Ultraviolet Light as an Adjunct Treatment to Pasteurization for Microbial Reduction in Milk Intended for Extended Shelf Life

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Abstract

The objective of this study was to determine whether ultraviolet light treatment of milk could be used in adjunct with pasteurization and to determine whether the microbial count in bulk tank raw milk was sufficient enough to receive significant results. Samples were collected from a bulk tank filled with 1600 gallons of raw Holstein and Jersey milk from the Cal Poly Dairy throughout the course of a week. Control (library) samples were also taken from the raw milk tank and stored without air or agitation for comparison. These samples were plated each day and examined 24 and 48 hours after preliminary plating to determine microbial load. The milk was then processed using extreme levels of UV light treatment in addition to pasteurization and traditional pasteurization controls in order to set boundaries for more specific testing. Samples were taken after each treatment stage for every process to determine microbial kill efficiency of each method. After treatment the finished product was bagged for final product evaluation. Bulk tank milk aerobic bacteria counts increased over the course of the week as compared to library samples without air space and agitation; therefore it seems as if the bulk tank does not need to be inoculated with bacteria in order to gain significant results. Aerobic bacteria and coliform counts were greatly reduced in the various combinations of pasteurization and UV light treatment. This work shows that UV light with pasteurization may be used to successfully lower the microbial counts of aerobic bacteria and coliforms in raw bulk tank milk.

Introduction

Ultraviolet (UV) treatment of milk is a huge step in alternative pasteurization methods. Since milk is a product with a relatively short shelf life, even with heat treatment, UV could help to increase consumer desire for tasteful milk products which last much longer. The trouble comes in finding a system of treatment that is effective in microbial disinfection and yet has flavor and nutrition that is desirable to the consumer. Since UV treatment of colloidal suspensions like milk has not yet been perfected it is extremely important to find a combination treatment with pasteurization that works well to ensure destruction of all possible pathogens and extend shelf life. Once it can be shown that UV light can effectively kill all pathogens in milk, it may potentially be used as a sole means of milk treatment.

Pasteurization has been the gold standard by which milk products have been made safe since the early 1900's (Hawthorne, 1978). Since this time no other method for preserving milk has been accepted by the United States government, partially because pasteurization is a great way to disinfect milk while leading to minimal flavor change characteristics. When UV light is used at maximum dosage it has been shown to be effective in microbial kill, including pathogenic organisms such as *Listeria monocytogenes* (Matak et al., 2005). Although effective, this high dose has also been shown to lead to extreme off flavors (Matak et al., 2007). New turbulent flow UV systems have allowed for the continuous treatment of milk, which is much more effective than in years past. Development of efficient UV treatment technology has become much more appealing as the use of this new system may give shelf life and flavor profiles which are much more appealing than those in the past.

By using different strengths of UV treatment combined with traditional High Temperature Short Time (HTST) pasteurization it is hoped to find a process that will fit all these criteria and produce an acceptable product. Using milk that is of high enough bacterial load to warrant sufficient kill rates along with sensory testing will enable one to examine both physical, chemical, and flavor attributes of the milk. By first finding a way to link UV treatment with pasteurization, it is hoped to gain recognition of the effectiveness of UV and one day challenge pasteurization as an alternative heat treatment which can stand alone.

Literature Review

The FDA currently holds high safety standards for the processing and sale of milk and milk products. Currently no other single treatment for milk is accepted for pasteurization by the FDA; however, alternatives, such as ultraviolet irradiation, are growing as research supports new evidence.

Pasteurization

Since the early 19th century households have been boiling milk to make it safer to consume and prevent illness. Although such treatments existed before this, it was not until 1957 that an entire state made pasteurization a law. Not long after Michigan became the first state to adopt pasteurization all other states adopted the process as well (Steele, 2000). As studies found that pasteurization improved milk safety and shelf life the process became a foundation of the dairy industry.

Pasteurization was originally performed in a large batch method, with milk being heated at lower temperatures for long lengths of time. As dairy technology advanced in the early 20th century many processors began turning to High Temperature Short Time (HTST) pasteurization.

This method was much more economical for large dairy processing facilities and maintained many of the qualities in milk that batch pasteurization lacked (Hall and Trout, 1968). HTST pasteurization treats milk in a continuous flow with product being heated to high temperatures for a short amount of time. The current regulation of holding milk at 161^{o} F for 15 seconds was originally introduced to destroy the pathogen *Mycobacterium tuberculosis* but has also been shown to inhibit all other viable pathogens in milk as well. This temperature is also preferable over higher holding temperatures since higher values have been shown to result in lower keeping quality (Smit, 2003). Pasteurization destroys organisms by inducing high temperatures to degrade biological matter needed for survival. Though all organisms have different heat tolerances the temperature used has been shown to be effective against all significant pathogens. While HTST treatment is very efficient, it is ineffective against spores which can germinate and spoil milk post pasteurization (Smit, 2003).

Even though HTST pasteurization makes milk safer for the consumer, a small amount of nutrition is lost during processing. These losses include a 10-25% loss in thiamine and a 20% loss in vitamin C; however, it is known that milk is a poor source of vitamin C and therefore insignificant (Hall and Trout, 1968). The flavor profile of milk stays quite similar to that of raw milk with slight changes in cooked flavor and mouth feel.

Ultraviolet Light

Ultraviolet light treatment is a relatively new process that is becoming more commonplace in the food processing industry. UV light treatment is already being used for the handling of water, fruit juices, and wine among many other non fluid products, and is more efficient than thermal milk treatment in many ways (Bintsis et al., 2000).

There are three types of ultraviolet light UVA, UVB, and UVC. UVA light has the longest wavelength of ultra violet light ranging from 320 to 400 nm in length and has very little affect on living cells, therefore it is not generally used for germicidal purposes. UVB light is medium wavelength ultraviolet light that ranges from 280 to 320 nm which is not used for disinfection, but is better known for darkening skin color. The final group of ultraviolet light is UVC known for wavelengths of 200 to 280 nm. 254 nm wavelength is the primary strength used for disinfecting air, surfaces, and foods because it is the wavelength at which maximum DNA absorption occurs (Bintsis et al., 2000).

UVC light works by altering microbial DNA so that genetic processes of transcription and replication cannot be carried out; this eliminates the ability of organisms to cause disease. Treatment using UVC light has be shown to be effective in treating liquids whose particulates have been removed (Franz et al., 2009). Therefore water has been verified to be readily disinfected by UVC light; however colloidal liquids such as milk do not have as high of disinfection rates. New studies have shown that using UVC light, along with clear glass quartz tubing and a constant turbulent flow, greatly increases the efficiency of microbial treatment in milk and similar liquids; however even with increased efficiency, microbial specificity is still a problem. Although microbial counts after this new treatment are comparable to traditional pasteurization, there have been few studies examining which strains survive the UVC process. Because of this there is high possibility for pathogenic bacteria to survive treatment, and specific strains may thrive and cause disease. Pasteurization completely destroys all pathogens in milk and leaves no risk for pathogenic bacteria in the final product (Bintsis et al., 2000).

Treating milk with UV uses much less energy and lower temperatures than traditional heating methods. Because lower temperatures are used UV does not cause off flavors that are

characteristic of heat treatment or degrade nutritional ingredients, such as vitamins (Keyser et al., 2008). Although UVC does not create off flavors after the treatment of water, tests have shown that some characteristics of milk are altered by UVC treatment. Studies have concluded that UV light exposure in goat milk causes unpleasant odors that are more stinky and goaty compared to controls (Matak et al., 2007). It was also noted that the UV treatment of milk caused a greater occurrence of oxidation byproducts and a change in conjugated lineoleic acid composition (Matak et al., 2007). Another study on the sensory effects of UV on bovine milk, showed that UV also caused cardboard flavor and odors along with burnt aromas (Jiminez et al., 2009). There are no known toxic byproducts that are caused by the UV process, although furan has been shown to form when the processing of fruit juice occurs. Furan is known to be carcinogenic in rats and it is unsure whether it is also a carcinogen in humans (Bule et al., 2010).

Raw Milk Microorganisms

There are many different pathogens which are naturally found in raw milk; therefore pasteurization was originally proposed in order to destroy these organisms so that they could not cause disease in the population from drinking milk. Typical pathogenic organisms found in raw milk are *Salmonella spp.*, *Listeria monocytogenes*, *Yersinia enterocolitica*, *Campylobacter jejuni*, *Staphylococcus aureus*, and Shigga toxin-producing *Escheria coli* (Jayaro et al. 2006). These bacteria usually cause symptoms of gastroenteritis (diarrhea and vomiting), however more serious diseases, such as stillbirth, can be caused by *Listeria monocytogenes* (Namminga, 1999). Other pathogens such as *Coxiella burnetii* and *Mycobacterium tuberculosis* can also cause serious disease and sometimes death.

Although there are pathogens in milk, there are also many microorganisms in milk which are known more for their spoilage properties in milk rather than disease. A large reason for milk spoilage comes from cold-tolerant endospore forming bacteria such as *Bacillus spp.* and *Paenibacillus spp.* (Huck et al., 2008). These organisms are not destroyed by the heat of pasteurization because spores form as protection from harsh environments. After milk has been treated these endospores turn into viable bacterial cells and thrive in the rich milk environment soon reaching levels that cause milk spoilage. Another spoilage organism that can occur in milk is *Pseudomonas*. This type of bacteria is generally known to cause ropy or slimy defects in milk products after extended storage. Since *Pseudomonas* is killed during the pasteurization process it is believed that its presence in pasteurized milk is caused by post pasteurization contamination (Ranieri and Boor, 2009).

Aerobic Bacteria and Coliforms

When checking for microbial growth in milk the two most common bacteria to look for are aerobic bacteria and coliforms. Aerobic bacteria are found on milk that is plated on a 3M Aerobic Plate Count (APC) petrifilm. These bacteria are aero-tolerant organisms that represent milk quality. The total aerobic bacteria count typically represents how clean or dirty milk is (Pritchard, 2010).

Coliforms on the other hand are bacteria which could be potential pathogens. Milk is usually plated on E.coli/Coliform plates to check for the levels of possible harmful bacteria or coliforms in milk. These plates can also differentiate for E.coli which can cause serious disease. Most coliforms in milk come from environmental or fecal contamination on the dairy

(Washington State Department of Health, 2010). It is important to remove all coliforms from milk in order to protect the health and safety of the public.

Extended Shelf Life

Extended shelf life (ESL) is in general milk that has lower bacterial counts than pasteurized milk and is hygienically packaged in order to create a product that lasts longer before spoiling. Ultra High Temperature (UHT) processed milk is the most common extended shelf life product in the international market; however, many consumers do not like the flavor of UHT milk because it has a characteristic cooked flavor that is much more pronounced than traditional HTST processed milk. In order to appease the consumers want for a better tasting ESL milk new technology must be considered for treatment (Rysstad and Kolstad, 2006).

One way to extend the shelf life of milk is to use microfiltration or a centrifuge (bactofuge) in order to remove bacteria and spores before the product is pasteurized. This process removes much more pyschrotrophic (cold-loving) bacteria than pasteurization alone which in return slows the growth of these spoilage organisms (Rysstad and Kolstad, 2006). High heat pasteurization can also be used to make an ESL milk product. High heat pasteurization is much more efficient at destroying spores in raw milk than centrifugation or microfiltration; however, the high heat used to treat the product can cause extreme cooked flavor and degradation of nutrients. The best way to heat treat milk for ESL is to use direct heat that heats the product very quickly at an extremely high temperature as to not create any sensory defects (Rysstad and Kolstad, 2006). UV treatment along with pasteurization could create a potential ESL product by treating milk in two different ways, using both light and heat to destroy microorganisms.

When creating an ESL product it is very important to eliminate post treatment contamination from organisms, such as *Pseudomonas*, and use aseptic packaging. The use of UV light for disinfecting packaging materials is already practiced and ensures hygienic packaging. These precautions along with proper storage temperature well help extend product shelf life no matter what process is used (Rysstad and Kolstad, 2006).

Since it is not yet believed that the FDA will accept ultraviolet light processes for the treatment of milk, the purpose of this trial is to set up a UV treatment process that can work as an adjunct with high temperature short time pasteurization. It is proposed that doing so will increase the shelf life of milk products while maintaining the microbial, sensory, and nutritional value of a high quality milk product. Using UV treatment along with pasteurization would be energy efficient and open up the possibility of exporting milk products due to extended shelf life. This opportunity would create new markets for milk and drive up the overall demand for milk and related foodstuffs.

Materials and Methods

Microbial Analyses of Raw Milk Tank

Microbial growth in raw bulk tank milk was monitored throughout the course of the designated processing week in order to determine the extremity of bacterial growth and whether the addition of bacteria was necessary for accurate UV treatment results. Bacteria counts were taken from samples that were withdrawn from the raw tank using Falcon plastic test tubes from the sampling spigot or a dipper. One sample was measured each day from the raw tank to ensure a significant reading in raw milk bacterial growth. This growth was recorded to determine

whether the agitation of processing milk throughout the week including the airspace available in the raw tank significantly enhanced microbial growth. Measurements were taken for a total of four days and included four samples. Four library samples of milk were also taken on the first day of milk reception to compare agitated oxygen exposed milk to stable sealed milk. The samples were kept individually in Falcon test tubes allowing minimum air space and stored in a refrigerator of similar temperature to the raw milk tank. Samples were kept in the refrigerator until bacterial testing was completed (figure 5).

Microbial growth was determined by plating milk samples on 3M Aerobic Plate Count and *E. coli*/Coliform Petrifilms. A sample of raw tank and control milk dilution of 10^{-1} was then plated on two *E. coli*/Coliform petrifilms and incubated for 24 hours at 37^{o} C. After 24 hours the petrifilm counts were recorded and colony forming units / mL (cfu/mL) were calculated. These samples were also checked after 48 hours for further verification. Samples of raw tank and control milk were also plated on 3M Aerobic Plate Count Petrifilm. Dilutions of 10^{-1}, 10^{-2}, and 10^{-3} mL of each sample were plated on two plates of each dilution and then incubated at 32^{o} C for 48 hours. Samples were then but in a refrigerator for a storage bacterial counts to be performed 3 weeks later. After 48 hours colony growth was observed and cfu/mL were calculated (figure 5).

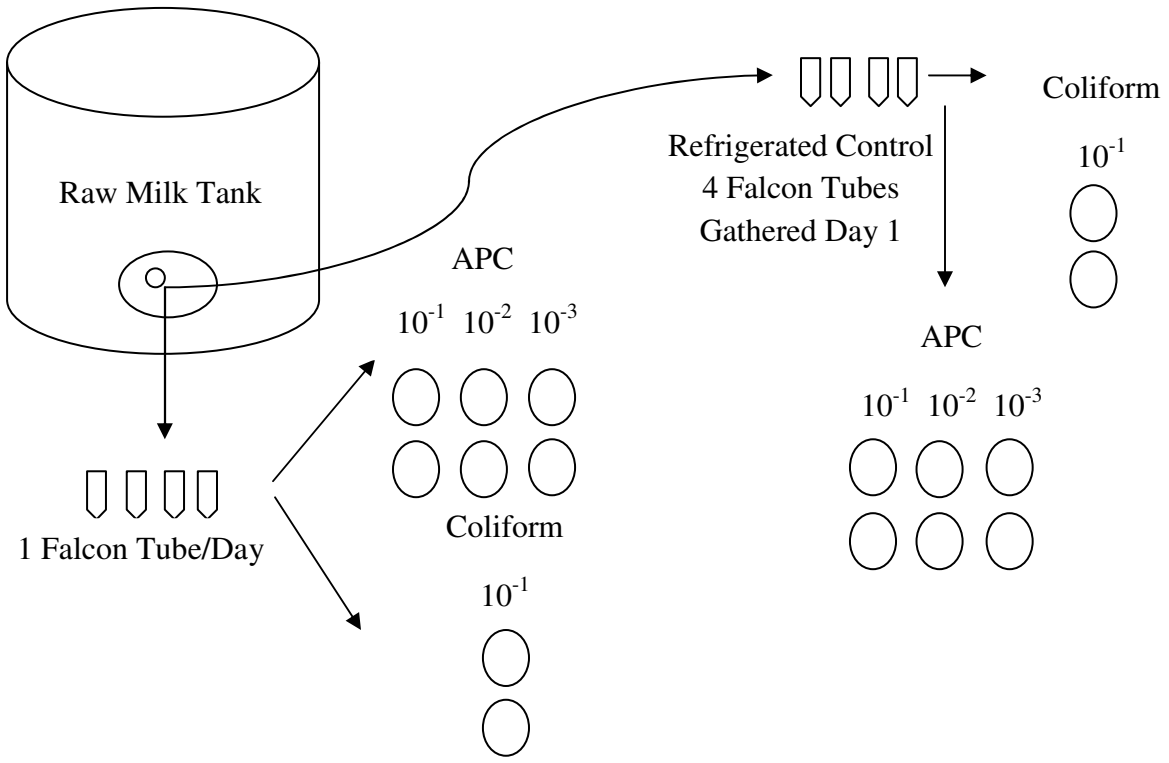


Figure 1. Sample collection procedure for raw agitated bulk tank and raw library samples along with plating procedures for aerobic plate count and coliform count

Milk Processing

Raw milk was picked up from the California Polytechnic Dairy the morning of day 1 in a single tank milk truck. 1600 gallons of milk were pumped into a raw milk silo using a receiving line and proper sanitary procedures. After the milk was pumped into the silo it was allowed to sit until the following day at a temperature of 39^{o} F. On day 2 the milk was standardized from approximately 4.0% milk fat to 3.5% milk fat using a 10 gallon/minute flow cream separator to remove approximately 25 gallons of cream. After the tank was standardized the cream was

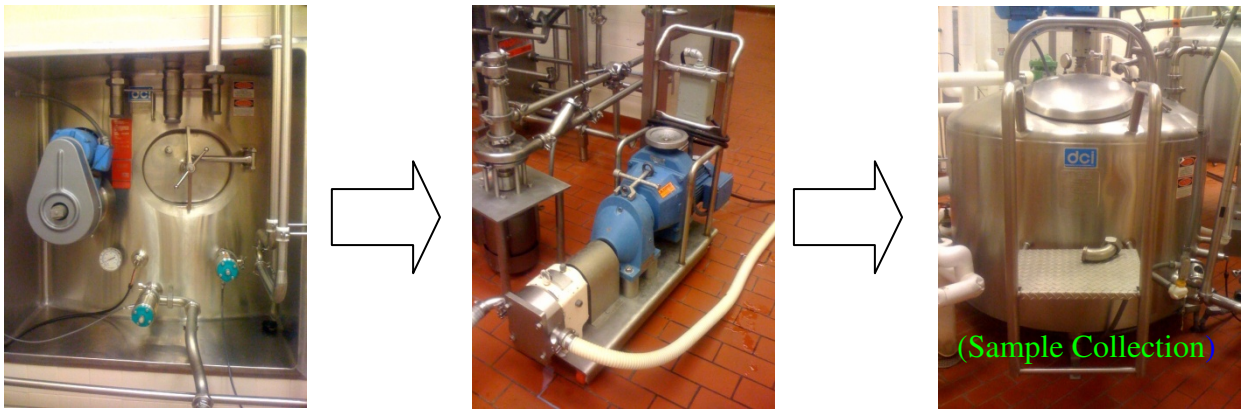
disposed and processing lines were set up for the first run. All runs were processed using an HTST at a temperature of approximately 165^{o} F and an UV system provided by SurePure (South Africa). The UV system had various strengths depending on how few or how many lamps were used.

In run number 1 milk was treated using UV 6 lamp (low) treatment followed by pasteurization; this would be our lowest dose treatment. During processing samples of milk were taken with sterile Falcon test tubes from various areas of the system in order to determine the microbial kill rates (see figure 1). Three people were available at all times to take care of processing, testing, and packaging of the finished product. Each run processed approximately 200 gallons of milk and all products were stored in Scholle bags for later use. Between each run the milk lines and equipment were rinsed with a water and chlorine solution. After all the runs were complete a CIP rinse was then performed using a caustic and an acid sanitizer was used to clean the system of residues.

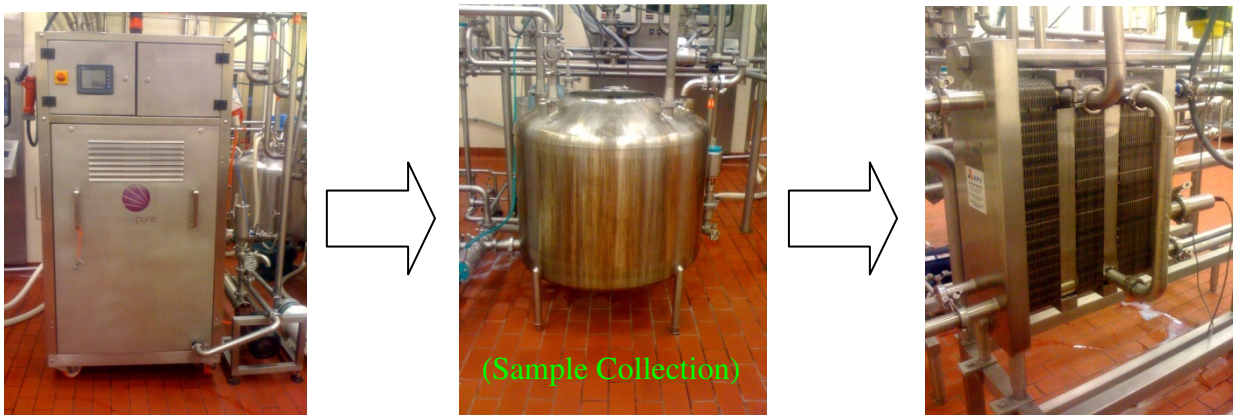
On day 5 three more trial runs were conducted (1) double pasteurization with holding time in UV equipment (no lamps) (figure 2), (2) UV 40 lamp (high) treatment followed by pasteurization (figure 3), (3) and pasteurization followed by UV 6 lamp treatment (figure 4). Samples of milk were then taken from different areas of the system using sterile falcon test tubes and tested for microbial changes. All runs were considered to be the extremes of the UV unit and processing order; therefore, these were the outer boundaries of the expected results. Each process used about 200 gallons of milk and the finished product was placed in Scholle bags using a Scholle bag filler. After each run was completed the system was flushed with a chlorine solution and water. When all runs were finished caustic and acid sanitizers were used in order to disinfect all equipment.

Processes	Day 2	Day 5
Run #1	UV 6 lamp + pasteurization	Double pasteurization with UV equipment
Run #2	-	UV 40 lamp + pasteurization
Run #3	-	Pasteurization + UV 6 lamp

Table 1. Processing variables for the four different product runs



Raw Milk Tank Positive Displacement Pump Cream Tank



UV 6 Lamp

Balance Tank

HTST

Homogenizer

HTST

Pasteurized Tank

Scholle Filler

Figure 2. Flow diagram of day 2 run #1 (UV 6 lamp + Pasteurization)

Raw Milk Tank Positive Displacement Pump Cream Tank

Positive Displacement Pump Balance Tank HTST

Homogenizer HTST Batch Tank

UV 0 Lamps

Balance Tank

HTST

Homogenizer

HTST

Pasteurized Tank

Scholle Filler

Figure 3. Flow diagram of day 5 run #1 (Double Pasteurization + UV equipment)

Raw Milk Tank

Positive Displacement Pump

Cream Tank

UV 40 Lamp

Balance Tank

HTST

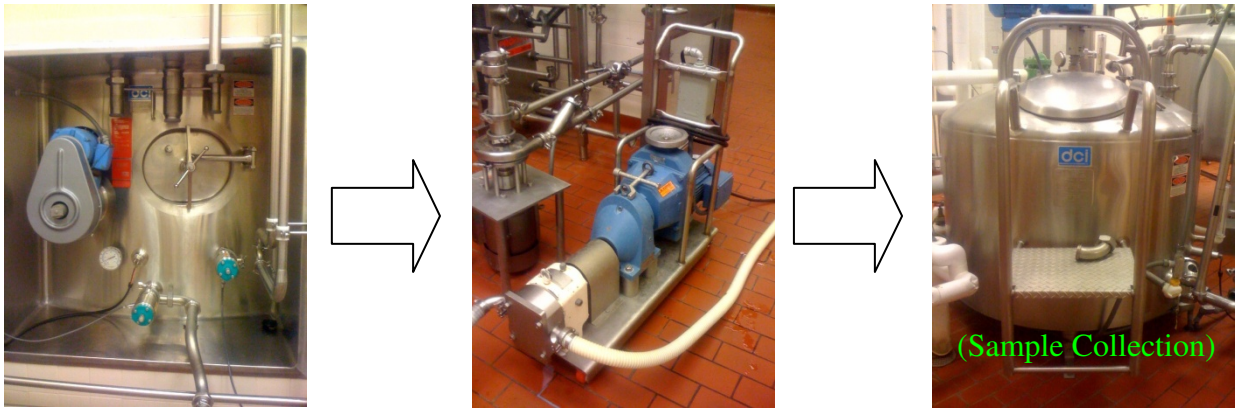
Homogenizer

HTST

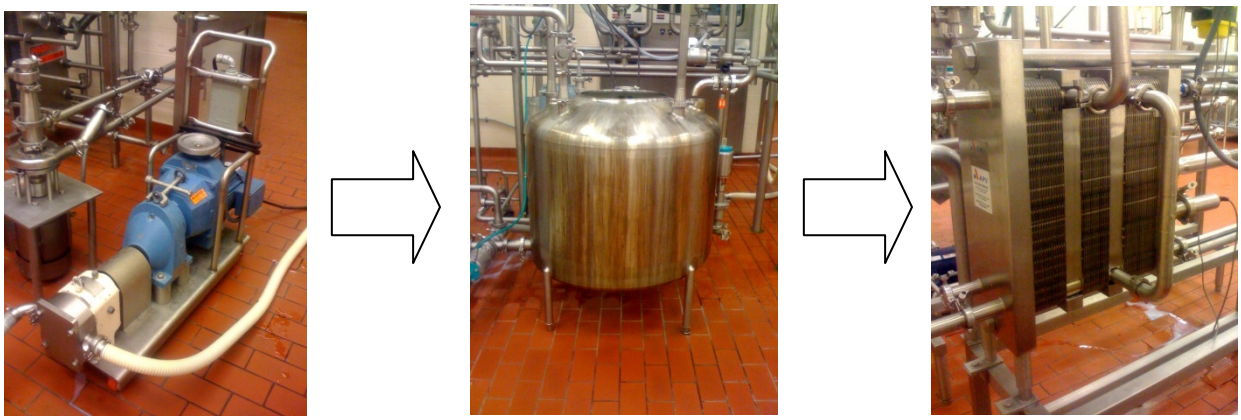
Pasteurized Tank

Scholle Filler

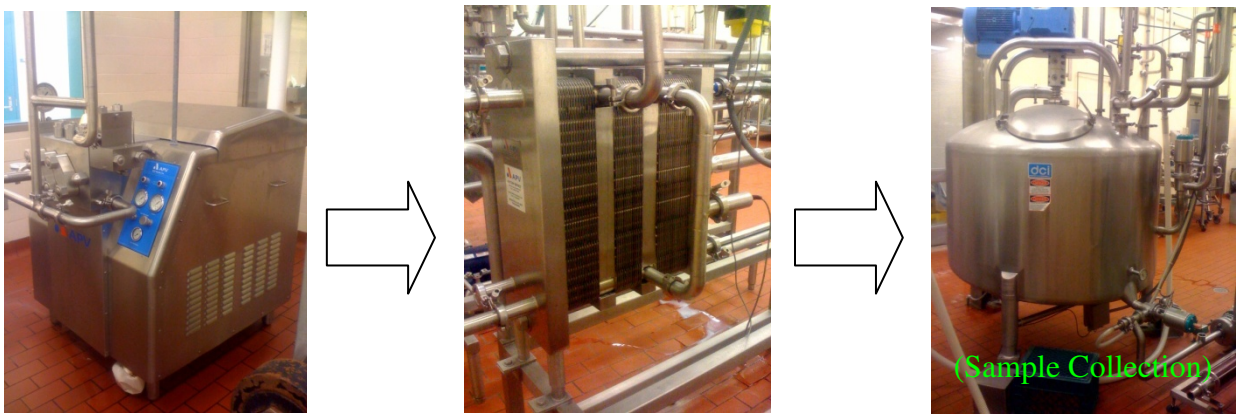
Figure 4. Flow diagram of day 5 run #2 (UV 40 lamp + Pasteurization)



Raw Milk Tank Positive Displacement Pump Cream Tank



Positive Displacement Pump Balance Tank HTST



Homogenizer HTST Batch Tank

UV 6 Lamp Pasteurized Tank Scholle Filler

Figure 5. Flow diagram of day 5 run #3 (Pasteurization + UV 6 lamp)

Plating of Processing Samples

All samples that were taken during milk processing were plated the same day as production. Since the milk used for all 3 runs on day 5 came from the same bulk cream tank only one sample was taken for three different variables.

Pre-treated raw milk samples that were collected were plated on two different 3M APC petrifilms in dilutions of 10^{-1}, 10^{-2}, and 10^{-3}, those samples which had received some sort of UV or pasteurization treatment were also plated on two different APC petrifilms but with direct, 10^{-1}, and 10^{-2} dilutions instead. The petrifilms were then incubated for 48 hours at 32^{o} C. After incubation the cfu/mL of each film was calculated and recorded. All samples were also plated on 2 3M E. coli/Coliform petrifilms with untreated milk being plated with a 10^{-1} dilution and treated milk being directly pipetted onto the petrifilm. The films were placed in a 37^{o} C incubator for 24 hours and the coliforms/mL were calculated. This number was then reaffirmed after 48 hours and recorded.

Detection of Spoilage

After 3 weeks samples were again plated to examine bacterial growth using dilutions of 10^{-1}, 10^{-2}, and $10^{-3}$for aerobic plate counts and direct plating for coliform counts. The same procedures for plating and incubation as previous were used and the colonies were counted and recorded.

Results

Raw Tank Bacterial Growth

Aerobic bacteria plate counts in the raw milk tank increased greatly over the course of 4 days. On day 1 the raw tank sample showed bacteria counts of 55,000 and 39,000 cfu/ml. By the end of the week on day 4 the aerobic plate count had increased almost a full log to 360,000 and 260,000 cfu/mL (Figure 6).

Aerobic bacteria plate counts in the control (library) samples that were not given air or agitation did not increase at all but rather stayed around the same amount of organisms. The aerobic plate counts on day 1 for the library sample were 12,200 and 10,300 cfu/mL. On day 4 the library sample aerobic plate counts were 6,500 and 8,500 cfu/mL. The amount of growth was not notably different to suggest any change (Figure 6).

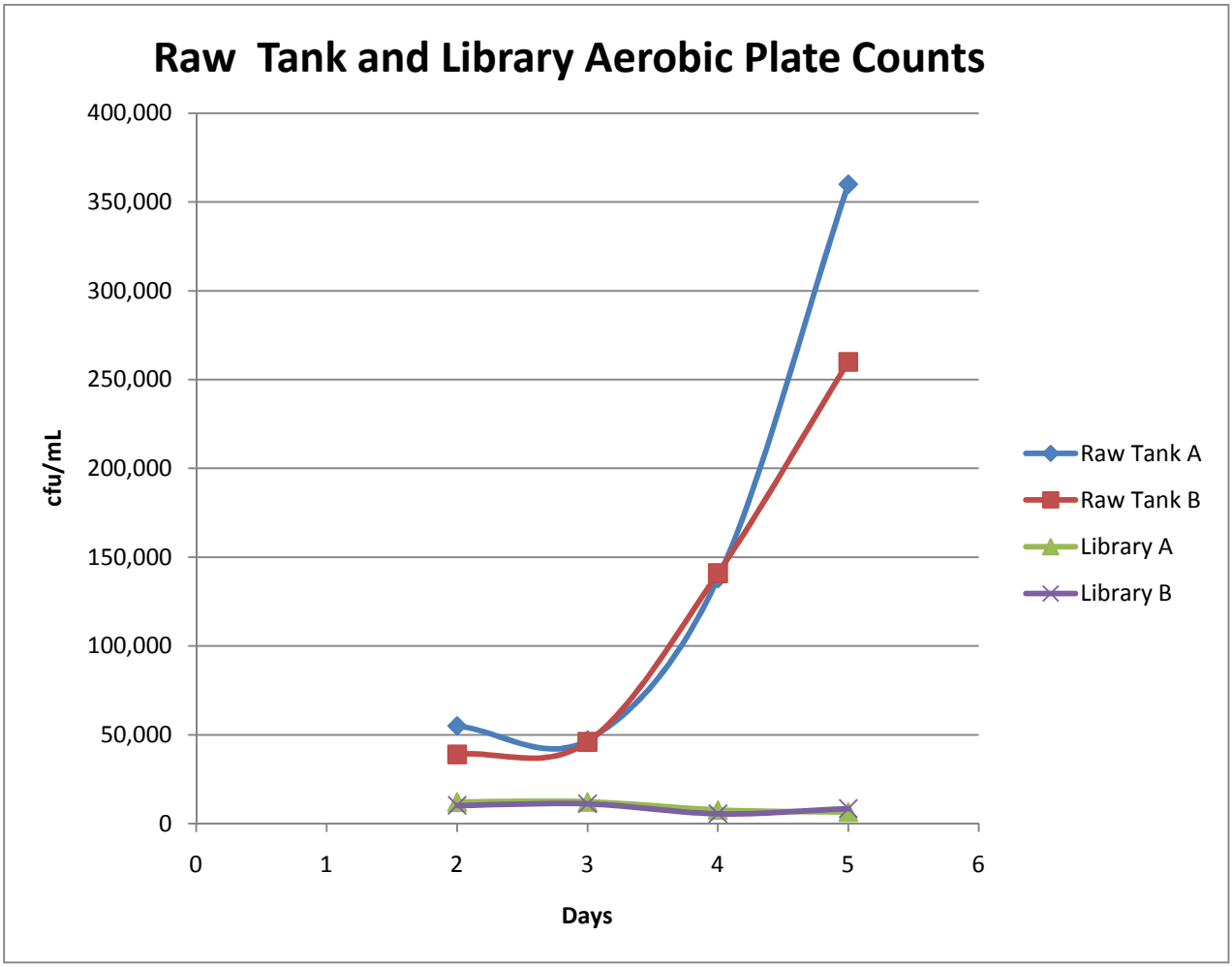


Figure 6. Aerobic plate counts of agitated raw tank and library samples over four days

Coliform counts for the 4 day period of processing for the raw tank sample did not change significantly. On day 1 raw tank sample contained 40 and 60 coliforms/mL and on day 4 the sample had 20 and 30 coliforms/mL (figure 7).

Results were similar for the library samples however slightly lower. Coliform counts for the day 1 library sample were 0 and 10 coliforms/mL and for day 4 were 10 and 0 coliforms/mL. Since there were less than 25 colonies on each plate these numbers may be less accurate however an estimate can still be made (figure 7).

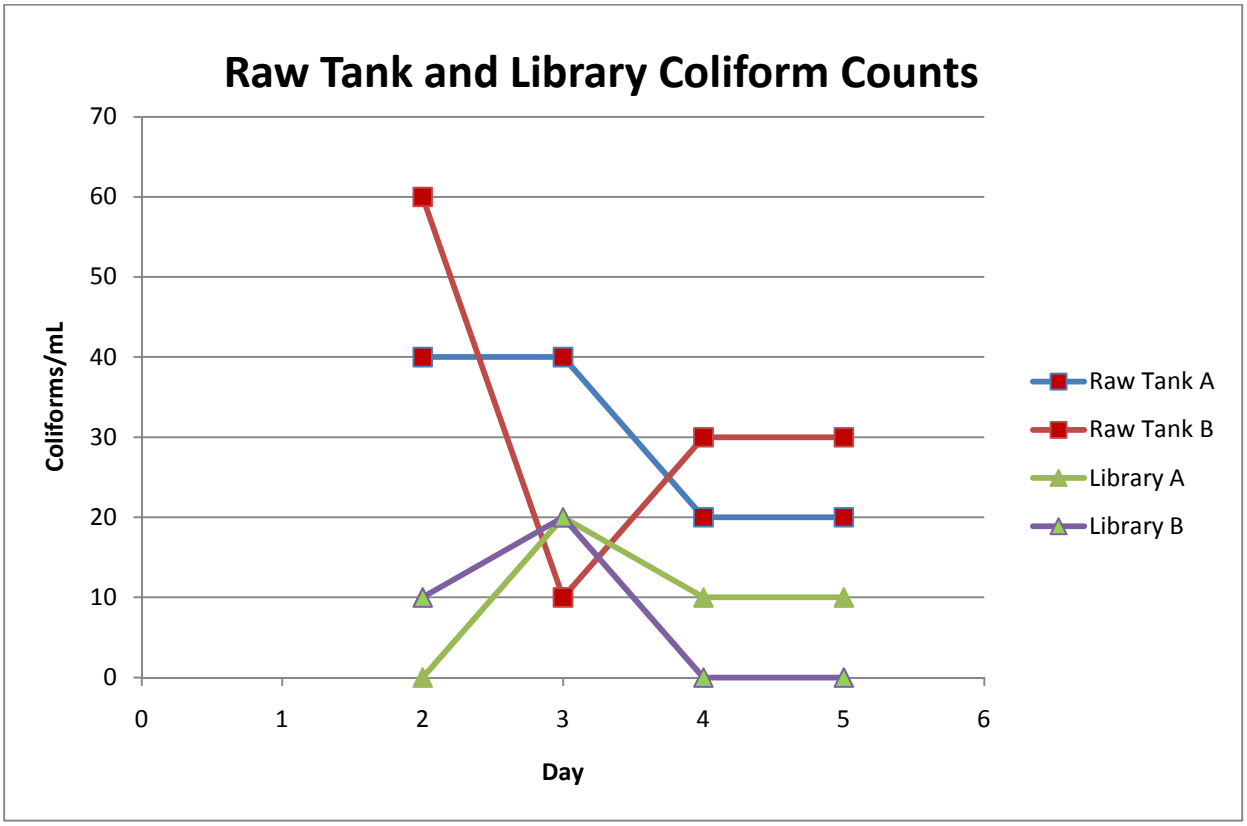


Figure 7. Coliform counts of agitated raw milk and library samples over 4 days

Processing Run Samples Bacteria Counts

UV 6 Lamp + Pasteurization Coliform Counts (cfu/mL)	
Batch Tank A	160
Batch Tank B	230
Balance Tank A	44
Balance Tank B	45
Pasteurized Tank A	0
Pasteurized Tank B	0

Table 2. Coliform counts for run #1 on day 2

UV 6 lamp + Pasteurization Aerobic Plate Counts (cfu/mL)	
Batch Tank A	30,000
Batch Tank B	48,000
Balance Tank A	16,800
Balance Tank B	15,800
Pasteurized Tank A	11
Pasteurized Tank B	14

Table 3. Aerobic plate counts for run #1 on day 2

Double Pasteurization + UV Equipment Coliform Counts (cfu/mL)	
Cream Tank A	2220
Cream Tank B	2130
Batch Tank A	0
Batch Tank B	0
Balance Tank A	0
Balance Tank B	0
Pasteurized Tank A	0
Pasteurized Tank B	0

Table 4. Coliform counts for run #1 on day 5

Double Pasteurization + UV Equipment Aerobic Plate Counts (cfu/mL)	
Cream Tank A	TNTC >500,000
Cream Tank B	TNTC >500,000
Batch Tank A	21
Batch Tank B	28
Balance Tank A	29
Balance Tank B	32
Pasteurized Tank A	21
Pasteurized Tank B	32

Table 5. Aerobic plate counts for run #1 on day 5

UV 40 Lamp + Pasteurization Coliform Counts (cfu/mL)	
Cream Tank A	2220
Cream Tank B	2130
Balance Tank A	1
Balance Tank B	2
Pasteurized Tank A	0
Pasteurized Tank B	0

Table 6. Coliform counts for run #2 on day 5

UV 40 Lamp + Pasteurization Aerobic Plate Counts (cfu/mL)	
Cream Tank A	TNTC >500,000
Cream Tank B	TNTC >500,000
Balance Tank A	4000
Balance Tank B	3400
Pasteurized Tank A	8
Pasteurized Tank B	11

Table 7. Aerobic plate counts for run #2 on day 5

Pasteurization + UV 6 Lamp Coliform Counts (cfu/mL)	
Cream Tank A	2220
Cream Tank B	2130
Batch Tank A	0
Batch Tank B	0
Pasteurized Tank A	0
Pasteurized Tank B	0

Table 8. Coliform counts for run #3 on day 5

Pasteurization + UV 6 Lamp Aerobic Plate Counts (cfu/mL)	
Cream Tank A	TNTC >500,000
Cream Tank B	TNTC >500,000
Batch Tank A	29
Batch Tank B	22
Pasteurized Tank A	37
Pasteurized Tank B	37

Table 9. Aerobic plate counts for run #3 on day 5

Aerobic bacterial levels in the initial milk for processing were over 500,000 cfu/mL on day 5 and much higher than the estimated 30,000 and 48,000 cfu/mL that were calculated from the batch tank on day 2. Initial coliform counts for day 5 were also much higher as compared with day 2 of processing with an estimated 160 and 230 coliforms/mL on day 2 compared to 2220 and 2130 coliforms/mL on day 5.

Microbial disinfection of milk worked well with all processing methods (Tables 2-9). Every variable of treated milk had an endpoint where 0 coliforms existed per mL and all aerobic plate counts were well under 50 cfu/mL at the end of treatment. Even the minimal 6 lamp treatment lowers both aerobic bacterial counts and coliforms greatly, however not to the extent of pasteurization (Tables 2 and 3). The use of 40 lamp UV treatment on the other hand does

come much closer to pasteurization in effectiveness. When milk was processed with UV light first, aerobic plate counts decreased greater than 2 log and coliforms were essentially eliminated from milk leaving but a few per mL if any (Tables 6 and 7).

During the double pasteurization trail elimination of aerobic bacteria was efficient reducing bacterial levels by greater than 3 log; however after the second treatment of pasteurization aerobic microorganism levels remained the same (Table 5).

Shelf Life Bacteria Counts

UV 6 Lamp + Pasteurization 3 Week Coliform Counts (cfu/mL)	
Pasteurized Tank A	0
Pasteurized Tank B	0

Table 10. 3 week coliform comparison for day 2 run #1

UV 6 Lamp + Pasteurization 3 Week Aerobic Plate Counts (cfu/mL)	
Pasteurized Tank A	<250
Pasteurized Tank B	<250

Table 11. 3 week aerobic plate count comparison for day 2 run #2

Double Pasteurization + UV Equipment 3 Week Coliform Counts (cfu/mL)	
Pasteurized Tank A	0
Pasteurized Tank B	0

Table 12. 3 week coliform comparison for day 5 run #1

Double Pasteurization + UV Equipment 3 Week Aerobic Plate Counts (cfu/mL)	
Pasteurized Tank A	<250
Pasteurized Tank B	<250

Table 13. 3 week aerobic plate count comparison for day 5 run #1

UV 40 Lamp + Pasteurization 3 Week Coliform Counts (cfu/mL)	
Pasteurized Tank A	0
Pasteurized Tank B	0

Table 14. 3 week coliform comparison for day 5 run #2

UV 40 Lamp + Pasteurization 3 Week Aerobic Plate Counts (cfu/mL)	
Pasteurized Tank A	<250
Pasteurized Tank B	<250

Table 15. 3 week aerobic plate count comparison for day 5 run #2

Pasteurization + UV 6 Lamp 3 Week Coliform Counts (cfu/mL)	
Pasteurized Tank A	0
Pasteurized Tank B	0

Table 16. 3 week coliform comparison for day 5 run #3

Pasteurization + UV 6 Lamp 3 Week Aerobic Plate Counts (cfu/mL)	
Pasteurized Tank A	62,000
Pasteurized Tank B	74,000

Table 17. 3 week aerobic plate count comparison for day 5 run #3

All of the samples except for the pasteurization + UV 6 aerobic plate counts reported values which were extremely similar to the microbial counts from 3 weeks earlier. All samples still did not have any growth of coliforms and all aerobic bacteria counts were below 250 cfu/mL besides pasteurization + UV 6 lamp which contained 62,000 and 74,000 cfu/mL (Table 17).

Discussion

Bulk Tank Bacterial Growth

The increase of aerobic bacteria in the bulk raw tank from 55,000 and 39,000 cfu/mL on day 2 to 360,000 and 260,000 cfu/mL on day 5 supports that the oxygen filled and agitated environment of a raw milk bulk tank allows bacteria to thrive even under 39^{o} F conditions. Even more validity is given to this point after seeing that aerobic bacteria in oxygen deprived refrigerated control samples with no agitation did not increase over the course of 4 days but rather fell slightly from 12,200 and 10,300 cfu/ml to 6,500 and 8,500 cfu/mL.

Coliform bacteria did not seem to grow very well at refrigerated temperatures in either the bulk tank or in the control library samples. All samples either decreased or stayed the same over the course of 4 days with growth in the library samples being slightly less than those in the bulk tank.

Processing Run Samples Bacteria Counts

After looking at bacteria counts throughout the treatment process it is made clear that pasteurization is a much better treatment for disinfecting milk; consequently all processing runs were efficient in killing desirable amounts of bacteria because they were all aided by pasteurization. Every variable also resulted in a finished product coliform count of 0 coliforms/mL. Pretreated milk with a high dose of UV light followed by pasteurization was the

best form bacterial disinfection. When milk was first processed using 40 lamps of UV light and then passed through the pasteurizer aerobic bacteria counts of the finished product were 8 and 11 cfu/mL, which was lower than any other method even compared to day 2 of production when initial bacteria levels were significantly lower.

Shelf Life Bacteria Counts

All aerobic bacteria counts in all samples besides the pasteurization + UV 6 lamp seemed as if bacterial levels were stabilized throughout the 3 week refrigerated storage period. As for the pasteurization + UV 6 lamp sample it is unsure what may have caused such a high level of 62,000 and 74,000 cfu/mL. The high counts may have been caused by contamination of the sample or other human error. Further testing must be done to ensure the counts have nothing to do with the treatment method.

Conclusion

Microbial levels in the raw milk bulk tank have showed that there is no need to inoculate the raw milk tank with microorganisms in order to receive credible bacteriocidal readings, as there are plenty of bacteria already growing in the bulk tank environment. Using UV as an adjunct system to pasteurization may prove to be a beneficial process as lower aerobic bacteria counts were achieved with combined treatment. Microorganisms in 3 week stored milk were also low however sensory testing must be done in order to ensure a satisfactory product. From a microbial point of view UV treatment of milk looks promising; the use of the extreme boundaries set with this experiment will allow for more specific testing to be performed in the future.

REFERENCES

Bintsis, T, R.K Robinson, and E Litopoulou-Tzanetaki. "Existing and Potential Applications of Ultraviolet Light in the Food Industry--a Critical Review." *Journal of the Science of Food and Agriculture*, 80.6 (2000): 637-645.

Bule, Mahesh, Peter Slade, Rekha Singhal, Alfredo Rodriguez, Kiran Desai, Brian Parisi, and Satish Parulekar. "Furan Formation During UV-treatment of Fruit Juices [Electronic Resource]." *Food Chemistry*, 122.4 (2010): 937-942.

"Coliform and Bacteria - Washington State Dept of Health." *Washington State Department of Health (DOH) Home Page*. Web. 15 Nov. 2010.

Franz, Charles M.A.P, Volker Graef, Mario Stahl, Ingrid Specht, and Gyu-Sung Cho. "UV-C-inactivation of Microorganisms in Naturally Cloudy Apple Juice Using Novel Inactivation Equipment Based on Dean Vortex Technology [Electronic Resource]." *Food Control*, 20.12 (2009): 1103-1107.

Hall, Carl W., and G. Malcolm. Trout. *Milk Pasteurization*. Westport: AVI Publ., 1968. Print.

Hawthorn J. (1978). A history of milk in the food industry. Proceedings of the Nutrition Society, 37, pp 211-215 doi:10.1079/PNS19780031

Huck, J.R, K.J Boor, and M Sonnen. "Tracking Heat-Resistant, Cold-Thriving Fluid Milk Spoilage Bacteria from Farm to Packaged Product." *Journal of Dairy Science*, 91.3 (2008): 1218-1228.

Jayarao, B.M, N.V Hegde, J.L Brown, A.A Sawant, S.C Donaldson, and B.A Straley. "A Survey of Foodborne Pathogens in Bulk Tank Milk and Raw Milk Consumption Among Farm Families in Pennsylvania." *Journal of Dairy Science*, 89.7 (2006): 2451-2458.

Jimenez, Rafael, Ammar Olabi, and John Walker. *The Characterization of the Physicochemical and Sensory Properties of UV Treated Milk Samples*. 2009.

Keyser, Maricel, Wihann Nel, Pieter Gouws, Ilze M?lller, and Frans Cilliers. "Ultraviolet Radiation as a Non-thermal Treatment for the Inactivation of Microorganisms in Fruit Juice [Electronic Resource]." *Innovative Food Science & Emerging Technologies*, 9.3 (2008): 348-354.

Matak, K.E, E Hovingh, C.R Hackney, M.D Pierson, J.J Churey, R.W Worobo, and S.S Sumner. "Efficacy of UV Light for the Reduction of Listeria Monocytogenes in Goat's Milk." *Journal of Food Protection*, 68.10 (2005): 2212-2216.

Matak, K.E, S.S Sumner, S.E Duncan, E Hovingh, R.W Worobo, C.R Hackney, and M.D Pierson. "Effects of Ultraviolet Irradiation on Chemical and Sensory Properties of Goat Milk." *Journal of Dairy Science*, 90.7 (2007): 3178.

Namminga, Kelly, (1999), “Health Risks of Drinking Raw (Unpasteurized) Milk,” http://www.abs.sdstate.edu/flcs/ecoli/milk.htm, South Dakota State University, Brookings, SD (reviewed by E. Kim Cassel, SDSU Extension Dairy Specialist).

Pritchard, Donald E. "Raw Milk Bacteria Tests – What Do They Indicate?" Web. 15 Nov. 2010

Ranieri, M.L, and K.J Boor. "Short Communication: Bacterial Ecology of High-temperature, Short-time Pasteurized Milk Processed in the United States." *Journal of Dairy Science*, 92.10 (2009): 4833.

Rysstad, G, and J Kolstad. "Extended Shelf Life Milk - Advances in Technology." *International Journal of Dairy Technology*, 59.2 (2006): 85-96.

Smit, Gerrit. *Dairy Processing: Improving Quality*. Cambridge: Woodhead, 2003. Print.

Steele, J.H. "History, Trends, and Extent of Pasteurization." *Journal of the American Veterinary Medical Association*, 217.2 (2000): 175-178.