# EVALUATION AND IMPROVEMENT OF RAW AND PASTEURIZED FLUID MILK QUALITY

A Thesis

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# ABSTRACT

Effective strategies for extending fluid milk product shelf-life by controlling bacterial growth are of economic interest to the dairy industry. To that end, the effects of addition of L-arginine, Nα-lauroyl ethylester monochloride (LAE) on bacterial numbers in fluid milk products were measured. Specifically, LAE was added (125, 170, or 200 ppm) to conventionally homogenized and pasteurized 3.25% fat chocolate or unflavored milk products. The treated milks and corresponding controls were held at 6°C and plated on standard plate count (SPC) agar within 24 hours of processing and again at 7, 14, 17 and 21 d of storage. Bacterial numbers in all unflavored milk samples treated with LAE remained below the Pasteurized Milk Ordinance (PMO) limit of 4.3 log cfu/mL for the entire 21 days. Bacterial numbers in unflavored samples containing 170 and 200 ppm LAE were significantly lower than those in the untreated unflavored milk at d 17 and 21 post-processing. Specifically, bacterial numbers in the milk treated with 200 ppm LAE were 5.77 log cfu/mL lower than in untreated milk at 21 d post-processing. Bacterial numbers in chocolate milk treated with 200 ppm LAE were significantly lower than those in the untreated chocolate milk at d 14, 17 and 21. In chocolate milk treated with 200 ppm LAE, bacterial numbers were 0.9 log cfu/mL lower than in the untreated milk at 21 d post-processing. Our results show that addition of LAE to milk can reduce bacterial growth. LAE addition is more effective at controlling bacterial growth in unflavored milk than in chocolate milk.


The dairy industry has a great deal of interest in tests that not only determine the quality of raw milk, but that will also help to predict the quality of the finished product processed from that raw milk. One test that has been used widely to test raw

milk quality is the Preliminary Incubation (PI) test which stresses raw milk at 12.8 degrees C for 18 hours prior to enumeration. An elevated PI count has been used as an indicator that cleaning, sanitization and cooling practices on the farm are inadequate as well as an indicator of pasteurized product quality. Samples of raw milk and corresponding commercially pasteurized milk were obtained from four New York State (NYS) fluid milk processors over a one year time period from October 2007 through September 2008 to assess the overall quality of raw and pasteurized milk in NYS as well as to determine the accuracy of raw milk tests in predicting pasteurized product shelf life. Standard plate counts (SPC) and sensory quality from commercially pasteurized milk samples at day 17 post-pasteurization were compared to corresponding raw milk PI counts, with resulting $R^2$ values of 0.2416 and 0.1007 respectively. When the confounding factor of post-pasteurization contamination (PPC) in the commercially pasteurized samples is accounted for, the $R^2$ values for both PI versus day 17 SPC and PI versus day 17 sensory drop to 0.1972 and 0.0726 respectively. These results indicate that the PI count is not a good predictor of pasteurized milk quality, and while raw milk quality is an important factor in providing consumers with high quality product it appears that plant factors including PPC and processing conditions have a major impact on product quality. More research is needed to develop a test that can accurately and rapidly assess raw milk quality as it pertains to pasteurized product performance.

## BIOGRAPHICAL SKETCH

Nicole (Woodcock) Martin grew up in Addison, NY on a small farm with her parents and older sister. After graduating from high school, Nicole went on to earn a Bachelor of Science degree in Food Science from Cornell University. Nicole began working as a technician in the Milk Quality Improvement Program where she also began graduate research through the Employee Degree Program in Food Science. Nicole enjoys baking, gardening and spending time with her husband, pets and family.

To my God, my husband, my family and my mentors; I wouldn't be here without any of you

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## LIST OF ABBREVIATIONS

LAE - L-arginine, Nα-lauroyl ethylester monohydrochloride

GRAS - Generally Recognized as Safe

NOAEL - No Observable Adverse Effect Level

SMEDP – Standard Methods for the Examination of Dairy Products

NYS – New York State

SPC – Standard Plate Count

PPC –Post-Pasteurization Contamination

PBC- Psychrotrophic Bacteria Count

LP –Laboratory Pasteurization

LPC –Laboratory Pasteurization Count

CC –Coliform Count

SCC –Somatic Cell Count

PI –Preliminary Incubation count

VJ –Vogel Johnson

CVTA –Crystal Violet Tetrazolium Agar

PMO –Pasteurized Milk Ordinance

HTST-High Temperature Short Time

DMC-Direct Microscopic Count

SP-Spore enumeration

# CHAPTER ONE

## RAW AND PASTUERIZED FLUID MILK QUALITY

As the US beverage industry continues to become increasingly competitive (IDFA, 2007), the fluid milk industry has focused on improving quality of both raw and pasteurized product thereby extending product shelf-life. Grade A pasteurized milk shelf-life is defined by the Pasteurized Milk Ordinance as the number of days for pasteurized product to reach 20,000 cfu/mL (FDA, 2009). In recent years the quality of commercial fluid milk products has improved, due mostly to strides taken to reduce post-pasteurization contamination (PPC) (Carey et al., 2005). In the absence of PPC the biological barrier to the extension of conventionally pasteurized fluid milk shelf life is *Paenibacillus* (Fromm et al., 2004; Huck et al., 2007b; Huck et al., 2008; Ranieri et al., 2009b). *Paenibacillus* is a gram positive spore-forming rod that is found ubiquitously in nature, survives pasteurization in spore-form and subsequently grows at refrigeration temperature. Strategies to extend fluid milk shelf-life in the absence of PPC must include methods that detect and address shelf-life limiting organisms in the farm to processing continuum or control these organisms in the finished product. The former strategy of detecting shelf-life limiting organisms in raw milk and predicting their effects on pasteurized milk shelf-life has been a goal of the dairy industry for many years and has recently become the subject of much debate. The latter strategy calls for the implementation of innovative methods, such as the use of antimicrobial compounds, to retard the growth of shelf-life limiting organisms.

The first study was focused on determining the effects of the antimicrobial N-α-Lauroyl-L-Arginine Ethyl Ester Monohydrochloride (LAE) on bacterial growth in

pasteurized fluid milk. This novel antimicrobial has been granted “generally recognized as safe” (GRAS) status by the FDA and has been shown to be an effective antimicrobial against a variety of microbes (Rodriguez et al., 2004). We hypothesized that the addition of LAE would reduce bacterial numbers in chocolate and unflavored pasteurized fluid milk, thereby extending product shelf-life. To test this hypothesis we held samples of chocolate and unflavored conventionally pasteurized milk treated with three different concentrations (125, 170 or 200 mg/L) of commercially prepared LAE along with untreated controls at refrigeration temperature (6°C) and monitored the bacterial growth over 21 days.

The results of this study show that chocolate milk treated with 200 mg/L of LAE had lower bacterial numbers than the control and the other two samples at days 14, 17 and 21. The unflavored samples treated with 170 and 200 mg/L LAE had lower bacterial numbers than the control and the other treated sample at days 17 and 21. These results show that the addition of LAE is capable of limiting bacterial growth in both chocolate and unflavored milk, suggesting a possible role for GRAS antimicrobials such as LAE for use in the dairy industry as a method to extend product shelf-life.

A second study focused on evaluating the ability of raw milk tests to predict pasteurized fluid milk quality. This work was initiated by the recent debate concerning the use of a raw milk test, the Preliminary Incubation (PI) count as a predictor of pasteurized milk quality. We hypothesized that the PI count would not be a good predictor of pasteurized milk quality. To test our hypothesis, 43 raw and corresponding pasteurized milk samples were collected from four New York State (NYS) fluid milk processors over a year long period. A myriad of tests were

performed to assess the quality of the raw and pasteurized milk collected. Bacterial isolates were also collected to analyze the bacterial ecology of the samples.

Our results indicate that the PI count has no ability to predict pasteurized milk shelf-life quality when defined by either bacterial numbers or sensory analysis. We also evaluated the ability of the other raw milk tests to predict pasteurized milk shelf-life, and we found that the coefficient of determination ($R^2$) was always low, indicating that none of the raw milk tests currently used in the dairy industry are adequate for predicting pasteurized milk quality. Control of PPC and other plant factors such as processing temperature are critical in providing high quality product to consumers. Assuming these factors are addressed, the industry would benefit from a raw milk test that would accurately detect the presence of the biological barrier to shelf-life extension, *Paenibacillus*.

# CHAPTER TWO

# N-α-LAUROYL-L-ARGININE ETHYL ESTER MONOHYDROCHLORIDE (LAE) REDUCES BACTERIAL GROWTH IN PASTERIZED MILK[1]

## INTRODUCTION

In recent years, the dairy industry has made strides in improving the quality of commercial fluid milk products (Carey et al, 2005). However, the highly competitive nature of the overall US beverage industry (IDFA, 2007) underscores the need for the dairy industry to employ novel and innovative methods to ensure retention and possible growth of the fluid milk market share. Specifically, to compete with the rapidly expanding market share enjoyed by such products as shelf-stable bottled water and fruit juice-based beverages (U.S. Census Bureau, 2008), fluid milk processors are striving to further improve product quality and extend shelf-life. The most important factor limiting the shelf-life of conventionally pasteurized fluid milk is bacterial growth. We hypothesized that use of a Generally Recognized As Safe (**GRAS**) antimicrobial in fluid milk might provide an effective means of extending pasteurized product shelf-life by controlling bacterial growth. L-arginine, Nα-lauroyl ethylester monochloride (**LAE**) is a novel antimicrobial substance derived from lauric acid and arginine. LAE has been demonstrated as an effective antimicrobial against a variety of microbes. Rodriquez et al. (2004) examined the effect of LAE on *Salmonella typhimurium* and *Staphylococcus aureus*. In combination, transmission electron microscopy, fluorescence microscopy, flow cytometry and ion-flux tests showed that LAE disrupts the structure of the cell membrane and consequently, membrane

potential, which results in bacterial cell death. Specifically, the compound disrupts the lipid bilayer in the bacterial membrane, thereby interrupting metabolic processes and inhibiting cellular proliferation (Bakal et al, 2005). Toxicological studies (Ruckman, 2004) showed that LAE has low acute toxicity, with systemic No Observable Adverse Effect Levels (**NOAELs**) established at 15,000 ppm. In addition, mammalian metabolic studies have shown that LAE is metabolized into the amino acid arginine, which is ultimately broken down into $CO_2$ and urea (Ruckman, 2004). In 2005, the FDA granted GRAS status to LAE for use as an antimicrobial in more than 20 foods, including meat and poultry products. Research by Luchansky et al. (2005) showed that *Listeria monocytogenes* populations inoculated onto the surface of Ready to Eat (**RTE**) ham were reduced 2.9, 4.6 or 5.12 log cfu/mL when the surfaces had been treated with 4, 6 or 8 mL of a 5% LAE solution, respectively. LAE is not currently approved for use in dairy products (Department of Agriculture, 2008c).

The objective of this study was to determine if addition of LAE to flavored and unflavored fluid milk products at levels currently approved for use in various food products would result in extension of product shelf-life relative to that of comparable untreated products. The U.S. Pasteurized Milk Ordinance (**PMO**) (FDA, 2009) specifies a bacterial limit of 20,000 cfu/mL for Grade "A" Pasteurized Milk while the milk is offered for sale, therefore, for the purpose of our study, "shelf-life" was defined as the number of days post-pasteurization that a fluid milk product can be held under refrigerated storage (6°C) prior to reaching 20,000 cfu bacteria/mL.

## MATERIALS AND METHODS

Samples of commercially processed homogenized and pasteurized chocolate and unflavored fluid milk products that had been packaged in paperboard gable top containers (polyethylene/paperboard/polyethylene) were obtained on processing day on each of three independent occasions from the Cornell University Dairy processing facility in Ithaca, NY. For each of the three trials, 946 mL (1 quart) of 3.25% fat unflavored homogenized milk and of 3.25% fat chocolate milk were obtained. Process temperatures were 79.5°C for 23 s for the unflavored milk and 82°C for 23 s for chocolate milk. Samples were collected and handled according to Standard Methods for the Examination of Dairy Products (**SMEDP**) (Laird et al, 2004). A commercially prepared 10% LAE solution (Mirenat-N) was obtained from A&B Ingredients (Fairfield, NJ).

To create sub-samples from the same product for testing at each 7, 14, 17 and 21 d post-processing, each milk sample was shaken as described in SMEDP and then the product was distributed aseptically among four sterile Pyrex bottles (Corning, Inc., Corning, NY) with screw caps. The following treatments were prepared for both the chocolate and unflavored milk samples: a control (no LAE), 100 mL of milk with 125 ppm LAE, 100 mL of milk with 170 ppm LAE and 100 mL of milk with 200 ppm LAE. As 200 ppm LAE was the upper limit suggested by the manufacturer (A&B Ingredients), it was chosen as the maximum LAE concentration for this study. The samples were held at 6°C and pour plated for bacterial enumeration according to SMEDP (Laird et al, 2004) within 24 hours of processing, and at 7, 14, 17 and 21 d post-processing. Bacterial colonies on the plates were counted after 48 hours of incubation at 32°C. All SPC data were $\log_{10}$transformed. Dunnett's method of statistical analysis was performed using JMP software (SAS Institute, Cary, NC, USA)

to compare bacterial numbers in the treated samples to those in the control sample in each group.

## RESULTS AND CONCLUSIONS

The effects of different concentrations of LAE on bacterial numbers in unflavored and chocolate milk products were evaluated over a 21 d period (Figures 2.1 and 2.2). Overall, our data show that LAE limited bacterial growth and extended the shelf-life of unflavored milk. Bacterial numbers in the unflavored milk without LAE were less than 2 log cfu/mL immediately post processing, and increased to over 7 log cfu/mL after 21 d of storage. In the unflavored milk treated with 125, 170, or 200 ppm LAE, bacterial numbers reached 3.64, 2.65, and 1.43 log cfu/mL respectively after 21 d of storage. Bacterial numbers in unflavored milk treated with 200 ppm LAE were 5.77 log cfu/mL lower than in untreated milk at 21 d post-processing. Bacterial numbers in unflavored samples containing 170 and 200 ppm LAE were significantly lower ($P<0.05$) than those in the untreated milk at d 17 and 21.

Bacterial numbers in chocolate milk without LAE were less than 2 log cfu/mL immediately after processing, but increased to nearly 8 log cfru/mL after 21 d of storage. Chocolate milk treated with 125, 170 or 200 ppm LAE had bacterial numbers of 7.96, 7.44, and 7.06 log cfu/mL respectively, after 21 d of storage. Bacterial numbers in 200 ppm-treated chocolate milk were 0.9 log cfu/mL lower than those in the untreated milk at 21 d post-processing. Bacterial numbers in the chocolate milk samples treated with 200 ppm LAE were significantly lower than the control samples ($P< 0.05$) at d 17 and 21, with a weakly significant difference ($P< 0.075$) between the

control and the chocolate sample containing 200 ppm LAE on d 14. There were no significant differences between the samples with 170 ppm and 125 ppm LAE and the controls on any test day (P> 0.05).

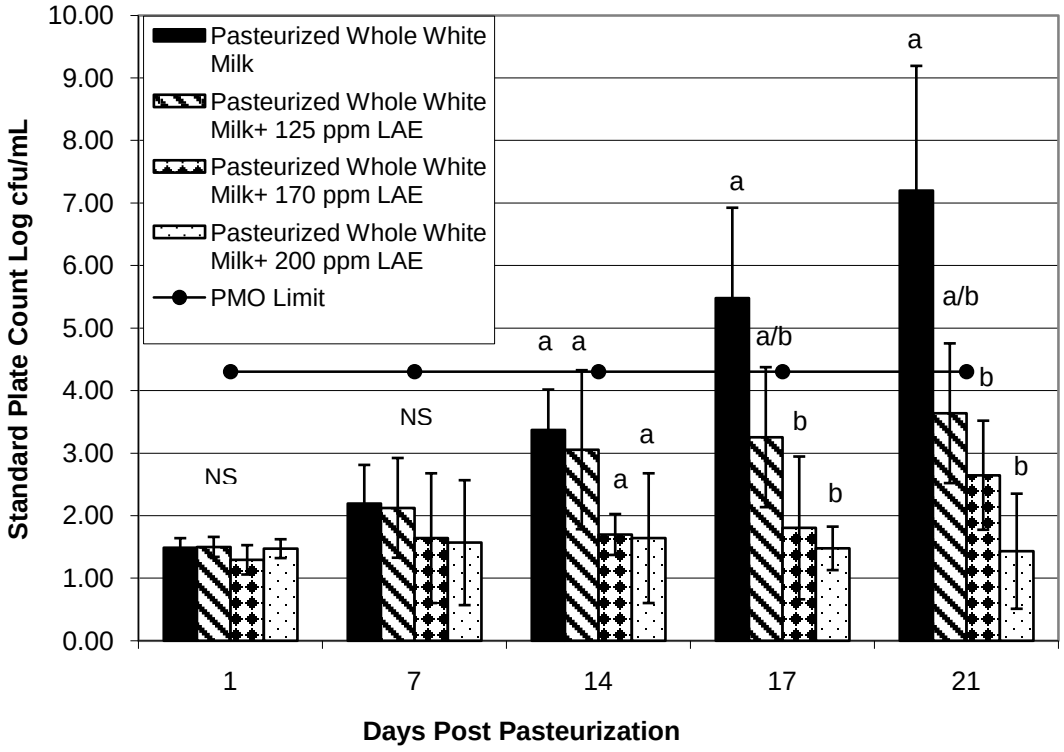



Figure 2.1. Mean standard plate count (SPC) for pasteurized unflavored milk treated with LAE (0, 125, 170, or 200 ppm) and held at 6°C for up to 21 d. The horizontal line at 4.3 log cfu/mL represents the PMO limit of 20,000 cfu/mL for pasteurized fluid milk. Error bars represent $\pm$ 1 SD from the mean of data collected from three independent experiments. Differing lower case letters within the same graph (i.e., a, b) indicate statistically significant differences in bacterial numbers ($P < 0.05$) by Dunnett's method of statistical analysis on $\log_{10}$ transformed data.

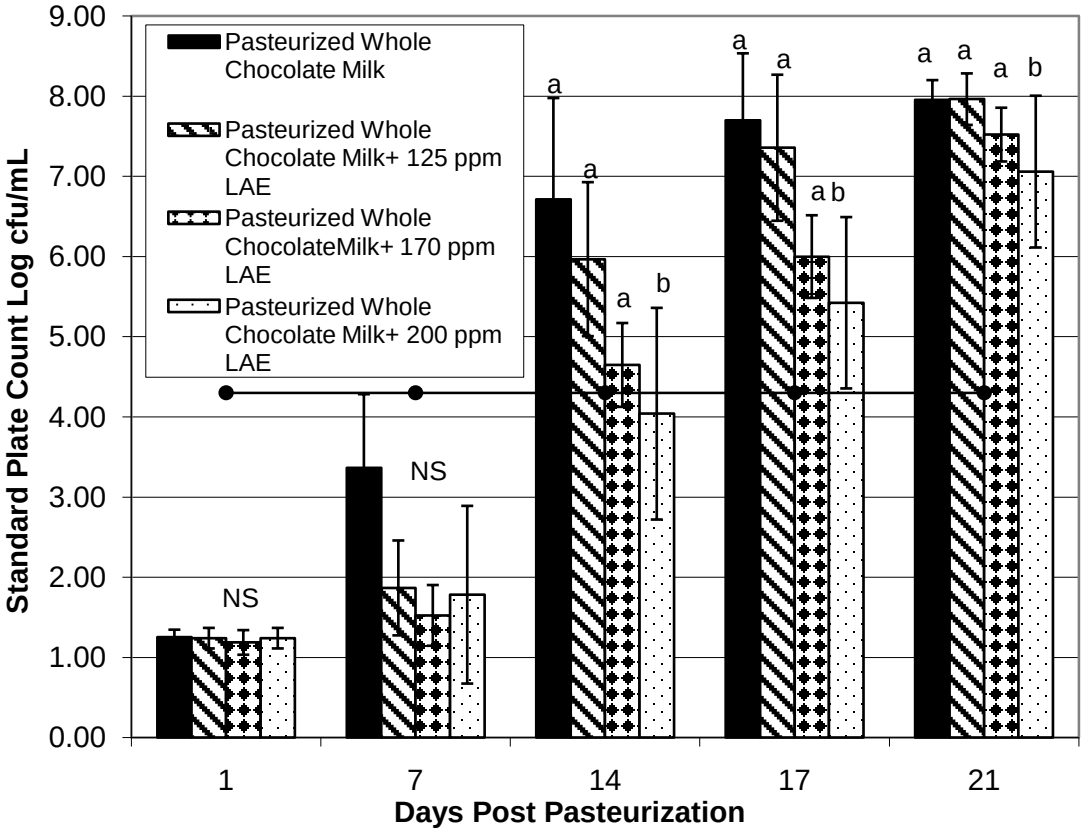



Figure 2.2. Mean standard plate count (SPC) for pasteurized chocolate milk treated with LAE (0, 125, 170, or 200 ppm) and held at 6°C for up to 21 d. The horizontal line at 4.3 log cfu/mL represents the PMO limit of 20,000 cfu/mL for pasteurized fluid milk. Error bars represent $\pm$ 1 SD from the mean of data collected from three independent experiments. Differing lower case letters within the same graph (i.e., a, b) indicate statistically significant differences in bacterial numbers ($P < 0.05$ or $P < 0.075$ for 200 ppm LAE on d 14) by Dunnett's method of statistical analysis on $\log_{10}$ transformed data.

Higher bacterial numbers in matched samples of chocolate and unflavored milk have been documented previously by Douglas et al. (2000). Specifically,

pasteurized chocolate milk had a greater relative increase in bacterial numbers over 21 d of shelf-life when compared to paired pasteurized unflavored milk. The chocolate powder that had been added to the milk was implicated as promoting more rapid microbial growth in the chocolate milk relative to that in the unflavored milk. Our results are consistent with the findings from this previous study. In the present study, the chocolate control samples (without LAE) reached 20,000 cfu/mL between 10-14 d post-processing, while the unflavored control milk reached 20,000 cfu/mL between 14-17 d post-processing. The addition of LAE more effectively retarded bacterial growth in unflavored milk than in chocolate milk. It is possible that the reduced effectiveness of LAE in chocolate milk is due to the presence of stabilizers in the chocolate powder (e.g. carrageenan), as the presence of stabilizers has been implicated as decreasing the effectiveness of LAE in retarding bacterial growth (A&B Ingredients, Fairfield, NJ, personal communication).

The cost of adding 200 ppm of LAE to fluid milk would be approximately $0.30 per gallon of finished product (as of 8/2008). Therefore, with the average retail price of a gallon of whole milk at $3.816 in 2008, the addition of LAE would result in at least a 9.6% increase in retail price. The addition of LAE to milk will also affect the labeling of milk products. According to the Department of Agriculture (2008a, 2008b), the addition of LAE to fluid milk would require labeling the product as “milk” with the addition of the common name of the ingredient and a statement describing the function of the ingredient, such as “preservative”, or “to retard spoilage” rather than simply labeling the product as “milk”. Consumer perspectives on this proposed designation would need to be assessed to gauge acceptance of this additive.

The present study did not assess the sensory consequences of LAE addition on product flavor or acceptability. Future testing will be needed of the effects of LAE on

sensory characteristics of milk. While the addition of the antimicrobial LAE to chocolate and unflavored milk was effective in retarding bacterial growth, it was considerably more effective in unflavored milk than in chocolate milk. Our results suggest a possible role for GRAS antimicrobials such as LAE for use in the dairy industry as a way to extend product shelf-life.

# CHAPTER THREE

# EVALUATION OF RAW MILK TESTS AS PREDICTORS OF FINISHED PRODUCT MILK QUALITY

## INTRODUCTION

One goal of the fluid milk industry in the United States and a number of other countries is to extend the shelf-lives of high-temperature-short-time (**HTST**) pasteurized milk products (Reneau, 2007). Post-pasteurization contamination, e.g., with psychrotolerant *Pseudomonas* species (Dogan and Boor, 2002), has been shown to be a major contributor to reduced shelf life of HTST products. In addition to implementation of strategies to prevent post-pasteurization contamination in HTST processing plants, processors also are interested in strategies that allow for identification of raw milk supplies that will facilitate production of extended shelf life HTST products. Raw milk somatic cell counts (**SCC**) are inversely related to cheese yield, composition and quality (Politis and Ng-Kwai-Hang, 1988; Klei et al., 1998), and thereby effect the economics of cheese production; SCC are thus a valuable measure of the quality of raw milk that is used for cheese manufacture. While raw milk with high SSC has shown some negative effect on HTST shelf life (Ma et al., 2000), SCC of most raw milk supplies is low enough to have limited effect on HTST quality, at least under currently typical HTST fluid milk shelf lives (i.e., $\leq$ 21 days). There thus is a particular interest in using tests that evaluate the microbiological quality of raw milk to help to identify a raw milk supply that allows for production of

extended shelf life HTST products. A number of raw milk tests have been used to assess raw milk quality, often with the goal of identifying specific farm practices that are associated with raw milk quality issues, such as poor udder hygiene, inappropriate cooling, or mastitis.

The most commonly used microbiological tests for raw milk quality include the Standard Plate Count (**SPC**), Psychrotrophic Bacteria Count (**PBC**), Coliform Count (**CC**), Laboratory Pasteurization Count (**LPC**), the Direct Microscopic Count (**DMC**) (Laird et al., 2004), and the Preliminary Incubation (**PI**) test (Wilson, 2002). The SPC involves plating of a raw milk aliquot on SPC agar, followed by incubation at 32°C for 48 h and therefore provides an estimate of total bacteria able to grow under these (aerobic) conditions. While PBC uses the same growth media as SPC, incubation occurs at 7°C for 10 days; this test thus provides a measure of the number of bacteria that can grow at low temperatures. CC involves specific enumeration, on a selective medium, of Coliforms, as an indication of fecal contamination. LPC involves heat treatment of raw milk at a time/temperature combination that mimic pasteurization, followed by enumeration of bacteria on SPC agar, thus providing an estimate of the number of bacteria that are likely to survive pasteurization. The PI test is conducted by holding raw milk at 55°F (12.8°C) for 18 hours prior to performing a SPC count. PI results are then compared to those from an SPC that had been conducted on the same raw milk sample, or in some cases, the results are compared to a pre-defined standard. The underlying theory behind the PI test is that bacteria typically associated with a clean, healthy cow (e.g. lactic acid bacteria) are not expected to reproduce under PI conditions, whereas other bacterial contaminants that typically originate from dirty equipment, the exterior of soiled cows, etc. may increase to significant numbers under PI conditions (Murphy, 2008).

The objectives of the present study were to characterize and statistically evaluate relationships between results obtained from a comprehensive set of raw milk tests and the sensory and microbiological characteristics of pasteurized fluid milk manufactured from the tested raw milk. These data should aid in scientifically valid selection of raw milk tests that can be used to predict the performance of raw milk in manufacture of HTST fluid milk products.

## MATERIALS AND METHODS

**Dairy Plants and Sample Collection.** Both (i) raw silo milk (sampled aseptically from raw milk silos into sterile 500 mL bottles [Thermo Fisher Scientific-Nalgene, Rochester, NY]) and (ii) corresponding pasteurized 2% milk fat fluid milk (sampled as one gallon (3.8 L) plastic containers) were obtained from four fluid milk processing plants in New York State (**NYS)** (plants A, B, C and D, Table 3.1). Pasteurization time/temperature combinations used for 2% milk ranged from a low of 76.7°C for 25 seconds (plant A) to a high of 80.3°C for 33 seconds (plant B) (Table 3.1), indicating that the pasteurized product sampled represents a range of pasteurization conditions.

**Table 3.1.** Relevant plant specifications and pasteurized milk quality parameters for plants A to D

| | Parameter for plant | | | |
|---|---|---|---|---|
| | **A** | **B** | **C** | **D** |
| Pasteurization time - temperature combination | 76.7°C for 25s | 80.3°C for 33s | 80.0°C for 30s | 77.6°F for 30s |
| Code date provided on finished product containers | 14 days | 17 days | 17 days | 20 days |
| No. of samples with PPC/total no. of samples | 0/12 | 4/12 | 4/7 | 3/12 |
| No. of samples with a Sensory Score <6.0 at day 17/total no. of samples | 0/12 | 1/12 | 1/7 | 4/12 |
| Samples with a SPC count >20,000 cfu/mL at day 21/total no. of samples | 3/12 | 9/12 | 7/7 | 9/12 |
| Average day 17 SPC (Log cfu/mL) for all samples | 2.50 | 6.07 | 6.08 | 5.86 |
| Average day 17 SPC for samples with no evidence for PPC[a] (Log cfu/mL) | 2.50 | 4.76 | 4.87 | 4.32 |

[a]PPC = post pasteurization contamination

In each plant, raw and pasteurized milk samples were taken monthly, by designated processing plant personnel, during 12 consecutive months from October 2007 to September 2008. While three of the plants were sampled once a month for the

full study duration (i.e, 12 months), plant C ceased operation in April 2008 and hence samples were only collected for 7 consecutive months. Samples of raw milk were collected immediately prior to processing while pasteurized samples were collected immediately post processing; all samples were shipped overnight (on the day of sample collection) to the laboratory in coolers packed on ice. Temperature controls were included in each cooler and temperature controls were evaluated immediately upon arrival to the laboratory; any samples with temperatures $\geq 6$°C were rejected.

**Microbiological Evaluation of Raw Milk.** Each raw milk sample was aseptically distributed into two sterile 250 mL glass bottles (~100 mL/bottle) and four 60 mL vials. While one 60 mL vial was sent to a commercial laboratory (Dairy One Cooperative, Ithaca, NY) to determine SCC, the other aliquots were used to perform a battery of raw milk microbiological tests on each raw milk sample. Each raw milk sample was tested by plating on (i) Edwards media (Northeast Laboratory Services, Winslow, Maine) for the enumeration of *Streptococcus* spp. (Zadoks et al., 2004); (ii) Vogel Johnson (VJ) media (Quality Milk Production Services, Cornell University, Ithaca, NY) for the enumeration of *Staphylococcus* spp. (Zimbro et al., 2009); and (iii) Crystal Violet Tetrazolium agar (CVTA) (Difco, BD Diagnostics, Franklin Lakes, NJ) for the enumeration of Gram negative organisms (Frank and Yousef, 2004). Further tests performed included (iv) SPC, performed by spiral plating raw milk on SPC agar (Difco, BD Diagnostics, Franklin Lakes, NJ), followed by incubation at 32°C for 48h (as described by Laird et al, 2004); (v) PBC, performed by spiral plating raw milk on SPC agar (Difco, BD Diagnostics, Franklin Lakes, NJ), followed by incubation at 7°C for 10 days (as described by Laird et al., 2004); (vi) Coliform Count (CC) performed by plating on Petrifilm™ Coliform Count plates according to manufacturer's instructions (3M, Saint Paul, MN); (vii) ropy milk test, performed by incubating 25

mL of each sample at 21°C for 48 h with evaluation for ropiness after both 24 and 48 hours; (viii) laboratory pasteurization (**LP**) count, performed by heating 100 mL of each sample at 62.8°C for 30 min, followed by plating on SPC agar and incubation at 32°C for 48h (Frank and Yousef, 2004) with the remainder of the LP sample held at 6°C and plated on days 7, 10, 14, 17 and 21; (ix) spore enumeration (**SP**), performed by heat treating 100 mL of sample at 80°C for 12 min, followed by spiral plating on SPC agar with the remainder of the SP sample held at 6°C and plated on days 7, 10, 14, 17 and 21(Huck et al., 2007a); and (x) PI count, performed by incubating 25 mL of raw milk at 13°C for 18 h, followed by plating on SPC agar and incubation at 32°C for 48h (Duncan et al., 2004). The PI count detailed by Duncan et al. (2004) is specifically described as a method for testing pasteurized milk; the PI count for raw milk was removed from Standard Methods for the Examination of Dairy Products after the 15$^{th}$ edition published in 1985. To further evaluate bacteria present after PI treatment (i.e., incubation at 13°C 18 h), raw milk after PI treatment was also spiral plated on Edwards media, VJ media, and CVTA; a 5 mL aliquot of PI treated milk was also subjected to LP test (i.e., laboratory pasteurization at 62.8°C for 30 min, followed by spiral plating on SPC agar).

**Microbiological and Sensory Evaluations of Pasteurized Milk.** Each pasteurized milk sample was aseptically distributed, after 25 complete inversions of the commercial container, among four sterile 500 mL glass bottles (Corning Inc, Corning, NY) (with approx. 400 mL milk/bottle), ten sterile 250-mL glass bottles (approx. 100 mL milk/bottle), and two sterile 60 mL vials. One of the 500 mL bottles was used for initial day sensory evaluation while the remaining 500 mL and 250 mL bottles were held at 6°C for subsequent sensory and microbiological testing at 7, 10, 14, 17 and 21 days post processing.

Microbiological evaluation of pasteurized milk, over shelf life, was performed on the initial day as well as days 7, 10, 14, 17 and 21 post processing. Tests performed included (i) SPC (performed as described above for raw milk) and (ii) CC, performed on Petrifilm™ Coliform Count plates (as described for raw milk above).

Pasteurized samples were also evaluated for sensory characteristics on days Initial, 10, 14 and 17. Sensory evaluations were performed in accordance with the guidelines of the American Dairy Science Association as previously described (Bodyfelt et al., 1988); individual scores for a given product were assigned by each member of a trained panel of 6 staff and graduate students from the Cornell Univ. Dept. of Food Science and an average acceptability score for each sampled was computed from the individual scores. This study was granted exempt status from obtaining human subject approval by the Cornell Univ. Committee on Human Subjects. The Compusense 5 (v4.6, Compusense Inc., Guelph, Ontario, Canada) computerized data collection program was used to determine order of sample presentation and to collect data. Milk samples were mixed by inversion in dim light, capped and presented to the panelists at 15°C, Samples were scored on a scale of 1–10, with scores of less than 6 considered “unacceptable”.

**Isolate Collection and Characterization.** For each sample of raw milk, bacterial isolates were collected from agar plates used for SPC, PBC, and SP as well as from plates used for selective enumeration of streptococci (i.e., Edwards). In addition, for raw milk samples treated by PI incubation, bacterial isolates were collected from SPC and Edwards plates as well as after LP of PI treated samples. For each sample of raw milk treated by LP or SP, bacterial isolates were collected from samples that reached bacterial counts of >20,000 cfu/mL or on the last day of plating (regardless of bacterial counts). For commercially pasteurized 2% milk, isolates were also collected

from samples that showed bacterial counts of >20,000 cfu/mL (in the SPC) or on the last day of plating (regardless of bacterial counts). In all cases, colonies representing each visually distinct morphology present (typically one to ten colonies per sample) on the plates described above were selected and streaked for purity on BHI agar. Isolates were frozen at -80°C in 15% glycerol.

A total of 1,745 isolates were collected over the duration of the study. All isolates obtained from commercially pasteurized milk samples were characterized by sequencing a 616 nt fragment of the 16S rRNA gene, performed as previously described (Huck et al., 2007a). 16S sequence data were used to characterize isolates to the genus and species level (where possible), using the RDP database. Further information on isolates collected in this study as well as 16S rRNA sequences can be found at www.pathogentracker.net.

**Statistical Analysis.** All statistical analyses were performed in JMP (Version 7.0, SAS Institute Inc., Cary, NC). Microbiological data were log transformed prior to linear regression and coefficient of determination analysis to determine $R^2$ values. $R^2$ values are a measure of how much of the data can be attributed to, or explained by a linear regression model. A $R^2$ value of 0.0 would indicate that 0% of the data can be explained by the regression model, while a $R^2$ value of 1.0 would indicate that 100% of the data could be explained by the regression model (Worster et al., 2007).

## RESULTS AND DISCUSSION

Results from the different raw milk tests evaluated here showed very limited correlation among each other, highlighting the fact that distinct microbial populations are targeted by these tests. Limited correlation was also observed between the raw milk microbiological test results and the results from analyses testing the sensory and microbiological quality of the processed milk that had been commercially pasteurized. Further analyses also showed limited correlations between raw milk quality parameters and (i) sensory and microbiological quality of commercial milk when excluding the 11 samples that showed evidence for post pasteurization contamination and (ii) microbiological quality of raw milk that has undergone lab pasteurization. We thus conclude that the raw milk tests evaluated here show limited ability to predict the quality of HTST fluid milk manufactured.

**The Raw Milk Tests Evaluated Here Show Very Limited Correlation among Each Other.** The relationship between all raw milk parameters used during this study was examined using a scatterplot matrix and $R^2$ values (Figure 3.1, Table 3.2). Overall, only two pairs of raw milk tests had $R^2$ values above 0.50, the remainder of the $R^2$ values were low (see Table 3.2). The best correlation (i.e., $R^2$ value) between different raw milk tests was between CVTA and PBC results ($R^2$=0.71). The high correlation between bacterial numbers on CVTA, a media that selects for Gram negatives and PBC, a test that selects for organisms that grow at refrigeration temperature suggests that the Gram negative organisms found in raw milk are capable of growing at refrigeration temperatures. Interestingly, the second highest $R^2$ value found when comparing raw milk tests ($R^2$= 0.68) was for PI results versus results for

plating on CVTA after PI incubation. This result indicates that the organism growing during the PI incubation are those that grow well on CVTA, typically Gram negatives. These findings are consistent with data reported by Johns and Landerkin (1969), who also concluded that Gram negative rods were the primary causative organism type responsible for increased PI counts. The presence of these organisms in raw milk can indicate mastitis, unsanitary conditions and/or improper cooling procedures on the farm (Jayarao and Wang, 1999; Murphy, 2008). While a number of Gram-negative bacteria could be responsible for increased PI counts, the Gram negative *Pseudomonas* spp. are of particular importance in the dairy industry and have been shown to exist in both the dairy farm environment (Jayarao and Wang, 1999) as well as the dairy processing environment (Ralyea et al., 1998; Dogan and Boor, 2002). Some *Pseudomonas* spp. have been shown to produce heat stable enzymes (e.g., proteases, lipases) that are not inactivated by pasteurization and can affect sensory quality of the milk post pasteurization; this generally is only an issue when *Pseudomonas* are present in raw milk at levels higher than the Grade "A" raw milk limit (for commingled milk) of 300,000 cfu/mL (Adams et al., 1975; Grieve and Kitchen, 1985). While high PI counts thus may be an issue for raw milk that has high numbers of *Pseudomonas*, the raw milk samples evaluated here were generally of high quality (e.g., average SPC of 18,000 cfu/mL, range 3,700 – 120,000 cfu/mL; average SCC of 220,000/mL, range 160,000 – 280,000 /mL), clearly indicating that organisms detected by the PI count should not be responsible for finished product defects. Overall, low correlation between different raw milk tests was similar to results from a previous study (Boor et al., 1998), which also, generally, indicated that there was no clear correlation between the results of different raw milk microbiological tests used.

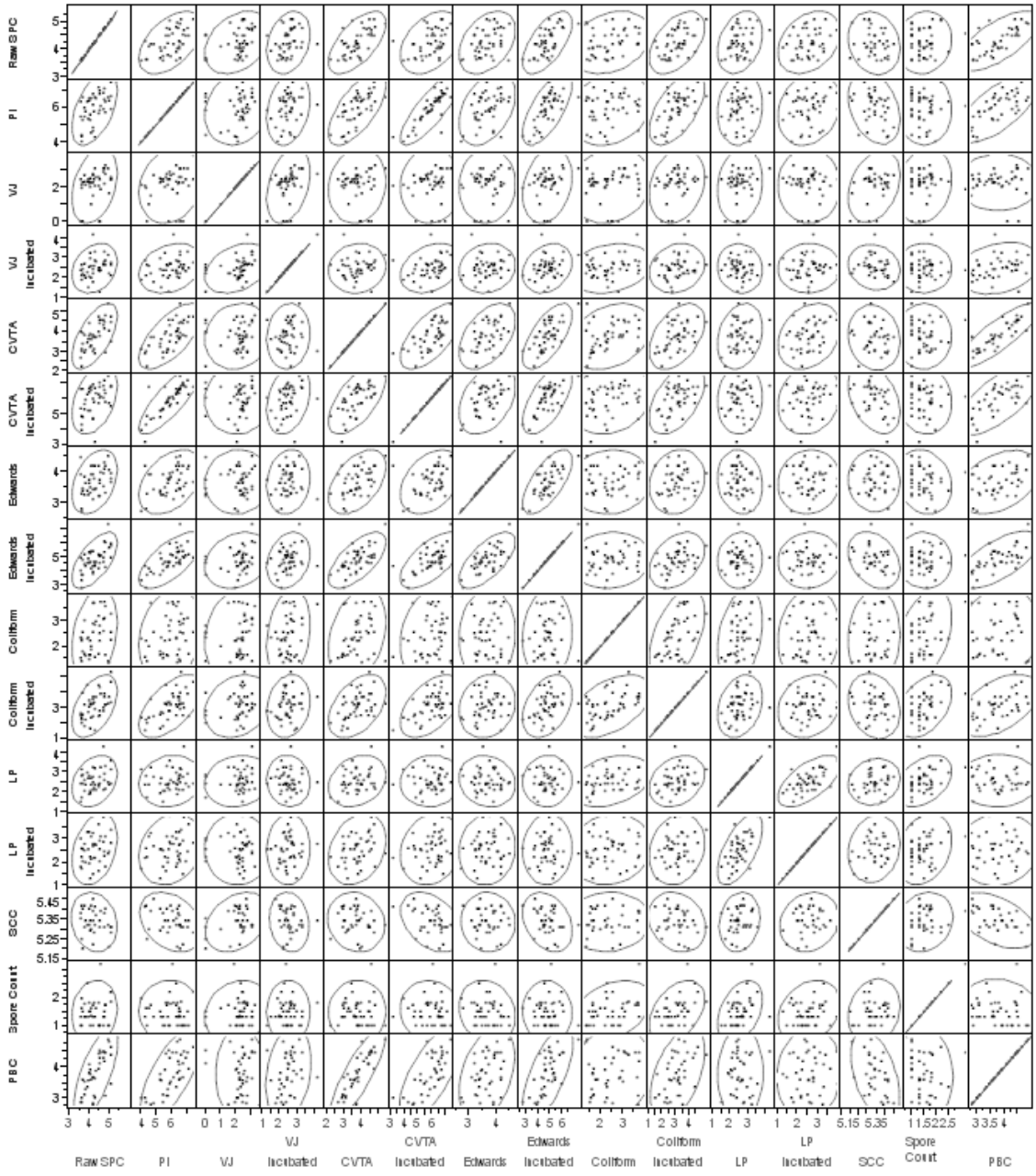



**Figure 3.1**. Scatterplot matrix depicting the relationship between results for different raw milk tests performed on 43 raw milk samples collected from four NYS fluid milk

processors. All data are shown as log transformed bacterial count data (log cfu/mL). Ellipses encompass 95% of data points.

| | $R^2$ for [1] | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Test** | **PI-LP** | **PI-Coliform** | **PI-Edwards** | **PI-CVTA** | **PI-VJ** | **PI** | **PBC** | **SP** | **LP** | **Coliform** | **Edwards** | **CVTA** | **VJ** | **Raw SPC** | **SCC** |
| **SCC** | 0.02 | 0.04 | 0.09 | 0.14 | 0.02 | 0.10 | 0.22 | 0.03 | 0.02 | 0.00 | 0.00 | 0.01 | 0.10 | 0.02 | 1.00 |
| **Raw SPC** | 0.10 | 0.24 | 0.39 | 0.16 | 0.06 | 0.26 | 0.48 | 0.01 | 0.09 | 0.08 | 0.15 | 0.39 | 0.11 | 1.00 | |
| **VJ** | 0.00 | 0.05 | 0.06 | 0.00 | 0.12 | 0.03 | 0.00 | 0.03 | 0.02 | 0.02 | 0.00 | 0.01 | 1.00 | | |
| **CVTA** | 0.07 | 0.20 | 0.44 | 0.40 | 0.01 | 0.45 | 0.71 | 0.00 | 0.06 | 0.09 | 0.23 | 1.00 | | | |
| **Edwards** | 0.01 | 0.02 | 0.44 | 0.15 | 0.01 | 0.16 | 0.15 | 0.02 | 0.01 | 0.00 | 1.00 | | | | |
| **Coliform** | 0.01 | 0.23 | 0.00 | 0.04 | 0.05 | 0.04 | 0.03 | 0.16 | 0.10 | 1.00 | | | | | |
| **LP** | 0.39 | 0.08 | 0.00 | 0.00 | 0.00 | 0.01 | 0.00 | 0.16 | 1.00 | | | | | | |
| **SP** | 0.07 | 0.17 | 0.00 | 0.01 | 0.00 | 0.00 | 0.00 | 1.00 | | | | | | | |
| **PBC** | 0.01 | 0.24 | 0.44 | 0.47 | 0.06 | 0.48 | 1.00 | | | | | | | | |
| **PI** | 0.04 | 0.34 | 0.46 | 0.68 | 0.07 | 1.00 | | | | | | | | | |
| **PI-VJ** | 0.00 | 0.03 | 0.06 | 0.09 | 1.00 | | | | | | | | | | |
| **PI-CVTA** | 0.03 | 0.26 | 0.40 | 1.00 | | | | | | | | | | | |
| **PI-Edwards** | 0.00 | 0.13 | 1.00 | | | | | | | | | | | | |
| **PI-Coliform** | 0.03 | 1.00 | | | | | | | | | | | | | |
| **PI-LP** | 1.00 | | | | | | | | | | | | | | |

[1] $R^2$ values for each raw milk test compared to themselves. Somatic Cell Count (SCC), Standard Plate Count (SPC), Vogel-Johnson (VJ), Crystal Violet Tetrazolium Agar (CVTA), Laboratory Pasteurization (LP), Spore Pasteurization (SP), Psychrotophic Bacteria Count (PBC), Preliminary Incubation (PI), PI milk plated on VJ (PI-VJ), PI milk plated on CVTA (PI-CVTA), PI milk plated on Edwards media (PI-Edwards), PI milk plated on Petrifilm Coliform Count plates (PI-Coliform) and PI milk that has undergone Laboratory Pasteurization (PI-LP)

**Table 3.2.** Coefficient of determination ($R^2$) values from linear regression models of log transformed raw milk test data.

**Raw Milk Tests do not Predict Microbiological and Sensory Performance and Shelf Life of Pasteurized Milk.** SCC and microbiological raw milk test data were used to assess their correlation with different parameters that indicate the quality and shelf life of the commercially pasteurized 2% milk produced, including (i) SPC at day 17 and day 21 of shelf life, and (ii) sensory scores at day 17 of shelf life (Table 3.3, Figure 3.2). Overall, correlations between different raw milk test results and pasteurized milk quality parameters were low (i.e., all $R^2$ values were <0.3). When comparing the different raw milk test results to day 17 SPC counts for pasteurized milk, $R^2$ values ranged from a low of 0.0031 (for SP), to a high of 0.2416 (for PI

count, see Figure 3.3a). Similarly low $R^2$ values were found when comparing the different raw milk test results to day 21 SPC counts; $R^2$ values ranged from a low of 0.0000 (for SP), to a high of 0.2211 (for PI count) (Table 3.3, Figure 3.3a). When comparing raw milk tests results to pasteurized milk sensory scores (at day 17 or day 21), correlations were even lower; $R^2$ values ranged from 0.000 to 0.1314 for day 17 sensory scores (Table 3.3). The numerically highest $R^2$ value for correlations between raw milk test results and day 17 sensory scores was 0.1314 (for ΔPI, i.e., the difference between the PI count and the SPC before PI incubation; see Table 3.3); the $R^2$ value of PI count versus day 17 sensory score was 0.1007 (Table 3.3, Figure 3.3b). Overall, these initial analyses indicate that none of the raw milk tests (when performed on silo raw milk) show sufficient correlation with pasteurized milk quality to allow for prediction of microbiological or sensory quality and shelf life of commercially HTST pasteurized fluid milk.

**Table 3.3.** Correlation between different raw milk tests and various measures of pasteurized milk quality

| | $R^2$ for [a] | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **Raw milk tests** | **D17 SPC** | **D17 SPC (w/o PPC)** | **D21 SPC** | **D21 SPC (w/o PPC)** | **D17 Sensory Score** | **D17 Sensory Score (w/o PPC)** | **D17 LP** | **D21 LP** |
| SCC | 0.0221 | 0.0005 | 0.0235 | 0.0016 | 0.1132 | **0.1571*** | 0.0014 | 0.0092 |
| SP | 0.0031 | 0.0001 | 0.0000 | 0.0058 | 0.0301 | 0.0223 | 0.0002 | 0.014 |
| Edwards | 0.0092 | 0.2637 | 0.0002 | 0.2186 | 0.0283 | 0.0212 | 0.0109 | 0.018 |
| VJ | 0.0011 | 0.095 | 0.0003 | 0.1274 | 0.0145 | 0.0229 | 0.0569 | 0.0266 |
| LP | 0.0299 | 0.1009 | 0.0365 | 0.1122 | 0.0004 | 0.006 | 0.0882 | 0.0509 |
| Raw SPC | 0.0544 | 0.2095 | 0.0463 | 0.1877 | 0.0000 | 0.0000 | 0.0896 | 0.0523 |
| PBC | 0.128 | 0.2173 | 0.0876 | 0.1228 | 0.0097 | 0.0038 | 0.0601 | **0.0798*** |
| CVTA | 0.1238 | **0.4169*** | 0.1125 | **0.3681*** | 0.0003 | 0.0092 | **0.0907*** | 0.0678 |
| Coliform | 0.1301 | 0.2641 | 0.1471 | 0.2413 | 0.0565 | 0.0461 | 0.0399 | 0.0064 |
| PI | **0.2416*** | 0.1973 | **0.2211*** | 0.1874 | 0.106 | 0.0725 | 0.0003 | 0.0003 |
| ΔPI | 0.1807 | 0.1524 | 0.1554 | 0.1291 | **0.1314*** | 0.0628 | 0.0436 | 0.027 |

[a] $R^2$ values were obtained from linear regression models of log transformed raw milk test data and various measures of pasteurized milk quality; $R^2$ values in bold and marked with a *indicate the highest $R^2$ values for a raw milk test associated with each pasteurized milk quality parameter. Raw milk quality tests are shown in the first column and include Standard Plate Count (SPC), Laboratory pasteurization at day 0 (LP), Somatic Cell Count (SCC), Spore Pasteurization (SP), Vogel Johnson (VJ), Psychrotrophic Bacteria Count (PBC), Crystal Violet Tetrazolium Agar (CVTA), Preliminary Incubation (PI), and the change in bacterial numbers from raw SPC to PI (ΔPI). Pasteurized milk quality parameters include (i) SPC at day 17 and day 21, (ii) average sensory score at day 17 and 21 as well as Laboratory pasteurization counts at days 17 and 21 (D17 LP and D21 LP). For pasteurized milk SPC and sensory scores $R^2$ values were calculated for all samples or only those samples that showed no evidence for post pasteurization contamination (PPC) (marked as "w/o PPC").

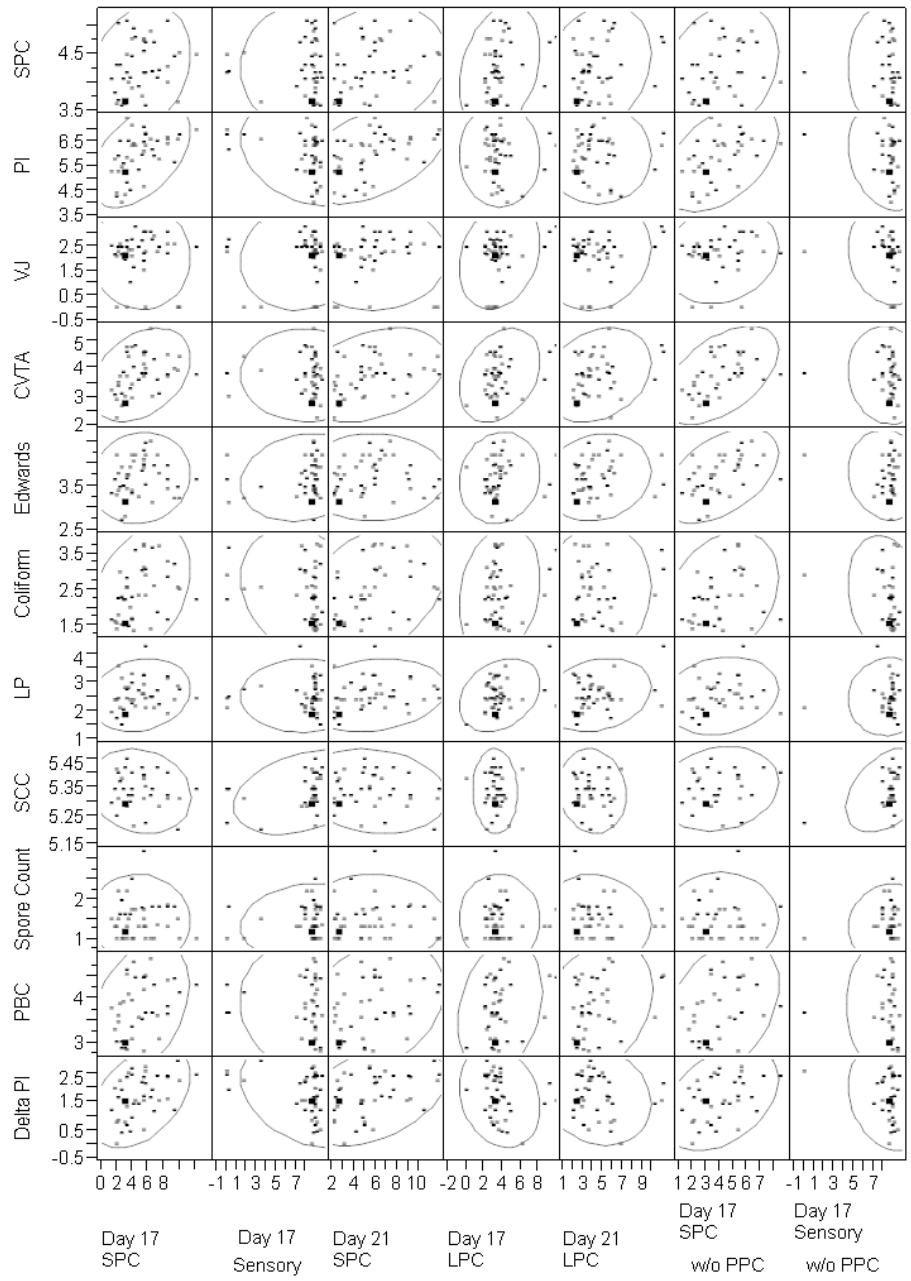
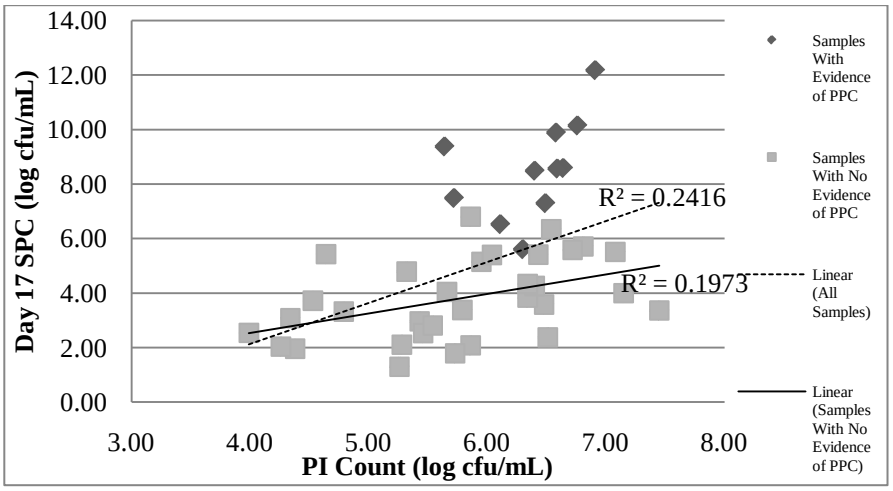


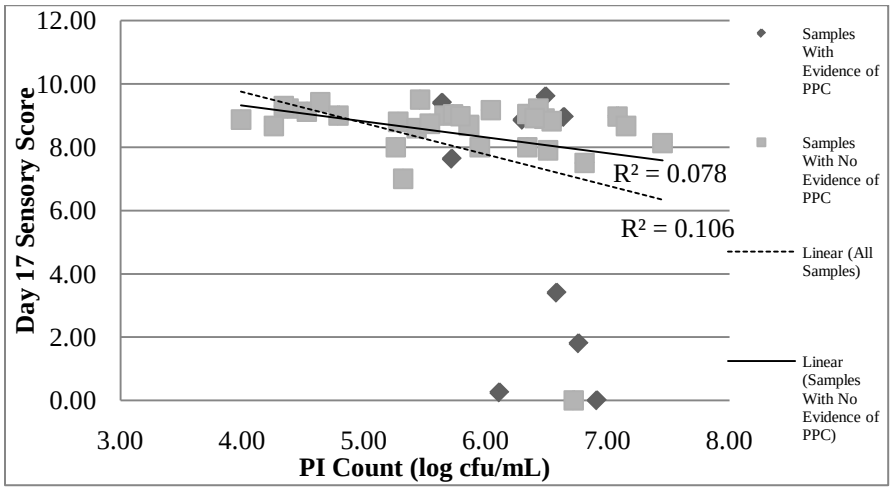

**Figure 3.2.** Scatterplot matrix of log transformed data depicting the relationship between raw milk test results performed and various measures of pasteurized milk quality (data points represent relationships for 43 raw milk and 43 pasteurized milk samples collected from four NYS fluid milk processors). Ellipses encompass 95% of data points.

**Figure 3.3.** Linear regression analysis of (A) log transformed day 17 SPC counts of 2% pasteurized milk versus log transformed raw milk PI counts; (B) day 17 Sensory scores of 2% pasteurized milk versus log transformed raw milk PI counts; and (C) log transformed LPC for day 17 and day 21 versus log transformed raw milk PI counts. Each point represents data from one of 43 samples collected from four New York State fluid milk processors. For panels A and B, data for sample pairs where the pasteurized milk sample showed evidence for post pasteurization contamination (PPC) are shown as a black diamond, while sample pairs where the pasteurized milk sample showed no evidence for PPC are shown as a grey square. Linear regression lines for all samples and for only those samples that show no evidence for PPC are also shown in panels A and B.

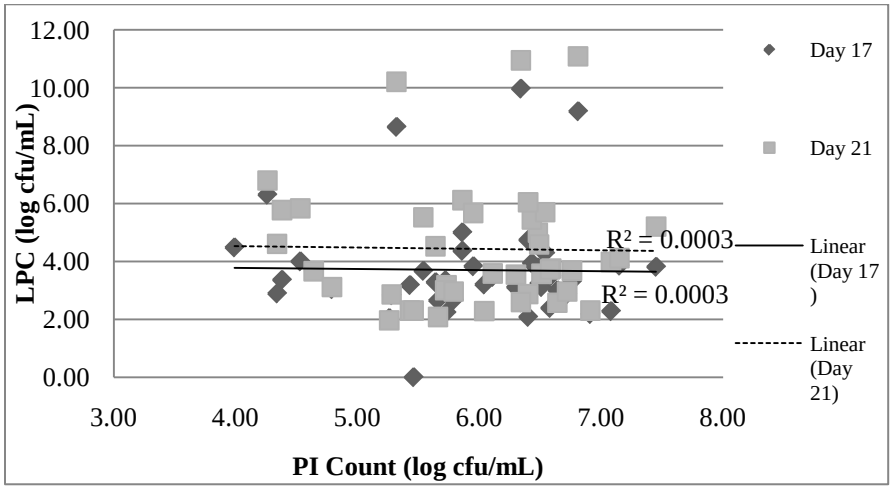
A


14.00
12.00
10.00
8.00
6.00
4.00
2.00
0.00
Day 17 SPC (log cfu/mL)
3.00
4.00
5.00
6.00
7.00
8.00
PI Count (log cfu/mL)
R² = 0.2416
R² = 0.1973
Samples With Evidence of PPC
Samples With No Evidence of PPC
Linear (All Samples)
Linear (Samples With No Evidence of PPC)

B


12.00
10.00
8.00
6.00
4.00
2.00
0.00
Day 17 Sensory Score
3.00
4.00
5.00
6.00
7.00
8.00
PI Count (log cfu/mL)
R² = 0.078
R² = 0.106
Samples With Evidence of PPC
Samples With No Evidence of PPC
Linear (All Samples)
Linear (Samples With No Evidence of PPC)

C.


12.00
10.00
8.00
6.00
4.00
2.00
0.00
LPC (log cfu/mL)
3.00
4.00
5.00
6.00
7.00
8.00
PI Count (log cfu/mL)
R² = 0.0003
R² = 0.0003
Day 17
Day 21
Linear (Day 17 )
Linear (Day 21)

**Raw Milk Tests do not Predict Pasteurized Milk Performance When Post Pasteurization Contamination is Controlled.** As commercially produced HTST fluid milk spoilage is affected by a variety of factors in addition to raw milk quality, most notably post-pasteurization contamination, we hypothesized that the poor correlation between raw milk test results and pasteurized milk microbiological and sensory quality measures, which is detailed above, may be due to the fact that some of the pasteurized milk samples tested here showed spoilage due to post-pasteurization contamination. To address this issue, we performed additional analyses that evaluated correlation between different raw milk test results and (i) sensory and microbiological quality of commercial pasteurized milk when excluding pasteurized milk samples that showed evidence for post pasteurization contamination (PPC) and (ii) microbiological quality of raw milk that has undergone lab pasteurization.

Overall, 11 of the 43 samples of pasteurized 2% milk evaluated here showed evidence for post-pasteurization contamination (PPC) (see Tables 3.1 and 3.4). In order to identify pasteurized milk samples with evidence of spoilage due to PPC, bacterial isolates obtained from all pasteurized milk samples were collected (for samples that showed >20,000 cfu/mL as well as all samples at day 21) and characterized by 16S rRNA gene sequencing, which allows for accurate identification of isolate genus and, in many cases, species. Overall, 243 bacterial isolates from 43 pasteurized milk samples were characterized with an average of 5 to 6 isolates characterized from each given sample. The most common genera found among the isolates characterized included *Bacillus* (43 isolates), *Paenibacillus* (90 isolates), *Pseudomonas* (55 isolates), other Gram-negative organisms (including *Acinetobacter* spp., *Stenotrophomonas* spp., and others for a total of 33 isolates) and other Gram-positive organisms (including *Staphylococcus* spp., *Streptococcus* spp. and others for a

total of 22 isolates). Eleven pasteurized milk samples with evidence for microbial spoilage due to PPC showed microbial spoilage profiles that were characterized by a predominance of *Pseudomonas* spp. (and other non spore-forming spoilage organisms that are not expected to survive HTST pasteurization, e.g., *Staphylococcus* spp. and *Streptococcus* spp.) (Table 3.4). Four of these eleven samples that were determined to be PPC had unacceptable sensory scores at day 17 (Table 3.4); all eleven of the PPC samples had > 20,000 cfu/mL by day 17, including 7 samples that had > 20,000 cfu/mL by day 10 (Table 3.1). Microbial profiles of the pasteurized milk samples without evidence for PPC were predominated by the presence of *Bacillus* spp. and *Paenibacillus* spp., representing sporeformers that have the ability to survive HTST pasteurization (Collins, 1981).

**Table 3.4.** Selected raw milk and finished product parameters for individual pairs of raw and pasteurized milk samples collected from four New York State processing plants over a 1-year period

| Plant | Month | Raw SPC (log cfu/mL) | Raw PI (log cfu/mL) | Day 17 Sensory Score | Isolates genus (no. of isolates) collected from pasteurized milk at Shelf-Life Day[a] | | | |
|---|---|---|---|---|---|---|---|---|
| | | | | | 10 | 14 | 17 | 21 |
| A | October | 3.66 | 5.28 | 8.8 | - | - | - | Ba (3), Pb (1), Ot (1) |
| | November | 5.04 | 5.86 | 8.68 | - | - | - | Ba (1), Pb (1) |
| | December | 4.04 | 5.26 | 8 | - | - | - | Pb (2), Ba (1), Ot (2) |
| | January | 3.74 | 5.43 | 8.6 | - | - | - | Ba (1), Pb (1) |
| | February | 3.58 | 5.46 | 9.5 | - | - | - | Ba (2), Pb (1), Ot (1) |
| | March | 3.61 | 4.38 | 9.22 | - | - | - | Pb (2), Ba (1), Ot (2) |
| | April | 3.79 | 5.73 | 9.04 | - | - | - | Ba (2), Pb (1) |
| | May | 4.04 | 5.54 | 8.74 | - | - | - | Ba (2), Pb (1) |
| | June | 3.92 | 5.32 | 7 | - | - | Ba (2), Pb (1), Ps (1), Ot (3) | Pb (1), Ba (1), Stp (1) |
| | July | 3.59 | 3.99 | 8.87 | - | - | Pb (1), Ba (1), Ot (2) | N/A |
| | August | 3.6 | 4.34 | NA | - | - | - | Pb (3), Ot (2) |
| | September | 4.28 | 4.26 | 8.67 | - | - | - | Ba (4), Pb (1), Ot (3) |
| B | October | 3.99 | 6.48 | 8.9 | - | - | - | Pb (1) |
| | **November [b]** | **5.08** | **6.64** | **8.95** | **-** | **Ps (1)** | **Ps (2)** | **Ps (2)** |
| | **December [b]** | **4.2** | **5.72** | **7.63** | **Ps (3)** | **N/A** | **Ps (3), Ot (2)** | **Ps (3)** |
| | January | 4.75 | 7.08 | 8.97 | - | - | Pb (3) | Pb (2) |
| | February | 4.46 | 7.15 | 8.67 | - | - | - | Pb (1) |
| | March | 4.08 | 4.53 | 9.12 | - | - | - | Pb (2) |
| | **April [b]** | **4.26** | **6.59** | **8.82** | **-** | **Ps (1)** | **N/A** | **Ps (1)** |
| | **May [b]** | **4.51** | **6.76** | **1.8** | **Ps (2)** | **Ps (5)** | **Ps (7)** | **Ot (1)** |
| | June | 4.69 | 6.81 | 7.5 | - | - | Ba (2), Ot (2) | Pb (1), Ps (1) |
| | July | 4.71 | 5.86 | 8.7 | - | Ba (2), Pb (2) | Pb (3) | N/A |
| | August | 4.15 | 4.64 | 9.42 | - | Pb (3), Ba (1), Ot (1) | Pb (5), Ba (1) | Pb (2), Ba (1) |
| | September | 4.15 | 4.79 | 9 | - | - | - | Pb (5), Ba (2), Ot (1) |
| C | October | 3.75 | 5.95 | 8 | - | - | Pb (2), Ba (1) | Pb (2), Stp (1) |
| | November | 4.76 | 5.66 | 9 | - | - | - | Ot (2) |
| | **December [b]** | **4.18** | **6.11** | **0.25** | **-** | **-** | **Pb (2), Ba (1), Ot (1)** | **Ot (1)** |
| | **January [b]** | **4** | **6.4** | **9.07** | **Ba (3), Ot (1), Str (1)** | **Pb (1), Str (1), Ot (1)** | **Pb (2), Ba (1), Str (1), Ot (1)** | **Pb (2), Str (1)** |
| | **February [b]** | **4.08** | **6.49** | **9.6** | **Ba (1), Ot (1)** | **Pb (2), Ot (1)** | **Pb (2), Ba (1)** | **Pb (2), Ps (1)** |
| | **March [b]** | **3.57** | **6.3** | **8.86** | **-** | **Ot (2)** | **Pb (1), Ps (1)** | **Pb (2), Ot (1)** |
| | April | 4.74 | 6.43 | 9.22 | - | - | Pb(1) | Pb(2) |
| D | October | 4.3 | 6.51 | 7.9 | - | - | - | Ba (1), Pb (1), Ot (1) |
| | November | 3.89 | 6.34 | 9.05 | - | - | - | Ba (1), Pb (1) |
| | December | 5.08 | 7.45 | 8.13 | - | - | - | Pb (2) |
| | **January [b]** | **4.48** | **6.91** | **0** | **Ps (1)** | **Ps (3)** | **Pb (2), Stp (1)** | **Ps (1), Str (1)** |
| | February | 4.15 | 6.72 | 0 | - | - | Pb (3) | Pb (1), Ba (2) |
| | **March [b]** | **3.64** | **6.58** | **3.4** | **Ps (2), Ot (1)** | **Ps (3)** | **Ps (5)** | **Str (1)** |
| | April | 4.9 | 6.54 | 8.82 | - | Pb (1) | Pb (1) | Pb (1) |
| | May | 4.96 | 6.4 | 8.92 | - | - | - | Pb (2) |
| | June | 4.79 | 6.34 | 8 | - | Ba (1), Str (1), Ot (2) | Ba (1) | Pb (1), Stp (1) |
| | **July [b]** | **4.46** | **5.64** | **9.4** | **Ps (1), Ot (1)** | **Ps (1)** | **Ps (1)** | **Ps (1)** |
| | August | 4.54 | 6.04 | NA | - | Pb (4), Ot (1) | Pb (3) | Pb (3) |
| | September | 4.48 | 5.79 | 8.98 | - | - | - | Pb (1), Ot (2) |

[a] These columns list the genus of bacterial isolates collected from pasteurized milk samples tested at shelf-life life days 10, 14, 17, and 21; isolates were collected and identified by 16S sequencing from samples that showed SPC > 20,000 cfu/mL and from all day 21 samples; - indicates samples for which no isolates were collected and N/A indicates samples which met inclusion criteria but for which isolates were not available for characterization. Isolates were characterized into the genera Pseudomonas (Ps), Streptococcus (Str), Bacillus (Ba), Staphylococcus (Stp), Paenibacillus (Pb); genera that were rarely isolated are listed as Others (Ot)

[b] rows with this superscript and bolded text across indicate sample pairs where the pasteurized milk samples showed microbiological evidence for Post-Pasteurization Contamination (PPC).

In order to evaluate the ability of raw milk tests to predict the performance of pasteurized milk, without the confounding effects of including data for commercially pasteurized milk that were spoiled due to post-pasteurization contamination, we determined correlations (i.e., $R^2$ values) between raw milk test results and corresponding quality and shelf life parameters for the commercially pasteurized 2% milk, including (i) SPC at day 17 and day 21 of shelf life, and (ii) sensory scores at day 17 of shelf life (Table 3.3, Figure 3.2), excluding the 11 pasteurized milk samples (and corresponding raw milk data) that showed evidence for PPC. When comparing the different raw milk test results to day 17 SPC counts for pasteurized milk, $R^2$ values ranged from a low of 0.0001 (for SP) to a high of 0.4170 (for CVTA, see Table 3.3). Similarly low $R^2$ values were found when comparing the different raw milk test results to day 21 SPC counts; $R^2$ values ranged from a low of 0.0016 (for SCC), to a high of 0.3681 (for CVTA). $R^2$ values for raw milk test results versus sensory scores at day 17 were also extremely low, ranging from 0.000 (for raw milk SPC) to 0.1571 (for SCC). The $R^2$ values for PI count results versus day 17 SPC, day 21 SPC, and day 17 sensory scores were all extremely low (0.1973, 0.1874, and 0.1060, respectively; see Table 3.3, Figure 3.3a,b). Overall, these data further support that the raw milk tests used here (when performed on silo raw milk) do not show sufficient correlation with pasteurized milk quality to allow for prediction of microbiological or sensory quality and shelf life of commercially HTST pasteurized fluid milk.

While the analyses evaluating correlations between raw milk tests and pasteurized fluid milk quality parameters, excluding fluid milk with apparent spoilage by post-processing contamination, should provide for an appropriate evaluation of the ability of raw milk tests to predict effects of raw milk quality parameters on HTST fluid milk quality and shelf life, we cannot exclude that processing plant specific parameters and factors have a considerable enough effect to obscure any correlations

between raw milk quality parameters and pasteurized fluid milk quality. In order to address this issue, all raw milk samples were also used to perform a lab pasteurization (i.e., batch treatment at 63°C [145°F] for 30 min), followed by incubation at 6°C over 21 days and bacterial enumeration on SPC agar on days 0, 7, 10, 14, 17 and 21 (i.e., an LPC). This laboratory treatment, which is also known as batch pasteurization, provides a level of human pathogen inactivation in raw milk that is equivalent to that of HTST pasteurization (FDA, 2009). We reasoned that, due to the ability to control PPC and other possible confounding factors, this test is a close approximation of the quality of pasteurized product when plant factors are controlled. Correlations between different raw milk test results and day 17 LPC ranged from $R^2$ values 0.0002 (for SP) to 0.0907 (for CVTA); correlations between raw milk test results and day 21 LP were similarly low, ranging from $R^2$ values of 0.0003 (for PI, see Fig. 3.3c) to 0.0798 (for PBC) (Table 3.3). These results further support that none of the raw milk tests evaluated here show appropriate relationships with pasteurized product quality to justify their use to predict the quality of pasteurized product produced from a given silo tank of raw milk.

While it may be tempting to propose that a raw milk test that showed, by relative comparison, the best correlation with pasteurized milk quality (e.g., CVTA, see Table 2) may represent an appropriate test, it is important to emphasize that none of the raw milk tests evaluated showed $R^2$ values of >0.45, suggesting that none of these test show an appropriate predictive power. In biological systems, an $R^2$ value of at least 0.50 to 0.80 would be considered a strong relationship between tests (Trigiano and Gray, 1999). In addition to statistically significant relationships between raw milk tests and pasteurized milk quality, the strength of biological relationships between organisms detected by a raw milk test and pasteurized milk spoilage also needs to be considered. For example, plating on CVTA predominantly detects Gram-negative

organisms (e.g., *Aeromonas* spp., *Klebsiella* spp., etc.) that are easily inactivated by HTST pasteurization and hence there is no clear biological link between raw milk bacterial counts on CVTA and HTST milk spoilage.

While our data convincingly demonstrate that raw milk tests do not seem to be able to predict the sensory and microbiological shelf life of HTST pasteurized milk, it is important to consider that raw milk evaluated here was generally of excellent quality (e.g., average SPC of 18,000 cfu/mL, range 3,700 – 120,000 cfu/mL; average SCC of 220,000/mL, range 160,000 – 280,000 /mL). Hence, it is feasible and not unlikely that some of the tests evaluated here may be appropriate to screen a raw milk supply for poor quality milk, which, if used for fluid milk production, may affect finished product quality. Similarly, some of the raw milk tests may be useful to identify farms that have problems with on-farm milk quality (which, as a matter of fact, is the intended use of many of the tests evaluated), even if they do not accurately and reproducibly predict finished product quality when applied on a population basis. Future research is also needed to further evaluate the correlation between HTST product quality and different raw milk tests, when applied to individual bulk tank milk samples (rather than raw milk collected from processing plant silos). The study design used here was critical though as it allowed for correlation between raw milk quality parameters and quality parameters for commercially produced HTST, which is not feasible when evaluating individual bulk tank samples (except for the rare small processing plants where one commercial processing run uses milk from a single bulk tank).

**Plant Factors Appear to Have a Major Effect on Pasteurized Milk Quality.** Our data evaluating microbial and sensory characteristics of raw and HTST pasteurized milk from 4 processing plants in New York also supports that, despite increased

interests on the effects of raw milk quality on HTST product quality, plant specific and in-plant factors still have an important effect on pasteurized milk quality. As detailed above, one key factor contributing to the quality of commercially produced HTST milk is post-pasteurization contamination (PPC); not only did 11 of the 43 pasteurized milk samples show evidence for PPC, occurrence of PPC also differed between plants with only plant A showing no evidence of PPC among all samples tested. Overall, these findings are consistent with previous studies suggesting that post pasteurization contamination is still a major cause of pasteurized HTST product spoilage across the US (Ralyea et al., 1998; Dogan et al., 2002; Carey et al., 2005, Ranieri et al., 2009a, 2009b).

In addition to controlling PPC, other plant factors that have been shown to have an effect on pasteurized milk quality are pasteurization conditions. Ranieri et al. (2009b) specifically showed, in controlled pilot plant pasteurization trails, that pasteurization temperature is inversely related to bacterial growth over refrigerated shelf-life. This phenomenon seems to largely related to outgrowth of Gram positive psychrotolerant endospore-forming bacteria belonging to the genus *Paenibacillus* (Ralyea et al., 1998, Fromm and Boor, 2004; Huck et al., 2007b; Ranieri et al., 2009a), which seems to be enhanced in milk that is pasteurized at higher temperatures, possibly due to enhanced inactivation of endogenous antimicrobial systems (e.g., the lactoperoxidase system) or enhanced germination. Interestingly, our data on commercially processed HTST milk are consistent with the findings from the pilot plant study reported by Ranieri et al. (2009a). Specifically, HTST milk samples from Plant A, which had the lowest pasteurization temperature, showed the lowest average bacterial numbers after 17 days of refrigerated storage, while milk from all other plants showed, on average, at least 2 log (100 fold) higher bacterial numbers at day 17 (Table 3.1, Figure 3.4). Importantly though, none of the pasteurized milk samples

from Plant A showed evidence of PPC, suggesting that, in general plant A, represents a facility with a high level of quality control; hence, overall high level of quality control may also be responsible for or contributing to the high quality finished product. The importance of plant specific factors, for HTST product quality, is also supported by the fact that, despite a wide range of microbial quality of commercially pasteurized milk among the samples collected during this study (Fig. 3.4), the LP counts after 17 days were on average under 20,000 cfu/mL and not significantly different between raw milk collected at different plants (see Fig. 3.4) indicating raw milk sampled from all plants was capable of performing well under refrigerated storage.

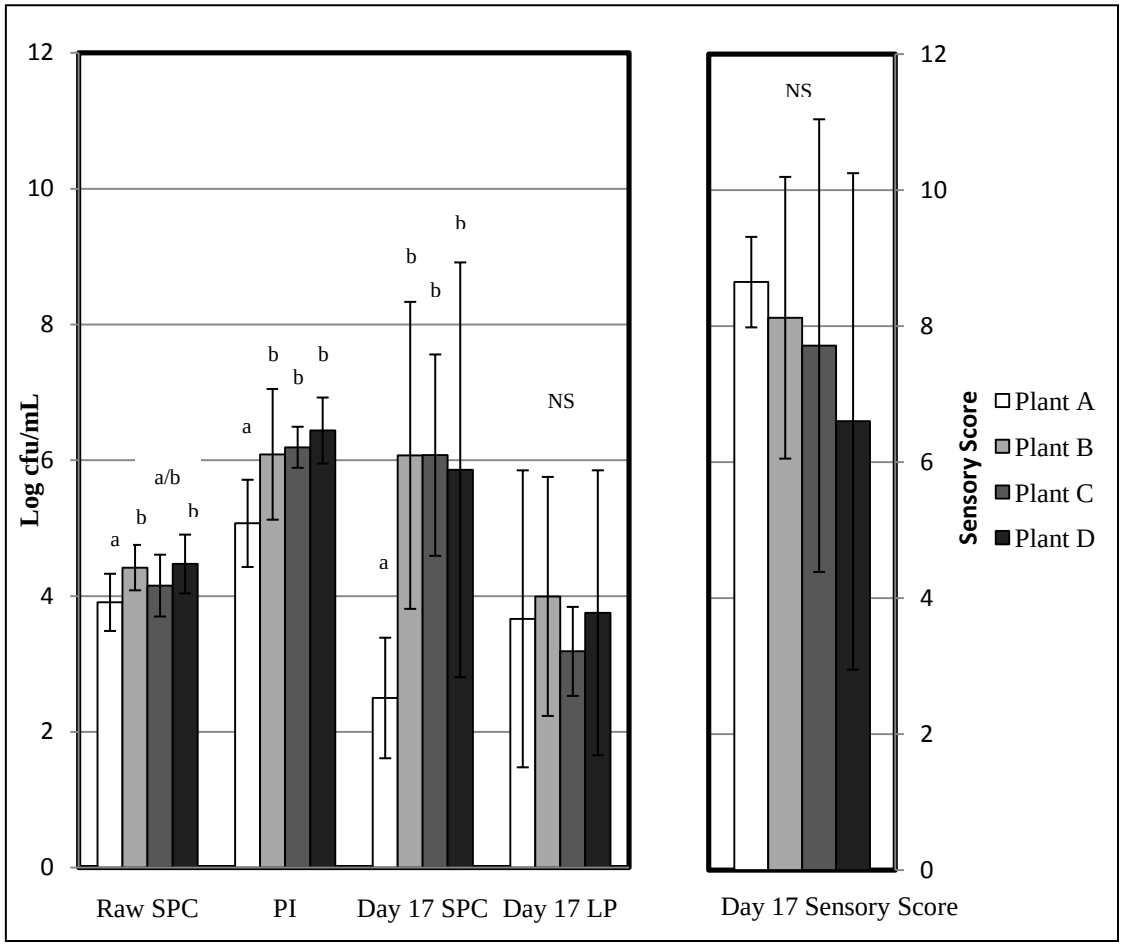



**Figure 3.4.** Selected mean raw milk and mean pasteurized milk quality parameters for samples collected from four New York State fluid milk processors. Raw milk quality parameters shown include log transformed mean SPC and PI counts; quality parameters shown for pasteurized 2% milk include mean log transformed day 17 SPC and mean sensory scores. Mean day 17 LPC data are also shown. Data represent the means for 12 samples (plants A,B, and D) and 7 samples (plant C); error bars represent ±1 standard deviations.

# CONCLUSION

Our results indicate that none of the tests commonly used by the fluid milk industry to screen raw milk have the ability to predict the bacterial or sensory quality of pasteurized milk. As plant factors, such as post pasteurization contamination, still seem to play a major role in the quality of pasteurized milk produced in many plants, further efforts to optimize in-plant quality assurance and contamination control will be critical to further improve fluid milk quality and shelf-life. Nevertheless, it is increasingly apparent that contamination with sporeforming bacteria, in particular *Paenibacillus* spp., and outgrowth of these bacteria, which often have the ability to grow at refrigeration temperatures, in pasteurized fluid milk represents a major factor limiting product shelf life to <21 to 24 days, if post pasteurization contamination is controlled. While raw milk tests are available that specifically enumerate spores in raw milk (i.e., **SP**, which involves enumeration of bacteria present after a heat treatment that kills most non-sporeforming bacteria), we found that SP counts also did not correlate with fluid milk spoilage. This finding is not as surprising as it may initially seem, as raw milk can contain heat resistant bacterial spores representing different genera, including *Bacillus* spp. and *Paenibacillus* spp. (Ralyea et al., 1998, Fromm and Boor, 2004; Huck et al., 2007b; Ranieri et al., 2009). While many vegetative *Bacillus* spp. cells do typically not grow under refrigeration temperatures, a considerable proportion of *Paenibacillus* spp. appears to have the ability to grow in milk, under refrigeration temperatures, although typically at growth rates lower than *Pseudomonas* spp. (Fromm and Boor, 2004). Initial loads of spore-formers in raw milk (and in particular *Paenibacillus* spp.) are also often very low and below the SP detection limit. SP thus lacks both the sensitivity and specificity needed to detect the sporeforming bacteria likely to affect fluid milk shelf-life. We conclude that the dairy

industry is still in need a scientifically sound raw milk test that will be able to predict the quality and shelf life of pasteurized milk that is not exposed to post-pasteurization contamination. Development of these, including possible molecular biology-based tests, seems feasible with recent advances in our understanding of *Paenibacillus* mediated fluid milk spoilage (Huck et al., 2007a,b; Ranieri et al., 2009a,b).

# CHAPTER FOUR

## CONCLUSIONS

Improving pasteurized milk quality and extending shelf-life is essential to the fluid milk industry as it competes in the US beverage industry. In the absence of PPC the extension of fluid milk shelf-life is predominantly limited by the spore-forming microorganism, *Paenibacillus* spp. (Fromm et al., 2004; Huck et al., 2007b; Huck et al., 2008; Ranieri et al., 2009b). *Paenibacillus* spp. survives pasteurization in spore form and subsequently grows at refrigeration temperature, limiting shelf-life. Strategies for extending shelf-life and improving the quality of conventionally pasteurized milk include evaluating raw milk for the presence of organisms that will limit pasteurized milk shelf-life and controlling these organisms after pasteurization.

Our work demonstrates the ability of the antimicrobial LAE to retard bacterial growth in conventionally pasteurized milk. At the highest concentration, LAE was effective in both chocolate and unflavored milk at retarding bacterial growth over 21 days at refrigeration temperatures. Despite the fact that using a GRAS antimicrobial would change the way the product would be required to be labeled, there may be a role for these compounds in extending pasteurized fluid milk shelf-life when other factors, such as PPC, are controlled.

The need for a raw milk test that will accurately and reliably predict pasteurized fluid milk shelf-life is undeniable. This type of test would be valuable in ensuring that high quality product is available to consumers. Raw milk tests currently employed by the fluid milk industry are not adequate for this purpose. Plant factors that cannot be detected by raw milk tests, including PPC, pasteurization parameters and storage temperature have a major effect on the quality of pasteurized product. If

these factors are controlled and optimized, the focus of raw milk testing should be on detecting *Paenibacillus* spp. as the limiting factor of pasteurized milk shelf-life.

As the dairy industry continues to advance in improving the quality of product available to consumers, it must be innovative in its strategies to extend product shelf-life. In addition to promoting stringent cleaning and sanitization programs and optimizing pasteurization parameters, future research efforts should focus on detecting *Paenibacillus* spp., controlling its entry into the milk system and developing novel methods for controlling its outgrowth after pasteurization.

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