Journal of Food Protection Vol. 40, No. 10, Pages 693-697 (October, 1977)

Microflora of Retail Fluid Milk Products[1]

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(Received for publication January 7, 1977)

ABSTRACT

Numbers and types of microorganisms in retail pasteurized fluid milk products were determined as well as the effect that type of product, brand, and season of the year had on counts of 13 different microbial types. *Clostridium perfringens* was the only pathogen detected and it averaged less than one organism per milliliter. Chocolate milk samples generally had the highest mean counts, followed by skim milk, low-fat (2%), and whole milk (3.25%). Most brands had means for the various microbial counts which were not significantly different from each other. Only three brands had counts which differed significantly from other brands. Psychrotrophic, coliform, staphylococcal, yeast and mold, and Standard Plate Counts were highest between May and October, while counts for spores, streptococci, and thermophiles were highest between December and March. No seasonal trends were detected for counts of anaerobes, *C. perfringens*, enterococci, or lactobacilli.

The last comprehensive survey of the microflora of retail milk was conducted in 1953 (*1*). Many changes have occurred in the dairy industry since that time. Use of farm bulk tanks, every-other-day pick-up of farm milk, five-day-a-week plant operation, decreased home deliveries, once-a-week purchase by the consumer, as well as other factors, may have affected numbers and types of microorganisms present in retail fluid milk products (*25*). While several researchers have noted that the microflora of retail fluid milk products has shifted toward a largely psychrotrophic population (*14,17*), the exact effect that changes in the dairy industry have had on composition of the microflora of retail milk products has not been fully established.

This study was undertaken to determine the following: (*a*) major groups of microorgansims which comprise the microflora of fluid milk products at time of purchase; (*b*) numbers and types of selected pathogens present in these products, and (*c*) influence of season, brand, and product on microflora.

[1]*Published with the approval of the Director of the Kentucky Agricultural Experiment Station as journal aritcle no. 76-5-93*
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MATERIALS AND METHODS

Samples

Half-gallon or quart cartons of whole (3.25% milkfat), low-fat (2%), skim and chocolate milks were purchased from retail outlets in Lexington, Kentucky during the first week of each month for 1 year. Samples were purchased before their expiration dates from outlets with rapid turnover of fluid dairy products. Samples pruchased from these outlets were placed on ice, transported to the laboratory, and held at 4 ± 1 C until analyzed. All samples were analyzed within 8 h of purchase.

Microbial analyses

Coliform, psychrotrophic, spore, thermophilic, yeast and mold, and Standard Plate Counts were done according to *Standard Methods* (*2*). The confirmed test described in *Standard Methods* was used to verify as coliforms colonies obtained from Violet Red Bile Agar. Except as described below, procedures in *Bacteriological Analytical Manual for Foods* (*8*) were used for enumeration of enterococci, staphylococci (surface spread plates of Vogel and Johnson Agar), salmonellae (pre-enrichment in nutrient broth), and *Clostridium perfringens* (overlaid pour plates of Sulfite Polymyxin Sulfadiazine Agar (SPS) incubated anaerobically). The method of Angelotti et al. (*3*) was used to confirm as *C. perfringnes* all black colored colonies on SPS plates. Anaerobic counts were determined using Reinforced Clostridial Agar plates incubated anaerobically in BBL Gas Pak jars at 37 C for 48 h. Lactobacilli were enumerated using LBS agar (BBL) enriched with 20% sterile tomato juice obtained by filtering canned whole tomatoes. Plates with LBS agar were incubated for 48 h at 37 C. Streptosel Agar plates incubated at 37 C for 48 h were used to enumerate streptococci.

Statistical analyses

Counts of less than 30 were used only when obtained on the lowest dilution plated. Data were analysed using the Statistical Analysis System of Barr and Goodnight (*4*). Differences among counts from various products, brands, and months were determined using a Least Significant Difference (LSD) procedure (*23*).

RESULTS AND DISCUSSION

The 377 fluid milk samples analyzed represented eight brands with 96 samples of whole milk (3.25% milkfat), 92 samples of low-fat milk (2%), 96 samples of skim milk, and 93 samples of chocolate milk.

Neither salmonellae nor coagulase-positive staphylococci were detected in any of the samples examined. *C. perfringens* was the only pathogen detected and it averaged less than one per milliliter. Results obtained for salmonellae were similar to those obtained by other investigators (*13,21*) and were not surprising since no outbreaks of *Salmonella*-related illness have been linked to consumption of pasteurized retail fluid milk products during recent years in the United States (*9*). However, failure to detect coagulase-positive staphylococci differs from results obtained by Foltz et al. (*7*) and Sheikh and Luedecke (*22*) who reported presence of coagulase-postive staphylococci in 3.4% and 4.9% of the retail dairy products they examined, respectively. Differences in results could have been due to differences in sources of samples as well as the medium used for enumeration and isolation of staphylococci.

Mean logarithms, standard deviations, and range of logarithms obtained for the various microbial counts for each of the four milk products are in Table 1. The Least Significant Difference (LSD) procedure was used to determine if the counts between products differed significantly (Table 1). Mean log counts obtained from chocolate milk samples for anaerobes, coliforms, enterococci, lactobacilli, psychrotrophs, spores, Standard Plate, staphylococci and streptococci were higher ($P < .01$) than those obtained from the three non-chocolate milk samples. Skim milk samples tended to have second highest values for most counts, while whole milk samples tended to have lowest values. However, few of the differences in counts among the non-chocolate products were significant. These results are in agreement with those obtained by Langlois et al. (*14*).

C. perfringens counts of whole milk samples were higher ($P < .01$) than those of the other three products. However, because of the small numbers detected in this study, it was not possible to determine if the higher counts represented a real difference or were due to random variation in counts because of errors in enumeration and confirmation procedures. Illness should not result from consumption of milk containing the low numbers of *C. perfringens* found in this study, since 5×10^8 to 5×10^9 cells are necessary to cause illness (*6,12*).

Spore and thermophilic counts were similar in that highest counts for both were obtained from chocolate milk samples and lowest counts from whole milk samples. The similarities between these two counts suggest that some thermophilic bacteria also were spore-formers. These results agree with those obtained by Hansen (*11*). Spore counts obtained from chocolate milk could be expected, since cocoa powders contain spores which survive pasteurization (*10*).

Staphylococcus counts were higher ($P < .01$) in chocolate milk samples than in non-chocolate milks. No significant differences were detected among staphylococcus counts from non-chocolate milks. In fact, average staphylococcus counts for chocolate milk samples were three times the counts for skim milk samples, almost four times those for whole milk samples, and five times those for low-fat milk samples. All staphylococcus colonies tested were coagulase-negative.

TABLE 1. *Mean logarithms, standard deviation, and range of microbial counts obtained from fluid milk products obtained from retail outlets*

Products:	Whole	Low-fat	Skim milk	Chocolate
No. of samples:	96	92	93	93
Microbial counts (log_{10}/ml)				
Anaerobic	1.92 ± 0.90^a (0.00 – 3.18)	2.11 ± 0.91^a (0.00 – 2.88)	2.11 ± 1.06^a (0.00 – 3.04)	2.77 ± 1.12^b (1.45 – 2.75)
Clostridium perfringens	0.06 ± 0.22^a (0.00 – 1.30)	0.04 ± 0.17^b (0.00 – 1.00)	0.00 ± 0.00^b (0.00 – 0.00)	0.02 ± 0.12^b (0.00 – 1.00)
Coliform	0.12 ± 0.52^a (0.00 – 3.67)	0.16 ± 0.70^a (0.00 – 5.89)	0.18 ± 0.54^a 0.00 – 2.95)	0.42 ± 1.05^b (0.00 – 3.65)
Enterococci	0.12 ± 0.40^a (0.00 – 2.15)	0.05 ± 0.31^a (0.00 – 1.30)	0.16 ± 0.53^a (0.00 – 1.70)	0.30 ± 0.67^b (0.00 – 2.79)
Lactobacilli	0.07 ± 0.27^a (0.00 – 1.30)	0.03 ± 0.18^a (0.00 – 1.00)	0.02 ± 0.18^a (0.00 – 1.78)	0.13 ± 0.52^b (0.00 – 3.11)
Psychrotrophic	1.88 ± 2.10^a (0.00 – 7.69)	1.90 ± 2.23^a (0.00 – 8.86)	2.74 ± 2.28^b (0.00 – 7.53)	3.76 ± 1.97^c (0.00 – 7.75)
Spore	1.88 ± 0.50^a (0.60 – 2.60)	1.93 ± 0.48^a (0.78 – 2.51)	1.88 ± 0.47^a (0.95 – 2.58)	2.27 ± 0.53^b (1.60 – 4.76)
Standard Plate	3.22 ± 1.27^a (1.70 – 7.30)	3.36 ± 1.55^a (1.30 – 8.51)	3.72 ± 1.60^a (2.00 – 6.46)	4.07 ± 1.43^b (1.90 – 7.26)
Staphylococci	0.08 ± 0.39^a (0.00 – 3.30)	0.06 ± 0.33^a (0.00 – 1.70)	0.10 ± 0.38^a (0.00 – 1.48)	0.30 ± 0.62^b (0.00 – 2.78)
Streptococci	1.41 ± 0.97^a (0.00 – 3.99)	1.70 ± 1.43^a 0.00 – 8.90)	1.44 ± 1.23^a (0.00 – 3.97)	2.11 ± 1.46^b (0.00 – 6.23)
Thermophilic	1.80 ± 0.62^a (0.00 – 3.34)	1.99 ± 0.60^b (0.00 – 2.79)	1.99 ± 0.72^b (1.00 – 5.69)	2.15 ± 0.56^b (1.00 – 3.15)
Yeast and mold	0.17 ± 0.49^{ab} (0.00 – 2.11)	0.06 ± 0.21^c (0.00 – 1.11)	0.10 ± 0.32^{ab} (0.00 – 1.00)	0.23 ± 0.75^b (0.00 – 6.30)

[a,b,c]Means with different superscripts are different ($P < .01$) within rows by LSD.

The ability of coagulase-negative staphylococci to produce enterotoxin was not determined. However, based on other studies (*5,16,19,24*) few, if any, coagulase-negative staphylococci would have been expected to produce toxin.

While yeast and mold counts obtained from low-fat samples were lower (P < .01) than counts from the other three products, counts obtained from the four milk products averaged less than 2 per milliliter. Thus, any differences in counts could have been due to error inherent in the enumraton procedure.

Flavor evaluations of milk samples were not included in this study. However, if the population levels of psychrotrophic bacteria established by Punch et al. (*18*) as indicating flavor defects are used, then 5.0% of the samples should have had off-flavors. These off-flavors should have been present in 5.2% of whole milk samples, 5.4% of low-fat samples, 5.3% of skim samples, and 4.3% of chocolate samples. Chocolate milk had the greatest number of samples exceeding both the 20,000/ml and 10 coliform/ml limits, but the smallest number of samples exceeding the off-flavor limits of Punch et al. This result is possible because the limits of Punch et al. were extremely high (5 million/ml) and were established using psychrotrophic counts rather than coliform or Standard Plate Counts.

Higher counts were obtained in a study conducted in 1953 (*1*) than were obtained in this study. Streptococcus and yeast and mold counts were more than one log higher, while spore and Standard Plate Counts were about one-half a log higher than corresponding counts obtained in this study. It is impossible to determine the exact reasons for the higher counts obtained in the earlier study. However, when the 1953 survey was done, farm bulk tanks were not yet in widespread use. Thus milk was not refrigerated as quickly after milking as it is today. In addition, in the 1953 study, plates were incubated at 30 C for 5 days which could have accounted for some discrepancy in counts.

The log values obtained for counts from all samples were averaged and plotted against month; results are in Fig. 1. Since no significant differences were observed among the monthly counts obtained for anaerobic, *C. perfringens*, enterococcus, or lactobacillus counts, these counts were omitted from the figure. Standard Plate Counts for the month of April were not determined. Counts obtained for spores and thermophiles were so similar that the two counts were averaged and represented by one curve.

Counts for psychrotrophs, coliforms, Standard Plate, staphylococci, and yeast and molds were highest from May through October, while counts for spores, streptococci, and thermophiles were highest from December through March.

Curves for psychrotrophic and Standard Plate Counts were similar, with highest values occurring in June and October and lowest counts in December and March. However, psychrotrophic counts showed a greater

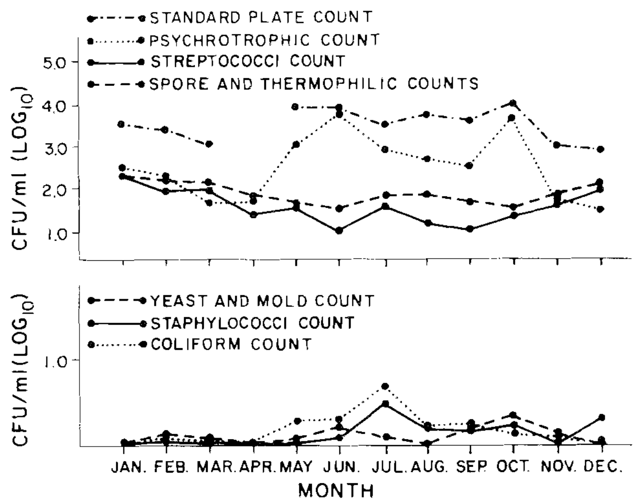

Figure 1. *Selected microbial counts obtained from retail fluid milk products purchased monthly.*

month-to-month variation than did the Standard Plate Counts. These findings disagree with those of Macy (*15*), who reported that season had no effect on Standard Plate Counts.

The curve for spore and thermophilic counts, which was highest during the winter months, roughly paralleled the curve for streptococcus counts. These counts may suggest a greater degree of contamination by dust during the winter months due to the greater amount of feed required to maintain animals during the colder weather. Higher spore counts obtained during the winter months are in agreement with the findings of Ridgeway (*20*).

A seasonal trend for yeast and mold counts is suggested by data in the figure. However, the small numbers obtained make it difficult to establish whether a season trend actually existed or if the differences obtained were due to errors inherent in the plating procedure. Likewise, enumeration errors could have masked any seasonal trends that may have been present for counts of anaerobes, *C. perfringens* enterococci, or lactobacilli.

Mean logarithms, standard deviations, and range of logarithms obtained for the various counts made on each of the eight brands are in Table 2. In addition, the LSD procedure was used to determine if counts between brands differed significantly (Table 2).

Mean log counts from products of brand C were lower (P < .01) than those from most other brands for anaerobic, psychrotrophic, spore, Standard Plate, streptococcus, and yeast and mold counts. In addition, this brand had the lowest mean log coliform count; however, only the difference between it and the value obtained for brand D was significant.

Counts obtained from samples of brand D were higher (P < .01) than those from most brands for anaerobic, coliform, and psychrotrophic counts, but were lower (P < .01) for thermophilic counts. These results suggest that products of this brand were produced under poor sanitary conditions or were improperly processed.

Counts of enterococci, lactobacilli, streptococci, and thermophiles were higher ($P < .01$) for products of brand A than they were from most other brands. These results could have been obtained if products of brand A were produced under insanitary conditions or were improperly processed.

The only statistically significant difference detected among the remaining brands (B, E, F, G, and H) was that spore counts from brand E were higher ($P < .01$) than those obtained from all but brand H.

In conclusion, retail fluid milk products should present no health hazard. Analysis of Standard Plate Counts showed that 15.6% of whole milk samples, 15.2% of low-fat milk samples, 30.1% of skim milk samples, and 39.7% of chocolate milk samples had counts that exceeded 20,000/ml. The following percentages of samples within each brand had Standard Plate Counts over 20,000/ml: brand A 19.1%; brand B, 18.7%; brand C, 2.3%; brand D, 51.2%, brand E, 13.6%, brand F, 31.7%; brand G, 34.0%; and brand H, 26.1%. The percentage of samples with counts over the 10/ml coliform limit were as follows: brand A, 8.3%; brand B, 10.4%; brand C 0%; brand D 16.6%; brand E, 2.0%; brand F, 8.3%; brand G, 2.0%; and brand H, 6.2%. Examination of coliform counts revealed that 4.1% of whole milk samples, 3.2% of low-fat samples, 6.4% of skim samples, and 12.9% of chocolate samples exceeded 10/ml.

TABLE 2. *Mean logarithms, standard deviation, and range of counts of different brands of fluid milk samples obtained from retail outlets*

Brand:	A	B	C	D	E	F	G	H
No. of samples:	48	48	47	42	48	48	48	48
Microbial count ($\log_{10}$/ml)								
Anaerobic	2.44 ± 1.21^{ab} (0.00 – 6.66)	2.38 ± 1.11^{ab} (0.30 – 5.27)	1.58 ± 0.73^{c} (0.00 – 3.47)	2.62 ± 1.28^{a} (0.00 – 5.77)	2.15 ± 0.50^{b} (1.00 – 4.47)	2.14 ± 0.84^{b} (0.00 – 3.96)	2.38 ± 1.03^{ab} (0.00 – 5.17)	2.08 ± 1.17^{b} (0.00 – 6.17)
C. perfringens	0.02 ± 0.14^{a} (0.00 – 1.00)	0.20 ± 0.14^{a} 0.00 – 1.00)	0.03 ± 0.16^{a} (0.00 – 1.00)	0.05 ± 0.25^{a} (0.00 – 1.30)	0.02 ± 0.10^{a} (0.00 – 0.60)	0.01 ± 0.09^{a} (0.00 – 0.60)	0.01 ± 0.09^{a} (0.00 – 0.60)	0.03 ± 0.16^{a} (0.00 – 1.00)
Coliform	0.28 ± 0.75^{a} (0.00 – 3.65)	0.25 ± 0.66^{a} (0.00 – 2.94)	0.01 ± 0.08^{a} (0.00 – 0.60)	0.58 ± 1.28^{b} (0.00 – 5.88)	0.07 ± 0.24^{a} 0.00 – 1.27)	0.23 ± 0.78^{a} (0.00 – 3.39)	0.13 ± 0.77^{a} (0.00 – 5.32)	0.19 ± 0.65^{a} (0.00 – 2.80)
Enterococci	0.43 ± 0.81^{a} (0.00 – 3.47)	0.12 ± 0.38^{b} (0.00 – 1.77)	0.13 ± 0.43^{b} (0.00 – 2.11)	0.16 ± 0.46^{b} (0.00 – 1.84)	0.16 ± 0.64^{b} (0.00 – 3.84)	0.07 ± 0.28^{b} (0.00 – 1.47)	0.05 ± 0.36^{b} (0.00 – 2.51)	0.09 ± 0.36^{b} (0.00 – 1.69)
Lactobacilli	0.21 ± 0.68^{a} (0.00 – 3.11)	0.06 ± 0.24^{b} (0.00 – 1.00)	0.04 ± 0.20^{b} (0.00 – 1.00)	0.06 ± 0.30^{b} (0.00 – 1.69)	0.09 ± 0.32^{ab} (0.00 – 1.30)	0.00 ± 0.00^{b} (0.00 – 0.00)	0.02 ± 0.14^{b} (0.00 – 1.00)	0.00 ± 0.00^{b} (0.00 – 0.00)
Psychrotrophic	2.96 ± 2.43^{ab} (0.00 – 7.74)	2.66 ± 1.84^{ab} (0.00 – 6.78)	1.17 ± 1.36^{c} (0.00 – 5.46)	3.75 ± 2.35^{d} (0.00 – 8.86)	1.65 ± 1.85^{bc} (0.00 – 6.53)	2.82 ± 2.47^{ab} (0.00 – 7.41)	3.34 ± 2.54^{ad} (0.00 – 7.82)	2.20 ± 2.07^{bc} (0.00 – 6.59)
Spore	2.01 ± 0.57^{a} (1.00 – 3.44)	2.00 ± 0.52^{a} (0.94 – 3.20)	1.82 ± 0.49^{b} (0.60 – 2.91)	2.00 ± 0.62^{a} (1.00 – 3.00)	2.18 ± 0.31^{c} (1.38 – 2.58)	1.88 ± 0.38^{ab} (1.04 – 2.55)	1.96 ± 0.48^{ab} (1.04 – 3.64)	2.02 ± 0.64^{ac} (0.00 – 3.20)
Standard plate	3.82 ± 1.41^{ab} (2.27 – 7.25)	3.73 ± 1.16^{ab} (2.20 – 6.78)	2.55 ± 0.80^{c} (1.30 – 5.34)	4.31 ± 1.71^{a} (2.00 – 8.50)	3.33 ± 1.22^{b} (1.95 – 7.94)	3.76 ± 1.61^{ab} (1.69 – 7.23)	3.86 ± 1.90^{ab} (1.30 – 8.27)	3.37 ± 1.36^{b} (2.04 – 7.30)
Staphylococci	0.26 ± 0.63^{a} (0.00 – 2.77)	0.17 ± 0.47^{ab} (0.00 – 1.95)	0.02 ± 0.15^{b} (0.00 – 1.04)	0.24 ± 0.67^{a} (0.00 – 3.30)	0.09 ± 0.35^{ab} (0.00 – 1.69)	0.00 ± 0.00^{b} (0.00 – 0.00)	0.09 ± 0.37^{ab} (0.00 – 1.95)	0.15 ± 0.51^{ab} (0.00 – 2.51)
Streptococci	2.24 ± 1.40^{a} (0.00 – 6.23)	1.44 ± 1.05^{bc} (0.00 – 5.14)	1.15 ± 1.07^{c} (0.00 – 3.34)	1.97 ± 1.83^{ab} (0.00 – 8.89)	1.55 ± 1.14^{bc} (0.00 – 5.04)	1.50 ± 1.06^{bc} (0.00 – 4.20)	1.90 ± 1.24^{ab} (0.00 – 5.44)	1.53 ± 1.30^{bc} (0.00 – 4.85)
Thermophilic	2.18 ± 0.34^{a} (1.47 – 3.14)	1.94 ± 0.57^{abc} (0.00 – 2.84)	1.96 ± 0.91^{abc} (0.00 – 5.69)	1.79 ± 0.86^{c} (0.00 – 3.34)	2.10 ± 0.33^{ab} (1.39 – 2.77)	1.88 ± 0.37^{bc} (1.00 – 2.74)	1.92 ± 0.56^{bc} (0.00 – 3.47)	2.02 ± 0.78^{ab} (0.00 – 3.50)
Yeast and mold	0.23 ± 0.95^{ab} (0.00 – 6.30)	0.13 ± 0.31^{abc} (0.00 – 1.11)	0.01 ± 0.06^{c} (0.00 – 0.30)	0.29 ± 0.56^{a} (0.00 – 2.11)	0.22 ± 0.52^{ab} (0.00 – 2.47)	0.08 ± 0.31^{abc} (0.00 – 1.71)	0.06 ± 0.26^{bc} (0.00 – 1.34)	0.06 ± 0.29^{bc} (0.00 – 1.74)

[a,b,c,d]Means with different superscripts are different ($P < .01$) within rows by LSD.

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