Effects of UV Irradiation on the Reduction of Bacterial Pathogens and Chemical Indicators of Milk

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(ABSTRACT)

Consumer demand for fresher and minimally processed foods has brought about a movement to find effective, non-thermal processing technologies for the treatment of milk. The influence of temperature on bacterial reduction in UV irradiated milk was tested. 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 using the CiderSure 3500 apparatus (FPE Inc., Macedon, NY). Bacterial concentrations before and after UV exposure were enumerated and the results indicated that processing temperature was not significantly related to bacterial reduction ($p > 0.05$). The results did indicate that skim milk samples had a greater bacterial reduction, regardless of processing temperature compared to reduced fat milk and whole milk samples ($p < 0.05$). Solids such as milk fat, protein, lactose and minerals, in the milk have a greater effect over bacterial reductions than processing temperatures.

Traditional goat cheeses are produced using unpasteurized milk, which increases the food safety concerns for these types of products. 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. 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 processed 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.

Organoleptic consequences of goat's milk treated with UV technology were assessed. Olfactory studies were conducted and a highly significant difference was determined

between the odor of fresh goat's milk and UV processed milk ($p < 0.05$). The extent of lipid oxidation and hydrolytic rancidity was measured by thiobarbituric acid reactive substances (TBARS) and acid degree values (ADVs). Results indicated that as the UV dose increased, there was a significant increase in TBARS values and ADVs of the milk samples ($p < 0.05$). Milk samples were processed using the UV processor under the same conditions as previously described without exposure to the UV source to determine if the agitation from pumping was causing off-flavors by way of hydrolytic rancidity. The ADVs from these samples increased at the same rate as the UV irradiated samples; however, sensory studies indicated that the increase of free fatty acids (FFA) was not enough to cause detectable off-odors in the milk. Solid phase microextraction and gas chromatography (SPME-GC) was utilized to quantify the production of volatile compounds that were formed due to UV processing. The formation of pentanal, hexanal and heptanal was identified after as little as 1.3 mJ/cm^2 UV dose. Peak areas were measured and analyzed after 7.8 mJ/cm^2 and 15.6 mJ/cm^2 and were determined to increase significantly as UV dose increased ($p < 0.05$). The chemical analyses supported the findings from the olfactory studies. The outcome of this research showed that UV irradiation at the wavelength 254 nm, was detrimental to certain chemical properties of fluid milk. The properties that were perceived as negative in fluid milk may be considered an attribute in certain types of cheese and future studies in the cheese production sector should be considered. Other applications for this technology could be for use in developing countries where milk is not typically processed because of the high costs of thermal pasteurization. On-farm applications for the treatment of replacement milk should also be considered.

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CHAPTER I

INTRODUCTION

The production of dairy products from raw milk is a traditional food practice and common in many cultures. Recent consumer trends toward “fresh” and “wholesome” dairy products have created a niche for the production of foods that have been processed in a fashion to minimize impact to the original product (Reed and Grivetti, 2000). It has been predicted that bacterial foodborne outbreaks will not deter consumption of unprocessed cheeses because consumer preference for raw dairy products is linked with perceived superior organoleptic characteristics that cannot be obtained when the milk is heat-treated (Reed and Grivetti, 2000; Buchin *et al*., 1998).

Of the total amount of milk produced globally, 3.5% is from small ruminants such as goats and sheep (FAO, 1999). Throughout the developing world, more people drink milk from goats then from any other animal (Haenlein, 1996; Park, 1990). The amount of commercially produced goat’s milk in the United States is estimated to be 24,000 tons compared to the world leader India with 2,000,000 tons and 520,000 tons in France; approximately half of the milk is commercially processed as fluid, powder, UHT or evaporated and the other half is used for commercial cheese production which has seen an increase in popularity and consumption per capita (Haenlein, 1996; Stern, 1992; Park, 1990).

There are many small-scale, non-commercial dairy goat farmers that represent over 1.5 million dairy goats in the US producing roughly 600,000 tons of milk worth over $500 million (Haenlein, 1996). These farms are mostly widely geographically scattered, therefore, dairy cooperatives are not typically organized. Farmers and producers of dairy goat products are forced to endure extremely high transportation costs to processing plants or must process their commodities on-site. A recent phenomenon in this sector is the launch of small-scale producers that process dairy commodities on-farm to sell at high-end restaurants, farmer’s markets and natural foods stores.

The importance of guidelines for the safe treatment of milk was recognized early on and in 1924, with collaboration between processors and regulators, the Public Health Service devised a series of voluntary recommendations called the *Standard Milk Ordinance* in an attempt to control and limit the outbreak of milk-borne diseases. After a series of revisions, the Grade "A" Pasteurized Milk Ordinance (PMO) came into existence. The U.S. Code of Federal Regulations (CFR) (21CFR 131.3) and the PMO for the treatment of milk are based on time/temperature requirements for the reduction of *Coxiella burnetii* and *Mycobacterium tuberculosis* (Enright *et al*., 1957; Jay 1996). However, both the CFR and the PMO address the possibility for processing alternatives to heat treatment (21 CFR 1240.61 and CFSAN, 2002). The PMO states "that nothing shall be construed as barring any other pasteurization process which has been recognized by the Food and Drug Administration to be equally efficient and which is approved by the regulatory agency" (CFSAN, 2002).

While thermal pasteurization of milk has been proven to be effective at reducing pathogenic bacteria and denaturing lipase enzymes, consideration of alternative methods that would be less costly for producers working with smaller volumes and would have less perceived effects on organoleptic properties has become of interest. A publication by Gallmann and Eberhard (1992) compiled a brief summary of alternative processing techniques that have been used for milk. The techniques 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 require a subsequent heat treatment to kill microorganisms (Sastry *et al*., 2000).

It has been well reported that both UV and visible light, with wavelengths between 280 nm and 700 nm, is a key factor in the creation of flavor defects and malodors in milk (Azzara and Cambell, 1992; Bekbölet, 1990; Borle *et al*., 2001; Cadwallader and Howard, 1998; Dimick, 1982; Frankel, 1980; Frankel, 1991; Min and Boff, 2002). However, historical studies that looked at the potential of UV irradiation for vitamin D enrichment at the specific germicidal wavelength of 254 nm did not report negative sensory data (Burton, 1951; Capstick *et al*., 1949; Caseiro *et al*., 1975).

The first commercial irradiation equipment was specifically designed for vitamin D enrichment and had little consideration for the limited penetration of UV rays through opaque substances. The desire was to make a machine capable of making milk films to maximize UV exposure, but was not practical with the technology of the time (Burton, 1951). In an early attempt to maximize the amount of UV exposure, equipment was designed where the milk would travel over the surface of several rollers with the UV lamps positioned around the cylinder. The addition of turbulence would bring all milk molecules to the surface for UV exposure (Burton, 1951). It was noted that most of the early studies demonstrated insufficient germicidal effects, presumably due to the lack of adequate doses of UV sufficient to kill all microorganisms (Gallmann and Eberhard, 1992). Research toward the development of UV irradiation-based processing methods that would meet the safety criterion set by the CFR and the PMO for milk was hard to justify because of the confirmed successes of thermal pasteurization. As a consequence, there was little motivation for development of effective alternative methods. Recently, it has been suggested that thermal pasteurization of milk is inadequate for certain pathogens (e.g., *Bacillus cereus* spores and *Mycobacterium avium* subspecies *paratuberculosis*) (Lin *et al*., 1998; Grant *et al*., 2002), and interest to find alternative methods are on the rise.

On January 19, 2001, FDA released the "Hazard Analysis and Critical Control Point (HACCP): Procedures for the Safe and Sanitary Processing and Importing of Juice, Final Rule" (66 FR 6137). These regulations mandated the adoption of HACCP principles (NACMCF, 1997) and the use of a process that would achieve a 5-log reduction of the pertinent pathogen identified for each type of fruit and vegetable juices. The FDA responded to requests to make thermal pasteurization compulsory by stating, "Mandating a specific intervention technology such as pasteurization would limit the development of new, potentially less costly technologies that may be as effective as pasteurization" (66 FR 6137). The imminent date for enforcement of these new regulations triggered a race to find cheaper alternatives to heat pasteurization that would win FDA approval. By using more sophisticated processing equipment then in the past, UV processing technology was reexamined and was effective for the microbial treatment of water and apple cider (Hanes *et*

al., 2002; Wright *et al*., 2000; Worobo, 1999). The methods and concepts used in these trials could prove to be valid for development of an alternative method to heat pasteurization of fluid milk for small dairy operations. The long-term objective of this project will be to study the effects of UV irradiation as an alternative processing method for dairy product manufacturers.

1.1 Specific Project Objectives

Study 1: To determine if processing temperature and milk fat concentration can effect reduction of *Escherichia coli* ATCC 25922 in commercially processed skim, 2% and whole milk.

Study 2: To determine the dose of UV irradiation necessary to achieve a 5-log reduction of *Listeria monocytogenes* in experimentally inoculated goat's milk.

Study 3: To assess the occurrence of oxidation and rancidity in UV processed goat's milk by chemical indicator analyses.

CHAPTER II

REVIEW OF LITERATURE

2.1 General Milk Microbiology

When milk is secreted by the mammary gland it is sterile, but as it flows through the udder it becomes contaminated by microorganisms which, except in mastitis cases, are non-pathogenic and few in numbers (ICMSF, 1998). Most contamination occurs after milk leaves the udder and is exposed to the outside environment, i.e. during milking, storage, transportation, etc. The microbial content of milk is used as an indicator of quality and safety.

There are a number of microorganisms commonly found in milk that contribute to the flavors and aromas of milk and milk products, but some microorganisms are the cause of food borne illnesses. Microorganisms that are normally associated with milk are usually from one of four general groups: lactic acid bacteria, coliforms, spoilage microorganisms, and pathogenic microorganisms (ICMSF, 1998). Lactic acid bacteria utilize lactose for lactic acid production and are often used as starter cultures for the production of fermented milk products like cheese and yogurt. Coliforms are aerobic or facultative anaerobic, non-sporeforming Gram-negative rods that ferment lactose, which produces acid and gas, at temperatures between 32 and 35°C within 48 hours. Coliforms in milk are considered an indication of initial product quality and suggest the possibility of the presence of pathogens. In general, microorganisms cause spoilage by degrading proteins, carbohydrates and fats and are responsible for the deterioration of product quality (ICMFS, 1998). Pathogenic organisms are usually introduced into milk by improper handling, storage, and employee hygiene. Some pathogenic bacteria associated with milk products are *Coxiella burnettii*, *Brucella* spp., *Salmonella* spp., *Mycobacterium tuberculosis*, *Yersinia enterocolitica*, *Camplybacter jejuni*, *Listeria moncytogenes* and *Escherichia coli* O157:H7 (ICMFS, 1998).

2.1.1 *Escherichia coli* O157:H7

In 1885, Escherich attempted to isolate the etiologic agent of cholera, but instead isolated and studied the microorganism that is now called *Escherichia coli* (Jay, 1996). This Gram-negative, facultative anaerobic, asporogeneous rod-shaped bacterium shares the family Enterobacteriaceae with the coliforms *Citrobacter*, *Enterobacter*, and *Klebsiella*. *E. coli* is commonly found in the intestinal tract of animals and humans, and therefore is a good indicator of fecal contamination in foods. This bacterium was first identified as having strains pathogenic strains for humans in 1971 after an outbreak attributed to imported cheeses. Five virulent groups are now recognized: enterotoxigenic (ETEC), enteroaggregative (EaggEC), enteropathogenic (EPEC), enteroinvasive (EIEC), and enterohemorrhagic (EHEC). There are over 60 serotypes that belong to the EHEC group, including *E. coli* O157:H7. Strains in this group adversely affect humans, producing Shiga-like toxins (verotoxins that closely resemble those produced by *Shigella dysenteriae*) in the colon (Cassin *et al*., 1998). The infectious dose to cause illness is estimated to be less then 100 cells (Meng *et al*., 2001). When *E. coli* O157:H7 is ingested there is an incubation period that lasts 3 to 4 days before symptoms of hemorrhagic colitis occur. Symptoms include the onset of severe abdominal cramping followed by watery and bloody diarrhea. Signs of illness may persist for over a week and complications may include hemolytic uremic syndrome (HUS) and thrombotic thrombocytopenic purpura (TTP) (Meng *et al.,* 2001). HUS is a life-threatening complication that typically occurs in children. It is classified by the destruction of red blood cells and platelets which could lead to acute kidney failure and permanent loss of kidney function. TTP is a complication that most often affects elderly individuals and symptoms include fever, neurological symptoms from blood clots in the brain and HUS (Meng *et al.,* 2001).

E. coli O157:H7 has long been associated with dairy cattle. In 1996, USDA's National Animal Health Monitoring System (NAHMS) Dairy'96 Study tested fecal samples from over 91 dairy operations (approximately 3,600 milk cows and 600 cull cows) and 97 cull dairy cow markets (over 2,200 cull cows) and discovered that 24.2% and 30.9%, respectively, had at least one cow that was fecal culture positive for the fecal shedding of verotoxogenic *E. coli*

O157:H7 (Wells *et al.*, 1996). Hancock and others (1997) suggested that if a study were conducted where repeated fecal sampling occurred possibly all dairy operations would be positive.

Typical test and cull methods that have been successful for controlling other pathogens such as *Mycobacterium bovis* and *Brucella abortus* will not likely control *E. coli* O157:H7 on-farm (Wells *et al*., 1996). Wells and colleagues (1996) suggested that identifying and removing the sources of new *E. coli* O157:H7 infections in cattle could be incorporated into herd maintenance and quality assurance plans. Withholding the sale and slaughter of cows that are shedding could also reduce adulteration of meat products. Pasteurization of milk is critical for controlling contamination of dairy products (Wells *et al*., 1996).

2.1.2 *Listeria monocytogenes*

Until 1981, listeriosis was a disease believed to only affect animals. It is now known that consumption of foods infected with *Listeria monocytogenes* may cause the disease in humans (ICMSF, 2002). If a lactating animal has the disease listeriosis, she may shed *L. monocytogenes* in milk thereby contaminating the raw milk product. Proper heat processing will destroy the bacteria, but contamination of ready-to-eat foods with *L. monocytogenes* has been shown to occur after processing (Hof, 2003). Possible post-processing sources of contamination of may include condensation on the floors in coolers, freezers, processing facility, drains, floor mats, cross-contamination with utensils and equipment, etc. (Hof, 2003).

L. monocytogenes is a Gram-positive, non-spore-forming, rod-shaped bacterium that is found widespread in nature including soil, fecal material, milk, silage, etc. It is facultatively anaerobic, and grows well at temperatures between –0.4 and 45°C, a pH between 4.39 and 9.4, a water activity at or above 0.92, and salt concentrations of less then 10% (ICMSF, 2002). There are 13 serotypes of *L. monocytogenes* that are known to cause disease, but 95% of human infection is due to three serotypes: ½a, ½b and 4b (Swaminathan, 2001).

Listeria monocytogenes is an opportunistic intracellular pathogen that primarily affects those with a compromised immune system: pregnant women, fetuses, young children, and the elderly. Although the infective dose for *Listeria* is unknown, the mechanism for infection occurs when the bacteria are ingested and then they invade the gastrointestinal epithelium. The bacteria are then engulfed by phagocytic cells in the host and are able to enter the blood stream (Hof, 2003). Infection by *L. monocytogenes* causes flu-like symptoms and may include other complications such as septicemia, meningitis, encephalitis, and cervical infections in pregnant women that could lead to spontaneous abortion or stillbirths. The clinical definition of listeriosis is when the organism is isolated from otherwise sterile environments such as blood, cerebrospinal fluid, placenta or fetus (CFSAN, 2003).

Listeria monocytogenes does not survive pasteurization but has demonstrated thermotolerance. The body temperature of a cow (including the udder) is normally 38.8^{o}C. Cows that are infected with *L. monocytogenes* may have a fever where the body temperature can reach as high as 42.8^{o}C (Doyle *et al*., 1987). In a study by Farber and colleagues, *L. monocytogenes* were grown at 30, 39 and 43°C, suspended in raw milk, and then passed through a high-temperature short-time system at 72, 69, 66, and 63°C (Farber *et al*., 1992). The results of the study showed that the largest number of survivors occurred with those cultures grown at 43°C and it was inferred that *L. monocytogenes* shed from infected, febrile cows would be more themotolerant than cells grown at lower temperatures (Farber *et al.*, 1992).

Linton *et al*. (1990) suggested that thermotolerance involves the response of the organism to heat shock. *L. monocytogenes* Scott A strains in log phase growth were suspended in tryptic soy broth plus 6% yeast extract (TSB+YE) and then exposed to temperatures of 40, 44, and 48°C for 3, 10, and 20 min, respectively. The samples were then subjected to heating at 55°C for 50 min to simulate a heat shock scenario. The results of the study showed that as the heat shocking time and temperatures increased; heat shocked *Listeria* was able to survive longer than cells that were not heat shocked (Linton *et al.*, 1990). With vat pasteurization , the amount of time it takes for milk to come up to processing temperature may contribute to the heat resistance of *Listeria* (Linton *et al*., 1990).

Knabel and Thielen (1995) proved that severely heat-injured *L. monocytogenes* in pasteurized milk could be recovered in an enrichment media using a strictly anaerobic method. The *Listeria* strain was obtained from Centers for Disease Control (CDC), Atlanta, GA. It was isolated from raw milk from a farm that produced dairy products suspected to contain *L. monocytogenes*. The cultures were grown for 18 h at 43°C in TSB + 6% YE and then re-suspended in raw milk. The inoculated samples were placed in sealed, thermal-death-time tubes and heated at 63.5^{o}C for 5 min. The most effective method of recovering the heat-injured cells was by purging the headspace of the thermal-death-time tubes with N_2 gas and the addition of filtered sterilized cysteine [final concentration 0.5 g/l]. This technique was shown to increase recovery from 0% to 60% with the USDA-University of Vermont modified enrichment broth and from 11 to 100% with the FDA Listeria enrichment broth (Knabel and Thielen, 1995).

Abou-Eleinin and colleagues (2000) assessed the incidence and seasonal variation of *Listeria* obtained from bulk tanks on 39 goat farms in Vermont. Researchers attempted to sample each farm once a month 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 in prevalence similar to cow's milk were observed (Fernandez Garayzabal *et al*., 1987), 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).

2.2 Raw Dairy Products and Foodborne Illnesses

The production of dairy products from raw milk is a traditional food practice and common in many cultures (Reed and Grivetti, 2000). The culture most famous for consumption of raw milk and raw milk products are the French (De Buyser *et al*., 2000). Consumer preference for raw dairy products is linked with perceived superior organoleptic characteristics that cannot be obtained when the milk is heat-treated (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. This was attributed to the preservation of heat sensitive enzymes and microbiota.

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, and of those, 48% were from raw milk products and 51% were from unspecified milk processing type (De Buyser *et al*., 2000). Fresh-style soft cheeses (Jalisco cheese) made from raw milk are popular within the United States Latin American population. These cheeses have been implicated in many recent outbreaks in the Hispanic community, including an outbreak in 1997 of salmonellosis that affected over 150 people in California and an outbreak in 2000 of listeriosis in 12 people in North Carolina (Reed and Grivetti, 2000; CDC, 2001). The Milk Dairy Foods Control Branch of the California Department of Food and Agriculture (CDFA) reported that over 21,000 tons of Jalisco cheese is illegally produced each year (Reed and Grivetti, 2000). It has been predicted that bacterial outbreaks will not deter consumption of unprocessed cheeses (Reed and Grivetti, 2000). It is supposed that the consumption of raw milk products will continue regardless of educational programs or increased governmental regulations to deter the consumption of raw milk products.

2.3 UV Light as an Alternative to Thermal Processing

The germicidal effects of UV light have been known since the late 1800's (Burton, 1951). The electromagnetic waves in UV light are shorter than those in the visible light spectrum and produce bactericidal effects and inactivate enzymes (Ing and Kessler, 1981). The rays begin where the violet end of the visible spectrum ends and extend to the boundaries of the X-ray region (Burton, 1951). The ultraviolet (UV) region of the electromagnetic spectrum ranges from 100 to 400 nm and can be sub-divided into four sections: UVA, UVB, UVC, and vacuum UV. UVA (315 – 400 nm) is responsible for changes in the color of human skin when exposed to sunlight (tanning); UVB (280 – 315 nm) is the cause for sunburns that may eventually lead to skin cancer; UVC (100 – 280 nm) is the germicidal range that inactivates enzymes, bacteria and viruses; and vacuum UV (100 – 200 nm) is the range absorbed by all substances when transmitted in a vacuum (IFT, 2000). The maximum germicidal effect is

achieved within the UVC range of 250 and 270 nm and the effect will decrease as the wavelength increases until it is non-existent (Bachmann, 1975).

UV light is natural energy from the sun and may be reproduced in the processing plant by mercury vapor lamps (Ing and Kessler, 1981). At 30°C, 85% of the light transmitted by mercury vapor lamps is at a wavelength of 254 nm making them ideal for sterilization (Capstick *et al*., 1949; Bachmann, 1975). The temperatures at which the mercury vapor lamps operate are important because below 30°C, the efficiency of UV light output is greatly reduced (Capstick *et al*., 1949).

Another form of UV technology has been successful in industries where sterilization need only be applied at the surface, i.e.; packaging materials, medical supplies, transparent pharmaceutical products, etc. is called "pulsed light technology". The treatment is administered by using quick, but intense, pulses of light onto the surface of an object. The range of electromagnetic energy is between 170 and 2600 nm and has an energy density in the range of 100 to 5×10^4 mJ/cm^2 (Barbosa-Canovas, *et al*., 2000). The spectrum of pulsed light covers the range of the ultraviolet region which causes photochemical changes, while the visible spectrum and infrared regions induce photothermal changes (Barbosa-Canovas *et al*., 2000). This technology has been most successful for water treatment and surface sterilization due to the limitations of light penetration beyond the surface of opaque substances.

2.3.1 Microbial Inactivation from UV Irradiation

Microbial inactivation is illustrated by a sigmoidal shaped curve (Sastry *et al*., 2000; IFT, 2000). The initial plateau indicates the commencement of cell injury. As exposure continues, maximum damage occurs to the cells and minimal additional treatment becomes lethal (IFT, 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 (Sastry *et al*., 2000; IFT, 2000).

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). In some cases, wounded cells are able to repair the damage caused by UV exposure and may be able to reactivate themselves through activity by repair-enzymes (Parrotta and Bekdash, 1998). Photoreactivation is the activation of these repair enzymes by visible light shortly after UV exposure. Parrotta and Bekdash (1998) suggest that the key to limiting photoreactivation is to determine the UV dose that would cause permanent inactivation of nucleic acids throughout the DNA chain. The exposure requirement for extensive mutations to occur has been suggested to be at least 40 mJ/cm^2 on all parts the product (Sastry *et al*., 2000). This level of exposure is difficult to achieve in most food products because of the penetration limitations of UV light beyond the surfaces of most substances (Jay, 1996; Sastry *et al*., 2000).

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).

2.3.2 UV Irradiation and Vitamin D Production

The connection between sunlight exposure and the development of rickets was made in the early 20th century. Huldschinsky (1919) demonstrated that ultraviolet light had antirachitic activity and early experiments identified the occurrence of a photochemical reaction that activated the "antirachitic factor" when edible products, including milk, were exposed to UV light (Burton, 1951). This unknown factor was later identified as vitamin D_3, a precursor of the steroid hormone [1α,25$(OH)_2$ D_3] with the primary function to regulate calcium metabolism (Norman, 2000). Vitamin D_3, or cholecalciferol, is produced naturally in higher animals when enzymes within the skin convert sterol cholesterol into 7-dehydrocholestoral which, when exposed to sunlight or wavelengths between 240 and 300 nm, is converted into vitamin D_3 (Norman, 2000; Burton, 1951). When sufficient sunlight is available, vitamin D_3 produced in the skin is sufficient to meet the nutritional requirements of the human body. Living indoors, wearing clothing, and living in regions where sunlight is inadequate are all factors that contribute to deficient vitamin D_3 production (Norman, 2000). Supplementing vitamin D in the diet became necessary to prevent the onset of rickets in children and osteomalacia in adults. Naturally occurring vitamin D in whole milk occurs in concentrations between 0.34 to 0.84 IU/g fat; therefore unfortified fluid milk is not considered a significant source of vitamin D. In the 1930s, producers began fortifying milk with vitamin D (McBean and Speckmann, 1988; Murphy *et al*., 2001). Many fortification methods, including UV irradiation, have been introduced over the years, but the direct addition of concentrated vitamin D supplements have proven to be the most consistent and reliant (Murphy *et al*., 2001).

2.3.3 Ultraviolet Processing Technology and Milk

Steenbock (1928) developed and patented a method to activate vitamin D_3 in some food products. An example of this primordial UV technology was to expose foods to a quartz mercury vapor lamp (Cooper-Hewitt, type BY) for varying lengths of time, from thirty minutes up to seventeen hours. Steenbock (1928) conceded that this method may be used, to a certain extent, on milk, but warned that care must be taken to avoid overexposure, which

would result in off-flavors. The bactericidal benefits of UV irradiated water were known before Steenbock submitted his patent in 1924, and the possibility of using this technology to reduce the microbial load in unpasteurized milk was addressed. Steenbock (1928) acknowledged that the limitations of the available equipment would require extreme exposure times before producing antibacterial benefits.

The first commercial irradiation equipment specifically for vitamin enrichment in milk was designed with little consideration for the limited penetration of UV rays through opaque substances. In an early attempt to maximize the amount of UV exposure, equipment was designed where the milk would travel over the surface of several rollers with the UV lamps positioned around the cylinder. The addition of turbulence would bring all milk molecules to the surface for UV exposure (Burton, 1951). Most early studies demonstrated insufficient germicidal effects due to insufficient UV doses and deleterious effects on sensory properties (Gallman and Eberhard, 1992).

A German scientist by the name of Lembke (1949) developed an apparatus capable of drastically reducing the bacterial load in raw milk. Early experimentation by Lembke demonstrated that the optimum wavelength for microbial lethality is at 253.7 nm (Capstick *et al*., 1946). The opacity of the milk restricted UV penetration to approximately 1 mm and therefore when the UV apparatus was developed, maximum turbulent flow was produced so that the microorganisms would surface often enough to be destroyed by the UV irradiation (Capstick *et al*., 1946). This apparatus, called Lembke Apparatus, was comprised of a series of vertical quartz tubes that totaled 100 m in length with an internal diameter of 1 cm. UV sources were arranged among the milk tubes and turbulence was introduced by moving the milk at a velocity that corresponded to a high Reynolds number (Re) which is calculated using the following equation:

$$Re = \frac{Dv\rho}{\mu}$$

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 ($N\ sm^{-2}$) (Burton, 1951; Fellows, 1997).

In spring of 1946, the British Intelligence Objectives Subcommittee (B.I.O.S.) sent a team of scientists into war-torn Germany to verify or disprove claims by German dairymen that "pasteurization" of milk by the Lembke Apparatus had been successful (Capstick *et al.*, 1946). In their study, raw milk was run through the UV apparatus at a velocity of 80 cm/sec and an exposure time of 120 sec (Capstick *et al.*, 1946). When the milk was processed at temperatures between 19°C and 22°C, a 3-log reduction in microbes was achieved, but as they lowered the temperature the bactericidal benefit of UV treatment decreased. When the same milk was thermally vat pasteurized at 63°C for 30 min, Capstick and colleagues (1946) were only able to achieve an approximate 2-log reduction in microbial load. Capstick and colleagues (1946) concluded that as the temperature of milk increases the reduction of bacteria due to UV irradiation increases. They also suggested that if milk is warmed to a temperature above 25°C and then exposed to UV light, the reduction of bacteria would be greater than that of milk processed by thermal vat pasteurization (Capstick *et al.*, 1946).

In a more recent study by Caserio and colleagues (1975) an apparatus that consisted of two stainless steel walls with two mercury lamps positioned between them was used to UV irradiate milk. The milk flowed down the walls in films roughly 0.2 mm thick at a flow rate of 1200 L/h. The wavelength of maximum UV exposure was 240 nm and dose rate was 0.18 mJ/cm^2. The milk was kept at room temperature (20-22°C) before treatment and after exposure, and the UV irradiated milk was made into Italian 'Grand Padano' cheese cakes. Cheese "clots" made from the UV irradiated milk had 1.47 logs fewer coliforms than "clots" made from the fresh milk (Caserio *et al.*, 1975). The number of other microorganisms was also reduced: mesophilic lactic acid bacteria (0.50 logs fewer), thermophilic lactic acid bacteria (0.56 logs fewer), caseinolytic fermenting bacteria (0.52 logs fewer) and citrate fermenting bacteria (0.40 logs fewer) (Caserio *et al.*, 1975). Sensory studies were conducted on the cheese cakes by both trained and untrained panels. Triangle tests and multiple comparison ranks were used to evaluate color, consistency, and flavor. The results of the sensory studies showed that cheese made from UV-treated milk had organoleptic properties typical of 'Grana Padano' cheese.

2.3.4 Oxidation and Hydrolytic Rancidity of Milk Lipids

The study of oxidation and hydrolytic rancidity of milk lipids is a widely studied topic and there have been many studies to examine the causes, effects and controlling methods of these reactions (Azzara and Cambell, 1992; Bekbölet, 1990; Borle *et al*., 2001; Cadwallader and Howard, 1998; Dimick, 1982; Frankel, 1980; Frankel, 1991; Min and Boff, 2002).

2.3.4.1 Lipid Oxidation

Lipid oxidation is considered the primary cause of non-microbial spoilage in foods and has been blamed for the most common and severe off-flavors in milk (Wong and Kitts, 2001; Cadwallader and Howard, 1998). Many factors are responsible for the degradation of lipids due to oxidation, and one of the major causes of this defect has been attributed to the oxidation of unsaturated lipids (Cadwallader and Howard, 1998). deMan (1999) explains that the rate of the oxidation is dependant upon "the amount of oxygen present, degree of unsaturation of the lipids, presence of antioxidants, presence of prooxidants, especially copper, and some organic compounds such as heme-containing molecules and lipoxidase, nature of packaging material, light exposure and temperature of storage." Lipid oxidation occurs by two major mechanisms; 1) free radical autooxidation of unsaturated lipids with triplet oxygen, and 2) the oxidation of milk lipids with singlet oxygen. The autoxidation reaction proceeds by a three stage radical process: initiation, propagation and termination (**Figure 1**). During the initiation phase, a free radical ($R^{\bullet}$) and hydrogen radical ($H^{\bullet}$) are formed by the dissociation of the hydrogen molecule (H) usually from the methylene group adjacent to the double bond of the fatty acid moiety (R) (O'Connor and O'Brian, 1994; deMan, 1999; Wong and Kitts, 2001). This reaction is most often initiated by light, oxygen, transition metals, radiation, heat and other free radicals (deMan, 1999; Wong and Kitts, 2001). The next phase is propagation when the free radical combines with oxygen to form peroxy radicals ($ROO^{\bullet}$) which in turn abstracts hydrogen from the RH group of other molecules to yield hydroperoxides (ROOH and $R^{\bullet}$) (**Figure 2**) (deMan, 1999). The $R^{\bullet}$ reacts with oxygen and the cycle is repeated until termination occurs because the reaction of the free radicals with other free radicals yields non-active products (deMan, 1999 and Fennema, 1996).

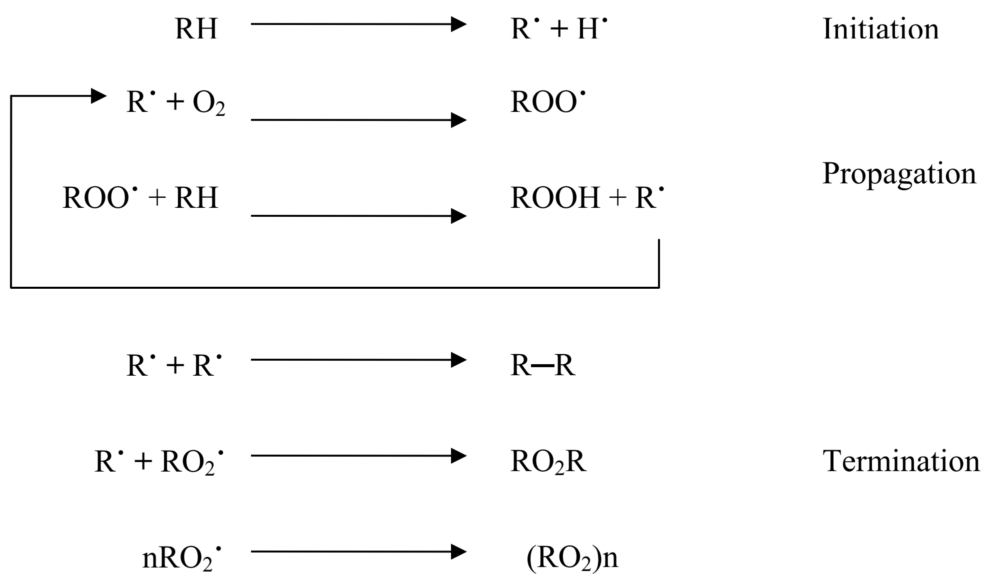

Figure 1. Autoxidation pathway of lipid oxidation.

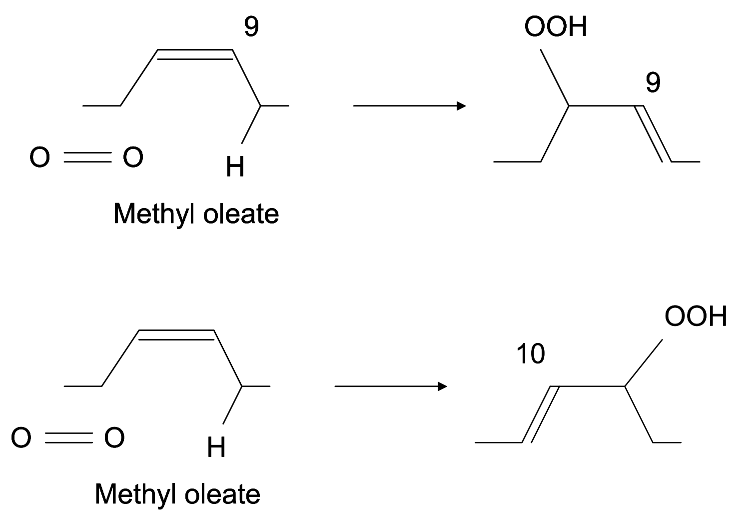

Figure 2. Hydrogen abstraction and hydroperoxide formation at the allylic carbons of methyl oleate in free radical oxidation (Hamilton, 1994)

2.3.4.2 Photooxidation

Degradation of foods due to light exposure occurs in the UV and visible light region of the spectrum, between 280nm and 780nm, but the blue to green band of the visible region (430-460nm) is considered the main band responsible for light-induced oxidation of milk and dairy products (Borle *et al*., 2001). Photochemically-induced off-flavors and odors are directly related to the source of light, the amount of exposure, and the wavelength; other constituents responsible for changes are proteins, amino acids such as methionine, riboflavin, and vitamins A and C (Bekbölet, 1990). Riboflavin is considered to play a significant role as a sensitizer in the photooxidation process of dairy products. Lee (2002) found that the concentration of riboflavin in the milk was directly related to the extent of off-flavors when milk was exposed to fluorescent light.

Light-induced oxidation occurs when a photosensitizer like riboflavin in the presence of light transfers the energy to triplet oxygen ($^3O_2$) exciting it to the highly reactive singlet oxygen ($^1O_2$) state (Borle *et al*., 2001). Hydroperoxides are formed from the singlet oxygen by way of the "ene" reaction involving the formation of a six-member ring transition state (Fennema, 1996; Hamilton, 1994; Frankel, 1980). Oxygen is then inserted at either end of the double bond of the unsaturated fatty acid to yield an allylic hydroperoxide (Hamilton, 1994; Frankel, 1980). Lipid hydroperoxides are very unstable and are broken down further into aldehydes and ketones that are responsible for the off flavors indicative of lipid oxidation by way of the autoxidation process described in Section 2.3.5.1.

2.3.4.3 Protein Oxidation

The burnt (or sunlight) flavor associated with oxidized milk is typically blamed on lipid oxidation, but protein oxidation has been shown to contribute significantly to the deterioration of milk quality and the production of similar off flavors (Hui, 1993). Jung *et al*. (1998) postulated that methionine reacts with singlet oxygen produced by riboflavin under lighted conditions to yield dimethyl disulfide, methyl sulfide and other sulfur-containing

compounds. By-products of this reaction are ammonia and carbon dioxide that give milk a similar odor to that of lipid oxidized milk (Hui, 1993). Østdal *et al*. (2000) investigated the reaction between lactoperoxidase (LPO) and H_2O_2 in the presence of bovine serum albumin (BSA), β-lactoglobulin, and casein for the formation of protein radicals. It was determined that LPO activity may lead to oxidative changes in the protein fraction of milk and since this reaction can trigger lipid oxidation, it may also play a role in the spontaneous oxidation of milk.

2.3.4.4 Hydrolytic Rancidity

Hydrolytic rancidity refers to hydrolytic changes in milk lipids that are catalyzed by lipases resulting in the production of free fatty acids (FFAs) that are likely to cause off-flavors in milk (**Figure 3**) (Hamilton, 1994; Deeth and Fitz-Gerald, 1995). Lipase enzymes may be inherent in the milk or from bacterial sources. Inherent goat's milk lipases are distributed equally between the cream and serum phases and only a minute amount is found in the casein; total activity is lower then in cow's milk (Deeth and Fitz-Gerald, 1995). Two types of lipolysis are recognized, "induced" and "spontaneous". Agitation and foaming, homogenization, temperature activation and freezing may initiate induced lipolysis (Deeth and Fitz-Gerald, 1994). Spontaneous lipolysis is affected by lactation stage, feed and nutrition, seasonal variation, and individual milk production (Deeth and Fitz-Gerald, 1994).

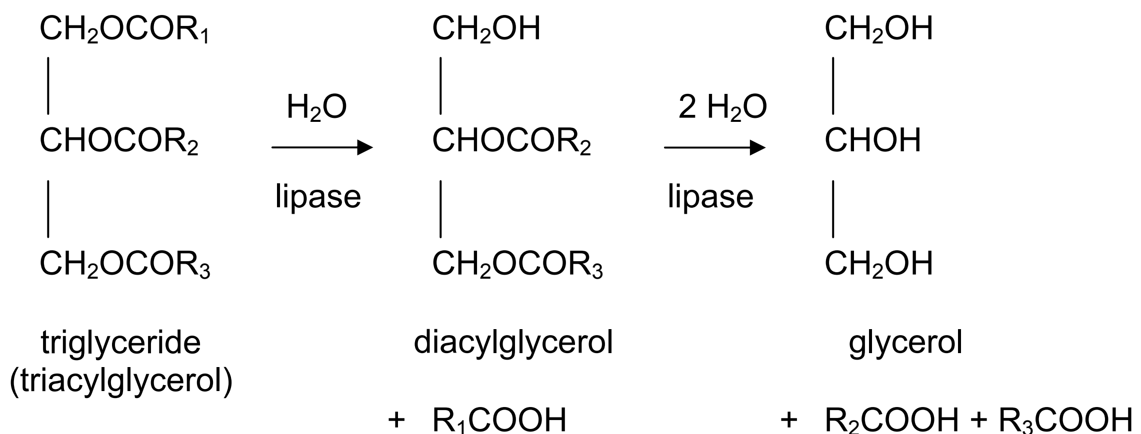

Figure 3. Hydrolysis of triglycerides and production of free fatty acids by the enzymatic action of lipase (Allen, 1994).

2.3.6 Assessment of Off-flavors from Oxidation

The flavor of fresh, quality milk has a light, almost bland taste, but with a characteristic sweet and salty flavor from lactose and milk salts (Walstra and Jenness, 1984). Many other compounds each present in undetectable amounts in combination contribute to the odor and flavor of milk. Since the flavor of milk is subtle, even a minor variation in the flavor or odor could render it unacceptable by consumers. Some common off flavors are acidic, astringent, unclean, cowy, bitter, cooked, feedy, weedy, fruity, foreign, lacking freshness, malty, rancid, salty and oxidized (Ogden, 1993). Off-flavors may be influenced by ingested feed, rancidity, denatured proteins and chemical reactions resulting from thermal processing, packaging, microbial activity, exposure to light, etc. The following table (**Table 1**) includes volatile compounds that are commonly associated with off-flavors of milk.

Table 1. Some Volatile Compounds Reported in Abused Milk with Off-Flavors (Marsili, 1999b)

Compound	Possible Abused Agent	Reference
3-methylbutanal	microbial contamination, Cu oxidation	Azzara and Campbell, 1992. Marsili and Miller, 1998
pentanal	light abuse, Cu oxidation	Cadwallader and Howard, 1998; Marsili and Miller, 1998
hexanal	light abuse, Cu oxidation	Cadwallader and Howard, 1998; Marsili and Miller, 1998
heptanal	light abuse, Cu oxidation	Forss et al., 1955a, 1955b; Marsili and Miller, 1998
octanal	Cu oxidation	Forss et al., 1955a, 1955b; Marsili and Miller, 1998
nonanal	Cu oxidation	Forss et al., 1955a, 1955b; Marsili and Miller, 1998
2-octenal	Cu oxidation	Forss et al., 1955a, 1955b; Marsili and Miller, 1998
2-nonenal	Cu oxidation	Forss et al., 1955a, 1955b; Marsili and Miller, 1998
2-pentanone	high heat, microbial contamination	Hawke, 1966
2-heptanone	high heat, microbial contamination	Hawke, 1966
2-nonanone	high heat, microbial contamination	Hawke, 1966
2-undecanone	high heat, microbial contamination	Hawke, 1966

1-octen-3-one	Cu oxidation, light abuse	Cadwallader and Howard, 1998; Marsili and Miller, 1998
acetic acid	microbial contamination	Azzara and Campbell, 1992.
butanoic acid	microbial contamination; lipases	Azzara and Campbell, 1992.
hexanoic acid	microbial contamination; lipases	Azzara and Campbell, 1992.
octanoic acid	microbial contamination; lipases	Azzara and Campbell, 1992.
3-methylbutanol	microbial contamination	Hawke, 1966
2-pentanol	microbial contamination	Hawke, 1966
dimethyl disulfide	microbial contamination, light abuse	Jung et al., 1998

Kim *et al*. (2003) conducted an experiment that would enable them to study light-induced volatile compounds in goat's cheese using solid phase microextraction (SPME)-gas chromatography (GC)-mass spectrometry (MS), headspace oxygen depletion, and sensory evaluation. Four Sylvania fluorescent lamps (General Electric, Cleveland, OH) with a total power output between 350-750 nm were used as a light source for exposure. The results of the study showed that 90% more volatile compounds were formed in samples exposed to the light at 30°C for 2 days then samples stored in the dark at 30°C (Kim *et al*., 2003). Volatile compounds such as 1-heptanol, heptanal, nonanal, and 2-decenal were formed in the cheese exposed to light and sensory evaluation by a trained panel scored samples with these compounds as having off-flavors. The researchers concluded that these light-induced volatile compounds were due to the presence of riboflavin that has been shown to be a photosensitizer that accelerated singlet oxygen oxidation of unsaturated fatty acids (Kim *et al*., 2003).

Cadwallader and Howard (1998) identified key aroma compounds that contributed to the oxidized flavor of milk. Two milk sample groups were tested: no fluorescent light exposure and light exposure for 18 h. The authors highlighted key volatiles that contribute to the light activated flavor of milk. These odor-active compounds were detected by dynamic headspace sampling, GC-MS. There was a significant increase in the concentration of acetaldehyde, pentanal, 1-hexen-3-one, hexanal and 1-octen-3-one in light activated samples and only a slight increase in 2-methylpropanal and 3-methylbutanal in light activated samples. There

was a decrease in dimethyl sulfide in light activated samples (Cadwallader and Howard, 1998).

With SPME-GC, Lee (2002) found the formation of pentanal, hexanal, dimethyl disulfide, and heptanal occurred only when milk was stored under lighted conditions. These compounds were not present in the samples that received zero light exposure. Dimethyl disulfide is formed when a sulfur containing amino acid like methionine is oxidized whereas pentanal, heptanal and hexanal are products of lipid oxidation. Marsili (1999a) was able to detect parts per billion levels of dimethyl disulfide, pentanal, hexanal, and heptanal using SPME-GC analysis. They concluded that this methodology was as sensitive as other methods, but the results were more precise and easier to interpret.

Acid degree values (ADVs) are used as a measurement of free fatty acids in milk fat recovered from an extraction method and titration. These values, coupled with sensory evaluation, can be used as an indicator of rancid off flavors in milk. Historically, an ADV of >1.0 meq/100g in milk is indicative of rancid off-flavors. Duncan and Christen (1991) conducted a study that evaluated the relationship between ADVs and rancid off-flavors. It was determined that short-chained fatty acids (C4 to C8) did not enter the fat phase recovered by the ADV procedure in quantities comparable with medium- (C10 to C16) or long-chain (C18:0 to C18:1) fatty acids. The results implied that the ADV procedure does not measure the fatty acids responsible for rancid flavor (C4 to C12) at the same rate as the longer chained fatty acids (Duncan and Christen, 1991). The authors recommend that milk not be considered rancid based on ADVs alone.

The thiobarbituric acid reactive substances (TBARS) test is used to measure carbonyl oxidation products as a result of lipid oxidation. The test measures the production of malondialdehyde which is a secondary product of oxidation (**Figure 4**). Results of this test are based on the reaction of lipid peroxidation products and thiobarbituric acid color absorbance and read spectrophotometrically at 532 nm. This test is non-specific because color reactions by other secondary oxidation products may occur (Frankel, 1998). Therefore, the TBARS test can only give a general measurement of lipid peroxidation.

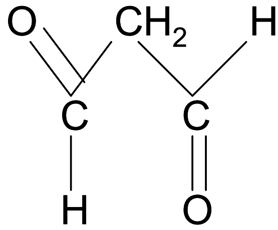

Figure 4. Chemical structure of malondialdehyde.

2.4 Current Studies using UV Irradiation and Disinfection

New regulations imposed by FDA call for the implementation of "Hazard Analysis and Critical Control Point" (HACCP) strategies in fruit and vegetable juice processing operations (66 FR 6137). This has sparked the need for the development of affordable technology that would achieve the 5-log reduction of pathogens required by the new policy. Recent studies have concentrated on the efficacy of UV irradiation to control pathogens in apple cider (Hanes *et al.,* 2002; Wright *et al.,* 2000; Basaran, 2004).

Wright and colleagues (2000) inoculated apple cider with a cocktail of five *E. coli* O157:H7 strains to an approximate level of 10^6 CFU/ml and pumped it in thin films through the Cider-10uv model (Ideal Horizons, Poultney, VT) UV disinfection unit at 254 nm (Wright *et al*., 2000). This unit consisted of a quartz sleeve that allowed the apple cider to pass through a series of 10 individual UV chambers. The flow rates ranged from 60 to 90 L/hour to create UV doses between 9.4 and 61 mJ/cm^2. The mean log reduction, i.e., the difference in microbial counts between the untreated and treated cider, was 3.8 log cfu/ml (Wright *et al*., 2000). UV irradiation significantly reduced *E. coli* numbers in the apple cider ($P<0.0001$) although the differences in UV-dosage did not result in statistically significant differences in *E. coli* survival (Wright *et al*., 2000). The bactericidal benefit of UV irradiation occurs only in the direct beam of the light, therefore, the lack of a dose-response relationship between *E. coli* survival and UV dose may have been due to limitations of the UV apparatus (Bachmann, 1975). Wright and colleagues (2000) suggested that if the machine were modified to increase the intensity of UV irradiation as well as the maximal flow rate, greater reduction would be achieved at faster rates (Wright *et al*., 2000).

Wright and colleagues (2000) discovered that the levels of yeasts and molds in the cider affected the reduction of microbial counts in the treated ciders. When the researchers pretreated the cider to minimize yeast and mold concentrations (less then 3 log cfu/ml), a microbial reduction of 5 log cfu/ml or greater was obtained regardless of UV dosage (Wright *et al*., 2000).

The CiderSure 3500 UV apparatus (FPE, Inc., Rochester, NY) was developed and studies using apple cider confirmed its ability to achieve a 5-log reduction of *Cryptosporidium parvuum* and *E. coli* (Hanes *et al*., 2002; Basaran, 2004). The apparatus is designed to allow the apple cider to pass by a series of eight germicidal UV lamps in thin films (**Figure 5**). The wavelength and intensity of the lamps inside the CiderSure apparatus is 254 nm and 14.3 mJ/cm^2 of UV irradiation respectively, with exposure times between 1.2 and 1.9 sec (Hanes *et al*., 2002). A computer monitored UV sensor placed within the apparatus to adjust the flow rate according to sensor readings ensured that all of the cider received the correct amount of UV light.

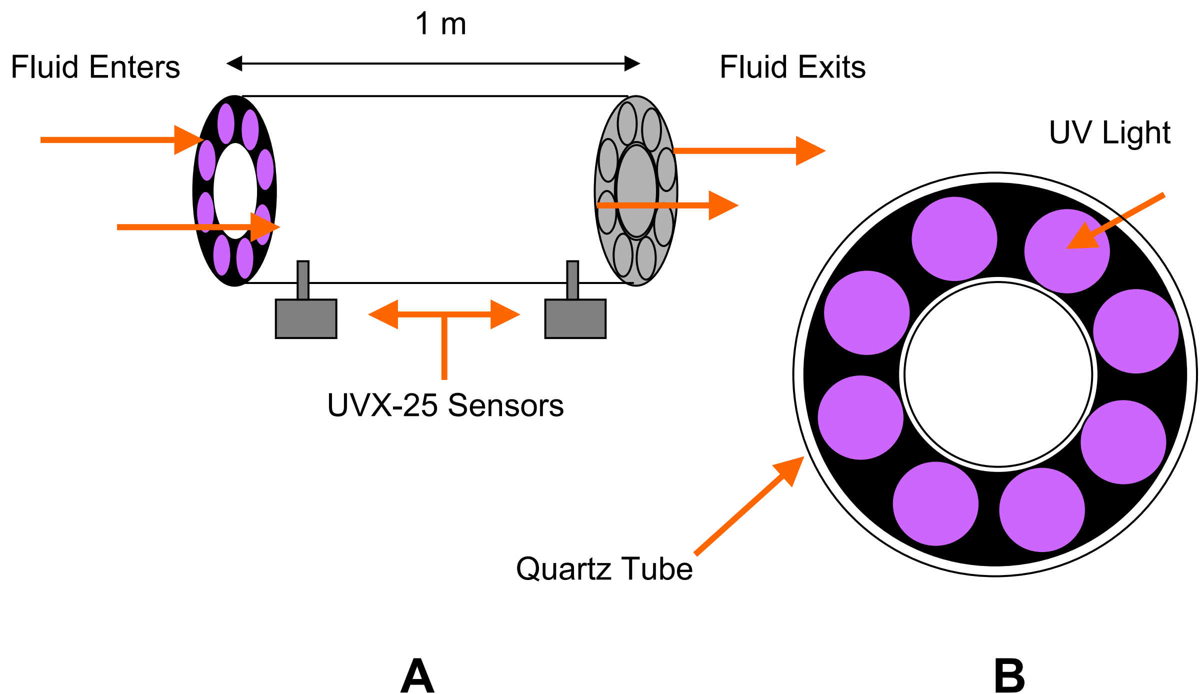

Figure 5. Diagram of UV chamber (A) and layout of UV lights (B) within the Cidersure 3500A UV Apparatus (FPE Inc., Rochester, NY).

In a study conducted by Basaran (2004), ciders with different solids compositions and concentration had been subjected to different filtration treatments resulting in lighter and darker ciders. Inclusion of these variables was deliberate to test the UV apparatus for its ability to overcome these differences and still achieve a 5-log reduction in *E. coli* O157:H7. Three strains of *E. coli* O157:H7 were utilized in the study (ATCC 43889, 43895, and 933). The apple cider samples were inoculated with the each of the strains and then passed through the CiderSure UV-irradiation unit at $4^{o}C$. Sampling of the cider for microbial analysis occurred immediately before and immediately after UV treatment. The results of the study showed a 6.12 +/- 0.36, 5.83 +/- 0.11, and 5.87 +/- 0.11 log reduction for the *E. coli* O157:H7 strains ATCC numbers 43889, 43895, and 933, respectively (Basaran, 2004).

The laboratory results by Basaran (2004) encouraged testing for the usefulness of the CiderSure 3500 UV apparatus in a large-scale commercial cider mill. The identification of a non-pathogenic strain to be used as a surrogate to the pathogenic strains of *E. coli* O157:H7 was necessary because the test cider mill would revert to commercial usage at the cessation of the study. Investigations conducted in the laboratory determined that the nonpathogenic *E. coli* strain ATCC 25922 had similar UV resistance and sensitivity as the pathogenic strains (Basaran, 2004), and accordingly, this strain was used in the large-scale study. The results of the study confirmed that the CiderSure 3500 was effective at achieving a reduction in *E. coli* ATCC 25922 of greater than 5 logs in apple cider at a typical cider mill and production setting (Basaran, 2004).

Quintero-Ramos *et al.* (2004) conducted experiments using the CiderSure 3500 to study the effects of UV light dose and pH on the inactivation of *E. coli* 25922 in apple cider. The results showed that pH of apple cider did not have a significant effect on microbial reduction. The results did show that a nonlinear relationship existed between survivorship and UV dose. These results are consistent with the sigmoidal-shaped curves from experiments done by 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 (Quintero-Ramos *et al*., 2004).

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