined for organic acid composition and variation (*5*). Changes were observed in acetic acid and PCA after processing. On storage, succinic, acetic, and PCA all increased, with PCA and acetic acid showing the greatest increase at higher storage temperatures. Pyruvic, glutaric, oxaloacetic, and malonic acids also were formed during processing. Alpha keto-glutaric acid was the only acid that disappeared.

Luh et al. (*6*) examined differences between retort and aseptic processing of strained carrots. Malic and galacturonic acids were the predominant acids and fumaric, succinic, and citric acids were present in small amounts. Galacturonic acid and PCA were found in larger concentration in the conventional retort product than in the aseptically processed product. Succinic, fumaric, and citric acids were essentially unchanged.

This investigation dealt with changes in organic acid content as a result of processing at different temperatures with the same F_0 value of 4.9. Samples were examined in duplicate for pH and organic acid content.

MATERIALS AND METHODS

Carrots from two different growing regions. California and Texas, were purchased from a local market. The carrots from each region were analyzed separately. Each batch was washed, peeled, and immediately comminuted in a Fitzpatrick Mill (Fitzpatrick Mill, Model M comminuting machine, W. J. Fitzpatrick Co., Chicago, Ill.) using a fine (No. 40) screen. A large amount of air was incorporated into the puree because of the nature of the comminuting process. The air was removed prior to filling Thermal Death Time (TDT) tubes by placing the puree under vacuum for 10 minutes in a vacuum dessicator. The samples were placed in each of eighty TDT tubes using a hypodermic syringe. Tubes were sealed and processed using the methods of Gupte and Francis (*3*). A temperature controlled glycerol bath was used for processing. The processing temperature ranged from 240-300 F with 20 F increments and an F_0 value of 4.9. Twenty tubes were used for each processing temperature and were divided into two groups of ten tubes each for analyses. Each group was analyzed for pH and organic acid content.

Organic acid analyses were carried out using an automatic organic acid analyzer (AOAA) (Waters Associates, Inc., Framingham, Mass.), using methods developed by Lin et al. (*4*). Such methodology produces results with an error of approximately 2%. All data obtained was based on determinations using this instrument on duplicate samples. The pH measurements were obtained with a Beckman Expandomatic pH meter (Beckman Instruments Inc. Fullerton, California 92634).

Statistical evaluation of data was performed according to Snedecor and Cochran (*11*).

RESULTS AND DISCUSSION

California Carrots

The greatest change in both batches of California carrots was observed for PCA (Table 1). Increases of

[1]*Paper No. 1034 of the Massachusetts Agricultural Experiment Station, University of Massachusetts at Amherst.*

[2]*This research was supported in part from Experiment Station Project No. 192 and the Glass Container Manufacturers Institute, New York, N.Y.*

TABLE 1. *Concentrations of organic acids present in California carrot puree processed over the temperature range of 240 F to 300 F with an $F_0 = 4.9$ and 20 F increments*

Process (F)	Acid concentration (μeq/g dry weight carrot puree)[3]									
	Propionic	Acetic	Pyruvic	Formic	Fumaric	Succinic	t-Aconitic	PCA	Malic	Citric
Batch 1										
Unheated	3.4	38.5	7.5	—	42.5[1]	t[4]	t	3.6[2]	156.3[1]	107.6[1]
240	3.7	32.1	20.3	—	50.3	t	t	15.4	207.8	123.3
260	15.6	36.8	2.6	34.9	52.5	t	t	8.1[2]	219.2	134.5
280	13.1	49.9	8.0	35.6	47.6	t	t	6.1[2]	150.8	92.6[1]
300	13.6[2]	8.2	2.9	—	51.8	t	t	4.4[2]	166.4	139.7
Batch 2										
Unheated	4.7	53.6	5.9	—	37.1[1]	t	t	3.4[2]	138.7[1]	95.0[1]
240	5.0	49.3	11.8	—	50.0	t	t	15.0	211.8	131.8
260	—	28.7	27.4	33.8	47.8	t	t	7.4[2]	142.6	128.9
280	—	7.0	9.4	—	55.5	t	t	6.3[2]	194.7	215.3[1]
300	12.0[2]	16.3	8.2	—	51.5	t	t	4.7[2]	193.8	124.4

[1]Significant difference from the concentration of 240 F at the 95% confidence level for combined batches.
[2]Significant difference from the concentration of 240 F at the 99% confidence level for combined batches.
[3]Results are the average of duplicate analyses.
[4]t = trace amount present.

TABLE 2. *Concentrations of organic acids present in Texas carrot puree processed over the temperature range of 240 F to 300 F with and $F_0 = 4.9$ and 20 F increments*

Process (F)	Acid Concentration (μeq/g dry weight carrot puree)[3]									
	Propionic	Acetic	Pyruvic	Formic	Fumaric	Succinic	t-Aconitic	PCA	Malic	Citric
Batch 1										
Unheated	11.3	31.5[1]	40.5[2]	—	50.2[2]	t[4]	t	7.4[2]	191.7[1]	117.4
240	11.8	9.0	4.6	34.0	71.8	t	t	25.5	349.8	88.8
260	8.2	14.2	10.8	32.0	82.8	t	t	15.2[2]	381.6	146.1
280	3.2	10.4	22.1[2]	46.8	82.1	t	t	9.0[2]	224.3	—
300	—	—	—	48.7	86.0[2]	t	t	7.7[2]	324.3	99.7
Batch 2										
Unheated	8.62	39.7[1]	31.0[2]	—	44.1[2]	t	t	7.2[2]	159.5[1]	136.0
240	—	21.7	9.5	—	71.2	t	t	26.6	456.4	123.3
260	2.5	0.6	9.8	33.3	80.3	t	t	14.1[2]	337.2	94.5
280	7.0	2.5	22.0[2]	49.1	82.8	t	t	10.5[2]	310.4	99.8
300	11.2	—	31.4	40.1	94.1[2]	t	t	8.6[2]	400.5	127.6

[1]Significant difference from the concentration at 240 F at the 5% confidence level for combined batches.
[2]Significant difference from the concentration at 240 F at the 1% confidence level for combined batches.
[3]Results are the average of duplicate analyses.
[4]t = trace amount present.

327% and 318% over the concentration of PCA in the fresh product were noted for samples processed at 240 F. The higher temperature processes were associated with smaller increases in PCA concentration.

Fumaric, citric, and malic acids exhibited significant increases between the fresh and the 240 F processed product. Citric acid also differed significantly between the 240 F and the 280 F process at the 95% confidence level, while propionic acid concentration showed a significant increase from the 240 F process to the 300 F process at the 99% confidence level.

While PCA, citric, malic, and fumaric acids all exhibited significant differences between the fresh and 240 F processed product, only PCA showed differences in concentration due to the different processing times and temperatures used. In general, citric, malic, and fumaric acids were effected by processing as compared to the fresh sample, but no differences were noted between the various temperatures used in processing.

Texas Carrots

Results from both batches of processed Texas carrots, shown in Table 2, show a significant increase in the concentration of PCA. Increases of 219% and 218% were noted over the fresh product concentration for the 240 F processed samples. Smaller increases were also noted for the higher temperature processes.

Fumaric acid showed changes similar to those observed for the California samples with a significant increase between the 240 F processed product and the fresh product. Texas samples also showed a significant increase in fumaric acid when processed at 300 F rather than 240 F. Malic acid concentration significantly increased when the fresh sample was processed, but no difference was noted between processes. a similar increase was noted during the processing of the California samples. A significant reduction in pyruvic acid content was noted for the 240 F processed product when compared to fresh samples processed at 280 F or higher. This was not observed with the California carrots. This change might be due to possible thermal degradation of the pyruvic acid, but no further work was carried out to test this hypothesis. Acetic acid concentration also decreased from the fresh product when processed at 240 F, but no changes were observed among the various processes.

Significant differences were observed between the fresh product and the 240 F process for PCA, citric, malic, fumaric, and acetic acids. While pyruvic and fumaric acids exhibited possible variation due to the type of thermal processing they received, only PCA definitely changed in concentration with changes in processing temperature.

pH Evaluation

The greatest change in pH from the fresh product occurred for the 240 F processed product (Table 3) for both the California and Texas samples. As the processing temperature increased, keeping the F_0 value constant, the change in pH from that of the initial sample decreased. The similarity of the pH readings for the carrot samples from the two different regions may indicate that despite different environmental conditions and genetic differences, the effects of processing on the two sets of samples were quite similar. These changes were similar to those observed by Lin et al. (*5*) for spinach puree, where the HTST processing caused the smallest change in pH and the conventional processing at 240 F, the greatest change.

The reasons for the changes in many of these acids are not known. Various hypothetical mechanisms have been proposed. One mechanism, the conversion of glutamine to PCA has received a great deal of attention (*1, 5, 7, 9*). This formation has also been cited as causing a reduction in product quality caused by off-flavor production (*1, 8, 10*). It has been determined that this particular formation may be controlled by varying the processing parameters (*1*). In order to produce the highest quality products for the consumer, more of these mechanisms responsible for the changes in organic acids should be evaluated so that it might be possible to better control such changes.

TABLE 3. *pH of California and Texas carrot puree processed from 240-300 F with an $F_0 = 4.9$ and initial samples.*

Process Temperature (F)	pH	
	California	Texas
Batch 1		
Unheated	5.90	5.90
240	5.40	5.43
260	5.75	5.68
280	5.78	5.73
300	5.80	5.80
Batch 2		
Unheated	5.95	5.91
240	5.42	5.42
260	5.70	5.64
280	5.75	5.74
300	5.80	5.75

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Some Functional Properties of Succinylated Single Cell Protein Concentrate

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ABSTRACT

Single cell protein concentrate was prepared by extracting dried *Candida utilis* with 0.2 N NaOH at 95 C for 10 min. The soluble protein was precipitated at pH 3.5, washed, and lyophilized. Portions of the protein isolates were succinylated so that 84% of the free amino groups were blocked. Alkaline extractions under optimum conditions gave a yield of 25 to 30% of total solids. Nucleic acid content of the isolates was 10.4% whereas protein comprised 67.3%. Succinylated (SI) and nonsuccinylated (NSI) single cell protein concentrate exhibited similar solubilities above pH 4, the apparent isoelectric point. At pH values below 4, SI was quite insoluble whereas NSI was very soluble at pH 2. Digestibilities as measured by percentage of lysine released from the protein by pepsin and pancreatin were 32.7%, 18.6%, and 3.3% for NSI, whole egg, and SI, respectively. Stabilities of emulsions prepared from corn oil (20-50%) tended to be lower when gelatin was used at the 1% level compared to NSI and SI. However, higher levels of gelatin tended to yield more stable emulsions compared to NSI and SI. Stabilities of emulsions prepared from NSI and SI tended to be comparable with NSI being slightly better. Viscosities of emulsions increased with increasing oil content and increasing emulsifier concentration.

Development of novel sources of protein such as single cell protein represents a challenge for the food scientist to effectively incorporate these proteins into foods. This challenge is evident on two different levels. For example, in the developing countries, because of economic reasons, it may be adequate to supplement traditional foods with single cell protein.

On the other hand, in the industrialized countries, more sophisticated procedures may be required to introduce single cell proteins into conventional foods or to develop completely new food systems. In these instances the functionality of the single cell proteins becomes of paramount importance. Briskey (*7*) has reviewed the functional properties of proteins often desirable for their use in foods. Among these functional properties are included solubility, emulsifying characteristics, gelation, adhesion, cohesion, etc. Lipinsky and Litchfield (*24*) have reviewed the production and utilization of single cell protein, and they point out the need for research on protein functionality. Very little has been done regarding the evaluation of the functionality of single cell proteins. Labuza and co-workers (*22, 23*) have studied the effect of various processing conditions on the functionality of single cell protein used in breadmaking. The rheological properties of single cell protein concentrate have been determined by Huang and Rha (*18*). Balmaceda and Rha (*5*) have studied the coagulation rate of single cell protein concentrate. Several studies have been published on spinning of microbial proteins into fibers (*11, 17, 19*). Rha (*28*) has recently reviewed the functionality of single cell protein concentrates.

The present paper describes the functionality of *Candida utilis* proteins in terms of their solubility as a function of pH and their emulsifying characteristics compared to gelatin. In addition, the single cell proteins were succinylated to study effects of this modification on the above functional properties.

MATERIAL AND METHODS

Extraction of protein

Several extraction procedures were initially applied to dried *C. utilis* (Edible Grade, Amber Laboratories, Juneau, Wis.) to prepare protein samples. Extraction procedures utilizing NaC1, $CaC1_2$, succinic anhydride, various temperatures, acidic and basic conditions, and various combinations of the above were tested to determine the condition or combination of conditions resulting in the greatest yield. The high temperature-high pH method described by Tannenbaum et al. (*31*) for fish protein concentrate was found to be most efficient. Preliminary investigation established the optimum conditions for the above extraction to be a 10% (w/v) suspension of *C. utilis* in 0.2 N NaOH at 95 C for 10 min, followed by centrifugation and precipitation of the soluble protein at pH 3.5 with 0.2 N HC1. Precipitated protein isolate was centrifuged, washed three times with distilled water, and freeze-dried. The protein isolate prepared by this method will be referred to as nonsuccinylated isolate (NSI).

Total nitrogen in NSI was determined by the Kjeldahl method (*4*), and the DNA and RNA concentrations in the isolate were determined according to the methods of Burton (*8*) and Kerr and Seraidarium (*21*), respectively. Total protein in the isolate was determined by calculating nucleic acid nitrogen in the total nitrogen and multiplying the difference by 6.25.

Modification of the protein

Protein isolates were modified by addition of succinic anhydride to free amino groups. A modification of the succinylation procedure of Habeeb et al. (*15*) was employed. Preliminary experiments defined the most satisfactory conditions as follows: Succinic anhydride, in an amount equal to 10% of the weight of the total protein in the solution, was added to a 5% solution of NSI, pH 7.5. Succinic anhydride was added gradually over a period of 30 min with continual magnetic stirring. The pH was maintained by gradual addition of 0.2 N NaOH from a pipette. At the completion of the reaction (as evidenced by a constant pH) the isolate was once again precipitated at pH 3.5 with 0.2 N HC1 and freeze-dried. These preparations will be referred to as succinylated isolate (SI).

The approximate degree of succinylation was determined by the method of Habeeb (*16*). In this procedure epsilon amino groups of

lysine and N-terminal amino groups of equal quantities of NSI and SI were reacted with 2, 4, 6-trinitrobenzenesulfonic acid (TNBS). Free lysine (amount bound by TNBS) was measured colorimetrically at 344 nm and the ratio of free lysine in SI to free lysine in NSI was the percentage of free or nonsuccinylated amino groups. Therefore, the converse is the approximate degree of succinylation.

Determination of protein digestibility

Protein quality was determined by a modification of the enzymatic procedure of Akeson and Stahmann (*3*). Modifications included the use of 0.2 M phosphate as the buffer at pH 8.0, 1% sulfosalicylic acid instead of 1% picric acid as the precipitant, and lysine as the variant (instead of all the amino acids) to determine the digestibility by pepsin and pancreatin. NSI, SI, and whole dried egg were compared in this manner. The total and free lysine content of both isolates and egg were determined by amino acid analysis with a Phoenix model M-7800 amino acid analyzer, according to the method of Spackman et al. (*30*).

Determination of protein solubility

Relative solubility of the two isolates was determined by dispersing equal amounts of isolate in aqueous suspension at various pH values while stirring magnetically for 15 min. The undissolved protein was separated by centrifugation and absorbance of the supernatant fluid was read at 280 nm in a Beckman DU-2 spectrophotometer.

Preparation of oil-in-water emulsions

Solutions containing 0.1 g isolate/ml were prepared for NSI, SI, and 250 Bloom gelatin. Aliquots of each solution were placed in 125-ml Erlenmeyer flasks with corn oil at 25 C. Distilled water was added to bring the final volume to 40 ml. Oil concentrations ranged from 20-50% (w/v) and isolate concentrations ranged from 1-5% (w/v).

The mixtures were shaken to sufficiently disperse the two liquids. The dispersion was immediately emulsified in a Potter-Elvehjem tissue homogenizer (3.0 × 11.5 cm) with three complete passes. Microscopic examination showed that each emulsion formed was an oil-in-water (O/W) emulsion.

Emulsion stability test

A stability test measuring initial and final moisture content in the emulsions was a modification of the procedure of Acton and Saffle (*1*). Modifications included: three samples instead of two, 2-g samples rather than 5-ml aliquots, shortening the time in the 37-C water bath from 24 h to 2 h, and Mojonnier total solids test (*26*) to determine moisture content replacing the AOAC method (*4*).

Viscometry of oil-in-water emulsions

The viscosity of the emulsions in centipoise was determined for O/W emulsions stabilized by NSI and SI. A Brookfield Synchro-Lectric Viscometer, Model LVF, was used at a shear rate of 30 rpm with the No. 1 and 2 spindles. Viscosities were converted to centipoise using a basic correction table. The emulsions were packed in ice immediately after preparation and the viscosities examined at 1-3 C.

Statistical analyses

Analysis of variance using a randomized complete block design (*27*) was done for emulsions prepared to study the effects of pH and fat source. If a statistical difference was found among means, Duncan's new multiple range test (*27*) was employed to determine which mean values were significantly different.

RESULTS AND DISCUSSION

The high temperature-high pH extraction process provides a soluble protein product which has a light brown color, a chalky texture, and a bland flavor. Extractions at optimum conditions gave a yield of 25 to 30% of total solids. Kjeldahl analyses of the isolate indicated that it was 12.4% nitrogen. RNA and DNA analyses were done because of high nucleic acid contents in whole yeast and because of possible health problems encountered by consuming high levels of these compounds (*32*). RNA and DNA contents of NSI were 7.5% and 2.8%, respectively. The extraction procedure reduced the nucleic acid content from 14% in the whole yeast to 10.4% in the isolate. From this knowledge, and the fact that nucleic acids are from 15-16% nitrogen (*33*), it was determined that 87% of the total nitrogen was proteinasceous. This value, multiplied by 6.25, indicated that the isolate was 67.5% protein.

The purpose of modification of NSI via succinylation was to alter the protein in such a way as to improve its functional properties. These properties include solubility, emulsification stability, and viscosity, and will be discussed later in the paper.

Acylation of food proteins has been used to alter their functionality. Modification of egg white with 3,3-dimethylglutaric anhydride resultedin increased heat stability (*13*). Succinylated egg yolk proteins have been found to be useful in the production of mayonnaises and salad dressings (*12*). Acylated soybean proteins used to prepare coffee whiteners result in a product with good flavor, odor, and dispersion characteristics (*25*). Plant proteins have been modified with succinic anhydride, among other acylating reagents, to yield products with improved functional properties (*9*). Groninger (*14*) prepared succinylated fish myofibrillar protein from undenatured myofibrillar protein. This modified fish protein hydrated fairly rapidly to yield viscous aqueous dispersions. It had good heat stability, relatively high

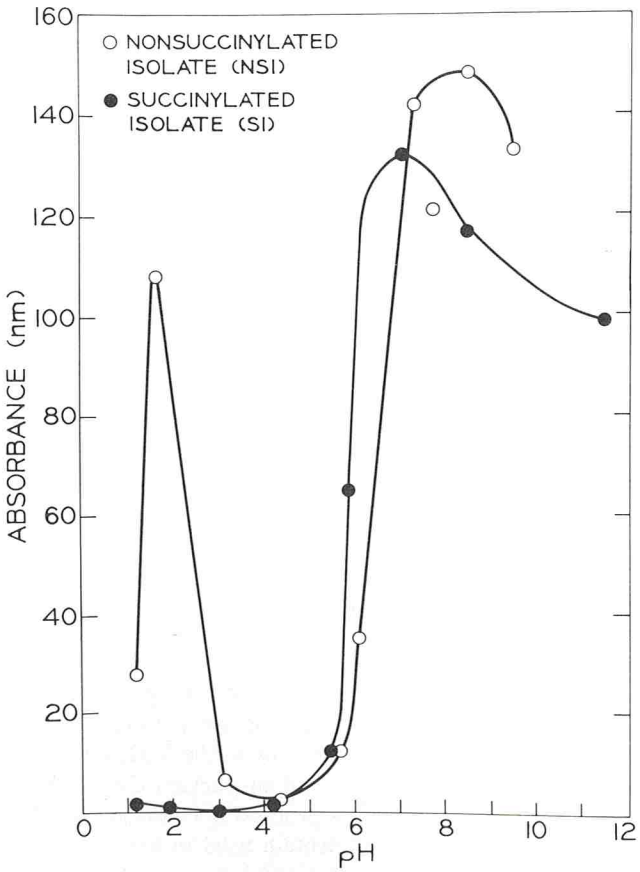

Figure 1. *Relative solubilities of yeast protein isolates.* ○—○ *Non-succinylated isolate (NSI),* ●—● *Succinylated isolate (SI)*

emulsification capacity, and bland odor and flavor. Recently, Chen et al. (*10*) studied some functional properties of succinylated proteins from fish protein concentrate.

Physical properties of SI were very similar to NSI with the exception of bulk density. NSI occupied less than half the volume of SI, presumably because addition of succinate to protein in solution prohibits its refolding upon drying.

Relative solubilities of NSI and SI are reported in Fig. 1. Both isolates exhibit similar solubility at pH values greater than pH 4, the apparent iso-electric point, but at lower pH values SI exhibits little or no solubility. Several factors can explain this observation. Succinylation adds succinate molecules to free amino groups, which leave amino groups bound and introduce free carboxyl groups. Analysis with TNBS indicated that 84% of the free amino groups were blocked by succinylation. Because the pK of the succinate radical is 4.16, approximately 84% of the carboxyl groups have no charge at pH 3.5 and even fewer are charged as the pH becomes more acidic. The amino groups of NSI have a positive charge at low pH values, which make NSI soluble in this range (its behavior at pH 1.0 is inexplicable). In the case of SI there are few free amino groups and hence, little charge. The hydrophilic groups have been bound, and therefore the protein is insoluble. Because of the decrease in total charge on the protein, repulsion forces that generally keep proteins separated are absent or insignificant, allowing the molecules to aggregate thus hindering solubilization.

Evaluation of protein quality or digestibility was determined to examine the effect of succinylation on the nutritional value of the protein. Whole egg was used as a standard because of its high biological value. Digestibility values for NSI, whole egg, and SI were 32.7%, 18.6%, and 3.3%, respectively. These data are surprising in the sense that NSI was almost twice as digestible as whole egg under the conditions chosen. Perhaps the NSI was highly denatured compared to whole egg and, thus, more readily digested. The fact that NSI was almost ten times as digestible as SI can be more easily explained. It was mentioned earlier that the degree of succinylation was approximately 84%.This leaves only 16% of the amino groups free. Apparently addition of succinate groups to amino groups inhibited the enzymatic hydrolysis of SI. More extensive in vivo studies are needed to determine whether the protein reacts in this manner in the intact animal. Recently an acyllysine deacylase which removes acyl residues from epsilon amino groups of lysine in proteins has been demonstrated in animal tissues (*20*).

Before the effects of fat or emulsifier concentration on emulsion stability were determined, the effect of type of oil was examined to determine which oils created the most stable emulsions. Stability ratings for emulsions containing commercial oil from five species of plants and stabilized by NSI and SI, all other variables constant, are given in Fig. 2. The type of oil used in conjunction with

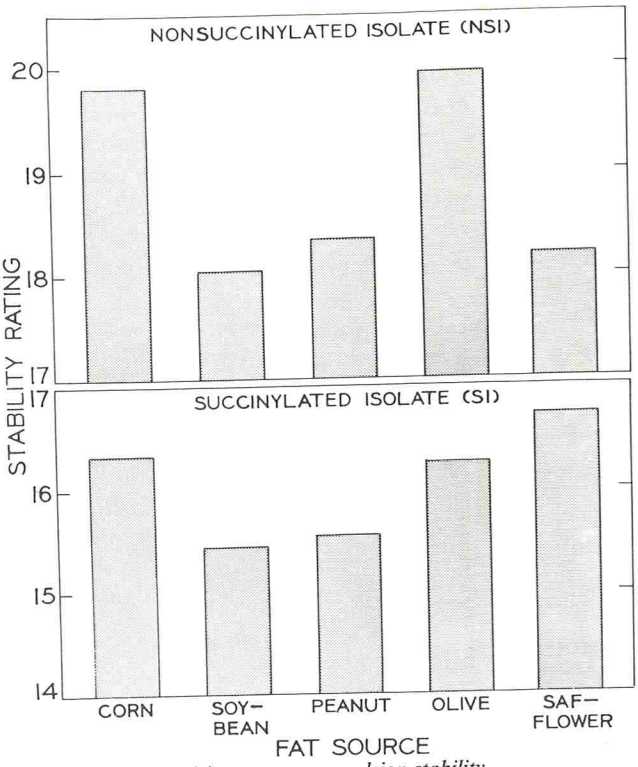

Figure 2. *Effect of fat source on emulsion stability.*

SI showed no significant difference at a 5% probability level. However, for NSI, soybean, peanut, and safflower oils had stability ratings significantly ($P \leq .05$) lower than

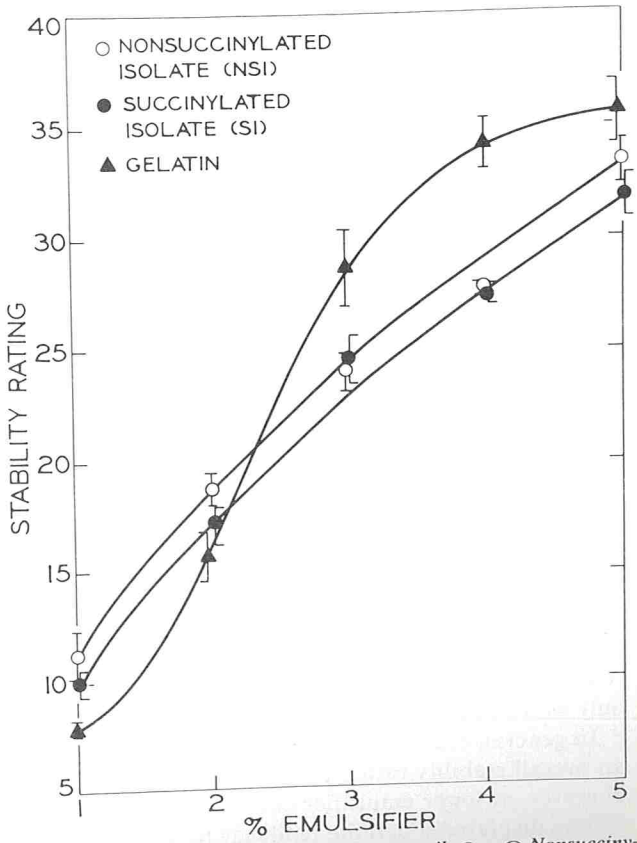

Figure 3. *Emulsion stability with 20% corn oil.* ○—○ *Nonsuccinylated isolate (NSI).* ●—● *Succinylated isolate (SI), and* ▲—▲ Gelatin.

olive oil, whereas soybean and safflower oils had ratings significantly ($P \leq .05$) lower than corn oil. These data correlate quite well with those of Acton and Saffle (*2*) except for high stability ratings of corn oil emulsions.

Preliminary investigations indicated that pH had little or no effect on emulsion stability. The stability of emulsions prepared with corn oil and NSI, SI, or gelatin at various concentrations and pH 7.4 is shown in Fig. 3, 4, 5, and 6. The formula used to calculate stability rating

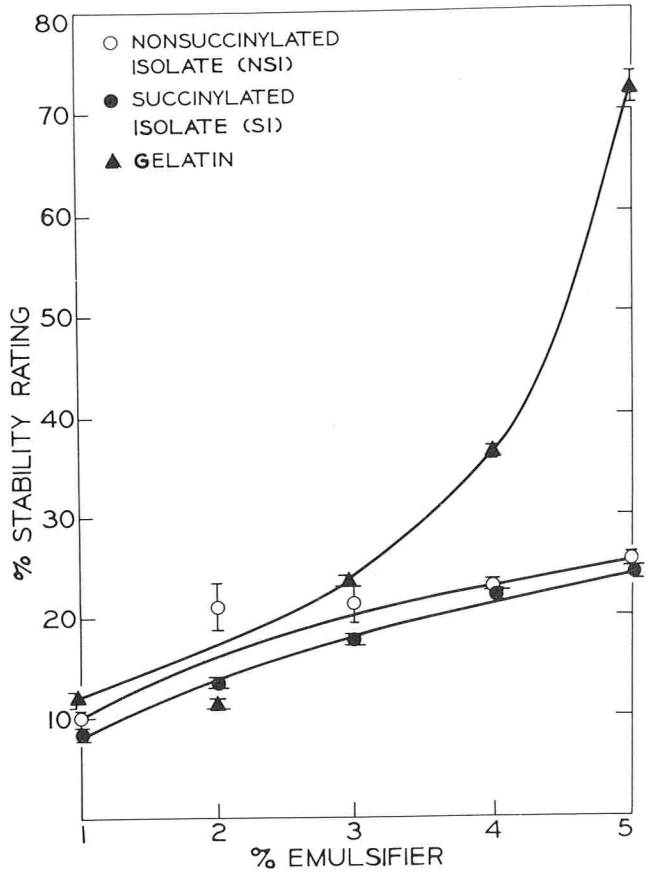

Figure 4. *Emulsion stability with 30% corn oil.* ○—○ *Nonsuccinylated isolate (NSI),* ●—● *Succinylated isolate (SI), and* ▲—▲ *Gelatin.*

$$(\text{S.R.} = \frac{100\text{-}M_t}{100\text{-}M_o} \times 100, \text{ where } M_o = \text{initial moisture content of the emulsion and } M_t = \text{moisture content in the bottom 2 ml after cooling 2 h at 4 C and heating 2 h at 37 C})$$

is valid for comparing emulsions at constant fat levels, but when used to compare the effect of fat level variation at a constant emulsifier concentration, the results conflict significantly with viscosity data. For this reason, the effect of fat concentration will be discussed only as it related to viscosity.

In general, emulsions prepared with gelatin exhibited an overall stability rating greater than either NSI or SI. However, at lower emulsifier concentrations (1 and 2%) gelatin displayed a definite tendency to be less stable. At emulsifier levels of 3% or greater, gelatin invariably produced more stable emulsions (one exception being in Fig. 6 where emulsions with greater than 2% gelatin were not tested because of the inability of the emulsifying apparatus to emulsify the preparations).

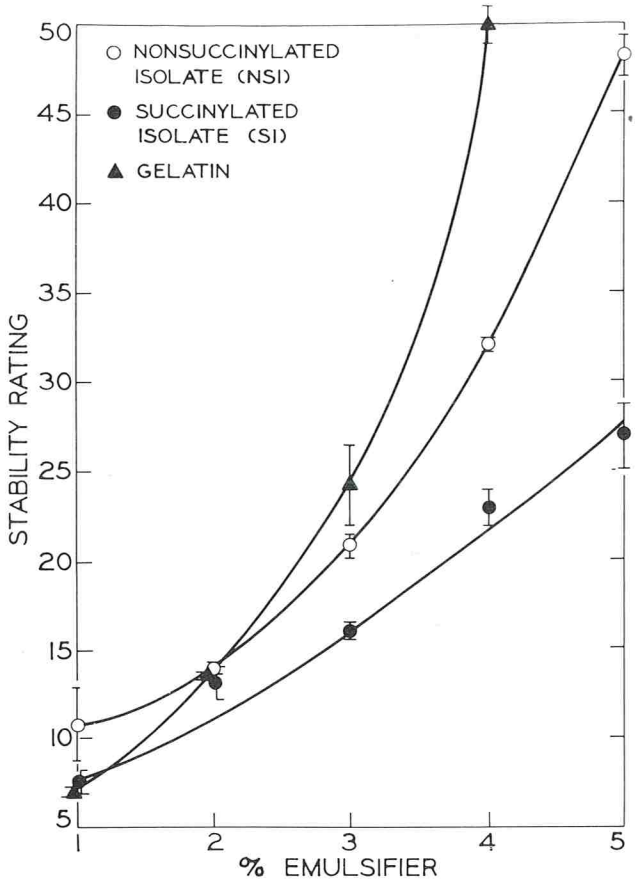

Figure 5. *Emulsion stability with 40% corn oil.* ○—○ *Nonsuccinylated isolate (NSI),* ●—● *Succinylated isolate (SI), and* ▲—▲ *Gelatin.*

NSI portrayed stabilities midway between those of SI and gelatin. While having consistently larger stability ratings than SI, the general shapes of the curves more closely approximated those of SI than of gelatin.

SI, as can be understood, had the lowest stability of the three emulsifiers. Because of the additional carboxyl groups present on this protein, the overall charge at pH 7.4 would be more negative than that of NSI. Since the charge on the fat globules is also negative (*29*), the repulsion of the two phases would be greater, and hence, the emulsion stability lessened.

It should be noted at this point that Acton and Saffle (*2*), in similar experiments, used sodium caseinate, gelatin (type A), and soy sodium proteinate as emulsifiers in concentrations half as great as those used in this study to obtain stable emulsions. Preliminary experiments indicated that emulsions prepared with NSI or SI at these levels were very unstable. This implies that NSI is not as good as emulsifier as sodium caseinate or soy sodium proteinate and SI is not as good as NSI.

Fig. 7 and 8 show the viscosities of the emulsions stabilized with NSI and SI, respectively. No viscosity data are available for gelatin because at 1 to 3 C (where

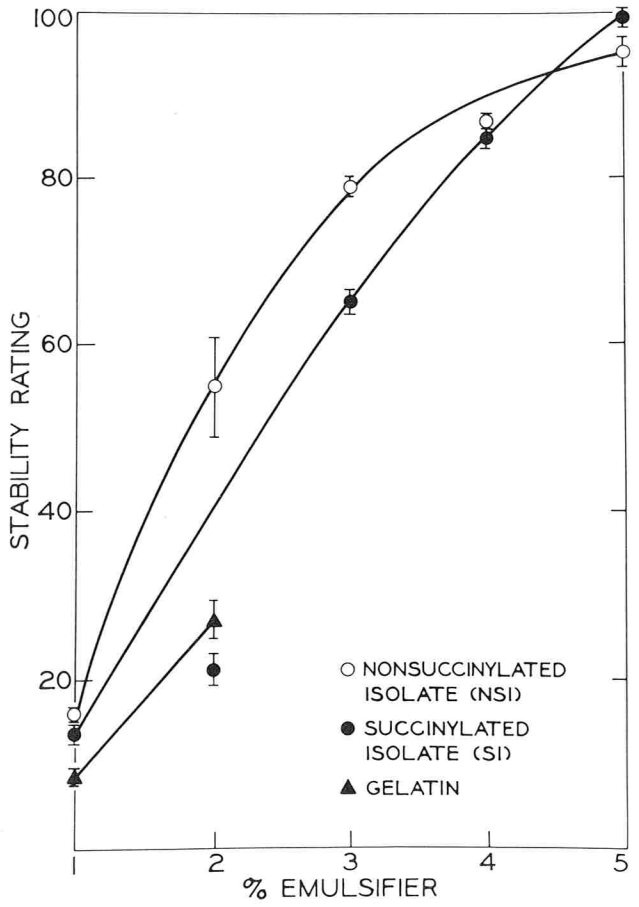

Figure 6. *Emulsion stability with 50% corn oil.* ○—○ *Nonsuccinylated isolate (NSI).* ●—● *Succinylated isolate (SI), and* ▲—▲ *Gelatin.*

viscosity was measured) the emulsions stabilized with gelatin solidified. Earlier work revealed that, in general, the higher the viscosity, the more stable the emulsion (*2, 6*). From these findings, it can be postulated from viscosity data that as the fat and/or emulsifier concentrations increase, so does the emulsion stability.

Comparison of Fig. 7 and 8 shows that SI emulsions have higher initial viscosities than NSI emulsions. This is probably because SI in solution has an unfolded, thread-like structure due to addition of succinate radicals, whereas NSI in solution has more of a globular, interwoven structure. The open structure of SI would cause emulsion viscosity to be greater. Emulsion stability, however, was lower for SI emulsions than for NSI emulsions. Apparently the greater negative charge of SI had a greater undesired effect on emulsion stability than desired effect on increased emulsion viscosity.

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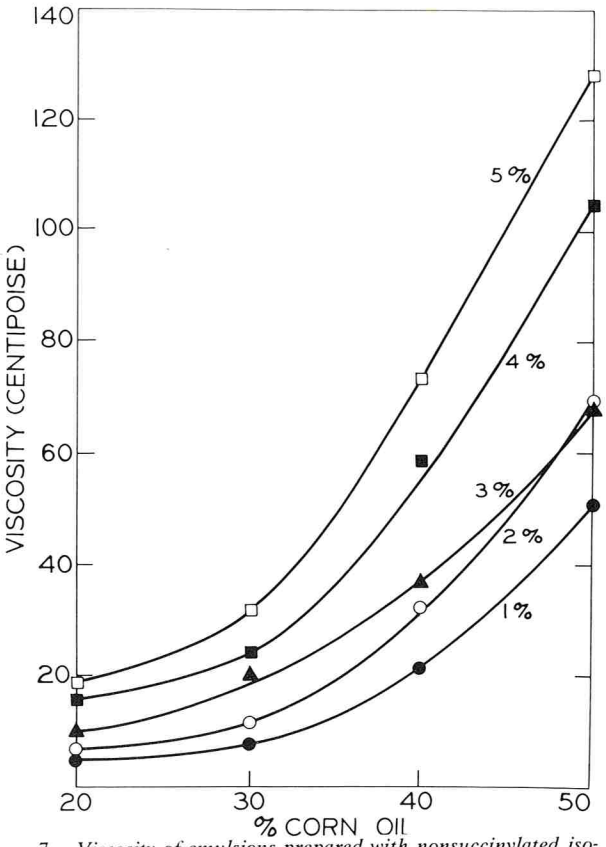

Figure 7. *Viscosity of emulsions prepared with nonsuccinylated isolate (NSI).* □—□ *5% isolate,* ■—■ *4% isolate,* ▲—▲ *3% isolate,* ○—○ *2% isolate, and* ●—● *1% isolate.*

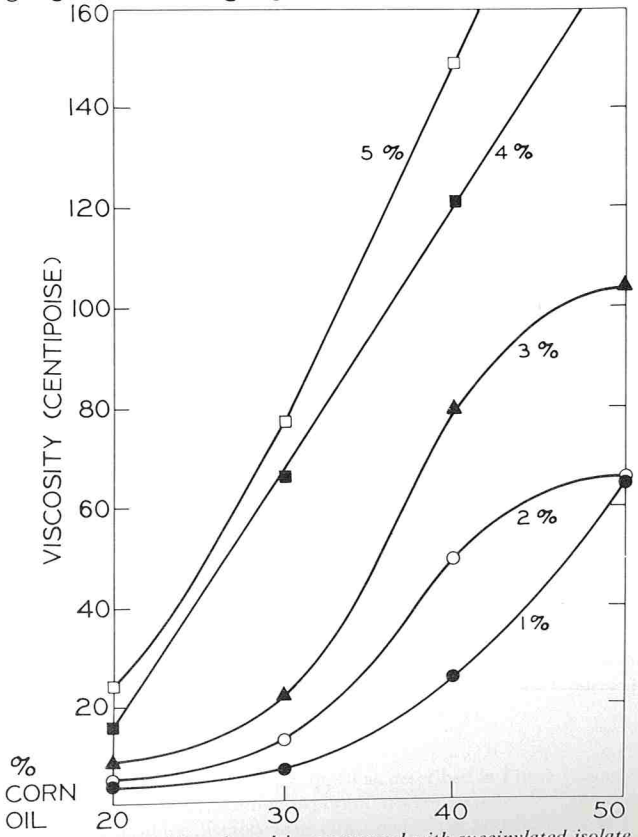

Figure 8. *Viscosities of emulsions prepared with succinylated isolate (SI).* □—□ *5% isolate,* ■—■ *4% isolate,* ▲—▲ *3% isolate,* ○—○ *2% isolate, and* ●—● *1% isolate.*

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A Field Study of Bulk Milk Transport Washing Systems[1,2]

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ABSTRACT

Mechanical spray devices commonly used to wash bulk milk transport tankers in Florida were evaluated. A drop-in unit and two types of spray balls operated according to manufacturers' recommendations were used with the cleaning system supplied by the dairy. A self-contained circulating single-use system was used with one type of spray ball. When operated according to manufacturers' recommendations any of the spray devices could clean a tanker. Pressure and volume relationships below those recommended resulted in inadequate cleaning. Excessive pressure and volume damaged one spray ball which resulted in poor cleaning and required repair. A 120-day period was required to make the necessary changes in the plant cleaning system before it was possible to operate the spray devices consistently at the recommended pressure and volume. A pressure-temperature recorder in the solution supply line was used to monitor tanker washing and detect problems associated with the supply systems. It was necessary to routinely examine the tankers and spray devices to insure cleanliness of the tankers and remove trash that can collect in the spray device.

All milk handling equipment must be thoroughly cleaned to prevent contamination of milk. During transportation of milk from dairy farms to processing plants one bulk transport tanker can contaminate milk from several producers. Since transports are mobile and do not always deliver milk to the same location, it is difficult to regulate their sanitary condition.

Milk transport tankers can be cleaned either manually or by circulation cleaning. Hand cleaning is difficult, unpleasant, and sometimes dangerous. It is subject to human error and results may be inconsistent. Several types of spray devices are available that can be used for circulation cleaning of milk transport tankers. At least two drop-in cleaning devices (*2, 3*) and several types of spray balls (*2, 4*) are manufactured. Each device requires a specific flow rate and pressure to function properly. Often it is not possible to use the various spray devices interchangeably with one solution supply system. Circulation cleaning is generally more desirable and less costly than manual cleaning.

In 1973 a hearing was held in Florida to consider specifications for cleaning and sanitizing bulk milk transport tankers. Considerable discussion concerning the merits of cleaning systems resulted in the formation of a committee to investigate tanker washing procedures. The committee consisted of representatives from the Florida Department of Agriculture and Consumer Services (Division of Dairy Industry), Florida Department of Health and Rehabilitative Services, independent contract milk haulers, equipment suppliers, and a milk processing representative. The committee proposed a field study at the T. G. Lee processing plant in Orlando, Florida, to evaluate drop-in units and fixed spray balls. The objective of the study was to evaluate mechanical cleaning devices commonly used in Florida.

EXPERIMENTAL PROCEDURE

Phase 1

Five 6000-gal stainless steel tankers (Walker) were selected from the T. G. Lee Transport fleet. Tankers 1 and 2 were cleaned with a drop-in unit (Girton TTW48). Single spray balls (SB-13, Economics Laboratory, Inc.) were installed in Tankers 3 and 5 exactly one-third the distance from either end of the tanker. Dumbell spray balls (BASF Wyandotte Corporation) were installed in Tanker 4 exactly one-third the distance from either end of the tanker. Tankers 1, 2, 4, and 5 were cleaned during this period with the dairy-owned C.I.P. system. Tanker 3 was washed with a single-use C.I.P. system (Klenzmation 1800, Economics Laboratory, Inc.).

Phase 1 lasted 120 days and was used to identify and correct problems in the washing systems so the spray devices could be operated according to manufacturers' specifications. Because of the many changes made during this period, it was impossible to report actual operating conditions. However, every effort was made to operate the spray devices properly.

Florida Department of Agriculture and Consumer Services (Dairy Division) personnel in the presence of industry representatives examined each tanker weekly for physical cleanliness. The inside surface of each tanker was swabbed to determine if microbial contamination was present. Designated areas for swabbing were the top 180° of the outlet valve, top of the tanker 2 ft from the backwall, top of the tanker 2 ft behind the manhole, top of the tanker 2 ft ahead of the manhole, top of the tanker 2 ft from the fron wall, and a random swab in the most suspicious area. An 8 in^2 area was swabbed in each selected position using Standard Methods buffer solution (*1*).

Phase 2

Tankers 1, 2, 3, and 5 were equipped as described in Phase 1. Single spray balls (BASF Wyandotte Corporation) were installed in Tanker 4 exactly one-third the distance from either end of the tanker. Tankers 1, 2, 4, and 5 were washed with the plant C.I.P. system. This 500-gal re-use system was also used to wash one 50,000 and two 25,000 gal silo

[1] *Florida Agricultural Experiment Station Journal Series No. 5738.*
[2] *Reference to commercial products is for identification purposes only and no discrimination nor endorsement is intended.*
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tanks on alternate days. The re-use tank was emptied twice weekly and automatically maintained the selected chemical concentration. Tanker 3 was washed with the single-use C.I.P. system described in Phase 1.

The volume of the wash solution and the pressure at the spray device used was the manufacturers' recommendation for the drop-in units (*3*) and the spray balls in Tankers 3 and 5 (*2*). Spray balls used in Tanker 4 were experimental and manufacturers' recommendations were not available. The volume of water used to wash Tanker 3 was determined by measuring water flow with a water meter during a timed interval. Water usage during washing of Tankers 1, 2, 4, and 5 was determined by measuring the volume of solution removed from the supply tank during 30-sec intervals. Pressures were determined at the spray device with a pressure gauge. The recommended and actual volume and pressure for each spray device is shown in Table 1.

TABLE 1. *Operating parameters for spray devices*

Unit	Volume		Pressure	
	Recommended	Actual	Recommended	Actual
	—(Gallons per minute)[a]—		—(lb/in²)[b]	
Drop-in	115	120	50	60
Spray ball[c]	80-100	81	25-30	26
Spray ball[d]	80-100	110	25-30	27
Spray ball[e]	—[e]	160	—[e]	34

[a]Total volume for 2 spray balls in each tanker.
[b]Determined at spray device.
[c]SB-13 used with single-use cleaning system.
[d]SB-13 used with plant cleaning system.
[e]Experimental spray ball. No recommended operating parameters specified.

Tankers 1, 2, 4, and 5 were cleaned with 10 separate 15-sec pre-wash (pre-rinse) bursts of 90 F water followed by a 20-min wash cycle at 150 F. The post-wash rinse (post-rinse) consisted of 10 separate 15-sec bursts of 90 F water. Tanker 3 was cleaned by pre-rinsing with three separate 21-sec bursts of 90 F water followed by a wash cycle of 12 min at 145 F. The post-rinse consisted of three separate 21-sec bursts.

The wash solution used in Tankers 1, 2, 4, and 5 contained 50 ppm chlorine and .06% active alkalinity expressed as NaOH. Acid sanitizer (140 ppm) was added during the final burst of the post-rinse for a final pH of 2.4. An air blow-down completed the cleaning cycle. The wash solution used in Tanker 3 contained 75 ppm chlorine and .23% active alkalinity expressed as NaOH. Enough acidifying chemical was added to the post-rinse to assure a pH of 3.0 in the final burst.

Areas in Tankers 1, 2, 4, and 5 that had surface film were washed manually at the initiation of Phase 2. Tanker 3 was mechanically cleaned with solutions of increased concentration early in Phase 1 and did not require additional cleaning. Each tanker was inspected and certified clean by Florida Division of Dairy Industry personnel and committee representatives. After the initial inspection, weekly inpections were made throughout Phase 2 which continued for 60 days. The criteria for cleanliness was a satisfactory sanitary rating after visual inspection by the regulatory agency. Tanker washing was monitored in both washing systems with pressure-temperature recorders.

RESULTS AND DISCUSSION

Phase 1

Bacterial counts from swabbed tanker surfaces did not reflect the sanitary condition of the tankers. They were usually negative for each 8 in² area. On occasion a residue could be removed from the tanker wall with the swab, yet, the bacterial count did not reflect this condition. The lack of relationship between bacterial counts and observed sanitary conditions could be either the result of the high wash temperature and the inspection schedule or the effect of the sanitizers. Wash solution temperatures for both the single-use and the plant cleaning systems were in excess of 140 F and the wash cycle exceeded 11 min. This time-temperature relationship would cause death or injury to many bacteria. All tankers were in use so were inspected and swabbed within 8 h of washing. A longer time interval between washing and inpection could have resulted in high bacterial counts if microbial growth occurred. However, under the conditions of this study, it was not possible to evaluate the relationship between bacterial counts at various time intervals following cleaning. Because of the lack of relationship between the swab test results and the physical examination, swab tests were discontinued after Phase 1.

To insure that the spray devices in Tankers 1, 2, 4, and 5 were consistently supplied with wash solution at the proper volume and pressure, changes had to be made in the plant cleaning system. A 10-hp sanitary-type centrifugal pump in use at the plant as the supply pump had to be replaced with a 15-hp industrial-type centrifugal pump. The volume of wash solution supplied by this pump exceeded the capacity of the return pump and caused flooding in the tankers. This problem was solved by using the previously mentioned 10-hp pump as a return pump. To maintain an adequate volume of solution to the supply pump, 3-inch piping had to be installed from the solution tank to the suction side of the pump. Foreign material that entered the solution tank would lodge in the pump and cause a pressure drop, Raising the outlet from the solution tank to 8 inches above the bottom of the tank eliminated this problem. A new chemical control system to the solution tank was installed during Phase 1.

The single-use cleaning system was installed immediately prior to the start of Phase 1. Adjustments made during Phase 1 were to increase the wash solution temperature from 132 to 145 F and extend the wash time from 6 to 12 min. Early in Phase 1 the chlorine activating mechanism failed when it became disconnected from its electrical supply.

Phase 2

At the initiation of Phase 2 all tankers were certified clean. Throughout this period no additional cleaning of the tankers in excess of the described washing procedure was permitted.

Every tanker passed regulation sanitation requirements regardless of the spray device or the cleaning system used (Table 2). However, small amounts of surface film were observed in the tankers during the inspection period. These films were never heavy milkstone or milkfat deposits. Frequently they covered only small areas and the location of these areas varied weekly within tankers. The films were not visible when inspected with a black-light (ultra-violet). Films were

observed during seven and six of the eight inspection periods for Tankers 1 and 2, respectively.

The spray device associated with the drop-in units is oscillated by an electric motor while cleaning and is independent of the solution supply system. The electrical supply to the motor became disconnected twice. It was not immediately detected and for several days Tankers 1 and 2 were not being adequately cleaned.

Tanker 4 contained a film at each inspection except inspection period 1. The spray balls in this tanker were experimental and no recommendations regarding installation or solution volume and pressure were available from the manufacturer. During Phase 2 it was necessary to raise the spray balls a total of nine inches. This was accomplished over a 4 week period by successively removing 2, 4, and 3 inches from a 40-inch shank. It was possible to evaluate the adjustment of the spray balls by interrupting the pre-rinse cycle after the first burst. Milk residue would then be visible on those areas of the tanker that were not being adequately rinsed.

Tankers 3 and 5 contained identical spray balls. Tanker 3 was washed with the single-use system and twice small areas of a light film were visible. A failure in a 110-volt plant lighting circuit inactivated the single-use system once and it was necessary to wash Tanker 3 with the plant cleaning system.

On three inspections a film was observed in Tanker 5. A problem with split or dislodged spray balls that occurred in this tanker was traced to a period during Phase 1 when excessive pressure was supplied to the spray balls. This caused deformed bails which could not hold the spray balls together during use. The bails were replaced 1 week before the end of the study.

It is significant that Tankers 3 and 5 were cleaned even though the spray balls were not placed according to manufacturers' recommendations. Manufacturers' recommendations suggest placing the spray balls one-fourth the distance from either end of the tanker. In this study the spray balls were located one-third the distance from either end of the tankers.

Data in Table 3 show the volume of water used to clean each of the tankers. The plant system required more water than the single-use system. The total volume of water used in pre-rinse and post-rinse cycles was 600, 600, 800, and 550 gal in Tankers 1, 2, 4, and 5, respectively. This is directly related to the number of rinse bursts in each cycle. It is possible to clean a tanker with fewer than 10 bursts. This is evident since Tankers 3 and 5 contained identical spray balls but were washed with different systems. However, this might not be true for drop-in units.

The plant cleaning system was also used to wash silo tanks. The dairy used 10 bursts in the rinse cycles for silo tanks and this procedure was used for tanker washing. This was continued because the scope of these trials did not include an investigation of the number of bursts that are required to satisfactorily rinse a tanker.

Since this system was used to wash both silo tanks and tankers it was impossible to calculate the volume of water used to wash each tanker. The plant re-use system contained 500 gal of wash solution and was dumped twice weekly. An average of five tankers were washed seven days each week and three silo tanks on alternate days. Consequently, about 25 tankers and seven silo tanks were washed each week with 1000 gal of wash solution. The water used in the pre-rinse, wash, and post-rinse cycles for Tanker 3 was 85, 45, and 85 gal, respectively. The wash solution was discharged to the drain after the tanker was washed.

TABLE 2. *Regulatory inspection reports for milk transport tankers during phase 2*

Inspection period	Met regulatory sanitation conditions Tanker number					Surface film present Tanker number				
	1	2	3	4	5	1	2	3	4	5
1[a]	yes	yes	yes	yes	yes	no	no	no	no	no
2	yes	yes	yes	yes	yes	yes	no	no	yes	no
3	yes	yes	yes	yes	yes	yes	yes	no	yes	no
4	yes	yes	yes	yes	yes	yes	yes	yes	yes	no
5	yes	yes	yes	yes	yes	yes	yes	no	yes	yes
6	yes	yes	yes	yes	yes	yes	yes	no	yes	yes
7	yes	yes	yes	yes	yes	yes	yes	no	yes	yes
8	yes	yes	yes	yes	yes	yes	no	yes	yes	no

[a]Weeks after certification of clean tankers at the beginning of Phase 2.

TABLE 3. *Gallons per minute, washing cycle time, and volume of water used in washing tankers*

Cycle	Gallons per minute Tanker number					Time in cycle (minutes) Tanker number					Volume of water in cycle (gallons) Tanker number				
	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5
Pre-rinse	120	120	81	160	110	2.5	2.5	1.05	2.5	2.5	300	300	85	400	275
Wash	120	120	81	160	110	20.0	20.0	12.00	20.0	20.0	*	*	47	*	*
Post-rinse	120	120	81	160	110	2.5	2.5	1.05	2.5	2.5	300	300	85	400	275
TOTAL						25.0	25.0	15.00	25.0	25.0	600	600	217	800	550

* Wash solution requirement for each tanker could not be determined in the re-use system.

Values in Table 3 showing total time do not reflect the actual time required to clean a tanker. They represent the time in a cycle and do not include the delay between bursts, cleaning cycles, and hook-up time. Approximately 35 and 25 min were required between the entry and exit of a tanker from the washing area for the plant system and the single-use system, respectively.

The time required to connect spray balls to the cleaning systems was drastically reduced by connecting the spray balls to a manifold with a single outlet (Fig. 1).

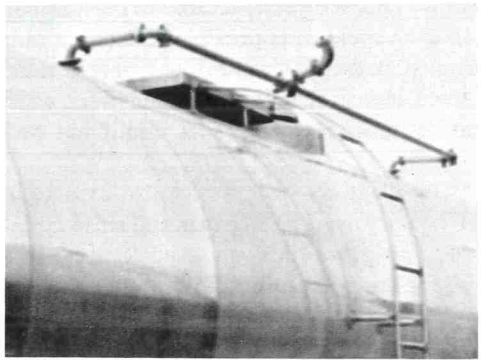

Figure 1. *Manifold used to provide a single connection for cleaning spray ball equipped tankers.*

This reduced both hook-up and removal time and the possibility of an accident by eliminating the need to make two connections on top of a tanker. The manifold performed satisfactorily and there were no sanitary problems associated with its use.

Advantages and disadvantages of drop-in and spray ball cleaning devices are shown in Table 4. Obviously, either unit has attributes that could make it the device of choice. The wide variety of cleaning systems that are used by individual plants make it difficult to suggest a unit of choice. For example, a plant that is equipped with only a drop-in unit could not clean a tanker carrying spray balls without manual cleaning of the interior of both the shank and spray balls. Conversely, a plant equipped to clean only tankers carrying spray balls could not clean a tanker without spray balls.

Further complications arise in that most spray devices have pressure and volume requirements within rather narrow limits. Difficulties arise when attempting to use cleaning systems with less demanding spray devices when the system was designed to deliver the pressure and volume requirements to the most demanding spray device. However, the T. G. Lee cleaning system was used to wash tankers equipped with either drop-in or spray balls. This was accomplished by placing a restrictor in the manifold built on Tanker 5. It was not determined in this study if this approach can be used in all dairy plants. It might be possible to incorporate an adjustable pressure relief valve into the supply line. The pressure could then be adjusted to the type of cleaning device used. However, this possibility was not investigated.

SUMMARY AND CONCLUSIONS

Milk transport tankers were adequately cleaned by either a drop-in unit or spray balls when used with the plant cleaning system.Tanker 3 was washed with a different supply system and different chemical concentrations. Therefore, it was impossible to make a direct comparison of the performance of spray balls used in the two systems. However, the proper pressure and volume at the spray device was maintained. Since the criterion of adequate cleaning was a satisfactory sanitary rating, it can be concluded that proper pressure and volume at the spray device is more important to the performance of a spray device than the type of system supplying it. Results presented in this paper indicate that any of the spray devices used in this study will adequately wash a tanker if the pressure-volume relationship is correct.

No spray device will clean a tanker unless the proper chemical concentration, time, temperature, pressure, and volume relationships are attained. If any of these parameters are inadequate, effective cleaning can not be achieved. The cleaning ability of any spray device is dependent upon the engineering system supplying the water and washing solution. If the pressure and volume requirements of a particular device are not achieved the device will not clean. Excessive pressure can damage the

TABLE 4. *Advantages and disadvantages of drop-in and spray ball cleaning devices*

Drop-In	Spray Ball
Advantages	
1. Can be used to wash any size tanker presently in use	1. Permanently mounted and not subject to improper alignment
2. Large spray openings not easily plugged	2. Minimal pressure and volume required for effective operation
3. Easy to check spray heads when installing or removing unit from tanker	3. Miminal maintenance is required
	4. Can be installed to wash any size tanker
Disadvantages	
1. Requires regular maintenance	1. Tanker must be entered to check condition of spray ball openings
2. Spray heads subject to damage during handling resulting in a change of cleaning pattern	2. If tanker is not cleaned with spray balls the spray balls and shank must be disassembled and the interior cleaned manually
3. Care is required in positioning unit in tanker to obtain proper coverage during washing	
4. Weight and need for a high ceiling sometimes makes it difficult to install and remove from a tanker	

device or change its hydraulics which will result in improper cleaning.

Regardless of the spray device used in circulation cleaning it is still necessary to routinely check the equipment, maintain it, and make corrections when needed. The temperature-pressure recorders were invaluable for monitoring the cleaning procedure. Pressure and temperature inadequacies of the supply solutions can readily be detected by examining the charts.

Foreign material lodging in the pumps was a problem. It was eliminated by raising the outlet from the solution supply tank. It seems possible that a screening device could be incorporated into the circulation system at some point and eliminate this problem in any washing system.

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Pollution Load of Cottage Cheese Whey and Wash Waters[1]

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ABSTRACT

Composition, waste characteristics, and potential pollution load of cottage cheese whey and wash waters were determined after draining and washing by the following methods: (*a*) partial draining of whey until the settled curd appeared, (*b*) complete draining of whey, (*c*) complete draining of whey with filtration, (*d*) partial draining with filtration, (*e*) partial draining with the remainder of whey cooled, recirculated through the curd, and completely drained, and (*f*) same as e with filtration. Whey and three wash waters were analyzed for temperature; pH; acidity; settleable, total, and suspended solids; BOD_5; COD; protein; nitrates; phosphates; and lactose. Temperature, pH, and acidity were controlled within the ranges expected. Settleable solids in whey and wash waters were negligible after filtering. Total and suspended solids, BOD_5, COD, protein, nitrates, phosphates, and lactose were reduced by about 50% when using the complete draining methods. A comparison was made using Method a as a control. A 20 to 35% reduction in BOD_5 could be achieved when including whey as a waste, while a 35 to 60% reduction could be obtained when only the three wash waters were included as a waste.

Current and past research on cottage cheese whey and wash waters is rather limited in the United States. Most of the work in progress or recently completed is centered primarily around whey recovery and utilization.

It is important, however, to obtain data showing the significance of the potential pollution load of not only whey and wash waters, but of the several different methods used to drain the whey and wash the curds, as some of these methods are being practice commercially. Nationally only about 50% of all whey (*2*) is utilized today. Complete removal of whey from waste-waters is a continuing problem. Cottage cheese wash waters, being too dilute, are not being utilized economically at this time and are second only to whey as a pollution source from the cheese industry.

Of special concern to the cottage cheese industry in pollution control are the following parameters: BOD_5 (biological oxygen demand), COD (chemical oxygen demand), suspended solids, total solids, settleable solids, and pH. Also important are the components that create this pollution problem such as nitrates, phosphates, protein, lactose, and lactic acid.

MATERIALS AND METHODS

Collection of samples

Samples were collected from the University of Vermont dairy plant Between 550 and 600 Kg of skim milk were used for each trial. Chemical composition and waste characteristics determinations were made on all samples. Whey was pumped from a 1,000 Kg cheese vat into a 900 Kg holding tank to measure the volume and to obtain a representative sample of the entire lot. The wash waters were metered into the cheese vat and then pumped into the holding tank for sampling.

Laboratory analysis

Tests were conducted to determine the amounts of BOD, COD, suspended solids, total solids, settleable solids, nitrates, phosphates, protein, and lactose in the whey and the wash waters. All tests were run in duplicate.

Nonconstituent analysis

The BOD and COD were estimated according to standard methods (*1*). Settleable, suspended, and total solids determinations were conducted according to recommended procedures (*8*).

Titratable acidity was determined as outlined by Newlander and Atherton (*7*). The pH was determined using a Fisher model 320 pH meter. Nitrates were determined by the Brucine-Sulfate method according to standard methods (*1*). The determination of phosphates was according to *Hawk's Physiological Chemistry* (*5*). Protein was determined by using the Kjeldahl procedure (*3*). The procedure for determining lactose was a modification of the Hycel method (*6*).

Six methods of draining and washing the curd were investigated to determine the potential pollution load of the cottage cheese whey and wash waters.

Process Method 1, which was used as the control in one part of this investigation, consisted of a partial draining of the whey until the curd particles appeared above the whey. The curd and remaining whey was then flooded with cold water (5 - 10 C). The curds were washed three times.

Method II was a complete draining of whey with the curds flooded with three washings. Each wash water volume was equal to about 75% of the total volume of skim milk used.

Method III was again a complete draining, except that the whey and wash waters were filtered through regular 16.5 cm strainer discs.

Method IV was a partial draining procedure with the same filtering as Method III.

Method V was a draining of about half of the total whey. The remainder was pumped through a plate cooler and then recirculated into the cheese vat. This cooling process continued until the curds were cooled to 10 C and then washed three times.

Method VI was the same as V except that all the whey and wash water samples were filtered before testing.

In all of the above procedures a plastic fiber cheese cloth was used to filter out the larger cheese particles in the whey and wash waters.

RESULTS AND DISCUSSION

STUDY I. Composition and waste characteristics of cottage cheese whey and wash waters

Data on composition and waste characteristics of whey are shown in Tables 1 and 2. Percent protein and lactose

[1]*University of Vermont Agricultural Experiment Station Journal article No. 338.*

TABLE 1. *Composition of whey*

Process method	Protein %	Lactose %	Nitrates mg/l	Phosphates mg/l	Protein/ lactose ratio
I.	0.85	4.20	1129.6	885.0	0.20
II.	0.86	4.47	739.5	808.1	0.19
III.	0.80	4.37	779.1	900.9	0.18
IV.	0.80	4.56	749.8	793.4	0.17
V.	0.83	4.60	980.1	805.6	0.18
VI.	0.84	4.46	583.3	748.2	0.19

TABLE 2. *Waste characteristics of whey*

Process method	BOD_5 mg/l	COD mg/l	Susp. S.[1] mg/l	Total S.[2] mg/l	Sett. S.[3] ml/l
I.	42,167	64,596	928.1	7,020	14.20
II.	37,883	64,415	645.0	6,840	7.50
III.	39,917	64,188	349.2	6,630	0.05
IV.	35,667	66,941	341.8	6,750	0.03
V.	34,500	67,213	905.1	6,950	8.50
VI.	36,283	66,281	419.9	6,790	0.30

[2]Suspended Solids
[2]Total Solids
[3]Settleable Solids

were within the ranges reported in the literature (*4*). Percent protein found in all methods ranged from 17 to 20% of the total amount of lactose. No apparent reasons were observed for the large variation in nitrate content of whey. Phosphates were within normal ranges as reported by Harper (*4*).

The BOD_5 (Table 2) ranged from 34,500 to 42,167 mg/1, with Method I having the greatest concentration. The COD, however, showed very little fluctuation (64,188 mg/1 in Method III to 67,213 Mg/1 in Method V) between the different methods, mainly because all the organic material is oxidized within a 2- to 3-hour period. Figure 1 graphically illustrates variation in BOD_5 and COD in each of the six methods.

There was no noticeable difference in the amount of BOD_5 and COD in filtration Methods III, IV, and VI. But there was a marked decrease in suspended and settleable solids (Table 2) content in the whey, with settleable solids being almost negligible as compared with nonfiltration. However, there was only a slight decrease in the total solids in the three filtration methods.

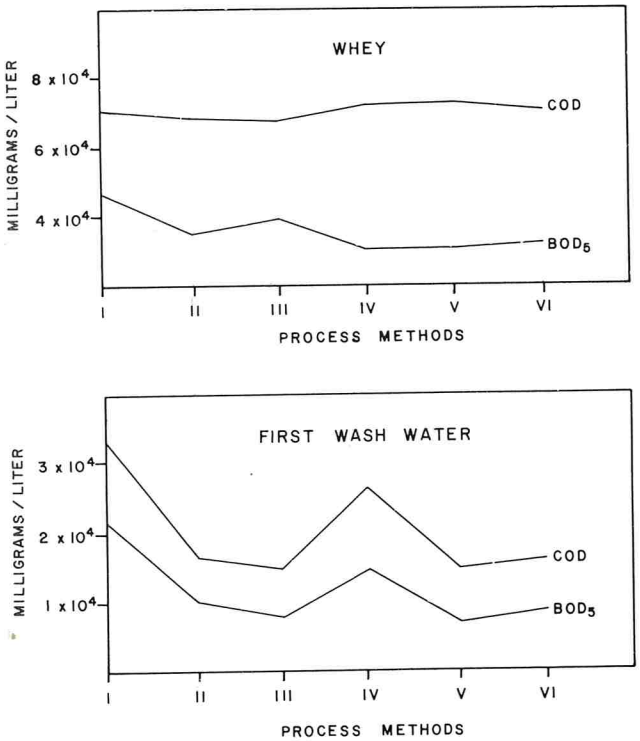

Figure 1. *BOD_5 and COD strengths of the whey and the first wash water.*

TABLE 3. *Composition of the first wash water treatment*

Process method	Protein %	Lactose %	Nitrates mg/l	Phosphates mg/l	Protein/ lactose ratio
I.	0.46	2.24	523.5	451.5	0.20
II.	0.23	1.33	174.4	223.5	0.17
III.	0.18	1.23	183.8	245.2	0.15
IV.	0.38	2.25	401.2	375.9	0.17
V.	0.23	1.01	198.4	191.8	0.22
VI.	0.20	1.10	134.4	198.4	0.18

In the first washing, all components (Table 3) were highest in Methods I and IV. This was caused by the partial draining where part of the whey remained in the vat when the first wash water, was added. Consequently, even though a smaller volume of water was used, this doubled the potential polution load (Table 4) of the first wash water as compared to the first wash water when complete draining was used. The BOD_5 and COD strengths are graphically illustrated in Figure 1.

TABLE 4. *Waste characteristics of the first wash water treatment*

Process method	BOD_5 mg/l	COD mg/l	Sus. S.[1] mg/l	Total S.[2] mg/l	Settl. S.[3] ml/l
I.	22,583	34,642	544.3	3,670	9.80
II.	10,983	18,066	340.9	1,820	5.00
III.	8,625	15,388	103.9	1,630	0.00
IV.	16,667	27,838	151.9	3,210	0.02
V.	7,758	15,350	641.1	1,520	7.70
VI.	9,500	15,985	237.7	1,580	0.00

[1]Suspended Solids
[2]Total Solids
[3]Settleable Solids

Protein and lactose in the whey are major contributors to the pollution load of the first wash water. Methods I and IV (Table 3) showed a twofold increase in the amounts of protein and lactose in the partial draining methods. The filtration methods (Table 4), as expected, reduced the suspended and settleable solids. Total solids in filtration Method IV, however, was double that of Methods III and VI, again caused by the remaining whey left in the first wash.

TABLE 5. *Composition of the second wash water treatment*

Process method	Protein %	Lactose %	Nitrates mg/l	Phosphates mg/l	Protein/ lactose ratio
I.	0.19	0.79	197.6	156.4	0.24
II.	0.11	0.46	53.2	89.1	0.24
III.	0.06	0.43	60.9	90.1	0.14
IV.	0.10	0.66	104.7	114.6	0.15
V.	0.13	0.41	84.7	92.9	0.32
VI.	0.08	0.43	53.9	84.7	0.19

The components in the second wash (Table 5) became more diluted with water. Percent lactose, which was still higher in Methods I and IV, decreased from 4.4% in the whey to 0.5% in the second wash water. Protein was reduced from about 0.85 to 0.15%. Nitrates and phosphates were <100 mg/1 except for Methods I and IV.

TABLE 6. *Waste characteristics of the second wash water treatment*

Process method	BOD_5 mg/l	COD mg/l	Sus. S.[1] mg/l	Total S.[2] mg/l	Sett. S.[3] ml/l
I.	6,775	13,580	488.5	1,340	6.00
II.	3,950	7,301	368.1	710	5.80
III.	3,923	5,963	79.6	630	0.00
IV.	4,440	9,249	72.1	900	0.00
V.	3.477	7,136	482.6	710	6.83
VI.	3,867	6,916	120.6	670	0.70

[1]Suspended Solids
[2]Total Solids
[3]Settleable Solids

Pollution load of the second wash remained high (Table 6). The BOD_5 ranged from 3,477 mg/1 in Method V to 6,775 mg/1 in Method 1. COD also was higher in Methods I and IV. Solids content of the second wash followed the same pattern as that of the whey and first washes. The lowest suspended and settleable solids content occurred in the filtration methods. Total solids in Method IV was higher than Methods III and VI due to part of the whey remaining in the first wash.

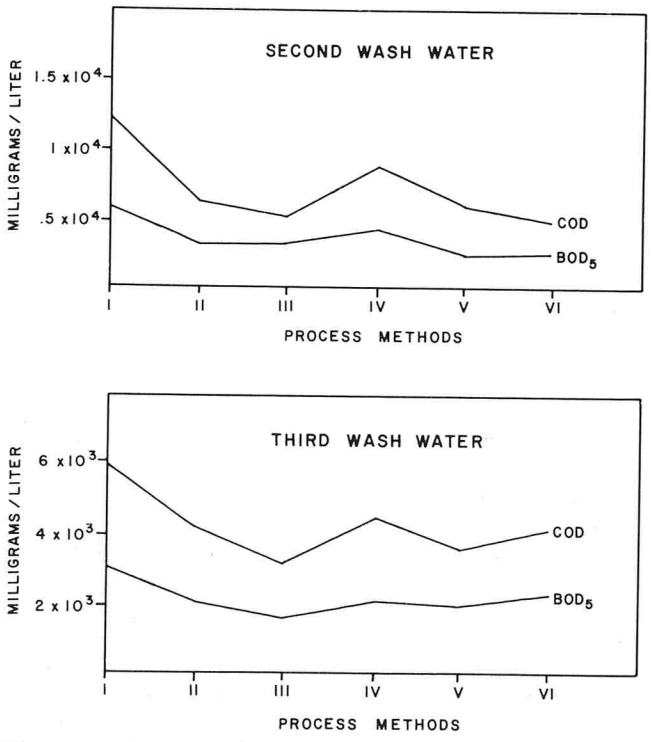

Figure 2. *BOD_5 and COD strengths of the second and third wash waters.*

Figure 2 illustrates the difference in BOD_5 and COD strengths in the second wash.

All components in the third wash showed little difference among the six methods (Table 7). Four components that were investigated were slightly higher in Method 1, probably because this was a partial draining method without filtration. Protein to lactose ratio ranged from 0.20 in Method IV to 0.35 in Methods II and V. Nitrates and phosphates ranged from 32 to 87 mg/1.

TABLE 7. *Composition of the third wash water treatment*

Process method	Protein %	Lactose %	Nitrates mg/l	Phosphates mg/l	Protein/ lactose ratio
I.	0.09	0.34	87.0	64.9	0.26
II.	0.08	0.23	32.1	49.8	0.35
III.	0.04	0.19	36.3	47.5	0.21
IV.	0.05	0.25	51.7	55.6	0.20
V.	0.07	0.20	51.6	49.6	0.35
VI.	0.06	0.23	32.2	52.1	0.26

TABLE 8. *Waste characteristics of the third wash water treatment*

Process method	BOD_5 mg/l	COD mg/l	Sus. S.[1] mg/l	Total S.[2] mg/l	Sett. S.[3] ml/l
I.	3,317	6,117	390.8	590	6.00
II.	2,066	4,004	344.3	400	6.50
III.	1,842	3,283	62.7	340	0.00
IV.	2,117	4,613	56.6	420	0.00
V.	2,085	3,773	331.9	390	6.33
VI.	2,267	4,359	116.5	430	0.00

[1]Suspended Solids
[2]Total Solids
[3]Settleable Solids

Even in the third wash, waste characteristics remained high (Table 8). The BOD_5 varied from 1,842 mg/1 in method III to 3,317 mg/1 in Method I. The COD ranged from 3,283 to 6,117 mg/1. The suspended solids in the filtration methods were about one-third that of the nonfiltration methods. Total solids did not vary significantly in the six methods as they ranged from 340 mg/1 in Method III to 590 mg/1 in Method I. The graph in Figure 2 is a comparison of the BOD_5 and COD strengths in the third wash water.

The pH gradually increased (4.30 to 4.50) starting with the whey to the final wash water treatment. Curd pH also increased gradually with each water treatment (4.30 to 4.40).

STUDY II. Potential pollution load of cottage cheese whey and wash waters

Inorder to develop a meaningful program to reduce wastewater BOD loads, it is helpful to know the strength of the waste coming from whey and each wash water involved in cottage cheese production. Values presented in Figure 3 are those that can be expected to occur in processing Methods I and VI for every 1,000 Kg of waste material utilized.

First, it was decided that Method I, the partial draining method, he used as a control. Percent reduction in Kg of BOD was determined for Methods II through VI (Figure 3). The formula used to calculate Kg of BOD is follows:

$$\frac{\text{Kg waste material} \times BOD_5\ (\text{mg/l})}{1{,}000{,}000} = \text{Kg of BOD}$$

Percent reduction was determined in two ways: first by including whey as a waste material (Figure 3) and second

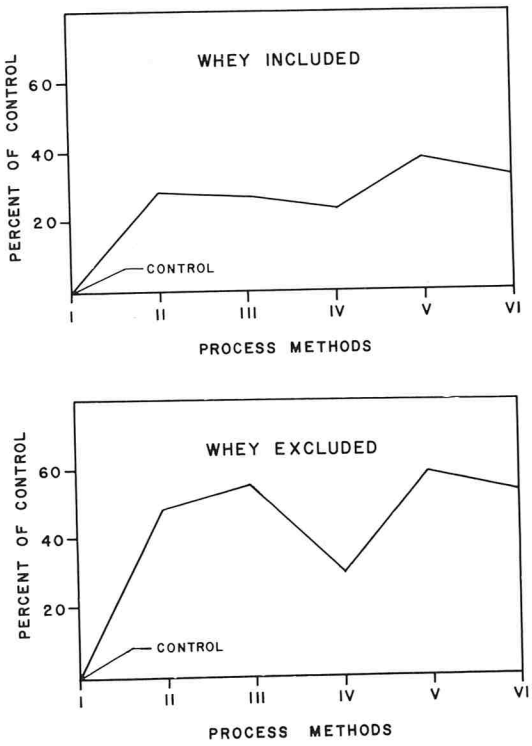

Figure 3. *Percent reduction of Kg of BOD_5 with method I used as a control.*

by excluding whey as a waste product. All three washings were considered as waste material.

By including whey as a waste, Method I (the control) contained 75.2 Kg/kkg of waste material. Method IV showed the least reduction containing 58.9 Kg/kkg of waste material, a 21% reduction in total pollution load. Methods V and VI showed the greatest reduction in terms of waste material, having 47.8 and 51.9 Kg/kkg of waste, respectively. This represented a 36.3 and 31.0% reduction, respectively.

By excluding whey as a waste material the percent reduction was even greater. The control contained 32.3 Kg/kkg waste load, while Method IV had 23.2 Kg/kkg of waste. This represents a 29% reduction (Figure 3). Methods II, III, V, and VI all ranged between 13 and 17 Kg/kkg of waste material. This represented a 50 to 60% reduction in total pollution load was compared to the control. Even though the partial draining Methods I and IV required a lesser amount of water, the remaining whey in the first wash created a higher total pollution load.

Next, the waste reduction using one wash and two washes as compared to three washes was determined

TABLE 9. *Percent BOD_5 reduction using one wash water as compared to three washings, (Kg/kkg waste material)*

Methods	First wash Kg	Second & third washes Kg	Reduction %
I.	22.58	10.09	30.9
II.	10.99	6.00	35.3
III.	8.63	5.76	40.0
IV.	16.60	6.56	28.3
V.	7.76	5.56	41.7
VI.	9.50	6.13	39.2

TABLE 10. *Percent BOD_5 reduction using two wash waters as compared to three washings (Kg/kkg waste material)*

Methods	First & second washings Kg	Third wash Kg	Reduction %
I.	29.35	3.32	10.2
II.	14.94	2.05	12.1
III.	12.55	1.84	12.8
IV.	21.04	2.12	9.2
V.	11.23	2.09	15.7
VI.	13.36	2.27	14.5

(Tables 9 and 10). Cottage cheese wash waters, as shown earlier in this discussion, possess relatively high waste loads, and utilizing all three washes makes total volume of wash water much greater than the total volume of whey. In order to eliminate one or two washings, a comparison was made to determine the waste load of one wash and two washes as compared to three washings. The parameter used was the 5-day BOD. The data in Table 9 show the percent reduction using only one wash water as compared to three washings. By eliminating two washes the reduction ranged from 28.3 to 41.7%, with the greatest reductions taking place in Methods III, V, and VI. Methods I and IV contained some whey, therefore raising their total pollution load. The major significance here is the possibility of eliminating the volume of two wash waters. If this is feasible, Methods V and VI seem to be the most probable, as the remaining whey (10 to 20%) and curds were cooled to 5 to 10 C before any wash water was added.

The percent reduction using two washings (Table 10) was decreased considerably, mainly because the pollution load of the third wash-even though quite high-was substantially less than the first and second washes. If only the third wash was eliminated there would be a 0 to 16% decrease in pollution load (Table 10). This would mean a savings of 1.84 to 3.32 Kg BOD/kkg of waste material.

TABLE 11. *pH of cottage cheese curd after draining of whey and after the first, second and third washing*

Method	Whey	After first washing	After second washing	After third washing
I.	4.36	4.35	4.36	4.36
II.	4.36	4.35	4.30	4.26
III.	4.28	4.28	4.31	4.33
IV.	4.35	4.36	4.40	4.28
V.	4.27	4.27	4.26	4.28
VI.	4.28	4.30	4.31	4.31

The data on the pH change of curd after draining the whey, after first wash, after second wash, and after third wash is reported in Table 11. When using draining and washing method V, the pH of the curd was 4.27 after draining whey, 4.27 after first wash, 4.26 after second wash and 4.28 after third wash. There were similar results when using method VI. The pH was 4.28, 4.30, 4.31, and 4.31 on the curd after whey draining, first, second, and third washing, respectively. With such a minimum change in pH it is felt that when the curd is cooled by the whey, only one washing with water is required.

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Detection of Penicillin in Milk by Bioluminescence[1]

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ABSTRACT

A procedure is described using the measurement of adenosine triphosphate (ATP) by bioluminescence for detection of penicillin in milk. Following a 3-h incubation period the method detected penicillin at 0.015 unit per ml when a 5% inoculum of *Bacillus subtilis* ATCC 6633 was used as the test culture.

Milk or commingled milk must currently be tested for antibiotics at least four times during any consecutive 6-month period (*6*). In addition, many manufacturers of cultured products utilizing milk as a substrate find it desirable to check their milk supply more frequently. The current edition of *Standard Methods for the Examination of Dairy Products* (*1*) lists two procedures which can be used to detect antibiotics in milk. These two methods and various modifications (*2, 4, 5*) are variations of a disc assay procedure for antibiotics which was first developed by Vincent (*7*).

Following the *Standard Methods* procedures (*1*) as close as possible, we have developed a test for penicillin in milk. The visual observation of inhibitory zones on seeded plates has been eliminated. Inhibition of the test organism is determined by measuring ATP.

MATERIAL AND METHODS

Test culture

Bacillus subtilis ATCC 6633 was obtained from Baltimore Biological Laboratories (BBL), Division of BioQuest, Cockeysville, Maryland. The spore suspension was stored at 5 C until used.

Assay media

Seed broth (SB) contained: 1.2% gelysate peptone, 0.8% trypticase, 0.6% yeast extract, 0.2% glucose and 0.3% yeast extract, all from BBL, and was autoclaved at 121 C for 15 min and refrigerated at 5 C until used. This broth is actually Antibiotic medium #1 (seed agar) described in *Standard Methods* (*1*) minus the agar. Nonfat milk (NFM) 11.0% was prepared, steamed at 100 C for 30 min, and refrigerated until used.

Testing of samples

Either a 1.0 or 5.0% inoculum of the spore suspension was made into the SB and heated at 80 C for 10 min. Equal volumes of the cooled inoculated SB and 11.0% NFM containing various concentrations of penicillin (BBL) were mixed and incubated in a water bath at 37 C for 0, 2, and 3 h.

Instruments and reagents

The photometer used was specifically designed for measurement of luminescent reactions and is commercially available from E. I. Dupont De Nemours and Company, Inc., Wilmington, Delaware. The firefly extracts, luciferin and luciferase, were also obtained from Dupont. Morpholinopropane sulfonic acid (MOPS) buffer was prepared by dissolving 2.09 g of MOPS/l of low response water (free of ATP). The buffer was adjusted to pH 7.4 with NaOH and sterilized by autoclaving at 121 C for 15 min. ATP standards were prepared by dissolving 100 mg of crystalline adenosine-5′-triphosphate-disodium salt in 100 ml of 0.01 M MOPS buffer (Boehringer and Mannheim). A more detailed description of the apparatus and procedure can be found in the Dupont 760 Luminescence Biometer Manual (*3*).

ATP determination

For analysis 0.1 ml of the sample was pipetted into a sterile disposable test tube. To each sample 0.9 ml of dimethylsulfoxide (90% in MOPS buffer) (Fisher Scientific) was added, shaken on a Vortex mixer, and allowed to stand for 2 min. Five milliliters of MOPS buffer was added to the extracted sample, mixed, and 0.01 ml injected into the biometer.

RESULTS AND DISCUSSION

Several instruments are commercially available to detect ATP by bioluminescence. We had access only to the Dupont apparatus. The Dupont biometer was designed to measure maximum light intensity produced and to provide a digital readout in 3 sec. Most reactions will have reached maximum light intensity within 3 sec. However, since we were working with a substrate (milk and SB) not previously reported, we felt it desirable to evaluate the relationship between maximum light intensity (as displayed by peak height on a recorder attached to the machine) and digital readout. Statistical analysis of the association between peak height and digital readout yielded an r value of 0.9989 and an r^2 of 99.8%.

To further evaluate the procedure and reproducibility of the machine we extracted and injected the same sample 100 times. The statistical analysis of data yielded the following values: $\bar{X}$ (mean) $= 7.55 \times 10^5$ Femtograms of ATP, S (standard deviation) $= 0.2687 \times 10^5$, SE (standard error) $= 0.0269 \times 10^5$ and CV (coefficient of variation) $= 3.558\%$.

ATP concentrations following incubation of samples containing penicillin when inoculated at the 1% level with *B. subtilis* spores are shown in Fig. 1. Similar data at the 5% inoculum level are presented in Fig. 2. When the inoculum level was 1%, detectable levels of ATP decreased in samples containing greater than 0.020 unit of penicillin/ml following 3 h of incubation at 37 C (Fig. 1). The same experiment repeated at the 5% level

[1]*Scientific Article No. A2029, Contribution No. 4981 of the Maryland Agricultural Experiment Station, Department of Dairy Science.*

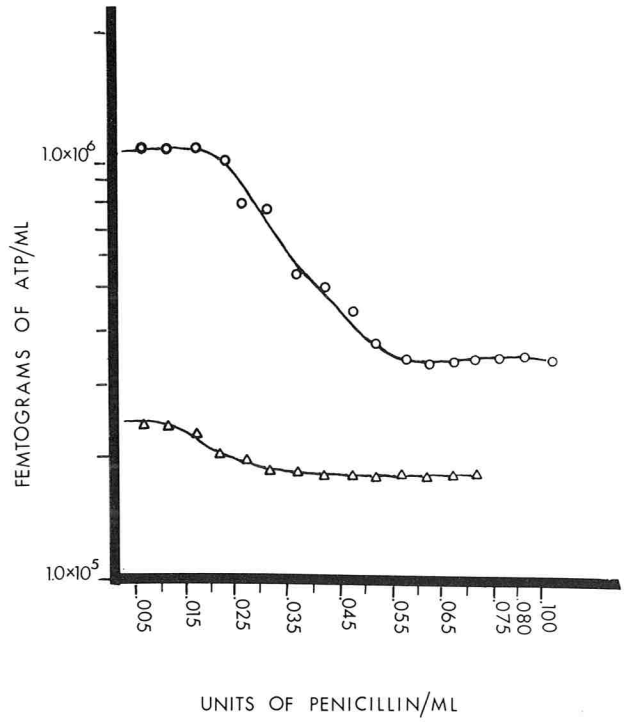

Figure 1. *Concentrations of ATP extracted from* Bacillus subtilis *(1% inoculum) following incubation at 37 C in milk containing penicillin. Triangles and circles are values following 2 and 3 h of incubation, respectively.*

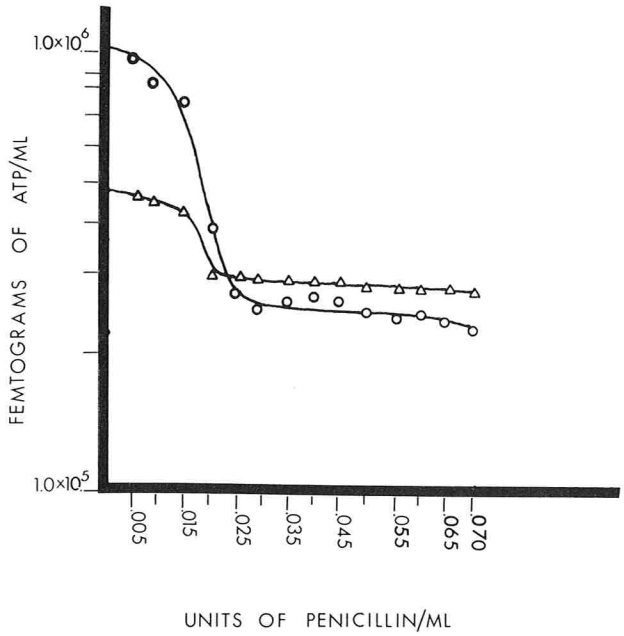

Figure 2. *Concentrations of ATP extracted from* Bacillus subtilis *(5% inoculum) following incubation at 37 C in milk containing penicillin. Triangles and circles are values following 2 and 3 h of incubation, respectively.*

resulted in a noticeable decrease in ATP in samples containing greater than 0.005 unit of penicillin/ml (Fig. 2). The sensitivity of the assay procedure, therefore, was increased by using a higher quantity of *B. subtilis* spores. In both instances, however, the detectable level of ATP continuously decreased and then leveled off (Fig. 1, 2), presumably indicating complete inhibition of the spore suspension.

In an attempt to shorten the assay time, samples were also analyzed following 2 h of incubation at 37 C. When the inoculum level was 1% the decrease in detectable levels of ATP was minimal with increasing concentrations of penicillin (Fig. 1). However, at the 5% inoculum level a noticeable difference in detectable ATP concentrations was observed in samples containing greater than 0.015 unit/ml (Fig. 2).

The reliability of several different combinations of incubation times and inoculum levels was tested. Four combinations were used: (*a*) 1% inoculum, 2 h of incubation, (*b*) 1% inoculum, 3 h of incubation, (*c*) 5% inoculum, 2 h of incubation and (*d*) 5% inoculum, 3 h of incubation. For the two procedures employing the 1% inoculum level, a concentration of penicillin of 0.025 unit/ml was selected, since this represented the breaking point in the curves depicted in Fig. 1. Similarly, at the 5% inoculum level, 0.015 unit of penicillin/ml was used. The reliability of each of the four different procedures was established by analyzing blind randomized samples with and without penicillin. These data are presented in Table 1. We had no difficulty in distinguishing between

TABLE 1. *The percent of samples identified correctly as containing penicillin when compared to controls*

Hours of incubation	Percent inoculum	
	1%	5%
2	100[a] (0.025)[b]	55 (0.015)
3	100 (0.025)	100 (0.015)

[a]Total number of samples analyzed = 100; 50 samples containing the respective penicillin levels, 50 samples with no penicillin. Percentage identified correctly of the number of positive samples are presented.
[b]Concentration of penicillin in the samples containing penicillin.

samples containing penicillin (0.025 unit/ml) and controls when a 1% inoculum was used with incubation for either 2 or 3 h (Table 1). Similarly, a combination of 5% inoculum and 3 h of incubation was 100% effective in distinguishing between penicillin-containing samples (0.015 unit/ml) and controls.

The latest estimate of cost for the Biometer reagents is about 50 cents per assay, not including the cost of the machine.

This study suggests the possible use of ATP measurements for detection of penicillin in milk. It is realized that many other antibiotics and/or test cultures could be investigated.

ACKNOWLEDGMENTS

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Understanding and Teaching the Most Probable Number Technique[1]

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ABSTRACT

The most probable number (MPN) technique is extensively used in food microbiology. However, because statistics involved often are cumbersome and it is difficult to obtain some of the widely scattered literature, many individuals are not able to delve into the subject to gain a thorough understanding of the method. To overcome these inherent difficulties, this review and discussion was prepared as an introduction and aid in presenting the MPN method to students and individuals working in food microbiology.

BACKGROUND

As we surveyed the literature dating back to Phelps' 1908 method (*14*) for calculating numbers of *Bacillus coli* (*Escherichia coli*) in dilution tests and continuing up to Parnows' 1972 publication (*13*) dealing with computer programs and the Most Probable Number (MPN), it was obvious that considerable time and effort has been spent in examining the statistical theory behind the method for determining bacterial densities by the tube dilution technique, i.e., the MPN method. What we propose to discuss is a teaching approach that will lend itself to the presentation of pertinent theory and data dealing with this subject to food microbiology oriented individuals. It is hoped that such an approach will enable us to convey both the advantages and disadvantages of this widely used method. We do not want to overlook the complexities of the statistics involved but, rather, we would like to suggest that the student not be overburdened with calculations that are not fundamental to understanding, using, and interpreting the MPN as a laboratory method.

It must be remembered that the mathematics of the MPN method are based on probability statistics and that results are directly related to the frequency of occurrence of a series of positive results most likely to occur when given numbers of bacteria are present in a sample.

Phelps (*14*) suggested that the number of *E. coli* in water samples be expressed as the reciprocal of the smallest portion of the sample in a geometric series of dilutions which gives a positive test. This method assumes that the dilutions of the sample are such that the final few dilutions give negative results. The method becomes confusing when "skips" occur in the sequence of dilutions and when more than one observation is obtained from a given sample.

A plate count can be converted directly into a number that corresponds to the most probable number of bacteria and this number is a linear function of the plate count. But, with a multiple tube result, the most probable number of bacteria is a logarithmic function of this result and further calculations are necessary to convert the results into the MPN value.

McCrady (*12*) was the first to discuss in depth the numerical interpretation of fermentation tube results in terms of both actual numbers and precision. In fact, the MPN tables he developed are still in use today. McCrady also emphasized the need to use many tubes, e.g., 10 per dilution to obtain meaningful (precise) results for accurate interpretation.

Since McCrady's early report, numerous authors have attempted to modify this cumbersome method of estimating bacterial densities. Greenwood and Yule (*5*), Wolman and Weaver (*19*), Stein (*17*), and Reed (*15*) emphasized the need to use large numbers of replicate tubes within series of dilutions to obtain accurate data. Halvorson and Ziegler (*6, 7, 8, 9*) and Halvorson and Moeglein (*10*) published articles dealing with the application of statistics to problems in bacteriology. In these articles not only was the role of large numbers of replicate tubes discussed, but also the effects of the nature of the bacterial population were stressed, in regard to experimental accuracy. All these workers pointed out that much of the previous work in this area dealt with estimates of bacterial densities determined by inoculating aliquots of a single dilution into a number of replicate tubes, and that such estimates did not yield accurate data. Greenwood and Yule (*5*) were given credit for first describing the general case of using several dilutions inoculated into a series of tubes to make such estimates. Halvorson and Ziegler (*7*) showed that when three effective dilutions are used to determine the bacterial population, the accuracy is independent of the number of organisms, and dependent only on the number of tubes per dilution. This is in contrast to the case in which only one dilution is used, the reason being that deviation from the mode (most probable value) is narrowed as more tubes are used. That is, the probability of a more accurate assessment of the bacterial density is

[1]*Florida Agricultural Experiment Stations Journal Series No. 5785.*

increased as the number of tubes is increased. In 1935, Halvorson and Ziegler (*9*) published a comparison of the multiple tube method (dilution method), the plate count, and the direct microscopic count. They found that, as a rule, the dilution method gave higher values for bacterial populations than did the plate count method. The direct count method gave the same value as the plating and dilution methods only when used on cultures before they had entered the death phase, indicating that viability is another variable that must be considered when evaluating bacterial densities.

The following formula for the estimation of the MPN was given by Thomas (*18*):

$$\text{M.P.N.} = \frac{\text{Number of positive tubes}}{\sqrt{(\text{Number of ml of sample in negative tubes}) \times (\text{Number of ml of sample in all tubes})}}$$

This formula is not restricted as to number of tubes and dilutions used. The author noted, however, that where increased precision is desired, a corresponding increase in the number of tubes tested is necessary. In addition, if tubes in the lowest dilution are all positive, the omission of this lowest dilution from the computation will improve the agreement between the formula and table values.

McCarthy et al. (*11*) demonstrated a substantial mathematical bias in MPN values relative to plate counts. With the agar plate count method as the control estimator, 10 replicate MPN's indicated an average bias of + 29%, + 10%, + 6%, and − 4% when compared to the plate counts' arithmetic average, geometric mean, median, and harmonic mean, respectively. The precision of 10 replicate plate counts was at least three times that of replicate MPN values. Perhaps Woodward (*20*) summed it up the best: "The lack of precision of MPN estimates of bacterial densities is generally recognized—at least by those who perform these tests."

With the foregoing discussion serving as a broad overview of the MPN method, let us then get to some definite features of the method that need to be related to the food microbiology student who may someday find himself or herself in a quality control or regulatory laboratory working with the MPN.

BASIC ASSUMPTIONS

Several assumptions are involved with the MPN as a method. First, it is assumed that organisms are distributed randomly throughout the sample. This means that an organism can be found in any portion of the sample and that clumping or attracting and/or repelling forces do not exist. This is important when one considers the various types of foods that are examined in most laboratories, especially when the sample is comprised of particulate matter. Second, one has to assume that each aliquot from the liquid will exhibit growth whenever such an aliquot contains one or more organisms and said aliquot is incubated in an "appropriate" growth medium. Freedom from contamination of supplies and equipment is also assumed, as is appropriate technical expertise.

APPLICATIONS

Disadvantages often associated with the MPN include time, space, and material considerations. However, the advantages of the MPN technique allow numerous useful applications: (*a*) Estimates can be made of a population using any number of dilutions—just as with the plate count method. (*b*) Accuracy can be adjusted by increasing the number of tubes per dilution—this consideration has been previously discussed. (*c*) Sample size can be quite large—this is becoming more important as limitations of the membrane filter technique become more widely known (*4, 16*). (*d*) Sensitivity and recovery with the MPN is generally better at low population levels than with the plate count (*2*)—the reason for this being that it is possible to use larger sample volumes than with the plate count method. (*e*) Recovery may be better in liquid than solid media, depending on the particular sample involved (*6*). (*f*) Materials can be prepared in advance and readily used under field conditions. (*g*) If the appropriate medium is available, estimates can be made of any organism(s).

USING MPN TABLES

Practically speaking, the most widely used form of the MPN involves three-tube and five-tube (per dilution) analyses for coliforms. Once the actual experimental manipulations of samples and materials are completed, regardless of the type of MPN analysis, tubes go into an appropriate incubator. After a prescribed incubation period, observations are recorded, further analyses may be conducted and then the appropriate MPN table is sought. The reader is referred to the 1960 edition of *Standard Methods for the Examination of Water and Wastewater* (*1*) for exhaustive tabular 5-tube MPN values and confidence limits. Some unusual sample volume combinations and their appropriate confidence limits are shown in Table 1; Table 2 presents MPN values and confidence limits for 3-tube series. Examples of MPN results and their interpretation are given in Table 3.

TABLE 1.[a] *MPN and 95 per cent confidence limits for various combinations of positive results in the following series:*
A—five 10-ml tubes
B—five 10-ml, five 1-ml & five 0.1-ml tubes
C—five 10-ml, one 1-ml & one 0.1-ml tubes
D—one 50-ml & five 10-ml tubes
E—one 50-ml, five 10-ml & five 1-ml tubes
F—five 50-ml, five 10-ml & five 1-ml tubes

	No. of Positive Tubes out of: Five 10-ml Tubes	MPN per 100 ml	Limits of MPN	
			Lower	Upper
A	0	0	0	6.0
	1	2.2	0.1	12.6
	2	5.1	0.5	19.2
	3	9.2	1.6	29.4
	4	16.0	3.3	52.9
	5	∞	8.0	∞

TABLE 1. (*Continued*).

	Five 10-ml Tubes	Five 1-ml Tubes	Five 0.1-ml Tubes	MPN per 100 ml	Limits of MPN	
					Lower	Upper
B	0	0	1	2	<0.5	7
	0	0	2	4	<0.5	11
	0	1	0	2	<0.5	7
	0	1	1	4	<0.5	11
	0	1	2	6	<0.5	15
	0	2	0	4	<0.5	11
	0	2	1	6	<0.5	15
	0	3	0	6	<0.5	15
	1	0	0	2	<0.5	7
	1	0	1	4	<0.5	11
	1	0	2	6	<0.5	15
	1	0	3	8	1	19
	1	1	0	4	<0.5	11
	1	1	1	6	<0.5	15
	1	1	2	8	1	19
	1	2	0	6	<0.5	15
	1	2	1	8	1	19
	1	2	2	10	2	23
	1	3	0	8	1	19
	1	3	1	10	2	23
	1	4	0	11	2	25
	2	0	0	5	<0.5	13
	2	0	1	7	1	17
	2	0	2	9	2	21
	2	0	3	12	3	28
	2	1	0	7	1	17
	2	1	1	9	2	21
	2	1	2	12	3	28
	2	2	0	9	2	21
	2	2	1	12	3	28
	2	2	2	14	4	34
	2	3	0	12	3	28
	2	3	1	14	4	34
	2	4	0	15	4	37
	3	0	0	8	1	19
	3	0	1	11	2	25
	3	0	2	13	3	31
	3	1	0	11	2	25
	3	1	1	14	4	34
	3	1	2	17	5	46
	3	1	3	20	6	60
	3	2	0	14	4	34
	3	2	1	17	5	46
	3	2	2	20	6	60
	3	3	0	17	5	46
	3	3	1	21	7	63
	3	4	0	21	7	63
	3	4	1	24	8	72
	3	5	0	25	8	75
	4	0	0	13	3	31
	4	0	1	17	5	46
	4	0	2	21	7	63
	4	0	3	25	8	75
	4	1	0	17	5	46
	4	1	1	21	7	63
	4	1	2	26	9	78
	4	2	0	22	7	67
	4	2	1	26	9	78
	4	2	2	32	11	91
	4	3	0	27	9	80
	4	3	1	33	11	93
	4	3	2	39	13	106
	4	4	0	34	12	96
	4	4	1	40	14	108
	4	5	0	41	14	110
	4	5	1	48	16	124
	5	0	0	23	7	70
	5	0	1	31	11	89
	5	0	2	43	15	114
	5	0	3	58	19	144
	5	0	4	76	24	180
	5	1	0	33	11	93
	5	1	1	46	16	120
	5	1	2	63	21	154
	5	1	3	84	26	197
	5	2	0	49	17	126
	5	2	1	70	23	168
	5	2	2	94	28	219
	5	2	3	120	33	281
	5	2	4	148	38	366
	5	2	5	177	44	515
	5	3	0	79	25	187
	5	3	1	109	31	253
	5	3	2	141	37	343
	5	3	3	175	44	503
	5	3	4	212	53	669
	5	3	5	253	77	788
	5	4	0	130	35	302
	5	4	1	172	43	486
	5	4	2	221	57	698
	5	4	3	278	90	849
	5	4	4	345	117	999
	5	4	5	426	145	1,161
	5	5	0	240	68	754
	5	5	1	348	118	1,005
	5	5	2	542	180	1,405

	Five 10-ml Tubes	One 1-ml Tube	One 0.1-ml Tube	MPN per 100 ml	Limits of MPN	
					Lower	Upper
C	0	0	0	0		5.9
	0	1	0	2	0.050	13
	1	0	0	2.2	0.050	13
	1	1	0	4.4	0.52	14
	2	0	0	5	0.54	19
	2	1	0	7.6	1.5	19
	3	0	0	8.8	1.6	29
	3	1	0	12	3.1	30
	4	0	0	15	3.3	46
	4	0	1	20	5.9	48
	4	1	0	21	6.0	53
	5	0	0	38	6.4	330
	5	0	1	96	12	370
	5	1	0	240	12	3,700
	5	1	1		88	

	One 50-ml Tube	Five 10-ml Tubes	MPN per 100 ml	Limits of MPN	
				Lower	Upper
D	0	1	1	<0.5	4
	0	2	2	<0.5	6
	0	3	4	<0.5	11
	0	4	5	1	13
	1	0	2	<0.5	6
	1	1	3	<0.5	9
	1	2	6	1	15
	1	3	9	2	21
	1	4	16	4	40

	One 50-ml Tube	Five 10-ml Tubes	Five 1.ml Tubes	MPN per 100 ml	Limits of MPN	
					Lower	Upper
E	0	0	1	1	<0.5	4
	0	0	2	2	<0.5	6
	0	1	0	1	<0.5	4
	0	1	1	2	<0.5	6
	0	1	2	3	<0.5	8
	0	2	0	2	<0.5	6
	0	2	1	3	<0.5	8
	0	2	2	4	<0.5	11
	0	3	0	3	<0.5	8
	0	3	1	5	<0.5	13
	0	4	0	5	<0.5	13

TABLE 1. (*Continued*).

1	0	0	1	<0.5	4
1	0	1	3	<0.5	8
1	0	2	4	<0.5	11
1	0	3	6	<0.5	15
1	1	0	3	<0.5	8
1	1	1	5	<0.5	13
1	1	2	7	1	17
1	1	3	9	2	21
1	2	0	5	<0.5	13
1	2	1	7	1	17
1	2	2	10	3	23
1	2	3	12	3	28
1	3	0	8	2	19
1	3	1	11	3	26
1	3	2	14	4	34
1	3	3	18	5	53
1	3	4	21	6	66
1	4	0	13	4	31
1	4	1	17	5	47
1	4	2	22	7	69
1	4	3	28	9	85
1	4	4	35	12	101
1	4	5	43	15	117
1	5	0	24	8	75
1	5	1	35	12	101
1	5	2	54	18	138
1	5	3	92	27	217
1	5	4	161	39	<450

	Five 50-ml Tubes	Five 10-ml Tubes	Five 1-ml Tubes	MPN per 100 ml	Limits of MPN	
					Lower	Upper
F	0	0	1	1	<0.5	2
	0	0	2	1	<0.5	2
	0	1	0	1	<0.5	2
	0	1	1	1	<0.5	2
	0	1	2	1	<0.5	2
	0	2	0	1	<0.5	2
	0	2	1	1	<0.5	2
	0	3	0	1	<0.5	2
	1	0	0	1	<0.5	2
	1	0	1	1	<0.5	2
	1	0	2	1	<0.5	2
	1	0	3	2	<0.5	4
	1	1	0	1	<0.5	2
	1	1	1	1	<0.5	2
	1	1	2	2	<0.5	4
	1	2	0	1	<0.5	2
	1	2	1	2	<0.5	4
	1	2	2	2	<0.5	4
	1	3	0	2	<0.5	4
	1	3	1	2	<0.5	4
	1	4	0	2	<0.5	4
	2	0	0	1	<0.5	2
	2	0	1	1	<0.5	2
	2	0	2	2	<0.5	4
	2	0	3	2	<0.5	4
	2	1	0	1	<0.5	2
	2	1	1	2	<0.5	4
	2	1	2	2	<0.5	4
	2	2	0	2	<0.5	4
	2	2	1	2	<0.5	4
	2	2	2	3	1	7
	2	3	0	2	<0.5	4
	2	3	1	3	1	7
	2	4	0	3	1	7
	3	0	0	2	<0.5	4
	3	0	1	2	<0.5	4
	3	0	2	2	<0.5	4
	3	1	0	2	<0.5	4
	3	1	1	2	<0.5	4
	3	1	2	3	1	7
	3	1	3	4	1	9
	3	2	0	3	1	7
	3	2	1	3	1	7
	3	2	2	4	1	9
	3	3	0	3	1	7
	3	3	1	4	1	9
	3	4	0	4	1	9
	3	4	1	4	1	9
	3	5	0	5	2	12
	4	0	0	2	<0.5	4
	4	0	1	3	1	7
	4	0	2	3	1	7
	4	0	3	4	1	9
	4	1	0	3	1	7
	4	1	1	4	1	9
	4	1	2	4	1	9
	4	2	0	4	1	9
	4	2	1	4	1	9
	4	2	2	5	2	12
	4	3	0	5	2	12
	4	3	1	5	2	12
	4	3	2	6	2	14
	4	4	0	6	2	14
	4	4	1	7	3	17
	4	5	0	7	3	17
	4	5	1	8	3	19
	5	0	0	4	1	9
	5	0	1	4	1	9
	5	0	2	6	2	14
	5	0	3	7	3	17
	5	0	4	8	3	19
	5	1	0	5	2	12
	5	1	1	6	2	14
	5	1	2	7	3	17
	5	1	3	9	3	21
	5	2	0	6	2	14
	5	2	1	8	3	19
	5	2	2	10	4	23
	5	2	3	12	4	28
	5	2	4	15	5	37
	5	2	5	18	6	53
	5	3	0	9	3	21
	5	3	1	11	4	26
	5	3	2	14	5	34
	5	3	3	18	6	53
	5	3	4	21	7	66
	5	3	5	25	8	78
	5	4	0	13	5	31
	5	4	1	17	6	47
	5	4	2	22	7	70
	5	4	3	28	9	85
	5	4	4	35	11	101
	5	4	5	43	14	118
	5	5	0	24	8	75
	5	5	1	35	11	101
	5	5	2	54	18	140
	5	5	3	92	27	218
	5	5	4	161	39	424

[a]This table taken from *Standard Methods for the Examination of Water and Wastewater* (*1*).

At this point it is questionable as to whether or not one needs to be able to derive the formula that is responsible for the tabular MPN values. We are going under the assumption that it is not necessary. It is important that one be able to read the table properly and to understand the significance of the results. Assuming that the tabulated numbers are correctly computed, the next consideration should be an appreciation of the "confidence interval" surrounding a given value in this table. That is, the tabular MPN value really represents a

TABLE 2.[a] *MPN and 95 per cent confidence limits for various combinations of positive results using three tubes each with volumes of 10, 1, 0.1 ML**

No. of Positive Tubes out of:					
Three 10-ml Tubes	Three 1-ml Tubes	Three 0.1-ml Tubes	MPN per 100 ml	Limits of MPN Lower	Limits of MPN Upper
0	0	0		0	
0	0	1	3		9
0	0	2	6		
0	0	3	9		
0	1	0	3	0.085	13
0	1	1	6.1		
0	1	2	9.2		
0	1	3	12		
0	2	0	6.2		
0	2	1	9.3		
0	2	2	12		
0	2	3	16		
0	3	0	9.4		
0	3	1	13		
0	3	2	16		
0	3	3	19		
1	0	0	3.6	0.085	20
1	0	1	7.2	0.87	21
1	0	2	11		
1	0	3	15		
1	1	0	7.3	0.88	23
1	1	1	11		
1	1	2	15		
1	1	3	19		
1	2	0	11	2.7	36
1	2	1	15		
1	2	2	20		
1	2	3	24		
1	3	0	16		
1	3	1	20		
1	3	2	24		
1	3	3	29		
2	0	0	9.1	1.0	36
2	0	1	14	2.7	37
2	0	2	20		
2	0	3	26		
2	1	0	15	2.8	44
2	1	1	20		
2	1	2	27		
2	1	3	34		
2	2	0	21	3.5	47
2	2	1	28		
2	2	2	35		
2	2	3	42		
2	3	0	29		
2	3	1	36		
2	3	2	44		
2	3	3	53		
3	0	0	23	3.5	120
3	0	1	39	6.9	130
3	0	2	64		
3	0	3	95		
3	1	0	43	7.1	210
3	1	1	75	14	230
3	1	2	120	30	380
3	1	3	160		
3	2	0	93	15	380
3	2	1	150	30	440
3	2	2	210	35	470
3	2	3	290		
3	3	0	240	36	1,300
3	3	1	460	71	2,400
3	3	2	1,100	150	4,800
3	3	3		460	

* For values not given, *approximate* lower and upper limits may be estimated as 21 per cent of the MPN for the lower and 395 per cent for the upper. The confidence limits given are more exact calculations for the tube results most likely to be encountered. The results for which confidence limits are not given my be expected to be less than 1 per cent of the results commonly observed.

[a]This table taken from *Standard Methods for the Examination of Water and Wastewater* (*1*).

TABLE 3. *Examples of determining MPN estimates*

Example	Dilution of sample 10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	Reported positive tubes	MPN (org/gram)
a	3/3*	2/3	0/3	0/3	0/3	3-2-0	93
b	3/3	3/3	3/3	1/3	0/3	3-1-0	43,000
c	3/3	2/3	2/3	0/3	0/3	3-2-2	210
d	3/3	3/3	0/3	1/3	0/3	3-0-1	3,900
e	3/3	3/3	3/3	3/3	3/3	3-3-3	<1,100,000
f	3/3	0/3	1/3	0/3	0/3	3-0-1	39
g	3/3	2/3	1/3	1/3	0/3	3-2-2	210
h	3/3	3/3	2/3	2/3	1/3	3-2-3	290
i	2/3	2/3	2/3	2/3	0/3	2-2-2	350
j	0/3	1/3	0/3	0/3	0/3	0-1-0	3

$$* \frac{\text{Numerator}}{\text{Denominator}} = \frac{\text{Number of positive tubes}}{\text{Number of tubes inoculated}}$$

Directions for use of MPN table (3-tube). When more than three dilutions are used in a series of dilutions, the results from only three of these dilutions are used in determining the MPN value. Select the highest dilution in which all three tubes are positive and the next two higher dilutions (examples: a,b,c,d,e,f). When a positive result is noted in a dilution higher than the three selected according to the rule, it should be added to the result for the highest dilution chosen (examples: g,h). If more than three dilutions are made and none of the dilutions show three positive tubes, use the data which incorporate the positve tubes that are present (example: i). For those cases in which the numbers of microorganisms are very low (example: j), incorporate the positive result such that it is represented by the middle dilution if possible. The MPN value for 0-1-0 or 1-0-0 is nearly identical and well within published confidence limits.

range and not an absolute value. MPN estimates are often credited with a precision they do not live up to.

Confidence limits for MPN estimates can be computed on the basis of a logarithmically normal distribution. As the MPN estimate is biased (as we have previously discussed), these confidence limits are not symmetric about the MPN estimate. Woodward (*20*) published tables of confidence limits for three- and five-tube multiple dilution assays and pointed out that for a three-tube test the 95% confidence limits cover a 33-fold range from approximately 14 to 458% of the actual MPN estimate. For a five-tube multiple dilution test, the 95% confidence limits cover a 13-fold range from approximately 24 to 324% of the MPN. It is highly doubtful that a student would have occasion to compute confidence limits, but they should understand that such limits exist.

Another point that should be made clear to students just becoming familiar with MPN tables is that you really need only one "master" table. That is to say, once the sample volume per tube is established, it's just a matter of moving the decimal point for the MPN value that corresponds to the dilution being reported.

TEACHING APPROACH

In teaching the application of the MPN method, we try to progress through a series of experiments which enable the student to grasp both the positive and negative

aspects of the method. A lecture approach using much of the previous discussion as background is given first. Then, in the laboratory, a water sample is analyzed using lactose broth rather than lauryl sulfate tryptose broth so that an "estimation of total numbers" from growth-positive tubes can be made as well as for presumptive coliforms (gas-positive tubes). Then the rationale for transfer of gas-positive tubes into Brilliant Green broth is given and a discussion of reading the tubes is presented. Final calculations are made and an understanding of the confidence limits to be placed on the results is stressed. A discussion of using the tables, with the possibilities of unusual combinations of sample volumes [Table 1 (*1*)], is presented and possible applications are discussed. The value of the MPN method in survey work, screening for low populations of organisms, and using large sample volumes is stressed.

Then a more involved experiment, quantification of low numbers of enteropathogenic *E. coli* from a meat sample, is done. Emphasis is placed on the care required in marking all tubes, plates, and individual isolates, so that following serological identification of the isolates, results may be related back to the original sample dilutions. As the course progresses, working with staphylococci, enterococci, and other organisms, the method becomes second nature to the student.

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Botulism in Commercially Canned Foods

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ABSTRACT

Occasionally, a problem thought to be well under control returns to plague us. This is the case with botulism in commercially canned foods. These foods have had a remarkably good record over the last 45 years with approximately 775 billion cans of commercially canned foods being consumed with only four known deaths through 1971. Beginning in 1971, however, botulinal toxin and/or *Clostridium botulinum* has been found in commercially canned vichyssoise, chicken vegetable soup, peppers, marinated mushrooms, tuna fish, beef stew, and in 41 cans of mushrooms from 20 lots packed by seven domestic and two foreign producers. The typical cause of botulism in canned foods is underprocessing which may result from inadequate equipment, improper operating procedures, and scheduled processes which are not appropriate for the actual operating conditions being used.

Botulism is a relatively rare neuroparalytic disease affecting man and animals. The fatality rate has been high, but prompt treatment with antitoxin and the use of respirators in recent years has somewhat mitigated this. Although there is increasing evidence that some cases may be attributed to wounds infected with *Clostridium botulinum* spores (*8, 25*), the disease is usually contracted by the ingestion of a food containing the toxin of one or more of the seven immunological types of the organism (A-G). The foods implicated are generally those of a type that have undergone some treatment intended for the preservation of the product such as canning, pickling or smoking, but one which failed to destroy the spores of *C. botulinum.* When the intended preservative treatment is inadequate, and is followed by storage under conditions which permit the germination and growth of *C. botulinum* (*1, 2*), one of the most lethal toxins known to man may be produced.

Commercial canners had many problems with *C. botulinum* in the early days of the industry, but through intensive study of processing procedures and the microbiology of spoilage, these problems were thought to be largely solved during the 1920's and 30's (*21, 31, 32*). Even with the problems of the industry in the early days, the bulk of the cases recorded since 1899 have resulted from home canning (*11, 28*). The record of outbreaks in the United States from both commercial and home processed foods is shown in Table 1. The full extent of the problem with home processed foods, however, is probably not known since it is likely that reporting is poor, especially in rural areas where most home processing is done. In addition, the clinical symptoms are often confused with those of other diseases, even by well

TABLE 1. *Outbreaks of foodborne botulism in the United States attributed to commercially processed or home processed foods 1899-1973*[1]

	Number of outbreaks									
Source of food	1899	1900-1909	1910-1919	1920-1929	1930-1939	1940-1949	1950-1959	1960-1969	1970-1973	Total
Home processed	1	1	48	77	135	120	50	42	21[2]	495
Commercially processed	0	1	14	26	6	1	2	10	2	62
Unknown	0	0	8	13	13	13	51	26	7	131
Total	1	2	70	116	154	134	103	78	30	688

[1]From reference 11.
[2]Includes one outbreak in which the vehicle was canned by the owner of a restaurant and sold to his customers.

TABLE 2. *Food products causing botulism outbreaks in the United States*[1,2] *1899-1973*

	Number of outbreaks									
Botulinum toxin type	Vegetables	Fish and Fish Products	Fruits	Condiments[3]	Beef[4]	Milk and Milk Products	Pork	Poultry	Other[5]	Total
A	96	7	22	16	3	2	2	1	5	154
B	24	3	5	4	1	2	1	2		42
E	1	19								20
F					1					1
A & B	2									2
Total	123	29	27	20	5	4	3	3	5	219

[1]From reference 11.
[2]Includes only outbreaks in which the toxin type was determined.
[3]Includes outbreaks traced to tomato relish, chili peppers, chili sauce, and salad dressing.
[4]Includes 1 outbreak of type F in venison, and 1 outbreak of type A in mutton.
[5]Includes outbreaks traced to vichyssoise soup, spaghetti sauce, and to corn and chicken mash.

trained physicians. Diagnoses such as acute poliomyelitis, streptococcal sore throat, small bowel obstruction, or cerebral vascular occlusion have been made in some cases before the correct diagnosis was made (*24*). It is impossible to say how many such erroneous diagnoses have been made and never corrected, but there is adequate evidence that such diagnostic errors do occur.

A great variety of foods has been involved as the vehicle for botulism. These, with few exceptions, are low acid foods (having a pH >4.6). A summary of the types of foods involved in outbreaks in the United States is shown in Table 2.

The spores of *C. botulinum* are widely distributed in nature. They have been found in garden soils and virgin forest soils (*26, 27*), the shore and bottom deposits of lakes (*11, 12, 29*), the littoral regions of oceans and seas (*13, 19, 20, 33*), fish, shellfish, and, though perhaps rarely, the intestinal tract of animals (*14, 15, 16, 17, 23, 26, 27, 30, 34, 35, 36, 37*). The contamination of the raw ingredients of many products is, therefore, highly probable and consequently the canning process must be designed to destroy the spores of *C. botulinum.* Other spoilage organisms of higher heat resistance (although causing economic losses to the canner, they are of no public health consequence) are the basis for design of commercial processes and, therefore, should provide a margin of safety.

Despite the fact that the essential parameters for processing canned foods had been established many years earlier, in 1971 commercially canned vichyssoise soup containing type A toxin was responsible for two cases of botulism, with one death (*3*). In 1973, canned mild hot peppers in glass jars, produced by a small cannery in West Virginia, were responsible for another outbreak involving eight people but causing no deaths (*6*). The peppers were found to contain type B toxin. Again in 1973, a Canadian outbreak was traced to marinated mushrooms packed in oil by a small cannery near Boston; the product contained type B toxin (*7*). Only one person became ill and, fortunately, recovered. Beef stew produced by another canner was found to contain type A toxin which resulted in two additional cases with one death in November, 1974 (*10*). In addition to the clinical cases, investigation of three other canned products, which were not responsible for any further outbreaks, showed the presence of botulinum toxins. One canner had swollen No. 10 cans of mushrooms contaminated with type B toxin in 1970. The second had swollen cans of chicken vegetable soup containing both type A and B toxins in 1971 (*4*), and in 1974 the third had type C toxin in a leaking can of tuna fish (*9*). Table 3 shows the commercially canned foods produced in the U.S. responsible for botulism outbreaks, or found to contain *C. botulinum,* from April 1970 through November 1974 with the exception of the 1973-1974 mushroom incidents.

During 1973-1974, commercially canned mushrooms were a particularly alarming problem, since the products

TABLE 3. *Commercially canned foods contaminated with botulinum toxin April 1970-November 1974*[1]

Date	Food	Company	Toxin	Number of clinical cases
4/70	Mushrooms	Wm. F. Lehmann, Inc.	B	0
6/71	Vichyssoise	Bon Vivant Soups, Inc.	A	2
8/71	Chicken vegetable soup	Campbell Soup Co.	A,B	0
5/73	Peppers	Felix & Son's Wholesale, Inc.	B	8
7/73	Mushrooms	Wirth Food Products, Inc.	B	1[2]
4/74	Tunafish	Star Kist Foods, Inc.	C	0
11/74	Beef stew	Kelly Foods, Inc.	A	2

[1]Exclusive of all 1973-1974 incidents of toxic canned mushrooms except marinated mushrooms of July 1973. Other mushrooms incidents presented in Table 4.
[2]Marinated mushrooms which involved Canadian citizen in Montreal.

of some canners were found to have either preformed toxin or viable spores of *C. botulinum.* The first toxic cans of mushrooms in 1973 were detected by a can company making an investigation of a spoilage problem in the product of one of the canners. The presence of botulinum toxin in the product was confirmed by the Food and Drug Administration (FDA) (*5*) and, in the end, 19 cans in 7 codes of this canner's mushrooms were found to contain *C. botulinum* type B toxin. About a month later, upon inspection of the plant following a consumer complaint, FDA found that a second lot of canned mushrooms from another canner contained viable spores of *C. botulinum* type B. In this case no toxin was found in the product. As a result of these two episodes, the inspection of mushroom canneries was intensified and in September 1973 the Commissioner of Food and Drugs ordered a complete survey of all domestic and imported canned mushrooms held in warehouses in the U.S. Inspections were completed by the end of 1973 and analyses were finished in 1974.

Thirty cans of mushrooms were found to be toxic and an additional 11 contained viable spores of *C. botulinum* without preformed toxin. These results are shown in Table 4. One of the toxic cans contained both toxin and

TABLE 4. *U.S. Experience with toxic clostridia in commercially canned mushrooms, 1973 and 1974*

Location of processor	Number of codes	Can size	Style	Toxic Cans: Toxic product	Toxic Cans: Toxin from cultures	Toxic Cans: Type of toxin[1]
U.S.	7	No. 10	Pizza cut Pieces and stems Sliced	19	19	B
U.S.	1	4 oz.	Pieces and stems	0	3	B
U.S.	2	8 oz.	Sliced	2	2	B
		16 oz.	Sliced	3	3	B
U.S.	3	No. 10	Pieces and stems	2	3	B
U.S.	1	4 oz.	Pieces and stems	0	1	A&B
U.S.	1	4 oz.	Buttons	1	1	B
U.S.	1	16 oz.	Pieces and stems	2	2	B
Ecuador	2	4 oz.	Sliced	0	3	B
France	1	16 oz.	Whole	1	1	*C. tetani*
France	1	16 oz.	Whole	0	4	A&B
Total	20			30	42	

[1]Botulinum toxin unless otherwise specified.

viable spores of *C. tetani* but no *C. botulinum.* The cans containing *C. botulinum* were from seven U.S. producers and two foreign plants. Half of the cans implicated were the institutional size No. 10 can, 20% were 16 oz. cans, and the remainder were smaller than 10 oz. Although this indicates a greater problem, numerically, with the larger sizes, 4 oz. and 8 oz. cans also were implicated. The majority of styles were involved and although the preponderance of all cans, regardless of size or style, were contaminated with *C. botulinum* type B, one can of U.S. origin and one of foreign origin were contaminated with type A as well as type B. To our knowledge, however, no cases of clinical botulism were traced to any of these mushrooms.

The spores of *C. botulinum* are frequently eaten with raw or fresh foods such as vegetables or berries that grow near the ground. Under these circumstances they are harmless. The danger of botulism results from the growth of the organism in a suitable food with the production of toxin. Since moisture, the absence of free oxygen, and a pH >4.6 are conducive to the growth of the organism, ideal conditions are provided in most low-acid canned foods. Whether vacuum packed or not, the little air remaining in a canned food at the time of sealing reacts chemically with the food during retorting and storage and is unavailable to microorganisms. The rate of reaction depends upon the composition of the food, type of container, and temperature to which the canned product is exposed. If the spores of *C. botulinum* are not destroyed by the canning process, there is a good probability that they will grow and produce toxin. Toxin, however, is only one of the many metabolic products of the growth of *C. botulinum.* Concomitant with its growth, gas is usually produced. Because of this, cans that contain botulinum toxin usually are swollen. Nevertheless, it is possible to have flat cans that are toxic either because enough growth to produce toxin has occurred but not enough gas has evolved to swell the can, or because the can leaks. As an example of the latter, in one outbreak of botulism involving canned tuna fish, the can apparently was not swollen and this was believed to be caused by leakage of gas. Many other cans in the same lot were found to be defective (*18*).

Although canned foods in which *C. botulinum* has grown often look and smell abnormal, this is not always the case. In addition to the seven immunologic types with respect to toxin production, *C. botulinum* also exists in two metabolic forms usually referred to as proteolytic and saccharolytic, or proteolytic and non-proteolytic. The proteolytic strains digest coagulated egg white, coagulated serum and meat. As a result of the breakdown of these substances, they not only alter the appearance of the protein but also produce the foul odors associated with the breakdown of proteins. The non-proteolytic strains are unable to attack these substrates but attack principally the sugars and therefore do not produce foul odors on growth. All known strains of type A are proteolytic whereas all known strains of types C, D, and E are non-proteolytic. Types B and F contain both proteolytic and non-proteolytic strains. Type G has not yet been found in a food product and, having been only recently discovered, has not been adequately characterized. However, it appears to be mildly proteolytic. Thus, the appearance and odor of product may not be appreciably altered, particularly if non-proteolytic strains are involved. Even with proteolytic strains, decomposition products may be masked by seasonings or the normal texture of the product. The problem of detection of spoilage by odor and appearance is further complicated by variation in individual tastes and sensitivity to off odors and flavors so that what may be unacceptable to one person may be perfectly acceptable to another. Thus, appearance and odor are not altogether reliable criteria for spoilage.

The non-proteolytic strains of *C. botulinum* are less likely to be found in canned foods than proteolytic strains because the spores are less heat resistant. They have, nevertheless, been responsible for outbreaks traced to canned foods.

The toxins of all strains are destroyed by heat; therefore, boiling thoroughly before serving provides an adequate margin for safety (*26*). Thorough heating, however, is most important since the ease with which the toxin can be destroyed depends upon how readily the heat penetrates to all parts of the product where toxin may be found. If the product contains large pieces of some ingredients, for example, these may offer protection and more thorough heating may be required to destroy the toxin. In this connection, experimental evidence showed that heating according to the instructions on at least one brand of frozen pizza would have been inadequate to destroy botulinum toxin had mushrooms in the topping contained botulinum toxin. In case of doubt, a canned food should never be tasted until after it has been thoroughly heated.

Low acid canned foods produced by commercial packers in the U.S. have had a remarkably good record during the last 45 years. Approximately 775 billion cans of food were produced through 1971. During that time, only three outbreaks of botulism involving death were traced to commercially canned foods. Mushroom sauce caused one death in 1941 (*22*); tuna canned in improperly sealed cans in 1963 caused two deaths (*18*); and in 1971, one person died from eating canned vichyssoise soup (*3*).

With all the study and research of the 1920s and 1930s resulting in the development of improved processes for canning (*21*), it can well be asked, "What went wrong with the mushroom canning industry?" Intensive inspections of the mushroom canneries during 1973 revealed a number of deficiencies in operating practices or conditions. A summary of the more significant of these is shown in Table 5.

In 1973, the Food and Drug Administration published a final order "Good Manufacturing Practice Regulations for Thermally Processed Low-Acid Canned Foods in Hermetically Sealed Containers." This regulation (Part

TABLE 5. *Some significant deficiencies found in equipment and operating procedures used to can mushrooms*

Equipment
1. Inaccurate or broken thermometers
2. No steam controller on retort
3. Inadequate steam supply
4. Faulty retort piping
5. Water leaking into retort during processing
6. Inaccurate timing devices
Operating Procedures
1. Poor fill control
2. Improper or no measurement of initial temperature
3. Inadequate venting
4. Improper timing of process
5. Using pressure gauge or recording thermometer rather than mercury thermometer as indicator of retort temperature
6. Opportunity for product to miss retort

128b) gives both the recommended and required practices for the operation of a cannery in order to provide assurance that the product will be safe and wholesome. It encompasses the quality of raw materials, sanitary practices of food handlers, sanitation and environmental conditions in the plant, processing methods, quality control, record keeping and training and qualifications of certain key employees.

Requirements of Part 90, published April 1, 1974, include (*a*) registration of every commercial processor engaged in manufacture, processing or packaging of thermally treated low-acid canned foods in hermetically sealed containers, (*b*) filing the processing procedure including time and temperature and other data needed to calculate the sterilizing value of the heat treatment (F_0) used for each low-acid product in each size can used, (*c*) reporting spoilage and any process deviations which could cause a health hazard if the food is in distribution, and (*d*) having a procedure for recalling products through all distribution channels down to the consumer level. If the processor fails to meet all of the above requirements, an emergency permit governing the conditions of manufacture, processing or packing of his products may be required.

These regulations are supplemented by an inspectional procedure by FDA which identifies critical control points in the processing and includes a hazard analysis of these points (HACCP). The inspectors thus look carefully at the processes used by the manufacturers along with their quality control program in an effort to prevent or detect any potentially hazardous condition which might arise.

The inspection of both domestic and imported canned foods has been intensified by FDA. Also, filed processes are reviewed by experts of FDA and if any appear to be suspect, they are more closely evaluated. If they then appear to be hazardous, corrective steps will be taken.

The problem of botulism continues to be of great concern to the consuming public, the industry and the regulatory agencies. We believe that the creation and application of the Good Manufacturing Practice Regulation for low acid canned foods coupled with an intensified inspectional program and canner registration will accelerate improvements in the canned food industry. We also believe that these measures should further reduce the incidence of *C. botulinum* in our commercially canned food supply.

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Holders of 3-A Symbol Council Authorizations on August 25, 1975

"Questions or statements concerning any of the holders of authorizations listed below, or the equipment fabricated, should be addressed to Earl O. Wright, Sec'y.-Treas., 413 Kellogg Ave., P.O. Box 701, Ames, Iowa 50010."

01-06 Storage Tanks for Milk and Milk Products As Amended

28 Cherry-Burrell Corporation (10/ 3/56)
575 E. Mill St., Little Falls, N.Y. 13365

102 Chester-Jensen Company, Inc. (6/ 6/58)
5th & Tilgham Streets, Chester, Pennsylvania 19013

2 CREPACO, Inc. (5/ 1/56)
100 C. P. Ave., Lake Mills, Wisconsin 53551

117 Dairy Craft, Inc. (10/28/59)
St. Cloud Industrial Park
St. Cloud, Minn. 56301

76 Damrow Company (10/31/57)
196 Western Avenue, Fond du Lac, Wisconsin 54935

115 DeLaval Company, Ltd. (9/28/59)
113 Park Street, So., Peterborough, Ont., Canada

109 Girton Manufacturing Company (9/30/58)
Millville, Pennsylvania 17846

114 C. E. Howard Corporation (9/21/59)
9001 Rayo Avenue, South Gate, California 90280

127 Paul Mueller Company (6/29/60)
P.O. Box 828, Springfield, Missouri 65801

31 Walker Stainless Equipment Co. (10/ 4/56)
Elroy, Wisconsin 53929

02-03 Pumps for Milk and Milk Products as Amended

214R Ben H. Anderson Manufacturers (5/20/70)
Morrisonville, Wis. 53571

212R Babson Bros. Co. (2/20/70)
2100 S. York Rd., Oak Brook, Ill. 60621

29R Cherry-Burrell Corporation (10/ 3/56)
2400 Sixth St., S. W., Cedar Rapids, Iowa 52406

63R CREPACO, Inc. (4/29/57)
100 C. P. Ave., Lake Mills, Wisconsin 53551

205R Dairy Equipment Co. (5/22/69)
1919 So. Stoughton Road, Madison, Wis. 53716

65R G & H Products, Inc. (5/22/57)
5718 52nd Street, Kenosha, Wisconsin 53140

145R ITT Jabsco, Incorporated (11/20/63)
1485 Dale Way, Costa Mesa, Calif. 92626

26R Ladish Co., Tri-Clover Division (9/29/56)
9201 Wilmot Road, Kenosha, Wisconsin 53140

236 Megator Corporation (5/ 2/72)
125 Gamma Drive, Pittsburgh, Pa. 15238

241 Purity S. A. (9/12/72)
Alfredo Noble #39, Industrial Pte. de Vigas
Tlalnepantla, Mexico

148 Robbins & Myers, Inc. (4/22/64)
Moyno Pump Division
1345 Lagonda Ave., Springfield, Ohio 45501

163R Sta-Rite Industries, Inc. (5/ 5/65)
P.O. Box 622, Delavan, Wisconsin 53115

72R L. C. Thomsen & Sons, Inc. (8/15/57)
1303 53rd Street, Kenosha, Wisconsin 53140

219 Tri-Canada Cherry-Burrell Ltd. (2/15/71)
6500 Northwest Drive, Mississauga, Ont., Canada L4V 1K4

175R Universal Milking Machine Div. (10/26/65)
National Cooperatives, Inc.
First Avenue at College, Albert Lea, Minn. 56007

52R Viking Pump Div.
Houdaille Industries, Inc. (12/31/56)
406 State Street, Cedar Falls, Iowa 50613

5R Waukesha Foundry Company (7/ 6/56)
Waukesha, Wisconsin 53186

04-03 Homogenizers and High Pressure Pumps of the Plunger Type, As Amended

247 Bran and Lubbe, Inc. (4/14/73)
2508 Gross Point Road, Evanston, Illinois 60201

87 Cherry-Burrell Company (12/20/57)
2400 Sixth Street, S.W., Cedar Rapids, Iowa 52404

37 CREPACO, Inc. (10/19/56)
100 C.P. Ave., Lake Mills, Wis. 53538

75 Gaulin, Inc. (9/26/57)
44 Garden Street, Everett, Massachusetts 02149

237 Graco Inc. (6/ 3/72)
60-Eleventh Ave., N.E., Minneapolis, Minn. 55413

256 Hercules, Inc. (1/23/74)
2285 University Ave., St. Paul, Minnesota 55114

05-11 Stainless Steel Automotive Milk Transportation Tanks for Bulk Delivery and/or Farm Pick-up Service, As Amended

131R Almont Welding Works, Inc. (9/ 3/60)
4091 Van Dyke Road, Almont, Michigan 48003

98R Beseler Steel Products, Inc. (3/24/58)
417 East 29th, Marshfield, Wisconsin 54449

70R Brenner Tank, Inc. (8/ 5/57)
450 Arlington, Fond du Lac, Wisconsin 54935

40 Butler Manufacturing Co. (10/20/56)
900 Sixth Ave., S.E., Minneapolis, Minn. 55114

66 Dairy Equipment Company (5/29/57)
1818 So. Stoughton Road, Madison, Wisconsin 53716

45 The Heil Company (10/26/56)
3000 W. Montana Street, Milwaukee, Wisconsin 53235

201 Paul Krohnert Mfg., Ltd. (4/ 1/68)
811 Steeles Ave., Milton, Ontario, Canada L9T 2Y3

80 Paul Mueller (Canada), Ltd. (11/24/57)
84 Wellington Street, So., St. Marys, Ont., Canada

85 Polar Manufacturing Company (12/20/57)
Holdingford, Minn. 56340

71 Progress Industries, Inc. (8/ 8/57)
400 E. Progress Street, Arthur, Illinois 61911

121 Technova Inc. Gosselin Division (12/ 9/59)
1450 Hebert c. p. 758
Drummondville, Quebec, Canada

47 Trailmobile, Div. of Pullman, Inc. (11/ 2/56)
701 East 16th Ave., North Kansas City, Mo. 64116

189 A. & L. Tougas, Ltee (10/ 3/66)
1 Tougas St., Iberville, Quebec, Canada

25 Walker Stainless Equipment Co. (9/28/56)
New Lisbon, Wisconsin 53950

08-09 Fittings Used on Milk and Milk Products Equipment, and Used on Sanitary Lines Conducting Milk and Milk Products

79R Alloy Products Corporation (11/23/57)
1045 Perkins Avenue, Waukesha, Wisconsin 53186

138R A.P.V. (Canada) Equipment, Ltd. (12/17/62)
103 Rivalda Rd., Weston, Ont., Canada

245 Babson Brothers Company (2/12/73)
2100 S. York Road, Oak Brook, Illinois 60521

82R Cherry-Burrell Company (12/11/57)
2400 Sixth Street, S.W., Cedar Rapids, Iowa 52406

260 CREPACO, INC. (5/22/74)
100 CP Ave., Lake Mills, Wis. 53551

124R DeLaval Company, Ltd. (2/18/60)
113 Park Street, South, Peterborough, Ont., Canada

255 The Duriron Company (1/18/74)
45 North Findlay Street, Dayton, Ohio 45401

67R G & H Products, Inc. (6/10/57)
5718 52nd Street, Kenosha, Wisconsin 53140

199R Graco, Inc. (12/ 8/67)
60 Eleventh Ave., N.E., Minneapolis, Minn. 55413

203R Grinnell Company (11/ 7/68)
260 W. Exchange St., Providence, R. I. 02901

218 Highland Corporation (2/12/71)
74-10 88th St., Glendale, N.Y. 11227

34R Ladish Co., Tri-Clover Division (10/15/56)
2809 60th St., Kenosha, Wisconsin 53140

239 LUMACO (6/30/72)
Box 688, Teaneck, N.J. 07666

200R Paul Mueller Co. (3/ 5/68)
P.O. Mox 828, Springfield, Mo. 65801

242 Purity, S.A. (9/12/72)
Alfredo Nobel #39 Industrial Pte. de Vigas, Tlalnepantla, Mexico

149R Q Controls (5/18/64)
Occidental, California 95465

89R Sta-Rite Industries, Inc. (12/23/68)
P.O. Box 622, Delavan, Wis. 53155

73R L. C. Thomsen & Sons, Inc. (8/31/57)
1303 43rd Street, Kenosha, Wisconsin 53140

191R Tri-Canada Cherry-Burrell, Ltd. (11/23/66)
6500 Northwest Drive, Mississauga, Ontario, Canada L4V 1K4

250 Universal Milking Machine Division (6/11/73)
Universal Cooperatives, Inc.
408 First Ave. S.
Albert Lea, Mn. 56007

86R Waukesha Specialty Company, Inc. (12/20/57)
Darien, Wisconsin 53114

266 Condor Manufacturing Company (8/ 1/75)
418 W. Magnolia Avenue
Glendale, California

09-00 Thermometer Fittings and Connections Used on Milk and Milk Products Equipment and Supplement 1, As Amended

32 Taylor Instrument Process Control,
Div. Sybron Corp. (10/ 4/56)
95 Ames Street, Rochester, New York 14601

206 The Foxboro Company (8/11/69)
Neponset Ave., Foxboro, Mass. 02035

246 United Electric Controls (3/24/73)
85 School Street, Watertown, Massachusetts 02172

10-01 Milk and Milk Products Filters Using Disposable Filter Media, As Amended

35 Ladish Co., Tri-Clover Division (10/15/56)
2809 60th Street, Kenosha, Wisconsin 53140

11-03 Plate-Type Heat Exchangers for Milk and Milk Products, As Amended

20 A.P.V. Company, Inc. (9/ 4/56)
137 Arthur Street, Buffalo, New York 14207

30 Cherry-Burrell Corporation (10/ 1/56)
2400 Sixth Street, S.W., Cedar Rapids, Iowa 52404

14 Chester-Jensen Co., Inc. (8/15/56)
5th & Tilgham Streets, Chester, Pennsylvania 19013

38 CREPACO, Inc. (10/19/56)
100 CP Avenue, Lake Mills, Wisconsin 53551

120 DeLaval Company, Ltd. (12/ 3/59)
113 Park Street, South Peterborough, Ont., Canada

17 The DeLaval Separator Company (8/30/56)
Dutchess Turnpike, Poughkeepsie, N.Y. 12602

15 Kusel Dairy Equipment Company (8/15/56)
100 W. Milwaukee Street, Watertown, Wisconsin 53094

12-04 Internal Return Tubular Heat Exchangers, for Milk and Milk Products, As Amended

248 Allegheny Bradford Corporation (4/16/73)
P.O. Box 264, Bradford, Pa. 16701

243 Babson Brothers Company (10/31/72)
2100 S. York Road, Oak Brook, Illinois 60521

103 Chester-Jensen Company, Inc. (6/ 6/58)
5th & Tilgham Street, Chester, Pennsylvania 19013

152 The DeLaval Separator Co. (11/18/69)
350 Dutchess Turnpike, Poughkeepsie, N.Y. 12602

217 Girton Manufacturing Co. (1/23/71)
Millville, Pa. 17846

252 Ernest Laffranchi (12/27/73)
P.O. Box 455, Ferndale, Calif. 95536

238 Paul Mueller Company (6/28/72)
P.O. Box 828, Springfield, Missouri 65801

96 C. E. Rogers Company (3/31/64)
P.O. Box 118, Mora, Minnesota 55051

13-01 Farm Milk Cooling and Holding Tanks, As Amended

240 Babson Brothers Company (9/ 5/72)
2100 S. York Road, Oak Brook, Illinois 60521

11R CREPACO, Inc. (7/25/56)
100 C. P. Ave.
Lake Mills, Wisconsin 53551

119R Dairy Craft, Inc. (10/28/59)
St. Cloud Industrial Park, St. Cloud, Minn. 56301

4R Dairy Equipment Company (6/15/56)
1919 S. Staughton Road, Madison, Wisconsin 53716

92R DeLaval Company, Ltd. (12/27/57)
113 Park Street, South Peterborough, Ontario, Canada

49R The DeLaval Separator Company (12/ 5/56)
Dutchess Turnpike, Poughkeepsie, N.Y. 12602

10R Girton Manufacturing Company (7/25/56)
Millville, Pennsylvania 17846

95R Globe Fabricators, Inc. (3/14/58)
3350 North Gilman Rd., El Monte, California 91732

179R Heavy Duty Products (Preston), Ltd. (3/ 8/66)
1261 Industrial Road, Preston, Preston, Ont., Canada

12R Paul Mueller Company (7/31/56)
P.O. Box 828, Springfield, Missouri 65801

249 Sunset Equipment Co. (4/16/73)
3765 North Dunlap Street
St. Paul, Minnesota 55112

216R Valco Manufacturing Company (10/22/70)
3470 Randolph St., Huntington Pk., Calif. 90256

42R VanVetter, Inc. (10/22/56)
2130 Harbor Avenue S.W., Seattle, Washington 98126

16R Zero Manufacturing Company (8/27/56)
Washington, Missouri 63090

14-00 Inlet and Outlet Leak Protector Plug Valves for Batch Pasteurizers, As Amended

122R Cherry-Burrell Company (12/11/59)
2400 Sixth St., S.W., Cedar Rapids, Iowa 52406

69 G & H Products Corporation (6/10/57)
5718 52nd Street, Kenosha, Wisconsin 53140

27 Ladish Co. - Tri-Clover Division (9/29/56)
2809 60th Street, Kenosha, Wisconsin 53140

78 L. C. Thomsen & Sons, Inc. (11/20/57)
1303 43rd Street, Kenosha, Wisconsin 53140

16-04 Evaporators and Vacuum Pans for Milk and Milk Products

254 Anhydro, Inc. (1/ 7/74)
130 S. Washington St., North Attleboro, Mass. 02760

132R A.P.V. Company, Inc. (10/26/60)
137 Arthur Street, Buffalo, New York 14207

164R Anderson IBEC (4/25/65)
19609 Progress Drive
Strongsville, Ohio 44136

263 C. E. Howard Corporation (12/21/74)
9001 Rayo Avenue
South Gate, California 90280

107R C. E. Rogers Company (8/ 1/58)
P. O. Box 118, Mora, Minnesota 55051

186R Marriott Walker Corporation (9/ 6/66)
925 East Maple Road, Birmingham, Mich. 48010

259 Pollution Control, Inc. (4/ 5/74)
P.O. Box 208, Wilson Place, South Barre, Vt. 05670

17-00 Fillers and Sealers of Single Service Containers, For Milk and Milk Products, As Amended

192 Cherry-Burrell Corporation (1/ 3/67)
2400 Sixth St., S.W., Cedar Rapids, Iowa 52404

137 Ex-Cell-O Corporation (10/17/62)
P.O. Box 386, Detroit, Michigan 48232

220 Hercules, Inc., Package Equipment Division (4/24/71)
2285 University Ave., St. Paul, Minnesota 55114

211 Twinpack, Inc. (2/ 4/70)
2225 Hymus Blvd., Dorval 740 P.Q.

19-00 Batch and Continuous Freezers, For Ice Cream, Ices and Similarly Frozen Dairy Foods, As Amended

141 CREPACO, Inc. (4/15/63)
100 C. P. Avenue, Lake Mills, Wisconsin 53551

146 Cherry-Burrell Company (12/10/63)
2400 Sixth Street, S. W., Cedar Rapids, Iowa 52404

22-03 Silo-Type Storage Tanks for Milk and Milk Products

168 Cherry-Burrell Corporation (6/16/65)
575 E. Mill St., Little Falls, N.Y. 13365

154 CREPACO, Inc. (2/10/65)
100 C.P. Ave., Lake Mills, Wisconsin 53551

160 Dairy Craft, Inc. (4/ 5/65)
St. Cloud Industrial Park
St. Cloud, Minn. 56301

181 Damrow Company, Division of DEC International, Inc. (5/18/66)
196 Western Ave., Fond du Lac, Wisconsin 54935

262 De Laval Company Limited (11/11/74)
113 Park Street South' Peterburough, Ontario

156 C. E. Howard Corporation (3/ 9/65)
9001 Rayo Avenue, South Gate, California 90280

155 Paul Mueller Co. (2/10/65)
P.O. Box 828, Springfield, Missouri 65801

195 Paul Mueller (Canada), Ltd. (7/ 6/67)
84 Wellington St., So., St. Marys, Ont., Canada

165 Walker Stainless Equipment Co. (4/26/65)
Elroy, Wisconsin 53929

23-00 Equipment for Packaging Frozen Desserts, Cottage Cheese and Milk Products Similar to Cottage Cheese in Single Service Containers

174 Anderson Bros. Mfg. Co. (9/28/65)
1303 Samuelson Road, Rockford, Illinois 61109

209 Doboy Packaging Machinery (7/23/69)
Domain Industries, Inc.
869 S. Knowles Ave., New Richmond, Wis. 54017

258 Hercules, Inc. (2/ 8/74)
2285 University Ave., St. Paul, Minnesota 55114

222 Maryland Cup Corporation (11/15/71)
Owings Mills, Maryland 21117

193 Triangle Package Machinery Co. (1/31/67)
6655 West Diversey Ave., Chicago, Illinois 60635

24-00 Non-Coil Type Batch Pasteurizers

161 Cherry-Burrell Corporation (4/ 5/65)
575 E. Mill St., Little Falls, N.Y. 13365

158 CREPACO, Inc. (3/24/65)
100 C. P. Avenue, Lake Mills, Wisconsin 53551

187 Dairy Craft, Inc. (9/26/66)
St. Cloud Industrial Park
St. Cloud, Minn. 56301

177 Girton Manufacturing Co. (2/18/66)
Millville, Pennsylvania 17846

166 Paul Mueller Co. (4/26/65)
P.O. Box 828, Springfield, Mo. 65601

25-00 Non-Coil Type Batch Processors for Milk and Milk Products

162 Cherry-Burrell Corporation (4/ 5/65)
575 E. Mill St., Little Falls, N.Y. 13365

159 CREPACO, Inc. (3/24/65)
100 C.P. Avenue, Lake Mills, Wisconsin 53551

188 Dairy Craft, Inc. (9/26/66)
St. Cloud Industrial Park
St. Cloud, Minn. 56301

167 Paul Mueller Co. (4/26/65)
Box 828, Springfield, Mo. 65801

196 Paul Mueller (Canada), Ltd. (7/ 6/67)
84 Wellington St., So., St. Marys, Ont., Canada

202 Walker Stainless Equipment Co. (9/24/68)
New Lisbon, Wis. 53950

26-00 Sifters for Dry Milk and Dry Milk Products

228 J. H. Day Co. (2/28/72)
4932 Beech Street, Cincinnati, Ohio 45202

229 Russell Finex Inc. (3/15/72)
156 W. Sandford Boulevard, Mt. Vernon, N.Y. 10550

173 B. F. Gump Division (9/20/65)
Blaw-Knox Food & Chem. Equip. Inc.
750 E. Ferry St., P.O. Box 1041
Buffalo, New York 14240

185 Rotex, Inc. (8/10/66)
1230 Knowlton St., Cincinnati, Ohio 45223

176 Sprout, Waldron & Co., Inc. (1/ 4/66)
Munsy, Pennsylvania 17756

172 SWECO, Inc. (9/ 1/65)
6111 E. Bandini Blvd., Los Angeles, California 90022

28-00 Flow Meters for Milk and Liquid Milk Products

253 Badger Meter, Inc. (1/ 2/74)
4545 W. Brown Deer Road, Milwaukee, Wis. 53223

223 C-E IN-VAL-CO, a division of Combustion Engineering, Inc. (11/15/71)
P.O. Box 556, 3102 Charles Page Blvd., Tulsa Oklahoma 74101

231 The DeLaval Separator Company (3/27/72)
350 Dutchess Turnpike
Poughkeepsie, New York 12603

265 Electronic Flo-Meters, Inc. (3/10/75)
12115 Self Plaza, Dallas, Texas 75218

226 Fischer & Porter Company 12/ 9/71)
County Line Road, Warminster, Pa. 18974

261 Foss America, Inc. (11/ 5/74)
Route 82
Fishkill, N.Y. 12524

224 The Foxboro Company (11/16/71)
Foxboro, Massachusetts 02035

29-00 Air Eliminators for Milk and Fluid Milk Products

251 The DeLaval Separator Company (12/10/73)
350 Dutchess Turnpike, Poughkeepsie, N.Y. 12603

30-00 Farm Milk Storage Tanks

257 Babson Bros. Co. (2/ 7/74)
2100 S. York Road, Oak Brook, Illinois 60521

32-00 Uninsulated Tanks for Milk and Milk Products

264 Cherry-Burrell Company, Division of Paxall, Inc. (1/27/75)
575 E. Mill St., Little Falls, N.Y. 13365

News and Events

Agricultural Engineering Society Announces Winter Meeting Theme and Site

The American Society of Agricultural Engineers (ASAE) will focus its attention on methods of increasing world food production at its Winter Meeting to be held at Chicago's Palmer House Hotel December 16-19, 1975.

In announcing plans for the Annual Meeting, ASAE President S.S. DeForest said agricultural engineers from all over the United States and around the world will examine new food production methods and agricultural technology at the meeting.

General Session and special Theme Session speakers will explore the meeting's theme, "Productivity—Key to Easing World Food Shortages," during the three-day conference which is expected to draw 2,000 agricultural engineers.

Over 400 research papers will be presented during the meeting's technical sessions. Papers will cover topics in all of the Society's major divisions: Power and Machinery, Soil and Water, Structures and Environment, Electric Power and Processing, and Food Engineering, said DeForest.

American Society of Agricultural Engineers is a non-profit, technical, scientific and educational society dedicated to the betterment of agriculture through improved application of engineering principles. Headquartered in St. Joseph, Mich., the Society's membership consists of 7,400 full members and 1,000 student members located in 50 states and 90 foreign countries.

Energy Savings Offered for Freezers/Coolers with Mars Air Doors

A complete line of air doors, especially designed for use over cooler and freezer room doors to prevent the escape of cold air, is now offered for immediate delivery from Mars Air Doors.

By providing an invisible curtain of high-velocity air across the opening, costly cold air is sealed inside when door is opened. As a result, moist air or frost build-up is minimized and operation and maintenance of the refrigeration system is lowered with significant cost savings.

Meeting NSF Standard 37 and USDA requirements, Mars Air Doors are ideal for installation in cold storage plants, canneries, meat packing and frozen food plants, dairies and all other similar food processing applications.

Nutrition and Drug Symposium

NUTRITION AND DRUG INTERRELATIONS, a three-day international symposium sponsored by the Nutrition Foundation and the Iowa State University Nutrition Sciences Council will be held on August 4-6, 1976 at Iowa State University. For further information write Dr. John N. Hathcock, Department of Food and Nutrition, Iowa State University, Ames, Iowa 50010.

Knuckles Wins Academy Award

Left to Right: Professor Morris A. Shiffman, Dean Bernard G. Greenberg, and Professor Emil T. Chanlett of the University of North Carolina's School of Public Health; and the recipient of the Award, Maurice E. Knuckles.

Maurice E. Knuckles, graduate student in environmental sciences at the University of North Carolina School of Public Health, is the recipient of the 1975 Sanitarian's Award of the American Intersociety Academy for the Certification of Sanitarians. The annual award recognizes a sanitarian graduate student for outstanding academic achievement and leadership qualities.

The winner is elected by the faculty of the Department of Environmental Sciences and Engineering.

Mr. Knuckles attended the University of Tennessee at Knoxville, and graduated from East Tennessee State University, Johnson City, Tennessee, in 1971 with a major in Environmental Health. His experience, before returning for graduate study in environmental management and protection, included the positions of sanitarian with the Indian Health, U.S.P.H.S., and with the Georgia Department of Public Health. Most recently, he was the environmental health director for Healthco, Inc., the comprehensive health care program in Soul City, N.C.

News and Events

USDA Announces Pamphlet on Judging and Scoring Milk and Cheese:

The art of judging and scoring milk and cheese is described in a new pamphlet released by the Agricultural Marketing Service (AMS) of the U.S. Department of Agriculture (USDA).

The pamphlet is intended for use by high school students taking vocational agricultural courses. An ability to judge and score milk and dairy products is important to anyone planning to enter the dairy and food industry because judging skills are basic to effective quality control on the farm and at the processing plant.

Farmers and processors need to know when off flavors or other conditions are present in the milk or dairy products so corrections can be made.

In the pamphlet, students can find tips on how to judge and score milk, sediment in milk, cottage cheese, milking machine parts, and how to identify various types of natural cheese.

The pamphlet also includes information on basic flavor evaluation and how to work with the score card used in the Milk Quality and Dairy Foods Contest sponsored by the Future Farmers of America. Such judging contests develop the skills of youthful participants and promote uniformity in judging practices.

Single copies of the pamphlet are available upon request from the Standardization Branch, Dairy Division, AMS, USDA, Washington, D.C. 20250. Ask for Farmers Bulletin 2111, "Judging and Scoring Milk and Cheese." Don't forget to include your zip code along with your name and address.

Student Dairy Products Contest Set for October 20

The 54th Annual Collegiate Dairy Products Evaluation Contest will be held on Monday, Oct. 20, 1975, at the Kraft Foods Plant, Montreal, Canada.

Invitations have been extended to colleges and universities with dairy or food science departments to enter student teams in the contest. The contest consists of the taste sampling of butter, cheddar cheese, cottage cheese, ice cream and milk donated by companies which process the products. Students record their ratings of the flavor of each product and, except for milk, evaluate them for body, texture and appearance. More than 1,000 highly exacting judgments are usually recorded on individual score cards.

USDA Offers Visual Aid for Dry Milk Products Quality Control

A visual aid used to identify the level of scorched particles in dry milk and other dairy products is being made available to the dairy industry by the U.S. Department of Agriculture (USDA).

Although developed by USDA's Agricultural Marketing Service (AMS) as a reference tool for dairy products inspectors, the visual aid can also be effectively used in plant quality-control programs.

The visual aid is a composite print showing four standard discs which contain 7.5, 15.0, 22.5, and 32.5 milligrams, respectively, of scorched particles.

These levels represent limits of scorched particles allowed by U.S. grade standards for regular and instant nonfat dry milk, dry whole milk, dry buttermilk, dry whey, and other dairy products.

To qualify for the U.S. Extra grade, for example, nonfat dry milk may contain no more than 15.0 milligrams of scorched particles.

The scorched particles content of a dairy product can be evaluated by comparing the four standard discs pictured in the composite print with a laboratory sample disc of the product being examined. The standard discs were prepared according to procedures specified in the U.S. Scorched Particles Standards for Dry Milks.

To obtain the composite print and a copy of the scorched particles standards, send $3.75 to the Standardization Branch, Dairy Division, AMS, USDA, Washington, D.C. 20250. Only checks or money orders can be accepted. Do not send stamps or cash.

Program in Continuing Education

1. Environmental Public Health 305, Water Borne Disease Control, Fond du Lac, WI; once monthly, January 16-May 21, 1975.
2. Environmental Public Health 302, Food Borne Disease Control, UW-Eau Claire, WI; once monthly, January 9-May 14, 1975.
3. Environmental Public Health 305, Water Borne Disease Control, Madison, WI; once monthly, January 16-May 7, 1975.
4. Environmental Health 112, Community Hygiene II, Green Bay, WI; once monthly, January 23-May 21, 1975.

Details for registration, charges, location and dates may be obtained from Dr. Gerberic, Division Allied Health Programs, University of Wisconsin-Eau Claire, Library 2045, Eau Claire, WI 54701.

News and Events

PCB Residues in Indiana's Milk Supply—An Emerging Problem

During the last four months, the Indiana State Board of Health has been reporting the presence of polychlorinated biphenyls (PCBs) in some Indiana milk supplies. The initial question is, "How did the PCBs get into the milk in the first place?"

To answer the above question, I would like to share some of my experiences involving PCBs with you. During my tenure with the Ohio Department of Agriculture, I did become involved with the PCBs problem relative to Ohio's raw milk supply and can convey to you some background information concerning this milk contaminant problem.

Most of you are aware that PCBs are commercially produced and are used primarily as heat transferring media in capacitors and electrical transformers. However, other uses for PCBs are as plasticizers, sealers, surface coatings and hydraulic lubricants.

Of importance, PCBs, when ingested, can be accumulated in the body fat. PCBs have been shown to cause live cell damage[1] and to induce liver enzymes which degrade oestradiol, a steroid involved in calcium physiology [2]. Therefore, the presence of a PCB in a raw milk sample at or above the 0.2 mg/1 (whole milk basis) or 2.5 mg/kg (fat basis) level is considered justification to eliminate that milk from the marketing channels.

The major PCB contamination pathway involved is as follows:

Epoxy (containing PCB) coated silo → fermenting silage → cow → adulterated milk

The sequence of events begins with the fermenting silage producing the leaching agent(s), acetic acid. The leached PCB collects at the bottom of the silo, thus contaminating the silage at that location site. The contaminated silage is fed to the dairy herd and metabolized by the individual cows. Principle accumulation of the PCB occurs in the body fat. However, during the milk synthesizing process, the PCB becomes translocated with the fat-like materials used in the fabrication of the fat globule and ultimately is excreted in the milk with some modifications of the PCB molecule.

An experience with one Ohio dairy herd provides some useful guidelines for natural elimination of PCBs from the cow's system. After the Ohio herd was identified as being contaminated with PCBs, immediate steps were taken to eliminate all contaminated feed and for the sequential 12 months the cows were fed uncontaminated feed. No aids or stimulants were used to increase the elimination of the PCB from the cow's system.

Initial individual cow PCB concentration levels were in the range of 0.3 up to 1.85 mg/1 of milk. Approximately 60 to 90 days were required after contaminated feed withdrawal to return 70% of the lactating cows to producing milk for marketing purposes. At the end of 270 days another 20% was added to commercial production. Eight cows continued to show PCB concentrations above the actionable level near the end of the 12-month study. Thus, body residual PCB can be eliminated from the cow, naturally. The rate of PCB elimination is dependent upon the cow's dietary intake, individual metabolism and milk fat excretion rate.

Literature Cited:

1. American Industrial Hygiene Association, 1965. Amer. Industr. Hyg. Assn. J. 26:92-94.
2. Risebrough, R. W. *et al.,* 1968. Nature 220:1098-1102.

Shelf-Life of Dairy Product Prolonged

Consumers noticing an extended shelf-life on whipping cream products are not being deceived by their eyes or their grocers. A new ultra-pasteurization technique, adopted by some of the dairies in New York State this year greatly prolongs the shelf-life of heavy cream and half-and-half products.

"It's the best thing that has happened to the dairy industry in a long while," said David Bandler, professor of food science at the N.Y. State College of Agriculture and Life Sciences, Cornell University.

He explained that ultra-pasteurization heats the cream to 280 degrees Fahrenheit for at least two seconds and yields an "almost sterile" product. Conventional pasteurization processes require heating to only 161 degrees Fahrenheit for 15 seconds. The higher pasteurization temperature destroys more of the microbes that are present in raw cream, and therefore, inhibits spoilage.

Products that have been ultra-pasteurized may remain on the supermarket shelf for as long as six weeks. Cream that is pasteurized conventionally is removed generally after one week. (In New York City, which has different health codes from Upstate, ultra-pasteurized cream can be sold for 15 days, a big improvement over the 66-hour limit set on pasteurized products.)

Bandler said that, for now, only cream products are being processed via the high heat technique of ultra-pasteurization.

"Ultra-pasteurized milk would not be acceptable to the consumer," said Bandler. "Under present technology, fresh, whole milk taken to 280 degrees Fahrenheit will retain the 'cooked' taste, sometimes characteristic of evaporated milk."

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Association Affairs

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Treas., Vincent Mattera, R. I. Dept. of Health, 2843 South County Trail, East Greenwich, R.I. 02818

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Financial Report
IAMFES

ASSETS

	Year Ended June 30, 1975	June 30, 1974
Current Assets:		
Cash on hand, in bank and savings and loan assoc.	$16,621.75	11,959.86
Accounts receivable-trade	3,820.49	2,452.06
Inventory-supplies	4,339.30	5,218.28
Prepaid expenses	78.05	76.56
Total current assets	24,859.59	19,706.76
Fixed Assets:		
Office equipment	3,768.12	3,627.83
Addressing and mailing equipment	3,214.36	3,214.36
	6,982.48	6,842.19
Less allowance for depreciation	6,144.02	5,885.95
Net fixed assets	838.46	956.24
Total assets	$25,698.05	20,663.00

LIABILITIES AND NET EQUITY

Current Liabilities:		
Accounts payable-trade	$ 812.22	3,960.97
Payroll taxes payable	557.51	551.27
Special purpose funds	2,735.27	1,573.24
Total current liabilities	4,105.00	6,085.48

Association Affairs

Equity:		
Balance-beginning of period	14,577.52	19,235.26
Net increase, decrease (-) during period	7,015.53	4,657.74-
Balance-end of period	21,593.05	14,577.52
Total liabilities and equity	$25,698.05	20,663.00

See Notes To Financial Statements—June 30, 1975

INCOME STATEMENT
For the Years Ended June 30, 1975 and 1974

	Year Ended June 30, 1975	June 30, 1974
Income:		
Affiliate dues	$18,515.87	16,938.00
Direct dues	10,021.82	10,053.66
Total dues received	28,537.69	26,991.66
Contributions received for awards	2,700.00	—
Convention and meeting income	2,500.00	1,468.54
Publications and pamphlets	1,881.76	2,376.76
Sale of 3-A Standards	2,474.46	3,467.92
Decals, buttons, misc. and expense reimb.	1,208.19	122.32
Expense reimbursement—3-A	4,940.27	1,694.18
Interest income	334.76	68.71
Total income	44,577.13	36,190.09
Expense:		
Salaries	24,590.62	19,534.99
Payroll tax expense	1,653.39	989.58
Travel	3,095.31	3,453.19
Executive Board-Travel and misc. expense	—	1,140.40
Office supplies	1,954.37	656.73
Box rent and postage	1,876.83	1,583.88
Telephone	924.00	911.94
Office rent	2,625.00	2,812.50
Insurance	149.11	130.93
Legal and professional fees	1,058.00	1,004.33
Dues and subscriptions	—	200.00
Depreciation-office equipment	216.96	190.69
3-A Standards expense	256.61	450.28
Citations and awards	2,594.45	50.00
Committee expense	—	95.25
Buttons and decals	263.28	—
Convention expense	1,332.72	531.84
Cost of printing pamphlets	342.16	1,669.65
Moving expense	—	759.09
Miscellaneous	258.17	169.71
Total expense	43,190.98	36,334.98
Net income (loss) of Association	1,386.15	(144.89)
Add-net (loss) of Journal-Exhibit B-1	5,629.38	(4,512.85)
Total net income (loss)	$ 7,015.53	(4,657.74)

See Notes To Financial Statements—June 30, 1975

Association Affairs

JOURNAL OF MILK AND FOOD TECHNOLOGY
INCOME STATEMENT
For the Years Ended June 30, 1975 and 1974

	Year Ended June 30, 1975	June 30, 1974
Income:		
Advertising	$12,303.81	10,710.67
Subscriptions	16,865.67	13,413.25
Sale of Journals	117.18	347.19
Sale of reprints	8,385.28	5,513.48
Page charges	10,850.00	5,928.00
Addressograph income	—	12.01
Total income	48,521.94	35,924.60
Expense:		
Editorial salaries	3,350.00	3,285.66
Printing and publishing	29,499.29	28,787.90
Plates, cuts, etc.	297.50	492.41
Mailing and postage	2,843.08	2,954.67
Reprint expense	3,971.74	3,452.20
Advertising commission	626.60	61.25
Copyright expense	—	72.00
Stationery and supplies	773.65	297.34
Travel expense	555.09	922.75
Addressograph expense	—	6.25
Depreciation-addressing equipment	41.11	41.11
Telephone	28.96	38.91
Consulting	525.00	—
Miscellaneous	380.54	25.00
Total expense	42,892.56	40,437.45
Net income (loss) of Journal	$ 5,629.38	(4,512.85)

See Notes To Financial Statements—June 30, 1975

Notes to Financial Statements—June 30, 1975

Inventory

Inventory of supplies is recorded at the lower of cost or market.

Fixed Assets

Office equipment and addressing and mailing equipment are recorded at cost. Depreciation is computed on the straight-line method over the estimated useful life.

Recognition of Revenues

Income from dues and subscriptions is recorded on the cash basis. All other income is recorded on the accrual basis.

Classified Ad

Single Service Milk Sample tubes. For further information and a catalogue please write Dairy Technology, Inc., P.O. Box 101, Eugene, Oregon 97401.

Index to Advertisers

THE ONLY *Approved*
SANITARY METHOD OF APPLYING
A U. S. P. LUBRICANT
TO DAIRY & FOOD
PROCESSING EQUIPMENT

Haynes-Spray

U. S. P. LIQUID PETROLATUM SPRAY

U.S.P. UNITED STATES PHARMACEUTICAL STANDARDS

CONTAINS NO ANIMAL OR VEGETABLE FATS. ABSOLUTELY NEUTRAL. WILL NOT TURN RANCID – CONTAMINATE OR TAINT WHEN IN CONTACT WITH FOOD PRODUCTS.

SANITARY—PURE

ODORLESS—TASTELESS

NON-TOXIC

This Fine Mist-like
HAYNES-SPRAY
should be used to lubricate:

SANITARY VALVES
HOMOGENIZER PISTONS – RINGS
SANITARY SEALS & PARTS
CAPPER SLIDES & PARTS
POSITIVE PUMP PARTS
GLASS & PAPER FILLING MACHINE PARTS
and for ALL OTHER SANITARY MACHINE PARTS which are cleaned daily.

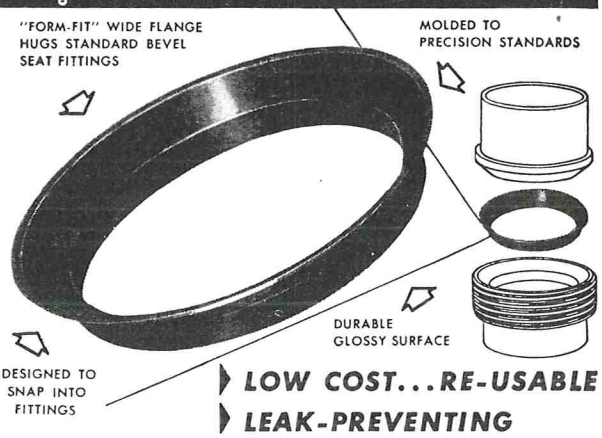
The Modern HAYNES-SPRAY Method of Lubrication Conforms with the Milk Ordinance and Code Recommended by the U. S. Public Health Service

The Haynes-Spray eliminates the danger of contamination which is possible by old fashioned lubricating methods. Spreading lubricants by the use of the finger method may entirely destroy previous bactericidal treatment of equipment.

PACKED 6-12 oz. CANS PER CARTON SHIPPING WEIGHT - 7 LBS.

THE HAYNES MANUFACTURING COMPANY
4180 LORAIN AVENUE • CLEVELAND, OHIO 44113

HAYNES SNAP-TITE GASKETS



LOW COST...RE-USABLE

LEAK-PREVENTING

NEOPRENE GASKET for Sanitary Fittings

Check these SNAP-TITE *Advantages*

- Tight joints, no leaks, no shrinkage
- Sanitary, unaffected by heat or fats
- Non-porous, no seams or crevices
- Odorless, polished surfaces, easily cleaned
- Withstand sterilization
- Time-saving, easy to assemble
- Self-centering
- No sticking to fittings
- Eliminate line blocks
- Help overcome line vibrations
- Long life, use over and over

Available for 1", 1½", 2", 2½" and 3" fittings.
Packed 100 to the box. Order through your dairy supply house.

THE HAYNES MANUFACTURING CO.
4180 Lorain Avenue • Cleveland 13, Ohio

HAYNES SELF-CENTERING SNAP-TITE® *Gaskets*

SIZES 1" - 1½"
2" - 2½" - 3"

*MADE FROM
TEFLON®

"The Sophisticated Gasket"

THE IDEAL UNION SEAL FOR BOTH VACUUM AND PRESSURE LINES

Gasket Color . . . slightly off-white

SNAP-TITE self-centering gaskets of TEFLON are designed for all standard bevel seat sanitary fittings. They SNAP into place providing self-alignment and ease of assembly and disassembly.
HAYNES SNAP-TITES of TEFLON are unaffected by cleaning solutions, steam and solvents. They will not embrittle at temperatures as low as minus 200° F. and are impervious to heat up to 500° F.

FOR A FITTING GASKET THAT WILL OUT-PERFORM ALL OTHERS...

Specify . . . HAYNES SNAP-TITES of TEFLON

• *TEFLON ACCEPTED SAFE FOR USE ON FOOD & PROCESSING EQUIPMENT BY U. S. FOOD AND DRUG ADMINISTRATION*

*Gaskets made of DuPont TEFLON® TFE-FLUOROCARBON RESINS

THE HAYNES MANUFACTURING COMPANY
4180 LORAIN AVENUE • CLEVELAND, OHIO 44113

A HEAVY DUTY SANITARY LUBRICANT

Available in both
SPRAY AND TUBE

All Lubri-Film ingredients are approved additives and can be safely utiiized as a lubricant for food processing equipment when used in compliance with existing food additive regulations.

ESPECIALLY DEVELOPED FOR LUBRICATION OF FOOD PROCESSING AND PACKAGING EQUIPMENT

For Use in Dairies — Ice Cream Plants — Breweries — Beverage Plants — Bakeries — Canneries — Packing Plants

SANITARY • NON TOXIC • ODORLESS • TASTELESS

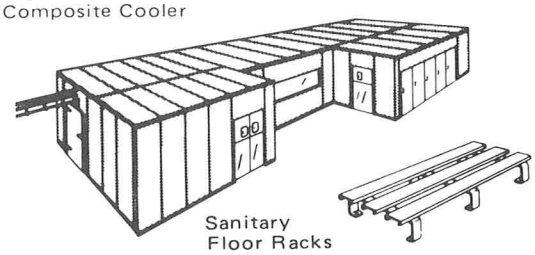
SPRAY — PACKED 6 — 16 OZ. CANS PER CARTON
TUBES — PACKED 12 — 4 OZ. TUBES PER CARTON

THE HAYNES MANUFACTURING CO.
CLEVELAND, OHIO 44113

ELIASON is committed to User and Public Health Sanitary, Safety and Performance SPECIFICATIONS

SANITARY walk-in coolers and freezers are rust proofed, fire proofed and made to outlast those "covered up" by minimum criteria.

Supermarket Multi-Room Composite Cooler

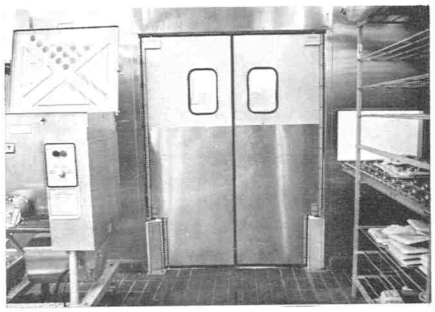


Sanitary Floor Racks

ELIASON SECTIONAL COOLERS will even outlast facilities and flammably hazardous, plastic labeled, unreinforced uniformity conformity. ELIASON WALL SHELVING ACCESSORIES are all hand removable for sanitizing without tools or easily cleaned in place and rust proofed in design. ELIASON FRESH MEAT TRACK SYSTEMS for interior need no exterior rods or reinforcements and are rust proofed too.

SAFETY in service, traffic, convenience doorways demanded patented Easy-Swing DOORS self-closing NO SPRING double action for coolers.

Easy Swing COOLER DOORS GASKETED (also available for food and sales service)

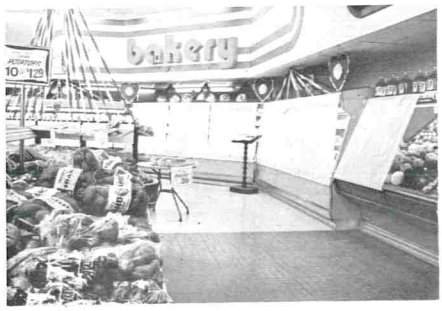


with DOOR JAMB GUARDS in nickel stainless steel meat processing or storage

ELIASON Easy Swing DOORS are exclusively safe and easy to walk through, push carts through and protect temperatures without age old "high resistance and high maintenance old fashioned doors hard to open," doors propped open and doors not designed for heavy duty traffic. ELIASON Easy Swing DOORS were first invented for walk-in cooler areas where elimination of dangerous spring hinge elbow banging, sidebinding and heel catching caused accidents and injuries. The safest occupationally useful doors.

PERFORMANCE that has made food refrigerator equipment make American Supermarkets world famous by ingenious upgrading and innovations is enhanced by patented ECONO-COVERS

NITE COVERS being pulled down from case top over vertical freezers

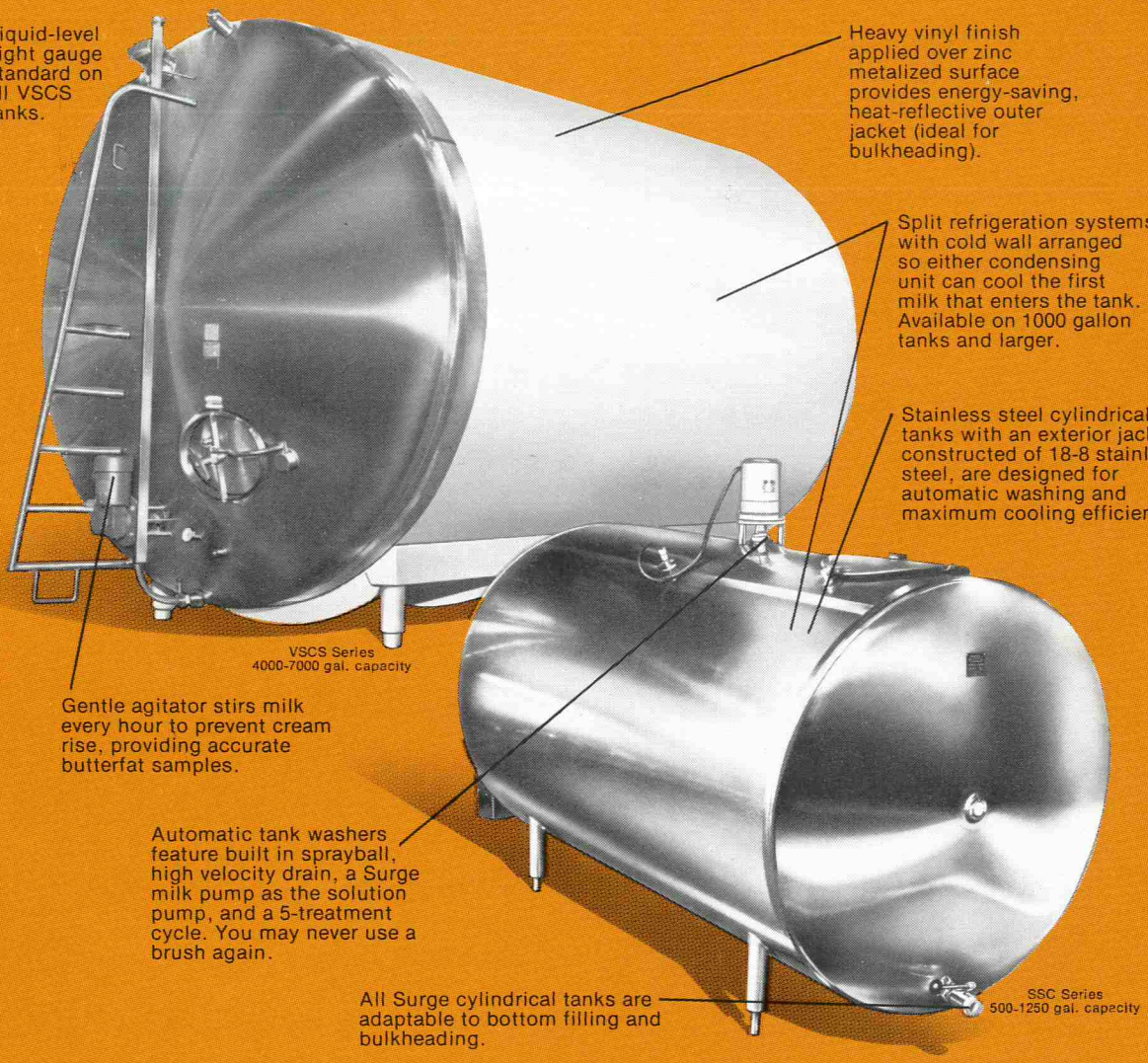


ELIASON ECONO-COVERS are perforated for "air make up and defrost air escape" but provide a FOOD PROTECTION safety shield NEVER dumped or lost in back rooms. ECONO-COVERS fit all size open top or open front refrigerator and freezer cases all over the world. Invaluable for perishables! SANITARIANS are urged to tell food stores how ECONO-COVERS protect their profits while they sleep, reduce ENERGY to pay for the covers in less than a year and cut FOOD SPOILAGE LOSSES by measureable hundreds and thousands of dollars in stores.

ELIASON a for profit, taxpaying, practitioner of "Lawful Competitive Free Enterprise" is an INDUSTRY MEMBER of NEHA, IAMFES and a few SANITARIAN STATE ASSOCIATIONS. ELIASON is not an affiliate of any MAXIMUM PRICING ASSOCIATION or MINIMUM STANDARDS LOBBY GROUPS. ELIASON is dedicated to ENVIRONMENTAL QUALITY PERFORMANCE.

write to . . . ELIASON CORPORATION, P.O. Box 2128, Kalamazoo, Michigan 49003

Select your new milk tank from America's fastest growing line of cooling equipment. SURGE.



Liquid-level sight gauge standard on all VSCS tanks.

Heavy vinyl finish applied over zinc metalized surface provides energy-saving, heat-reflective outer jacket (ideal for bulkheading).

Split refrigeration systems, with cold wall arranged so either condensing unit can cool the first milk that enters the tank. Available on 1000 gallon tanks and larger.

Stainless steel cylindrical tanks with an exterior jacket constructed of 18-8 stainless steel, are designed for automatic washing and maximum cooling efficiency.

VSCS Series
4000-7000 gal. capacity

Gentle agitator stirs milk every hour to prevent cream rise, providing accurate butterfat samples.

Automatic tank washers feature built in sprayball, high velocity drain, a Surge milk pump as the solution pump, and a 5-treatment cycle. You may never use a brush again.

All Surge cylindrical tanks are adaptable to bottom filling and bulkheading.

SSC Series
500-1250 gal. capacity

The fast growth and acceptance of Surge cooling tanks is due to several things: First of all, Surge tanks are built with a unique concept called "dimple wall' construction. The result is more evenly spaced precision cooling walls that help promote more uniform distribution of refrigerant.

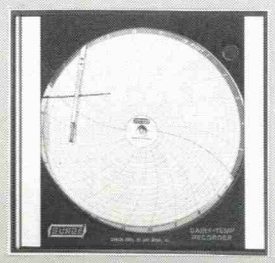
Then there's the length of the Surge line; a total of 21 models and sizes to choose from. Result: Your dealer can install a tank that precisely fits your dairy and your milk pick-up schedule.

Finally, these tanks all come with genuine Surge service and expert installation performed by an experienced, trained dealer backed by a company respected throughout the industry for quality and service.

VSC Series
1500-4000 gal. capacity

VSA Series
500, 600, 800 gal. capacity

VSA Series
300-400 gal. capacity



Surge Dairy-Temp recorders feature the latest developments in measurement and recording. Enclosed capillary system impervious to milk room conditions. Sensing bulb fits most tanks without alterations. Also available as a controller.

"You're a step ahead with Surge"

SURGE

For more information see your Surge Dealer or write to Babson Bros. Co.
2100 S. York Road, Oak Brook, Illinois 60521