Characterization of Pasteurized Fluid Milk Shelf-life Attributes

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ABSTRACT: Pasteurized fluid milk samples were systematically collected from 3 commercial dairy plants. Samples were evaluated for microbial, chemical, and sensory attributes throughout shelf life. In general, product shelf lives were limited by multiplication of heat-resistant psychrotrophic organisms that caused undesirable flavors in milk. The predominant microorganisms identified were Gram-positive rods including *Paenibacillus*, *Bacillus,* and *Microbacterium*. Principal component analysis of sensory data collected using quantitative descriptive analysis showed that attributes related to milk flavor defects explained the largest amount of variance. These findings highlight the need to develop specific strategies for excluding bacterial contaminants from milk to further extend product shelf lives.

Keywords: shelf life, fluid milk, spoilage, quantitative descriptive analysis, principal component analysis

Introduction

Per capita consumption of fluid milk in the United States has decreased steadily over the past 30 years (ERS/USDA 2001). Highly perishable fluid milk products must compete in the marketplace against shelf-stable beverages that have captured a large proportion of the beverage market in recent years (IDFA 2003). Extending fluid milk shelf life may enable processors to maintain a competitive position in the beverage market by facilitating the production of high-quality milk products that allow distribution over wider geographic areas and through new outlets (for example, vending machines).

Currently, bacterial spoilage is the most limiting factor in extending the shelf life of conventionally pasteurized high-temperature short-time (HTST) processed fluid milk products beyond 14 d (Boor 2001). Microbial growth and metabolism shorten the shelf life of milk by producing undesirable changes in aroma and taste attributes that influence consumer acceptability of the products. Processed fluid milk microbial spoilage may be attributed to either Gram-negative bacteria that contaminate milk postpasteurization or to Gram-positive organisms, some of which are able to survive pasteurization temperatures (Ternström 1993; Boor and Murphy 2002). Psychrotrophic Gram-negative organisms have been reported as common postpasteurization contaminants of commercial fluid milk, with filling machines identified as the main contamination reservoir (Schröder 1984; Cromie 1991; Gruetzmacher and Bradley 1998). Control and elimination of these Gram-negative postpasteurization contaminants in the processing environment has not only led to product shelf-life improvement, but has also uncovered the next bacterial hurdle to further extending HTST fluid milk shelf life (Ralyea and others 1998). The presence of high numbers of Gram-negative postpasteurization contaminants has typically masked the presence of smaller numbers of heat-resistant, psychrotrophic Gram-positive bacteria such as *Bacillus* spp. and *Microbacterium* spp. These bacteria generally cause spoilage in milk products processed in dairy plant environments where Gram-negative postpasteurization contamination has been controlled and longer product shelf lives are expected (Champagne and others 1994; Ralyea and others 1998). These Gram-positive organisms can be present in raw milk, but they also may enter milk products at various points during production and processing (Griffiths and Phillips 1990; Schraft and others 1996; Svensson and others 1999). Further extension of product shelf lives will require elimination of these heat-resistant, Gram-positive contaminants. To that end, these contaminants and their reservoirs must be definitively identified. As a 1st step toward further extending fluid milk shelf lives, the objectives of this study were to isolate and identify the predominant microorganisms present in fluid milk products initially and at 7 d, 14 d, and 17 d postprocessing and to develop a quantitative descriptive analysis (QDA) approach to determine the key sensory attributes associated with HTST milk throughout shelf life.

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Materials and Methods

Milk sample collection and handling

Three fluid milk processing plants in New York state were sampled 4 times each between October 2002 and February 2003, with an approximate 1-mo interval between sampling times. The plants were selected on the basis of performance history in the ongoing Cornell Univ. Milk Quality Improvement Program (MQIP; Boor 2001). Processing conditions for 2% HTST milk at each of the selected plants are listed in Table 1.

A plant sampling consisted of the collection of multiple 2% fat HTST pasteurized fluid milk samples that had been packaged in 3-layer (polyethylene, paperboard, polyethylene) gable-top 1-qt volume (0.946 L) cartons and 1 raw milk sample. Per plant visit, 1 118-mL raw milk sample was collected into a 4-oz. vial from the silo containing the milk used to manufacture the pasteurized products analyzed in the study. A total of 9 1-qt processed milk cartons were randomly selected for each of the 3 plants (A to C) on the same day. The sampling strategy consisted of selecting 4 products from the early part of the packaging run, 2 from the middle, and 2 from the latter part to account for sample variability that occurs during a packaging run. Milk samples from plants A and B were collected the day after production, placed into a plastic container with ice or

a cooler with freezer ice packs, respectively, and immediately transported to the laboratory. Plant C milk samples were collected by plant personnel 1 d after production and placed into a cooler with freezer ice packs for overnight shipping to the laboratory. In addition to the samples collected for testing, a 1-qt carton was included in each cooler to serve as a reference temperature check. Raw and processed milk samples were transported in coolers kept below 4.0 °C. Upon receipt, the milk samples were held at 6 °C until plating, which occurred within 3 h of arrival.

Before microbial, chemical, and sensory analyses, the pasteurized milk samples for each plant were aseptically commingled and distributed in duplicate into 3 sterile 1000-mL glass bottles for a total of 6 bottles per plant (900 mL of milk/bottle). These bottles were labeled, stored at 6 °C, and left unopened until the appropriate times for microbiological, chemical, and sensory testing. The samples remaining in the commingling containers were used for initial day testing, which began within 24 h of collection for plants A and B and within 48 h for Plant C. Plant C did not process 2% milk on the same day as Plants A and B. To enable all sensory work to be conducted on the same day for all comparable samples, for each test period, Plant C samples were collected the day before samples were collected at the other plants. Sample testing was carried out at 7 d, 14 d, and 17 d postpasteurization, except for Plant C samples, which were tested at 8 d, 15 d, and 18 d postpasteurization. For simplicity, however, results from all plants are reported throughout this article as obtained on initial, 7 d, 14 d, and 17 d postpasteurization. On each evaluation date, milk from duplicate bottles was used for all the chemical, microbial, and sensory tests. Samples for microbial tests were aseptically drawn 1st to avoid contamination.

Microbiological analyses

Raw milk samples from each of the 3 plants were evaluated for total bacterial count (TBC), coliform count (CC), laboratory pasteurized count (LPC) (Marshall 1992), and somatic cell count (SCC). TBC and CC were also performed on pasteurized milk samples on each shelf-life testing day. For TBC, samples were serially diluted in Butterfield phosphate buffer (Weber Scientific, Hamilton, N.J., U.S.A.) and spread plated on plate count agar (Difco, Sparks, Md., U.S.A.). CC was obtained by pour-plating samples on violet red bile agar (Difco). Laboratory pasteurization consisted of heating raw milk samples to 63 °C for 30 min, immediately cooling to 10 °C, and plating on plate count agar. All samples were plated in duplicate and incubated at 32 °C. The numbers of colony-forming units (CFU) per milliliter were counted and recorded after the appropriate incubation times, according to standard methods (Marshall 1992; method number 6.2, 7.8A). Raw milk samples were submitted to a local analytical lab (DairyOne, Ithaca, N.Y., U.S.A.) for SCC measurement. The samples were analyzed using a Fossomatic 5000 (FOSS, Eden Prairie, Minn., U.S.A.).

Chemical analyses of processed milk

Chemical analyses were performed in duplicate on shelf-life test day (for example, initial, 7 d, 14 d, 17 d postprocessing) for all processed milk samples. The free fatty acid content, expressed as acid degree value (ADV) meq FFA/100 g of fat, was used to determine the extent of lipolysis in processed milk samples throughout shelf life. The ADV was determined by a modified copper soap method (Shipe and others 1980; Ma and others 2003). The Kjeldahl method was used to determine total nitrogen (TN) (AOAC 2000; method number 991.20; 33.2.11), nonprotein nitrogen (NPN) (AOAC 2000; method number 991.21; 33.22.12), and noncasein nitrogen (NCN) (AOAC 2000; method number 998.05; 33.2.64). Nitrogen results were expressed as a protein equivalent using a conversion factor of 6.38. True protein (TP) and casein nitrogen (CN) were calculated as (TN-NPN) × 6.38 and (TN-NCN) × 6.38, respectively. The calculation for casein as a percentage of TP (CN/TP) was (CN/TP) × 100%. CN/TP served as an indicator of proteolysis.

Table 1—Processing parameters for plants A, B, and C

Processing parameter	Plant A	Plant B	Plant C
Pasteurization conditions	79 °C/18 s	79.4 °C/22 s	79.4 °C/28 s
Average days in code	21	15	15
Frequency of processing	1 d/wk	5 d/wk	4 d/wk
Average volume of milk processed (kg/y)	544000	54432000	73025000
Volume of unflavored milks (kg/y)	408000	53071000	55784000
Packaging equipment	Tetra Pak TR7	Cherry Burrell Q-7	Evergreen Q-11
Order of 2% packaging	2nd	Varies	2nd

Bacterial isolation and identification

Between 1 and 4 colonies representing the predominant microflora on a given TBC plate were selected through visual observation from each processed and lab-pasteurized milk sample plate that was used for enumeration. The selected colonies were streaked to isolation on brain heart infusion agar plates (Difco). The strategy consisted of picking 1 colony to represent every visibly different morphology on each plate, resulting in a maximum of 8 colonies obtained per milk sample. Each of the 223 isolates collected was characterized by classical biochemical tests, including Gram determination using a 3-step Gram stain kit (Becton, Dickson and Co., Sparks, Md., U.S.A.) and the KOH test, oxidase test with BBL® DrySlide™ (Becton, Dickson and Co.), and catalase reaction using 3% H_2O_2.

Identification of the isolates to the genus and species level was carried out using API 50 CH strips and CHB medium (bioMérieux Inc., Hazelwood, Mo., U.S.A.) and by partial 16s rDNA sequencing. Lysozyme/proteinase-K DNA lysates were prepared as previously described (Furrer and others 1991). A 2-mL volume of the lysates was used for polymerase chain reaction (PCR). Fragments of approximately 700 bp of the 16s rDNA sequence were amplified using primers PEU 7 (5′-GCA AAC AGG ATT AGA TAC CC-3′) (Rothman and others 2002) and DG74 (5′-AGG AGG TGA TCC AAC CGC A-3′) (Griesen 1994). PCR conditions consisted of 30 cycles of 94 °C for 1 min, 50 °C for 1 min, and 72 °C for 1.5 min. The PCR products were purified with the QIAquick PCR purification kit (Qiagen Inc., Valencia, Calif., U.S.A.) and were directly sequenced with an Applied Biosystems ABI 3700 automated DNA sequencer using primers PEU7 and 16S-P3SH (5′- CTA CGG TTA CCT TGT TAC GAC TT-3′) (Ralyea and others 1998). Microbial identification was accomplished through Basic Local Alignment Search Tool (BLASTN; available from: http://www.ncbi.nlm.nih.gov/BLAST; accessed 18 Sept. 2004) search analysis to compare the obtained sequence data with 16s rDNA sequence data stored in GenBank (Altschul and others 1990).

Sensory panel recruitment and training

A group of 13 panelists (3 males and 10 females between 22 and 50 y of age), consisting of staff and graduate students from the Cornell Univ. Dept. of Food Science, was recruited and trained to evaluate processed fluid milk products using a quantitative descriptive analysis (QDA) methodology (Stone and Sidel 1993). The use of human subjects in this study was reviewed and approved by the

Cornell Univ. Committee on Human Subjects. Panelists were recruited on the basis of perceived verbal skills, interest, and availability. All panelists received payment for participation. A total of 9 1-h working sessions were held during a 4-wk period. Training sessions consisted of tasting 2% fluid milk samples at various points in shelf life, ranging from freshly pasteurized to code date, to provide a range of flavor characteristics. A descriptive language to evaluate aroma, taste, and aftertaste of fluid milk products was developed through group discussions. Subsequent training sessions centered on familiarizing the panel with the newly generated ballot and on refining terminology. Panelists were provided with physical reference standards for each descriptor, as shown in Table 2, which were determined by panel consensus. After language development, the panel evaluated samples of HTST pasteurized 2% fat fluid milk products. Evaluation consisted of tasting and scoring the perceived intensity of aroma, taste, and aftertaste attributes in milk samples varying in degree of freshness by use of the descriptive terms previously developed. Attributes were quantified using an intensity scale ranging from 0 to 15 (0 = not detected and 15 = strong). The scale was anchored on the left and on the right with the terms "none" and "strong," respectively. During this phase of training, panelists were allowed to discuss attributes and to become familiar with the use of the scale. The category "other" was included to allow panelists to describe any perceived aroma, taste, and aftertaste characteristics for which descriptors were not listed in the ballot. The final training session introduced the use of computerized sensory data collection software (Compusense® *five*, release 4.6, Compusense Inc., Guelph, Ont., Canada).

Table 2—Descriptive terms and references used to evaluate pasteurized fluid milk

Attribute	Reference
Aroma	
Cheese	Colby cheese
Cooked	Freshly pasteurized milk (79 °C/18 s)
Cream	Heavy cream
Hay/Grain	Hay
Sulfur	Boiled egg
Sour/Fermented	Sauerkraut
Putrid	Limburger cheese
Taste	
Baby formula	Baby formula
Butter	Butter
Cooked	Freshly pasteurized milk (79 °C/18 s)
Flat	2% fat milk with 3% added water
Nutty	Peanuts
Rancid	Strong provolone cheese
Sweet	1:1 100% lactose free Lactaid and 2% fat milk
Aftertaste	
Cardboard	Cardboard box
Sweet	1:1 100% lactose free Lactaid and 2% fat milk
Sour	Cultured buttermilk
Metallic	$FeSO_4$ (0.445 g/L)
Drying	Unsweetened tea
Lingering	—

Sample preparation for sensory evaluation

The glass bottles containing milk samples were removed from refrigerated storage and mixed by inverting 25 times. Approximately 50 mL were poured into 148-mL translucent plastic cups labeled with a randomly assigned 3-digit code. Samples were poured in dim light to minimize light oxidation of the product, and the cups were covered with a plastic lid. Milk samples were then placed in cardboard boxes designated for each panelist and stored at 6 °C until evaluation, which occurred within 5 h. Just before evaluation, samples were removed from refrigerated storage and warmed up to approximately 15 °C by placing the cardboard boxes in a microwave oven and heating for 22 s at a predetermined setting. Each panelist independently scored a set of 6 samples presented in a randomized order at each testing session. Panelists evaluated milk samples according to the method described by Chapman and others (1998). Spring water and unsalted crackers were provided to cleanse the palate between samples.

During evaluation, panelists were provided with definitions for each term as well as a complete list of the attributes and references (Civille and Lyon 1996). References were provided when needed to describe or clarify an attribute. As proposed by the panel, references for cheese, cooked, cream, sweet, and baby formula attributes were provided at each tasting session.

Statistical analysis

All statistical analyses were performed using the SAS system ver. 8 (SAS Inst. Inc., Cary, N.C., U.S.A.). The microbial and somatic cell count data were log10 transformed. Raw milk microbial counts among plants were analyzed using a mixed model (PROC MIXED), with plant as a fixed effect and sampling period and its interaction with plant as random effects. Least square means were used to examine significant differences. A mixed model, with plant and day as fixed effects, was also used to analyze differences in mean microbial counts, free fatty acid levels, and casein content of the fluid milk products among shelf-life test day and plants. Where the F-test indicated a significant difference between means for day, plant, or day by plant interaction, Tukey paired comparisons were used to determine where the means were different. Significant differences were defined as $P < 0.05$.

Panelist consistency was assessed by estimating the variability between duplicate samples on each test day. The variance estimate of the residual after estimating the contribution of all random and fixed effects was used as an estimate of the variability between duplicate sample scores for each panelist. Data for 3 of the 13 panelists were not included in the analysis because of the high level of discrepancy observed in duplicate samples for all descriptors for these panelists. Principal components analysis was applied to the sensory data using PROC FACTOR in SAS. The analysis was used to obtain a smaller set of principal components (PCs) from the initial 20 descriptors used in milk product evaluation. The Kaiser criterion (eigenvalue > 1) and proportion of variance accounted for by each component were criteria applied to determine the final number of components to retain (Hatcher 1994). The factors were orthogonally rotated following the Varimax method. The resulting factor scores were used for further analysis to test for differences among means of test day and plants by the mixed procedure.

Results and Discussion

Raw milk

The bacterial quality of raw milk was assessed for each plant during each of the 4 sampling periods. Raw milk samples were taken from silos containing milk used to manufacture the pasteurized products analyzed in this study. Bacterial numbers in raw milk samples before and after laboratory pasteurization did not differ significantly among plants ($P > 0.05$) (Figure 1). Average raw milk bacterial counts were below the regulatory limit of 300000 CFU/mL before pasteurization and ranged between 12000 and 66000 CFU/mL. No differences in SCC were found among milk samples from different plants, with numbers typically ranging between 230000 and 283000 cells/mL. A total of 26 isolates were obtained from LPC

plates from sampling periods 3 and 4. The organisms isolated from LPC plates included Gram-positive cocci (42%), Gram-positive rods (38%), and Gram-variable rods (19%). Gram-variable rods were identified as *Microbacterium lacticum*. No Gram-negative organisms were isolated from lab-pasteurized milk samples. Other organisms identified included *Bacillus* spp., *Kocuria varians* (formerly *Micrococcus varians*), and *Streptococcus* spp. (Table 3). For plant A, organisms found in LPC plates included only *Bacillus* spp. and *Kocuria varians*. *Streptococcus* isolates were found only in laboratory-pasteurized milk samples and not in commercial samples.

Spoilage of processed milk

The bacterial quality of processed milk samples did not differ significantly ($P > 0.05$) among plants on the initial testing day, with no samples exceeding the 20000 CFU/mL legal limit (USFDA 2001). A significant plant by day interaction indicates that some plants had greater bacterial count differences among test day than others (Table 4). To illustrate, bacterial numbers were significantly different after 14 d of storage at 6 °C, relative to numbers present on initial day, for products from plants B and C, whereas no significant differences in microbial numbers were found for plant A products until day 17 testing (Figure 2). These results suggest that the processing plant at which products were collected significantly influenced the final microbial numbers.

At 7 d postprocessing, 8% of the samples tested had counts greater than 20000 CFU/mL. By day 14, 58% of the samples had greater than 20000 CFU/mL, and 42% had counts higher than 10^6 CFU/mL. Results show a rapid increase in bacterial numbers after 14 d postprocessing, as illustrated by 92% of samples with >20000 CFU/mL and 50% of samples with $>10^6$ CFU/mL after 17 d of refrigerated storage.

The most predominant microorganisms found were Gram-positive rods, which made up 87% of the processed milk microbial collection. Gram-positive cocci and Gram-negative rods made up 7% and 6% of the total processed milk bacterial isolates, respectively. Isolate distribution by shelf-life test day for all plants is shown in Table 5. The most common genera were *Paenibacillus* (39%), *Bacillus* (32%), and *Microbacterium* (14%). The majority of Gram-positive cocci identified were *Kocuria* (5%). The Gram-negative bacteria consisted of *Pseudomonas* (3%) and *Acinetobacter* (1%). Organisms belonging to a species for which 3 or fewer isolates were collected were grouped in the "other" category. Isolates classified as "unidentifiable" could not be characterized using API strips or 16s rDNA sequencing methods. Initial grouping into categories solely based on Gram stain showed that 43% of the total microorganisms isolated were Gram-positive rods, 38% Gram-negative rods, and 13% Gram-positive cocci. Further characterization to the genus and species level by a combination of API strips and 16s rDNA sequencing revealed that the majority of organisms initially categorized as Gram-negative rods were actually Gram-positive *Paenibacillus* spp., which yield variable Gram stain results. The relative proportion of

Table 3—Bacterial isolates collected from laboratory pasteurized count (LPC) plates

Species	Nr of isolates	% of isolates[a]
Bacillus	8	31
cereus	3	12
licheniformis	3	12
pumilus	1	4
lentus	1	4
Microbacterium lacticum	7	27
Kocuria	7	27
varians	6	23
kristinae	1	4
Streptococcus macedonicus	3	12
Other	1	4
Total	26	101

[a]Percentages were rounded to the nearest whole integer, resulting in a sum of 101.

Table 4—Mixed model results for microbial and chemical tests

	F value		
Source of variation	**TBC**	**CN/TP**	**FFA**
Day	54.96[a]	234.16[a]	23.11[a]
Plant	17.15[a]	33.66[a]	16.02[a]
Day × plant	3.14[a]	0.68[b]	0.50[b]

[a]$P < 0.01$.
[b]$P > 0.05$.

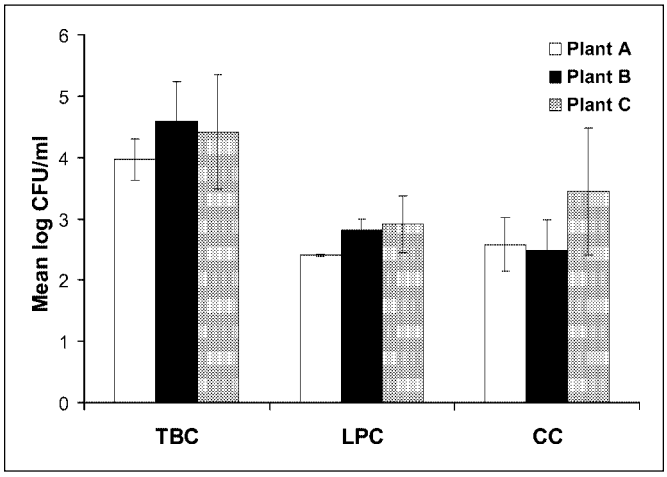

Figure 1—Bacterial numbers in raw milk samples as determined by total bacterial count (TBC), laboratory pasteurized count (LPC), and coliform count. Bars indicate standard deviations.

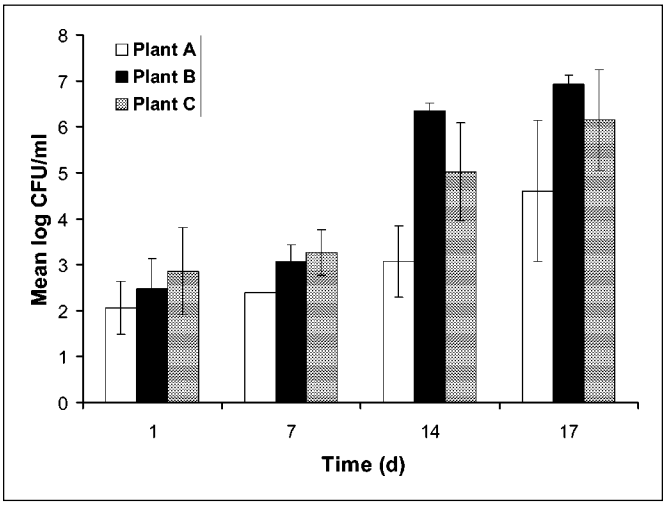

Figure 2—Bacterial numbers in commercial fluid milk samples stored at 6 °C throughout 17-d shelf life. Bars indicate standard deviations.

Table 5—Distribution of bacterial isolates collected from commercial fluid milk samples throughout shelf life

Bacterial isolate	Initial day	Day 7	Day 14	Day 17	Total nr isolates	% of isolates[a]
Paenibacillus	3	11	33	30	77	39
amylolyticus	0	3	6	5	14	7
spp	3	8	27	25	63	32
Bacillus	26	15	14	8	63	32
cereus	6	7	4	1	18	9
licheniformis	11	5	0	0	16	8
mycoides	1	0	3	4	8	4
stearothermophilus	3	1	0	0	4	2
pumilus	3	0	0	0	3	2
lentus	1	1	0	0	2	1
circulans	0	0	2	0	2	1
subtilis	1	0	0	0	1	1
spp	0	1	5	3	9	5
Microbacterium lacticum	16	10	1	0	27	14
Kocuria varians	4	5	0	0	9	5
Gram-negatives	1	1	0	6	8	4
Pseudomonas	1	1	0	4	6	3
Acinetobacter	0	0	0	2	2	1
Unidentifiable	1	2	1	3	7	4
Other	2	1	0	3	6	3
Total	53	45	49	50	197	101

[a]Percentages were rounded to the nearest whole integer, resulting in a sum of 101.

Paenibacillus isolates obtained from the milk samples increased over shelf life, whereas the number of *Bacillus* spp. isolates decreased, with the exception of *Bacillus mycoides* ($n = 8$), which were found mainly at day 14 and day 17. Similarly, the majority of *M. lacticum* isolates were collected in the early shelf-life test days; none were isolated at 17 d postpasteurization. This result suggests that *M. lacticum* has the ability to resist pasteurization temperatures but is less able to grow in fluid milk under refrigerated storage conditions than *Paenibacillus.* In the same way, *Bacillus* spp. was possibly outcompeted by *Paenibacillus*, which were the predominant isolates collected on the last shelf-life test day. These findings are consistent with those of Ternström (1993), who identified the largest group of Gram-positive spoilage bacteria in pasteurized fluid milk as *Bacillus polymyxa*, which is presently classified in the genus *Paenibacillus* (Jay 2000).

The distribution of the 223 total isolates by plant was as follows: 23% of the isolates were collected from plant A, 41% from plant B, and 35% from plant C. The difference in number of isolates collected for each plant reflects the differences in visually observable colony morphologies on plates from each plant during selection for isolation. Plant comparisons show that a higher proportion of *Paenibacillus* isolates were collected from plant B than plant A or C. Only 8% of the total number of *Paenibacillus* isolates collected in the study originated from plant A milk samples, representing 12% of the total isolates collected from this plant. *Bacillus* was the most common organism isolated from plant A samples, and approximately 56% of these *Bacillus* isolates were *B. cereus*. *B. licheniformis* was the only species of *Bacillus* isolated from all 3 plants. A small number of *B. mycoides* were identified in samples from plants A and B. For plants B and C, *Paenibacillus* spp. was the most common isolate collected. None of the isolates obtained from plant A were identified as *M. lacticum*, which was more commonly found in plant C. *Kocuria varians* was isolated only during initial and day 7 testing, and not during later test days.

Chemical analyses

To assess the extent of lipolysis and proteolysis in processed milk samples throughout shelf life, free fatty acid (FFA) content and casein as a percent of true protein (CN/TP) were measured. The FFA content of samples from all plants significantly increased throughout shelf life ($P < 0.001$). The effect of test day on FFA levels was not significantly different among plants, as demonstrated by the similar trend observed for all plants throughout shelf life (Figure 3). No significant differences in FFA content were found between samples tested on the initial day and day 7, but increases were significant at 14 d and 17 d. Increased FFA levels are a consequence of milkfat lipolysis and may contribute to rancid off-flavors (Shipe and Senyk 1981; Bishop and White 1986; Champagne and others 1994). Santos and others (2003) found that at a concentration of 0.25 meq of FFA/kg, 34% of sensory panelists were able to detect lipolyzed or rancid off-flavors in 2% fat milk. This proportion increased to 63% at levels of 0.35 meq FFA/kg. In our study, FFA content levels ranged between 0.08 and 0.50 meq FFA/kg of fat, with significantly higher levels found at the end of shelf life. Sensory results show that the rancid attribute measurements by the panel had a strong influence on the "defects" group of descriptors, as demonstrated by the attribute's high factor loading in principal component 1 (Table 6). As with FFA content, scores for this "defects" group of attributes also increased significantly toward the end of shelf life. *Pseudomonas fluorescens* has been identified as a major contributor to lipolytic spoilage (Fitz-Gerald and Deeth 1986; Champagne and others 1994); however, while FFA levels increased in all samples by day 17 postprocessing, *Pseudomonas* spp. were isolated from only 8% of the samples tested. Although rancid off-flavors in milk are often associated with the growth of lipolytic microorganisms, they may also result from the presence of indigenous lipoprotein lipase (Bodyfelt and others 1988).

A significant change in CN/TP was observed over time for samples from all plants ($P < 0.001$). Casein levels were predicted to decrease over time as a result of proteolytic activity. The average decrease in CN/TP was approximately 2% after 17 d of refrigerated storage for samples from all plants. As with FFA content, the effect of days on casein levels was not significantly different among plants. Figure 4 illustrates the changes in casein levels. An overall significant decrease in casein levels was found when comparing each of the shelf-life test days for all plants, indicating a relatively rapid decrease over time (Figure 4). Plant A was different than plants B and C, having higher levels of casein initially and throughout the duration of refrigerated storage. Proteolytic activity as a result of psychrotrophic microbial growth has been associated with off-flavors in fluid milk, particularly bitterness (Shipe 1978; Bodyfelt and others 1988; Ma and others 2000).

Quantitative descriptive analysis

A total of 5 PCs were retained from the PCA applied to the ratings of the 20 attributes used for milk sensory evaluation. Combined, these components accounted for 74% of the total variance and each displayed an eigenvalue greater than 1. The principal axis method was used to extract the components, followed by a Varimax (orthogonal) rotation to obtain uncorrelated components and facilitate interpretation of the solution (Hatcher 1994). The Varimax rotated factor loadings, which correspond to the correlation between the PC and the original attribute ratings (Chapman and others 2001), are listed in Table 6. An attribute was considered to load on a given component if the factor loading was greater than 0.50 for that component (shown in bold type) and less than 0.50 for all other components.

The attributes and the corresponding component on which they

Table 6—Varimax rotated principal component factor loadings for high-temperature short-time (HTST) pasteurized fluid milk attributes[a]

Attributes	PC1	PC2	PC3	PC4	PC5
Aroma					
Cooked	–11	6	6	**92**	3
Hay/grain	**78**	28	–7	17	–5
Sulfur	17	–12	31	33	**52**
Sour/fermented	**80**	2	13	–25	16
Putrid	5	17	–14	–11	**83**
Taste					
Baby formula	**66**	41	11	12	–15
Butter	42	25	**68**	19	–13
Cooked	–14	32	12	**85**	–3
Flat	17	**86**	16	5	–7
Nutty	**84**	5	18	–2	4
Rancid	**88**	13	4	–13	21
Sweet	13	31	**85**	7	–1
Aftertaste					
Cardboard	14	**65**	–9	45	–5
Drying	22	**73**	29	34	5
Lingering	15	**65**	22	22	14
Metallic	**83**	11	3	–7	2
Sour	14	**69**	22	–28	18
Sweet	–6	10	**87**	–1	6
Variance explained (%)	23.6	17.7	13.1	12.9	6.3

[a]Values are multiplied by 100 and rounded to the nearest integer. Factors whose unadjusted values are greater that 0.5 are shown in bold type.

Table 7—List of descriptors loaded on each principal component

PC1	PC2	PC3	PC4	PC5
Defects	Aftertaste sensations	Butter/sweet	Cooked flavor	Sulfur/ putrid
Hay/grain	Cardboard	Butter	Cooked aroma	Sulfur
Sour/fermented	Drying	Sweet taste	Cooked taste	Putrid
Baby formula	Lingering	Sweet aftertaste		
Nutty	Sour			
Rancid	Flat			
Metallic				

loaded were labeled based on representative characteristics and are presented in Table 7. PC1, labeled as defects, is related to undesirable attributes in 2% HTST milk: hay/grain and sour/fermented aroma, baby formula, nutty and rancid taste, and metallic aftertaste. PC2 has large loadings for flat taste and the following unfavorable aftertaste sensations: cardboard, drying, lingering, and sour. PC3 is related to butter notes and sweetness: butter taste, sweet taste, and sweet aftertaste. PC4 is strongly influenced by cooked attributes: cooked aroma and cooked taste. Finally, PC5 is related to objectionable odors: sulfur and putrid aroma. Cheese and cream aroma data showed high standard deviations, inconsistent panel ratings over duplicates and did not load on any 1 component. Therefore, these attributes were not selected for further analysis.

Differences between the means of the factor score values obtained from the PCA were analyzed by applying the mixed procedure to each PC. Table 8 summarizes the results of this analysis. For the defects component, a significant day effect was found. Differences were found between the initial day and day 17. Differences were also detected between day 7 and day 17. These results indicate the panel was able to perceive differences in levels of defects between recently pasteurized milk (initial to 7 d postprocessing) and milk samples approaching the end of shelf life (17 d), although differences among plants were not apparent. The rancid attribute correlated well with the defects component, as shown by its high factor loading in this PC. Mean scores for the defects component increased over time, indicating defects were more detectable by the panel toward the end of shelf life.

Differences in perceived aftertaste sensations, grouped in PC2, were evident among days and plants. Initial day samples differed from day 14 and day 17 samples, and day 7 samples differed from those evaluated on day 17. Similar to PC1, the mean scores for PC2 increased over time. Plant A, which had the lowest mean PC2 scores, was significantly different from both plants B and C. As with PC2, the butter/sweet component (PC3) mean scores differed between fresh samples (initial to 7 d) and those evaluated at the end

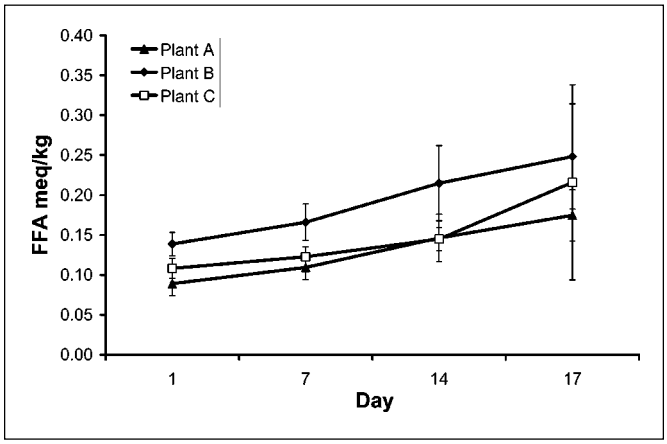

Figure 3—Free fatty acid content of processed milk samples throughout 17-d shelf life as determined by acid degree value (ADV). Bars indicate standard deviations.

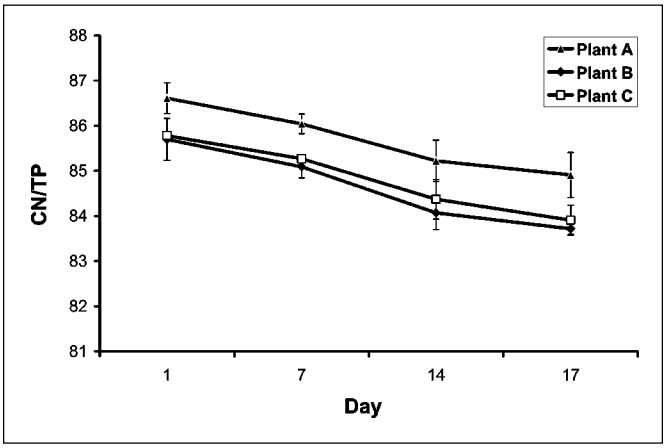

Figure 4—Casein as a percentage of true protein in processed milk samples as determined by Kjeldahl analysis. Bars indicate standard deviations.

of shelf life (14 to 17 d). Unlike the defects and aftertaste components, mean scores for the butter/sweet component decreased over shelf life. Plant A samples showed the highest butter/sweet component scores, which may reflect lower microbial numbers observed in these products throughout shelf life.

The cooked flavor and sulfur/putrid components had significant plant by day interactions. For the cooked flavor component, day effect was present only in plant A. For this plant, the initial day was significantly different from days 7, 14, and 17. Plant A had the highest overall cooked flavor scores. Cooked flavors appear to dissipate over time, as demonstrated by the decrease in mean scores over time. The intensity of the cooked flavor reflects pasteurization time and temperature and is more pronounced when products are processed at higher temperatures (Shipe and others 1978). A significant difference between plant A and plants B and C for the cooked component was found on the initial day only, when the cooked notes were most evident. This difference may be attributed to the difference in the amount of time the products were held at pasteurization temperature and the rate at which the milk was cooled after pasteurization. Although Plant A has an 18-s hold, the heated milk is transported to the homogenizer before reaching the regeneration section of the pasteurizer. This additional time before cooling may lead to the more pronounced cooked flavor found in these milk samples. The sulfur/putrid component, which explains the least amount of the variance (6.3%), had a significant day effect for plant C. Differences were detected between the first 3 test days and day 17. As predicted, sulfur/putrid mean factor scores were highest on day 17.

The flavor of high-quality milk has been described as bland, pleasantly sweet, and free of defects (Shipe 1980). Growth of psychrotrophic organisms during refrigerated storage results in a number of off-flavors such as bitter, fermented, rancid, and putrid (Shipe 1978; Bishop and White 1986; Bodyfelt and others 1988). The production of off-flavors and odors directly affects the quality and shelf life of fluid milk products. Organoleptic changes that determine consumer acceptability generally become apparent at bacterial levels above 10^6 CFU/mL (Bishop and White 1986). Sensory defects for dairy products have traditionally been grouped into categories based on their cause. The quantitative descriptive analysis approach centers on developing a flavor profile of the product, which determines the intensity of both desirable and undesirable flavor characteristics through a trained panel. Through PCA, attributes loading on a given component share a similar conceptual meaning and are grouped into a single category, such as "defects." It is not possible based on our data to establish a direct causal relationship between the attributes loaded on each component and the specific source of the flavor characteristics. The flavor profile of fluid milk products throughout shelf life is determined by a combination of factors including processing parameters, microbial growth levels, and the extent of lipid and protein degradation. The usefulness of QDA lies in establishing a benchmark profile that reflects high-quality milk throughout shelf life, influenced mainly by the components relating to favorable attributes such as the butter/sweet or cooked while minimizing the effect of attributes relating to defects.

Fluid milk shelf-life extension

The plants selected for this study had demonstrated high-quality performance in routine microbial, chemical, and sensory testing carried out by the MQIP at Cornell Univ. Raw milk samples collected from the plants had bacterial counts and somatic cell counts that were well below the regulatory limits. No differences were detected in raw milk microbial counts among plants, suggesting that differences in pasteurized milk quality throughout shelf life may be attributed to factors other than raw milk quality. The most predominant organisms isolated from processed milk were Gram-positive rods. Less than 5% of the total isolates identified from samples collected over a 4-mo period were Gram-negative rods. One half of the *Pseudomona*s isolates that were collected originated from the same processed milk sample. Our findings suggest that Gram-positive rods have considerable spoilage potential in products manufactured in the participating plants, which appear to have relatively little postpasteurization contamination by Gram-negative rods. These plants currently face the challenge of developing strategies to eliminate heat-resistant psychrotrophic spoilage organisms in milk. It is hypothesized that these organisms are present in raw milk and survive pasteurization. However, these organisms may also be present in the dairy plant environment and therefore also may enter the product as postprocessing contaminants (Griffiths and Phillips 1990; Schraft and others 1996; Svensson and others 1999). Although these organisms are present in low numbers following pasteurization, in general their levels increase significantly over time, thus limiting product shelf life.

Table 8—Mixed model results for principal component factor scores

Source of variation	*F* value				
	PC1	PC2	PC3	PC4	PC5
Day	5.69[a]	14.09[a]	10.00[a]	12.98[a]	3.95[a]
Plant	0.01[b]	8.57[a]	5.50[a]	4.68[b]	0.45[b]
Day × plant	0.11[b]	1.65[b]	0.30[b]	2.89[a]	4.41[a]

[a]$P \leq 0.05$.
[b]Not significant, $P > 0.05$.

When tested initially following pasteurization, there were no differences in bacterial numbers among plants, although the final microbial numbers were significantly influenced by processing plant. Previous studies have shown a similar plant influence on microbial numbers (Douglas and others 2000). Plant A, for example, had smaller differences in bacterial counts among shelf-life test days. Factors that influence the greater bacterial count differences among shelf-life test days in 2 of the plants appear to be related to processing conditions, production volume, and sanitation practices. Smaller production volumes and less frequent processing may allow more thorough sanitation between runs, and result in reduced postprocessing contamination. The large influence of plant on the bacterial quality of milk samples over shelf life is an indicator that individual processing parameters and postpasteurization handling play a key role in determining product shelf life. Bacterial counts ranging from 3.0×10^5 to 1.0×10^7 CFU/mL at 17 d postprocessing found in this study demonstrate there is a need to identify and eliminate psychrotrophic heat-resistant spoilage organisms in fluid milk.

Conclusions

Although *Paenibacillus, Bacillus,* and *Microbacterium* were over all the most commonly isolated organisms found in 2% HTST fluid milk, the microflora identified in each of the 3 plants tested varied in species and proportion. The variability observed among plants suggests that plant-specific strategies will be needed to identify and reduce or eliminate sources of contamination. Development of these strategies might be achieved through systematic sampling of the dairy plant environment, including areas such as milk contact surfaces, equipment, floors, and drains. Environmental sampling should facilitate the identification of bacterial reser-

voirs, which must be targeted to reduce contamination at identified entry points and contribute to extended shelf life in fluid milk products.

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