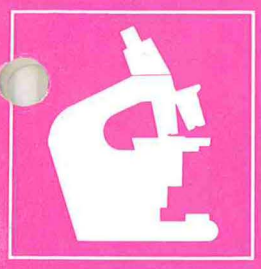

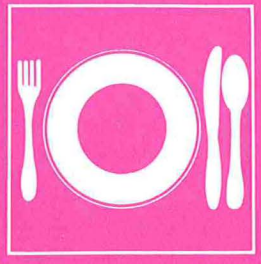

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An International Journal Concerned With An Adequate Food Supply That Is Safe, Wholesome, Nutritious, and Palatable

64th Annual Meeting
Sioux City Hilton Hotel
Sioux City, Iowa
August 14-18, 1977

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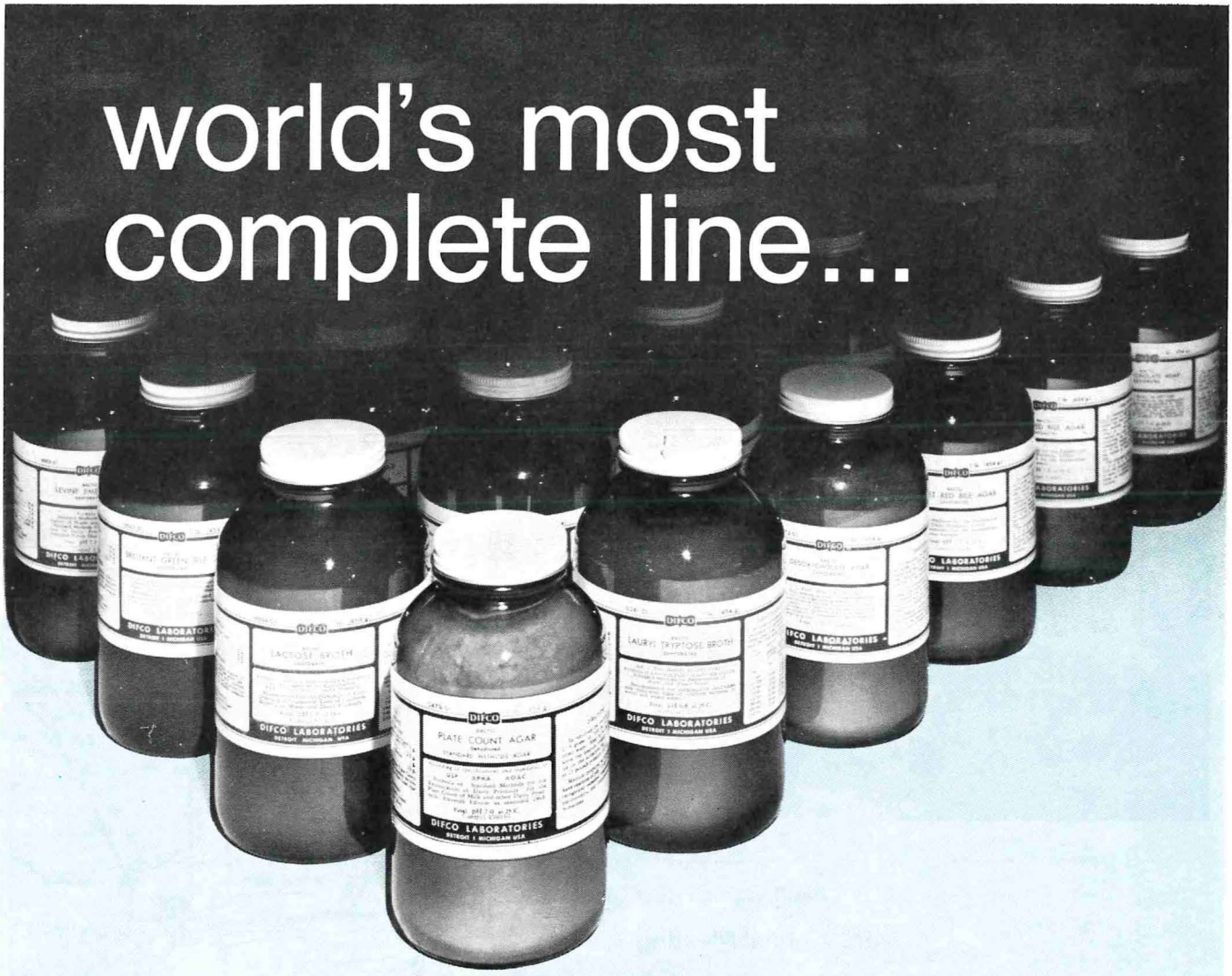

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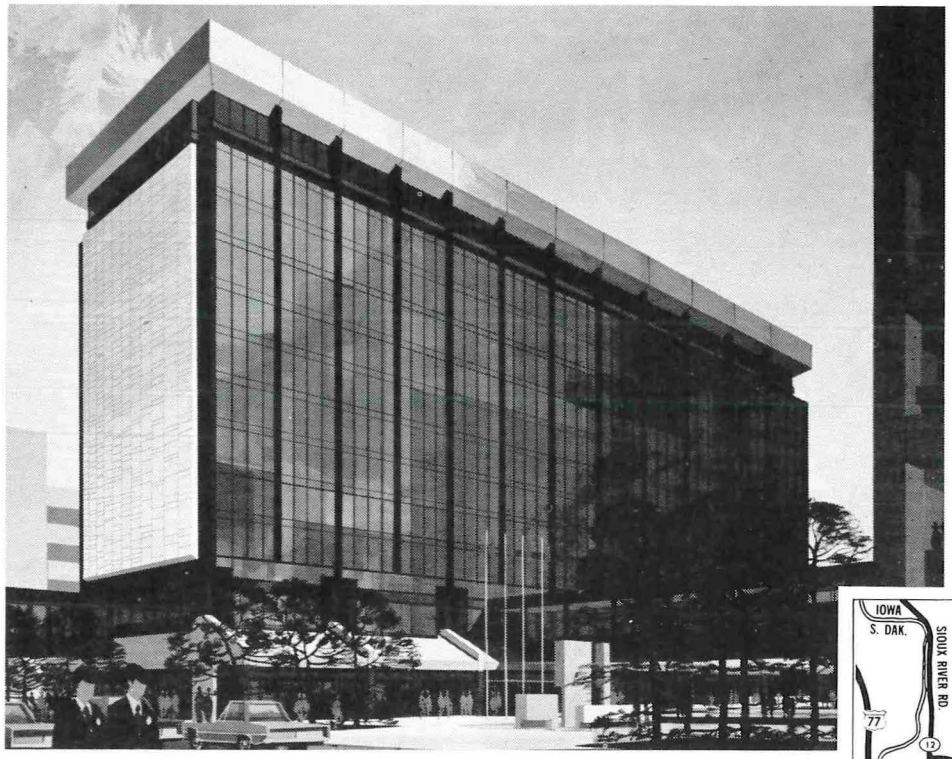

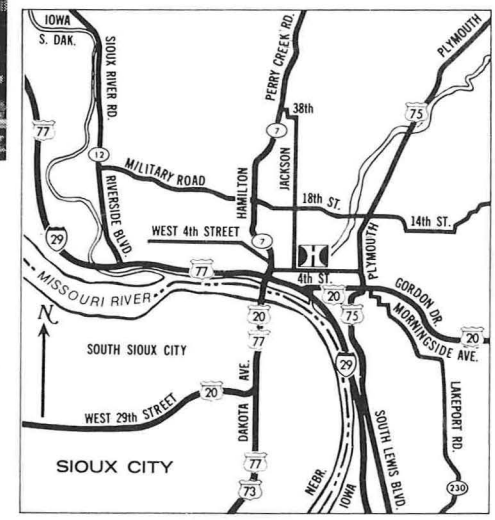

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Vol. 40 February, 1977 No. 2

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Distribution of Added Iron in Milk of Cows and Buffaloes and its Effect on Oxidized Flavor Development

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ABSTRACT

Association of added ferrous and ferric forms of iron in raw and pasteurized cow and buffalo milk is discussed in connection with its effect on oxidative deterioration of milk. Association of iron with sulfhydryls is shown to accelerate oxidized flavor development. Heat treatment or a suitable reducing agent capable of rendering the metal to the reduced state appears to promote the catalytic activity of added iron in milk. Buffalo milk does not differ from cow milk in its resistance to iron-induced oxidative deterioration.

Recent reports on iron deficiency anemia (*1, 12, 16, 17*) have attracted the attention of nutritionists in finding effective means of enriching the human diet with sufficient iron. Since iron deficiency is highest in children, iron fortification of milk has attained special significance in recent years. Controversy has arisen over the effectiveness of different iron compounds that have been used for food fortification. The reduced forms of iron compounds such as ferrous sulfate are generally favored, presumably because of their greater solubility and probable availability. However, the dairy industry has accepted the idea of iron fortification with caution as it is well known that iron salts produce undesirable flavors in milk and dairy products, though to a lesser extent than copper. In this respect, ferrous iron has been shown to be more detrimental than ferric iron (*2, 3, 7*). The structure of the iron salt itself, in part at least, determines its potency in effecting lipid oxidation (*15*). Taking these points into consideration, recent workers have compared the suitabilities of certain iron salts in fortification of cow milk. Pla and Fritz (*9*), based on biological availability, considered ferrous sulfate and ferric chloride as good sources of iron. The work herein reported deals with distribution of these salts in cow and buffalo milks and their influence on oxidized flavor development.

MATERIALS AND METHODS

Milk of individual animals form the herds maintained at the institute farm was obtained. The cow herd consisted of Jersey, Tharparkar, and cross bred animals while the buffaloes were of Surti breed. The animals selected were all in the middle stage of lactation. Samples of milk representing middle portions during the milking of an animal were directly collected into glass bottles which were thoroughly cleaned with nitric acid and repeatedly washed with glass-distilled water. Extreme care was taken to prevent metal contamination of the samples.

Pasteurization of samples was conducted in glass bottles. Samples were first heated in a boiling water bath to 81 C and maintained at this temperature for 1 min. After this holding time, bottles were removed to a cold water bath for immediate cooling. One set of samples was pasteurized at 63 C for 30 min for comparision. Samples were stored under refrigeration at a temperature of 5 ± 1 C.

Ferric iron as chloride and ferrous iron as sulfate solutions were added to the milk samples before or after pasteurization as experiments warranted. The concentration of iron solutions in either case was such as to give a final concentration of 10 ppm when 1 ml was added to 100 ml milk.

To follow migration of iron between cream and skim milk, samples were centrifugally separated at room temperature (25 C) in an International Centrifuge, model SBV size 1., at 1500 × g. Centrifuged samples were cooled in an ice water bath and the hardened cream layer was punctured with a pointed glass rod and the skim milk drawn off. The percentage of fat in the cream was adjusted to 55% in each instance by adding the required quantity of skim milk obtained during the separation. The fat content of cream was determined by the Gerber method. Cream was washed by diluting with glass-distilled water (45 C) to the volume of the original milk and reseparating. Cream was washed thrice in this manner. The first wash water, which contained most of the skim milk associated with cream was added to the skim milk portion which was used for further fractionation. Casein was removed from the skim milk by isoelectric precipitation at pH 4.5, with 1 N HCl. The casein obtained was washed repeatedly with glass-distilled water and the washings added to whey. Alternatively, casein was obtained from skim milk by super centrifugation for 2 h at 25,000 × g in the International centrifuge. Part of whey proteins was precipitated by heating isoelectric whey at 85 C for 10 min. This precipitate was removed from whey by centrifugation and decantation.

Estimation of iron in milk fractions was done by a colorimetric method using 1, 10 phenanthroline (*10*). Protein estimation was done by micro-Kjeldahl method. Active sulfhydryls were estimated by the method of Narang et al. (*8*) with the following modification: The absorbance was measured at 310mμ instead of 300 mμ as at this wave length there was less interference from substances that absorbed at 300 mμ. Milk fat oxidation was followed by thiobarbituric acid (TBA) values suggested by King (*4*). A difference in the absorbance of 30×10^{-3} indicated oxidized flavor.

RESULTS AND DISCUSSION

Distribution of added iron in raw milk fractions is given in Table 1. The difference in iron content between the fractions of iron-enriched samples and the corresponding fractions of non-enriched samples is taken to represent 'added iron' concentration. From data in the table it will be seen that about 9% of the added iron was found in the total cow cream, while about 12.5% was

associated with total cream in buffalo milk. Though the cream samples were standardized to 55% fat, buffalo milk contained larger amounts of skim milk because of its higher initial fat content. Therefore, the higher percentage of added iron found in buffalo cream could be due to larger amount of skim milk associated with it. This is supported by the fact that there was no significant difference between the added iron concentrations of cow and buffalo washed creams. However, the concentration of added iron associated per gram of washed cream was found to be significantly lower in buffalo samples, compared to cow cream samples. This may be of importance as it has been suggested that the concentration of the metal associated with fat globules is an important factor in the oxidative deterioration of milk (*5*).

More than 90% of the added iron was associated with skim milk, mostly bound to casein; about 20% of the added iron remained with the whey. King et al. (*6*) found that added iron was partly dialysable at low pH. A part of the added iron dissociated from casein when the pH of milk was lowered to that of isoelectric point of casein. This would explain why the concentration of iron per unit weight was slightly more in centrifuged casein than in isolectric casein. No attempt was made to determine the iron content of whey protein fractions, as it has been shown that low pH treatments and denaturation change the association of the whey proteins with metals (*5, 6*).

Table 2 gives the distribution of iron added to milk before pasteurization. A comparison of data in Tables 1 and 2 shows that the distribution pattern of added iron, ferrous or ferric, in both cow milk and buffalo milk was unaffected by subsequent pasteurization. The pasteurization process employed in this study would denature the whey proteins which might result in their coprecipitation with isoelectric casein. Protein determinations in whey showed a loss of about 5% of the protein due to pasteurization; since this constitutes only about 1% of the weight of casein, analytical results would not be influenced noticeably.

Data on distribution of iron added to pasteurized milk are in Table 3. Though the amount of iron associated with cream was unaffected by the stage of addition, less iron was bound to casein in milk, if added after

TABLE 1. *Distribution of iron added*[a] *to raw milk*

	Cow milk				Buffalo milk			
	Percent of added iron		μg of added iron/g of fraction		Percent of added iron		μg of added iron/g of fraction	
Fraction	Fe^2	Fe^3	Fe^2	Fe^3	Fe^2	Fe^3	Fe^2	Fe^3
Cream	8.9 ± 0.5	8.9 ± 0.7			12.5 ± 2.3	12.6 ± 2.1		
First wash water	3.2 ± 0.5	3.2 ± 0.4			6.8 ± 1.1	6.9 ± 0.8		
Washed cream	3.4 ± 0.6	3.4 ± 0.7	4.3 ± 0.9	4.3 ± 0.8	3.6 ± 0.6	3.7 ± 0.7	2.5 ± 0.4	2.6 ± 0.6
Skim milk	92.5 ± 1.6	92.2 ± 2.6			90.2 ± 3.3	90.4 ± 2.6		
Centrifuged casein			261 ± 17	255 ± 21			248 ± 10	253 ± 16
Isoelectric casein	69.7 ± 8.4	70.1 ± 7.0	239 ± 33	240 ± 28	72.3 ± 6.4	71.8 ± 5.6	227 ± 20	226 ± 19
Isoelectric whey	21.4 ± 6.7	20.9 ± 6.2			20.1 ± 6.0	20.1 ± 5.3		
Whey proteins			98 ± 11	104 ± 14			90 ± 14	97 ± 10

[a]Iron was added to a concentration of 10 ppm. Results are expressed as mean of 15 samples ± standard deviation.

TABLE 2. *Distribution of iron added to milk prior to pasteurization*[a]

	Cow milk				Buffalo milk			
	Percent of added iron		μg of iron/g of fraction		Percent of added iron		μg of iron/g of fraction	
Fraction	Fe^2	Fe^3	Fe^2	Fe^3	Fe^2	Fe^3	Fe^2	Fe^3
Cream	8.9 ± 0.6	8.9 ± 0.5			12.4 ± 2.1	12.6 ± 2.0		
Washed cream	3.4 ± 0.5	3.6 ± 0.5	4.4 ± 0.7	4.5 ± 0.8	3.6 ± 0.5	3.6 ± 0.6	2.5 ± 0.4	2.5 ± 0.4
Skim milk	91.2 ± 1.1	90.9 ± 1.1			89.7 ± 1.5	89.0 ± 1.6		
Isoelectric casein	69.7 ± 7.5	70.1 ± 6.6	237 ± 30	238 ± 27	72.4 ± 5.6	71.9 ± 4.6	225 ± 16	224 ± 17
Isoelectric whey	20.7 ± 6.6	20.2 ± 7.1			18.6 ± 6.1	19.5 ± 5.1		
Whey proteins			104 ± 11	96 ± 16			94 ± 10	99 ± 7

[a]Iron was added to a concentration of 10 ppm. Results are expressed as mean of 15 samples ± standard deviation.

TABLE 3. *Distribution if iron added to pasteurized milk after pasteurization*[a]

	Cow milk				Buffalo milk			
	Percent of added iron		μg of iron/g of fraction		Percent of added iron		μg of iron/g of fraction	
Fraction	Fe^2	Fe^3	Fe^2	Fe^3	Fe^2	Fe^3	Fe^2	Fe^3
Cream	8.9 ± 0.5	8.9 ± 0.4			12.3 ± 2.2	12.2 ± 2.0		
Washed cream	3.4 ± 0.5	3.4 ± 0.5	4.3 ± 0.7	4.3 ± 0.7	3.5 ± 0.7	3.5 ± 0.6	2.4 ± 0.3	2.5 ± 0.4
Skim milk	91.7 ± 1.2	91.4 ± 1.4			88.7 ± 1.2	88.1 ± 1.7		
Isoelectric casein	66.9 ± 7.0	66.2 ± 7.1	227 ± 29	225 ± 27	68.6 ± 4.9	67.6 ± 5.2	213 ± 14	210 ± 16
Isoelectric whey	23.6 ± 6.6	24.2 ± 6.4			22.6 ± 5.8	22.9 ± 4.8		
Whey proteins			101 ± 15	96 ± 14			96 ± 9	94 ± 13

[a]Iron was added to a concentration of 10 ppm. Results are expressed as mean of 15 samples ± standard deviation

pasteurization. This might be due to the strong affinity of iron towards sulfhydryl groups released from whey proteins during pasteurization. This difference in distribution of iron might also mean that iron once bound to casein is not released to combine with whey fractions at the temperature employed in this experiment. Estimation of iron in whey proteins precipitated by heat coagulation of isoelectric whey at 85 C for 10 min showed this fraction to be low in iron concentration. Further, no significant difference was noticed between iron concentrations of whey proteins isolated by this method from raw milk samples or samples to which iron was added either before or after pasteurization. However, this may not present a true picture of the association of iron with whey proteins in milk as the heat treatment employed to denature the proteins could have affected changes in their association.

The data presented do not give evidence of any effect of the oxidation state of iron on the distribution pattern of its salts, either in cow milk or in buffalo milk.

The oxidative stability of iron-enriched milk samples is given in Table 4. The TBA values were measured 48 h after addition of iron salts to milk. Raw milk was found to be more resistant to iron-catalysed lipid oxidation. Neither ferrous nor ferric iron produced noticeable oxidized flavors in raw milk even after 48 h of storage, as indicated by TBA values. However, iron salts added to pasteurized samples produced oxidative deterioration in both cow milk and buffalo milk within this storage period. This enhanced activity of iron in pasteurized milk should be considered in the context of the difference in the distribution of iron in these two sets of samples (Tables 1 and 2). Though there was no significant difference in the amount of iron associated with cream, the amount associated with whey was more in pasteurized milk, probably, as already pointed out, because of the preferential association of iron with sulfhydryl groups activated in whey proteins. To block these sulfhdryl groups 50 ppm N-ethyl maleimide (NEM) was added to samples of pasteurized milk before addition of iron. Analysis showed that significantly less iron had associated with the whey fraction of milk in the presence of NEM; in such samples, the TBA absorbance was less than 30×10^{-3} even after 48 h of storage (Table 4). Demott (*2*) had noted less cooked flavor in ferric iron enriched milk samples which were subsequently pasteurized. The present study on pasteurized samples enriched with ferric iron showed a decrease of about 80% within 24 h in active sulfhydryl groups estimated by the NEM method. This would imply rapid oxidation of sulfhydryls by iron, getting itself reduced in the process to a reportedly stronger pro-oxidant ferrous form. Scanlan and Shipe (*11*) in a study of homogenized milk also showed that steam-vacuum treatment reduced ferric iron. Addition of hydrogen peroxide retarded the reduction of iron and prevented development of oxidized flavor. However, presence of iron in the reduced stage did not appear to be the controlling factor in determining its oxidative potency. This is evident from the fact that ferrous iron in raw milk was less potent than ferric iron added to pasteurized milk. This might mean that iron-sulfhydryl association has a more specific role than the mere conversion of ferric iron to the ferrous form. This appears analogous to the role of ascorbic acid in copper catalyzed oxidation of milk (*13, 14*). In this case ascorbic acid is not involved in milk lipid oxidation catalyzed by iron (*15*).

TABLE 4. *Thiobarbituric acid values of milk samples stored 48 h at 5 ± 1 C after addition of ferrous or ferric forms of iron*[a]

Sample	Treatment	A x 10^3 State of iron added Fe^2	A x 10^3 State of iron added Fe^3
Cow milk	A[b]	24 ± 7	25 ± 6
	B[c]	39 ± 7	37 ± 5
	C[d]	63 ± 7	44 ± 8
	D[e]	76 ± 9	61 ± 7
	E[f]	28 ± 6	22 ± 8
Buffalo milk	A	24 ± 5	24 ± 6
	B	42 ± 8	42 ± 6
	C	61 ± 8	45 ± 7
	D	81 ± 8	66 ± 7
	E	27 ± 11	28 ± 5

[a]Results are expressed as mean of 15 samples ± standard deviation.
[b]Iron was added to raw milk.
[c]Iron was added to milk after pasteurization at 81 C for 1 min.
[d]Iron was added to milk before pasteurization at 81 C for 1 min.
[e]Iron was added to milk before pasteurization at 63 C for 30 min.
[f]Iron was added to milk after pasteurization at 81 C for 1 min and addition of 50.0 ppm NEM.

Pasteurization of milk after addition of iron increased the catalytic activity of both forms of iron. Statistical analysis of the data indicated that ferrous iron was significantly more potent than ferric iron in producing oxidative deterioration ($P<0.05$) (Table 4). Comparison of data in Tables 1 and 2 shows no marked difference in the distribution pattern of added iron. Demott (*2*) who employed a lower temperature for pasteurization (63 C for 30 min) which would not activate measurable quantities of sulfhydryls, also noticed oxidized flavor development in samples enriched with iron before pasteurization. This enhanced oxidative deterioration of milk appears to be due to activation of added iron during heat treatment. TBA values were higher in iron-enriched samples pasteurized at 63 C for 30 min compared to samples pasteurized at 81 C for 1 min. Prolonged heating at lower temperatures in comparison to short time heating at higher temperature appears to activate iron to induce oxidized flavors.

From the foregoing observations it may be concluded that heat treatment or the presence of a suitable reducing agent capable of rendering iron to its reduced form enhances the catalytic activity of iron. The TBA values show that unlike with copper, buffalo milk does not differ from cow milk in its resistance to oxidative deterioration induced by iron.

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Preparation of ^{14}C-Labeled Penicillic Acid with High Specific Activity and Yield

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ABSTRACT

^{14}C-Labeled penicillic acid was produced by stationary culture incubation of *Penicillium cyclopium* (NRRL 1888) on a modified Raulin-Thom broth medium containing ^{14}C-labeled acetate. Approximately 1.2 g of radioactive compound, with a specific activity of 23.0 μCi/mmole, was produced in 9 days in 1500 ml of the broth. Incorporation of the isotope into penicillic acid was 11.9%. Production of the radiolabeled compound with high specific activity was achieved by correlating the monitoring of expired ^{14}C-CO_2 with production of penicillic acid during the fermentation. The effects of various growth substrates, pH, and incubation times on production of non-labeled penicillic acid also were investigated. Results show that sterile rice is an excellent substrate, that among liquid media examined, higher yields were obtained in stationary rather than in shake cultures, and that higher yields of penicillic acid were obtained at pH 3.5 or lower. Simultaneous monitoring of penicillic acid production and ^{14}C-label incorporation is essential to detect and isolate a high yield of labeled compound with high specific activity.

Penicillic acid was first isolated in 1913 by Alsberg and Black (*1*) from a culture of *Penicillium puberulum*. Many mold species, in addition to *P. puberulum*, also are reported to produce penicillic acid (*4*). Penicillic acid has antibiotic properties; however, it is also reported to be toxic to a number of laboratory animals (*4*), having an LD_{50} in mice (subcutaneous injection) of 100 mg/kg (*9*). Subcutaneous injection in rats has resulted in malignant tumors at the site of injection (*6*). Penicillic acid induced cytomorphological changes in cultured cells from rat liver, kidney, and lung (*12*) and in HeLa cells (*13*).

Penicillic acid has been isolated from corn, rice, barley, sorghum, oats, and wheat substrates inoculated in the laboratory with molds known to produce penicillic acid (*5*), in addition to being found in naturally contaminated dried beans, corn (*11*), and tobacco (*8*). The extent of toxin production varied with strain of mold, commodity, and growing conditions. Maximum production of toxin resulted when cultures were grown at 15-20 C; however, accumulation of toxin was increased when cultures were grown at even lower temperatures (1-10 C) (*5*). These facts plus data from studies reporting the toxicological nature of penicillic acid (*4, 6, 9, 12, 13*) reflect the potential health hazard of penicillic acid-contaminated food products resulting from mold infestation. This paper reports experiments designed to give optimum conditions for production of ^{14}C-labeled penicillic acid with high specific activity and yield for use in in vivo studies with penicillic acid.

MATERIALS AND METHODS

Organism

Penicillium cyclopium (NRRL 1888) was used throughout this study. The culture was maintained on Czapek-Dox agar and the inoculation suspension of conidia was prepared by transferring conidia of *P. cyclopium* from an inoculated Czapek-Dox agar plate (maintained at room temperature for 5 days after inoculation) to 100 ml of sterile phosphate buffer solution.

Media and culture conditions

Raulin-Thom medium supplemented with diammonium tartrate as described by Bentley and Keil (*2*) was used to produce ^{14}C-labeled penicillic acid. The medium (1500-ml portions) was sterilized in 2.8-liter Fernbach flasks and each flask was inoculated with 2×10^7 conidia. Sterile rice (50-g aliquots), sterile rice broth (prepared by grinding rice in a Wiley mill, placing in cheesecloth, cooking in water for 25 min, and allowing to drain) (1500 ml), Czapek-Dox broth (1000 ml), and potato dextrose broth (300 ml) were also evaluated as substrates suitable for penicillic acid production.

All inoculated substrates were incubated at 26 C except for Czapek-Dox broth, which was incubated at 16 C. Stationary and shake conditions (150 rpm, controlled environment incubator shaker, New Brunswick Scientific Co., Inc.) were evaluated using the Czapek-Dox and the modified Raulin-Thom broths.

Extraction and purification of penicillic acid

The culture media were extracted twice with chloroform. Each extract volume equaled the amount of medium being extracted. Since we noted that higher yields were obtained if the pH of the medium was <3.5 before extraction, the medium was acidified to the desired pH with 1 N HCl when necessary. The mycelial pad and solid rice were extracted by blending the pad or solid rice and 200 ml of chloroform in a Waring blender at medium speed for 5 min before filtration. The blended mixture was filtered through S&S 588 filter paper (Schleicher and Schuell, Inc.). All chloroform extracts were evaporated to dryness and redissolved in 25 ml of ethyl acetate. Penicillic acid was purified by the liquid-liquid partition and column chromatographic procedures described by Thorpe and Johnson (*11*). This purification procedure is

applicable when the concentration of penicillic acid is <75 mg/extraction. Extractions containing larger amounts were proportionately subdivided.

Determination

The penicillic acid concentrations were estimated on two thin-layer chromatographic (TLC) systems by comparison with reference standards as described by Wilson et al. (*14*) and Ciegler and Kurtzman (*5*).

Confirmation of penicillic acid

Penicillic acid was confirmed by high-performance liquid chromatography (HPLC), mass spectrometry (MS), nuclear magnetic resonance spectroscopy (NMR), and infrared (IR) and ultraviolet (UV) spectrometry. The HPLC system consisted of a DuPont Model 840 instrument, using a 1 m × 2.1 mm SAX (strong anion exchange) column; 0.01 M sodium borate eluate; 0.040-0.036 ml/min flow rate; and a 254 nm UV detector (*10*). The mass spectrum was obtained on a Finnigan 3300F Quadropole mass spectrometer equipped with a Model 6100 data system and using a 100 C source temperature, solid probe inlet, 2×10^{-7} torr pressure, and scanning m/e 10-250. The NMR spectrum was obtained in an aqueous solution on a Varian A-60 NMR spectrometer. The IR spectrum was obtained on a Perkin-Elmer Model 180 IR spectrophotometer, 1 mg of sample in 200 mg of KBr on a 30 mm disk; the UV spectrum was obtained in ethanol on a Cary 14 recording UV spectrophotometer.

Radiolabeling of penicillic acid

Studies of the biosynthetic pathways of penicillic acid have indicated that orsellinic acid is a precursor which can be formed by the condensation of four acetate units (*2, 3*). Thus, [1-^{14}C] sodium acetate (Amersham/Searle Corp., Arlington, Ill.) (specific activity 57 mCi/mmole with 91% isotopic abundance in the C-1 atom) was used in this study. The labeled acetate was dissolved in sterile water and the ^{14}C-acetate solution was sterilized by filtration (13-mm Swinex Millipore filter, 0.22 μm pore size) before addition to the medium. A Mark III 6880 liquid scintillation spectrometer (Searle Analytic Inc.) and Permafluor-1 scintillation fluid (Packard Instrument Co.) were used for all radioactive counting analyses. The trap solutions were analyzed by using 0.5 ml of NCS-tissue solubilizer (Amersham/Searle)/0.2 ml of trap solution with subsequent dilutions as necessary to avoid chemiluminescence.

RESULTS AND DISCUSSION

Penicillic acid production and pH change in different substrates

Culture techniques described above were compared for the effect of pH on yields of penicillic acid. Results in Table 1 indicate that higher levels of penicillic acid were obtained if the pH was 3.5 or below. The pH of the medium decreased as production of penicillic acid increased, and then rose as the concentration of penicillic acid decreased. Stationary culture incubation produced substantially higher yields of penicillic acid than shake cultures.

The modified Raulin-Thom medium produced the greatest amount of penicillic acid [about 2 mg/ml of media, as reported earlier by Bentley and Keil (*2*)]. Penicillic acid usually was first detected between 3 and 4 days after inoculation, with maximum production occurring between 14 and 19 days.

Incorporation of ^{14}C-labeled acetate into penicillic acid

To determine the optimum time for the addition of ^{14}C-labeled acetate and for the harvesting of the ^{14}C-labeled penicillic acid, two Fernbach flasks, each containing 1500 ml of modified Raulin-Thom medium and an inoculum load of 2×10^7 conidia, were maintained under identical conditions. Both flasks were

TABLE 1. *Effect of culture medium, pH, and incubation time on the production of penicillic acid (μg/ml of medium) at 26 C using* Penicillium cyclopium [NRRL 1888]

	Incubation time, days													
	3		5		6		10		11		12		14	
Culture medium	μg/ml	pH	μg/ml	pH	μg/ml	pH	μg/ml	pH	μg/ml	pH	μg/ml	pH	μg/ml	pH
Raulin-Thom broth, stat.	0.4	3.8	25	3.4	—[a]	—	170	3.35	—	—	425	3.3	400	3.15
Raulin-Thom broth, shake	—	—	—	—	0.2	3.25	—	—	0.2	2.75	—	—	—	—
Czapek Dox broth, stat.[b]	ND[c]	7.35	ND	6.9	—	—	ND	6.4	—	—	ND	6.15	ND	5.7
Czapek Dox broth, shake[b]	ND	6.1	ND	3.3	—	—	0.3	3.5	—	—	0.2	3.55	0.1	3.6
Rice, sterile[d]	4.4	—	—	—	547	—	6940	—	—	—	—	—	4920	—
Rice broth, stat.	ND	5.75	0.4	5.2	—	—	0.6	4.05	—	—	2.0	3.55	2.0	3.25
Potato dextrose broth, stat.	—	—	—	—	5.0	4.2	—	—	2.4	3.9	—	—	—	—

	17		19		24		27		28		35		42	
	μg/ml	pH	μg/ml	pH	μg/ml	pH	μg/ml	pH	μg/ml	pH	μg/ml	pH	μg/ml	pH
Raulin-Thom broth, stat.	1400	3.15	2000	3.1	1200	3.1	—	—	800	3.7	800	5.9	600	6.6
Raulin-Thom broth, shake	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Czapek Dox broth, stat.[b]	—	—	ND	4.45	ND	4.05	ND	4.05	—	—	ND	3.7	—	—
Czapek Dox broth, shake[b]	—	—	0.3	3.6	0.4	3.6	0.4	3.8	—	—	0.6	4.1	—	—
Rice, sterile[d]	1.3	—	—	—	—	—	—	—	—	—	—	—	—	—
Rice broth, stat.	60.0	3.1	200	3.2	160	3.8	—	—	120	3.95	200	5.2	160	5.45
Potato dextrose broth, stat.	—	—	—	—	—	—	—	—	—	—	—	—	—	—

[a] Analyses were not done.

[b] Incubation temperature was 16 C.

[c] None detectable at 50 ng.

[d] Values for rice are given in μg/g, and pH was not monitored.

housed at room temperature (26 C) in a glove box. The first flask was monitored for production of penicillic acid (Table 2) to determine the appropriate time for the addition of ^{14}C-labeled acetate to the second flask and the subsequent harvesting of ^{14}C-labeled penicillic acid. The second flask was equipped with a positive air flow intake. The air entering the system was filtered to avoid contamination of the fermentation. Expired air was passed, in a closed system, through four NaOH traps for collecting and monitoring any expired radioactive CO_2 (Fig. 1). [1-^{14}C] Acetate (1.354 mCi) was added to the

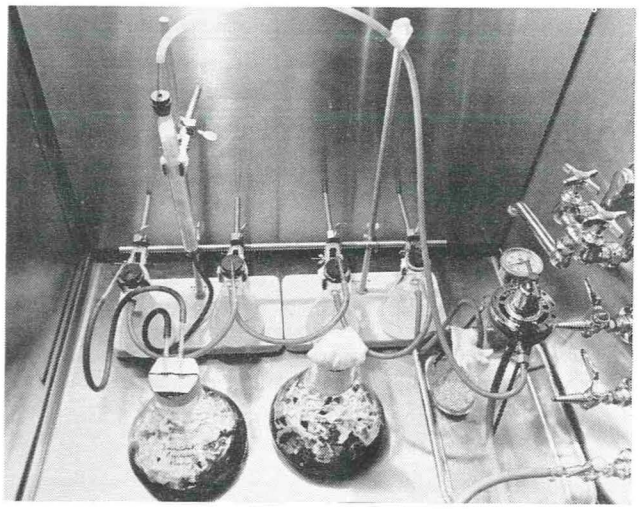

Figure 1. *Apparatus utilized to produce ^{14}C-labeled penicillic acid (26 C), including fermentation flasks (1500 ml of Raulin-Thom broth) and ^{14}C-CO_2 trap flasks (NaOH).*

second flask on the fourth day after inoculation, at which time 4 μg of penicillic acid/ml of medium was detected in the first flask. The ^{14}C-penicillic acid was harvested 9 days later (13 days after original inoculation) because: (*a*) the detectable level of penicillic acid in the first flask had reached 800 μg/ml; and (*b*) monitoring of the CO_2 traps for expired ^{14}C-CO_2 showed that radioactivity had leveled off, indicating that most of the ^{14}C-labeled acetate added to the second flask had now been metabolized (Table 2).

TABLE 2. *Monitoring of radioactivity of CO_2 traps and production of penicillic acid in Raulin-Thom broth medium during ^{14}C labeling of penicillic acid with [1-^{14}C] acetate*

Days after addition of ^{14}C label	Radioactivity in CO_2 traps[a] (DPM X 10^3)		Penicillic acid produced	
	No. 1	No. 2	μg/ml	DPM/μg
0	0.0	0.0	4	—[b]
3	6.6	0.0	200	—
6	13.6	0.0	400	—
7	26.9	9.9	—	—
8	21.0	5.8	800	294
9	24.6	7.9	—[c]	239

[a] CO_2 traps 3 and 4 and a flask monitoring room atmosphere (Fig. 1) did not contain any evidence of radioactivity.

[b] Analyses were not performed.

[c] ^{14}C-Penicillic acid was harvested on day 9.

Based on data in Table 1, the incubation time of the second flask could have been extended beyond day 13 to increase the yield of ^{14}C-labeled penicillic acid. However, this probably would have resulted in a product with a lower specific acitvity due to subsequent production of non-labeled penicillic acid. Monitoring of both the expired ^{14}C-CO_2 in the CO_2 traps and penicillic acid production in the first flask indicated that the thirteenth day after inoculation was the optimum for harvesting the ^{14}C-penicillic acid with both high specific activity and yield. The yield and specific activity of the ^{14}C-penicillic acid were 1.2 g and 23.0 μCi/mmole, repectively. The specific activity of the final product was approximately 30 times greater than previously reported (*2*). The actual amount of crystalline ^{14}C-labeled penicillic acid recovered was 0.935 g (about 78% recovery). An isotope incorporation of 11.9% was realized. The technique could be applicable to similar labeling experiments to obtain compounds with both high specific activity and yield.

Purity of ^{14}C-labeled penicillic acid

The radiochemical purity of ^{14}C-penicillic acid was confirmed on the two TLC systems previously described. When cochromatographed with the standard on the two TLC systems, the product gave only one compact spot, with the R_f matching that of the penicillic acid standard. Radiographs of the developed TLC plates indicated that all detectable radioactivity was within the adsorbent zone containing penicillic acid. The distribution of the radioactivity on the developed TLC plate was determined by scraping 1-cm increments of the adsorbent from the plate and counting.

The mass spectrum of the crystals matched the spectrum for penicillic acid (170 M^+) contained in the Mycotoxins Mass Spectral Data Bank (*7*). The IR spectrum showed absorptions at 3472, 3416 (OH in COOH), 3260 (broad band, t-OH hydrogen bonded to carbonyl of the lactone), 3100 (CH on the C=C), 2950 (CH_3), 1740 (C=O lactone), 1710 (C=O, acid), and 1630 cm^{-1} (C = C). These results support the hypothesis that penicillic acid exists in two tautomeric forms in the solid phase. The NMR spectrum displayed the following signals: 1.74 (d, J = 0.5 Hz), 3.94 (s), 5.42 (m), and 5.52 ppm (m). The UV spectrum in ethanol solution showed a maximum at 220 nm. The melting point of the crystals was 83 C.

In summary, results of this study indicate that stationary cultures produce higher amounts of penicillic acid than shake cultures. Higher production and extraction results were realized when the pH of the media was <3.5. The modified Raulin-Thom broth and sterile rice media produced the highest amounts of penicillic acid; however, the procedures for isolation and purification of penicillic acid resulted in higher recoveries with the modified Raulin-Thom medium. Conditions favoring the highest yield of ^{14}C-labeled penicillic acid will not necessarily result in the highest specific acitivity. To achieve both high yield and high specific activity, it is recommended that production of penicillic acid as well as incorporation of the ^{14}C-label be monitored. Expired ^{14}C-CO_2 can be efficiently used to monitor label incorporation in a fungal fermentation.

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Characteristics of Psychrotrophic, Gram-Positive, Catalase-Positive, Pleomorphic Coccoid Rods from Vacuum-Packaged Wholesale Cuts of Beef

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ABSTRACT

Morphological, physiological, and biochemical characteristics are presented for yellow-pigmented and non-pigmented psychrotrophic, gram-positive, catalase-positive, pleomorphic rods from vacuum-packaged wholesale cuts of beef. These isolates were essentially species of *Corynebacterium* and *Microbacterium thermosphactum.*

Gram-positive, catalase-positive, pleomorphic rods often constituted a significant part of the psychrotrophic microbial flora of vacuum-packaged wholesale cuts of beef (knuckles, ribs, chucks) stored for 0-35 days at 1-3 C (*7*). This was particularly true during the early phases of storage (0-21 days), whereas *Lactobacillus* spp. became dominant after 28-35 days. Pleomorphic rods of this type, often referred to as coryneform bacteria (or sometimes more specifically as species of *Corynebacterium, Arthrobacter,* or *Microbacterium*) are ubiquitous in nature. They have been isolated from various foods such as poultry, eggs, and meats (*1, 5, 6*), frozen vegetables (*9*), and seafood (*10*). This report describes the characteristics of yellow-pigmented and non-pigmented, gram-positive, catalase-positive, pleomorphic rods of vacuum-packaged beef.

MATERIALS AND METHODS

Source of cultures

Isolates described in this study were from surface swabs of vacuum-packaged wholesale cuts of beef (33 knuckles, 41 ribs, 15 chucks) stored for 0 to 35 days of 1-3 C (*7*). The surface of the meat samples was swabbed with sterile cellulose sponges wetted in sterile 0.1% peptone broth. After sampling, sponges were rinsed in 100 ml of sterile 0.1% peptone broth. Appropriate dilutions of this broth were plated on plate count agar. Details of this procedure are presented in a previous paper (*7*). The isolates were detected among colonies picked from plate count agar (PCA) plates which were incubated at 7 C for 10 days. Thirty to 40 colonies were randomly picked from countable plates and placed on trypticase soy agar (TSA) slants. Incubation of slants was at 25 C for 2-3 days. The 155 isolates examined in this study constitute a collection of gram-positive, catalase-positive, pleomorphic rods from vacuum-packaged beef (*7*), 92 from ribs, 30 from knuckles, and 33 from chucks. *M. thermosphactum* 11509 was purchased from the American Type Culture Collection, Rockville, Md. for comparison purposes.

Culture characteristics

The following tests were included: pigmentation on TSA or PCA (48 h at 25 C); growth at 4, 7, 25, and 36 C on TSA slants; mortility on Motility S medium (Difco, 48 h at 25 C) and hanging drop procedure with growth from TSA slants incubated at 25 C for 24 h; acid from carbohydrates in phenol red broth base with 1% filter-sterilized carbohydrates (reactions were recorded daily up to 30 days at 25 C, control tubes were pre-incubated to assure sterility); Hugh-Leifson (OF medium, glucose, lactose, or sucrose, 4 days at 25 C); oxidase (PathoTec-CO); nitrate reduction (Nitrate broth 48 h at 25 C); catalase (24 h growth from TSA slants at 25 C with 3% H_2O_2); Christensen's urea (1-4 days at 25 C); gelatin liquefaction (Nutrient gelatin, 14 days at 25 C); Indole (1% tryptone broth, 24 h at 25 C); Simmon's citrate (4-7 days at 25 C); starch hydrolysis (Nutrient agar with 1% potato starch, 48 h at 25 C); methyl red (MR-VP medium, 48 h at 25 C); H_2S production (TSI agar, 18-24 h at 25 C); litmus milk (BBL, 1-7 days at 25 C); and benzidine test (*4*). Heating of cultures was done in sealed glass ampules containing 1 ml of inoculated 10% skimmilk. The ampules were heated while submerged in a constant temperature shaker-water bath at the desired temperature. Before and after desired intervals of heating, ampules were removed from the bath, cooled in ice water, and the contents were plated on TSA. Plates were incubated for 3 days at 25 C. When colonies were absent or slow in development (small or pin-point) plates were re-incubated for several days and counted again if necessary.

RESULTS AND DISCUSSION

Two types of pleomorphic coccoid rods were often isolated from psychrotrophic agar plates of vacuum packaged beef (*a*) yellow to buff, non-fermentative (51 isolates) and (*b*) white fermentative (OF-glucose), 104 isolates.

Yellow to buff, non-fermentative isolates

Colonies on TSA plates incubated for 3 days at 25 C were circular, entire, convex, smooth and 0.5-1 mm in diameter. Three types were recognized (*a*) pale yellow, (*b*) deep yellow, and (*c*) buff-colored colonies. The pale yellow isolates consisted of gram-positive, pleomorphic rods in palisades and coccoid forms which destained easily (Fig. 1,A). The dark yellow isolates were slender, gram-positive to gram-variable rods in palisades (Fig. 1, , B). The buff-colored isolates were long, gram-positive, pleomorphic rods in palisades (Fig. 1, C) which did not destain as easily as the yellow-pigmented isolates. Major characteristics of the 3 groups are presented in Table 1.

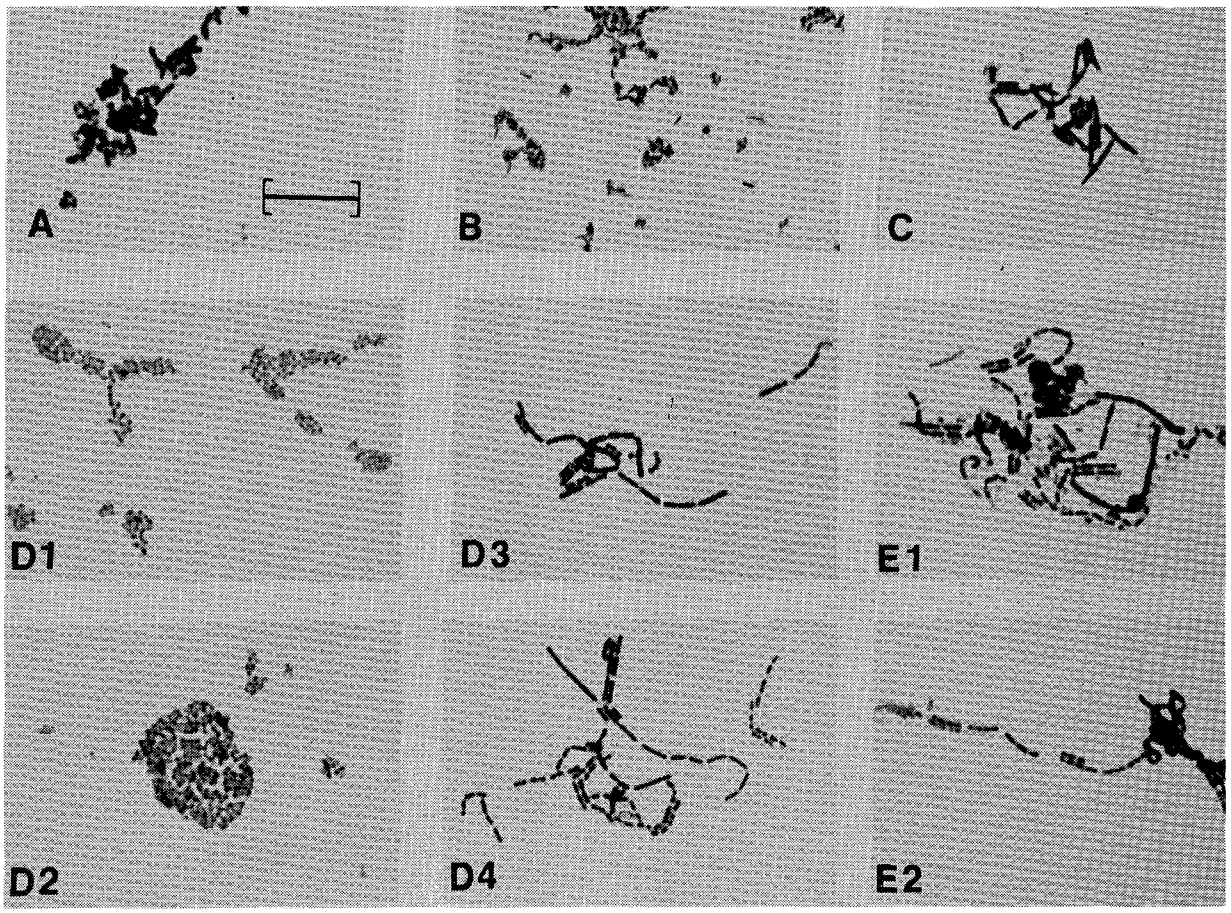

Figure 1. *Morphological characteristics of pleomorphic isolates from beef and* M. thermosphactum *ATCC 11509. Buff to yellow pigmented rods: A = Group 1, B = Group 2, C = Group 3. D-1 and D-3 represent coccoid and long rod forms of* M. thermosphactum *ATCC 11509, D-2 and D-4 are similar forms of our isolates. E-1 represents "large bodies" of* M. thermosphactum *ATCC 11509, E-2 of our isolates. Bar indicates 10μm, all photographs are of same magnification.*

TABLE 1. *Characteristics of yellow to buff, gram-positive, catalase-positive, non-fermentative pleomorphic rods from vacuum packaged beef*

Tests	Group 1 9 cultures	Group 2 14 cultures	Group 3 19 cultures
Oxidase	–	–	–
Catalase	+	+	+
Hugh Leifson Oxid.	+	+	–
(Glucose) Ferm.	–	–	–
Growth at 7 C	+	+	+
25 C	+	+	+
36 C	(±)[a]	+	+
Nitrate reducation	+	–	–
Gelatin liquefaction	+	+	–
Acid from:			
lactose	–	–	–
sucrose	–	+	–
glucose	+	+	–
H_2S from TSI	–	–	–
Starch hydrolysis	+	+	–
Litmus milk	NC[b]	NC or Red.	Alk
Hugh Leifson			
lactose Oxid.	–	–	–
Ferm.	–	–	–
sucrose Oxid.	–	+	–
Ferm.	–	–	–
Pigment	p. yellow	d. yellow	buff
Motility	–	–	–

[a]Limited growth
[b]NC = no change

In addition to morphological characteristics and pigmentation, separation of the groups was based on differences with respect to raction in OF glucose medium, growth at 36 C, nitrate reduction, gelatin liquefaction, starch hydrolysis, and acid production from sugars (glucose, sucrose). Nine of the 51 isolates did not fit any of the three groups. The heat resistance of representative isolates of the three groups is presented in Fig. 2. Isolates of group 1 were the least heat resistant, those groups 2 and 3 were more heat resistant but not as much as *Microbacterium* spp. (15 min at 72 C in skimmilk). A comparison of the properties of the beef isolates with those of the coryneform bacteria (Groups I-VI) from frozen vegetables as reported by Splittstoesser et al. (*9*) indicates that groups 1, 2, and 3 in this study related best to groups III or V, IV, and I, respectively. According to Splittstoesser et al. (*9*) their groups I-IV resembled the types more commonly isolated from egg, meat, and milk products (*5, 6, 8*) and are usually described as species of *Corynebacterium* or *Microbacterium*. Isolates of beef also related best to group I of six groups of coryneform bacteria isolated from pond-reared shrimp and pond water by Vanderzant et al. (*10*)

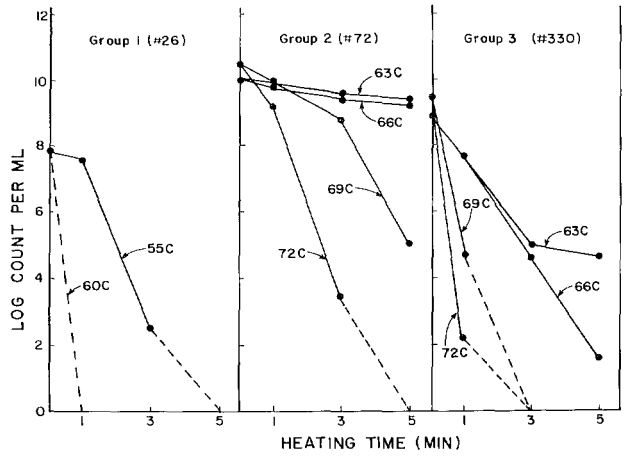

Figure 2. *Heat resistance of yellow to buff, gram-positive, catalase-positive, pleomorphic rods from vacuum-packaged beef.*

According to the 8th ed of *Bergey's Manual* (*2*), it seems unwise to recognize *Corynebacteriaceae* and instead a working concept is presented of a "Coryneform group of bacteria" to include *Corynebacterium, Arthrobacter,* (with the related genera *Brevibacterium* and *Microbacterium* as *genera incertae sedis*), *Cellulomonas,* and tentatively *Kurthia.*

If an attempt were made to further classify the meat isolates, those of group 1 which showed both rods and coccoid forms and were nitrate-positive, liquefy gelatin, and hydrolyze starch, resemble more closely the pleomorphic species described as *Arthrobacter* (*3*). In a previous paper by Seideman et al. (*7*) some of these isolates were included in *Corynebacterium.* Isolates of groups 2 and 3 which were non-fermentative in OF glucose medium and lacked the heat resistance of *Microbacterium* spp. probably could best be included in *Corynebacterium.*

White, fermentative (OF glucose) isolates

On TSA medium after incubation for 48 h at 25 C, colonies were white, circular, entire, raised, smooth, glistening and 1-2 mm in diameter. They consisted of gram-positive coccoid to short rods to long rods in palisades, occasionally in chains (Fig. 1 D-2, 4). When grown in liquid media and sometimes on TSI agar filaments, long slender rods and large bodies were observed (Fig. 1E-2). The filaments, coccoid and long rods consist of gram-positive and gram-negative cells. The large bodies were gram-positive. A comparison of various characteristics of our isolates with *M. thermosphactum* ATCC 11509 and those of McLean and Sulzbacher (*6*) are presented in Table 2. The isolates of the present study did not survive heating in skimmilk at 72 C for 15 min, 63 C for 5 min, or 55 C for 10 min. The log survivor curves for meat isolate 467 and *M. thermosphactum* ATCC 11509 at 55 C were similar (Fig. 3). Morphological, physiological and biochemical characteristics of the white fermentative isolates were identical to those of *M. thermosphactum* ATCC 11509. They were also similar to those of *M. thermosphactum* of

TABLE 2. *Characteristics of isolates referred to* Microbacterium thermosphactum

Tests	*M. thermosphactum* ATCC 11509	*M. thermosphactum* (this study)	*M. thermosphactum* (McLean and Sulzbacher, 1953)
Growth at 4 C	+	+	+
25 C	+	+	+
36 C	(±)[a]	(±)[a]	—
Gelatin liquefaction	—	—	—
Indole	—	—	—
Urea	—	—	—
Nitrate reduction	—	—	—
Citrate utilization	—	—	—
Methyl Red	+	+	NR[b]
Gas from glucose	—	—	—
Acid from:			
D-Glucose	+	+	+
Sucrose	+	+	+
Lactose	—	—	+
Maltose	+	+	+
Melibiose	—	—	NR
Dulcitol	—	—	+
D-Fructose	+	+	NR
D-Galactose	—	—	NR
D-Mannitol	+	+	+
D-Xylose	—	—	+
L-Arabinose	—	—	+
D-Cellobiose	+	+	+
Starch hydrolysis	—	—	NR
OF-glucose (Ferm.)	+	+	+
Motility Medium S and hanging drop	—	—	—
Oxidase	—	—	—
Benzidine test	+	+	+

[a](±) = limited growth
[b]NR = not recorded

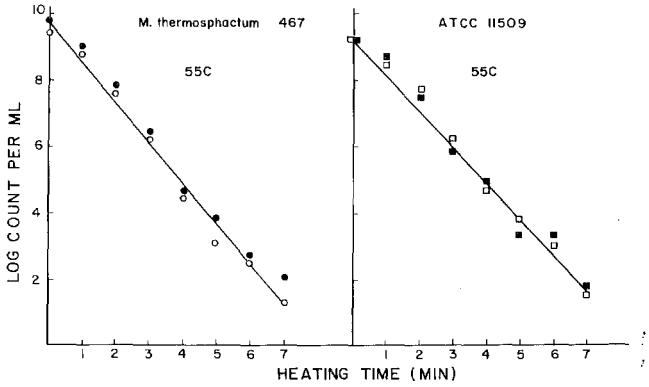

Figure 3. *Log survivor curves (2 trials) at 55 C of isolate 467* (M. thermosphactum) *and* M. thermosphactum *ATCC 11509.*

McLean and Sulzbacher, except that our isolates did not produce acid from lactose, dulcitol, xylose, or arabinose. The heat resistance of culture 467 at 55 C was similar to that of *M. thermosphactum* 49 of McLean and Sulzbacher (*6*).

Identification of the white and yellow to buff gram-positive, psychrotrophic, pleomorphic, catalase-positive rods from beef to the generic level is difficult. In view of the recommendations of the 8th edition of

Bergey's Manual (2) it probably would be best to refer to them as species of the coryneform group of bacteria. However, certain distinct differences can be recognized among the meat isolates particularly with respect to morphological characteristics, pigmentation, reaction in OF glucose medium, growth at 36 C, and heat resistance. If further classification were desired, the yellow to buff isolates could be referred to as species of *Arthrobacter* (group 1) and *Corynebacterium* (groups 2 and 3) and the white, fermentative isolates to *M. thermosphactum.*

Based upon this classification, the percentage of ribs (7) that contained *M. thermosphactum, Corynebacterium,* or *Arthrobacter* sp. was respectively, 80.5, 31.7, and 0%. Similar figures for knuckles were 45.5, 15.1, and 0%; for chucks 66.6, 40 and 6.7%. The level of *M. thermosphactum* and *Corynebacterium* sp. on ribs during refrigerated storage expressed as percentage of the total psychrotrophic population was respectively, 0.5-93% (avg. 19.4%) and 0.6-71.4% (avg. 20%); similar figures for knuckles were 0.9-70.8% (avg. 10.2%) and 0.6-63.6% (avg. 15%). *M. thermosphactum, Corynebacterium,* and *Arthrobacter* sp. in chucks constituted respectively 1.4-100% (avg. 31.9%), 4.2-37% (avg. 16.6%) and 25% of the total psychrotrophic population.

ACKNOWLEDGMENTS

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Microbial Flora of Vacuum-Packaged Lamb with Special Reference to Psychrotrophic, Gram-Positive, Catalase-Positive Pleomorphic Rods

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ABSTRACT

Wholesale cuts of lamb (loins, legs) were packaged in polyvinyl chloride (PVC) film and in two other types of packages with oxygen transmission rates (cc/100 in^2/24 h) of 0.41-0.75 and 2.28 respectively. Psychrotrophic counts were low (2×10-1.1×10^3 per in^2) intially and after storage for 21 days increased to 10^3-1.6×10^5 per in^2. Initially *Corynebacterium* species and *Microbacterium thermosphactum* were dominant. Upon refrigerated storage for 21 days, *Lactobacillus, Pseudomonas,* and *Moraxella-Acinetobacter* sp. became more significant. Among the *Corynebacterium* sp., buff-colored isolates, consisting of long, gram-positive pleomorphic rods in palisades were dominant.

In a previous paper (*7*) it was shown that the initial psychrotrophic microflora of vacuum-packaged wholesale cuts of beef (ribs, knuckles, chucks) consisted primarily of *Moraxella-Acinetobacter* sp. and coryneform bacteria. During refrigerated storage for up to 35 days, *Lactobacillus* sp. became more significant, particularly on ribs and knuckles. A. more detailed account by Hanna et al. (*3*) of the coryneform group of bacteria of vacuum-packaged beef indicated that they were essentially species of *Corynebacterium* and *Microbacterium thermosphactum.* Coryneform bacteria and more specifically pecies of *Corynebacterium, Microbacterium,* and *Arthrobacter* are often found in significant numbers in various foods, including meats, eggs, milk products, frozen vegetables, and seafoods (*1, 5, 6, 8, 9, 11*). Barlow and Kitchell (*1*) reported that a significant part (47.6-100%) of the microflora of lamb chops during refrigerated storage, whether held in air or under vacuum, was composed of gram-positive, catalase-positive rods in chains, often containing parts which stained gram-negative. They most closely resembled descriptions of *M. thermosphactum.* However, when they stored beef steaks under similar conditions a predominantly (81.3%) gram-negative microflora developed under aerobic conditions. The balance (18.7%) was made up of *M. thermosphactum.* Under anaerobic condit ..s, the microflora consisted of gram-negative rods (68%), lactobacilli (24%), and *M. thermosphactum* (8%). This report discribes changes in psychrotrophic plate counts and distribution of the microflora of lamb loins and legs during refrigerated storage for up to 21 days at 1-3 C. Special emphasis is given to the characteristics of isolates commonly referred to as the coryneform group of bacteria.

MATERIALS AND METHODS

Samples

Twenty loins and 10 legs were obtained from U.S. Choice lamb carcasses processed in a commercial operation. Following slaughter, carcasses were washed with cold water and chilled for 24 h at 1-3 C. Samples designated as treatment PVC were wrapped in polyvinyl chloride film (no vacuum). Those of treatment A were vacuum packaged with a nozzle type-clip seal machine in a barrier bag of the following characteristics: Moisture Vapor Transmission Rate (MVTR) of 0.66 g/100 $in.^2$/24 h/37.7 C/70% RH and Oxygen Transmission Rate (OTR) of 2.28 cc/100 $in.^2$/24 h/23.9 C/50%RH. In treatment B, cuts were packaged with a chamber-type, heat seal machine in barrier bags with a MVTR of 0.18-0.20 and OTR of 0.41-0.75. Packages of treatment B were passed through a dry-air type heat tunnel, while packages in treatment A were shrunk by passing through a hot-water type tunnel. "Master-Pac" samples consisted of pre-cut loins (cut into individual chops) which were re-formed and then packaged in the systems described as A and B. Five loins and five legs were examined initially (0 day), the remainder of the samples after 7-21 days of storage at 1-3 C (Tables 1, 2).

Microbiological

Bacteriological evaluation of the lamb samples was carried out by sampling the surface with a sterile cellulose sponge ($5 \times 5 \times 1.3$ cm) wetted in sterile 0.1% peptone broth. Each cut was sampled in the same manner, first the lean surface (64.5 cm^2) with one side of the sponge and then the fat cover (64.5 cm^2) with the other side. The sponge then was rinsed thoroughly into 100 ml of sterile 0.1% peptone broth. Psychrotrophic plate counts were made by plating appropriate dilutions on plate count agar with plate incubation at 7 C for 10 days. Distribution of the microbial flora was determined by randomly picking 30-40 colonies from countable plates and placing them on trypticase soy agar slants. These slants were incubated at 25 C for 2-3 days. Details of the above procedure are presented in a previous paper (*7*). General diagnostic schemes and procedures to identify the isolates are those published by Vanderzant and Nickelson (*10*). Procedures for isolation and identification from beef of *Enterobacteriaceae,* in particular *Yersinia enterocolitica* and of coryneform bacteria are presented by Hanna et al. (*3, 4*).

RESULTS AND DISCUSSION

Psychrotrophic plate counts and percent distribution of the microbial flora of lamb loins and legs at 1-3 C for 0-21 days under various conditions of packaging are presented in Tables 1 and 2. Psychrotrophic counts of lamb loins and legs initially were low (2×10-1.1×10^3 per in.^{2}) and increased after 21 days of storage to 10^3-1.4×10^5 per in.^{2} for loins and to 2×10^3-1.6×10^5 for legs. Initially *Corynebacterium* sp. and/or *M. thermosphactum* predominated on loins and legs. Upon storage these species became less dominant and *Lactobacillus, Pseudomonas* or *Moraxella-Acinetobacter* species became more significant. *Enterobacteriaceae* were not significant at any time during the storage period, except that *Y. enterocolitica* was presented in 2 of 30 samples at a level of 10^3 per in.^{2} *E. liquefaciens* and *E. herbicola* were found occasionally. Except for one sampe, yeasts were not significant. *Micrococcus* and *Staphylococcus* sp. were not detected.

Of the psychrotrophic, pleomorphic, gram-positive, catalase-positive rods, 86 isolates were examined more closely for morphological and biochemical characteristics as described previously (*3*). *Corynebacterium* sp. usually

TABLE 1. *Psychrotrophic plate count and microbial flora of lamb loin stored in various film bags for 0-21 days at 1-3 C*

Day	Sample[a]	PPC/in.^{2} (6.45 cm^2)[b]	*Pseudomonas*	*Moraxella-Acinetobacter*	*Flavobacterium*	*Aeromonas*	*Enterobacteriaceae*: *Y. enterocolitica*	*E. liquefaciens*	*E. herbicola*	*Lactobacillus*	*Microbacterium thermosphactum*	*Corynebacterium*	*Arthrobacter*	Yeasts
0	1	1.0×10^2		10.0[d]							30.0	50.0-(III,II)[c]	10.0	
	2	1.1×10^3		1.8	0.9					22.7		74.6-(II,III)		
	3	2.2×10^2		12.5							12.5	56.2-(III,II)	9.4	9.4
	4	6.2×10^2		17.7							41.9	33.9-(II,III)	4.8	1.6
	5	6.0×10		16.7								83.3-(II,III)		
7	PVC	9.4×10^2	64.9	31.9								1.1-(III)		2.1
	PVC	1.7×10^3	3.0	83.6	1.8						3.0	8.5-(III)		
	B	5.5×10^2	5.5	25.5	18.2	32.7					1.8	14.5-(III)		1.8
	B	9.3×10^2		22.9					4.3	68.8	3.9			
	B	6.4×10^2		56.3	1.6					12.5	12.5		12.5	4.6
14	B	2.0×10^2	50.0	50.0										
	B	1.6×10^4	36.4			17.4		38.6		2.5	2.5		2.5	
21	A	5.7×10^4	87.7							8.7		1.8-(III)		1.8
	A	1.0×10^3	100											
	B	2.0×10^3								100				
	B	1.5×10^4					6.7	20.0	6.7	66.6				
	Mp-A	1.3×10^4	7.7	7.7						7.7	76.9			
	Mp-A	1.2×10^4		25.0		41.7				33.3				
	Mp-B	1.4×10^5				0.7				98.6	0.7			
	Mp-B	2.9×10^4		79.3						6.9	13.8			

[a]1-5 = before packaging; film characteristics: PVC = polyvinyl chloride (no vacuum), film A: MVTR = 0.66, OTR = 2.28, film B: MVTR = 0.18-0.20, OTR = 0.41-0.75, Mp = Master-Pac (precut loins and then reformed and packaged).
[b]PPC = Psychrotrophic plate count.
[c]II and III represent groups of *Corynebacterium* listed in order of decreasing significance (see text for description).
[d]Percent.

TABLE 2. *Psychrotrophic plate count and microbial flora of lamb legs stored in various film bags for 0-21 days at 1-3 C*

Day	Sample[a]	PPC/in.^{2} (6.45 cm^2)[b]	*Pseudomonas*	*Moraxella-Acinetobacter*	*Flavobacterium*	*Y. enterocolitica*	*Lactobacillus*	*Corynebacterium*	*Arthrobacter*	Yeasts
0	1	2.1×10^2		10.0[d]	45.0			40.0-(III)[c]		5.0
	2	1.6×10^2		18.8	6.2			62.6-(III,II)	12.4	
	3	2.0×10							50.0	50.0
	4	1.5×10^2						53.3-(III, II)	46.7	
	5	9.3×10^2						98.9-(III,II)	1.1	
14	A	3.1×10^3	100							
	B	1.0×10^2	100							
21	A	2.0×10^3		50.0			50.0			
	B	1.6×10^5	3.7			0.6	95.7			
	B	1.3×10^5	15.4				76.9	7.7-(III)		

[a]1-5 = before packaging; film characteristics: film A: MVTR = 0.66, OTR = 2.28, film B: MVTR = 0.18-0.20, OTR = 0.41-0.75.
[b]PPC = Psychrotrophic plate count.
[c]II and III represent groups of *Corynebacterium* listed in order of decreasing significance (see text for description).
[d]Percent.

were dominant, primarily before refrigerated storage began (Tables 1, 2). Of the 86 isolates, 19 were assigned to *M. thermosphactum* and 67 to *Corynebacterium*. Of the latter, 12 belonged to group I, 14 to group II, and 35 to group III. Six of the 67 isolates could not be assigned to any of the 3 groups. Among the *Corynebacterium* sp., Group III was more dominant than either Group II or I. Group III were buff-colored colonies which consisted of long, gram-positive, pleomorphic rods in palisades (*3*) with the following characteristics: OF glucose, no reaction; growth on TSA at 36 C, positive; nitrate reduction, negative; gelatin liquefaction, negative; starch hydrolysis, negative; no acid produced from glucose, lactose, and sucrose. Isolates of Group II were deep yellow-pigmented colonies, consisting of slender, gram-positive to gram-variable rods in palisades. Principal reactions were as follows: OF-glucose, oxidative; growth on TSA at 36 C, positive; nitrate reduction, negative; gelatin liquefaction, positive; starch hydrolysis positive; acid was produced from glucose and sucrose but not from lactose.

Isolates of group I which showed both rods and coccoid forms were nitrate positive, hydrolyzed starch, and liquefied gelatin and resembled more closely the pleomorphic species described as *Arthobacter* (*3*).

A comparison of the initial microbial flora of seven wholesale cuts of beef (*7*) with 10 lamb cuts (five loins, five legs, this study) shows that gram-negative rods (*Moraxella-Acinetobacter*) were less significant on lamb than on beef. Both species were obtained from the same commercial establishment. On beef these bacterial types were present in five of seven cuts and represented an average of 38% of the total population (range 0-92.3%). They were present on seven of 10 lamb samples with an average of 9% of the total population (range 0-18.8%). Initially, *Corynebacterium* sp. appeared more consistently significant on lamb than on beef. The increased significance of *Lactobacillus* sp. in the microbial flora of lamb after storage for 21 days at 1-3 C is similar to the pattern reported for vacuum-packaged beef by Seideman et at. (*7*).

Barlow and Kitchell (*1*) reported significant percentages of micrococci (31.3-33.3%) and *M. thermosphactum* (47.6-100%) in prepacked lamb chops stored overnight at 0 C or held for 3-6 days at 5 C. In the present study, neither micrococci nor staphylococci were detected and *Corynebacterium* sp. (Groups III, II) usually were more dominant than *M. thermosphactum*. In the present study bacteriological evaluation was made by surface swabs primarily of wholesale cuts whereas Barlow and Kitchell (*1*) reported on lamb chops. In addition, conditions of plate incubation were different. In the present study, samples were plated on plate count agar and incubation of plates was for 10 days at 7 C, whereas Barlow and Kitchell employed 5 days at 20 C. Differences in composition of plating medium and conditions of plate incubation may cause significant differences in distribution of the microbial flora. The number of lamb loins and legs was too small to draw conclusions about the effect of differences in packaging conditions on the psychrotrophic plate count or on the distribution of the microbial flora. In a previous study (*7*) wholesale beef cuts (knuckles, ribs) were stored for 0-35 days at 1-3 C in vacuum packages similar to Treatments A and B. Differences in psychrotrophic, mesophilic, or lactobacilli counts because of differences in type of packages were not statistically significant. *Lactobacillus* sp. were dominant after 21 days at 1-3 C both on knuckles and ribs.

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Heat-Resistant Psychrotrophic Bacteria Isolated from Pasteurized Milk[1]

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ABSTRACT

Psychrotrophic bacteria were isolated from 227 pasteurized milk samples which had a shelf life in excess of 20 days at 7.2 C. Of 700 cultures isolated, 135 were resistant to heating at 72 C for 16 sec and were able to re-establish growth at 7.2 C. Thirty-five cultures, representing 15 different types were subjected to detailed examination to determine their actions on refrigerated milk, growth temperatures, thermal resistance at various temperatures, and their identities. The spore-forming genus *Bacillus* occured most frequently. The non-sporing types were assigned to the genera *Arthrobacter, Microbacterium, Streptococcus,* and *Corynebacterium.*

More than a decade ago psychrotrophs in milk were called psychrophiles and were reported to be destroyed by proper pasteurization (*2,3,38,41,50,53*). Some organisms were observed to survive laboratory pasteurization and grow at 10 C, but failed to grow at 7.2 C (*4,28*). Olson et al. (*37*) and Davis (*13*) claimed that a critical difference in growth response of microorganisms existed between 7.2 C and 10 C and that use of growth at 10 C as a criterion for grouping would lead to the inclusion of "thermodurics" in the psychrophilic count. Boyd et al. (*9*) made the same observation in comparing 5 and 10 C incubation temperatures. Those researchers who did isolate bacteria that could survive pasteurization of milk and grow at 7.2 C or below found them to be rather uncommon. Mikolajcik and Burgwald (*33*) found only 8 of 150 thermoduric isolates could grow at 7.2 C and Boyd et al. (*9*) reported that only 17.9% of thermoduric isolates they obtained grew at 5 C. An earlier study by Stark and Scheib (*47*) revealed 4 of 486 isolates from butter were thermoduric and could grow at 5 C.

Over the past decade changes in production methods, farm bulk tank storage of milk, long distance hauling of milk, silo storage of raw milk at the processing plant, and the trend in the dairy industry toward higher pasteurization temperatures have caused a definite change in the microbial flora of milk. This change is evident in both raw and pasteurized milk and may be the result of disruption of the ecosystem of milk as it passed on from the cow to the consumer (*46*). The trend toward higher pasteurization temperatures and longer refrigerated storage of milk before comsumption have brought to attention selected flora that may be described as "thermoduric psychrotrophs". This paper presents a study of identification, pasteurization resistance, growth characteristics and possible commercial impact of several such organisms isolated from a large number of processed milk samples.

MATERIALS AND METHODS

Samples

During an 18-month period samples of pasteurized milk at various stages of processing were subjected to a shelf life test at 7.2 C. Two hundred and twenty seven samples showing no organoleptic signs of spoilage at 20 days were retained until off flavors developed, while samples spoiling before 20 days were discarded.

Cultrue isolation

Thermoduric psychrotrophs were isolated from the spoiled pasteurized milk samples by inoculating a loopful of each sample into a 16-mm screw capped test tube containing 10 ml of sterile litmus milk and subjecting the inoculated milk to laboratory pasteurization at 71.7 C for 16 sec. Tubes were cooled and held at 7.2 C for 14 days, streaked on Plate count age (Difco) and incubated at approximately 25 C until colonies developed. Two colonies of each pre-dominant type were picked into litmus milk. After incubation at approximately 25 C for 5 to 7 days, one tube of each type of reaction was saved for further study.

The isolated cultures were grouped according to staining characteristics, colony morphology, cell morphology and reaction in litmus milk. From each group, depending on the number of isolates involved, one to five cultures were selected for futher study.

Thermal resistance

All cultures were subjected to laboratory pasteurization at 71.7 C for 16 sec at least four times, and some as many as nine times, followed by incubation at 7.2 C for 14 days.

In separate trials, cultures from each group were heated at 71.7 C for 20,30,40, and 50 sec. In additional trials cultures were heated for 16 sec at 74.4, 77.2, and 80 C. A low temperature exposure of 60.7 C for 30 min and a high extreme of 93.3 C for 5 min were also used.

Trials involving temperatures of 71.7 C or higher were accomplished by placing the test tube support and test tubes, containing one loopful of culture in 5 ml of previously sterilized litmus milk, in a container with 12 mm of water, a depth not sufficient to touch the bottoms of the tubes. The container was covered and a thermometer inserted through the lid into a control tube. The water was heated and the generated steam served to heat the tubes. When the thermometer reached a

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temperature 5° below that desired, the support and tubes were transferred to a water bath at the desired temperature. The latent heat in the tubes and the thermometer lag were compensated for in this fashion. The come-up time of 30 sec for the 71.7-C treatment closely approximated that of the dairy plant pasteurizer. At the end of the exposure time tubes were immediately cooled in an ice water bath.

Determination of spoilage

One hundred-ml quantities of fresh, pasteurized, homogenized milk were dispensed into glass bottles and brought to 82.2 C with flowing steam before cooling to 7.2 C. After cooling, bottles were inoculated with 0.5% from litmus milk cultures. Two bottles were reserved as controls. All bottles were stored at 7.2 C and tasted after 18 h to detect any carry over flavors from initial inoculation. Each bottle was examined organoleptically at two day intervals until a defect developed and then on one additional day to confirm the defect.

Growth on agar at 7.2 C

To determine whether the cultures could form countable colonies under conditions used for psychrotrophic counts, each culture was streaked on a PCA plate and the plate incubated at 7.2 C for 14 days. Sterile litmus milk was added to the agar at the rate of 10% before pouring to compensate for the milk normally added in routine plating. The plates were observed at intervals to determine the rate and extent of colony development.

Identification of isolates

Media, procedures, and stains (*5,14,24,45*) used were those frequently associated with unknown identification. The compiled results of tests, stains, and reactions were compared with those traits presented in *Bergey's Manual* (*10*) for identification of cultures. Since this work was completed, a new edition of *Bergey's Manual* (*11*) has been published, but all the data reported here are based on criteria established by the 7th edition of *Bergey's Manual* (*10*).

RESULTS

From 227 different samples of pasteurized milk, which had been held at 7.2 C for various periods, more than 700 bacterial cultures were isolated from agar plates inoculated with the milk. These samples were all from milk pasteurized at 73.9 C for 16 sec in the Oklahoma State University dairy plant during a period of 18 months. From these isolates, 357 cultures selected as representing the dominant types and the remaining cultures, which appeared to be duplicates, were discarded.

A number of these 357 isolates were eliminated because they failed to survive HTST laboratory pasteurization at 71.7 C for 16 sec. Only those which were able to survive this exposure four or more times in separate trials were selected as being thermoduric. Additional cultures were discarded because they failed to grow readily at 7.2 C within 14 days. Finally 135 cultures were selected which survived the heat treatment and which grew at 7.2 C. These were considered to be thermoduric psychrotrophs.

The gram staining reaction, cell morphology, colony characteristics, and reaction in litmus milk were used to classify the cultures into 15 types. From one to five cultures of each type, for a total of 35 cultures, were selected for more detailed study. These 35 cultures were then submitted to various tests commonly used for identification of bacterial (*10*). On the basis of the results obtained from these tests the cultures were divided into spore producing and non-spore producing types. The spore producers were further subdivided into seven types, designated as Types I, II, III, IV, V, VI, and VII. The non-spore producers were subdivided into eight types, designated as Types A,B,C,D,E.F,G, and H.

Spore producing types

Eighty-two spore producing cultures were isolated from pasteurized milk which had been held at 7.2 C. These were classified into seven types. Number of cultures in each type, average shelf of the samples from which they were isolated, defects produced in milk by the cultures, and growth on agar are shown in Table 1.

Data show that type I with 58 cultures was most frequently encountered, followed by Type II with 11, Type VI with seven, and Type III with three. Only one culture of each of the remaining three types was isolated. It should be noted that self life of samples from which cultures had been isolated was long, ranging from 22.7 to 68 days.

Action in refrigerated milk. All spore producing cultures caused spoilage of milk at 7.2 C in from 2 to 12 days. Type I produced a distinct fruity flavor and odor

TABLE 1. *Growth of spore producing types in milk and on agar at 7.2 C*

Type	Number of isolates	Average shelf life of source samples (days)	Development of defects in milk		Development of visible colonies on agar (days)			
			Days	Defects	5	7	10	13
I	58	33.3	4-10	Fruity, sour	+	+	+++	++++
II	11	28.2	4-6	Sour, yeasty, gassy	+	+	++	+++
III	3	27.3	4-12	Sweet curdling, unclean, bitter	+++	++++	++++	++++
IV	1	68	2-4	Sweet curdling, Bitter	–	+	+	++
V	1	50	6	Sour	–	+	++	+++
VI	7	22.7	6	Sweet curdling, bitter	–	–	+	+
VII	1	31	12	Unclean, sour	+	+	+	+++

– = No visible growth.
+ = Visisble growth but no countable colonies.
++ = Barely countable colonies.
+++ = Good growth; distinct colonies.
++++ = Abundant growth.

followed by souring. Type II produced a yeasty, sour flavor defect, which was rather objectionable, accompanied by gas formation. Three types, III, IV, and VI, caused sweet curdling followed by bitterness. Type IV required only 2 to 4 days to sweet curdle the milk. Type V resulted in souring in 6 days while Type VII appeared rather inert, requiring 12 days to produce an unclean, slight sour flavor defect.

Although the time required for occurrence of spoilage was only 2 to 12 days, a relatively heavy inoculation (0.5%) was used. Since the numbers of these organisms in plant pasteurized milk would ordinarily be expected to be small, spoilage of plant pasteurized milk by these types would likely occur only in milk which was heavily contaminated with these organisms or which had a long shelf life due to the absence of the common non-thermoduric spoilage types.

Growth temperatures. The seven types of spore producers all grew at 7.2 C and at 37 C and Types IV, V, and VI also grew at 45 C.

Six of the seven types formed countable colonies on agar at 7.2 C in 13 days. Type VI showed growth, but the colonies were very small and would likely not be included in psychrotrophic counts. The psychrotrophic count according to the *Standard Methods* (2) requires 10 day incubation at 7 C. Type III grew well at 7.2 C, producing visible colonies in 5 days. Types I, II, and V formed countable colonies within 10 days. These results indicated that, with the exception of Type VI and possibly IV and VII, the psychrotrophic plate counts would include the spore producing thermoduric psychrotrophs.

Thermal resistance. Results obtained from heat resistance trials (Table 2) showed that the seven types of spore producers were definitely thermoduric. All survived exposures of 20,30,40, and 50 sec at 71.7 C and also exposures of 74.4, 77.2, and 80 C for 16 sec. These exposures cover the entire range of HTST pasteurization exposures normally used in dairy plants. However, Type VII and one of four cultures of Type II were destroyed by exposure to 60.7 C for 30 min. Type VII and 3 of 4 cultures of Type II failed to survive an exposure to 93.3 C for 5 min.

The general results of the heat treatment trials indicate that the cultures were able to survive plant pasteurization and that they could pose a problem if present in large numbers in raw milk.

Identification. The spore producing types were placed in genus *Bacillus* because they were all aerobic, gram positive or gram variable, spore forming rod-shaped organisms which were catalase positive. On the basis of the results of various morphological, cultural and biochemical tests (Tables 3 and 4), Types I through VI were tentatively assigned the species designations given in Table 5, which also presents the determined characteristics which varied from those given in Bergey's

TABLE 2. *Thermal resistance of spore producing types*

Type	Heated at 71.7 C for sec				Heated for 16 sec at			Heated at	
	20	30	40	50	74.4 C	77.2 C	80 C	60.7 C for 30 min	93.3 C for 5 min
I	+	+	+	+	+	+	+	+	+
II	+	+	+	+	+	+	+	±[a]	±[b]
III	+	+	+	+	+	+	+	+	+
IV	+	+	+	+	+	+	+	+	+
V	+	+	+	+	+	+	+	+	+
VI	+	+	+	+	+	+	+	+	+
VII	+	+	+	+	+	+	+	−	−

[a] 3 of 4 cultures survived.
[b] 3 of 4 cultures were destroyed.

TABLE 3. *Morphological and cultural characteristics of spore producing types*

Type	Gram stain	Cell morphology	Size (microns)	Motility	Colony characteristics	Reaction in litmus milk
I	±	Rods	0.5-0.7 × 4.7	+	Thin, flat, translucent, greyish white, granular	Reduced, acid, fruity
II	±	Rods	0.5-0.7 × 2-6	+	Thin, flat, translucent, greyish white, granular	Reduced, coagulated, gassy, yeasty, Sl. proteolysis
III	+	Rods	0.7-1.0 × 2-6	+	Large, moist, translucent, grey, turning brown, thin dull grey margin; agar turns brown	Upper region turns brown followed by complete alkaline proteolysis
IV	+	Rods	0.6-0.8 × 2-5	+	Large, moist, translucent, grey, turning black; agar turns black	Reduced, complete proteolysis, Sl. alkaline, membranous pellicle
V	+	Rods	0.6-0.7 × 2-4	+	Small, circular, entire, greyish white; more dense center	Reduced, acid, may or may not coagulate
VI	+	Rods	0.6-1.0 × 3-4	+	Small, circular, entire, dense, shiny white	Reduced, acid, coagulated, shrunken curd, proteolysis
VII	+	Rods	0.5-0.7 × 3-13	+	Small, circular, entire white, more dense center	Partial reduction, slight acid

TABLE 4. *Biochemical characteristics of spore producing types*

Characteristic	Types I	II	III	IV	V	VI	VII
Catalase	+	+	+	+	+	+	+
Urease	−	−	−	−	−	−	−
Acetylmethyl-carbinol	−	+	±	+	+	−	−
H_2S	+	+	+	+	+	+	−
Nitrates reduced	±	+	±	+	−	+	−
Indol	−	−	−	−	−	−	−
Starch	±	+	−	+	+	+	−
Gelatin	−	+	+	+	−	+	−
Dextrose	+	AG	+	+	+	+	−
Lactose	+	AG	−	−	+	−	−
Maltose	+	AG	±	−	+	+	−
Sucrose	+	AG	±	+	+	+	−
Arabinose	+	+	−	−	+	+	−
Glycerol	+	AG	−	+	−	+	−
Inulin	+	AG	−	−	+	+	−
Raffinose	+	AG	−	−	−	−	−
Salicin	+	AG	−	−	+	+	−
Xylose	+	AG	−	+	+	+	−

+ = Positive reaction
− = Negative reaction
± = Some cultures positive; some negative
AG = Acid plus gas formation.

TABLE 5. *Tentative identification of spore producing types*

Type	Tentative identification	Variations from Bergey's
I	*Bacillus macerans*	No gas in milk
II	*B. polymyxa*	
III	*B. laterosporus*	Brown water soluble pigment formed
IV	*B. subtilis* var. *aterrimus*	No acid from arabinose
V	*B. lentus*	Urease negative; slow acid in litmus milk
VI	*B. cereus*	Acetylmethylcarbinol negative; acid from arabinose and xylose
VII	*B. sphaericus*	Slow acid in litmus milk; starch hydrolyzed

Manual (*10*). The failure of Type VI to produce a positive Voges-Proskauer reaction caused concern in assigning to it the identity *Bacillus cereus*. However, it has been shown (*18*) that the standard medium (*43*) for the V-P reaction will frequently produce a negative reaction with *B. cereus* if the glucose and K_2HPO_4 are autoclaved together, as was done in this study.

The psychrotrophic *Bacillus* cultures isolated in this study caused spoilage of milk at 7.2 C within 12 days. The types of spoilage included sweet curdling, bitterness, yeastiness, fruity flavor, and sourness. Labots and Hup (*29,30*) and Labots et at. (*31*) reported that a large number of psychrotrophic *B. cereus* types, which cause sweet curdling and "bitty" defect in unhomogenized cream, are found in Holland in autumn, Shehata et al. (*43*) also reported that many of the psychrotrophic *Bacillus* strains they isolated produced fruity, rancid, sour, and unclean flavors in milk stored at 7.2 C. In conducting keeping quality tests on milk at 0.5 and 4.4 C, Boyd et al. (*8*) stated that the milk with the best keeping quality was eventually spoiled by spore-forming bacilli.

Non-spore producing types

Fifty three non-spore producing cultures were isolated from pasteurized milk which had been held at 7.2 C. These were classified into eight types. The number of cultures in each type, average shelf life of the samples from which they were isolated, defects produced in milk by the cultures, and growth on agar are shown in Table 6.

Data show that Type A with 17 cultures, Type D with 13 cultures, and Type B with 10 cultures were the most commonly encountered types, followed by Type H with four, Type E with two, Types C and F with three each, and Type G with one. The average shelf life of the samples from which cultures had been isolated was rather long, ranging from 22 to 44 days.

Action in refrigerated milk. Results of tests conducted in which milk was inoculated with each culture, stored at 7.2 C, and changes observed, given Table 6. Types A,B,D, and E spoiled milk in 8-14 days with an unclean, sour defect. Type F caused ropiness plus a slight cheesy flavor in 14 days. Type H also caused a cheesy flavor although no ropiness was present. Type G caused sweet curdling within 6 days. Type C was relatively inert, causing an unclean, sour, malty defect only after 14 to 24 days.

The fact that it required from 6 to 24 days for spoilage to occur after a heavy inoculation with the organisms would seem to indicate that the presence of these types would not ordinarily be important in milk spoilage unless

TABLE 6. *Growth of non-spore-producing types in milk and on agar at 7.2 C*

Type	Number of isolates	Average shelf life of source samples (days)	Development of defects in milk		Development of visible colonies on agar (days)			
			Days	Defects	5	7	10	13
A	17	33.4	12-14	Unclean, sour	−	+	+	+
B	10	27.9	8-12	Unclean, sour, feedy	+	+	+++	++++
C	3	31.0	14-24	Unclean, sour, malty	−	+	+	++
D	13	35.4	12-14	Unclean, sour, cheesy	+	++	+++	++++
E	2	35.5	14	Unclean, sour	−	+	+	+++
F	3	44.0	14	Ropy, sl. cheesy	+	+	++	++++
G	1	22.0	6	Sweet curdling, bitter	−	+	++	+++
H	4	33.3	12	Cheesy	+	+	+++	++++

− = No visible growth
+ = Visible growth but no countable colonies
++ = Barely countable colonies
+++ = Good growth, Distrinct colonies
++++ = Abundant growth

relatively large numbers were present in raw milk and competition from the non-thermoduric spoilage organisms was at a minimum.

Growth temperatures. All eight types of non-spore producers grew at 7.2 and 37 C, but only Types G and H, which were cocci, grew at 45 C.

Seven of the eight types developed countable colonies on agar at 7.2 C in 13 days. Four of these, Types B,D,F. and H, showed visible growth after 5 days and large colonies at 13 days. Only one, Type D, had colonies large enough to be countable in 7 days, while five, Types B, D, F, G, and H, were considered countable after 10 days. Type A showed visible growth at 7 days, but only very small colonies after 13 days. These results indicate that many of these non-spore forming bacteria would not be included in routine psychrotrophic counts.

Thermal resistance. The effects of various heat treatments on non-spore producing types is shown in Table 7. Types A,B,C, and F survived heat treatments covering the range commonly used for pasteurization of milk. They survived 71.7 C for 50 sec, 80 C for 16 sec, and 61.7 C for 30 min. One of the two cultures of Type F failed to survive 80 C for 16 sec. Types E and H were resistant to pasteurization in that they survived 71.7 C for 50 sec, 74.4 C for 16 sec, and 61.7 C for 30 min. Types D and G were the least heat resistant. They survived 71.7 C for 20 but not for 30 sec; they failed to survive 74.7 C for 16 sec, and three of four cultures failed to survive 61.7 C for 30 min.

Identification. On the basis of results of various morphological, cultural, and biochemical tests (Tables 8 and 9) the non-spore producing types were tentatively identified to species designations shown in Table 10. Variations from descriptions given in *Bergey's Manual* (*10*) are also shown in the same table.

Due to the marked diversity of cellular forms observed with Types E and F, the appearance of straight, curved, and swollen rods with some showing snapping division, and the presence of coccoid forms, they were considered to be members of either the genus *Corynebacterium* or the genus *Arthrobacter.*

However, because most of the *Corynebacterium* are pathogenic to either plants or animals and generally have high minimum growth temperatures, and because the genus *Arthrobacter* contains several heat resistant species and generally lower minimum growth temperatures, these two types were placed in the genus *Arthrobacter.*

DISCUSSION

All spore-forming types encountered in this study were identified as *Bacillus* species. This is in agreement

TABLE 7. *Thermal resistance of non-spore-producing types*

Type	Heated at 71.7 C for sec				Heated for 16 sec at			Heated at 61.7 C for 30 min
	20	30	40	50	74.4 C	77.2 C	80 C	
A	+	+	+	+	+	+	+	+
B	+	+	+	+	+	+	+	+
C	+	+	+	+	+	+	+	+
D	+	−	−	−	−	−	−	±[b]
E	+	+	+	+	+	−	−	+
F	+	+	+	+	+	+	±[a]	+
G	+	−	−	−	−	−	−	+
H	+	+	+	+	+	−	−	+

[a]One of two cultures did not survive.
[b]3 of 4 cultures were destroyed.

TABLE 8. *Morphological and cultural characteristics of non-spore-producing types*

Type	Gram stain	Cell morphology	Size (microns)	Motility	Colony characteristics	Reaction in litmus milk
A	+	Rods	0.6-1.5 × 0.2-0.3	−	Circular, entire, convex, shiny yellow, translucent to slight dense	Reduction, slight acid
B	+	Rods	0.2-0.3 × 0.6-1.0	−	Smooth type, circular convex translucent, yellow, rough, opaque granular depressed into agar	Reduced, yellow ring and sediment, sl. proteolysis
C	+	Rods	0.2-0.3 × 0.6-2.0 ×	−	Small circular, entire, convex, dull white	Reduced, acid, coagulation
D	+	Rods	0.3-0.8 × 2.0-5.0 (coccoid on old slant)	−	Large, moist, smooth, dense, pulvinate, tannish white, circular to irregular, entire, tends to run	Alkaline in 5-7 days, grey flocculent pellicle
E	+	Rods	0.6-0.8 × 2.0-3.0 (coccoid on old slant)	−	Small, dense, circular, white	Alkaline in 5-7 days, grey flocculent pellicle
F	+	Rods (capsules)	0.3-0.7 × 1.5-4.0	−	Circular, dense, moist, pulvinate, white, tends to run or drip	Thick, ropy, mucus-like, grey pellicle, reduced or alkaline
G	+	Cocci	0.6-1.0	−	Punctiform, translucent, grey	Reduced, coagulated, sl. acid slight proteolysis, shrunken curd
H	+	Cocci	0.6-0.7	−	Small, shiny, white, convex, circular dense	Reduced, acid, coagulation

TABLE 9. *Biochemical characteristics of non-spore-producing types*

	Type							
	A	B	C	D	E	F	G	H
Catalase	+	+	+	+	+	+	–	–
Urease	–	–	–	+	–	+	–	–
Acetylmethyl-carbinol	–	–	–	–	–	–	+	–
H_2S	±	+	±	+	+	+	+	±
Nitrates reduced	–	+	–	±	–	+	–	–
Indol	–	–	–	–	–	–	–	–
Starch	–	±	–	–	–	±	–	–
Gelatin	–	+	–	–	–	–	+	–
Dextrose	+	+	+	–	–	–	+	+
Lactose	+	+	+	–	–	–	+	+
Maltose	+	+	+	–	–	–	+	+
Sucrose	+	+	+	–	–	–	–	+
Arabinose	+	±	–	–	+	–	+	+
Glycerol	±	+	±	–	–	±	+	+
Inulin	±	–	–	–	+	–	+	+
Raffinose	+	±	–	–	+	–	–	+
Salicin	+	±	±	–	+	±	+	+
Xylose	+	+	+	–	+	–	+	+

+ = Positive reaction
– = No reaction
± = Some cultures were positive and some negative

with the observations of several other workers. In a recent paper, Stadhouders (*46*) listed some of the *Alcaligenes, Streptococcus, Micrococcus, Microbacterium, Bacillus,* and *Clostridium* species as the thermoresistant (thermoduric) bacteria commonly found in milk. Of these, he singled out psychrotrophic spore-forming *Bacillus circulans* types to be important in the spoilage of uncontaminated pasteurized milk during storage between 0 and 6 C. Between 6 and 20-C holding, he attributed deterioration of uncontaminated pasteurized milk to *B. cereus,* a spore-former, and *Alcaligenes tolerans* a non-sporing, gram negative rod. Martin (*32*) reported that *Bacillus* spp. accounted for about 95% of the spore-forming bacteria in the U.S. market milk; of these, 43% belonged to the species *Bacillus licheniformis,* 37% were *B. cereus* types, and the remaining 20% comprised other *Bacillus* spp. Among these, *B. cereus* strains are considered to be the predominant psychrotrophic types. The first study dealing with the presence of psychrotrophic *Bacillus* spp. from milk was conducted by Washam (*52*). This report was soon substantiated by other workers (*19,42,44*). Shehata et al. (*43*) used representative psychrotrophic strains (all isolated from milk) of *Bacillus subtilis, B. circulans, B. cereus, Bacillus coagulans, Bacillus megaterium, Bacillus laterosporus, Bacillus brevis, B. licheniformis,* and *Bacillus pumilus* to study their temperature characteristics, which has been suggested by other workers (*20,22,23*) to be important in distinguishing between psychrophiles and mesophiles. Several of the strains of *Bacillus* spp. used by Shehata et al. (*43*) exhibited μ (mu)-values comparable to *Pseudomonas* strain 21-3 C, a true psychrophile. The other exhibited μ-values half way between those for *Pseudomonas* 21-3 C and *Escherichia coli,* a know mesophile.

Some *Bacillus* isolates in our study failed to yield identical biochemical reactions as the designated species description (*10*) to which they were assigned. It is possible that the milk isolates were variants of the assigned species. Shehata and Collins (*42*) also reported that the *Bacillus* cultures they isolated were variants that had gained the ability to grow at low temperatures. Overcast and Atmaram (*39*) also expressed similar observations when they found that their psychrotrophic milk isolate *B. cereus,* was a specific temperature-variant of the type culture of *B. cereus* (ATCC strain 14579). Their psychrotrophic strain although identical in cultural, morphological, and biochemical properties to the ATCC strain varied in its ability to produce sweet curdling of milk held at 5 to 7 C within 8 to 10 days; the ATCC strain failed to produce this defect at 5 to 10 C even after 20 days of incubation.

Variations in biochemical reactions probably represent metabolic adaption to cope with low temperature growth. In a recent study, Chung and Cannon (*12*) examined the glucose dissimilation patterns of a psychrotrophic strain of *B. cereus* at different incubation temperatures. They found that vegetative cells of the psychrotrophic *B. cereus* strain metabolized glucose by the simultaneous operation of the Embden—Meyerhof—Parnas (EMP) pathway and the pentose phosphate (HMP) pathway at 32 C, but as the temperature decreased from 32 C, data collected at 20 and 7 C showed that glucose was metabolized with increased participation of HMP. They also noted that cells grown at 32 C failed to oxidize acetate through the tricarboxylic acid cycle (TCA); those grown at lower temperature actively oxidized acetate through the TCA cycle. At 7 C, very little acid was produced from glucose relative to 20 and 32 C. At 7 C, the culture failed to sporulate readily, but at 20 and 30 C good sporulation was observed.

Although all the spore-forming organisms isolated in this study were identified as *Bacillus* species this should not infer an absence of anaerobic spore-formers. The techniques were not designed for their isolation. Clostridia have been reported (*10*) to survive pasteurization, but they are generally present in milk in very small numbers. Bhadsavle et al. (*7*) isolated four from 48 raw milk samples and Martin (*32*) reported only 5% of the spore-formers in milk to be the genus *Clostridium.*

All the genera listed in Table 10, to which the non-sporing isolates were assigned, are recognized to be thermoduric (*16,34,40,49*). Among these, the enterococcus species are considered capable of growth at low temperatures (*25, 48*). The other types are not generally considered to by psychrotrophic. These bacteria also may represent variants that have acquired the ability to grow at low temperatures. With the exception of *S. faecalis* var. *liquefaciens*, these bacteria are not considered detrimental to flavor or quality of milk properly refrigerated under normal conditions of usage in the home.

The significance of thermoduric psychrotrophs, both

TABLE 9. *Biochemical characteristics of non-spore-producing types*

	Type							
	A	B	C	D	E	F	G	H
Catalase	+	+	+	+	+	+	–	–
Urease	–	–	–	+	–	+	–	–
Acetylmethyl-carbinol	–	–	–	–	–	–	+	–
H_2S	±	+	±	+	+	+	+	±
Nitrates reduced	–	+	–	±	–	+	–	–
Indol	–	–	–	–	–	–	–	–
Starch	–	±	–	–	–	±	–	–
Gelatin	–	+	–	–	–	–	+	–
Dextrose	+	+	+	–	–	–	+	+
Lactose	+	+	+	–	–	–	+	+
Maltose	+	+	+	–	–	–	+	+
Sucrose	+	+	+	–	–	–	–	+
Arabinose	+	±	–	–	+	–	+	+
Glycerol	±	+	±	–	–	±	+	+
Inulin	±	–	–	–	+	–	+	+
Raffinose	+	±	–	–	+	–	–	+
Salicin	+	±	±	–	+	±	+	+
Xylose	+	+	+	–	+	–	+	+

+ = Positive reaction
– = No reaction
± = Some cultures were positive and some negative

with the observations of several other workers. In a recent paper, Stadhouders (*46*) listed some of the *Alcaligenes, Streptococcus, Micrococcus, Microbacterium, Bacillus,* and *Clostridium* species as the thermoresistant (thermoduric) bacteria commonly found in milk. Of these, he singled out psychrotrophic spore-forming *Bacillus circulans* types to be important in the spoilage of uncontaminated pasteurized milk during storage between 0 and 6 C. Between 6 and 20-C holding, he attributed deterioration of uncontaminated pasteurized milk to *B. cereus,* a spore-former, and *Alcaligenes tolerans* a non-sporing, gram negative rod. Martin (*32*) reported that *Bacillus* spp. accounted for about 95% of the spore-forming bacteria in the U.S. market milk; of these, 43% belonged to the species *Bacillus licheniformis,* 37% were *B. cereus* types, and the remaining 20% comprised other *Bacillus* spp. Among these, *B. cereus* strains are considered to be the predominant psychrotrophic types. The first study dealing with the presence of psychrotrophic *Bacillus* spp. from milk was conducted by Washam (*52*). This report was soon substantiated by other workers (*19,42,44*). Shehata et al. (*43*) used representative psychrotrophic strains (all isolated from milk) of *Bacillus subtilis, B. circulans, B. cereus, Bacillus coagulans, Bacillus megaterium, Bacillus laterosporus, Bacillus brevis, B. licheniformis,* and *Bacillus pumilus* to study their temperature characteristics, which has been suggested by other workers (*20,22,23*) to be important in distinguishing between psychrophiles and mesophiles. Several of the strains of *Bacillus* spp. used by Shehata et al. (*43*) exhibited μ (mu)-values comparable to *Pseudomonas* strain 21-3 C, a true psychrophile. The other exhibited μ-values half way between those for *Pseudomonas* 21-3 C and *Escherichia coli,* a know mesophile.

Some *Bacillus* isolates in our study failed to yield identical biochemical reactions as the designated species description (*10*) to which they were assigned. It is possible that the milk isolates were variants of the assigned species. Shehata and Collins (*42*) also reported that the *Bacillus* cultures they isolated were variants that had gained the ability to grow at low temperatures. Overcast and Atmaram (*39*) also expressed similar observations when they found that their psychrotrophic milk isolate *B. cereus,* was a specific temperature-variant of the type culture of *B. cereus* (ATCC strain 14579). Their psychrotrophic strain although identical in cultural, morphological, and biochemical properties to the ATCC strain varied in its ability to produce sweet curdling of milk held at 5 to 7 C within 8 to 10 days; the ATCC strain failed to produce this defect at 5 to 10 C even after 20 days of incubation.

Variations in biochemical reactions probably represent metabolic adaption to cope with low temperature growth. In a recent study, Chung and Cannon (*12*) examined the glucose dissimilation patterns of a psychrotrophic strain of *B. cereus* at different incubation temperatures. They found that vegetative cells of the psychrotrophic *B. cereus* strain metabolized glucose by the simultaneous operation of the Embden—Meyerhof—Parnas (EMP) pathway and the pentose phosphate (HMP) pathway at 32 C, but as the temperature decreased from 32 C, data collected at 20 and 7 C showed that glucose was metabolized with increased participation of HMP. They also noted that cells grown at 32 C failed to oxidize acetate through the tricarboxylic acid cycle (TCA); those grown at lower temperature actively oxidized acetate through the TCA cycle. At 7 C, very little acid was produced from glucose relative to 20 and 32 C. At 7 C, the culture failed to sporulate readily, but at 20 and 30 C good sporulation was observed.

Although all the spore-forming organisms isolated in this study were identified as *Bacillus* species this should not infer an absence of anaerobic spore-formers. The techniques were not designed for their isolation. Clostridia have been reported (*10*) to survive pasteurization, but they are generally present in milk in very small numbers. Bhadsavle et al. (*7*) isolated four from 48 raw milk samples and Martin (*32*) reported only 5% of the spore-formers in milk to be the genus *Clostridium.*

All the genera listed in Table 10, to which the non-sporing isolates were assigned, are recognized to be thermoduric (*16,34,40,49*). Among these, the enterococcus species are considered capable of growth at low temperatures (*25, 48*). The other types are not generally considered to by psychrotrophic. These bacteria also may represent variants that have acquired the ability to grow at low temperatures. With the exception of *S. faecalis* var. *liquefaciens*, these bacteria are not considered detrimental to flavor or quality of milk properly refrigerated under normal conditions of usage in the home.

The significance of thermoduric psychrotrophs, both

spore forming and non-spore forming, depends on several factors, including culture identity, cell numbers, competive flora, expected shelf life, storage temperature, etc. The dairy industry has considered these organisms primarily from the standpoint of commercial pasteurized milk (*15,21,36,49*). This may well be their most important area of involvement, especially in light of the trend toward longer shelf life milk and increased usage of lower refrigeration temperatures. However, the report of Nashif and Nelson (*35*) that the lipase of *Pseudomonas fragi* required 20 min at 99 C for complete destruction should direct attention to the vast uncharted expanse of enzymes produced by psychrotrophs, both heat sensitive and heat resistant.

Gillies (*17*), in a study of thermoduric organisms isolated from Cheddar cheese, found little effect on cheese flavor. However, the fact that these organisms remained static in numbers over 6 months of storage might suggest they were not psychrotrophic. The weak proteolytic and lipolytic nature of these organisms may have gone unexpressed at the 7-C ripening temperature of the cheese. Jensen et al. (*25,26,27*) reported a significant contribution of thermoduric psychrotrophs to the final flavor and quality of Cheddar cheese. Perhaps other thermoduric psychrotrophs play a role, either desirable or undesirable, in the ripening of cheese. Little is known of their ecological realtionship to dairy starter cultures or what factors affect this relationship. Is there a relationship between the presence of proteolytic thermoduric psychrophiles or their proteases (*1,6*) and the failure in recent years of some vats of milk to set even at a titratable acidity of 0.70 to 0.80%?

What implications do the increased use of enzymes in the cheese industry have on the role of this minority group of organisms? The potential use of lactase (*51*) at low levels in the cheese vat to give a continuing effect in both the milk and the whey could greatly alter the number of thermoduric psychrotrophs in the ripening product or in the resultant lactose-free whey. These questions, along with a multitude of other questions, some not yet asked, will eventually require answers as technology advances. Time has already changed our concept of the heat resistant nature of some "psychrophiles" and the "psychrophilic" nature of some *Bacillus* spp.

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Bacteriological Survey and Refrigerated Storage Test of Vacuum-Packed Sliced Imported Canned Ham

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ABSTRACT

At the time of slicing and packaging, 179 of 180 retail-sized vacuum-packed sliced ham samples collected from 12 establishments had aerobic plate counts of 2,000 or fewer/g. Coliforms were isolated from only three of the samples, and all samples were negative for *Escherichia coli, Staphylococcus aureus,* and salmonellae. When stored at 3 C, bacterial growth in vacuum-packed, sliced ham proceeded at a rate similar to that of non-vacuum-packed, cross-sections cut from hams just before slicing. However, after 68 days of refrigerated storage, there was no bacterial growth in a non-vacuum-packed, cross-section cut from a ham removed from a can with strict aseptic techniques. These results suggest that the slight contamination upon opening canned hams by conventional methods may have as great an effect on the bacteriological stability of sliced ham as any additional slight contamination from the slicing-packaging operation.

A survey was conducted to determine the bacterial levels of imported canned cooked ham as sliced and vacuum-packed in retail sizes for shipment from establishments under Federal inspection in the United States. The 16 or 21 pound refrigerated hams in pullman-type cans were imported from Denmark, Holland, and Poland. In most establishments, the hams were removed from cans and stored under refrigeration from 2 to 4 h for partial drying of the surfaces before slicing. The internal temperature of hams just before slicing ranged from -3 to 4.5 C.

Hams were sliced mechanically by machines adjusted to discharge slices in stacks of the desired net weights. In nine establishments, the stacks were deposited in plastic film-lined molds of a conveyor leading to a machine that overlays a plastic film cover, then evacuates and seals each resulting plastic package of sliced ham. In three establishments, each stack of slices was slipped manually into a preformed plastic bag, and the filled bags were placed manually in a vacuum-sealing machine. Various retail-sized packs, ranging from 4 to 12 oz, were prepared.

The finished product was packed in cardboard cartons and placed under refrigeration. Generally, the product was shipped to outlets within 48 h.

Conditions of sanitation in the 12 establishments visited were very good. In every firm, the canned hams and finished vacuum-packs were held at 4.5 C or below and the slicing-packaging rooms were at 10 C or below. All equipment contacted by the product was treated with a sanitizing agent after being cleaned. When slicing more than 5 h, the equipment was either cleaned and sanitized during midshift breaks or swabs of various sites of the slicing line were taken at regular intervals to demonstrate an absence of bacterial build-up. In general, employees appeared well trained in washing and sanitizing hands routinely and at the proper intervals. Six of the firms used an iodophor at 25-50 ppm for a handsanitizing solution, five used a quaternary ammonium compound at 100-200 ppm, and one firm used a hypochlorite solution at 100 ppm Cl_2. In seven of the firms, employees used disposable gloves. As required by regulations, slicers and packing lines used for cooked products were not used for other purposes. The sole activity in seven of the 12 firms was slicing and vacuum-packaging imported canned hams. Four of the 12 firms sliced ham and other cooked products (bologna, luncheon meats, etc.) in a room isolated from all other activities. A fresh pork sausage stuffing line was in the slicing room of one firm. However, the stuffing line was along a wall of the room and at least 25 ft from the ham slicing lines. Also, the employees at the stuffing line were required to wear frocks of a different color than those worn by the other employees to readily isolate them from cooked products.

MATERIALS AND METHODS

Sampling

At intervals from 1971 to 1974, samples were collected from 12 establishments that slice and vacuum-package imported canned hams. Six of the firms are located in the Northeast, two in the Mid-Atlantic states, two on the West Coast, one in the South, and one in the Southwest. The 12 firms slice almost 500,000 lbs./week; a substantial portion of the production in this country.

Samples taken from each firm included cross-sections (approximately 2 inches thick) cut aseptically from at least two hams just before slicing, and 10 vacuum-packs of sliced ham from the same lot. Two groups of samples were collected from one of the plants. The vacuum-packs were collected at intervals so that each pack represented sliced portions of a different ham. Samples of 10 vacuum-packs prepared the day before the visit were collected from five of the firms.

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The samples were frozen promptly and shipped under dry ice to the laboratory. A total of 38 cross-sections from unsliced hams and 180 vacuum-packs of sliced ham were collected and analyzed. Generally, analysis was begun 3 to 4 weeks after collection.

The additional samples collected from one of the firms for a refrigerated storage test were placed under wet ice for delivery to the laboratory.

Laboratory methods

Methods used for aerobic plate counts (APC), coliforms, *Escherichia coli, Staphylococcus aureus,* and salmonellae have been described (*7*).

The 50-g portion from each cross-section of unsliced ham was removed from the center. Similarly, 50 g from each vacuum-pack were removed through the center of the stack to obtain portions of each slice of ham.

The ham samples for the refrigerated storage test were examined by the same methods and were also examined for APC's at 4 C (10 day incubation).

To identify the dominant genera appearing during refrigerated storage, representative colonies were picked from the countable APC plates and streaked on Plate Count Agar slants. The isolates were categorized by gram reaction, morphology, and catalase production. Gram positive, non-sporeforming, catalase negative rods were considered *Lactobacillus.* Gram positive, catalase negative cocci with a positive reaction in KF broth were considered enterococci.

RESULTS AND DISCUSSION

Product at time of slicing and packaging

All 38 samples of sections cut from unsliced hams had APC's below 1,000/g (nine or fewer colonies on the 1:100 dilutions). Of the 180 samples of vacuum-packed sliced ham, 174 had APC's below 1,000/g and five had APC's of 1,000 to 2,000/g (10 to 20 colonies on the 1:100 dilutions). All samples were negative for *E. coli* and *S. aureus* in 0.1-g portions, and all were salmonellae-negative in 25-g portions. Coliforms in 0.1-g portions were isolated from only three of the 180 vacuum-packs, and from none of the 38 sections of unsliced hams.

One of the vacuum-packs of sliced ham had an APC of 7×10^6/g and was normal organoleptically; the dominant organisms isolated from the sample were identified as enterococci. The other nine vacuum-packs in the same set had APC's below 1,000/g. It is likely that the canned ham represented by the vacuum-pack with the high APC had been slightly underprocessed, or was a leaker, or had not been stored under proper refrigeration at one or more points along the distribution chain from the cannery in Europe to the slicing line in the U.S.

The APC's of 16 sections from unsliced hams and 70 vacuum-packs of sliced ham were determined at both 35 C (2-day incubation) and 20 C (4-day incubation). The counts were the same at both incubation temperatures.

Refrigerated storage test

Bacterial growth during storage at 3 C was determined in vacuum-packs of sliced ham and in non-vacuum-packed sections from unsliced hams.

Nine cross-sections (about 1 inch thick) were cut aseptically from a ham at the head of a slicing-packaging line. Each was wrapped in a sterile plastic bag so that a different "unsliced" ham section was examined at each interval of refrigerated storage. Also, 45 vacuum-packs of sliced ham from the same lot were collected at the end of the same slicing-packaging line. The hams had been removed from the cans by employees of the firm only 0.5 h before slicing (normally, this firm removed hams from cans 2 to 3 h before slicing). A 50-g portion of a section and 50-g portions of each of five vacuum-packs were examined bacteriologically and organoleptically at intervals up to 68 days of storage at 3 C.

One cross-section was cut from a ham which had not been previously removed from the can by the employees. Strict aseptic techniques were followed in removing the ham from the can. The cross-section (about 1 inch thick) was wrapped in a sterile plastic bag for examination at the end of the refrigerated storage period.

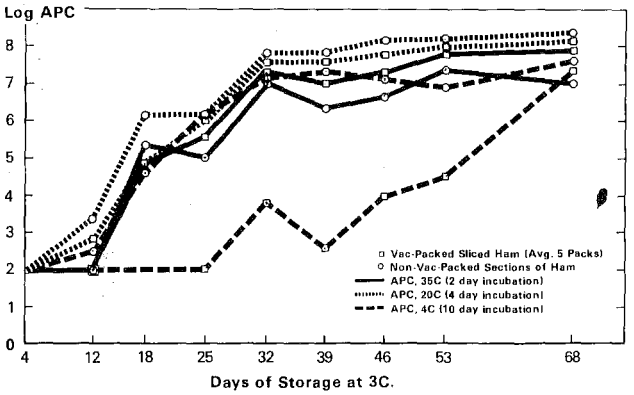

Figure 1. *Aerobic plate counts/g of vacuum-packed sliced ham and non-vacuum-packed sections of ham during storage at 3 C.*

The results (APC's) are presented in Fig. 1. The APC's determined at 35 C (2-day incubation) and at 20 C (4-day incubation) were similar. Kempton and Bobier (*3*), determining APC's at 30 C (3-day incubation), showed a similar rate of bacterial growth in refrigerated vacuum-packed cooked ham.

Figure 1 also shows that, except for APC's measured at 4 C, the rate of bacterial growth in the non-vacuum-packed sections cut from a ham opened by the firm was similar to that in the vacuum-packed sliced ham. However, after 68 days, no bacterial growth was detected at the three incubation temperatures in the non-vacuum-packed section of the ham removed from the can using strict aseptic techniques. These results suggest that the slight contamination upon opening cans by conventional methods may, in itself, have as great an effect on bacterial growth during refrigerated storage as the additional slight contamination from the slicing-packaging operation.

After the 68-day storage period, the vacuum-packed sliced ham and the "sterile" section of ham were acceptable organoleptically. However, the non-vacuum-packed sections cut from a ham removed from the can by conventional methods had developed an off-odor at the 32nd day of refrigerated storage. The factors suppressing proteolytic activity in vacuum-packed meats have been discussed by Allen and Foster (*1*).

Of the 45 vacuum-packs and 10 non-vacuum-packed sections examined during refrigerated storage, none were

positive for *E. coli, S. aureus,* or salmonellae. Coliforms in 0.1-g portions were recovered from only three of the vacuum-packs, and from none of the sections.

The dominant microorganisms isolated from the stored ham were enterococci and *Lactobacillus* sp., in that order. Other investigators (*1-6*) have presented more detailed studies on the bacteria appearing in refrigerated, cured meats.

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Total Aerobic and Coliform Counts in Beef-Soy and Chicken-Soy Patties During Refrigerated Storage

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ABSTRACT

Three commercial texturized soy proteins (TSP-A, TSP-B, TSP-C) and one commercial soy protein concentrate (SPC) were each added to raw ground beef or chicken at a 10% or 30% level to form patties which were stored at 4 C. During storage up to 8 days, aerobic plate counts of beef were significantly higher with than without the TSP's. Coliform counts were higher with the beef containing TSP-B and TSP-C. During storage up to 10 days chicken patties containing TSP-A or TSP-C exhibited higher aerobic plate counts than controls, whereas patties with TSP-B or SPC did not. No increases occurred in coliform counts. In beef-soy mixtures, the counts were higher for the 30% than for the 10% level but the reverse was true in chicken-soy mixtures. Coliforms were identified by the Analytab Products, Inc. System. In the beef control and beef-soy mixtures, *Serratia* was the predominant genus at 0 and 8 days of storage. In the chicken control, *Escherichia* was the predominant genus at 0 day of storage, but *Enterobacter* was predominant in the control and in chicken-soy mixtures after 10 days of storage.

Soy proteins are used increasingly in fabrication of synthetic meats and as extenders for ground meat. The effects of added soy on textural and physical properties of ground meat have been reported (*6, 9*), but little has been published describing the effect of soy proteins on storage quality of ground meats. Judge et al. (*7*) found no significant effect of soy protein additives on the aerobic plate count (32 C for 48 h) of ground beef patties stored at 4 C for 7 days. This research investigates the effect of soy supplements on the bacterial population in refrigerated ground beef and chicken.

MATERIALS AND METHODS

Boneless round beef purchased from a local supermarket was ground in a meat grinder (Sears 400.8260). Mean fat level of the beef was 25% and mean protein level was 21% (*1*). Raw, ground chicken was prepared from 65% broiler breast meat, 22% chicken skin, and 13% chicken fat mixed and ground in the meat grinder. Mean fat level was 23% and mean protein level was 16%. Beef or chicken samples were selected from the same source but at different times for the two trials. Total aerobic plate counts of beef differed initially by one log factor and of chicken by 0.2 log factor for the two trials.

Each of the soy protein additives was hydrated 1:3 with water and added to samples of ground beef and chicken to yield products containing 10% (wt/wt) or 30% (wt/wt) of the hydrated soy. Commercial soy proteins evaluated in this study were textured soy protein A (TSP-A), textured soy protein B (TSP-B), textured soy protein C (TSP-C), and a soy protein concentrate (SPC). TSP-B contained as additives ferrous sulfate, niacinamide, calcium pantothenate, vitamin B_6, riboflavin, vitamin B_1, and vitamin B_{12}.

Patties of beef or chicken with and without soy proteins and patties of hydrated soy proteins only were prepared by placing 25-g portions of the samples in 40 × 15 mm sterile petri dishes. Patties were stored at 4 C and sampled periodically by placing the 25-g portions into 225 ml of 0.1% peptone water and blending at low speed on an Oster blender (Model #497) for 1 min. Pour plates were prepared from serial dilutions. Violet red bile agar (VRBA, Difco) plates were poured, overlayed, and incubated at 35 C for 24 h for enumeration of coliforms. For total aerobic plate counts, Standard Methods Agar (SMA, Difco) was incubated at 21 C for 72 h.

For identification of coliforms from each of two trials 10 colonies were picked from VRBA plates of each sample representing storage of 0 day and 8 days for beef and 0 day and 10 days for chicken. Colonies were streaked for isolation on VRBA plates (35 C for 24 h). Isolated colonies were picked with sterile applicator sticks and suspended in 5 ml of sterile distilled water. API (Analytab Products Incorporated) strips containing 20 biochemical tests were inoculated with a capillary pipet, incubated at 35 C, and examined after 18 h. Those bacteria that were successfully isolated were identified to genus by use of the API decoder. When available data did not allow identification, the organism was inoculated into the API 50 biochemical strips and identified according to Edwards and Ewing (*4*).

Data were treated by analysis of variance. The multiple range test (*3*) was used to compare overall means of bacterial counts between patties with and without soy protein.

RESULTS AND DISCUSSION

Analysis of data from both trials showed that, except for SPC, during storage at 4 C aerobic plate counts increased faster in beef patties with than in those without soy proteins ($P < .05$). Data from one trial are shown in Fig. 1. The counts in those three beef-soy mixtures exceeded counts in beef alone by one log factor or less but were significant. Comparison of mean squares of mean log total plate counts shows that counts were greater at the 30% than at the 10% level of soy ($P < .05$). Counts increased over the storage period for all samples ($P < .01$).

Judge et al. (*7*) showed no significant difference in total aerobic bacterial counts (32 C for 48 h) between beef patties with or without 16 or 24% of soy flour or soy protein concentrate and stored at 4 C. Judge et al.,

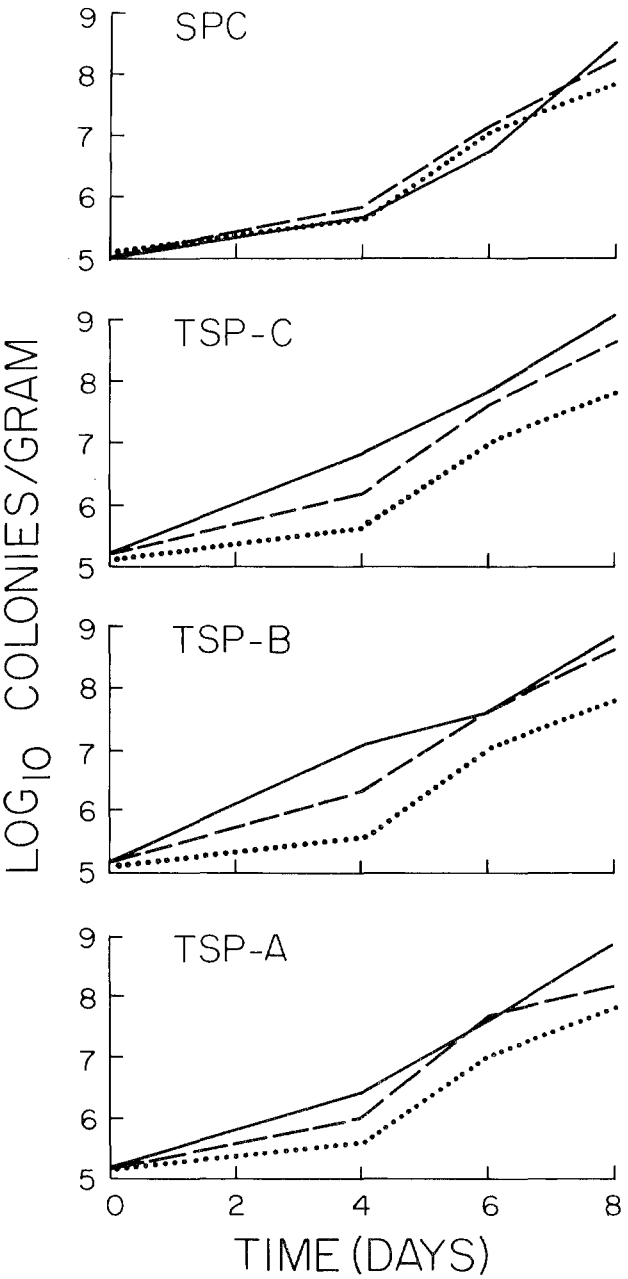

Figure 1. *Total aerobic plate count (21 C) in beef and beef-soy patties stored at 4 C. Beef (• • •), 10% soy protein (— —), 30% soy protein (——).*

however, reported counts after 7 days of storage only when the range was $5.0 \times 10^7 - 1.0 \times 10^8$ bacteria/gram. In our study, the differences in counts were greatest at days 4 and 6 when the range was $10^6 - 5.0 \times 10^7$/gram. With soy protein concentrate our results were similar to those of Judge et al.

Analysis of variance indicated that, in general, coliform counts increased more rapidly in beef patties with than without soy during storage at 4 C (Fig. 2). Statistically significant increases ($P < .05$) occurred in patties with TSP-B and TSP-C. Again increased counts generally exceeded counts for the control by less than one log except for patties with 30% TSP-B for which the difference was greater than one log. Coliform counts

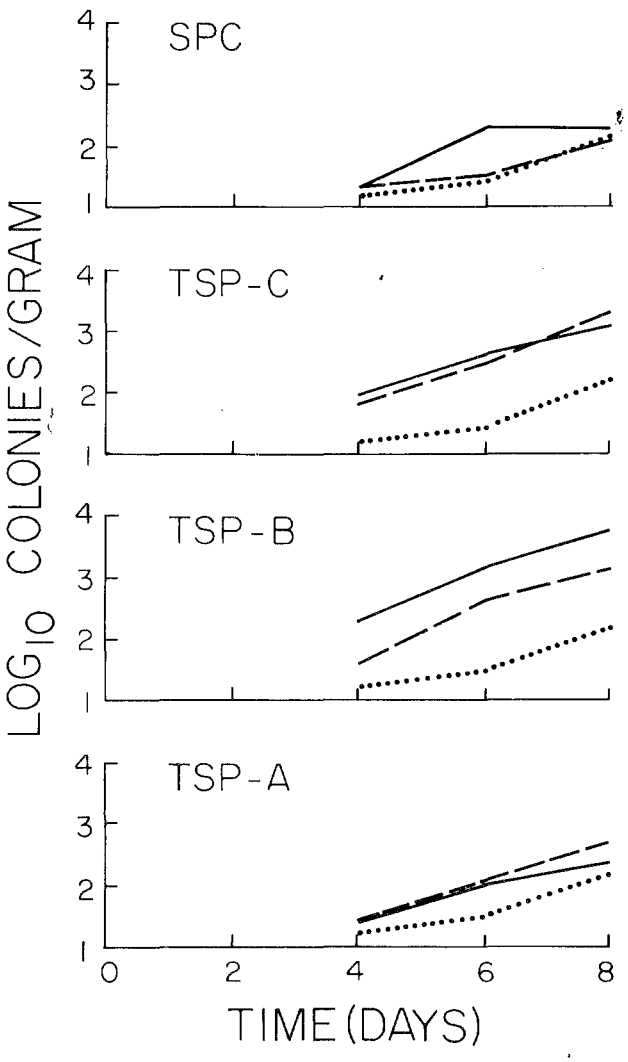

Figure 2. *Coliform counts (35 C) in beef and beef-soy patties stored at 4 C. Beef (• • •), 10% soy protein (— —), 30% soy protein (——).*

were not significantly higher at the 30% level than the 10% level of soy ($P < .05$).

Total aerobic plate counts in chicken with TSP-A and TSP-C exceeded those in controls but counts in chicken with TSP-B and SPC did not ($P < .05$, Fig. 3). Differences were less than those in the beef-soy patties. Counts were higher in chicken with 10% soy than with 30% soy ($P < .05$). Duncan's test showed that coliform counts in chicken with any of the four soys were no higher than chicken alone (Fig. 4). Coliform counts were no higher at the 10% than the 30% level of soy ($P < .05$).

The higher counts of total aerobes and, in some instances, coliforms in patties of beef and chicken with soy proteins prompted us to determine whether bacteria had been introduced into the patties with the hydrated soy and whether soy protein alone was a good growth medium. In some samples of meat with soy, counts on day 0 were higher than for meat alone (Fig. 1, 2, 3, and 4). However, initial total aerobic plate counts for hydrated TSP-A, TSP-B, TSP-C, and SPC (Fig. 5) were

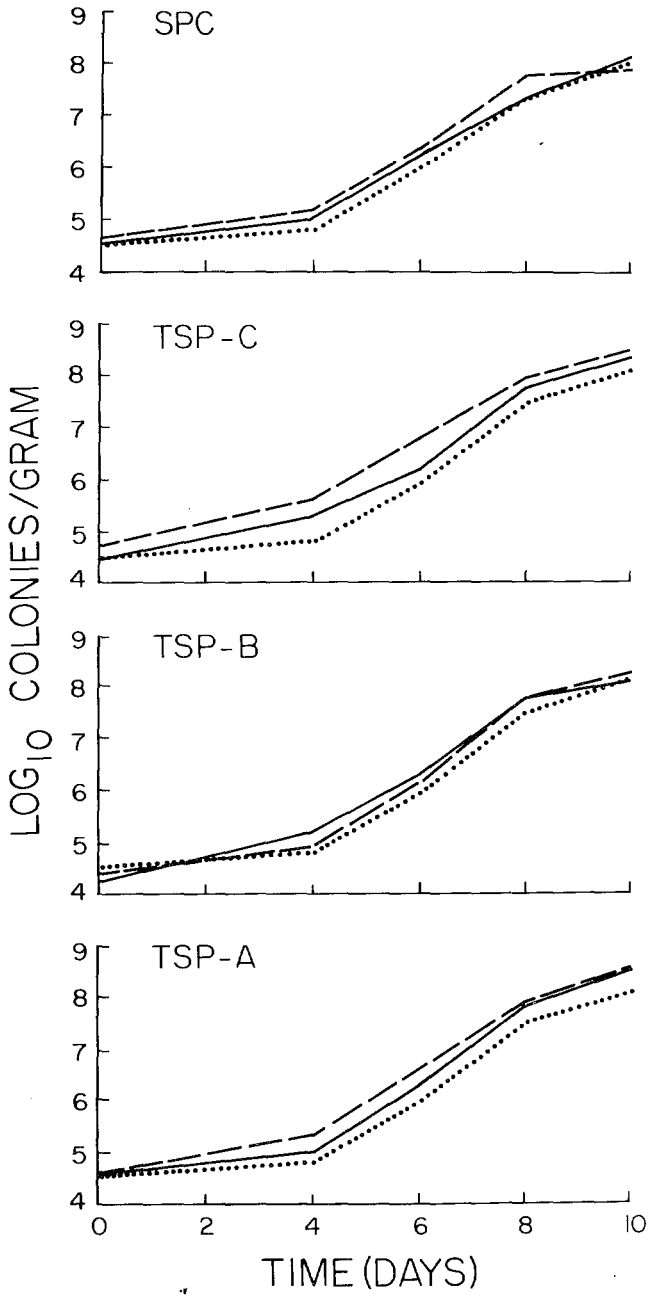

Figure 3. *Total aerobic plate count (21 C) in chicken and chicken-soy patties stored at 4 C. Chicken (• • •), 10% soy protein (— —), 30% soy protein (——).*

lower than for beef and chicken without soy. Also, the hydrated soy proteins alone supported growth at 4 C of total aerobes, but at a slower rate than in beef and chicken patties. Total aerobic counts of hydrated soy increased in 11 days one log for SPC, 2.5 logs for TSP-A, 3 logs for TSP-B and TSP-C. Increases for meat alone were 4 logs for beef in 8 days (Fig. 1) and chicken in 10 days (Fig. 3). No coliforms were isolated from any of the hydrated soy mixtures during the 11 days of storage.

Further investigations are needed to explain increased bacterial counts upon addition of soy proteins. Vitamin supplements in TSP-B may explain increased bacterial numbers in ground meat with TSP-B. Additions of

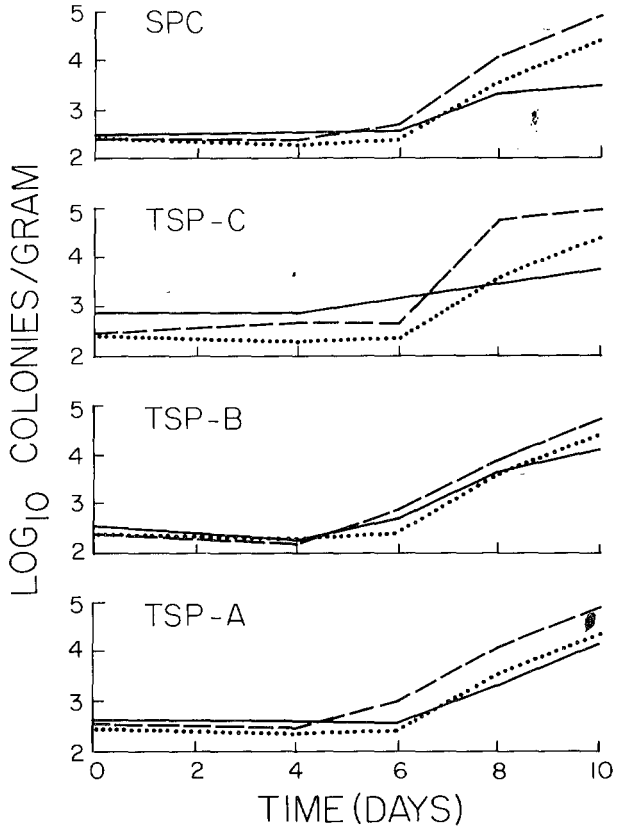

Figure 4. *Coliform counts (35 C) in chicken and chicken-soy patties stored at 4 C. chicken (• • •), 10% soy protein (— —), 30% soy protein (——).*

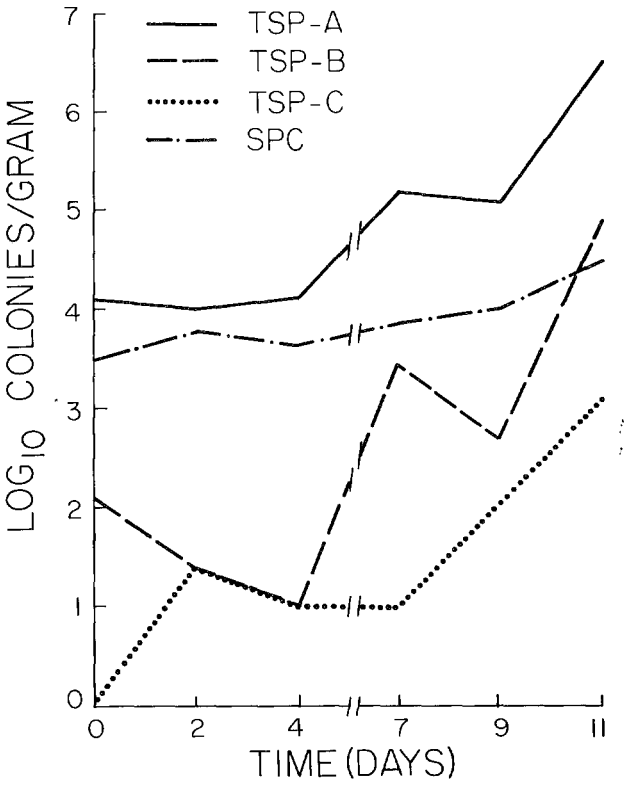

Figure 5. *Total aerobic plate counts (21 C) in hydrated soy proteins stored at 4 C.*

TSP-A and TSP-C also increase numbers of bacteria, and these soys contain no known supplements. In our study, addition of TSP at the 30% level did not appreciably alter the protein percentage. Ground beef with 30% TSP was 20 to 21% protein compared to 21% for the beef while ground chicken with 30% TSP was 16 to 17% protein as compared to 16% for chicken. Busta and Schroder (*2*) proposed that the stimulatory effect of some soy proteins on growth of *Clostridium perfringens* may be due to availability of certain growth factors not present in meat. The lower fat level (*6, 9*) and increased binding capacity (*7, 9*) of ground meats with added textured soy proteins may affect bacterial growth.

To determine whether the addition of soy proteins altered the predominant genera of coliforms upon storage at 4 C, we identified coliforms isolated from days 0 and 8 of storage for beef and from days 0 and 10 of storage for chicken (Table 1). For beef patties at days 0 and 8 *Serratia* was the predominant genus followed by *Enterobacter* and *Escherichia.* In beef patties with added soy proteins, *Serratia* was again the predominant genus and *Enterobacter* was less common.

TABLE 1. *Genera of coliforms isolated from ground beef and chicken supplemented with 30% soy protein and stored at 4 C*

Sample	Day of storage	Genus	Percentage[1] of total strains isolated
Beef	0	*Serratia*	40
		Enterobacter	40
		Escherichia	20
Beef	8	*Serratia*	67
		Enterobacter	27
		Escherichia	6
Beef and textured soy protein A	8	*Serratia*	88
		Enterobacter	6
		Escherichia	6
Beef and textured soy protein B	8	*Serratia*	84
		Enterobacter	11
		Unidentified	5
Beef and textured soy protein C	8	*Serratia*	78
		Enterobacter	22
Beef and soy protein concentrate	8	*Serratia*	69
		Enterobacter	25
		Unidentified	6
Chicken	0	*Escherichia*	47
		Enterobacter	26
		Serratia	11
		Klebsiella	11
		Citrobacter	5
Chicken	10	*Enterobacter*	55
		Serratia	28
		Escherichia	11
		Unidentified	6
Chicken and textured soy protein A	10	*Enterobacter*	52
		Serratia	48
Chicken and textured soy protein B	10	*Enterobacter*	59
		Serratia	41
Chicken and textured soy protein C	10	*Enterobacter*	56
		Serratia	38
		Shigella	6
Chicken and soy protein concentrate	10	*Enterobacter*	63
		Serratia	38

[1]Percentages based on 15-20 isolates from VRBA per sample.

For chicken patties *Escherichia* was the predominant genus at day 0. After 10 days of storage, for chicken with and without soy *Enterobacter* and *Serratia* predominated. Only one strain was isolated and identified by the API system as *Shigella.* No further tests (motility, serology) were used to confirm the identification. Atypical forms of *Escherichia coli* are sometimes incorrectly identified as *Shigella* (*5*). Since the API system is only 93% effective as compared to conventional systems (*8*) and, to our knowledge, *Shigella* is not known to occur in poultry, incorrect identification was likely.

Genera of predominant coliforms in beef and chicken differed on day 0 as compared to the last day of storage but did not differ between patties with and without soy. Although coliform counts were often higher in beef with than without soy proteins, the proportional distribution of coliform genera did not differ markedly. This finding suggested that any stimulating effect of soy proteins on growth of coliforms was non-selective among genera. The results of our study indicate that meat and poultry products containing soy additives might spoil sooner under extended refrigerated storage or under conditions of temperature abuse than similar products without such additives. We are investigating the effect of soy additives on the growth of food-poisoning bacteria in such products.

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Relation of Code Dates to Quality of Milk Sold in Retail Markets

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ABSTRACT

Milk collected at retail markets and in schools in Connecticut in 1970-71, 1974, and 1975 was examined for flavor quality, temperature at collection, code date (last day product is intended to be offered for sale), and age of sample (days from bottling). The relationships between quality and age of sample and the processor's code date were tested. Regression analysis of 1975 samples showed that, on the average, processors overestimated shelf life by about 2 days, but this overestimate varied from 0 to 7 days for individual dairies. Although the temperature of samples collected in 1974 and 1975 was lower than in the 1970-71 sampling, there was no diminution in the percentage of samples of unsatisfactory quality.

To allow consumers to assess the age of products at the time of purchase, a date is placed on the container that indicates either the date of packaging or the last date the product may be sold or offered for sale. The consumer, therefore, expects that a product purchased on any date up to the last date it may be sold or offered for sale is of acceptable quality. Also, if properly treated, it should remain acceptable beyond the last date of sale.

Since January, 1974, Connecticut law has required that the last date that pasteurized milk may be sold or offered for sale to consumers through retail stores (usually called the "code date") be clearly marked on the container. However, each dairy establishes its own code date and can change it without notifying any regulatory agency. Before 1974 some dairies coded their products but usually with a code not recognizable to the consumer.

We investigated the following: (*a*) What was the actual age of milk offered for retail sale and purchased by consumers? (*b*) Was the milk of acceptable quality when purchased? (*c*) What was the relationship between quality and age of sample at purchase? (*d*) Were the code dates used by processors realistic? Milk samples collected during 1970-71 and in 1974 and 1975 allowed us to investigate these questions. In addition the data provided a basis for determining the percentage of milk offered for sale after expiration of the code date, and which off-flavors predominated.

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METHODS

Milk samples were collected during 1970-71, 1974, and 1975 at retail markets in Connecticut by inspectors of the Dairy Division of the Connecticut State Department of Agriculture. No market was sampled more than once yearly. The temperature of the sample was determined at the time of collection and samples were refrigerated in an insulated container containing ice until delivered to the laboratory within 24 h, where flavor score and criticism were obtained as previously described (*2, 3, 4*). Scores ranged from 40 to 30; the higher score indicating a sample with no off-flavor. A flavor score of less than 36 was considered unsatisfactory. The number of samples examined were 450 in 1970-71, 528 in 1974, and 864 in 1975. Additionally, in 1975, 192 samples were collected from schools.

In this report "code date" is used to mean the date marked on the container designating the last day on which the milk may be sold or offered for sale. "Code period" is defined as the number of days between the date of bottling and the code date. "Age" means the number of days between bottling and collection, and "day 0" denotes that the sample was collected on the same day it was bottled. Individual dairies provided information on the length of their code period, and this permitted us to calculate the date of bottling, and thus the age of each sample.

RESULTS AND DISCUSSION

Age of retail milk samples

The age of milk collected in retail stores and schools, varied from 0 to 16 days (Table 1). The average age of samples in 1970-71 was 4.3 days and in 1974 and 1975, 4.7 days. Also shown in Table 1 is the distribution of age of samples offered for sale. However, age or distribution of age (Table 1), does not provide information on quality. Although the percentage of samples with unacceptable flavor scores increased from 10% in 1970-71 to 15% in 1974-1975 (Table 2), the difference is not statistically significant. The 450 samples of 1970-71 represented mostly large processors and were the only samples with code dates in a larger set of 1180 samples (*3*). Previously we reported that 16.7% of the 1180 samples collected in 1970-71 had unacceptable flavor scores (*3*). Thus, the

TABLE 1. *Age[1] of milk samples from retail outlets and schools*

Age (Days)	Year collected 1970-71 % of total	1974 % of total	1975 % of total	1975 schools % of total
0[2]	1.8	2.7	3.2	7.8
1	5.8	4.7	7.1	28.6
2	10.2	11.9	11.7	18.8
3	20.9	12.3	11.1	8.9
4	19.8	16.0	17.0	19.7
5	16.9	18.8	17.2	9.4
6	10.2	15.7	13.9	6.8
7	6.0	8.0	7.2	
8	3.1	3.4	4.9	
9	1.8	3.0	3.6	
10	1.8	1.7	1.4	
11	1.1	0.4	0.9	
12	0.4	0.6	0.3	
13	0.2	0.4	0.3	
14		0.4	0.1	
15		0		
16		0.2		
Total no. of samples	450	528	864	192
Avg. age[1] of milk collected	4.3	4.7	4.7	2.6

[1]Number of days from bottling to collection.
[2]Day 0 indicates sample collected the same day it was bottled.

TABLE 2. *Percentage of samples with unacceptable flavor scores (<36) and of samples beyond code date when collected*

Category	Year collected 1970-71	1974	1975	1975 Schools
Unacceptable flavor (score <36)	10.4	14.4	15.2	7.8
Past code date	5.1	1.5	0.5	0
Past code date and unacceptable flavor	1.6	1.1	0.5	0

percentage of samples with unacceptable flavor scores remained nearly the same during the 5 years.

The percentage of samples still offered for sale after expiration of the code date declined from 1970-1971 to 1974 and 1975 (Table 2). The decline may be explained in part by a change in the length of the code period for individual dairies. Of the five dairies examined in both 1970-71 and 1974-75, four increased their code period by an average of 2.5 days. Of the 18 sampled in 1974-75, two increased their code period by 4 days between 1974 and 1975. The average code period of dairies examined in 1970-71 was 8 days and in 1974-75 the average had increased to just over 10 days.

Refrigeration temperatures of samples

Poor quality milk cannot always be attributed to the processor. Retail stores must adequately protect perishable foods with good refrigeration. Although the average age of all samples from bottling to collection in 1975 was 4.7 days, the average age of samples with an unsatisfactory flavor score was 6.2 days, indicating a longer residence at the market. Temperatures at time of collection are shown in Table 3. Samples above 7.2 C (45 F) declined from 22.9% in 1970-71 to 5.4% in 1975. Further, the percentage of samples below 4.4 C (40 F) increased, and the percentage of samples at 10.0 C (50 F) or higher declined from about 4% in 1970-71 to 0.7% in 1975. Even though some samples had a temperature of >7.2 C when collected, they were still of acceptable quality (Table 3). Thus, despite an apparent improvement in milk refrigeration in retail stores, the percentage of samples with unacceptable flavor scores did not decline.

TABLE 3. *Temperature of retail milk samples at collection and relationship to flavor score*

Year collected	Temp. at collection	% of Samples	% of Total flavor score <36	% of Total flavor score ≥36
1970-71	<4.4C[1]	29.1	2.7	26.4
	4.4-7.2C	48.0	5.8	42.2
	>7.2C	22.9	2.0	20.9
	Total no. of samples	450	47	403
1974	<4.4C	36.0	3.8	32.0
	4.4-7.2C	56.4	8.1	45.8
	>7.2C	7.6	2.5	7.8
	Total no. of samples	528	76	452
1975	<4.4C	41.2	6.9	34.3
	4.4-7.2C	53.4	7.1	46.3
	>7.2C	5.4	1.2	4.3
	Total no. of samples	864	131	733

[1]4.4 and 7.2 C are 40 and 45 F respectively.

TABLE 4. *Flavor criticisms of retail and school milk samples*

Flavor criticism	1970-71 % of total	1970-71 % with flavor score <36[2]	1974 % of total	1974 % with flavor score <36	1975 % of total	1975 % with flavor score <36	1975 schools % of total	1975 schools % with flavor score <36
OK (no criticism)	0.2	0	0.2	0	2.0	0	2.6	0
Cooked	23.1	0	2.8	0	3.2	0	4.2	0
Cooked and feed	48.2	0	5.3	0	7.9	0	8.9	0
Feed	3.6	0	71.6	0	57.3	0	66.7	0
Oxidized	4.0	2.9	4.7	3.8	2.7	2.5	0.5	0.5
Lacks freshness (old)	17.3	5.6	10.4	8.0	15.9	9.4	10.9	6.3
Putrid	0.4	0.4	1.5	1.5	1.2	1.2	0.5	0.5
Rancid	0.7	0.2	0.4	0.4	0	0	0	0
Malty	0.9	0.9	0.2	0.2	0.2	0.2	0	0
Chemical	0.2	0	0.2	0	0.7	0.5	0	0
Unclean (barny)	0.4	0	0.4	0	1.0	0.2	0	0
Burnt (scorched)	0.2	0	1.7	0.2	6.5	0.2	3.6	0
Misc.[1]	0.7	0.5	0.6	0.3	1.6	1.0	2.1	0.5
Total no. of samples	450	47	528	76	864	131	192	15

[1]Misc. includes the criticisms bitter, sour, fruity, yeasty, curdled, onion, musty and salty.
[2]A flavor score of <36 is unsatisfactory.

Flavor criticism.

Flavor criticisms changed somewhat from 1970 to 1975 (Table 4). The number of samples criticized as being cooked declined dramatically, probably indicating better control of pasteurization procedures. The largest increase was in samples criticized as having a feed flavor. During 1974-75 there was a substantial increase in feed prices as well as a shortage of some feeds because of drought. Use of alternative feed could have caused the feed flavor. None of the samples criticized only for feed flavor had an unsatisfactory flavor score.

For samples with unsatisfactory flavor scores there was a rise in those designated as lacking freshness or old (Table 4). This may indicate increased activity of psychrotrophic bacteria and especially psychrotrophic pseudomonads (*1, 4*). Samples criticized as being putrid also increased slightly. The increase in these off-flavors could be related to the longer time the sample is held for sale (Table 1) even though temperatures were lower (Table 3), and again may indicate the presence of psychrotrophic organisms. Of interest is the large number of 1975 samples with a burnt or scorched flavor. Just over 75% of these were traced to one dairy. Faulty processing or too high a temperature used in sealing paper cartons is probably the cause.

School samples.

The 192 samples collected from schools in 1975 were somewhat different from those collected at retail markets. Only 7.8% of the school samples had an unacceptable flavor score compared to 15.2% for the samples from markets (Table 2). Essentially the same flavor criticisms were given to both groups of samples and the distribution of collection temperatures was also the same. No samples from schools were past the code date. The largest discrepancy between the two groups was in the average age of the sample (i.e., the days from bottling to collection), 2.6 days for schools and 4.7 days for stores (Table 1), and likely represents a difference in distribution. The average age of school samples below flavor score 36 was 2.9 days compared to 2.6 days for all school samples. Thus the samples were probably unsatisfactory before delivery. The same dairies supplying milk to both schools and retail markets had 13.8% of samples with an unsatisfactory flavor score in schools and 15.5% in retail markets.

Milk presently available to Connecticut consumers is older than in the past. In 1974-75 fewer samples were still offered for sale after the expiration of the code date, but the code period of samples increased an average of 2.5 days. Even though the milk was older when offered for sale, it was not of lower quality since the percentage of unacceptable samples did not increase during 1974-75. If anything, milk appears to be stored at a lower temperature, which shows an awareness of the necessity for proper refrigeration. We have also found that milk from stores chiefly selling dairy products is of a lower average age than from other stores, and that milk from smaller, local dairies is of a lower age than from larger dairies.

Relation of age to flavor score.

As the age of the milk increased, the flavor score decreased. In all 3 years there was a statistically significant negative correlation of flavor score with age among samples from retail outlets (Table 5). For samples collected in schools in 1975 there was no significant correlation of flavor score and age (Table 5), probably because of the shorter time between bottling and collection.

TABLE 5. *Correlation coefficient (r) of flavor score and age of milk samples collected at retail markets and schools*

Variables	Year collected			
	1970-71 (450 samples)	1974 (528 samples)	1975 (864 samples)	1975 Schools (192 samples)
Flavor score vs. age	-.301***[1]	-.261**	-.291***	-.128
Flavor score vs. days remaining in sale period	-.381***	.300**	.240***	.194**

[1]**Significant at 1% level; ***Significant at .01% level.

The consumer cannot test milk quality before purchase, nor does the consumer know how much time has elapsed since bottling. The only apparent indicator of milk quality is the time remaining before the code date expires. Is this remaining time a reliable indicator of quality? To test this we examined the correlation between flavor score and the time remaining to the code date. As shown in Table 5 flavor score decreased as the time remaining for sale decreased. Thus the longer the time remaining to the code date, the higher the flavor score of the samples.

If we accept a flavor score of less than 36 as indicating milk of unacceptable quality, the question becomes, when during the code period does milk deteriorate to this level? To determine this the regressions of flavor score on days remaining to the code date were calculated for the 3 years of samples from markets and the 1975 school samples (Fig. 1). The intersection of the regression line

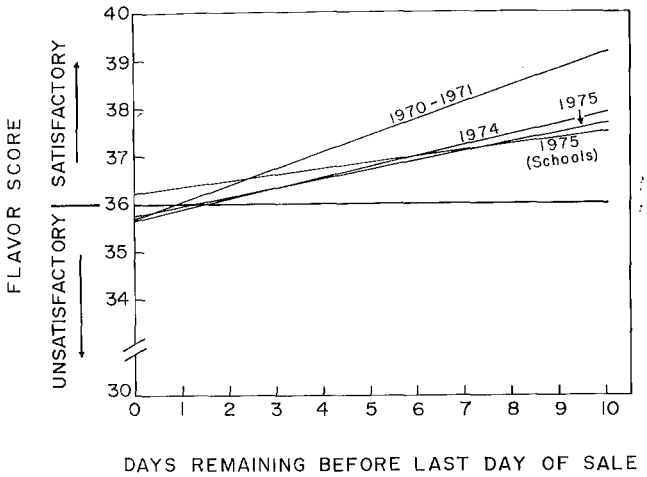

Figure 1. *Regression of flavor score on time (days) remaining before last day of sale for all milk samples collected at retail markets in 1970-71, 1974, 1975 and at schools in 1975. R^2 values for each regression are; 0.145 (P = <.001) for 1975, .090 (P = <.001) for 1974, .058 (P = <.001), and .038 (P = .008) for 1975 school samples.*

and the flavor score line of 36 indicates the number of days overestimated on the code period. On the average in 1974 and 1975, milk with about 2 days remaining to the code date had already reached a flavor score of 36. In 1970-71 the minimum time was about 1 day. Apparently, if the consumer is to obtain milk of acceptable quality at purchase, at least 2 days must remain before the expiration of the code date.

A similar analysis was made on samples from the individual dairies. As examples, the regressions for three major dairies with code periods from 7 to 14 days, are shown in Fig. 2. Overestimates of the code period ranged from less than 1 up to 7 days. Dairy A uses a code period of 12 days, yet the minimally acceptable score is reached in about 8 days. Hence, the code period is over estimated by 4 days. Dairy C with a code period of 14 days appears to be overestimating its code period by about 7 days. Dairy D with a code period of 7 days appears to be making an accurate appraisal since a flavor score of 36 is reached with 0.8 day remaining in the code period.

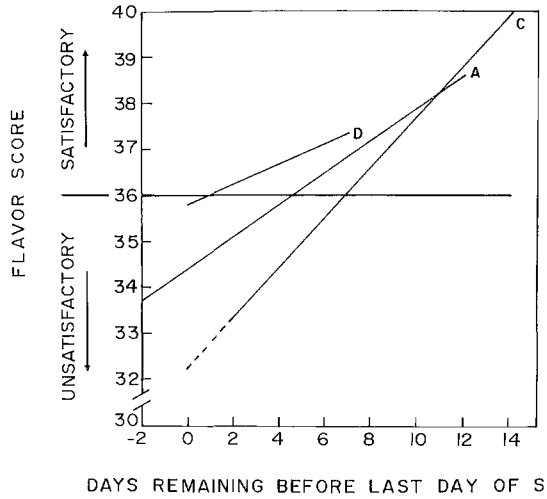

Figure 2. *Regression of flavor score on time [days] remaining before last day of sale for milk from three dairies (A, C, D) sampled in 1975. Solid line indicates actual range of values encountered. Dotted portion of line C is an extrapolation. R_2 values for each regression are; .174 ($P = <.001$) for A, .359 ($P = <.001$) for C, and .071 ($P = .006$) for D.*

Processors in Connecticut generally appear to estimate their code periods better for milk than they do for cottage cheese. We have previously found that processors of cottage cheese overestimated code periods about a third of the time (5). For milk, this value is about 15%.

From the consumer viewpoint, our data (Fig. 1) point out that on the average milk with less than 2 days remaining to the code date may not have an acceptable flavor, but there is great variability among dairies as well as within individual dairies (Fig. 2). This study should enable dairies to assess their quality control programs and if necessary make improvements for the benefit of consumers.

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The Health Profession's Attitudes toward Single-Use Food and Beverage Containers

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ABSTRACT

A national survey was conducted to determine the attitudes of the public health profession toward single-use food and beverage containers. Data summarized in this report were obtained from 2,760 of 3,000 questionnaires mailed to survey participants. Results of this study reveal that public health workers identify single-use food and beverage containers as beneficial in terms of reducing the potential for cross-infection and disease transmission among users. Respondents also recognize the importance of these products in eliminating the need for dishwashing facilities and in conserving energy. Accordingly, most public health workers are of the opinion that single-use products "contribute very much" to sanitation levels in food service facilities. While realizing the benefits derived from paper and plastic food service products, public health professionals recognize that they contribute to solid waste problems. However, most of the respondents agree that the public health benefits of paper and plastic food service products are greater than the possible disadvantages, such as the contribution of these products to the character and volume of urban solid waste.

Since passage of the Solid Waste Disposal Act of 1965 and its Amendment, the Resource Recovery Act of 1970, attention has been drawn to reduction, recovery, and recycling of material from the urban solid waste stream. This attention has resulted in a plethora of activity at the federal, state, and local levels of government.

Most notably, banning of non-returnable beverage and food containers has been forcefully advocated as a practical approach to source reduction. Several such bills have been introduced in the U.S. Congress but more activities have taken place at the state and local levels. Legislation banning single-service containers has been introduced in 50 state legislatures and numerous county and city legislative bodies since 1971.

Other proposals to stimulate source reduction and resource recovery have advocated guaranteed loans for recycling facilities, price support for scrap materials, ending depletion allowances for virgin material and eliminating biases against recycled materials in rail freight rates. The primary rationale for these proposals is that less solid waste will be generated by reducing the amount of material contained in products, increasing the lifetime of products, and substituting reusable for single-use products.

However, solid waste control, as a compenent of environmental quality, is also related to at least three major national goals: economic growth, public health and safety, and amenities and natural ecological values. Therefore, in developing public policies and programs for reduction of solid waste, it is necessary to evaluate them in terms of their contribution to all three goals. In addition their effects on other national goals such as employment and income distribution must be examined to reveal to policymakers the incidental consequences of proposed actions concerning strategies for solid waste management that appear to be quite unrelated.

Industry, labor unions, and conservation groups have respectively addressed in clear and concise terms the questions of economic growth and development, and amenities and natural ecological values, as these may be affected by a reduction in use of single service products such as beverage and food containers.

But with the exception of a resolution concerning "the U.S. Environmental Protection Agency's Solid Waste Management Guidelines for Beverage Containers," adopted by the International Association of Milk, Food and Environmental Sanitarians, Inc. at its 62nd Annual Meeting in 1975, the public health and safety goals have not been examined in any significant detail, and the input of professional public health workers has been virtually nil.

In many ways public health workers are in a unique position to evaluate the health impact, real or potential, of various policies and programs, such as solid waste reduction as one approach to improvements of the physical environment. They are continuously exposed to the end results of these developments and moreover must translate scientific, technical, and epidemiological evidence into programs and services for prevention of the occurrence and of the progression of disease and disability.

Therefore, constructive and balanced policies dealing with the entire spectrum of environmental quality issues must take into consideration the knowledge and attitudes of professional public health workers. In this direction a survey was undertaken to determine the

attitudes of professional public health workers toward the proposed elimination of single-use food and beverage containers. Questionnaires were mailed to 3,000 workers in organized community health programs in the United States. A summary of the results of the survey are presented in this report.

METHODS

Data used in this study were obtained from a national survey of public and environmental health professionals who are responsible for administration, management, and implementation of food protection programs in the United States. Also participating in this study were public health academicians and public health officials in federal agencies such as the Food and Drug Administration and the Center for Disease Control. Upon completion of a pretest the questionnaire was mailed to 3,000 public health professionals who were randomly chosen from the directory of state food and drug officials (1976) and the current (1975-76) membership of public and environmental health organizations. These organizations included the National Environmental Health Association; the Association of Food and Drug Officials of the United States; the Conference of Local Environmental Health Administrators; the Association of State and Territorial Health Officers; the International Association of Milk, Food, and Environmental Sanitarians, Inc.; and the American Public Health Association (Section on the Environment). Cross checks were made to ensure that persons who were members of more than one organization were not selected twice for inclusion in the survey. The findings in this study are based on the return of 2,760 questionnaires, providing a response rate of 92%.

RESULTS

Positions and organizations of respondents

Information on the positions held by those public health professionals who were surveyed is shown in Table 1. Forty-five percent (1,245) of the respondents are public health administrators who direct and manage 46 state and 1,218 local public and environmental health agencies. These officials also include middle-level managers such as supervisors of food protection programs at the local level. One percent (18) of those surveyed are health officials of professional public and environmental health organizations. In addition, 41% (1,145) of those responding are sanitarians who are responsible for implementing food protection and public health education programs. While 9% (240) of those responding are environmental health scientists, 2% (67 respondents) are academicians who are responsible for training public and environmental health practitioners and an additional 2% (45 respondents) are public health officials working in federal agencies.

TABLE 1. *Positions and organizations of respondents*

Position and organization	Number of respondents	Percent[a] of respondents
Public/Environmental Health Administrators (State and Local)	1,245	45
Officials of Professional Public/Environmental Health Organizations	18	1
Sanitarians (Field Level-State and Local Agencies)	1,145	41
Public/Environmental Health Academicians	67	2
Environmental Health Scientists (State and Local)	240	9
Public Health Officials (in Federal Agencies)	45	2
TOTAL	2,760	100

[a]Percentages are rounded to the nearest integer.

A further characteristic of the public health workers participating in this study is that of experience. Fifty-seven percent (1,582) of the respondents have 11 or more years of experience in the public health field. Twenty-six percent (715 resondents) have been public health professionals between 6 and 10 years and the remainder, 17% (463) of those responding, have between 3 and 5 years of experience.

TABLE 2. *Public health benefits derived from paper and plastic single-use products*

Benefit[a]	Number of respondents	Percent[b] of respondents
Reduce possibility of cross-infection	421	15
If properly stored and handled, reduce transmission of diseases	866	31
Practical and economical means for food service facilities to operate when reusable products are impractical	208	8
Eliminate need for dishwashing facilities	426	15
Provide consistently high level of food sanitation	385	14
Reduce human involvement required for cleaning and sanitizing	243	9
Convenience	128	5
Conserve energy	47	2
No real public health benefit	36	1
TOTAL	2,760	100

[a]Benefits were listed by respondents.
[b]Percentages are rounded to the nearest integer.

Public health benefits of single-use products

Table 2 summarizes responses to the open-ended question on the "public health benefits derived from paper and plastic single-use products." Forty-six percent of the public health workers agree that these products reduce the possibility of cross-infection and when properly stored and handled, they reduce transmission of diseases. In addition, 24% of those responding state that single-use products eliminate the need for dishwashing facilities and reduce human involvement that is required for cleaning and sanitizing multi-use products. Complementing these views is the judgment of 14% of the public health experts that single-service products provide a consistently high level of food sanitation in food service facilities. However, other veiwpoints relate to non-health benefits. For example, 8% of the respondents feel that "disposables are a practical and economical means for facilities to operate when reusable products are impractical," while 7% list such reasons as convenience and energy conservation. The remaining 1% of those responding believe that there is no real public health benefit derived from paper and plastic food service products.

Recognizing the possibility of the existence of disadvantages associated with single-use products, respondents were asked to enumerate these possibilities. The responses in Table 3 reveal that 54% of the public health workers are of the opinion that paper and plastic disposable products contribute to solid waste disposal problems, adding to the volume and bulk of solid waste,

TABLE 3. *Disadvantages derived from paper andplastic single-use products*

Disadvantage[a]	Number of respondents	Percent[b] of respondents
Contribute to solid waste disposal problems	782	28
Add to volume and bulk of solid waste	485	18
Increase litter	474	17
Contribute to disposal problems, especially with plastics that are non-biodegradable	229	8
Increase need for additional storage space	237	9
Poor quality of some disposable products	98	4
Limited acceptance in all restaurants by consuming public	396	14
Increasing cost of disposable products	59	2
TOTAL	2,760	100

[a]Disadvantages were listed by respondents.
[b]Percentages are rounded to the nearest integer.

especially with plastics that are non-biodegradable. On the other hand 40% of the responses include such disadvantages as the increase in litter, need for additional storage space in facilities, and limited acceptance of paper andplastic single-use products in all restaurants by the consuming public.

TABLE 4. *Comparison between benefits and disadvantages of single-use products*

Comparison	Number of respondents	Percent[a] of respondents
Benefits are greater than disadvantages	2,208	80
Disadvantages are greater than benefits	173	6
Benefits and disadvantages are fairly equal	297	11
Undecided about comparison	82	3
TOTAL	2,760	100

[a]Percentages are rounded to the nearest integer.

The relationship between the public health benefits and the disadvantages derived from single-use products is shown in Table 4. As perceived by 80% of the respondents, benefits are greater than disadvantages. However, 6% of the public health workers view the disadvantages as having a greater impact on environmental quality than the health benefits. While 11% of those responding suggest that the benefits. While 11% of those responding suggest that the benefits and disadvantages are fairly equal, 3% support an indecisive position in comparing benefits and disadvantages derived from single-use products.

Single-use products and sanitation levels

Table 5 reviews the position of the public health profession on the contribution of paper and plastic cups and plates to sanitation levels in food service facilities. Accordingly, 74% of those responding concur that these products "contribute very much" to sanitation levels, while 16% replied that they "contribute somewhat." In addition 9% agree that the contribution is slight. Only 1% of those responding feel that paper and plastic food service products do not contribute to sanitation levels in food service operations.

To further analyze these opinions, cross-tabulations were made between the attitude toward the contribution of paper and plastic food service products on sanitation levels and the position held by the respondents. As seen in the table, most respondents in each position believe that the relationship between single-use products and sanitation levels in food establishments is significant. For example, an overwhelming majority (70%) of public and environmental health administrators agree that these products "contribute very much" to sanitation levels.

When queried about their opinion regarding the impact on sanitation levels if single-use products were eliminated, respondents remained consistent. Seventy-four percent were adamant that sanitation levels in food establishments would definitely decrease. It is interesting to note, however, that 9% of the public health workers qualified their opinions by inserting that "sanitation levels would definitely decrease in facilities where reusable products are not practical."

Complementing these viewpoints is the issue of the impact on sanitation levels in food establishments if single-use products were required. The responses reveal that 74% of the public health professionals agree that "sanitation levels definitely would improve."

On the question of the recommended use of single-service products in public facilities, data collected indicate that public health workers feel it necessary to increase the level of use of these products in such facilities as fast food restaurants, hotels and motels, other restaurants, hospitals, and schools. In addition, many respondents added the need for an increase in use

TABLE 5. *Contribution of paper and plastic cups and plates to sanitation levels in foodservice facilities*

	Contribute very much		Contribute somewhat		Contribute slightly		Do not contribute at all		Total	
Public health professional	(No.)[a]	(%)[b]	(No.)	(%)	(No.)	(%)	(No.)	(%)	(No.)	(%)
Public/Environmental Health Administrators	876	70	207	17	153	12	9	1	1,245	45
Officials of Professional Public/ Environmental Health Organizations	10	56	6	33	—	—	2	11	18	1
Sanitatians	978	85	129	11	30	3	8	1	1,145	41
Public/Environmental Health Academicians	38	57	14	21	9	13	6	9	67	2
Environmental Health Scientists	112	47	69	29	50	21	9	4	240	9
Public Health Officials in Federal Agencies	29	64	7	16	7	16	2	4	45	2
TOTAL	2,043	74	432	16	249	9	36	1	2,760	100

[a]Number of respondents.
[b]Percent of respondents; percentages rounded to nearest integer.

of single-service products in jails and prisons (938 responses) as well as in day care centers (1,314 responses).

There is one advantage of using paper and plastic food service products which many public health officials tend to overlook. This advantage is the safety factor associated with these products. Fifty-one percent of the respondents view this safety aspect (non-breakage) as "very important," while 27% perceive it as "somewhat important." Although 14% responded to a slight importance, only 8% do not attribute any importance to the safety derived from the use of paper and plastic cups and plates.

Contribution of disposables to solid waste

As a cross-check of the attitude of the public health profession toward use of paper and plastic food service products, respondents were asked to summarize the impact of single-use containers on their community's solid waste problems. Thirty-two percent of the respondents stated that there is "somewhat" of a contribution to the solid waste problem. While 17% said that they contribute very much to their communities' solid waste problems, 29% qualified this viewpoint by stating that the contribution of disposable containers to solid waste problems is only in terms of volume. Additionally, 14% feel that the contribution is slight and 8% feel that these products do not contribute to solid waste problems.

Attitudes toward restricting disposable food and beverage containers

In summarizing the attitude of the public health profession toward restricting the use of disposable products, over half (52%) of those responding "disagree somewhat" that restrictive measures against use of disposables are effective methods for reducing litter. Twenty-seven percent of those responding "disagree completely" with this position. However, 15% of the public health workers "agree somewhat" with this policy and 6% "agree completely" with this judgment.

When asked to develop suggestions for dealing with the problem of litter, respondents varied in their opinions. Forty-two percent of the public health professionals suggested development of educational programs for the public. An overwhelming majority (90%) of these respondents injected that the problem of litter stems from public attitudes and behavior. Other suggestions for treating the litter problem include stricter enforcement of litter laws (25% of the responses), increasing the number and convenience of litter containers (14% of the responses), prohibiting non-returnable bottles and cans (8% of the responses), hiring more trash collectors (3% of the responses), developing a special tax for non-returnables (4% of the responses) and increasing the severity of fines for littering (4% of the responses).

DISCUSSION

Given the desirability of reducing the volume and changing the character of the urban solid waste stream, a central question arises-is elimination of single-use food and beverage containers, specifically plastic and paper cups and plates, which are used in food service operations, a desirable approach from a public health and safety viewpoint? The data presented in this report indicate that it is not.

Public health workers identified single-use food and beverage containers as beneficial in terms of reducing the potential for cross-infection and disease transmission among users. The theoretical basis for these views is the well-recognized sequence of factors which are essential to development of the infectious disease process (*1*). These are: (*a*) causative or etiological agents (viruses and bacteria), (*b*) a reservoir or source of the causative agent, (*c*) a mode of escape from the reservoir, (*d*) a mode of transmission from the reservoir to the potential new host, (*e*) a mode of entry to the new host, and (*f*) a susceptible host.

Elimination of one of the links, or where possible attacking simultaneously all six links, is the guiding principle for application, on any appreciable scale, of disease prevention measures to the animate or inanimate environments of man. This precept has been described as the principle of multiple barriers, the foundation on which rests much of the past and current successes in controlling waterborne and foodborne diseases. It recognizes as axiomatic the fact that all human efforts, no matter how well conceived or consciously applied, are imperfect and fallible (*5*). To be sure, dishwashing in food service operations has its "good and bad days" and is subject to occasional lapses, primarily due to the human element which controls both the dishwashing procedures and equipment.

Walker, in a 4-year study of washing and sanitizing procedures of reusable beverage glasses in 66 American hotels, found that over 90% of the "washed and sanitized" containers were "unacceptable" from the standpoint of both bacteriological and esthetic standards; 50% contained pathogenic microorganisms. Substandard dishwashing practices were identified as a major factor in the bacterial content of the 500 glasses analyzed (*7*).

In this setting use of single-service food and beverage containers would essentially remove one link, the vehicle, from the chain of disease transmission and reduce the need for dishwashing, and its impingement on the community's water resources, which was identified as another public health concern by the respondents.

On the water conservation issue, Salvato (*6*) has calculated the hot rinse water requirements for food service establishments of varyious sizes. For example, a restaurant which serves a maximum of 450 meals, and washes dishes over a period of 1½ h requires 181 gal. of water per hour. If no storage of hot water is provided, the required gas heater is 376,000 BTU/h, a factor to be considered in assessing energy consumption. This spent water contains inorganic chemicals-the basic ingredients

of dishwashing compounds-which are ultimately discharged into natural watercourses, and which may become concentrated enough to interfere with water uses. They may also contain end products which are toxic to man and animals.

Litter on streets and highways, a continuing issue in urban communities, was recognized by public health workers as a problem that is exacerbated by single-use products. But the respondents indicated that people litter not only single-use food service items but all kinds of single and multi-use materials and products.

A three-year research study of littering in Charlotte, N.C.; Tampa, Florida; and Macon, Georgia listed five sources of litter: pedestrians and motorists, improper storage of household refuse, mismanagement of commercial trash and garbage, loading and unloading docks, uncovered trucks, and construction and demolition sites (*4*).

Littering is a "behavior problem" influenced primarily by the knowledge and attitudes of those who indiscriminately deposit their used products on open space, on streets and highways. In many urban neighborhoods, which are evolving through the packing and thinning stages of urban growth, buildings designed in earlier years have been subdivided into numerous crowded living units with little provision for solid waste storage. Receptacles are often non-existent, makeshift, or in poor condition-all leading to a situation in which wind, animals, and vandals spread litter throughout houses and neighborhoods. The abundance of vacant lots and abandoned structures, already strewn with refuse, encourages further junk, garbage, and other debris.

An intensive national program is currently underway to reduce litter through an educational campaign. This comprehensive, voluntary system pioneered by Keep America Beautiful, an industry-sponsored group, takes into account the interrelationships among varied factors and it has been successful in reducing, by 65-70%, litter in at least three major urban centers and in the Washington, D.C. metropolitan area.

Finally, it is evident that solid waste management is an environmental quality problem, with many ramifications, which must be solved. But moving from the identification of an environmental quality problem to a clear-cut "solution" to that problem must not circumvent the often difficult task of defining the applicable goals to be served by public policies and programs designed to improve environmental conditions.

To be sure, the "problem-goals-alternatives-best solution" approach is far more productive in these situations than the "problem-solution" approach. Hufschmidt (*3*) cites an example: "the *problem* of proliferation of DDT, which adversely affects wildlife and ecological systems, is perceived, and a *"solution"* is adopted involving selective or total prohibition of the use of DDT. The solution never considered the adverse effects of such action on the public health objective." These adverse effects have been identified by Handler (*2*) and the World Health Organization (*8*).

Unquestionably, the current health, economic, and social settings require that environmental improvement efforts, such as solid waste control, recognize the importance of fitting the means to be used to the goals to be achieved. (A copy of the detailed study may be obtained from the authors.)

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Somatic Cells in Milk-Physiological Aspects and Relationship to Amount and Composition of Milk

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ABSTRACT

Somatic cells in milk include epithelial cells from the gland and leukocytes from the blood. Epithelial cells are elevated in very early and late lactation. Leukocytes increase during mastitis infection or injury. They have phagocytic properties and combat invading organisms. Mean somatic cell counts of each milking over a 1-month period for cows with no udder infection, non-pathogens, or pathogens, were 169,500, 225,800, and 997,800 cells per ml, with coeffecients of variation of 94, 66, and 82%. Advanced age, late lactation, and a previous history of mastitis are related to elevated cells. Milk loss in subclinical mastitis is related to somatic cell counts. On a quarter basis, loss started at 500,000 cells per ml, progressed to 7.5% at 1 million, and 30% at 5 million. In cell counting programs associated with monthly testing of individual cows, those cows with two cell counts over 1 million cells per ml produced over 1,000 pounds of milk per lactation less than other cows in the same lactation whose cell count never exceeded 500,000 per ml. Use of cell counting on an individual cow basis improves its usefulness as a management tool for the dairyman compared to bulk tank counts. Literature data suggest the following changes in the milk composition from quarters definitely positive to mastitis screening tests based on somatic cell counts compared to normal quarters (values represent percent of normal): total solids (92), lactose (85), fat (88), total protein (100), caseins (82), whey protein (162), chloride (161), sodium (136), potassium (91), pH (105), lipase activity (116), and acid degree value (183).

Regular monitoring of the number of somatic cells in bulk milk has become a rather universal practice in the United States and western Europe. Improved methodology now permits good accuracy in this determination. This development has permitted adoption of regulatory programs and has given general information on the mastitis status of herds. However, it has been of limited value to most dairymen. In the small herd, one high cow can have a disproportionate effect on the bulk tank count. In large herds the milk from several bad cows can be diluted out to acceptable levels in the bulk tank. Problem cows are not identified. There seems to be a general lack of understanding of how to interpret somatic cell counts. Often they are confused with bacterial counts.

But, somatic cell counts are here to stay and increasing use can be made of them. Use on individual cows in production testing programs is increasing rapidly and markedly improves their value as a management tool. About 400 herds are now on such programs in Wisconsin. This was made possible by development of an accurate method for estimating cells (*4*) which can be used on preserved milk after the fat test has been completed. It should be emphasized that accuracy beyond the common screening tests is needed to maximize the usefulness of somatic cell counts at either the bulk tank or cow level. Another use of somatic cell counts is in incentive programs for quality. These are being tried and should stimulate increased interest.

It is the purpose of this paper to summarize current information on somatic cells — their origin and function, fluctuation in levels, factors causing them to increase, and relationship of elevated levels to changes in amount and composition of milk. All of the data will be based on bucket or quarter samples and reflect what is happening at the individual cow level.

ORIGIN AND FUNCTION OF SOMATIC CELLS IN MILK

Somatic or body cells in milk are of two types, sloughed epithelial cells from the udder and leukocytes from the blood. Epithelial cells are present in normal milk as a result of normal breakdown and repair, but increase in late lactation as the gland prepares to be non-functional or as a consequence of injury in mastitis. Increases with advancing lactation are minimal in the first lactation but increase with successive lactations in which mastitis incidence is higher. Estimates of the percentage of total cells made up of epithelial cells are variable, ranging from about 35 to 70% in most reports. Normal milk has reported levels of 65 to 70%, chronic mastitis milk around 50%, and more severely mastitic milk lower levels (10 to 45%) due to dilution with leukocytes from the blood (*9, 18, 19*). Although there may be certain bactericidal materials contributed by the sloughed epithelial cells, they are generally considered simply worn out or destroyed cells on their way out. Of major importance in considering elevated somatic cell levels are the leukocytes.

The leukocytes, particularly the neutrophil or polymorphonuclear (PMN) leukocyte, enter milk from blood, being attracted by chemical substances released from injured mammary tissue. The first leukocytes entering presumably release specific substances that

cause an increase in premeability of vascular and secretory systems. This permits fluid and those substances which normally move from blood to milk in limited amounts to pass into milk in incraeased amounts. The fluids tend to dilute and neutralize the toxic products. The PMN leukocytes function in engulfing and digesting the bacteria in a process known as phagocytosis. Usually, when PMN leukocyte counts are high, the numbers of mastitis-causing bacteria are reduced. In persistent infections, opposite patterns of rise and fall of bacteria and leukocyte numbersin milk are common.

Factors affecting the phagocytic ability of PMN leukocytes are not well understood. Leukocytes are more efficient in blood than in milk (*14*), and addition of immune serum as well as glucose improve the activity of milk leucocytes in vitro (*11, 12*). Cow differences in the phagocytic ability of both blood and milk leukocytes have been demonstrated (*12, 14*); the propensity of milk leukocytes to engulf fat globules (*14*) may reduce their efficiency in milk. Further research is needed to improve our understanding of the factors which inhibit or stimulate the phagocytic ability of leukocytes.

Simply increasing leukocyte numbers would obviously increase the chances of phagocytosis. It has been suggested that even a modest elevation of leukocytes in a quarter (500,00 cells/ml) gives some protection against infection when small numbers of organisms pass the teat canal barrier (*18*). But elevation beyond this point is also associated with some milk loss, so a price is being paid for protection with higher cell counts. The protection aspect, however, argues for somewhat liberal limits under practical conditions where exposure to environmental organisms such as coliforms may be unpredictable and difficult to control.

DISTRIBUTION OF SOMATIC CELL COUNTS

In a study (*2*) based on 15,733 monthly somatic cell counts of bucket milk in 16 herds over a 2-year period, average cell count was 692,000 cells per ml with a standard deviation of 1,121,000. This average is misleading because the distribution of cell counts was skewed toward the higher values. Thus a small number of very high cell counts had an undue influence and elevated the average. A better figure is the median which was 390,000 cells per ml. Of the total counts, 18% were over 1 million cells per ml, 20% were between 0.5 and 1 million, and 62% were below 0.5 million. Current data, based on about 400 herds in the cell counting program in conjunction with production testing, shows figures of 10, 18, and 72% in each of the above categories.

DAILY FLUCTUATION IN SOMATIC CELL COUNT

Because the lactating mammary gland is a very active metabolic organ, it is logical to expect variation in cell counts from day to day or milking to milking. In a study in our laboratory (*5*), bucket samples were taken at each milking daily for 1 month from 12 cows with 4, 3, and 5 cows falling into the following infection categories: (*a*) non-infected, (*b*) infected with non-pathogens, and (*c*) infected with pathogens. Mean cell counts for the three groups were 169,500, 225,800, and 997,800 cells per ml, with coefficients of variation of 94, 66, and 82%. Only 2½% of the time did the first two groups exceed 500,000 cells per ml. The third group exceeded this level 75% of the time. Of concern in predicting infection from cell count is the other 25% of the time when infected cows may have a low cell count. Some have used a definition of infection requiring both isolation of a pathogen and a high cell count. Increasing the number of samples as is done in the production testing program also increases accuracy. It is likely that these cases where the organism is isolated but the cell count is low are due to early infections or teat canal infections where cytological damage has not occurred as well as cases where the sample was taken in the low ebb of the cell count cycle. In these cases it is likely that udder damage is more closely related to cell count than presence or absence of pathogens. Preliminary data from our laboratory suggest very good accuracy in predicting that cows with one bucket cell count over 1 million or two cell counts over 750,000 are infected. There appears to be a higher proportion of the false negative samples (low cell count with isolation of organisms) in the herds with a low incidence of mastitis.

RELATIONSHIP TO TYPE OF ORGANISM

There is good agreement that the major factor responsible for elevation of somatic cells in milk is the presence of microorganisms falling in the group considered to be pathogenic (*Staphylococcus aureus, Streptococcus agalactiae, Streptococcus dysgalactiae, Streptococcus uberis,* and gram-negative rods). The major efforts in mastitis control have been prevention of new infections due to these organisms along with elimination of existing infections.

There is a second group of organisms classified as non-pathogens or secondary pathogens which also elicit some cell response. Whether the presence of this latter group of organisms in the udder is good or bad has not been completely resolved. There are limited data to suggest a lower rate of new infection with pathogens when the quarter has a secondary pathogen. Possibly this is due to the elevated number of leukocytes (*3*).

In a study (*24*) involving 874 quarters milked with a quarter milker and sampled bacteriologically, mean cell count where no bacteria were found was 310,000. For the common pathogens, corresponding mean cell counts were: *S. agalactiae,* 900,000; *S. uberis,* 1,630,000; *S. aureus,* 1,500,000. For two others normally classified as non-pathogens, mean cell levels were: micrococci, 360,000, and coagulase negative Staphylococci, 1,400,000. The latter figure was surprisingly high, but was reduced to 710,000 if only quarters with no previous mastitis were included. With all pathogens there was also a rather marked increase in cell response in those quarters with prior occurrence of clinical mastitis. Even in those quarters with no organisms, mean cell count was

600,000 if there had been prior occurrence of clinical mastitis.

When a cow with mastitis is treated and the organisms eliminated, cell counts decrease, but the time required will vary depending upon the organism and the amount of tissue damage. With *S. agalactiae* it may take only a few days, with other streptococci a few days to a few weeks, while with *S. aureus* it may be a few weeks to a few months.

RELATIONSHIP TO AGE AND STAGE OF LACTATION

It has been known for many years that cell counts are highest in early and late lactation and in older cows. The explanation has been an elevation of epithelial cells in colostrum and late lactation milk, and a higher percentage of infection in older cows.

Data from use of somatic cell counts in the production testing program (*2*) suggest little problem with high cell counts due to early lactation. Samples cannot be taken before the sixth day, counting the day of calving as the first day. Elevation of cells in late lactation was related to age of cow and amount of milk. Cell numbers did not increase until after six months in lactation. Cell numbers in milk of younger cows did not rise as sharply in late lactation as in the milk of older animals. The cell count of 983 cows averaged 280,000 cells higher in the last month of lastation than in the second last month. Average cell numbers were significantly higher in the milk of cows with a yield on last test day of less than 10 lb.

These data suggest that higher cell counts should be tolerated in older cows (about 100,000 more cells per ml for each lactation). It also suggests that an elevation of cell count of about 250,000 cells per ml the last month of lactation is normal, with a lesser response in young cows and a greater response when milk production drops below about 10 lb.

HERD MANAGEMENT FACTORS

Because of the many factors influencing somatic cell counts, either through an influence on infection rate or through other mechanisms, it is difficult to establish the importance of specific management factors as they relate to cell count. However, the increased availability of field data permits use of a statistical model to compare various management practices. This was done on two sets of data on bucket milk, one involving 16 herds sampled monthly for 2 years and another involving 134 herds sampled only once (*2*). Herd size varied from 11 to 220 cows and no important relationship to herd size was apparent. Parlor milking systems had the lowest cell counts and around-the-barn pipelines the highest. Parlor systems may have had more modern equipment, while elevation of milk in around-the-barn pipelines may have contributed to higher cell counts compared to buckets. Use of individual towels for udder preparation was related to lower cell counts in the 16 herd sample but not in the other group. Herds practicing post milking teat dipping had lower cell counts than those that did not, which supports other data on the benefits of teat dipping in reducing new infection rate. A rather surprising observation in both groups of herds was the observation that those herds using selective dry therapy had lower cell counts than those dry treating all cows. Our interpretation of these data is not that the complete dry therapy caused an increase in cell counts, but rather that dairymen attempting to control mastitis with dry therapy without other good management practices were unable to maintain cell numbers comparable to other groups. Care must be exercised in interpreting these types of field data because the relationships observed do not necessarily establish cause and effect. It is difficult to separate the many factors involved. However, the results do represent general associations existing in the field.

SPECIAL ENVIRONMENTAL AND PHYSIOLOGICAL FACTORS

If there were factors other than infection which had important influences on somatic cell levels in milk, this would tend to negate the value of somatic cell counting in a mastitis control program. Thus far, aside from age and lactation stage, the effects of which can be predicted, existing data suggest that other influences are not likely to change the mastitis interpretation.

Trauma, due to injury such as a stepped-on teat, will elevate cells, but the cause is usually obvious. Although data are limited, problems with other common disorders such as retained placenta, milk fever, and ketosis do not appear to elevate somatic cells in milk. Daily samples taken through the estrus perios did not show significant changes in cell levels in a limited experiment in our laboratory. Although stress (*27*) or hot weather (*25*) have been related to elevated cells, there is a lack of convincing evidence that the relationship is significant enough to alter the mastitis interpretation under field conditions. Some seasonal relationships have been reported but interpretation is difficult (*2, 10*).

RELATIONSHIP TO MILK PRODUCTION

Losses in milk production associated with clinical mastitis are readily apparent. Not so apparent are losses due to subclinical or "hidden" mastitis. Many estimates have been made comparing herds with low and high infection levels, but these data create some uneasiness due to the many other factors influencing herd production.

With the advent of accurate cell counting and statistical techniques, it is possible to make these comparisons on a within-cow or with-herd basis, which markedly improves the accuracy of the estimates by correcting for many of the other influences.

Two studies along these lines have been conducted in our laboratory. One was based on a comparison of opposite quarters within a cow (*24*). Cows were milked with a quarter milker and milk production and cell counts were recorded for 874 quarters. Overall, 21% of the milk came from each fore quarter and 29% from each rear quarter. Using this correction for anatomical difference, it was possible to statistically estimate differences in milk production related to cell counts.

Milk loss on a quarter basis started at about 500,000 cell/ml. At 1 million cells it was 7.5%, at 2 million 15%, and at 5 million 30%. Losses on a bucket or bulk tank basis would start at lower levels because of dilution with milk from normal quarters.

More recent data on milk loss have been calculated from bucket cell count data obtained in the production testing program (*22*). Based on the concept that a high cell count in early lactation should cause more loss than one appearing later, an index was set up using bucket cell counts as indicated in Table 1.

TABLE 1. *Somatic cell count index for estimating milk loss*

Somatic cells[a] (thousands/ml)	Number of months since calving			
	<2	3 or 4	5 or 6	>
>1,000	4	3	2	1
750-1,000	3	2	1	1
500-750	1	1	0	0
<500	0	0	0	0

[a]Bucket cell counts observed during lactation in production testing program. Designed for application on a bi-monthly basis.

Using these index values, milk loss was calculated on a lactation basis using 3,277 complete lactations averaging 13,312 lb. of milk and 523 lb. of fat. Each lactation had to have at least four cell counts. The results appear in Table 2.

TABLE 2. *Milk loss associated with somatic cell index values*

Lactation	No. lactations	Loss per index point per lactation	
		Milk (lb.)	Fat (lb.)
1	1,028	126	3.9
2	740	131	5.8
3	543	140	6.1
4 and over	966	116	6.7

These data show a consistent loss across all lactations associated with high cell counts. The overall mean loss within lactation was 126 lb. of milk and 5.5 lb. of fat for each index point. This means that on the average, a cow with an index value of 10 indicating a bucket cell count over a million cells throughout the lactation would lose 1,260 lb. of milk and 55 lb. of fat. These are conservative figures because they are based on the assumption that no milk loss occurred with cell counts under 500,000 per ml.

In another method of analyses based on comparisons of lactation production of cows with two cell counts exceeding given levels, the results were comparable but not as consistent since the time at which the high cell counts occurred was not considered. As an example, first lactation cows with no more than one count over 500,000 cell/ml produced 11,952 lb. of milk per lactation compared to 10,825 for those cows with two or more cell counts over 1 million. Again we see the loss of over 1000 lb. of milk per lactation for cows with subclinical mastitis, translating into over $100 per cow.

In the future in our production testing program, we are contemplating calculating the estimated milk loss from mastitis based on cell count and reporting it along with the cell count data. This would emphasize the returns which could be expected from an effective mastitis control program.

RELATIONSHIP TO MILK COMPOSITION

Changes in milk composition induced by mastitis have been reviewed by Wheelock et al. (*26*) and more recently by Schultz (*21*). Most of the information to be reported here is based on comparisons of milks which are positive or negative to mastitis screening tests which estimate numbers of somatic cells in milk. It is the changes due to subclinical mastitis which are of concern because milk from clinical cases would be withheld from the market. As methods for estimating somatic cells become more accurate, it should be possible to improve the accuracy of predicting the accompanying changes in milk composition.

General concepts

There are two general physiological explanations for changes in milk composition associated with mastitis and elevated somatic cells: (*a*) injury to udder cells which reduces the synthesis of those milk components synthesized in the udder; typical examples are lactose and most of the casein, and (*b*) changes in permeability of membranes which permit increased "leakage" of materials from the blood to the milk. There appears to be increased permeability of both the vascular and secretory epithelia. Typical examples of materials which increase due to this pheomenon are sodium, chloride, and immunoglobulins. When lactose is decreased, compensation must be made to insure that milk and blood maintain the same osmotic pressure. Most of this compensation is accomplished by increases in sodium and chloride.

Another factor complicating milk composition changes with increased somatic cells is the accompanying decrease in milk production. If total yield of milk and synthesis of lactose, for example, were decreased equally, lactose percentage would stay the same. Usually synthesis is reduced more than yield, so percentage of the synthesized component drops. Fat may sometimes be an exception where milk yield is reduced more than fat synthesis and fat percentage goes up. For those components which increase, the decrease in milk yield accentuates the change.

Table 3 illustrates the general changes in milk composition associated with elevated somatic cells. Specific changes in the individual components are discussed later.

TABLE 3. *General changes in milk composition associated with elevated somatic cells*

Decrease	*Reason of change*
Lactose	Reduced synthesis
Fat	Reduced synthesis
Casein (total)	Reduced synthesis
Minor change	
Total protein	Opposite changes in components
Increase	
Whey proteins (total)	Leakage from blood
Chloride	Leakage from blood
Sodium	Leakage from blood
pH	Alkaline materials from blood

Milk protein

There is a common belief that mastitis and associated increases in somatic cells are accompanied by a decrease in the percentage of total protein in milk. The experimental evidence does not support this belief, most results suggesting either no significant change (*8*) or small increases (*1*). The confusion arises from the fact that some protein components decrease while others increase. In general, those components synthesized in the gland (a_S-casein, β-casein, β-lactoglobulin, α-lactalbumin) decrease while those coming in from the blood (mainly immunoglobulins and serum albumin) increase (*8, 17*). The increases about balance the decreases so the total stays the same or may increase slightly. Of course, from a processing standpoint the changes are undesirable because the total casein is decreased while the less valuable whey proteins are increased. The immunoglobulins have a special significance since they are high in colostrum milk and include antibodies which are absorbed by the newborn calf and give protection against disease. Normally there is a selective transfer of I_gG_1 relative to I_gG_2 from blood to milk. However, during experimental mastitis both rise and the selective mechansim appears to be overridden, allowing movement of all blood serum protein components into milk (*13*). Local production of antibodies is also a possibility and could account for a small amount of the increased immunoglobulins. This would help to explain the observation that immunoglobulins appear in milk later than serum albumin and persist longer, despite their larger molecular size.

TABLE 4. *Average protein composition (%) of quarter samples grouped by ranges of somatic cell counts (Holstein cows)*[a]

Measurement	Estimated somatic cells (thousands) <250	500-1000	>1000	Correlation with WMT
Total protein	3.61	3.59	3.56	
Casein	2.79	2.65	2.25	
Whey proteins	.82	1.10	1.31	
Casein: total protein ratio	.77	.71	.69	−.70
α_{S1}-casein	1.33	1.09	.85	−.54
β-casein	1.06	.92	.65	−.58
K-casein	.16	.20	.19	+.36
β-lactoglobulin	.25	.31	.22	−.58
α-lactalbumin	.28	.30	.23	−.51
Serum albumin	.15	.23	.35	+.46
Immunoglobulin	.14	.26	.51	+.61

[a]Data from reference (*8*).

Table 4 gives an example of specific changes in milk proteins based on quarter samples with increasing somatic cell counts (*8*). Most components having positive correlations with the Wisconsin Mastitis Test (WMT) are those which come in from the blood and those with negative correlations are those which are synthesized in the gland. There is also a high negative correlation of cells with the casein: total protein ratio. Haenlein et al. (*7*) earlier calculated the following regression equation relating WMT to casein and total protein percentages:

$$\text{WMT (mm)} = 63.46 - 0.678X$$

$$\text{where } X = \frac{100 \text{ (casein \%)}}{\text{total protein \%}}$$

The equation indicates that with a WMT of 10, the casein: total protein ratio would be .78. A similar equation based on the California Mastitis Test (CMT) has been calculated by Ashworth et al. (*1*). Caution should be exercised in attempting to use these equations on bulk milk because they are based on quarter milk and the screening tests are not very precise. Additional work should be done with more accurate cell counting to more accurately establish this relationship.

Milk fat

Most workers have reported a decrease in fat content with subclinical mastitis. Ashworth et al. (*1*) found fat contents of 4.2 and 3.7% in opposite CMT-negative and CMT-3 quarters. Randolph and Irwin (*16*) reported values of 3.45 and 3.20% fat in opposite quarters with WMT values of 10 and 20. The decreases reported for fat (about 10%) are generally less than those for lactose and casein (about 15%) on comparable samples. In the quarter samples we are comparing negative with definitely positive samples so these decreases are greater than would be expected in bulk tank samples with elevated cell counts.

In addition to a drop in total fat content, there are also changes in fat composition associated with elevated cells. Randolph and Irwin (*16*) and Irwin and Randolph (*6*) reported that the phospholipid content of milk with elevated cells was somewhat lower while there was an increase in lipase activity and free fatty acid content, as indicated by acid degree value. Short chain and C18:1 fatty acids increased somewhat while the C16:0 and C18:0 acids decreased. Table 5 shows some of the changes in milkfat and related components as reported by Randolph and Irwin (*16*).

TABLE 5. *Influence of mastitis on milk fat and related components*[a]

Measurement	WMT <10	WMT >20	Change
Milk fat (%)	3.45	3.20	−
Phospholipid (mg/g fat)	4.78	3.55	−
Free fatty acids (mg/g fat)	23.27	34.10	+
Lipase activity	1.49	1.73	+
Acid degree value	.64	1.17	+

[a]Data from reference (*16*).

The explanation for the significance of the milkfat changes are not completely clear. Decreased fat content could be explained by reduced fat synthesis due to injury to secretory cells. Phospholipids appear to be synthesized in the galnd also, so the same explanation could be applied to their decline. Phospholipid content of the fat globule membrane is also reduced (*6*) and thus may reduce protection of the fat against lipolysis. This idea is supported by increased free fatty acids and acid degree values, although increased lipase activity may also be involved. The increased lipase may be related to changes in the concentration of milk lipase secreted or to

the increased leucocytes, which apparently have lipase activity (*16*). The lowered phospholipid and increased lipase content would support the suggestion that mastitis may contribute to development of hydrolytic rancidity in milk.

Lactose

There is good agreement that the lactose and solids-not-fat (SNF) content of milk decrease when somatic cells are elevated. Ashworth et al. (*1*), using data from opposite quarters, reported lactose contents of 4.88, 4.83, 4.72, 4.50, and 3.95% for milks rated negative, trace, one, two, and three on the California Mastitis Test (CMT). It is obvious that the drop becomes steeper as the reaction increases. Comparable figures for non-fat solids determined gravimetrically were 8.99, 8.97, 8.90, 8.73 and 8.32%. Since lactose and protein are the major components, it appears that lactose accounts for essentially all of the drop in SNF. The fact that SNF drops less than lactose is explained by small increases in protein. The total drop in lactose for CMT-3 samples was 16.3% for lactose compared to 10.8% for fat, with a 6.% increase in total protein.

The explanation for the drop in lactose is the logical one of decreased synthesis. Lactose in milk is synthesized only in the udder from glucose removed from the bloodstream. It is of interest that one of the milk proteins, *a*-lactalbumin, is a component of an enzyme involved in the final step of lactose synthesis. Since *a*-lactalbumin is also reduced in mastitis, this could be a partial explanation for decreased lactose synthesis. It is clear that decreases in lactose associated with increased somatic cells are of the greatest magnitude of the major milk components.

Minerals

The major changes from normal in mineral content of milk with elevated somatic cells are increases in sodium and chloride and a decrease in potassium. Ashworth et al. reported a 61% increase in chloride in CMT-3, compared to negative milk. Tallamy and Randolph (*23*) reported a 38% increase in sodium and a 9% decrease in potassium comparing milk with low and high WMT reactions. These changes may be partially explained by "leaking in" of sodium and chloride and possibly "leaking out" of potassium. Blood levels of sodium and chloride are higher and those of potassium lower than milk. Thus, the old adage that milk becomes like the blood during mastitis can be applied here.

Calcium and phosphorous content of milk is considerably higher than that of the blood and both decrease slightly with elevated cells. Most likely this is a reflection of a decrease in the components with which they are complexed, such as casein. Magnesium changes only slightly in an upward fashion and does not fit the simple "leakage" explanation, since levels in blood are lower than in milk.

The trace minerals, iron, copper, and zinc, are very low in milk and increase slightly in mastitis (*23*). Possibly this is due to decreased milk yield. Table 6 gives a comparison of blood levels of minerals compared to milk with low and high cell counts.

TABLE 6. *Minerals (ppm) in blood compared to normal and high cell count milk*

Mineral	Blood[a] plasma	Milk[b] Mastitis negative	Milk[b] Mastitis positive
Sodium	3.400	436	603
Chloride	3.500	910	1,470
Potassium	300	1,725	1,573
Calcium	90	1,298	1,243
Phosphorus (inorganic)	45	695	642
Magnesium	22	121	128

[a]Schmidt (*20*)

[b]Chloride data from Ashworth et al. (*1*), remaining from Tallamy and Randolph (*23*).

SUMMARY OF MILK COMPOSITION RELATIONSHIPS

Table 7 summarizes the differences in milk composition between normal and high cell count milk.

TABLE 7. *Summary comparing milk composition of normal and high cell count milk*

Measurement	Milk Composition (%) Normal	High cells	Difference	% of Normal
From Ashworth et al. (*1*)				
Total solids	13.1	12.0	−1.1	92
Lactose	4.7	4.0	−0.7	85
Fat	4.2	3.7	−0.5	88
Chloride	0.91	0.147	+0.056	161
From Haenlein et al (*8*)				
Total protein	3.6	3.6	0	100
Caseins	2.8	2.3	−0.5	82
Whey protein	0.8	1.3	+0.5	162
From Tallamy and Randolph (*23*)				
Sodium	0.044	0.060	+0.016	136
Potassium	0.172	0.157	−0.015	91
Other				
pH	6.6	6.9	0.3	105
Lipase activity	1.49	1.73	0.24	116
Acid degree value	0.64	1.17	0.53	183

Although most of the changes in milk composition in high cell count milk can be related to decreased synthesis or increased "leakage" due to damage to udder tissue, these explanations are obviously over simplified and much more complex phenomena are involved in the total changes occurring.

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Potential Health Hazards Associated with Reusable Foodservice Utensils

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ABSTRACT

Health hazards of reusable foodservice utensils are reviewed, beginning with Crumbine's studies in Kansas City in 1907. Reports of transmission of communicable diseases through improperly sanitized utensils are included. Despite determined efforts by public health personnel and foodservice managers, utensil washing practices in public eating places continue to be unsatisfactory, with many utensils exceeding bacterial limits.

Cognizant of the continuing demand for improved management of the nation's solid waste problem, the Environmental Protection Agency (EPA), in its Third Report to Congress, recommended "source reduction" as one solution to the problem. The report (*11*) noted that generation of waste could be reduced by restricting consumption of materials and products and product reuse was offered as one of the technological options. Although a preliminary comparison between reusable plates and paper plates was included in the report, the EPA has contracted with Midwest Research Institute for a comparative study of the environmental impacts of certain disposable and reusable food service utensils, including cups and plates. Because public health aspects are frequently overlooked in these kinds of studies, we believe it is important to provide a review of the literature dealing with the health hazards of reusable food service utensils.

EARLY STUDIES

In 1907, Crumbine (*6*) made daily swabbings from the common drinking cups and glasses of all trains entering Union Station at Kansas City and from cups used at a surburban public school. He found that the cups "were a grave potential danger, for many who used them were capable of transmitting disease and undoubtedly did so."

Other isolated reports (*32*) were found in the early medical/health literature concerning the transmission of communicable diseases through improperly cleaned and disinfected eating utensils. Lynch and Cumming (*21*) described experiments conducted with Army troops during World War I which demonstrated that improperly washed and sterilized ("sanitized", as currently defined) dishes could carry bacteria, and were conveyers of disease. Cumming et al. (*7, 8, 9*) investigated transmission of influenza, pneumonia, and tuberculosis and concluded that one of the foremost avenues of transmission of these saliva-borne diseases at that time was through eating utensils. Other investigators (*29*) described the extremely poor methods of hand dish cleansing practices prevalent in many public eating places. Such reports led many public health officials to conclude that there was a lack of appreciation on the part of the public of the possible danger of disease transmission through improperly sterilized eating utensils, and that the sanitation of many restaurants, hotels, etc. was far below the accepted standard of cleanliness and safety.

CLEANING METHODS INADEQUATE

By the 1930's it was realized (*23*) that a definite need existed for development of methods for effective cleaning and sterlization of public eating utensils as well as educational and regulatory programs to insure the appropriate use of these methods. Repeal of Prohibition and proliferation of taverns and roadhouses in the mid-30's was accompanied by poor sanitary practices in the dispensing of alcoholic beverages. Concurrent with revelations by Mallmann (*28*) of inadequacy of cleansing techniques for drinking glasses in taverns were reports of a marked increase in Trench mouth among the general population, a disease that can be transmitted by inadequately cleaned eating utensils and glassware. Surveys (*22*) of glassware in alcoholic beverage dispensing places showed the presence of the bacteria which caused Trench mouth in significantly large numbers, even in places that were making every effort to clean glassware effectively.

In the late '30's many articles appeared in the literature discussing the ineffectiveness of commercially available "disinfectants" (*10, 25, 24*); the continued widespread ignorance concerning proper and effective methods of sanitizing dishes and glassware (*5, 16*); and the lack of a reliable method to enable the public health investigator to determine whether glasses and dishes have been properly handled (*12*). Another problem was

the frequency of improper operation of mechanical dishwashers due to ignorance of the most effective holding time and temperature of the wash and rinse waters, and the type and amount of chemical disinfectant to use. Despite determined efforts by public health personnel and some restaurant proprietors to improve sanitizing operations, most food service personnel felt that dishwashing was satisfactory as long as the dish appeared reasonably free of visible soil and few complaints were made by the clientele. Many of the articles (*26*) published in the '40's focused on the importance of proper dishwashing procedures. Techniques for obtaining bacteriological standards were sought (*13*). Surveys (*15*) of dishwashing practices in public eating places still showed that the quality of washing (as measured by bacteriological tests) was generally very poor throughout the country. Occasional instances of disease outbreaks in institutional populations were traced (*31*) to inadequate sanitization and storage of dishes.

MINIMUM STANDARDS

In 1950, the official minimum requirements for effective machine dishwashing were promulgated (*30*) as not more than 100 microorganisms per utensil surface, and it was felt that this standard could be consistently maintained by proper machines or manual methods. Although minimum dishwashing standards were now set, there were many instances (*3, 27*) where management failed to train and supervise food service workers in proper cleaning operations.

Since restaurants are frequented for the most part by persons in good health, the danger of infection from eating utensils is relatively low, except during epidemics. In hospitals, however, the possibility of infections from eating utensils is much greater because patients may carry large populations of infectious bacteria. Therefore, emphasis shifted toward sanitation problems in the dietary departments of hospitals (*1*). Articles (*17*) exploring this problem in hospitals stressed the importance of proper sanitary habits of kitchen workers. Reports (*19*) in the '60's suggested that the increasing number of illnesses in hospital patients might be traced to consumption of foods that were contaminated during preparation and service. Many workers failed to employ basic principles and practices of sanitation (*2, 18*) and the unsanitary storage of clean dishware (*14, 20*) was another problem which emerged.

RECENT OBSERVATIONS

More recent reports indicate the existence of many of the same unsanitary practices noted in the past. Walker (*33*), in a bacteriological study of reusable beverage glasses in 66 American hotels and motels, found that over 80% of the "washed and sanitized" glasses exceeded the standard of 100 organisms per glass. Sub-standard glasswashing practices were identified as a major factor in the bacterial content of the 430 glasses tested. In an extensive survey, conducted in 1974 at the request of the Comptroller General of the United States (*4*), the Food and Drug Administration (FDA) inspected 185 restaurants selected at random in nine metropolitan cities and found 90% unsanitary, 54% with inadequate facilities for washing and sanitizing equipment and utensils. They concluded that "equipment and utensils that are not thoroughly cleaned, sanitized, and maintained in good repair can harbor accumulations of food and other residues that support harmful bacterial growth, which may be transmitted to customers and employees."

It is extremely difficult to develop epidemiological evidence to support the thesis that lack of sanitization and cleanliness in handling utensils in food establishments is likely to result in demonstrable disease. The contacts are transitory, the sources are not readily identified, and the illnesses are frequently not properly diagnosed or reported. Further, as Walker (*33*) notes, "Questions involving the health effects of environmental bioloads are particularly prone to uncertainty and the health impact of various environmental levels of microorganisms on food or beverage contact surfaces are often unknown." Yet, as Walker states, "public health students recognize that the larger the number of bacteria present, the greater the probability of some of them being harmful."

We believe that the potential health hazards considered above must be included in any comparative study of disposable and reusable food service utensils.

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Professional Sanitarians and the Law

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ABSTRACT

Increased communications between sanitarians and lawyers are important as the public becomes more conscious of sanitation matters, the food distribution chain lengthens, and governmental budgets increase. Federal and state laws prohibit shipment of "adulterated" food, including food packed or held under insanitary conditions, even though the food itself is perfectly clean. Penalties for violation are seizure, injunction, and criminal prosecution, including prosecution of "responsible" individuals. In addition, recalls, which are not mentioned in the federal law, are frequently used. All of these actions require close cooperation between sanitarians and lawyers, whether on the industry side or the governmental side. Preparation for, conducting, and following up on inspections by FDA or other governmental agencies also require cooperation between sanitarians and lawyers.

Our two disciplines — yours as sanitarians and mine as a lawyer — have been coming into contact more frequently and more intimately in recent years, and it is extremely important that we open the door to further communications between us. Incidentally, it is much better for us to communicate on *your* grounds — in a manufacturing plant or warehouse, or even better at a meeting than it is to meet on *my* grounds — at an administrative hearing or worse yet in a court room.

REASONS FOR COMMUNICATIONS

In analyzing why it is increasingly important for us to have good communications, it appears to me that there are three reasons.

First, our country — including particularly the consuming public — has become increasingly conscious of sanitation and related matters. The recent "Philadelphia incident" was a dramatic example which captured public imagination and focused attention on the necessity for perpetual environmental vigilence, including sanitation. As we learn more about bacteria, yeasts, molds, and other even more exotic instrumentalities which are perpetually ready to assail the food supply of the human race, it becomes increasingly clear that sanitarians have an enormously important role to play. And it is also clear that the significance of this role is going to increase in the future. As it increases, I suspect you will be dealing more and more with lawyers, and I know from my own experience that lawyers are going to need you more and more.

Second, the role of sanitarians, and our mutual need to cooperate, seems to increase as the length of the food distribution chain increases. When food was produced primarily for home consumption on the farm a sanitarian would not have been a particularly welcome visitor. But as the producer and consumer have moved further and further apart, both in terms of geography and of middlemen, the role of the sanitarian has become a vital factor in protection of the public health.

Third, FDA, and other federal and state agencies have greatly increased budgets, which means that sanitarians and lawyers will be working much more closely together either for the government or for industry, and hopefully in solving mutual problems before they get to the litigation stage. FDA's budget, for example, was $5,000,000 in 1955; Commissioner Schmidt estimates that by 1980 it will be $500,000,000. This means more scientists, including sanitarians, working for the government; it means more comprehensive and intensive statutes, like the impending bill, S. 641; it means more knowledgeable and technical inspections at food plants; and it means more and better equipped FDA inspectors.

I cannot over emphasize to you the importance which professional sanitarians are going to be playing in food, milk, and environmental matters in the immediate future. The public health needs you, and we lawyers view you and your burgeoning profession with a great deal of respect.

REVIEW OF FOOD LAW

I would like to give you a brief review of food law as it applies to your profession. For some my comments may be quite elementary, while for others they may be exposure to a new field and new ideas.

The basic concept of the Federal Food, Drug, and Cosmetic Act (although this is an intentional over-simplification designed to let us zero in on our specific topic) is that adulterated food may not be shipped or received in interstate commerce. The word "adulteration" has several very specific definations in the Act, but the one which is most important for us is in Section 402(a) (4) (21 U.S.C. 342(a) (4)) which provides:

> "A food shall be deemed to be adulterated — (a) . . . (4) if it has been prepared, packed, or held under insanitary conditions whereby it may have become

contaminated with filth, or whereby it may have been rendered injurious to health . . . ."

The importance of this section is that a food is adulterated under this definition if the plant or warehouse in which it is packed or held is insanitary, even though the food itself is perfectly clean. And Section 402 (a) (4) is one of the sections of the Act used most frequently by the FDA in its enforcement proceedings.

PENALTIES FOR ADULTERATION

Next, let's take a brief look at the penalties for violating this adulteration section. Three remedies are provided in the Act:

(*a*) Seizure of the adulterated goods. This is by far the most common remedy used by FDA, and by state agencies enforcing state laws similar to Section 402 (a) (4). The maximum penalty in a seizure action is loss of the goods which have been seized, although of course there may be sequential problems such as what to do with similar goods not seized.

(*b*) An injunction against the firm and/or individuals to restrain them from some form of future action. For example, FDA has exercised this authority to effectuate closing of plants which it deemed to be so insanitary that they could not be corrected.

(*c*) Criminal prosecution against the offending firm and/or its individual officers or other responsible individuals. This is an extremely serious remedy, which involves a fine of $1,000 on each count for the firm and individuals, and/or a maximum of 1 year in jail on each count for individuals for a first offense, and more serious penalties for subsequent offenses.

Last year the United States Supreme Court decided the case of *U.S.* v. *Park*, 44 L.Ed. 2d 480 (1975), in which the president of Acme Markets, a large retail chain in the East with 16 warehouses and 874 retail outlets, was found guilty of violating the Act because of insanitary conditions in the firm's Baltimore warehouse. While the case involved only a misdemeanor, and the court assessed only a $250 fine, the principle was so important that Mr. Park appealed the case all the way to the United States Supreme Court which affirmed his conviction on the ground that Mr. Park was a responsible official, and was not "powerless" to have prevented the violation.

One effect of the *Park* case has been to get the very clear attention of corporate officials all over the United States. They are more than ever, and some for the first time, looking to sanitarians and lawyers to assure them that they are not in serious jeopardy of a jail sentence. If you ever had the slightest doubt about the importance of a sanitarian, you can now erase it. If you are a sanitarian in private industry or a consultant to private industry, the president of your company is in jeopardy of a criminal prosecution if you do not do your job properly; if you are a sanitarian for a governmental agency, the success of your agency's prosecutions may very well depend on the accuracy and detail of your work.

Let me be more specific. Just last month (July, 1976), I defended a criminal prosecution against a partnership which operated a food wholesale warehouse. Three of the four individual partners were also defendants. The government had charged each of the four defendants with eight separate violations of the Act, which could have resulted in a $32,000 fine and 8 years in jail for each of the three individuals. Five of the counts charged all four defendents with holding food which itself was contaminated, but the other three counts charged only that the food was held under insanitary conditions at the warehouse. Indeed, the government's own tests showed that the food involved in these three counts was not itself contaminated in any way. Thus Section 402 (a) (4) was the sole charge in these three counts.

You can imagine the importance of sanitarians in a case like this, both before the case was filed and as witnessess. I am pleased to say that the jury found the three individual defendants not guilty on all eight counts, and vindicated the warehouse's sanitation practices by finding the partnership not guilty on the three counts which involved Section 402 (a) (4) alone. The government prevailed on the five counts for which FDA had photographs taken by the inspectors 2 years and 1.5 years before the trial.

(*d*) A fourth remedy used with increasing frequency, although it is not mentioned in the Act, is a recall. FDA has recently published, in the *Federal Register* for June 30, 1976, a comprehensive proposal concerning recalls in which you may be interested.

INSPECTIONS

Finally, I would like to discuss inspections by federal and state officials, again an area in which sanitarians and lawyers must cooperate. If you are a sanitarian working with an inspected firm, it is crucially important that you work with your firm's lawyers in designing a suitable protocol covering preparation for the inspection, conduct of the inspection itself, and followup on the inspection. Obviously legal questions are involved in each of these three steps, so your profession and mine will again be working together. Let me emphasize particularly the importance of proper followup after the inspection is concluded. This followup should include not only internal changes, but also a thorough report to the inspecting agency. Again, a job for sanitarians — who are the experts in knowing what must be done to effectuate necessary changes — and lawyers, who hopefully are the experts in translating action into words which will be persuasive to the regulatory agency in its decision as to whether further regulatory action is needed.

If you are a sanitarian working for a governmental agency, you must of course be thoroughly prepared for the inspection, know how to conduct the inspection, and help carry out your dedication to the protection of public health by sharing your views with the firm at the conclusion of the inspection. If the firm fails to carry out what you believe to be necessary steps, you will undoubtedly be talking to lawyers who will be preparing a case, and again our two professions will be working

together.

One potential bone of contention in the handling of inspections is the taking of photographs. FDA inspectors are instructed to attempt to take pictures of any violative condition which they find, and they have authority to do so *unless* the inspected firm requests that the pictures not be taken. In other words, the inspected plant has the option of prohibiting the taking of pictures, and because pictures can so distort a condition, it is my strong recommendation that the inspector be requested to leave his camera in the car — politely but firmly.

I hope that these comments have reaffirmed your awareness of the importance of sanitarians and the work which you do. You are absolutely essential not only to the protection of public health, but your role is becoming increasingly important as technology develops. As a lawyer, I particularly thank you for the expertise which you have brought to the field of food technology, and look forward to working with you in the protection of the public health — hopefully on your field of battle but if necessary also on mine!.

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A Field Topic

Testing for Antibiotics in Milk - Field Observations

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ABSTRACT

Four tests are currently being used at several locations throughout New England. They are the Disc Assay, Hood Modified Disc Assay, Delvotest-P, and Cylinder Plate Method. The Disc Assay Methods are used mainly for fluid milk, while the Delvotest-P and Cylinder Plate methods are used for manufactured products. The incidence of positive antibotics tests on fluid milk is lower in percent now than 2 years ago; however, the total number of positives is about the same. Most positive tests are being found now with the 14-h Disc Assay only and not the 4-h Disc Assay as they were two years ago. This suggests that producers, through education and penalties, are trying to do a better and more conscientious job than was previously done. The sensitivity of the Disc Assay methods has been increased during this same period.

Yankee Milk, Inc. is a cooperative with 4500 dairy farmers throughout New England and eastern New York. It operates three manufacturing plants and 11 receiving stations or non-manufacturing plants. Its' members produced 3.2 billion pounds of milk in 1975.

Yankee Milk operates seven laboratories at its's plants and has facilities at all receiving stations to test for milkfat, DMC, and antibiotics. All laboratories and testing facilities are open 7 days a week with two laboratories open 24 h a day and the remaining open 12 to 16 h per day.

Testing for antibiotics is done at all locations using the 4- and 14-h disc assay methods on all farm pickup tank loads. Universal samples are taken daily on milk from all producers and these samples are available along with the farm pickup tank samples. In many instances, milk is picked up one day and delivered early the next morning therefore giving the laboratories time to test by the 4-h disc assay method before delivery to a plant. If a positive result is obtained, milk from all producers on the load is tested and the violator is detected. He is then shut off for further pickup until a negative sample can be obtained from his bulk tank thus clearing him to resume shipping. At the end of each month Yankee Milk assesses a penalty on all violators during the month.

PENALTY

The current antibiotic penalty adopted by our Board of Directors, September 1, 1975 reads as follows: "Any member or affiliated member who, by permitting the shipment of milk subsequently determined by test to be adulterated with antibiotic residue shall be subject to assessment calculated on the basis of the applicable Association pay price and the hundredweight of adulterated milk shipped and to further conditions as follows:

1. For the first violation in any 6-month period, beginning with the date of such violation; one and one half times the value of 2 days production.
2. For the second violation in the same period; two times the value of 2 days' production.
3. For the third violation in the same period; four times the value of 2 days' production. Within 5 days of such violation, the violator shall also be notified to appear before the Executive Committee or Board of Directors of the Association to show cause why his Producer Membership and Marketing Contract or other entitlement to market his milk through the Association should not be immediately terminated.
4. As used above, the term "2 days' production" shall, in the case of every-other-day shipper, mean the poundage in the shipment determined to be adulterated. In the case of an every-day shipper, said term shall mean two times the poundage in the shipment determined to be adulterated. In either such case, the term "shipment" shall include all milk produced by an individual member or affiliated member for storage in the same milk house and for shipment in the same bulk truck regardless of the number of farm bulk tanks from which shipment is made.
5. Following any violation of these standards, no milk produced by a violator shall be picked up unless and until a test for antibiotic residue on an official sample of his milk provides a negative result.

Any bulk load found to be positive is diverted by our supply division to our nearest manufacturing plant. There it is separated and the cream is tested in triplicate. If positive, the cream is dumped. If negative, it is sold. The skimmilk is condensed and depending on the original amount of antibiotic present in skimmilk, it is either made into powder or reconstituted into skimmilk and then recondensed and dried. Any powder still containing antibiotics can further be reconstituted to skimmilk and condensed and dried. This usually has eliminated the antibiotic residues in the powder, thus making it saleable. All reprocessed powder is tested by the Cylinder Plate Method.

One of our laboratories is equipped to do the Cylinder Plate Method for all our other locations. It is used as a means of determining the exact concentration of antibiotic in a suspected sample as well as clearing reprocessed powder.

DELVOTEST-P

The Delvotest-P is used by one of our laboratories to determine whether milk shipped to a particular cheese plant is free of inhibitors. Swiss cheese is made and very low levels of inhibitors, even those undetected by disc assay method, interfere with manufacturing of a satisfactory Swiss cheese.

The Delvotest-P is also used by our powder plants to screen loads of milk

going into powder. Very low levels of antibiotics that are undetected by Disc Assay Method can be detected, therefore preventing our drying such milk and ending up with positive powder. This was a serious problem until about 2 years ago. At that time it wasn't uncommon for us to have all incoming milk test negative and end up with positive powder. This was due to concentrating the antibiotic by reconstituting the powder 3:1 for testing as recommended by FDA. Through use of the Delvotest we have eliminated this problem.

The Delvotest is also used in conjunction with the Disc Assay Method in our laboratories as an aid in determining for producers whether milk will be picked up when there is a question of possible adulteration with antibiotics. If the Delvotest is negative, the Disc Assay has always been negative and we accept the milk. Last month (July, 1976), Dairylea instituted a program of testing their producers' milk by Delvotest at no charge when they were unsure whether antibiotics may have gotten into their supply. A phone call to their nearest plant division office, sanitarian, or field representative and an employee will come to the farm, sample, and test the milk. A sample brought to the nearest office or plant would be tested even quicker. To date, I haven't heard how their program is working.

MODIFIED DISC ASSAY

Dr. Harry Wildesin of the Hood Co. has modified the 4- and 14-h Disc Assay Methods to make them more sensitive. He has obtained sensitivities of .02 and .01 unit penicillin/ml, respectively. The modification includes touching the edge of the paper disc into a well mixed sample of raw milk previously heated to 40-50 C (104-122 F). Milk is allowed to completely wet the disc by capillary action and is removed as quickly as possible, thereafter to avoid excess milk on the disc. Raw milk is used to prevent reduction in penicillin concentration. In addition to making possible better capillary action, heating prevents a coating of fat from collecting on the disc, possibly preventing diffusion of antibiotic into the agar. There is also no natural inhibitor from fat using this method. The incubation temperature is changed to 41 C in the short method, so normally observations can be made after 3-4 h.

SAMPLES FROM PRODUCERS

We have, for the past 2 years, encouraged our members to bring into our laboratories or receiving stations samples when they believe that they may have accidently adulterated their tank of milk. We test it for them free of charge and, if positive, we won't pick up the milk. If negative, we would accept the milk. This helps to prevent them from being penalized if antibiotic did get into our milk supply.

An example: Recently a member noticed after milking that a cow that had been dry-treated was with the milking herd. He didn't believe that it had been milked, but he didn't want to take a chance. He brought in a sample and it was negative. This is how we expected the service to be used. This service, however, has been abused by many members in an attempt to beat the withholding time on treated animals. It is not uncommon to receive five or more cow samples every day from the same member. In an attempt to eliminate this misuse, our Board of Directors has just adopted this month a $3.00 fee on all producer samples brought or sent in by members.

Unfortunately, some farmers have tried to "beat the game" by substituting water for the milk they have dumped. I heard of one such case where a farmer dumped milk from three of his cows and substituted water for the dumped milk. Fortunately, the handler receiving the milk checked and found about 15% added water. The farmer then admitted what he had done.

POSITIVE TEST RESULTS

Our experience has been that the incidence of positive antibiotic tests in fluid milk is lower in percent now than 3 years ago, but the total number of positive results is about the same. For the 3-month period, January-March, 1973:

Bulk Loads		
2,427 samples	21 positives	0.87%
Producer		
768 samples	14 positives	1.82%

In August 1974 we expanded our program to include taking universal fresh milk samples on *all* of our producers even those we were not responsible for testing, as a means of eliminating antibiotics. We also began testing direct delivered bulk loads at this time. For the first 8 months, from August 1974 through March 1975 we had detected 133 member violations or an average of 17 per month. These violations were committed by 115 different members. The actual number of violations ranged from 14 to 23 per month. Of the 115 members, 103 had single violations, eight had two, three had three, and one had five. The total adulterated milk from these 115 members amounted to 621,000 pounds which in turn adulterated much larger amounts of milk.

Currently, during the 3 months of April through June 1976

Bulk Loads		
24,521 samples	45 positives	.18%
Producer		
23,226 samples	55 positives	.24%

In a little over 3 years we increased bulk load testing from 2,427 to 24,521 samples with the percentage of positives dropping from .87 to .18%. Producer testing increased from 768 to 23,226 samples with the percentage of positive test results dropping from 1.82 to .24%.

Most positive tests are being found now with the 14-h Disc Assay Method only and not with the 4-h method as they were 2 years ago. The sensitivity of the Disc Assay Methods have been increased in our laboratories through more careful preparation of plates during this same period. This leads me to believe that our members, through education and penalties are trying to do a better and more conscientious job than was previously done.

PREVENTING ANTIBIOTICS IN MILK

At member meetings we have continued to stress 15 recommendations for prevention of antibiotics in milk. They are:

1. Know the kind of drug being used and what its' effects are expected to be. Don't use questionable preparations. Experienced suppliers have spent large sums of money in attempting to assure that their label declarations are correct. All antibiotics such as penicillin, tetracycline, streptomycin, and combinations of these drugs MUST be kept out of market milk. This also includes eyedrops, ointments, and other preparations containing these drugs or combinations of them. Some teat dilators and uterine boluses also cause positive test reactions.

2. Read the label for dosage requirements. DO NOT EXCEED PRESCRIBED DOSAGE. If, for example, two tubes instead of one are used, the drug may not be eliminated from the cow in the usual time. Dose rate and excretion rate of some drugs are related. Sometimes even at normal treatment rates a drug can stay with a cow longer than label warnings indicate. It has happened!!

3. Read the label to determine the length of time milk must be withheld from sale. DO NOT CUT THIS TIME SHORT OR DEPEND ON DILUTION EFFECT. All labels specify the amount of antibiotic to administer and the appropiate withholding time for milk, usually 72-96 h. If a drug will not be eliminated in 96 h, it cannot be used on lactating cows.

4. Identify all cows treated either by herdsman or veterinarian so that all persons having anything to do with the milking operation know what cows should not be milked in the routine manner. A means of identifying treated animals is to mark both flanks or milking side with day of week and morning or afternoon milking when milk is safe to add to supply — for instance Wed./am or Sun./pm. Marking crayons, magic markers in vivid colors, and ankle bands are available for this purpose. Lack of communication is perhaps the most common cause of antibiotic-contaminated milk. According to one study, 40% of accidental adulteration occurs in this manner.

5. Treated cows should be milked *last*. One should make certain that all milk contact surfaces are thoroughly cleaned and sanitized after milking treated cows. This avoids some problems of carryover of treated milk on equipment to untreated milk. A single medicine dropper of milk from a treated cow can cause detectable levels in the next cow's milk passing through the same equipment. In milking parlors and pipeline set-ups, separate bucket type milkers should be provided so that the milk from treated cows can be kept out of the milk supply line to the bulk tank.

6. Even if only one quarter has been treated, discard *all* milk from that treated cow. Milk from the other three quarters may be contaminated, to a lesser degree perhaps, but contaminated nonetheless.

7. Milk from treated cows should not be fed to calves or other animals within 5 days of salughtering for human consumption. It is not a good practice to feed milk from treated cows to calves being raised for the milking herd.

8. One person should be responsible for administering all drugs and identifying all treated animals. This should reduce accidental contamination due to poor communication. If the veterinarian treats a cow in the absence of the herdsman, he should provide adequate information for the herdsman before leaving the farm.

9. Dry cows should not be treated within 4 weeks of freshening, and milk should not be added to the supply for a least 96 h after freshening. Milk may be contaminated from cows calving prematurely after dry treatment. Dry treatment antibiotic formulations are long lasting and generally require a 30-day withholding period. The 30-day withholding period extends through freshening for the full period. Even when cows freshen on time, a milk withholding period is often required. Read the label! Dry cow treatment should never be used on lactating cows. If a cow is treated by accident, its milk should be withheld until checked and found negative by a laboratory.

10. Intramuscular injections of antibiotics are absorbed into the bloodstream and eventually contaminate the milk. They generally require a longer withholding period than udder infusion. Withhold this milk from market for the prescribed period.

11. Milk from lactating cows just purchased or under treatment should be checked before adding the milk to the supply. Handle new cows in the herd as though they had just been treated by withholding milk from the bulk tank for at least 5 days or until proven negative by test. This is probably the second most common source of antibiotic contamination.

12. Do not add to the bulk milk tank any milk from a cow which has received any kind of medication — oral (including medicated feeds), intramuscular injection, udder infusion, intrauterine infusions, or even treatment for pink eye.

13. Treated cows should be milked completely. The more completely a cow is milked, the faster any drug will clear out.

14. When treated cows are shipped to slaughter, remember that the withdrawal time following treatment is much longer for tissue residues than is the withholding time for milk. It may be up to 60 days. Check the label, and do not ship them until the recommended time has elapsed.

15. If there is any question about mistakes that may have occurred, contact the milk handler or fieldman before milk is collected so he can have the tank and/or cow samples tested at the laboratory.

SUMMARY

In summary, it is essential for animal health that antibiotics continue to be available. Misuse of antibiotics jeopardizes this continued availability. Antibiotics are an aid to but not a substitute for good

herd management. The saying, "If all else fails, read the label," should be reversed to "Read the label first — and not just read the label — but HEED the label." If this is done, and treated cows are segregated and tested before returning to the milking herd, we will have gone a long way toward eliminating antibiotic residues from milk and milk products.

ACKNOWLEDGMENT

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Instructions for Authors

Journal of Food Protection

SCOPE OF THE JOURNAL

The *Journal of Food Protection*, an international monthly journal in the English language, is intended for publication of research and review papers on all topics in food science and on the food aspects of the animal (dairy, poultry, meat, seafood) and plant (cereals, fruits, vegetables) sciences. Major emphases of the journal will be on: (*a*) cause and control of all forms (chemical, microbial, natural toxicants) of foodborne illness, (*b*) contamination (chemical, microbial, insects, rodents, etc.) and its control in raw foods and in foods during processing, distribution, and preparing and serving to consumers, (*c*) causes of food spoilage and its control through processing (low temperatures, high temperatures, preservatives, drying, fermentation, etc.), (*d*) food quality and chemical, microbiological, and physical methods to measure the various attributes of food quality, (*e*) the foodservice industry, and (*f*) wastes from the food industry and means to utilize or treat the wastes. Prospective authors with questions about the suitability of their material for publication are invited to request an opinion from the Editor.

SUBMITTING MANUSCRIPTS AND OTHER ITEMS

All manuscripts, including "Letter to the Editor," should be submitted *in duplicate*, in flat form, and by first class mail to the Editor, Dr. E. H. Marth, Department of Food Science, University of Wisconsin, Madison, Wisconsin 53706. Revised manuscripts also should be submitted *in duplicate* to the Editor.

All materials intended for the "Association Affairs" and "News and Events" sections of the *Journal* should be submitted in flat form by first class mail to the Managing Editor, Mr. E. O. Wright, Box 701, Ames, Iowa 50010. Subjects suitable for inclusion in the "News and Events" section include: announcements of meetings, short courses, or other events of interest to the readership; notices of position changes and promotions; announcements of new products of interest to the readership; and notices of death and obituaries of members. Any questions on suitability of material can be answered by the Managing Editor.

Correspondence dealing with membership in the International Association of Milk, Food, and Environmental Sanitarians, Inc., subscriptions, advertising (including classified advertising), etc. should be sent to the Managing Editor at the address given above.

PUBLICATION OF MANUSCRIPTS

Manuscripts are accepted for publication, subject to editorial review. Most papers are reviewed by two members of the Editorial Board or by other specialists who may be called on by the Editor when in his opinion the subject of a paper is outside of the specialties represented by Editorial Board members. After review, a manuscript generally is returned to the author for revision in accord with suggestions made by reviewers. Authors can hasten publication of their papers by revising and returning them promptly. With cooperation by authors, research papers nearly always are published within 6 months after they are received and often they appear sooner.

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

Membership in the International Association of Milk, Food, and Environmental Sanitarians, Inc. is *not* a prerequisite for acceptance of a manuscript for publication. Nonmember scientist from the U.S. and from other countries are invited to submit papers for consideration for publication.

Papers, when accepted, become the copyrighted property of the *Journal* and its sponsoring association. Reprinting of any material from the *Journal* or re-publishing of any papers or portions of them is prohibited unless permission to do so is granted by the Editor or Managing Editor.

Submission of a paper implies that all authors and their institution(s) have agreed to its publication. It also implies that the paper is not being considered for publication in another domestic or foreign journal.

MANUSCRIPT SERVICE CHARGE

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

Most institutions accept the manuscript service charge as a necessary cost of conducting research and communicating the results. Nevertheless, it is realized that some authors may not have funds available for this purpose and hence exceptions can be made when necessary. *Inability to pay the service charge shall not prohibit publication of an acceptable manuscript*. An author will be informed of the manuscript service charge when proofs are sent. The manuscript service charge does not apply to review papers or other general interest papers that are not research papers.

REPRINTS

Reprints of a paper may be ordered by the author when he returns the proofs. An appropriate form for this purpose is attached to proofs. Reprints may be ordered with or without covers in multiples of 100. The cost varies according to the number of printed pages in the paper. No free reprints are provided.

Reprints also may be ordered after a paper has been published. The IAMFES office can supply reprints of any papers published in the *Journal of Food Protection, Journal of Milk and Food Technology,* or *Journal of Milk Technology*. Arrangements to obtain such reprints should be made with the Managing Editor.

TYPES OF PAPERS

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

Research paper

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

Research note

A research note is a short paper which describes observations made in a rather limited area of investigation. Negative results are sometimes best reported in the form of a research note. The research note should

not be used as a vehicle for reporting inferior research. A research note generally consists of less than 5 double-spaced typewritten pages of text together with appropriate figures or tables. Organization of a research note is the same as that of a research paper. The designation, "A Research Note" will appear above the titles of these papers since the *Journal* does not devote a separate section to research notes.

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

Review papers

Well written, thorough, well documented review papers on subjects of concern to the readers of the *Journal* are encouraged and will be published promptly. If a review paper substantially exceeds approximately 35 double-spaced typewritten pages, it may be necessary to subdivide the manuscript so that it will appear in several issues of the *Journal.* Authors are invited to discuss their plans for review papers with the Editor.

General interest-nontechnical

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

Letter to the Editor

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

Book reviews

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

PREPARATION OF MANUSCRIPTS

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

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

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

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

E. *Organization of research papers and research notes*

1. The *title* should appear at the top of the first page. It should be as brief as possible, contain no abbreviations, and be truly indicative of the subject matter discussed in the paper. Care should be exersised by the author in preparing the title since it is often used in information retrieval systems. *Good information can be lost through a poor title!*
2. *Name(s) of author(s) and affiliation(s)* should follow under the title. If an author has changed location since the work was completed, his new address should be given in a footnote.
3. The *Abstract* appears at the beginning of the paper. It should be brief, factual, and not exceed 200 words. The abstract should be intelligible without reading the remainder of the paper. Generally, an abstract should not contain abbreviations. Abstracts of papers are reprinted by abstracting journals and so will be disseminated beyond the readership of the *Journal* to people who often do not have access to the entire paper. Hence abstracts should be prepared with great care.
4. The *text* should contain: (*a*) introductory statements, objectives or reasons for research, and related literature, (*b*) materials and methods (*c*) results, (*d*) discussion (may be combined with results), (*e*) conclusions (only if needed; should not repeat the abstract) (*f*) acknowledgments, and (*g*) references.
5. *Citation of references* must follow the style of the *CBE Style Manual.* Several examples of proper citations are given below.

A. *Paper in a journal*

Alderman, G. G., and E. H. Marth. 1974. Experimental production of aflatoxin in citrus juice and peel. J. Milk Food Technol. 37:308-313.

b. *Paper in a book*

Marth, E. H. 1974. Fermentations. pp. 771-882. In B. H. Webb, A. H. Johnson, and J. A. Alford (eds.) Fundamentals of dairy chemistry (2nd ed.), Avi Publishing Co., Westport, Conn.

c. *Book*

Fennema, O. R., W. D. Powrie, and E. H. Marth. 1973. Low-temperature preservation of foods and living matter. Marcel Dekker, Inc., New York. 598 p.

d. *Patent*

Hussong, R. V., E. H. Marth, and D. G. Vakaleris. 1964. Manufacture of cottage cheese. U. S. Pat. 3, 117, 870. Jan. 14.

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

F. *Organization of review and general interest papers*

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

G. *Preparation of figures*

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

should appear in the picture.

H. *Preparation of tables*

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

Letters to the Editor

Comments on comparative study of potential health hazards associated with disposable and reusable food service utensils

DEAR SIR:

Elsewhere in this journal, Ms. Jean Katz and I provide a review of the literature on "Potential Health Hazards Associated with Reusable Food Service Utensils." Recently, an unpublished study comparing the potential health hazards associated with disposable and reusable food service items was conducted in Syracuse by the Food Protection Laboratory of Syracuse Research Corporation, a U.S. Public Health Service certified laboratory. Food service utensils were tested for total bacterial content and for specific bacteria (staphylococci, streptococci, *Escherichia coli*) commonly associated with disease. Fifteen sites (seven restaurants, two cafeterias, two fast food establishments, two hospitals and two schools) were randomly selected and samples collected from disposable and reusable plates, bowls, cups, and glasses and tested according to recommendations in *Standard Methods for the Examination of Dairy Products* (13th edition). I have reviewed the results of this study (Table 1) and offer the following comments. Because the two fast food facilities (Establishment No. 9 and 11) did not utilize reusable utensils, they were eliminated from the evaluation, although note should be made that the mean total plate count (TPC) was only 3 per utensil and only one potential pathogen (staphylococcus) was detected on the 66 samples tested. In the remaining 13 establishments, reusable utensils showed mean TPCs 13 times higher than disposables (275:21), in fact the mean TPC of 275 for reusables exceeded the maximum of less than 100 allowed by the standards for any individual sample. Eliminated from these calculations were two samples from reusable utensils which gave TPCs too numerous to count (greater than 325,000). Eighty five (or 21.2%) of the reusables, but only 11 (or 4.1%) of the disposables exceeded the TPC standard. With respect to potential pathogens, 102 (25.5%) of the reusables and 19 (7.0%) of the disposables were positive. It was found that 125 (31.2%) of the reusables and 25 (9.2%) of the disposables were contaminated because of excessive TPCs and/or the presence of pathogens. The authors observe that single service items are packed and stored in protective wrappers and imply that contamination of these disposable items resulted from mishandling at the service establishment.

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TABLE 1. *Microbiological examination of utensils*

Establishment number & type	Kind of utensil[a]	Number samples	Gross TPC	Mean TPC	Number of Samples: Exceeding TPC standard	With potential pathogens	Regarded as contaminated
1 Cafeteria	D	21	205	10	1	0	1
	R	27[b]	33,600	1244	9	8	10
2 Restaurant	D	19	235	12	1	1	2
	R	33	9,565	290	7	5	8
3 Restaurant	D	13	765	59	2	2	3
	R	28	3,915	140	8	13	13
4 Restaurant	D	14	190	14	1	0	1
	R	41[b]	1,270	31	4	5	8
5 Restaurant	D	14	100	7	0	4	4
	R	35	4,415	126	10	13	14
6 Restaurant	D	21	50	2	0	1	1
	R	35	9,945	284	19	21	25
7 Cafeteria	D	21	275	13	1	3	3
	R	35	5,500	157	9	13	15
8 Restaurant	D	28	265	9	1	1	1
	R	33	33,325	1010	8	7	10
10 Restaurant	D	21	440	21	1	4	4
	R	14	335	24	1	4	5
12 Hospital	D	35	380	11	1	0	1
	R	35	910	26	3	2	3
13 Hospital	D	33	2,780	84	2	3	4
	R	28	55	2	0	2	2
14 School	D	21	10	1	0	0	0
	R	28	4,660	166	1	4	4
15 School	D	10	0	0	0	0	0
	R	28	2,450	87	6	5	8
Total	D	271	5,695	21	11	19	25
	R	400	109,945	275	85	102	125

[a]D-disposable; R-reusable
[b]Not including one sample "Too Numerous to Count" (TNTC), > 325,000.

Bacteria not destroyed at 10-40 C

DEAR SIR:

The graph in Fig. 2 of the article entitled "Cooking Inoculated Pork in Microwave and Conventional Ovens" *Journal of Milk and Food Technology* 39:771-773 (1976) is misleading. As presented, one could easily infer that *Pseudomonas putrefaciens* subjected to heating in a conventional oven was destroyed as follows:

At 10 C-destruction was about 10%
At 20 C-destruction was about 15%
At 30 C-destruction was about 40%
At 40 C-destruction was about 50%

It is obvious that at temperatures between 10-40 C this organism will not die but will actually grow. The line as depicted before the initial point (60 C) is incorrect; it should not indicate a decrease but rather remain at the 100% survival level.

A similar observation can be made for each line on the graph. The only valid lines on the graph start at the 60 C point and extend to the 85 C point. The interested reader should read the graph utilizing only the specific points indicated by the temperatures of 60, 68, 77, and 85 C.

Finding a difference between a conventional oven and a microwave cook still remains an intersting observation.

OLIVER W. KAUFMANN
Division of Federal-State Relations
EDRO, Food and Drug Administration
550 Main Street
Cincinnati, Ohio 45202

Figure may be unclear but text gives information

DEAR SIR:

I agree that the graph is not as clear as it could have been. In the original graph symbols were located on the lines at the temperature observation points. When the graph was redone for publication by our art department and photographed the symbols were inadvertently omitted. This problem was recognized at that point but was considered minor since the five temperature points where microbiological analyses were done were clearly stated in the written text and certainly were suggested by the change in direction (in at least four out of six lines at each temperature point) of the lines in the graph. In a line graph, straight lines between observation points indicate that no information is available in this area. If one tries to extrapolate information between observation points this is an error in interpretation and not the fault of the graph.

Additional research in this area, with a different microwave oven, with different organisms, and with different conventional oven temperatures, has again suggested that cooking meat to the same internal temperature by the two techniques will yield less microbial destruction by the microwave technique.

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News and Events

Food Microbiology Lab Courses Scheduled

Two laboratory short courses for food technologists and scientists in food industries will be held in September at the University of Minnesota, St. Paul. The two courses run on consecutive weeks, and any person registering for both will receive a registration fee reduction.

Microbiological Examination of Food, Sept. 12-16, will cover laboratory procedures and safeguards; media preparation; isolation and identification of salmonellae, *Staphylococcus aureus,* and *Clostridium perfringens* from food; total numbers of microorganisms; foodborne diseases; indicator organisms, and interpretation of results. Registration fee is $200.

The Food Mycology Workshop September 19-23 will stress the identification of yeasts and molds common to food products. The course will include lecture and laboratory excercises with an emphasis on identification techniques and taxonomy.

The fee for anyone registering for both sessions is $350. Fees cover supplies and instructional materials. Participants may bring their own microscope sample for analysis to each course.

Contact the Office of Special Programs, 405 Coffey Hall, University of Minnesota, St. Paul 55108, for further information. Preregistration for either or both courses is possible with a $50 deposit and letter of intent. As registration will be limited, early application suggested. These short courses are sponsored by the University's Departments of Food Science and Nutrition and Plant Pathology in cooperation with the Agricultural Extension Service.

New Plastic Packaging Closure is Tamperproof and Dripless

Marketers of liquid food products as well as many other types of liquid products are now being offered a new plastic closure which provides two unique consumer benefits . . . visible evidence of prior tampering and dripless pouring.

The new closure is being introduced by the recently formed Breskin Industries Corporation which holds the exclusive rights to manufacture and market this and other closure designs in the North American market. Originally developed and perfected in Europe, the tamperproof-dripless resealable closure is molded in two separate pieces from FDA-approved medium density polyethylene. It will fit standard bottle crown neck finishes in 29mm, 33mm and 38mm diameters. It can be readily capped on conventional high-speed equipment with only minimum modification to operate at equivalent speeds.

The tamperproof feature provides direct, visual evidence of prior opening of the closure. Access to the product cannot be achieved until an integrally-molded internal diaphragm is removed. When the diaphragm is missing, the purchaser knows that the container has been opened by someone else. The closure is easily resealed with the air-tight, snap-fit overcap.

To prevent dripping, the closure has a curved pouring lip which assures a positive cut off of product flow where the container is returned to a vertical position. In this way, messy product residues are prevented from accumulating on the closure and the neck of the container.

The closure is ideally suited for edible oils, syrups, vinegar, salad dressings, honey, sauces, wine, fruit juices, pharmaceuticals, toiletries, household chemicals and similar liquid products.

For further information, contact Mr. T. B. Breskin, Breskin Industries Corporation, 5 Corporate Park Drive, White Plains, NY 10604, (914) 694-8730.

1977 Crumbine Award Competition Now Open to Entries from Local Public Health Agencies

Entries are now being accepted for the 1977 Samuel J. Crumbine Consumer Protection Award, given annually to a local government public health or environmental protection agency for "outstanding achievement in the development of a program of food and beverage sanitation."

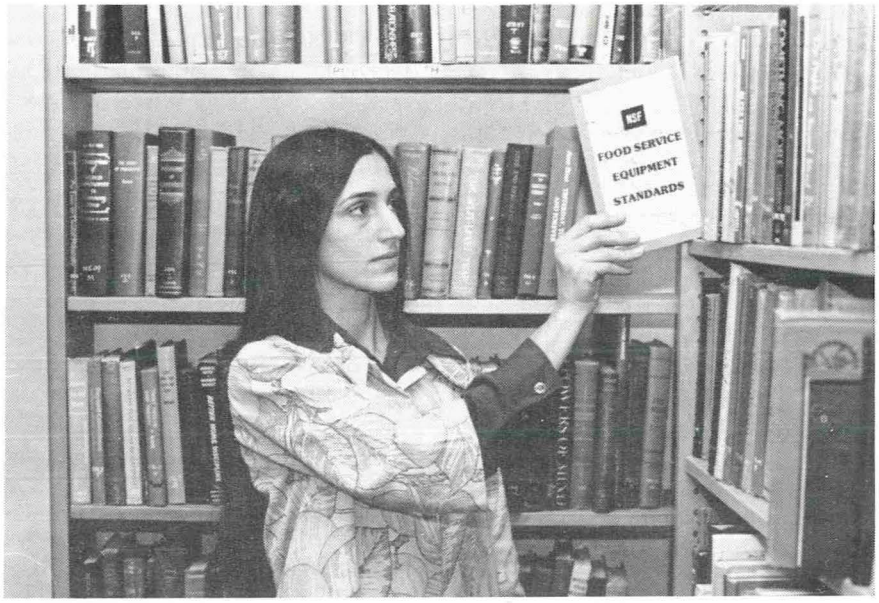
Announcement of the Award was made by its sponsor, the Single Service Institute, national trade association of manufacturers of single-use products for food service and packaging.

The 1977 Award will focus on program development in the area of public education relating to food service sanitation activities. According to Charles W. Felix, Director, Environment, Health and Communications of the Single Service Institute, this embraces "the continuing information and education activities the local regulatory agency has used to bring about the behavioral changes by food service managers and consumers which will help to achieve the food service sanitation program objectives."

Entries are now being sought from local units of government (such as counties, districts, cities, towns and townships) which are responsible for establishing and enforcing sanitation standards and procedures designed to protect the consumer in public eating and drinking establishments. Although state regulatory agencies as such are not eligible for the Award, a particular unit of a state agency may compete if it has its own separate budget.

Deadline for entries is May 15, 1977. Each entry must cover program activities in 1976 or fiscal year 1975-76. Application forms and further information may be obtained from the Single Service Institute, 250 Park Avenue, New York, N.Y., 10017.

NSF Compiles Equipment Standards



Food service equipment used in restaurants has long been a special concern of public health authorities. In their efforts to prevent foodborne illness, health officials have relied greatly on a group of booklets containing the 17 food service equipment standards of the National Sanitation Foundation. Few members of the general public have ever seen these NSF standards, but now they have been collected in a single volume and placed on the shelves of America's 1,300 largest libraries. In addition, NSF has presented copies of the 312-page book to the libraries of all U.S. universities and schools of public health. The National Sanitation Foundation is a non profit, non governmental environmental agency located in Ann Arbor, Michigan. It was originally housed in the school of public health of the University of Michigan.

'NSF Food Service Equipment Standards' is unlikely to make the best seller lists even though the 17 standards are a bulwark of defense against environmental hazards in the preparation, holding and serving of foods. The NSF standards contain hundreds of technical specifications and drawings which may be of little interest to lay persons, but which are professionally significant to public health officials and their staffs.

The NSF food equipment standards are enforced by law or policy in thousands of U.S. health jurisdictions. Each standard is the result of the combined efforts of health officers, industry experts and consumer representatives working as volunteers on NSF committees. Among the committee members are representatives of the U.S. Food and Drug Administration, numerous health jurisdictions at state and local levels, liaison officers from all of the U.S. armed forces and the Veterans Administration plus official representatives of such organizations as the American Dietetics Association, the American Public Health Association, the National Restaurant Association and the National Environmental Health Association.

Although NSF requirements for food service equipment do contain specifications for design and materials, they center around performance. All NSF standards stress "cleanability"—purposeful elimination of hiding places for food particles and vermin. The standards also require the maintenance of sanitizing temperatures in dishwashing equipment, adherence to temperture minimums for hot foods and temperature maximums for chilled or frozen foods. NSF specifications relating to construction include prohibitions against the use of toxic materials.

During the past 25 years, NSF has intensively distributed individual standards to public health officials and food service establishments," Robert M. Brown, president of NSF, stated. "Now, with the publication and free distribution of *'NSF Food Service Equipment Standards,'* we are able to give health educators, health science students and technical representatives of American consumers equal access to this information which affects the health of so many people."

NSF still maintains close ties with the school of public health at the University of Michigan. It also operates a national sanitation education program by means of seminars, publications and audio-visual materials.

Headquarters and testing laboratories of the National Sanitation Foundation are located in the NSF Building in Ann Arbor, Michigan. In addition to standards for food service equipment, there are more than 30 other NSF standards in such fields as plastics pipe, swimming pool filters and sanitation equipment for ships, mobile homes and recreational vehicles.

Affiliate Meetings

FLORIDA — March 15-17, 1977. Kayhler Plaza, Orlando.

IOWA—March 21, 1977. Scheman Continuing Education Building, Ames.

KENTUCKY — February 22-23, 1977. Stouffer's Inn, Louisville.

MINNESOTA—April 19 and April 21, 1977. Outstate educational meetings at Alexandria and Albert Lea.

ONTARIO—March, 1977. Holiday Inn, Highway #427, Etobioke.

PENNSYLVANIA — June 13-15, 1977. State College.

VIRGINIA — March 8-9, 1977. Donaldson Brown Center, VPI & SU, Blacksburg.

Shahani to Launch ACDPI Clinic

Dr. Khem Shahani, cultured product expert at the Department of Food Science and Technology, University of Nebraska, will keynote the 1977 Kultures and Kurds Klinic with the topic, "Cultured Food Products of the Future."

The Klinic-sponsored by the American Cultured Dairy Products Institute-will be held at the Holiday Inn-North, Cincinnati, Ohio on March 21-23. Purpose for the training school is to update cultured products plant personnel and allied tradesmen on new technology, culture developments, and effective quality control programs.

A national judging contest will be held in conjunction with the Klinic. Buttermilks, sour creams, yogurts, and cottage cheeses submitted by processors will be evaluated by experts. Winner of this event will receive the prestigious Neil C. Angevine Superior Quality Award.

Confirmed speakers for the Klinic at this time include: Dr. James Martin, South Dakota State University; Dr. Ebenezer Vedamuthu, Microlife Technics; Dr. Charles White and Bill Born, Dean Foods Co.; Erik Lundstedt and Dr. H. C. Olson, dairy consultants; Dr. Ronald Richter, University of Florida; Vance Grosser, Food Producers, Inc.; Dr. Ed Custer, Mississippi State University; Neil Angevine, Angevine-Funke; George Murphy, Abcor, Inc.; Dr. Emil Mikolajcik, Ohio State University; Charles Yeager, Beatrice Foods; Ed Sing, Moseley Laboratories; William Lane, Marschall Div.-Miles Labs; Dr. Douglas Emmons, Food Research Institute (Ottawa Canada); Dr. Harry Wildasin, H. P. Hood, Inc.; Joe Biltekoff, Bison Foods Co.

For additional information and/or advance registration forms, contact John Speer, ACDPI Treasurer, 910-17th Street, N.W., Washington, D.C. 20006 or Dr. C. B. Lane, ACDPI Secretary, P.O. Box 7813, Orlando, Florida 32804.

Calendar of Events

March 3-5, 1977. BAKING INDUSTRY SANITATION STANDARDS COMMITTEE MEETING. Hyatt Regency Chicago Hotel, Chicago, IL.

March 16, 1977. NRA ALLIED MEMBER SEMINAR. O'Hare Marriott Hotel, Chicago, IL.

March 21-25, 1977. MIDWEST WORKSHOP IN MILK AND FOOD SANITATION. Center for Tomorrow, Ohio State University, Columbus, Ohio. For information contact: John Lindamood, Department of Food Science and Nutrition, 2121 Fyffe Road, Ohio State University, Columbus, OH 43210.

March 28-April 1, 1977. SHORT COURSE ON MAXIMIZING CONSUMER SATISFACTION BY COMPUTERIZED FOOD MANAGEMENT AND DIET CONTROL. For food service and dietary executives. For information contact: Dr. Joseph L. Balintfy, College of Business and Management, University of Maryland, College Park, MD 20742.

March 29-31, 1977. WESTERN FOOD INDUSTRY CONFERENCE. Freeborn Hall, University of California at Davis. Registration fee: $18.00. For information contact: Robert C. Pearl, Food Science & Technology Department, University of California, Davis, CA 95616 (916) 752-0980.

April 5-7, 1977. NATIONAL CONTROLLED ATMOSPHERE RESEARCH CONFERENCE. Kellogg Center for Continuing Education, Michigan State University, East Lansing, Michigan. For further information contact: D. H. Dewey, Department of Horticulture, Michigan State University, East Lansing, MI 48824, or W. G. Chace, U.S. Department of Agriculture, ARS, Beltsville, MD 20705.

April 20-22, 1977. DAIRY AND FOOD INDUSTRIES SUPPLY ASSOCIATION 58TH ANNUAL MEETING. Beach Club Hotel, Naples, Florida. For information contact: DFISA, 5530 Wisconsin Ave., Washington, D.C. 20015 (301) 652-4420.

May 10-12, 1977. SECOND INTERNATIONAL POWDER & BULK SOLIDS HANDLING & PROCESSING SHOW. O'Hare International Trade & Exposition Center and the Regency O'Hare, Rosemont, Illinois. For information contact: Aaron Kozlov, Industrial & Scientific Conference Management, Inc., 222 West Adams St., Chicago, IL 60606 (312) 263-4866.

May 21-25, 1977. NRA RESTAURANT HOTEL - MOTEL SHOW. McCormick Place, Chicago, IL.

June 5-8, 1977. 37TH ANNUAL MEETING AND FOOD EXPO, INSTITUTE OF FOOD TECHNOLOGISTS. Philadelphia Civic Center, Philadelphia, Pennsylvania. More information is available from Dan Weber, Director of Convention Services, Institute of Food Technologists, 221 N. LaSalle St., Chicago, IL 60601.

June 9-11, 1977. INTERNATIONAL SYMPOSIUM ON SALMONELLA IN POULTRY AND PROSPECTS FOR CONTROL. University of Guelph, Guelph, Ontario, Canada. For further information contact: Office of Continuing Education, Johnston Hall, University of Guelph, Guelph, Ontario N1G 2W1, Canada.

June 13-15, 1977. ANNUAL CONFERENCE, NATIONAL ASSOCIATION OF COLLEGES AND TEACHERS OF AGRICULTURE. The Pennsylvania State University, University Park, Pennsylvania. For further information contact: Dr. Robert E. Swope, 217 Agricultural Administration Building, University Park, PA 16802.

October 5-7, 1977. SOUTHEASTERN NATIONAL FOODSERVICE SHOW. Atlanta Marriott Hotel, Atlanta, GA.

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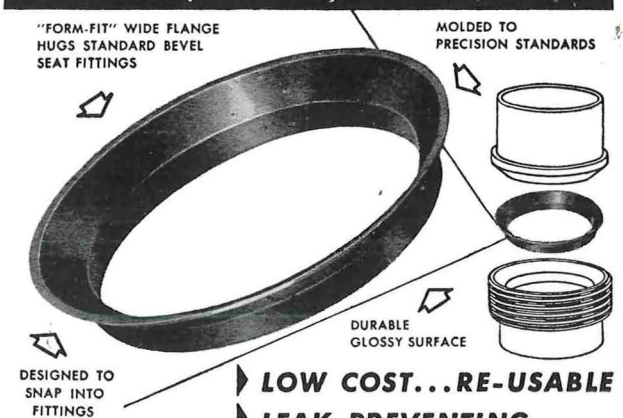

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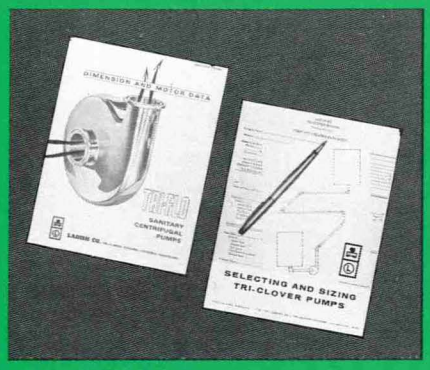

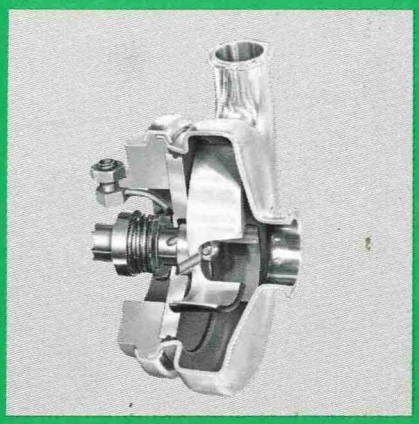

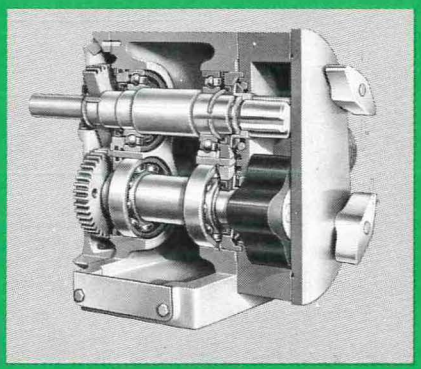

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P375

Dairy authorities speak out on better cow milking

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What's your score on vacuum?

An inadequate vacuum pump can affect the operation of the milking units drastically. Excessive "drop-off," slower milking, incomplete milking and an increase in the incidence of mastitis are likely results.

As a dairyman, your net dollar income depends on the vacuum pump and it probably is used more hours each year than most farm tractors.

Few people would hitch six plows behind a four plow tractor and head for the field to do a day's work. Yet many of these same people milk cows with a short vacuum supply and never question whether the pump is adequate.

Vacuum pumps used to be rated according to the number of units that could be operated. Today we measure the pump capacity in terms of Cubic Feet of air per Minute (CFM). Just as "horsepower" is more reliable than "plow rating" as an indication of tractor size, "CFM" is more reliable than "unit rating" when sizing a vacuum pump.

CFM output depends upon vacuum level. As vacuum level increases, the CFM output decreases. It's important to operate the system at the vacuum level specified by the manufacturer or the output of the pump will be altered.

The important consideration of any vacuum pump is the CFM output at the milking vacuum level. The pump must have adequate CFM output to meet the vacuum requirements of the system and provide sufficient reserve to maintain a constant vacuum level.

Vacuum Requirement
The milking unit is the most important of the machine components which admit air into the system. The air consumption of milking units varies depending upon shell and inflation size, pulsation rate and length and size of pulsated air tubes. Typically, the air requirement of a milking unit while it is not milking is three to four CFM. The pulsator consumes 50 to 70% of this volume. Considerably larger air pumping capacity (a reserve) must be provided to make a milking system operational. Other components which consume air are such things as vacuum operated door openers, milk metering devices, and the vacuum regulator. The requirements of each component must be added together to determine the system requirements.

Vacuum Reserve
The vacuum reserve is the air pumping capacity which remains after the vacuum requirement of all components has been satisfied. That's the problem. We've thought in terms of the vacuum reserve as the amount of CFM capacity that's *left over*. We really should be thinking in terms of a *base reserve* for the operator(s) before we begin to compute the system requirements.

The reserve is all-important in order to maintain vacuum stability. The reserve is necessary in order to make allowances for operator usage and possible leaks in the system or other contingencies.

The most important reason for an adequate vacuum reserve is to provide for the amount of air that the operator will use. The operator is the largest user of the vacuum reserve. Some operators are very wasteful of the available reserve. This occurs as units are being attached and removed. Improper unit adjustment is also a significant factor. When teat cups start to leak and "squeal" during milking, the vacuum reserve is depleted rapidly.

Some operators may deplete vacuum reserve as much as 30 or more CFM for short periods of time. The careful operator will use but half that amount during the milking process. The real test of any milking system is when a milking unit falls off. It takes huge reserves of air just to keep the remaining units on the cows. Reserve tanks aid a little during these occurrences but basically the vacuum pump must be relied upon to maintain vacuum level. It all adds up to the fact that an adequate pump is a *must* for every dairyman.

Research in Ireland, Wisconsin, Pennsylvania and California indicates that inadequate vacuum reserve is associated with higher leucocyte counts. In plain language it means that mastitis can result if your vacuum pump isn't large enough.

What's your Vacuum Score?
Don't make a mistake and just assume that your pump is putting out enough air. Have it checked with an air flow meter once a year. Many dealers are equipped to do this for you.

And how will you know for sure that they're not just trying to sell you a pump? Frankly, I've found most dealers to be very reliable in this respect.

There have been many different recommendations about pump sizes. It's hard to give one that's exactly right for each system. Here's a guide for you to check your vacuum needs. It's based upon the New Zealand Standard. The American Standard would give values equal to one-half of the New Zealand Standard.

For bucket users:
Allow 4 CFM per unit + 20 CFM base reserve.

For pipeline users:
Allow 5 CFM per unit + 40 CFM base reserve for the first operator and 20 CFM for each additional operator.

The resulting CFM values would give you the minimum size vacuum pump capacity. If your system has more than this, fine. If you have less vacuum capacity than this you should carefully investigate your vacuum needs.

This method of determining vacuum capacity is different from what you may have seen before. A 50 percent reserve is commonly used. While a 50 percent reserve may be satisfactory on a system of six or more units, our field studies indicate that using a 50 percent reserve is not adequate for the smaller system.

Remember this: There is no substitute for an adequate vacuum system. Make sure you know your score on your vacuum needs.