CHAPTER III

Inactivation by UV Irradiation of *Escherichia coli* in Milk of Different Temperatures and Milk Fat Percentages

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ABSTRACT

The purpose of this study was to examine the influence of temperature on bacterial reduction in UV irradiated milk of different milk fat concentrations. Commercially processed skim, reduced fat (2%), and whole milk samples were inoculated with a naladixic acid resistant *E. coli* O157:H7 surrogate (ATCC 25922), maintained at or brought to 4°C and 20°C, respectively, and then exposed to a UV light dose between 5.3-6.3 mJ/cm^2 for approximately 1.5 sec. Bacterial populations before and after UV exposure were enumerated and the results indicated that no significant statistical differences in bacterial reductions occurred when skim milk samples were processed at 4°C or 20°C, as determined by Tukey's HSD test ($p > 0.05$). These results were the same for reduced fat milk samples processed at 4°C and 20°C. A significant difference was found in whole milk samples processed at the different temperatures; bacterial reductions were greater at 20°C ($p < 0.05$). At 4°C, skim milk and reduced fat milk showed a statistically significant bacterial reduction compared to whole milk ($p < 0.05$), whereas at 20°C there was a significant bacterial reduction in the skim milk samples ($p < 0.05$) but no significant reduction in the reduced fat and whole milk samples ($p > 0.05$). Turbidity was measured for each milk type. Skim milk was the least turbid, followed by reduced fat and whole milk, respectively. This decrease in turbidity of skim milk samples may have contributed to the greater reduction of pathogens in the skim milk samples. Solids in the milk have a greater effect over bacterial reductions than processing temperatures.

Key words: UV irradiation, milk, *Escherichia coli*, temperature, milk fat

INTRODUCTION

The thermal treatment of foods is a practice that has been used throughout documented history; consequently, limited research has been done to examine alternative methods to the thermal pasteurization of milk. A publication by Gallmann and Eberhard (1992) compiled a brief summary of alternative processing techniques that have been used for milk. Those listed were microwaves, ohmic heating, infrared irradiation, ultraviolet irradiation, gamma irradiation, extrusion cooking, high-pressure homogenization, and combined operations. UV irradiation is an effective treatment that does not involve heat or a subsequent heat treatment to kill the microorganisms (Sastry *et al*., 2000). UV treatment is effective against numerous microorganisms found in drinking water (Parrotta and Bekdash, 1998) and is effective for the treatment of apple cider (Hanes *et al.*, 2002; Wright *et al.*, 2000; Basaran *et al*., 2004).

Microbial inactivation from UV treatment is associated with photochemical changes that take place in proteins and nucleic acids when UV light is absorbed (Jay, 1996). Mutations occur that disrupt DNA transcription and replication, which ultimately causes death of the microorganism (Miller *et al.*, 1999). Three factors are used to determine the bactericidal effectiveness of UV irradiation: wavelength, applied intensity, and contact time (Bachmann, 1975; Parrotta and Bekdash, 1998). The following equation is used to express UV dosage (intensity x time):

$$D = L\,(T)$$

where "D" represents the dosage of UV light, "L" refers to the applied intensity, and "T" is for the exposure time (Bachmann, 1975). This equation does not consider temperature when determining UV dose however, in studies where milk was processed using UV irradiation, higher processing temperatures were shown to have increased bacterial reduction (Caserio *et al.*, 1975).

The purpose of this research was to determine if a bacterial reduction in milk was possible using UV irradiation. The death kinetics of an acid resistant *Escherichia coli* O157:H7 surrogate (*E. coli* ATCC 25922) exposed to UV irradiation using a UV fluid processor are well documented (Quintero-Ramos et al., 2004; Basaran *et al*., 2004). The

same strain of E. coli (ATCC 25922) was used for the present study so that reduction results may be compared to previous studies with apple cider. The Grade "A" Pasteurized Milk Ordinance (PMO) (Section 7; Item 18r) states that raw milk should be cooled to 10^{o}C within 4 h from the commencement of milking and to 7°C or cooler within two hours after milking is completed (CFSAN, 2002). The PMO recommends that bulk tanks are held at temperatures ≤ 4^{o}C so the temperatures compared in the study were room temperature (20^{o}C) and a likely bulk tank temperature (4^{o}C). The effect of milk fat concentration on bacterial reduction was also assessed.

MATERIALS AND METHODS

Bacterial Strain

Nalidixic acid resistant *Escherichia coli* ATCC 25922 was obtained from the culture collection at the Food Science and Technology Department of Virginia Tech. This strain exhibited a similar response to UV irradiation as *E. coli* O157:H7 (Basaran *et al*., 2004) and therefore it was used as a non-pathogenic surrogate. The nalidixic acid resistant properties facilitated identification of recovered bacteria. Bacteria were grown at 35°C and stored at 4°C on tryptic soy agar (TSA) slants supplemented with 50 μg nalidixic acid/ml (NA) (Difco Laboratories, Detroit, MI). Cultures were twice transferred to tryptic soy broth (TSB) containing 50 μg NA/ml (Difco Laboratories, Detroit, MI) and incubated at 35°C for 18-24 h to the stationary phase of growth.

Sample Preparation

Commercially pasteurized and homogenized milk (Skim, 2% and Whole Milk) was maintained in a cooler at 4°C until processing. A one liter sample of each type of milk was treated by UV irradiation at two temperatures, the PMO-recommended bulk tank temperature (4°C) and at room temperature (20°C). Samples processed at 20°C were gradually brought up to temperature using a hot plate (Thermix Stirring Hot Plate, Model 310T, Fisher Scientific, USA) and sterile stir bar (< 5 min). Once the target temperature was reached (4°C or 20°C) an aliquot of the prepared *E. coli* ATCC 25922 was added, as described below, to the sample of milk to yield an initial inoculum level of 10^5 cfu/ml. Non-inoculated samples of each milk type were also tested. Immediately after inoculation, samples were passed through a UV apparatus (CiderSure 3500A, FPE Inc., Rochester, NY) at a fixed flow rate of 151 L/h.

Conditions of UV Irradiation

The non-inoculated and inoculated milk was pumped from the outer housing of the UV apparatus into thin films (<1 mm) through a quartz tube where eight germicidal, low-pressure mercury lamps were situated along the same axis within the cylinder to guarantee uniform UV exposure throughout the liquid passing through the tube. The Reynolds number was calculated to be 1371, which corresponds to laminar flow. The equation to calculate this is as follows: $Re = Dv\rho/\mu$, where D is the diameter of the tubing (m), v is the average velocity of flow (ms^{-1}), ρ is the density of the fluid ($kg\ m^{-1}$), and μ is the viscosity of the fluid ($N\ sm^{-2}$). The numbers used for the calculation were: $D = 0.009$ m, $v = 0.674$, $\rho = 1030\ kg/m^3$, and $\mu = 0.004505\ Ns/m^2$, respectively (Maduko, 2004; Simos *et al*., 1991).

Microbial Analysis

One-liter samples of each type of milk (skim, 2%, whole) were inoculated with *E. coli* ATCC 25922 and passed through the CiderSure 3500 UV apparatus. A one liter sample of non-inoculated milk was also UV processed and assessed for microbial load. Samples of inoculated and non-inoculated milk were collected aseptically before and after UV treatment and serially diluted in dilution blanks made up of sterilized phosphate and magnesium chloride in distilled water, or phosphate-buffered saline (PBS) which were prepared according to Marshall (1993). Aliquots of the appropriate dilutions were spread-plated in duplicate onto TSA supplemented with 50 μm NA/ml (Difco Laboratories, Detroit, MI) (TSAN) and incubated at 35^{o}C for 24 hr. The initial background flora of each milk type was also determined using the methodology previously described. After incubation, colony-forming units from replicate plates were counted and the number of cfu/ml were averaged and converted into logarithmic units.

Turbidity of Milk

Turbidity is a measurement of the degree to which light is scattered by suspended particles and soluble solids in water and the effectiveness of UV disinfection systems is

directly related to the turbidity and total suspended solids in wastewater (Mahmoud and Ghaly, 2004 and EPA, 1999). Turbidity of the three types of commercially pasteurized and homogenized milk (skim, 2% and whole) was measured in duplicate using a nephelometer (TA1 Nephelometer, Monitek Liquid Monitoring Specialist, USA). The range of the turbidimeter was up to 200 nephelometric turbidity units (NTU) therefore samples had to be diluted prior to measurement. Measurements were read at 1:1000 dilution and reported both by actual readings for the diluted samples and by calculating the value of the original samples. The transmittance of each milk type was measured using a spectrophotometer (Shimadzu UV 2101 UV Scanning Spectrophotometer, Shimadzu Corporation, Japan) at wavelengths between 253 and 255 nm. Samples were diluted up to 1:1000. Deionized distilled water was used to standardize the machine at 100% transmittance.

Statistical Analysis

The experiment was replicated four times for each type of milk (skim, 2%, and whole) at two temperatures (4^{o}C and 20^{o}C). Each type of milk at each processing temperature was processed on the same day. Microbial analysis of each sample was conducted before and after UV exposure. The microbial counts (CFU/ml) were converted into logarithmic units and the difference between the initial and final microbial concentration was calculated per average UV exposure. Data were analyzed by one-way analysis of variance using Jmp In (Version 4.04, SAS Institute, 2001) software. Tukey's Honestly Significant Difference (HSD) test was used to determine statistical difference in bacterial reductions between processing temperatures. Duplicate turbidity and transmittance measurements were evaluated during the final repetition of the study. Data were analyzed using bivariate fit of the average NTU by milk fat percentages.

RESULTS AND DISCUSSION

Inactivation of *Escherichia coli* by UV Light

The initial population of *E. coli* in the inoculated milk ranged from 1.5 x 10^5 to 8.7 x 10^6 CFU/ml. **Table 1** shows that the average log reductions of *E. coli* ATCC 25922 in skim milk and reduced fat milk were not significantly different when each was processed at 4^{o}C and 20^{o}C ($p > 0.05$). The bacterial reductions for the skim milk were approximately 2.29 and 2.27 logs at 4^{o}C and 20^{o}C, respectively and the reductions for the reduced fat milk were approximately 1.82 and 1.43 logs at 4^{o}C and 20^{o}C, respectively. The results are not considered statistically different by Tukey's HSD test ($p > 0.05$). Contrary to the skim and reduced milk results, whole milk samples processed at 4^{o}C had significantly less bacterial reduction then samples processed at 20^{o}C ($p < 0.05$). At 4^{o}C, an average bacterial reduction of 0.73 logs was achieved whereas at 20^{o}C an average reduction of 1.44 logs was achieved. Even though one-way analysis of variance of bacterial reduction by milk type at the two different temperatures showed a slight relationship between milk fat content and bacterial reduction (4^{o}C: $R^2 = 0.67$; 20^{o}C: $R^2 = 0.58$), for each temperature tested, there were significant differences between the bacterial reductions in skim milk and the bacterial reductions in whole milk ($p < 0.05$). The greatest bacterial reductions occurred in the skim milk that was processed at 4^{o}C, and the lowest reductions were in whole milk processed at 4^{o}C. These results are illustrated in **Figure 1**. At a similar UV dose, Quintero-Ramos *et al.* (2004) was able to achieve an approximate 3.45 log reduction of the same bacteria when it was suspended in apple cider and processed at 4^{o}C. The difference in bacterial reduction in the milk samples appeared to be influenced by a combination of fat content and product temperature. As temperature increases, so does the solubility of fat, therefore, at lower temperatures the fat may have flocculated causing a shadowing effect that protected the bacteria from the UV rays. Turbidity may have also influenced the amount of UV required for a comparable reduction in bacteria. It was theorized that the differences in bacterial reduction are due to the lower transmittance of milk and more particulates in milk (such as fats, proteins, etc.) may protect organisms from UV penetration so that as the amount of fat and other solids increase, the average bacterial reduction would decrease.

Physical Characteristics of Milk

Skim milk was the least turbid and turbidity increased with reduced and whole milk samples, respectively ($R^2 = 0.97$). Diluted milk samples (skim, reduced fat, and whole) had an average NTU value of 12, 29, and 49, respectively (**Table 2**). The NTU values demonstrate that milk is extremely turbid, which is perhaps the reason it is rarely considered in most studies dealing with milk, however, one of the aims of this study was to identify factors that may contribute to the ability of ultraviolet light to penetrate opaque substances. Koutchma, *et al.* (2002) investigated the efficacy of UV technology to achieve a 5-log reduction of *E. coli* in apple cider and reported that "the critical factors affecting inactivation efficiency of UV light were absorbance of the apple juice and turbidity or scattering properties due to particles of apple cider: the higher the absorbance component, the lower the UV inactivation rate." In milk suspended solids such as fat, proteins, and other solids, like lactose and minerals, influence turbidity. Since skim milk has a lower percentage of fat and total solids, it follows that it is the least turbid and therefore bacterial reductions in skim milk would be predicted to be greater then reduced and whole fat milk.

The transmittance of ultraviolet light through milk to was shown to be non-existent. All of the milk samples demonstrated 0% transmittance at wavelengths between 253 and 255 nm. Mahmoud and Ghaly (2004) found similar transmittance results in cheese whey tested at ten wavelengths within the germicidal UV range (195, 216, 240, 253, 254, 265, 270, 300, 315 and 330), except for the wavelength 300 nm where the transmittance was 0.1%. The authors of the study attributed this lack of transmittance through the whey as a consequence of high turbidity resulting in most of the radiation being absorbed or scattered (Mahmoud and Ghaly, 2004). Suspended solids in the milk may be responsible for absorbing and scattering the UV radiation, and therefore suggesting that UV irradiation would only be effective for microbial reduction if all of the microorganisms within the milk were directly exposed to the UV. Introducing turbulence into the system may facilitate the rise of more bacteria to the surface of the quartz tubing resulting in the required exposure.

CONCLUSIONS

A reduction of *E. coli* ATCC 25922 is possible in commercially pasteurized and homogenized milk exposed to UV irradiation. The amount of bacterial reduction is influenced by processing temperature and milk fat concentration. Suspended solids in milk such as fat, proteins, and other solids, like lactose and minerals, may influence bacterial reduction due to limitations in UV penetration. Future studies would assess the effect of increasing the flow rate, which should increase the turbulence of the milk through the UV apparatus. The addition of turbulence would theoretically bring all microorganisms to the surface for UV exposure, resulting in an increased bacterial reduction.

The use of the CiderSure 3500 UV apparatus for the reduction of *E. coli* ATCC 25922 was validated for commercially pasteurized and homogenized milk. This microbial strain was chosen because it exhibited similar UV death kinetics as the pathogen *E. coli* O157:H7 in apple cider (Basaran *et al*., 2004; Quintero-Ramos *et al*., 2004). Studies are needed to determine the UV dose required for a 5-log reduction of the pertinent pathogen in milk and to assess physical and chemical changes that occur as a result of this exposure. Evaluation of ultraviolet light on sensory aspects such as smell, color, flavor, shelf stability, etc. would also need to be assessed on milk exposed to this dose.

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Table 1: The reduction of *E. coli* ATCC 25922 in milk samples exposed to an average UV dose of 5.8 mJ/cm^2 for 1.5 sec.

Milk Type	Log Reduction Factor (log CFU/ml)	
	4 ^{o}C	20 ^{o}C
Skim (0%)	2.29 +/- 0.17[a]	2.27 +/- 0.10[a]
Reduced (2%)	1.82 +/- 0.18[a,b]	1.43 +/- 0.10[b]
Whole (3.25%)	0.73 +/- 0.17[c]	1.44 +/- 0.10[b]

[a, b, c] Values designated with the same letter within a column or row are not significantly different ($p<0.05$) as determined by Tukey's HSD test.

Table 2: Mean turbidity of milk samples (n=2) with different fat concentrations (skim, 2% and whole). All samples were measured at a dilution of 1:1000.

sample	milkfat (%)	turbidity (NTU)[a]	
		diluted	raw[b]
Skim	<1	12	12,000
Reduced	2	29	29,000
Whole	3.25	49	49,000

[a] Nephelometric Turbidity Units
[b] Calculated values of original sample.

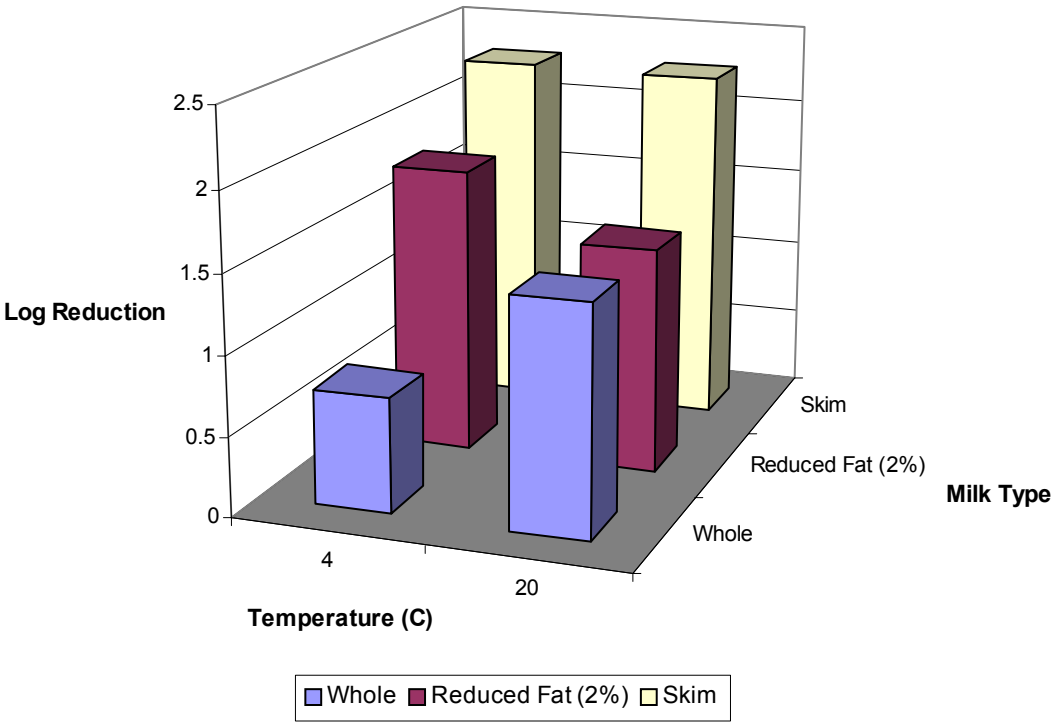

Figure 1. Average reduction comparison of *Escherichia coli* in skim, reduced fat (2%) and whole milk in response to an average UV dose of 5.8 mJ/cm^2 for 1.5 sec at different processing temperatures (4^{o}C and 20^{o}C).