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Determinants of fluid milk quality

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DETERMINANTS OF FLUID MILK QUALITY

A Thesis

Submitted to the Graduate Faculty of the
Louisiana State University and
Agricultural and Mechanical College
in partial fulfillment of the
requirements for the degree of
Master of Science

in

The Interdepartmental Program in The School of Animal Sciences

By
Jose L. Vargas
B.S., Escuela Agrícola Panamericana Zamorano, 2004
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# TABLE OF CONTENTS

ACKNOWLEDGMENTS .............................................................................................................. ii

LIST OF TABLES ...................................................................................................................... v

LIST OF FIGURES .................................................................................................................. vi

ABSTRACT ................................................................................................................................. viii

CHAPTER 1. INTRODUCTION ................................................................................................... 1

CHAPTER 2. LITERATURE REVIEW ........................................................................................ 5
  2.1. Standard Plate Counts ........................................................................................................ 5
  2.2. Coliform Counts .................................................................................................................. 5
  2.3. Psychrotrophic Counts ....................................................................................................... 5
  2.4. Aerobic Spores ................................................................................................................... 6
  2.5. Heat-Resistant Spore-Forming Psychrotrophs ................................................................. 6
  2.6. Fat Percentage, Protein Content and Somatic Cell Counts ............................................ 7
  2.7. Sensory Evaluation .......................................................................................................... 8

CHAPTER 3. MATERIALS AND METHODS .......................................................................... 10
  3.1. Experimental Design ........................................................................................................ 10
  3.2. Analytical Procedures ...................................................................................................... 10
    3.2.1. Standard Plate Counts ............................................................................................. 10
    3.2.2. Coliforms Counts .................................................................................................... 11
    3.2.3. Psychrotrophic Counts ............................................................................................ 11
    3.2.4. Heat-Resistant Spore-Forming Psychrotrophic Counts .......................................... 12
    3.2.5. Aerobic Spores Counts ........................................................................................... 12
    3.2.6. HR Tests .................................................................................................................. 12
    3.2.7. Fat % ....................................................................................................................... 13
    3.2.8. Protein % ................................................................................................................. 13
    3.2.9. Somatic Cell Count ................................................................................................. 13
  3.3. Sensory Evaluation .......................................................................................................... 13
  3.4. Statistical Analysis ........................................................................................................... 13

CHAPTER 4. RESULTS AND DISCUSSION ............................................................................ 15
  4.1. Standard Plate Counts and Coliform Counts ................................................................... 15
    4.1.1. Standard Plate Counts ............................................................................................. 15
    4.1.2. Coliform Counts ...................................................................................................... 18
  4.2. Psychrotrophic Bacteria Counts ....................................................................................... 18
  4.3. Heat-Resistant Spore-Forming Psychrotrophs and Aerobic Spores ............................... 21
    4.3.1. Heat-Resistant Spore-Forming Psychrotrophs ......................................................... 21
    4.3.2. Aerobic Spores ........................................................................................................ 24
  4.4. HR Stress Tests ................................................................................................................ 26
LIST OF TABLES

Table 1. Correlation Coefficients Found Among Analyses.......................................................... 43
LIST OF FIGURES

Figure 1. SPC Counts Day 0 for 2% Milk Samples ................................................................. 15
Figure 2. SPC Counts Day 0 for Whole Milk Samples ............................................................. 16
Figure 3. SPC Counts Day 14 for 2% Milk Samples ................................................................. 17
Figure 4. SPC Counts Day 14 for Whole Milk Samples ............................................................ 18
Figure 5. Psychrotrophic Counts for 2% Milk Samples (Traditional Method) ......................... 19
Figure 6. Psychrotrophic Counts for Whole Milk Samples (Traditional Method) ..................... 20
Figure 7. Psychrotrophic Counts for 2% Milk Samples (Rapid Method) ................................. 22
Figure 8. Psychrotrophic Counts for Whole Milk Samples (Rapid Method) ............................. 22
Figure 9. Heat-Resistant Spore-Forming Counts for 2% Milk Samples ................................. 24
Figure 10. Heat-Resistant Spore-Forming Counts for Whole Milk Samples ......................... 25
Figure 11. Aerobic Spores Counts for 2% Milk Samples .......................................................... 26
Figure 12. Aerobic Spores Counts for Whole Milk Samples .................................................... 27
Figure 13. HR-1 Test Results for 2% Milk Samples ................................................................. 28
Figure 14. HR-1 Test Results for Whole Milk Samples ............................................................. 28
Figure 15. HR-2 Test Results for 2% Milk Samples ................................................................. 30
Figure 16. HR-2 Test Results for Whole Milk Samples ............................................................. 30
Figure 17. HR-3 Test Results for 2% Milk Samples ................................................................. 32
Figure 18. HR-3 Test Results for Whole Milk Samples ............................................................. 32
Figure 19. Fat Percentage for 2% Milk Samples ..................................................................... 34
Figure 20. Fat Percentage for Whole Milk Samples ............................................................... 35
Figure 21. Protein Percentage for 2% Milk Samples ............................................................... 36
Figure 22. Protein Percentage for Whole Milk Samples .......................................................... 36
Figure 23. Somatic Cell Counts for 2% Milk Samples ............................................................. 38
Figure 24. Somatic Cell Counts for Whole Milk Samples ........................................................... 39
Figure 25. Sensory Evaluation Scores for 2% Milk Samples for Day 0................................. 40
Figure 26. Sensory Evaluation Scores for Whole Milk Samples for Day 0 ....................... 41
Figure 27. Sensory Evaluation Scores for 2% Milk Samples at Day 14 ............................... 42
Figure 28. Sensory Evaluation Scores for Whole Milk Samples at Day 14 ......................... 42
ABSTRACT

The objectives of this study were to provide an overview of fluid milk quality in the U.S., determine if there is a correlation between rapid and traditional tests, determine if results from different analyses correlate with a sensory analysis and to determine any correlation between any of the analyses done in this study. Whole and 2% milks were received from 17 dairy processing plants located in the west, midwest, and southern regions of the U.S in duplicates. Samples were pasteurized at the processing plants by HTST method (161-175°F for 15-25 seconds). For standard plate counts at day 0 no significant differences were found. The mean value was 1.89 log CFU/ml. In day 14, significant differences were found. The mean was 7.58 log CFU/ml. No coliforms were found in any of the samples on either day 0 or day 14. Psychrotrophic counts had no significant differences for the traditional method. 5% of the samples showed psychrotrophic counts. For the rapid method no significant differences were found. The mean value was 0.63 log CFU/ml. 10% of the samples showed psychrotrophic counts. For heat-resistant spore-forming psychrotrophs no significant differences were found. 10% of the samples showed psychrotrophs. For aerobic spores no significant differences were found. The mean value was 1.94 log CFU/ml. HR-1 tests showed no significant differences. The mean was a pink coloration. HR-2 test results did result in significant differences at 48 and 60 hrs, with a mean of a pink color. HR-3 tests showed a mean of pink. Significant differences were found among samples. Fat% results had no significant difference for either milk samples. 2% samples had a mean of 2.08%. Whole milk samples had a mean of 3.43%. Protein content showed no significant differences. The mean was 3.15%. No significant differences were found for somatic cell counts. The mean value was 1.77 log SCC/ml. Regarding sensory evaluation, for day 0 no significant
differences were found. The mean score was 7. Day 14 showed significant differences with a mean score of 4.39. Both test methods of psychrotrophic counts shared a high correlation coefficient but a t-test confirmed otherwise. We can conclude that the fluid milk quality in the U.S. needs to be improved.
CHAPTER 1. INTRODUCTION

In 2006, U.S. farm milk production volume hit an all-time high, reaching 181.8 billion pounds, 2.75% higher than 2005, with 32% of that milk being used to make fluid milk, cream and related products. 2006 was the first year that per capita consumption of fluid milk products in the United States grew, reaching 181.1 pounds, or about 21 gallons per capita, of mainly lower-fat milks. With consumption growing every year, it is no wonder quality and safety are of major concern to the consumer. (IDFA, 2007)

In the last 25 years, science and technology devoted to milk and milk products has produced major advances in mechanization, automation, and hygiene within the processing plant. Quality and safety, extensions in shelf life, and new product introductions have brought variety and convenience for the consumer. (Goff and Griffiths, 2006)

According to Muir (1996) the shelf life of a dairy product is best defined as the time during which the product remains wholesome and exhibits no physical or sensory defects. At least three aspects of quality contribute to shelf life: product safety together with sensory and physical properties. There are many ways to evaluate the quality of pasteurized milk. From general microbial testing such as standard plate counts, coliform counts, and tests for groups of organisms such as lipolytic bacteria and somatic cell count, to specific tests like detection of antibiotic or drug residues in milk and dairy products are just a few ways to evaluate quality. Of course, some methods are very meticulous and take a lot of time (which in turn has given way to new rapid test versions of original testing procedures). For example, the original method to test for psychrotrophic bacteria found in older versions of the Standard Methods for the Examination of Dairy Products took 10 days to give back results. According to Marshall
In the 16th Edition, the psychrotrophic bacteria count method now only takes 25 hours to get reliable psychrotrophic bacteria counts.

Microbial testing is an integral part in the analysis of pasteurized milk quality. One microbial test used to determine pasteurized milk’s quality is the standard plate count, which determines the total number of bacteria in a specified amount of milk, generally a milliliter. Coliform counts, which analyze the sample for the presence of either Escherichia or Aerobacter bacteria (the two genera of bacteria included in the coliforms group) is another test used to determine milk quality (Marth and Steele, 2001). Somatic cell counts are also being used to determine the quality of pasteurized milk. For example, according to Diamond V (1997) the somatic cell count of milk is the sum of two cell sources: myoepithelial cells (cells that convert nutrients to milk) and white blood cells, which represent the phagocytes, and other similar cells, that are released by the inflammation process in response to an invading organism or a mechanical stress.

Microbial testing is not the only way to determine the quality of milk. Other factors like fat percentage, protein percentage and testing for antibiotics are very important not only to the producer/processor, but also to the consumer. The colloidal nature of cow’s milk is a crucial structural feature that affects final product quality as well as its processing behavior. The colloidal system can be divided into two compositional domains, the casein micelle and the milk fat globule. These colloidal domains comprise nearly 80% of the approximate 12.7 g total solids 100 g\(^{-1}\) in milk. (Tamime, 2007)

Milk is a precursor for many food products. Its value has been enhanced by an enormous amount of research, especially over the past 50 years, to support the development and commercialization of dairy-based products with an increasing variety of
flavor, texture and shelf life. (Tamime, 2007) According to the International Dairy Foods Association (2007), milk products contain high quality proteins. The whey proteins constitute about 18% of the protein content of milk. Casein, a protein found only in milk, contains all of the essential amino acids. It accounts for 82 percent of the total proteins in milk and is used as a standard for evaluating protein of other foods. Protein is needed to build and repair body tissues and to form antibodies, which circulate in the blood and help fight infection. Another quality determinant of pasteurized milk is the absence of antibiotics. Testing for antibiotics is of high importance because not only does it cause failure of milk to have “starter” reaction in cheese making (causing costs of processors to sky-rocket due to the low yields of cheese) (Lam, 2008) but also the exposure to small levels of antibiotics causes modification of “good” bacteria in intestine leading to vitamin and mineral deficiencies and often “superinfection” (increased tendency to contract infection) in humans. (Lam, 2008)

Three tests developed by Randolph Associates that analyze different types of bacterial contamination in milk samples are also available to help evaluate the quality of fluid milk. For instance, the HR-1 Test is a stress test for contamination. It is a quick and simple qualitative test that helps pinpoint coliform and other contaminating bacteria in finished or line-sampled products. The HR-2 Test is a presumptive shelf life test. It is a qualitative test that helps to predict the shelf life of fluid dairy products within 48-60 hours of production. The HR-3 Test is a stress test screening for thermoduric Gram-positive microbial contamination in raw milk or pasteurized samples taken aseptically at the pasteurizer discharge. (Randolph Associates, Inc. 2006)

Even though pasteurized milk has come a long way in the last couple of decades there is still much to be accomplished. Not only is the consumer becoming more educated
on all matters of safety and nutrition, but also federal organizations are implementing ordinances, such as the Pasteurized Milk Ordinance (PMO) and institutions are funding programs for the improvement of raw milk (Milk Quality Improvement Program funded by the New York State Dairy Promotion Board), which are demanding from both the producer and the industry a safer, more shelf-stable product. Barbano and others (2006) state that the PMO requirements for maximum bacteria and somatic cell count are to ensure public health. Today, the typical refrigerated fluid milk shelf life for high-temperature short-time (HTST) milk in the United States is about 14 days (Boor, 2001). In these HTST milks, shelf life is typically limited by the growth of psychrotrophic bacteria and production of off-flavors associated with this microbial growth. (Barbano et al, 2006)

In this study we are looking to:

- Provide an overview of fluid milk quality in the U.S.
- Determine if there is a correlation between rapid tests and traditional tests.
- Determine if results from different analyses correlate with a sensory analysis.
- Determine any correlation between any of the analyses done in this study.
CHAPTER 2. LITERATURE REVIEW

2.1. Standard Plate Counts

The standard plate count is suitable for estimating bacterial population in most types of dairy products, and it is the reference method specified in the Grade A Pasteurized Milk Ordinance to be used to examine raw and pasteurized milk and milk products. (Marshall, 1992)

2.2. Coliform Counts

According to Tatini and Kauppi (2002) coliform bacteria such as those from the genera Enterobacter, Escherichia and Klebsiella are used as indicators of sanitation conditions during the processing of foods. Coliform tests are conducted following pasteurization to detect recontamination. According to Marth and Steele (2001), coliforms are defined as aerobic and facultatively anaerobic, asporogenous, gram-negative rods that ferment lactose with acid and gas production within 48 h at 32 or 35°C. They include the genera Escherichia, Enterobacter, Citrobacter, and Klebsiella. Aerobic gram-negative rods commonly found in milk include Pseudomonas fluorescens, P. putida, P. fragi, P. putrefaciens, and less frequently P. aeruginosa. The coliform count, in which samples are plated on the selective and differential medium Violet Red Bile Agar and incubated for 24 h at 32°C, estimates the number of coliform organisms present. The presence of these organisms can also indicate unsanitary production and processing practices. (Marth and Steele, 2001)

2.3. Psychrotrophic Counts

According to Meer and others (1991) though poor quality raw milk can result in defective products, post-pasteurization contamination with psychrotrophic spoilage bacteria is most detrimental. In most cases, product contamination is the result of
insufficient cleaning and sanitation of the processing equipment and plant environment. Product contamination may occur even when it appears that a well-designed sanitation and quality control program is in place. In the absence of post-pasteurization, certain strains of microorganisms (i.e. *Bacillus spp*) that are capable of surviving pasteurization and growing under refrigeration (thermoduric psychrotrophs) can eventually grow and cause spoilage, generally later in shelf life. (Meer et al., 1991) According to Eneroth and others (2001), in the absence of post pasteurization contamination byGram-negative psychrotrophic bacteria, growth of psychrotrophic spore-forming bacteria such as Bacillus cereus will limit the keeping quality of pasteurized milk.

### 2.4. Aerobic Spores

According to Scheldeman and others (2005), raw milk represents a very suitable medium for the growth of bacteria, and the quality of milk is dependent on its microflora. Bacillus species and their spores, often present in raw milk, play an important role in the bacterial deterioration of milk and milk products. According to Marth and Steele (2001) it might be concluded that the higher the quality of raw milk, the higher will be the incidence of gram-positive spore-forming bacteria. Bacillus species account for 95% of the total spore-forming bacteria in milk, with Clostridium species comprising the remaining 5% in raw milk. In the United States, 43% of Bacillus organisms are B. licheniformis and 37% are B. cereus; however, in other countries, B. cereus is predominant. Spore-forming bacteria are expected to be present in almost all raw milk supplies. (Marth and Steele, 2001)

### 2.5. Heat-Resistant Spore-Forming Psychrotrophs

To control the growth of Bacillus species various kinds of heat treatments are used. Although these processes are designed to result in commercially sterile products,
spoilage infrequently occurs because of recontamination during filling. Still, massive contaminations of heat-treated milk caused by heat-resistant spore-forming bacteria have been reported. (Scheldeman et al., 2005) According to Marshall (1992), product defect associated with the growth of aerobic spore-forming bacteria include the sweet-curdling defect in pasteurized milk and coagulation of canned evaporated milk.

According to Magnusson and others (2007) bacterial spore contamination of milk can cause processing problems for the dairy industry because spores can survive pasteurization. Presence of _Bacillus cereus_ is a limiting factor for the shelf life of pasteurized milk and a potential food poisoning agent. It is a spore-forming bacterium commonly found in soil and is most frequently found in milk during the grazing season when the risk of teat contamination with the soil is greatest. As the dairy processing industry becomes more involved with extended shelf life (ESL) products, the problem with spore-forming bacilli will probably increase. Thus, an aerobic spore count (80°C for 12 min followed by rapid cooling and plating on plate count agar (PCA) with incubation of plates at 32°C for 48 hours) will become a vital microbiological test for raw milk. (Marth and Steele, 2001)

2.6. Fat Percentage, Protein Content and Somatic Cell Counts

Fat percentage of milk or butterfat has a significant economic impact for dairy producers. In recent years the fat increment in milk has varied from less than seven cents per tenth of a percent butterfat above or below 3.5 percent fat in the milk to over 20 cents per tenth of a percent of fat. Recently, the fat content of milk accounted for approximately 50 percent of the total value of the milk. Although fat rarely accounts for 50 percent of the value of milk, small variations in fat percentage can significantly affect economic returns to dairy producers. (Pennington, 2008)
Cow's milk is a good source of low-cost high-quality protein, providing 8.1 grams of protein (16.3% of the daily value for protein) in one cup. The structure of humans and animals is built on protein. We rely on animal and vegetable protein for our supply of amino acids, and then our bodies rearrange the nitrogen to create the pattern of amino acids we require. (George Mateljan Foundation, 2008)

According to Pennington (2008) federal law allows milk to be sold only if the bulk tank has a somatic cell count of less than 750,000/ml. The primary reason for dairy producers to reduce somatic cell count is because somatic cell count relates to milk losses due to mastitis. On the other hand, processors want a decreased somatic cell count because it reflects increased cheese yield and keeping quality of the milk.

2.7. Sensory Evaluation

The importance of milk grading lies in the fact that dairy products are only as good as the raw materials from which they were made. It is important that dairy personnel have knowledge of sensory perception and evaluation techniques. The identification of off-flavors and desirable flavors, as well as knowledge of their likely cause, should enable the production of high quality milk, and subsequently, high quality dairy products. (Goff, 2008) Flavors of milk may be caused, in general, by five factors: Health of the cow, feeds consumed by the cow, bacteriological action, chemical changes, and absorption of foreign flavors after the milk is drawn. (USDA, 1975)

According to Delahunty (2002), the sensory properties of dairy products, categorized as flavor, texture and appearance attributes, determine consumer acceptability and willingness to repeat purchase of a product, with some additional contribution from their nutritional value and wholesomeness. A majority of sensory properties are complex by definition as they are stimulated by the integrated involvement
of many different compositional and structural properties of the product which means that they cannot be adequately detected or represented by instrumental or chemical techniques. However, due to the sophisticated functioning of the human sensory systems, even a slight change in composition can be detected as a change in sensory character and, therefore, sensory evaluations, in one form or another, has become routinely applied in the dairy industry, in particular for quality control. (Delahunty, 2002)
CHAPTER 3. MATERIALS AND METHODS

3.1. Experimental Design

Whole and two percent milks were received from 17 dairy processing plants located in the west, midwest, and southern regions of the U.S in duplicates. All milks were shipped overnight in Styrofoam coolers filled with ice to maintain the temperature of the samples. The samples were pasteurized at the processing plants by high-temperature short-time pasteurization (161-175°F for 15-25 seconds). The first set of milk samples was evaluated for standard plate count, coliforms, psychrotrophic counts using a standard method as well as a rapid method, heat-resistant spore-forming psychrotrophs, aerobic spores, HR testing (HR-1, HR-2 and HR-3), fat percentage, protein percentage, somatic cell count and a sensory evaluation immediately upon arrival. The duplicate set was evaluated for standard plate count, coliform count and a sensory evaluation at the end of two weeks storage time at 45°F±1°F. Three replications were conducted.

3.2. Analytical Procedures

3.2.1. Standard Plate Counts

Standard Plate Count was determined according to Houghtby and others; Chapter 6 in the Standards Methods for the Examination of Dairy Products (Marshall, 1992). This analysis was conducted at week 0 (day of arrival) and week 2 (day 14 after holding at 45°C) on all samples. At week 0, samples were first diluted in phosphated microbiologically suitable water (1.25 ml/L KH₂PO₄ and 5 ml/L MgCl₂) up to 10⁻² dilution and plated, on Standard Methods agar, in duplicate, which is composed of pancreatic digest of casein (5 gm/L), yeast extract (2.5 gm/L), glucose (1 gm/L) and agar (13.5 gm/L). Later, the samples were incubated at 32°C ± 1°C for 48 hours. After the incubation period, the colonies were counted, with the aid of magnification, and later, the
results were recorded. For week 2 the samples were diluted up to $10^{-10}$ and plated, in duplicate, on Standard Methods agar. The Petri dishes were incubated at $32^\circ C \pm 1^\circ C$ for 48 hours after which the colonies were counted and recorded for later analysis and interpretation.

### 3.2.2. Coliforms Counts

Coliforms counts were determined according to (Richardson, 1985). Coliform counts were determined at week 0 and week 2. For week 0 samples were diluted in phosphated microbiologically suitable water (1.25 ml/L KH$_2$PO$_4$ and 5 ml/L MgCl$_2$) up to $10^{-1}$ dilution and inoculated, in duplicate, in Coliform Count Plate Petrifilm$^\text{TM}$ (3M, St. Paul, MN) that contains violet-red bile agar. All Petrifilm$^\text{TM}$ was incubated at $32^\circ C \pm 1^\circ C$ for 24 hours. After the incubation period was over the Petrifilm$^\text{TM}$ were analyzed for coliform presence and the results were recorded. For week 2 samples were diluted in phosphated microbiologically suitable water (1.25 ml/L KH$_2$PO$_4$ and 5 ml/L MgCl$_2$) up to $10^{-2}$ dilution and inoculated, in duplicate, in Coliform Count Plate Petrifilm$^\text{TM}$ (3M, St. Paul, MN). All Petrifilm$^\text{TM}$ was incubated at $32^\circ C \pm 1^\circ C$ for 24 hours. After the incubation period was over the Petrifilm$^\text{TM}$ were analyzed for coliform presence and the results were recorded for later analysis.

### 3.2.3. Psychrotrophic Counts

The psychrotrophic bacteria count was determined according to Frank and others; Chapter 8 in the Standards Methods for the Examination of Dairy Products (Marshall, 1992). This analysis was carried out at week 0. Samples were diluted in phosphated microbiologically suitable water (1.25 ml/L KH$_2$PO$_4$ and 5 ml/L MgCl$_2$) to the $10^{-2}$ dilution and later inoculated, in duplicate, on Standard Methods agar on Petri dishes that were incubated for 10 days at $7^\circ C$ (standard method) and for 25 hours at $21^\circ C$ (rapid
method). At the end of the incubation period the colonies were counted with the help of magnification and the results were recorded for later analysis.

3.2.4. Heat-Resistant Spore-Forming Psychrotrophic Counts

Heat-resistant spore-forming counts were performed according to Frank and others; Chapter 8 in the Standards Methods for the Examination of Dairy Products (Marshall, 1992). Samples were inoculated, in duplicate, directly from the sample on Standard Methods agar with 0.1% added soluble starch (C₆H₁₀O₅ from MCB Manufacturing Chemists, Inc) and later incubated at 7°C for 10 days. At the end of the incubation period the colonies were counted and the results were recorded for later analysis.

3.2.5. Aerobic Spores Counts

Aerobic spores counts were performed according to Frank and others; Chapter 8 in the Standards Methods for the Examination of Dairy Products (Marshall, 1992). Samples were diluted in phosphated microbiologically suited water up to 10⁻² dilution and inoculated, in duplicate, on Standard Methods agar with 0.1% added soluble starch (C₆H₁₀O₅ from MCB Manufacturing Chemists, Inc). Later, the samples were incubated at 32°C for 48 hours, after which the colonies were counted with the help of magnification and the results were recorded.

3.2.6. HR Tests

HR stress tests have three types of tests: HR-1, HR-2 and HR-3. All were conducted according to Ralph Associates Inc. (2006). For the HR-1 stress test the samples were combined with 3 ml of HR-1A and 3 mL of HR-1B reagents in 2 oz. vials, then incubated at 32°C for 18 to 22 hours. The HR-2 stress test samples were combined with 6 ml of HR-2 reagent in 2 oz. vials and later incubated at 21°C. Samples were
checked for color change at 24, 36, 48 and 60 hours. The HR-3 stress test samples were combined with 3 mL of HR-3 reagent, followed by an incubation period of 13 to 14 hours at 32°C.

3.2.7. Fat %

Fat percentage was determined using Bentley 2000 Fat, Protein and Somatic Cell Count Analyzer (Bentley Instruments Inc., Chaska, MN).

3.2.8. Protein %

Protein percentage was determined using Bentley 2000 Fat, Protein and Somatic Cell Count Analyzer (Bentley Instruments Inc., Chaska, MN).

3.2.9. Somatic Cell Count

Somatic cell count was determined using Bentley 2000 Fat, Protein and Somatic Cell Count Analyzer (Bentley Instruments Inc., Chaska, MN).

3.3. Sensory Evaluation

In this study the samples were evaluated for flavor by a trained 10-member panel. Panelists were trained using triangle tests using buttermilk (to train the panelists to detect acidic off-flavors) and ultra-pasteurized milk (for cooked off-flavor) using triangle tests. These were conducted once a week over a five week period. The official American Dairy Science Association intercollegiate dairy products evaluation scorecard was used. The scorecard consists of a 1-10 point scale for flavor, 10 indicating no defect.

3.4. Statistical Analysis

All the data was analyzed using the General Linear Models (PROC GLM, SAS version 9.1, Cary, NC) and the Univariate procedure (PROC UNIVARIATE, SAS version 9.1, Cary, NC). The sensory evaluation, standard plate count and HR-2 results were analyzed with PROC GLM with a repeated measure in time. Also, all data was
analyzed using a paired correlation (PROC CORR, SAS version 9.1, Cary, NC). An
independent t-test was also conducted to establish whether or not real correlation existed
between the analyses. Significance was established at $p < 0.05$. An independent t-test was
used to determine if the correlation found among certain analyses with PROC CORR was
a real correlation.
CHAPTER 4. RESULTS AND DISCUSSION

4.1. Standard Plate Counts and Coliform Counts

4.1.1. Standard Plate Counts

Standard plate counts for day 0 are reported in Figure 1 and Figure 2. There was no significant difference (p>0.05) between the 2% milk samples. The mean was 1.92 log CFU/ml (~80 CFU/ml). The counts ranged from 0 CFU/ml to 4.08 log CFU/ml (~12,000 CFU/ml). Whole milk samples also reported no significant difference (p>0.05) among them for standard plate counts at day 0. The mean for whole milk samples for standard plate counts at day 0 was 1.85 log CFU/ml (~70 CFU/ml) with counts ranging from 0 CFU/ml to 3.22 log CFU/ml (~1,620 CFU/ml). When all the samples were statistically analyzed together no significant difference (p>0.05) was found. The mean value was 1.89 log CFU/ml (~78 CFU/ml). Only 10% of the samples yielded no SPC counts. With the standard limit for standard plate count at 20,000 CFU/ml (FDA, 2008) we can see that all samples are well under the limit.

*sample number given for the study (all even numbers)

Figure 1. SPC Counts Day 0 for 2% Milk Samples
Results for the standard plate counts for day 14 can be found in Figure 3 and Figure 4. 2% milk samples were significantly different (p<0.05) at day 14. The mean value was 7.65 log CFU/ml (~4.50 x 10^7 CFU/ml). The lowest SPC at day 14 for 2% milk samples was 6.70 log CFU/ml (~5.01 x 10^6 CFU/ml) which is well above the established standard. At day 14 whole milk samples yielded similar results to those of the 2% milk samples. The samples differ significantly (p<0.05) and showed a mean value of 7.51 log CFU/ml (~3.20 x 10^7 CFU/ml). The lowest observed value was 6.69 log CFU (~5.00 x 10^6 CFU/ml) while the highest was 9.06 log CFU/ml (~1.15 x 10^9 CFU/ml).

When all the samples were statistically analyzed together at day 14, significant difference (p<0.05) was found among the samples. The mean was of 7.58 log CFU/ml (~3.8 x 10^7 CFU/ml). The lowest count was 6.70 log CFU/ml (~5.01 x 10^6 CFU/ml), while the highest count was 9.06 log CFU/ml (~1.15 x 10^9 CFU/ml). These high counts at day 14 of standard plate count demonstrate that the samples do not meet the standard for the SPC.
limit of 20,000 CFU/ml and could be considered an important factor in reducing the shelf life of fluid milk. According to Murphy (1998) the initial day standard plate count of fresh pasteurized milk is not a good indicator of the numbers of psychrotrophs present since most bacteria that survive pasteurization are not psychrotrophic (a few types of thermoduric bacteria will grow slowly under refrigeration conditions). A significant increase in the standard plate count after 7-10 days of refrigeration storage is evidence of psychrotrophic growth and suggests that post-pasteurization contamination has occurred and that shelf life will be shortened. Generally, when the standard plate count exceeds 1 - 100 million, the product will become unacceptable due to flavor defects related to bacterial growth. The key to preventing spoilage and extending the shelf life of a product is to prevent post-pasteurization contamination through a well-designed quality assurance program. It only takes a single psychrotrophic bacterium per container of milk to cause spoilage.

*sample number given for the study (all even numbers)

Figure 3. SPC Counts Day 14 for 2% Milk Samples
**4.1.2. Coliform Counts**

There were no coliforms found in any of the samples on either day 0 or day 14, results which fall under the coliform limits of <1 CFU/ml (FDA, 2008). Usually, the presence of coliforms in milk samples demonstrates post pasteurization cleaning and sanitizing practices. The fact that no coliforms were found in any of the samples shows the good hygiene of the plants used in this study.

**4.2. Psychrotrophic Bacteria Counts**

Figure 5 through Figure 8 show the results for both the traditional (Psy) and rapid methods (mPsy) for psychrotrophic counts in the samples. For Psy no significant difference (p>0.05) was found among 2% milk samples or whole milk samples. Only 5% of the 2% milk samples presented psychrotrophic counts (three samples in the first replication of the study, two samples in the third replication) with a mean value of 0.29 log CFU/ml (~2 CFU/ml). Whole milk samples yielded similar results. There was no
significant difference (p>0.05) found between samples and only 1% of the samples showed psychrotrophic counts. This was found only in the third replication of the study.

When statistically analyzed together (both the 2% and the whole milk samples) no significant difference (p>0.05) was found. It should be noted that in the first replication of the study only samples 4, 6 and 12 presented a count on psychrotrophic bacteria, while on the second and third replications they showed no counts. The rest of the samples showed no psychrotrophic counts at all. Samples 26, 27 and 28 showed counts of psychrotrophic bacteria only in the third replication. Only 5% of the samples showed to have psychrotrophic counts. This could have been the result of carelessness on the processor’s behalf regarding sanitation during the processing of the samples we obtained for replications of the study. Based on the results from the other replications where no psychrotrophic bacteria were found, we can conclude that these problems were acted upon and taken care of.

*sample number given for the study (all even numbers)

Figure 5. Psychrotrophic Counts for 2% Milk Samples (Traditional Method)
Figure 6. Psychrotrophic Counts for Whole Milk Samples (Traditional Method)

For mPsy no significant difference (p>0.05) was found among either samples (2% and whole milk) but replication did result in significant differences (p<0.05). 2% milk samples had a mean of 0.64 log CFU/ml (~4 CFU/ml) with only 10% of the samples showing counts of psychrotrophic bacteria in the study’s first replication. Whole milk samples yielded similar results with a mean of 0.61 log CFU/ml (~4 CFU/ml) and also only 10% of the samples, in the first replication of the study, showing psychrotrophic bacteria population. As it will be noted in Section 3.7 of this study, a high correlation coefficient between the traditional method and the rapid method for the testing of psychrotrophic bacteria was found but a t-test proved that this correlation was false. Based on these results we cannot conclude that the rapid method is of true efficiency and the use of the traditional method is still considered as being the most reliable one.

When all samples were analyzed together statistically, the results were no different. No significant difference (p>0.05) was found between samples but significant differences (p<0.05) were found between replications in the study. The mean value was
0.63 log CFU/ml (~4 CFU/ml) with only 10% of the samples presenting psychrotrophic bacteria counts, and these counts were found only in the study’s first replication. This could have been the result of carelessness on the processor’s behalf regarding sanitation during the pasteurization of the samples we obtained for the first replication of the study. Based on the results from the second and third replications, we can conclude that these problems were acted upon and taken care of.

Usually psychrotrophic bacteria are killed by HTST, which could explain the low counts found in this study. The testing of psychrotrophic bacteria is very important because these bacteria are able to produce heat stable, extracellular proteases, as well as lipases that, even at refrigerated storage conditions (where psychrotrophic bacteria may proliferate in milk), can reduce the shelf life of milk. (Jost, 2007) Even though that might sound advantageous, a high level of these organisms in raw milk could contribute heat stable enzymes that may produce off-flavor issues later during the shelf life of pasteurized milk. However, if the raw milk used for fluid milk processing conforms to the Grade A total bacteria count standards in the PMO, then raw milk psychrotrophic bacteria count should not be a problem in a good quality milk supply. On the other hand, post pasteurization contamination of milk with psychrotrophic bacteria has typically limited shelf life of conventionally pasteurized milk (HTST) to 14 to 17 days. (Barbano et al., 2006)

4.3. Heat-Resistant Spore-Forming Psychrotrophs and Aerobic Spores

4.3.1. Heat-Resistant Spore-Forming Psychrotrophs

Heat-resistant spore-forming psychrotrophs counts can be found in Figure 9 and Figure 10. No significant difference (p>0.05) was found for either of the milk samples
*sample number given for the study (all even numbers)

Figure 7. Psychrotrophic Counts for 2% Milk Samples (Rapid Method)

*sample number given for the study (all odd numbers)

Figure 8. Psychrotrophic Counts for Whole Milk Samples (Rapid Method)
(2% and whole milk). 2% milk samples had a mean value of 0.13 log CFU/ml (~1 CFU/ml) with only 10% of the samples showing a population. Whole milk samples had a mean value of 0.14 log CFU/ml (~1 CFU/ml) as well with only 5% of the samples yielding results that show psychrotrophs.

When statistically analyzed together (2% and whole milk samples), there was no significant difference (p>0.05) found for heat-resistant spore-forming psychrotrophs with only 10% of the samples showing at the most 0.14 log CFU/ml (~1 CFU/ml), mainly in the second replication of the study. The rest of the samples showed no count of heat-resistant spore-forming psychrotrophic bacteria. This could have been the result of carelessness on the processor’s behalf regarding sanitation during the pasteurization of the samples we obtained for the second replication of the study. Based on the results from the third replication, we can conclude that these problems were acted upon and taken care of.

On a previous study conducted by Boor and others (1998) raw milk samples were collected from bulk tanks from 855 randomly selected farms in New York State. The milk was examined for chemical and microbial qualities, including heat-resistant spore-forming psychrotrophs. They reported that in 95.9% of their samples the heat-resistant spore-forming psychrotrophs count was <1 CFU/ml, indicating a low level of heat-resistant spore forming psychrotrophs. These results concur to the results in this study of having 90% of the samples showing no heat-resistant spore-forming psychrotrophs counts.

Eneroth et al. (2001) conducted a study on pasteurized milk samples from Sweden. They tested for counts of *Bacillus cereus* from two dairy plants on samples from
four sampling sites along the production line of pasteurized milk, just after the pasteurized, from the buffer tank of pasteurized milk, just before the filling machine and filled and sealed consumer packages. In out study we did not test for *B. cereus* specifically but since *B. cereus* is a heat-resistant spore forming psychrotroph we can compare the results from Eneroth et al. (2001). Focusing on the results obtained from their study on the samples from the filled and sealed consumer packages, our results were much lower. Eneroth and others (2001) reported counts of *B. cereus* ranging from 10 to 1200 CFU/ml in the samples taken from consumer packages.

4.3.2. Aerobic Spores

The results for aerobic spores can be found in Figure 11 and Figure 12. There was significant difference (p<0.05) found between the 2% milk samples. These samples had a

*sample number given for the study (all even numbers)*

Figure 9. Heat-Resistant Spore-Forming Counts for 2% Milk Samples
mean of 2.06 log CFU/ml (~115 CFU/ml) with a standard deviation of 0.60 log CFU/ml (~4 CFU/ml). On the other hand, the whole milk samples did not have significant difference between them ($p>0.05$) with a mean of 2.00 log CFU/ml (~100 CFU/ml) with a standard deviation of 0.55 log CFU/ml (~4 CFU/ml). When statistically analyzed together (2% milk samples with whole milk samples) no significant difference ($p>0.05$) was found. A mean value of 1.94 log CFU/ml (~87 CFU/ml) with a standard deviation of 0.70 log CFU/ml (~5 CFU/ml) was found.

Boor and others (1998) reported values of 49 CFU/ml. The values in our study were higher. It should be noted that Boor and others’ study was done on raw milk and one would expect the counts to be higher. (Boor et al., 1998) Barbano et al. (2006) states that spores are typically present in low numbers in raw milk but they can survive HTST pasteurization, even at temperatures well above minimum (78°C for 15 to 30 s). This could explain why in our pasteurized milk samples the aerobic spore counts are higher...
than the ones found in Boor et al. (1998), which consisted of raw milk samples. Spores that originate from raw milk present a difficult challenge for achieving a long shelf life for refrigerated HTST milk and is likely that removal of spores from raw milk by microfiltration may be a solution in the future for this problem. (Barbano et al., 2006)

4.4. HR Stress Tests

4.4.1. HR-1 Stress Test

Figure 13 and Figure 14 show the results for HR-1 stress test. Colors were assigned a number for input into SAS. Number 1 represented a purple color (no major contamination), number 2 represented a pink color (moderate contamination) and number 3 represented white (major contamination).

HR-1 stress test results show that there is no significant difference (p>0.05) for either the 2% milks samples or the whole milk samples. The mean for 2% milk samples as well as the whole milk samples was 2. For both 2% and whole milk samples 25% of

![Graph showing Aerobic Spores Counts for 2% Milk Samples](image)

*sample number given for the study (all even numbers)

Figure 11. Aerobic Spores Counts for 2% Milk Samples
*Sample number given for the study (all odd numbers)

Figure 12. Aerobic Spores Counts for Whole Milk Samples

The samples had major contamination (number 3). Only 10% demonstrated results of 1. This is an indication that the quality of milk is sub-standard, with only 10% of the samples passing this test. Although samples can pass the HR-1 stress test they could still go bad at 14+ days due to thermoduric Gram-positive microbial contamination. Randolph Associates, Inc. (2006) recommend that Sanitation Standard Operation Procedures (SSOPs) and Good Manufacturing Practices (GMPs) should be reviewed if results like the ones seen in this study (90% of samples showing at least moderate contamination) are found.

4.4.2. HR-2 Stress Test

The results for HR-2 stress test can be found in Figure 15 and Figure 16. Colors were assigned a number for input into SAS. Number 1 represented a white color (negative to post pasteurization contamination of psychrotrophic bacteria) and number 2 represented a pink color (positive to post pasteurization contamination of psychrotrophic bacteria).
*sample number given for the study (all even numbers)

Figure 13. HR-1 Test Results for 2% Milk Samples

*sample number given for the study (all odd numbers)

Figure 14. HR-1 Test Results for Whole Milk Samples
Regarding the 2% milk samples there were significant differences (p<0.05) found between samples in all the intervals (24, 36, 48 and 60 hrs) of the HR-2 stress test. Of the 2% milk samples, 50% of them showed to have a good shelf life (yielding a positive result up to 60 hrs). At the end, only 25% of the samples showed an excellent shelf life.

Whole milk samples showed no significant difference (p>0.05) at any of the intervals for the HR-2 Stress Test. For the first 36 hrs 10% of the samples had a poor shelf life. By the 48 hr interval 25% of the samples had at least a marginal shelf life. Towards the last interval (60 hrs) half of the samples showed to have an excellent shelf life.

When both the 2% and the whole milk samples where statistically analyzed together no significant difference (p>0.05) was found for the 24 and 36 hrs. At the 48 and 60 hrs intervals significant differences (p<0.05) were found among samples. According to the results of the HR-2 stress test 75% of the samples had at least a marginal shelf life, with the remaining 25% having a poor shelf life. Although samples can pass the HR-2 test they can still go bad at 14+ days due to thermoduric Gram-positive microbial contamination. (Randolph Associates Inc., 2006) Randolph Associates, Inc. (2006) recommend that SSOPs and GMPs should be reviewed if results like the ones obtained with this analysis are found.

4.4.3. HR-3 Stress Test

HR-3 stress test results are shown in Figure 17 and Figure 18. Colors were assigned a number for input into SAS. Number 1 represented a purple color (no major microbial growth), number 2 represented a pink color (moderate to major microbial growth) and number 3 represented white (major microbial growth).
*sample number given for the study (all even numbers)

Figure 15. HR-2 Test Results for 2% Milk Samples

*sample number given for the study (all odd numbers)

Figure 16. HR-2 Test Results for Whole Milk Samples
The 2% milk samples did show significant differences (p<0.05) between samples with a mean value of 2.18, which is a pink coloration after 13-14 hours of incubation at 32°C. Pink coloration means that a moderate to major microbial growth exists. 25% of the samples showed to have a white coloration, which is a major microbial growth while only 5% had no major microbial growth, or a purple coloration.

For the whole milk samples no significant differences (p>0.05) were found between samples. With a mean value of 2.40 these samples also showed a moderate to major microbial growth in 50% of the samples. Like the 2% milk samples 25% had a major microbial growth with no samples passing the HR-3 Test.

When all the samples were statistically analyzed together, significant differences (p<0.05) were found among samples in HR-3 testing. 25% of the samples had a major microbial growth while only 1% passed the stress test yielding a purple result (no major microbial growth). This test analyzes thermoduric/psychrotrophic Bacillus bacterial contamination that compliments previous HR testing (Randolph Associates Inc., 2006). Randolph Associates, Inc. (2006) recommends that Sanitation Standard Operation Procedures (SSOPs) should be reviewed if results like the ones obtained with the HR-3 stress test in this study are found.

Similar results can be found in a previous study (Boeneke et al., 2008). In Boeneke et al. (2008) seven out of 12 samples that were non-organic high-temperature short time pasteurized had a mean score of 2, which indicated a moderate to major microbial growth.
*sample number given for the study (all even numbers)

Figure 17. HR-3 Test Results for 2% Milk Samples

*sample number given for the study (all odd numbers)

Figure 18. HR-3 Test Results for Whole Milk Samples
4.5. Fat %, Protein % and Somatic Cell Count

4.5.1. Fat %

Fat results can be seen in Figure 19 and Figure 20. No significant differences (p>0.05) were found between the 2% milk samples or the whole milk samples regarding fat. 2% samples had a mean of 2.08% fat with a standard deviation of 0.22% fat. The lowest value observed was 1.70% fat while the highest was 2.73%. For whole milk samples the mean was 3.43% fat with a standard deviation of 0.34% fat. The lowest value observed was 3.02% fat while the highest was 4.52%. When statistically analyzed together significant difference (p<0.05) was found with a mean value of 2.76% fat with a standard deviation of 0.74% fat.

As mentioned before, fat percentage of milk has a significant economic impact for dairy producers, and small variations in fat percentage can significantly affect economic returns to them. (Pennington, 2008) This is from the producers’ point of view but switching over to the processors point of view, it is always advantageous to keep your fat percentages in fluid milk, be it whole or 2%, because of economic returns as well. According to 21 the FDA (2008) whole milk fat percentage should be 3.25%. The samples in this study reported a mean 3.43% fat, which means the plants are loosing money because they could be using the extra fat to standardize other dairy products such as premium ice cream or various cheeses.

From a health point of view research done in the past milk fat contains a number of potential anticarcinogenic components including conjugated linoleic acid, sphingomyelin, butyric acid and ether lipids. Conjugated linoleic acid inhibited proliferation of human malignant melanoma, colorectal, breast and lung cancer cell lines. (Parodi, 1997) Parodi (1997) also states that the dairy cow also has the ability to extract
other potential anticarcinogenic agents such as β-carotene, which later converts to vitamin A, which is important for vision and bone growth. As a consumer, you would want to get for the fat that you are paying for.

4.5.2. Protein %

Figure 21 and Figure 22 show the results for protein percentage. No significant differences (p>0.05) were found for protein on either milk samples. 2% milk samples showed a 3.18% protein mean value with a standard deviation of 0.27%. Only 25% of the samples had at least 3.31% protein, which is the standard established by the FDA in the 21CFR131.110 (FDA, 2008) regarding protein content. Whole milk samples had a mean value of 3.13% protein with a standard deviation of 0.40%. In the 2% milk samples, only 25% of the samples met the 3.3% protein standard. When statistically analyzed together (2% and whole milk samples) the mean was 3.15 % protein with a standard deviation of 0.34 % protein. Again, the results showed that only 25% of the milk samples concur with the protein content standard of 3.3% as stated by the 21CFR131.110 (FDA, 2008).

*sample number given for the study (all even numbers)

Figure 19. Fat Percentage for 2% Milk Samples
According to Klopcic and others (2003) protein is one of the most important components that dictate the purchase price of milk. Protein content is influenced by various genetic as well as environmental factors, such as nutrition, stage of lactation, age on the animal, season, climatic effects, milking system, milking time, udder health, etc. Energy supply has the strongest impact on the protein content. Sufficient energy supply enables maximal synthesis of proteins in the rumen, representing as much as 60 to 80% of all proteins that digest in the small intestine. (Klopcic et al., 2003)

4.5.3. Somatic Cell Counts

Somatic cell count results can be seen in Figure 23 and Figure 24. There were no significant differences (p>0.05) for somatic cell count among 2% milk samples or whole milk samples. Somatic cell count mean for 2% milk samples was 1.73 log SCC/ml (~55 cells/ml) with a standard deviation of 0.51 log SCC/ml (~3 cells/ml). Whole milk samples had a mean of 1.83 log SCC/ml (~68 cells/ml) with a standard deviation of 0.51
log SCC/ml (~3 cells/ml). When statistically analyzed together (2% and whole milk samples) the mean was 1.77 log SCC/ml (~59 cells/ml) with the highest count being 2.48 log SCC/ml (~300 cells/ml).

*sample number given for the study (all even numbers)

Figure 21. Protein Percentage for 2% Milk Samples

*sample number given for the study (all odd numbers)

Figure 22. Protein Percentage for Whole Milk Samples
When comparing these results to a previous study carried out by Ma and others (2000) we can tell that the results are below the limits (<750,000 cells/mL) and also lower to those results yielded by pre-infection milk samples. It should be noted that in Ma et al. (2000), raw milk samples were evaluated, while on this study pasteurized milk was used. Cows infected with mastitis (inflammatory reaction within the udder of a cow, resulting from bacterial infection) produce milk that is characterized by increased raw milk somatic cell count. Almost all of the major and minor components in milk are affected by mastitis; increased proteolytic and lipolytic activities, reduced curd firmness in cheese making, compromised sensory quality, among others. (Ma et al, 2000) This alone is a good reason to seek low somatic cell count not only as a processor, but as a producer as well, because the lower the somatic cell count, the longer the shelf life of the product.

4.6. Sensory Evaluation

The results for the sensory evaluation are shown in Figure 25 through Figure 28. No significant differences (p>0.05) were found between 2% milk samples at day 0. The mean score was 6.98 with a standard deviation of 1.25. 90% of the samples had a score of at least 6 with only 1% of the samples having the highest score of 10 (no defect). The lowest score observed was 5 and only 5% of the samples had this score. At day 14 significant differences (p<0.05) were found between the 2% milk samples. The mean score was 4.23 with a standard deviation of 2.25, with 10% of the samples having the lowest score possible (1) and 25% of the samples with a score of at least 6 with a maximum score of 8.

Whole milk samples in day 0 showed no significant differences (p>0.05). The mean score was 7.04 with a standard deviation of 1.27. Less than 1% of the samples had
a score of 10 while 5% of the whole milk samples for day 0 had a score of 5. Significant differences (p<0.05) were found between the whole milk samples on day 14. The mean score was 4.55 with a standard deviation of 2.28. The highest score was 8 and only 5% of the samples yielded this score. 25% of the samples had a score of at the most 3 with the lowest score being 1. When all the samples were statistically analyzed together, no significant differences were found between samples for day 0. The mean value was 7 with a standard deviation of 1.26. The highest value perceived was 10 while the lowest was 3. 90% of the samples had a score of ≥6 while 5% presented a score of at the most 5. The most common flavor criticism among samples was that of a cooked off-flavor. Day 14 yielded significant difference (p<0.05) among the samples with a mean score of 4.39. 10% of the samples had a score of 1 while 25% of the samples had a score of at least 6 with the highest being 8. In Boeneke et al. (2008) lower scores were reported for HTST samples. HTST samples had mean values from about 1 to 3 after two weeks of storage.

*sample number given for the study (all even numbers)

Figure 23. Somatic Cell Counts for 2% Milk Samples
*sample number given for the study (all odd numbers)

Figure 24. Somatic Cell Counts for Whole Milk Samples

This could be due to the fact that in Boeneke et al. (2008) the samples were gathered from local groceries stores, where refrigeration might have become an issue, as well as oxidation from artificial lighting, whereas in our study we received the samples directly from the processing plants.

The most common flavor criticism found by the panelists was a cooked off-flavor as well as rancid and oxidized criticisms. Cooked flavor is obtained from heating milk. It may appear when all or part of the milk has been heated too high or too long. Normally, the higher the heating temperature, the more intense the cooked-off flavor. (USDA, 1975) According to Goff (2008) the cooked-defect is a function of the time-temperature of heating and especially the presence of any "burn-on" action of heat on certain proteins, particularly whey proteins. Whey proteins are a source of sulfide bonds that form
sulphhydril groups that contribute to the flavor. The defect is most obvious immediately after heating but dissipates within 1 or 2 days.

The oxidized flavor embraces many other flavors that represent various stages of oxidation or partial changes in the fatty portion of milk. This is one of the most troublesome milk flavors that develop when milk is placed in a glass or plastic container and left in the sun for a short time or for a longer time under artificial light in a store cabinet. Contact with copper or iron also contributes to this flavor. (USDA, 1975) Goff (2008) states that rancidity arises from the hydrolysis of milk fat by an enzyme called the lipoprotein lipase (LPL). The flavor is due to the short chain fatty acids produced, particularly butyric acid. LPL can be indigenous or bacterial. It is active at the fat/water interface but is ineffective unless the fat globule membrane is damaged or weakened. This may occur through agitation, and/or foaming, and pumping. For this reason, homogenized milk is subject to rapid lipolysis unless lipase is destroyed by heating first; the enzyme (protein) is denatured at 55-60°C. Therefore, always homogenize milk

![Graph](image)

*sample number given for the study (all even numbers)

Figure 25. Sensory Evaluation Scores for 2% Milk Samples for Day 0
*sample number given for the study (all odd numbers)

Figure 26. Sensory Evaluation Scores for Whole Milk Samples for Day 0

immediately before or after pasteurization and avoid mixing new and homogenized milk because it leads to rapid rancidity. (Goff, 2008)

4.7. Correlation

The results for the correlation can be seen in Table 1. Significant correlations (>0.95000) were found between the variables Psy and mPsy as well as standard plate count for day 0 with both Psy and mPsy. An independent t-test was conducted to prove if there was real correlation between these analyses. The results showed that there was no real correlation (p>0.05) between any of these analyses. Nonetheless, Boor and others (1998) report a correlation coefficient between standard plate count and mPsy of 0.7685, which agrees with the findings in this study where the standard plate count and mPsy share a correlation coefficient of 0.96545. Having such low correlation coefficients
between the other variables further illustrates the lack of predictive value between results among microbiological tests currently used to assess milk quality.

*sample number given for the study (all even numbers)

Figure 27. Sensory Evaluation Scores for 2% Milk Samples at Day 14

*sample number given for the study (all odd numbers)

Figure 28. Sensory Evaluation Scores for Whole Milk Samples at Day 14
Table 1. Correlation Coefficients Found Among Analyses

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*Table 1. Correlation Coefficients Found Among Analyses*
CHAPTER 5. CONCLUSIONS

After analyzing all the data we can conclude that the fluid milk quality in the U.S. needs to be improved, mainly in the microbial sense. SPC counts were far too high when compared to the standards, which would be a great starting point to the improvement of the milk’s quality. Better pasteurization methods, better packaging or ensuring a better raw milk quality are all ways to improve the quality of milk. For processors to be able to manufacture a final product that can satisfy the consumer in regards to an acceptable shelf life requires more care in raw product as well as processing methods. Microbial counts must be lowered and probably the implementation of new methods such as ultrafiltration or pulse-electrical field (PEF) pasteurization could contribute to this aspect.

In this study one traditional method was compared to its rapid predecessor. In regards to the psychrotrophic bacteria counts, the traditional and the rapid methods shared a high correlation coefficient, which leads us to believe that the rapid method can give accurate microbial counts, therefore producing reliable results.

The poor correlation between the analyses that were carried out in this study and the sensory evaluation carried out illustrates the lack of predictive value that these analyses have together. We can conclude that both the microbial analyses and the sensory evaluation compliment each other but there is no correlation between them.

The high correlation coefficient that the methods to determine psychrotrophic bacteria (traditional and rapid methods) share with the results for standard plate count for week 0 could be interpreted as methods that compliment each other. It should be noted that the standard plate counts measures a general bacterial population and psychrotrophic bacteria are included, which could lead to similar results between methods. Having such low correlation coefficients between the other variables further illustrates the lack of
predictive value between results among microbiological tests currently used to assess milk quality.
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Jose Luis Vargas was born in the capital of Honduras, Tegucigalpa. He graduated from high school in the year 2000. In spring of 2001, he joined the Escuela Agricola Panamericana “El Zamorano” where he obtained his Bachelor of Science in Food Science and Technology degree in fall 2004. He joined Louisiana State University in the summer of 2006 and is set to obtain his degree of Master of Science in Animal and Dairy Sciences in May 2009.