CHAPTER IV

Efficacy of UV Light for the Reduction of *Listeria monocytogenes* in Goat's Milk

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ABSTRACT

Traditional goat's cheeses are produced using unpasteurized milk, which increases the food safety concerns for these types of products. Popularity and consumption of goat's milk products has increased and the niche market includes gourmet goat's cheeses. The U.S. Code of Federal Regulations and the Pasteurized Milk Ordinance both address the possibility for processing alternatives to heat treatment and the use of ultraviolet processing may be a viable alternative while still ensuring the safety of the product. Fresh goat's milk was inoculated to 10^7 cfu/ml with *Listeria monocytogenes* (L-2289) and exposed to UV light using the CiderSure 3500 apparatus (FPE Inc., Macedon, NY). Inoculated milk was exposed to an ultraviolet dose range between 0 and 20 mJ/cm^2 to determine the optimal UV dose. A greater than 5-log reduction was achieved ($p < 0.0001$) when the milk was passed through the machine 12 times for a cumulative exposure time of roughly 18 sec and a cumulative UV dose of 15.8 +/- 1.6 mJ/cm^2. The results of this study indicate that UV irradiation could be used for the reduction of *L. monocytogenes* in goat's milk.

Key words: UV irradiation, goat's milk, *Listeria monocytogenes*

INTRODUCTION

The amount of commercially produced goat's milk in the United States is estimated to be 24,000 tons compared to 2,000,000 tons in India (Haenlein, 1996). Approximately half of the milk is commercially processed as fluid, powder, UHT or evaporated milk and the rest is used for commercial cheese production (Stern, 1992). Of the total amount of milk produced globally, 3.5% are from small ruminants such as goats and sheep and throughout the developing world, more people drink milk from goats than from any other animal (FAO, 1999; Haenlein, 1996; Park, 1990). In the US, dairy goat farmers are small in number, but cheeses made from goat's milk have seen a recent increase in popularity and consumption (Park, 1990). The market for goat's milk is expanding to include gourmet goat's cheeses that may only be found at natural food stores, farmer's markets and high-end restaurants. In addition to the commercial producers, there are many small-scale, non-commercial dairy goat farms with over 1.5 million dairy goats in the US and it is estimated that these produce roughly 600,000 tons of milk worth over $500 million (Haenlein, 1996). These farms are widely geographically dispersed. Dairy cooperatives are not typically organized. Farmers and producers of dairy goat's products often process their commodities on-site or face high transportation costs to processing plants.

Dairy products were implicated in 5% of the 3,839 total foodborne outbreaks attributable to bacteria that were reported in France between 1988 and 1997. Of those, 48% were from raw milk products and 51% were from unspecified milk processing type (De Buyser *et al*., 2000). Raw milk, un-aged soft cheeses (Jalisco cheese) are also produced in the United States and are especially popular with certain ethnic groups. Jalisco cheese has been implicated in many outbreaks in the Hispanic community, including a salmonellosis outbreak in 1997 that affected over 150 people in California and a listeriosis outbreak in 2000 that affected 12 people in North Carolina (CDC, 2001; Reed and Grivetti, 2000).

Traditionally, goat's cheese is produced using raw milk. It is expected that the consumption of raw milk products will continue regardless of educational programs or increased governmental regulations, since consumer preference for raw dairy products is

linked with perceived superior organoleptic characteristics (Buchin *et al*., 1998). Grappin and Beuvier (1997) found that cheeses made with raw milk versus those made with pasteurized milk developed a stronger flavor and ripened faster. These findings were attributed to the preservation of heat sensitive enzymes and microbiota. The Milk Dairy Foods Control Branch of the California Department of Food and Agriculture reported that over 21,000 tons of Jalisco-style cheeses are illegally produced each year (Reed and Grivetti, 2000). Reed and Grivetti (2000) predicted that bacterial outbreaks will not deter consumption of unprocessed cheese.

Methods that are less costly and have fewer organoleptic consequences then thermal pasteurization are being studied; one such method is ultraviolet irradiation. UV irradiation of milk was first examined in the mid-1900s for the purpose of vitamin D enrichment to prevent the development of rickets in infants (Steenbock, 1928). Irradiation and other methods proved to be effective at increasing the vitamin D content in milk, but the most reliable method of fortification was found to be direct addition of concentrated vitamin D (Murphy *et al*., 2001). The dairy industry therefore adopted this method over UV irradiation. Due to the confirmed success of thermal pasteurization, studies pertaining to potential processing alternatives for milk are limited.

The CiderSure 3500 UV apparatus (FPE, Inc., Rochester, NY) has been shown to be effective for the reduction of *Escherichia coli* O157:H7 and *Cryptosporidium parvum* oocysts in apple cider (Hanes *et al*., 2002; Basaran, 2004). The methods and concepts used in those trials could prove to be valid for the development of an alternative method to heat pasteurization of fluid milk for small-scale dairy producers and manufacturers of Jalisco-style cheeses that satisfy FDA safety standards. The efficacy of this methodology to produce a microbially safe product must first be demonstrated before evaluation of the effect of UV treatment on organoleptic properties.

Abou-Eleinin and colleagues (2000) assessed the incidence of *Listeria* obtained from bulk tanks on 39 goat farms over a one-year period. Thirty-five of the 450 raw milk samples were shown to have *Listeria* spp. present, and 17 of those were positive for *L.*

monocytogenes. Seasonal variations that were similar to cow's milk were observed, with *Listeria* isolation rates of 14.3% and 10.4% in the winter and spring, and 5.3% and 0.9% in autumn and summer (Abou-Eleinin *et al*., 2000).

Matak (2004) found that a reduction of *E. coli* ATCC 25922 in commercially pasteurized and homogenized whole cow's milk could be achieved by UV processing. The objective of this research was to conduct an investigation of the efficacy of UV light as a non-thermal pasteurization process for goat's milk with *Listeria monocytogenes* as the target pathogen.

MATERIALS AND METHODS

Milk

Fresh goat's milk was purchased from a commercial dairy goat farm. Temperature of the milk was maintained at $\leq 4^{o}$C until processing. The gross composition of the milk (total fat, protein, total solids) was evaluated to assess consistency among replications. The percent milkfat was determined using the modified Babcock procedure (Marshall, 1993). Protein content was determined by the Bradford method (Bio-Rad protein assay, Bio-Rad, Hercules, CA) using a spectrophotometer (Spectronic 20 Colorimeter, Bausch & Lomb Inc., Rochester, NY). Moisture content and total solids were determined using an infrared analyzer (Infrared Analyzer 115 Vac, Denver Instrument Company, Arvado, CO).

The initial background flora of the raw milk was assessed for each repetition by serial dilution in dairy dilution blanks made up of phosphate and magnesium chloride in distilled water, or phosphate-buffered saline (PBS) (Marshall, 1999). A series of dilutions were pour-plated in duplicate with tempered tryptic soy agar (TSA) (Difco Laboratories, Detroit, MI) and mesophilic plates were incubated at 35^{o}C for 48 h. Selective culture medium was used to test for initial *Listeria* spp. concentration in the raw milk before inoculation.

UV Irradiation

Milk was exposed to UV light for a period of time using an apparatus designed to increase UV penetration throughout semi-opaque liquids (CiderSure 3500A, FPE Inc., Rochester, NY). Multiple passes through the unit were necessary for significant bacterial reductions; therefore, after each consecutive pass the machine was cleaned and sanitized. Cleaning time was less then 2 min. The configuration of the apparatus consisted of a quartz tube encased in a stainless steel outer housing. The inoculated milk flowed in thin films from the outer housing into the quartz tube where eight germicidal, low-pressure mercury lamps (254 nm) were situated along the same axis within the cylinder to guarantee uniform UV exposure throughout the milk passing through the tube (**Figure 1)**. UV dose was calculated by multiplying exposure time by irradiance.

Enumeration of Microorganisms

Microbial load was assessed immediately after inoculation and after each consecutive pass through the UV unit. Aliquots of the appropriate dilution were pour-plated in duplicate onto TSA + 6% yeast extract (YE) (Difco Laboratories, Detroit, MI) and incubated at 20^{o}C for 5 h to facilitate the repair and growth of injured cells. After 5 h, plates were given a modified oxford overlay (MOX) (Difco Laboratories, Detroit, MI) and incubated at 35^{o}C for 48 h. Following incubation, colony-forming units from replicate plates were counted and the number of cfu/ml were averaged and converted into logarithmic units.

Bacterial Strains

Five strains of *L.monocytogenes* were obtained from Dr. Martin Wiedmann's Food Safety Laboratory culture collection in the Department of Food Science at Cornell University and included: FSL J1-119, human isolate from 1995 California Jalisco-style bovine cheese outbreak; FSL X1-003 (L-2289), lab strain; FSL R2-501, human isolate from 2000 North Carolina Jalisco-style bovine cheese outbreak; FSL H4-154, goat's milk isolate; and FSL K2-121, New York City Jalisco-style bovine cheese isolate. The strains were tested against each

other for UV sensitivity. Individual stock cultures of each *Listeria* strain were grown in TSB+ YE tryptic soy broth + 6% yeast extract (TSB+YE) at 35°C and stored at 4°C on TSA+YE (Difco Laboratories, Detroit, MI). Prior to use, individual strains were twice transferred by loop inocula to TSB+YE and incubated for 18-24 h to stationary phase of growth. The approximate level of inoculum obtained by this procedure was 10^9 cfu/ml.

One liter batches of raw goat's milk were inoculated to a target inoculum level of 10^7 cfu/ml with each strain and individually tested for bacterial reduction after UV exposure. The inoculum was dispersed in the milk using a stir plate (Thermix Stirring Hot Plate, Model 310T, Fisher Scientific, USA) and magnetic stir bar. Inoculated samples were passed through the Cidersure 3500 UV apparatus 3 times at 75% running capacity (567 L/h) for an approximate UV dose of 3.9 mJ/cm^2. After each pass a 50 ml aliquot was collected for evaluation of survivors (log cfu/ml) by the methodology previously described. Data were analyzed by one-way analysis of variance (ANOVA) using Jmp In (Version 4.04, SAS Institute, 2001) software. Tukey's Honestly Significant Difference (HSD) test was used to determine if there was a statistical difference in bacterial reductions between *Listeria* strains.

Processing Parameters

The flow rate through the UV processor that would achieve maximum microbial reduction was ascertained. Flow rates tested were 20% running capacity (182 L/h), 50% capacity (455 L/h), and 75% capacity (567 L/h). These rates correspond to a velocity of 0.674, 1.705, and 2.552 m/s, respectively. At 75% running capacity turbulent flow was achieved, therefore full running capacity was not tested. Milk was inoculated with *L. monocytogenes* X1-003 (L-2289) to an initial inoculum level of approximately 10^7 cfu/ml for each of the 3 flow rates tested. The inoculum was dispersed as previously described. Two liters of inoculated milk were passed through the UV processor for 3 consecutive passes. After each pass, approximately 50 ml of milk was collected as it exited the UV machine. The aliquot was diluted in PSB and assessed for microbial load using the method previously described. Each flow rate was tested once. Microbial analysis was performed in duplicate. Data were analyzed by one-way ANOVA and linear regression using Jmp In (Version 4.04,

SAS Institute, 2001) software. Tukey's HSD test was used to determine if a statistical difference existed in bacterial reductions between processing speeds. The greatest microbial reductions occurred when operating the apparatus at 75% capacity; therefore all repetitions of the study to determine the UV death kinetics of *L. monocytogenes* were conducted at this speed. At this flow rate, the lamps generated approximately 1.3 mJ/cm^2 at exposure times between 1 and 2 sec measured by two UVX-25 sensors (UVP Inc., CA).

UV Death Kinetics

L. monocytogenes X1-003 (L-2289) was added to 4 L goat's milk to yield an initial inoculum level of 10^7 cfu/ml. The inoculum was dispersed by magnetic stirring. Immediately after inoculation, an aliquot of the milk was taken to be evaluated for initial microbial load. The inoculated milk was then passed through the UV apparatus (CiderSure 3500A, FPE Inc., Rochester, NY) at a fixed flow rate of approximately 567 L/h. Multiple passes through the UV apparatus were necessary to achieve the targeted 5-log bacterial reduction. These passes were conducted consecutively and aliquots to be evaluated for microbial reduction were taken after each pass through the unit. For each pass, the UV dose was recorded and bacterial reduction was assessed using the enumeration methods previously described. Total processing time (including cleaning) was less then 30 min for 12 consecutive passes. The experiment was replicated in triplicate. 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 polynomial regression using Jmp In® software (Version 4.04, SAS Institute, 2001).

RESULTS AND DISCUSSION

Composition of the Raw Goat's Milk

The composition of the milk consisted of 88% moisture and 12% solids. The mean fat content was 4.13 ± 0.45% and the average protein content was 2.3 ± 0.17%. The average mesophilic counts were less than 15,000 cfu/ml SPC and *Listeria* spp. were not detected in any of the raw milk samples.

Bacterial Strains

Five strains of *L. monocytogenes* were tested against each other to determine if there was a difference in UV sensitivity between strains. No statistically significant differences were observed between strains ($p > 0.05$); therefore strain FSL X1-003 (L-2289) was used for the remainder of the study (**Figure 2**).

Effect of flow rate on bacterial reduction

The reduction of *L. monocytogenes* was related to the flow rate of the milk through the UV machine. **Figure 3** shows that as the flow rate increased, less UV dose was required to achieve the same amount of bacterial reduction ($p < 0.05$). When operating the UV apparatus at 20% running capacity (181.8 L/h) approximately 15 mJ/cm^2 irradiation was necessary to achieve a 1-log reduction of the pathogen. At 50% capacity (454.6 L/h) the amount of irradiation needed for a reduction of one log dropped nearly half to approximately 7.3 mJ/cm^2. At 75% capacity (567 L/h) the amount of irradiation dropped further to approximately 3.9 mJ/cm^2 for a 1-log reduction of *L. monocytogenes*.

The Reynolds Number was calculated using the following equation: $Re = Dv\rho/\mu$, 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}) (Fellows, 1997). The values used to calculate Reynolds Numbers for each operating mode were: 20% capacity $v = 0.674$,

50% capacity v = 1.705, and 75% capacity v = 2.552 m/s, respectively, and D = 0.009 m, ρ = 1030 kg/m^3 (Simos *et al*., 1991), μ = 0.004505 Ns/m^2 (Maduko, 2004). The calculated Reynolds Numbers for 20%, 50% and 75% capacity were 1369, 3465, and 5187, respectively; these numbers translate into a laminar, transient and turbulent flow, respectively (Fellows, 1997). In laminar flow, the milk is streamlined in flow through the quartz tube with the fastest movement in the middle of the cylinder. The least amount of movement would occur near the outer walls resulting in limited UV exposure. In turbulent flow the movement of the milk is unpredictable because of mixing and agitation. Transient flow shares laminar and turbulent flow properties.

The dramatic reduction of irradiation requirements is possibly due to the increase in turbulence as flow rates increase. Turbulent flow is dependent on both flow velocity and vessel diameter. The diameter of the tubing is constant therefore when the velocity of the milk through the system was increased the amount of turbulence also increased. The opacity of the milk restricts UV penetration and therefore increased turbulence may contribute to microbial reduction by bringing the microorganisms to surface often enough to be exposed to and destroyed by the UV irradiation. This may have resulted in lower UV dose requirements because it was no longer necessary to penetrate through the opaque milk to have detrimental effects on the pathogen.

Inactivation of *Listeria monocytogenes*

The initial population of *L. monocytogenes* in the inoculated goat's milk ranged from 2.0 to 2.8 x 10^7 cfu/ml. **Table 1** shows that a greater then 5-log reduction was achieved when milk was passed through the *CiderSure* 3500 (FPE Inc, Macedon, NY) approximately 12 times for a cumulative exposure time of roughly 18 sec and a cumulative dose of approximately 15.8 +/- 1.6 mJ/cm^2. Quintero-Ramos *et al.* (2004) observed similar results with the reduction of *E. coli* ATCC 25922 in apple cider using the same commercial UV processing unit where an average UV dose between 7 and 18 mJ/cm^2 was sufficient to achieve a greater then 5-log reduction (Quintero-Ramos *et al*., 2004). Hanes and colleagues (2002) were able to show that *C. parvum* oocysts in inoculated apple cider were inactivated

after exposure to 14.32 mJ/cm^2 UV irradiation for 1.2-1.9 seconds using the same commercial UV apparatus (Hanes *et al*., 2000). The susceptibility of different microorganisms to UV was tested and similar death kinetics to this present study were displayed. In these studies, the microorganisms were suspended in water and displayed lesser UV dose requirements then those for microorganisms suspended in apple cider and milk (Chang *et al*., 1985; Clancy *et al*., 2004; Sastry *et al*., 2000; Parrotta and Bekdash, 1998). Vegetative bacteria (*E. coli* (ATCC 11229), *Shigella sonnei, Salmonella typhi, Streptococcus faecalis* and *Staphylococcus aureus*) suspended in water and exposed to UV light each demonstrated similar susceptibility to UV light, requiring almost the same dose (approximately 10 mJ/cm^2) to achieve a 3-log reduction of the microorganisms (Chang *et al*., 1985). *C. parvum* oocysts in water were also susceptible to low levels of UV light and a 4-log reduction was achieved after a UV dose of 10 mJ/cm^2 (Clancy *et al*., 2004). It is possible that greater UV doses are necessary for a 5-log reduction of *E. coli* ATCC 25922 and *C. parvum* oocysts in apple cider than in water because of the suspended solids in the apple cider. It follows that a lesser UV dose would be required for the inactivation of microorganisms in apple cider then in goat's milk because of a greater amount of suspended solids in milk, i.e. fat and protein.

Figure 3 illustrates the reduction of *L. monocytogenes* at increasing UV exposure. As UV exposure increased, the population of the pathogen was significantly reduced ($p<0.0001$). The death curve exhibited a slight lag and tailing phase at the commencement and termination of UV exposure, respectively. The initial plateau has been attributed to the commencement of cell injury and as the exposure to UV is continued, maximum damage occurs to the cells and minimal additional treatment becomes lethal (Sastry *et al*., 2000). The survivor numbers decline rapidly and a lag phase due to UV resistance and experimental components (i.e. suspended solids that would protect the microorganism from direct exposure) is noticed (Hanes *et al.*, 2000; Sastry *et al*., 2000). These results are also consistent with the sigmoidal-shaped curves from experiments done by Quintero-Ramos *et al.* (2004) and Wright *et al*. (2000) for the reduction of *E. coli* in apple cider. The non-linear relationship between ultraviolet dose and bacterial population has been explained as

"multiple hit kinetics" where the deaths of microorganisms are due to the culmination of exposure to ultraviolet light (Chang *et al*., 1985; Quintero-Ramos *et al*., 2004).

As depicted in **Figure 3**, there was some variability in reduction of *L. monocytogenes* as UV dose increased as a function of time and exposure. This is consistent with variations in bacterial reductions reported by Wright *et al*. (2000) and Quintero-Ramos *et al*. (2004). Duffy *et al.* (2000) also reported a high degree of variability in the mean reduction of *E.coli* ATCC 25922 in individual tubes of inoculated apple cider. Variations in microbial inactivation were said to be due to slight inconsistencies that occur with any food processing technology. The authors offered that consistency of a 5-log reduction in the pertinent pathogen might be improved if compliance for processing requirements were made more rigid by increasing the target log reduction (Duffy *et al*., 2000).

CONCLUSIONS

The results of this study indicate that UV light would achieve a greater then 5-log reduction of *Listeria monocytogenes* in raw goat's milk when processed 12 consecutive times at a fixed flow rate of approximately 567 L/h. These results may be the first step towards gaining FDA approval for the use of ultraviolet irradiation as an alternative processing method to traditional thermal pasteurization. The development of an apparatus that can achieve maximum turbulence within the quartz tube with a holding time long enough to administer the proper amount of UV irradiation would be beneficial if this technology were approved for commercial use. Further research that would assess lipid and protein oxidation, lipase activity, and organoleptic properties of the milk would also be needed.

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Table 1. The effect of UV dosage on the reduction of *Listeria monocytogenes* strain FSL X1-003 (L-2289) in fresh goat's milk.

Number of Observations (n)	Mean cumulative UV exposure[a] (mJ/cm^2)	Mean log reduction factor[b] (log cfu/ml)
2	16.9	5.45
3	14.8	5.62
5	13.1	4.45
5	10.9	3.29
4	9.03	2.70
5	7.02	1.95
4	5.11	1.25
5	3.16	0.73
3	1.37	0.27

[a] Average UV exposure for samples processed within a range of >2.00 mJ/cm^2.
[b] Average difference in microbial counts on tryptic soy agar + 6% yeast extract with a Modified Oxford Agar overlay between un-treated and treated fresh goat's milk.

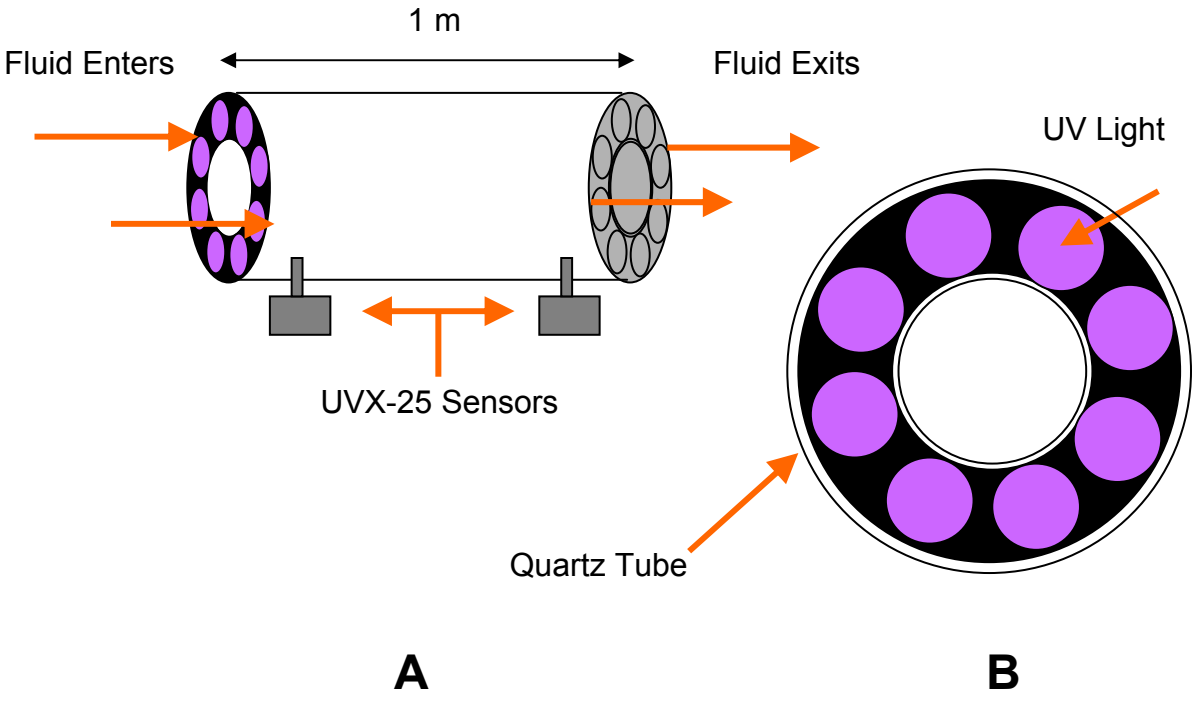

Figure 1. Diagram of UV chamber (A) and layout of UV lights (B) within the Cidersure 3500A UV Apparatus (FPE Inc., Rochester, NY).

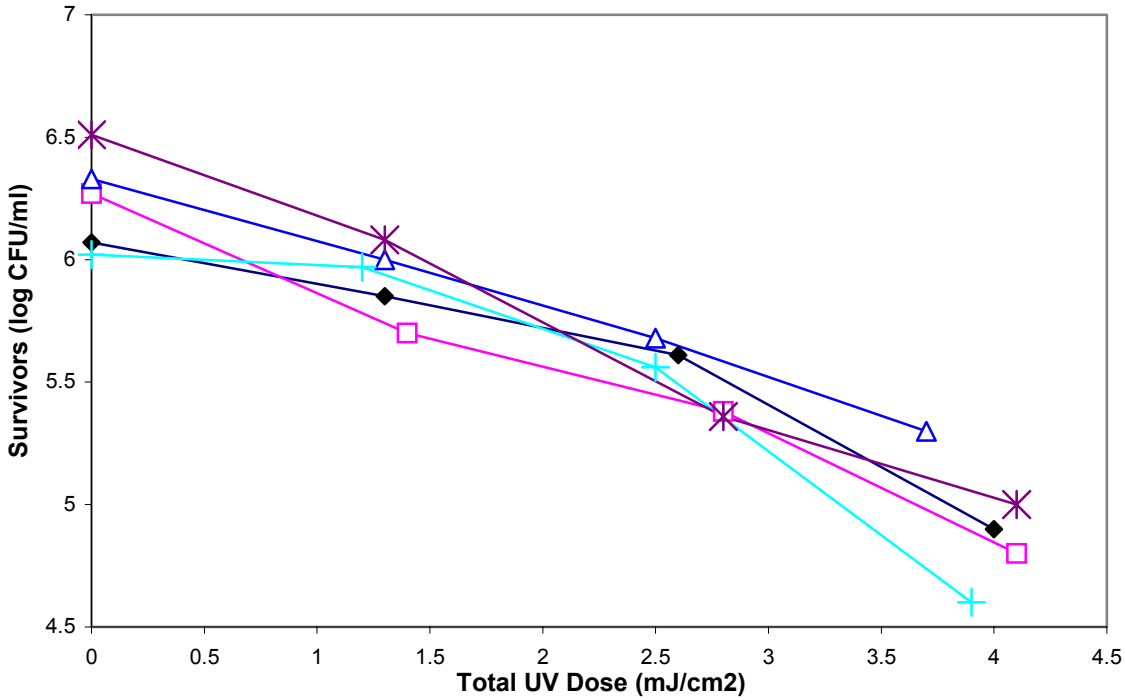

Figure 2: Reduction comparison of different *Listeria monocytogenes* strains in fresh goat's milk in response to UV light. No significant statistical difference was observed between the dose response and strain as determined by Tukey's test ($p<0.05$). Strains R2-501 (♦), X1-003 (□), K2-121 (Δ), J1-119 (+), and H4-154 (*).

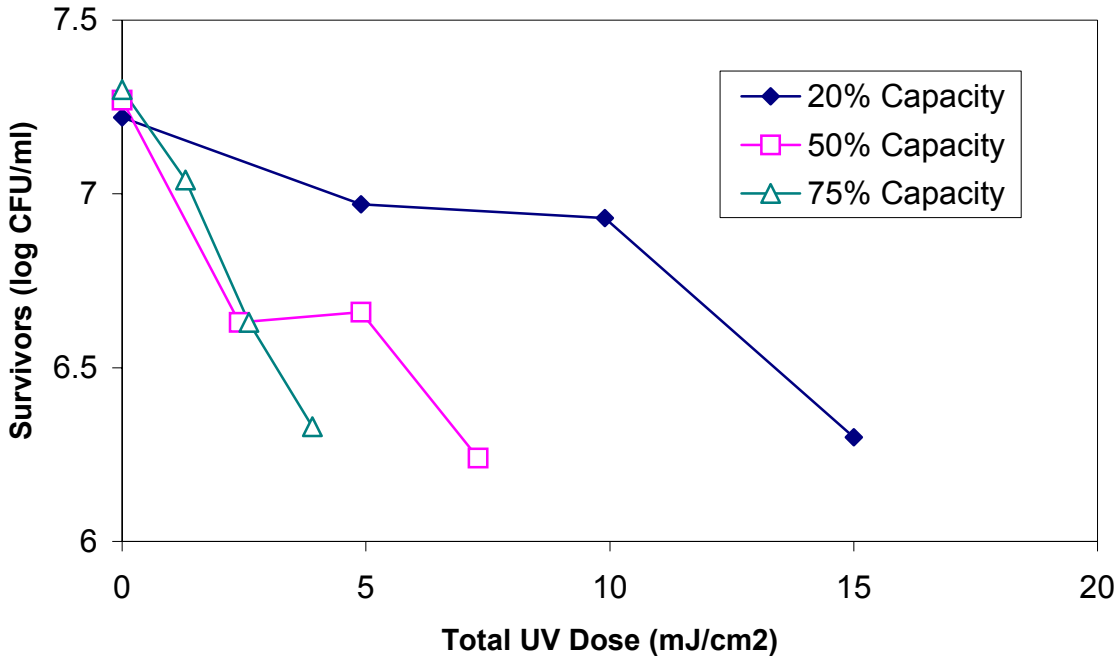

Figure 3: The relationship between the reduction of *Listeria monocytogenes* strains in fresh goat's milk in response to UV light at different processing speeds. As processing speeds increased, L. monocytogenes populations decreased ($p<0.05$) [20% capacity (181.8 l/h)(♦): $Y = 7.27 – 0.056X$, $R^2 = 0.86$; 50% capacity (454.6 l/h)(□): $Y = 7.16 – 0.13X$, $R^2 = 0.86$; 75% capacity (681.9 l/h)(Δ): $Y = 7.32 – 0.26X$, $R^2 = 0.99$)].

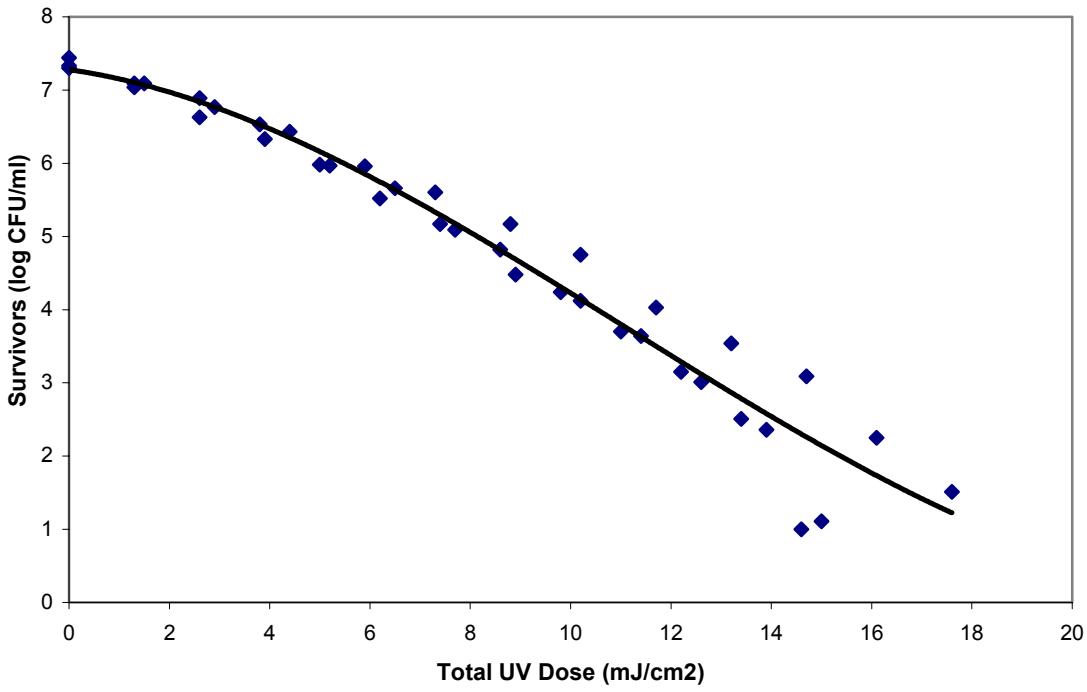

Figure 4: Reduction of *Listeria monocytogenes* strain FSL X1-003 (L-2289) in fresh goat's milk in response to UV light [$R^2 = 0.96$; $Y = 8.26 - 0.0004X - 8.5e\text{-}9\ (X - 7933)^2 + 8.82e\text{-}13\ (X - 7933)^3$; Polynomial Fit Degree = 3]