The Pennsylvania State University

The Graduate School

Department of Agricultural and Biological Engineering

DECONTAMINATION OF HARD CHEESES BY PULSED UV LIGHT

A Thesis in

Agricultural and Biological Engineering

by

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Submitted in Partial Fulfillment
of the Requirements
for the Degree of

Master of Science

December 2013

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ABSTRACT

Effectiveness of pulsed UV- light to inactivate *Penicillium roqueforti* and *Listeria monocytogenes* on packaged and unpackaged cheeses was investigated in this study. Treatment times and the distance from the UV strobe were evaluated to determine optimum treatment conditions. Packaged and unpackaged cheeses were treated from 5, 8, or 13-cm distances. The treatment was performed up to 40, 50 and 60-s from 5, 8 and 13-cm distances, respectively. For *P. roqueforti,* maximum log reduction was 1.32 $\log_{10}$ CFU/cm^2 after 40-s treatment at 5-cm distance on unpackaged cheese and 1.24 $\log_{10}$ CFU/cm^2 on packaged cheese at same treatment conditions. The optimum UV-light treatment condition for *P. roqueforti* inactivation for both packaged and unpacked cheeses were 8-cm distance for 30-s, which yielded ~1.1 $\log_{10}$ reduction. Log reductions of *L. monocytogenes* at the same treatment conditions were about 2.9 and 2.8 CFU/cm^2 packaged and unpackaged cheeses, respectively. The temperature changes and total energy increased directly proportional with treatment time and inversely with distance between UV lamp and samples. The changes in color and lipid oxidation extent were determined at mild (5-s at 13-cm), moderate (30-s at 8-cm) and extreme (40-s at 5-cm) treatments. The color and chemical quality of cheeses did not show significant difference after mild treatments ($p>0.05$). The plastic material (polypropylene) was evaluated in terms of mechanical properties after mild, moderate and extreme treatments, as well. There was a decreasing trend between elastic modulus data, however there was no significant difference between untreated, mild and moderate treatments ($p>0.05$). Overall, these results demonstrated that pulsed UV- light has potential to inactivate *P. roqueforti* and *L. monocytogenes* on the surface of hard cheeses.

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ACKNOWLEDGEMENTS

I would like to thank my family, parents and friends for their endless support and encouragement throughout my M.S. program.

I would like to thank my thesis committee, Dr. Ali Demirci, Dr. Virendra M. Puri and Dr. Hassan Gourama for their help and guidance. I would also thank and express my appreciation to Dr. Hojae Yi for his help and time on plastic analysis.

This work was supported in part by Turkish Ministry of Education by providing scholarship to Fidan Ozge Can and the Pennsylvania Agricultural Experiment Station. Also thank to the Xenon Corporation (Wilmington, MA) for their technical assistance for the pulsed UV system and plastic material analysis.

I would also thank everybody in Department of Agricultural and Biological Engineering for being kind and helpful.

CHAPTER 1

INTRODUCTION

Food borne outbreaks are still major concern for millions of people in the United States and around the world. The Centers for Disease Control and Prevention (CDC) estimates that each year in the United States, 48 million people gets sick, 128,000 are hospitalized, and 3,000 die of food borne diseases (NIAID, 2012). Food-borne outbreaks usually arises from improper production, handling, preparation, processing, or food storage. It is important to have good hygiene practices before, during and after food preparation in order to reduce the chances of food borne diseases.

Among the known foodborne pathogens, nontyphoidal *Salmonella*, *Campylobacter,* and *Toxoplasma* caused the most hospitalizations (CDC, 2011). The foods most associated with foodborne illness are usually raw meat and poultry, raw eggs, unpasteurized milk, and raw shellfish. Fruits and vegetables can also be concern for food safety, since contamination can occur with animal droppings on the farm or when manure is used to fertilize produce in the field, or unclean water can be used for irrigation or washing the produce. Raw sprouts are particularly concerning because the conditions under which they are sprouted are ideal for growing microbes (MDH, 2013).

Due to the increase in outbreaks of food borne illnesses associated with dairy products, decontamination of dairy products is getting more attention. According to USDA cheese is the most consumed dairy product. Fluid milk follows cheese consumption with 29.1% (skim solid base) (USDA, 2013). In spite of the fact that cheese was made from adequately pasteurized milk, it is often subsequently contaminated with pathogenic and spoilage microorganisms during post-processing and handling. Therefore,

there is an urgent need to decontaminate cheese at the post-packaging stage in order to avoid illnesses and extend shelf life of cheese products.

As many as 8.8 billion pounds of cheese were consumed in America in 2003 valued at $39.9 billion. Of these figures, commodity cheese made up about eight billion pounds valued at $33.5 billion and specialty cheese made up around 815 million pounds worth close to $6.4 billion (Business Journal, 2004). Per capita consumption of cheese in the US rose to new highs in 2010 to 33.29 pounds, surpassing the previous record set in 2007 of 33.16 (IDFA, 2012). Similarly, the data for American-type cheese consumption also registered an increase by 0.13 pounds to 13.83 pounds per person, setting a new record for that category of cheese.

Cheese is a ready-to-eat food easily contaminated on the surface by undesirable microorganisms at the production, packaging, and post-packaging processes. Some are spoilage microorganisms such as *Yarrowia lipolytica, Pseudomonas aeruginosa* and *Penicillium* spp., which may produce uncharacteristic visual appearance and diminish the commercial value of the cheeses.

Penicillium roqueforti is a common saprotrophic fungus that can be isolated from soil, decaying organic matter, and plants (EPA, 1997). *P. roqueforti* is also commonly found on cheese surfaces at refrigerator temperatures as the most common spoilage microorganism. *Listeria monocytogenes* has been associated with food-borne listeriosis by consumption of cheese (McLauchlin et al., 2004). *Listeria monocytogenes*, a facultative, anaerobe, is the causative agent of listeriosis. It is one of the most virulent food borne pathogens, with 20 to 30 percent of clinical infections resulting in death (Ramaswamy et al., 2007). Infection with *L. monocytogenes* was the fourth most

common cause of death due to bacterial indigenous food borne disease in England and Wales during the period 1992–2000 despite a very low incidence (0.003 cases/1000 people annually) (Adak et al., 2002). Listeriosis is responsible for approximately 2,500 illnesses and 500 deaths in the United States (U.S.) annually (Dharmarha and Smith, 2008). Listeriosis is the leading cause of death among foodborne bacterial pathogens, with fatality rates exceeding even *Salmonella* and *Clostridium botulinum* (Dharmarha and Smith, 2008).

Current decontamination methods for cheese are more focused on initial stages of production like heat treatments and addition of chemicals. However, there is a need for treatment at the post-packaging stage to avoid spoilage and pathogen microorganisms on the surface of products. Methods for enhancing shelf-life of various cultured milk products could be applied with the adoption of a single or a combination of the following techniques: Bacteriocins, nisin, microgard, natamycin, lactoperoxidase, microwave, and UV-light (Sarkar, 2006).

Out of these methods, ultraviolet (UV) light treatment can be accomplished in two modes; continuous or pulsed UV (Krishnamurthy et al., 2007). Continuous mode is conventional one, which is commonly used but has some disadvantages, such as poor penetration and low dissipation power. On the other hand, pulsed UV-light has been reported to be more effective than conventional UV-light due to greater instantaneous energy (Bialka et al., 2008). Pulsed UV-light is a non-thermal method for food preservation that involves the use of intense, short duration pulses to ensure microbial decontamination on the surface of either foods or packaging materials (Elmnasser et al., 2007). UV treatment offers inactivation of microorganisms on food surfaces in short

times. Pulsed UV-light involves a broad-spectrum between 100 and 1100 nm. During pulsed UV-light processing, the energy is stored in a high power capacitor and released constantly and in this way it supplies much higher instantaneous energy. At the post-packaging stage, pulsed UV treatment can enhance safety and further extend shelf life of foods at refrigerator temperatures.

Therefore, this study is undertaken to evaluate efficacy of pulsed UV-light on decontamination of cheese for the purpose of extending shelf life, enhancing quality, and reducing cheese-associated foodborne diseases. Pulsed light was applied to packaged and unpackaged artificially contaminated cheeses with *P. roqueforti* and *L. monocytogenes*. The pulsed UV-light treatment was optimized by the number of pulses and distance between the food product and the UV lamp and quality analysis of treated cheeses and plastics were performed.

CHAPTER 2

LITERATURE REVIEW

2.1. Food Safety

As world population increases, supplying fresh, safe and nutritious food becomes more of a challenge for food producers (Keklik, 2009). Failing in food safety not only causes money and prestigious loss for the food industry, but also could risk the public health due to food borne illnesses (sometimes called “food-borne disease”, “food-borne infection”, or “food poisoning”) is a common and costly public health problem. Each year, 1 in 6 Americans gets sick by consuming contaminated foods or beverages. There are many different food borne infections since many different type pathogens can contaminate foods. In addition, poisonous chemicals, or other harmful substances can cause food borne diseases if they are present in food (CDC, 2012a).

Proper food preparation can be vital since it prevents many foodborne diseases. World Health Organization (WHO) identified the need to communicate a simple global health message, based on scientific evidence, to educate all types of food manufacturers, and food handlers as well as consumers (WHO, 2012). The Five Keys to Safer Food message is a global health message, which explains the fundamental principles to ensure safe food handling practices and prevent foodborne diseases. The Five Keys of Safer Food are: Keep clean, separate raw and cooked, cook thoroughly, keep food at safe temperatures, and use safe water and raw materials (WHO, 2012). FDA defines “hazardous foods” as natural or synthetic food and that require temperature control, because it is in a form capable of supporting the rapid and progressive growth of infectious or toxigenic microorganisms, the growth and toxin production of *Clostridium*

botulinum or the growth of *Salmonella* Enteritidis. "Potentially hazardous food" is the one with the water activity (a_w) value of 0.85 or more and a food with a pH level of 4.6 or below when measured at 24°C (75°F) (FDA, 1999). Some of the food groups that meet the definition of hazardous foods include poultry (chicken, turkey, duck), fish, shellfish, and crustaceans, eggs (except those treated to eliminate *Salmonella*), milk and dairy products, mushrooms, cut tomatoes (when pH is 4.6 or above), cut leafy greens, raw sprouts, tofu, and other soy-protein foods, etc. (FDA, 1999).

Since 1973, CDC has maintained data on the occurrences and causes of foodborne diseases outbreaks in the United States (Keklik, 2009). One of the major outbreaks that occurred in the United States was in 2009. The food borne illness outbreak was associated with *Salmonella* in peanut products. That outbreak resulted in one of the largest food safety recalls ever in the United States. The scope of the recalls was enormous because the peanut products were used as ingredients in more than 3,900 products. Consumer purchases of peanut containing products initially slowed during the recalls but the recalls do not appear to have had a lasting impact on peanut demand and production (USDA, 2012). One of the recent outbreaks was reported by CDC in May 30, 2013. CDC reported that a total of 35 people infected with the strain of Shiga toxin-producing *Escherichia coli* O121 (STEC O121) in 19 states (Alabama, Arkansas, California, Colorado, Florida, Illinois, Indiana, Michigan, Mississippi, Missouri, New York, Ohio, Pennsylvania, South Dakota, Texas, Utah, Virginia, Washington, and Wisconsin) associated with Farm Rich chicken quesadilla product (CDC, 2013a). In order to prevent these kinds of outbreaks that could cost human life, required attention should be given to food production, packaging, and storage to supply safe food.

2.2. Cheese and Significance of Cheese Industry in the United States

Cheese is the generic name for a group of fermented milk-based food products, in a wide range of flavors and forms throughout the world (Fox and McSweeny, 2004). It is produced by coagulation of the milk protein casein by decreasing the pH to the isoelectric point. The milk is acidified and addition of the enzyme rennet causes coagulation. The solids are separated and pressed into final form. Processed cheeses are the most common commercial cheese types in the market. They are being sold as individually wrapped cheese slices or loaves and called as “processed cheese” (Linton and Harper, 2008).

Cheese represents the most varied subcategory of dairy products. As such, it presents many avenues of interest for academic research. In contrast to other dairy products, cheese products are biologically and biochemically dynamic. This inherent instability presents a significant challenge, but also great potential and interest for research (Fox et al., 2004).

Market research underscores cheese's ongoing status as a beloved foodstuff. According to the U.S. Department of Agriculture (USDA), per capita cheese consumption is estimated at 15.25 kg, reflecting a slight yet continued increase (USDA/ERS, 2013) (Fig 2.1). The International Dairy Food Association (IDFA) indicates that U.S. Food and Drug Store sales of processed cheese products reached 760 million pounds in 2011, with a value of $2.6 billion dollars (IDFA, 2012). Volume sales of natural cheese in U.S. Food and Drug Stores reached approximately 2.3 billion pounds in 2011, with a value of over $11 billion dollars (IDFA, 2012).

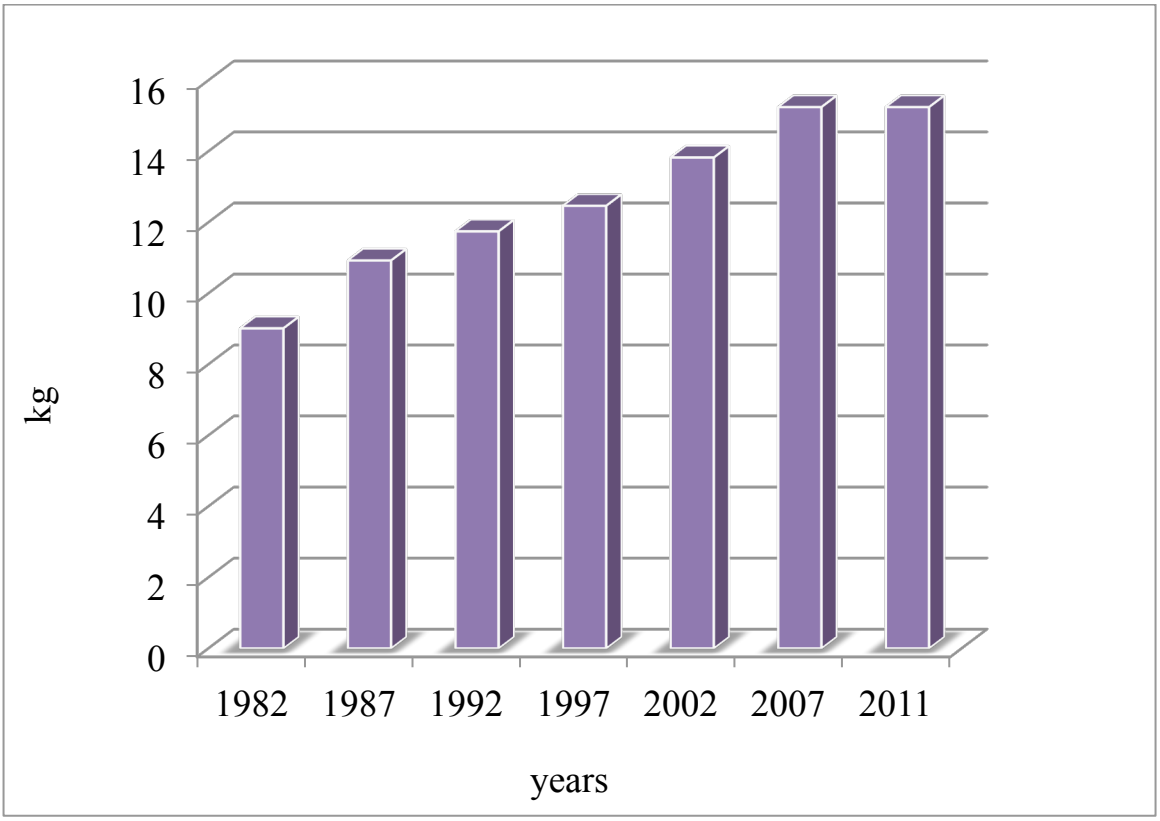

Figure 2.1. Per capita cheese consumption in the U.S., 1982-2007 (USDA/ERS, 2013).

* Year 2011 is an estimated value

2.3. Microbiological Significance of Cheeses

Color, taste, and texture are among the main quality determinants for cheese products. Color is affected by the temperature during processing and the fat content of the milk. Lactic acid produced during the fermentation stage augments the taste and texture of the resulting cheese. However, this can be altered through changes in the amounts of inoculum mixed in the milk and the heat treatment during its incubation period (Anonymous, 2008). Major sources of cheese spoilage have been traced to soiled equipment, contaminated milk, poor employee hygiene, and improper processing and storage conditions. As a result, it is highly recommended that all equipment be

thoroughly cleaned and sanitized after exploitation and examined before production recommences. Temperatures and duration of milk heating should be carefully monitored and adjusted accordingly to ensure proper heating. Incubation times and temperatures should be similarly controlled to ensure correct fermentation processing, where appropriate (Anonymous, 2008). One way to reduce food poisoning through cheese is through pasteurization of cheese milk. This means that raw milk, even has good quality, may be inappropriate for cheese manufacture. Thus, pasteurization has many beneficial effects on public health, but also has the benefit of improving the consistency of cheese (Fox et al., 2004).

Food spoilage microorganisms are those which upon growth in a food, produce undesirable flavor, odor, texture and appearance, and make food unsuitable for human consumption (Bhuina, 2010). Cheese is very susceptible to mold growth and is normally kept under refrigeration conditions; many retail packs are either vacuum-packed or flushed with an inert gas (Fox et al., 2004). Therefore, spoilage generally results from psychrotolerant molds that can grow at low oxygen tensions. Mold growth during ripening and storage often necessitates trimming. All molds causing cheese spoilage grow very well at refrigeration temperatures, and many can grow near freezing or slightly below (Fox et al., 2004). *Cladosporium cladosporioides* has been reported to grow at -10°C, but -2 to -5°C is probably a more realistic minimum. All of *Penicillium* species found in cheese grow well at 1-5°C, with maximum approximately 30°C (Pitt and Hocking, 1997).

One of the earliest microbial studies on pasteurized cheese spreads was performed in order to determine *Clostridium botulinum* growth and ability of toxin production

(Linton and Harper, 2008). The model created by Tanaka, the "Tanaka model", is still used by the industry when formulating cheese spreads (Linton and Harper, 2008). Gould et al. (2011) reviewed and analyzed the data obtained from CDC database on outbreaks associated with cheese consumption between the years 1998-2008. They reported 68 outbreaks, with the result of 1519 illnesses, 178 hospitalizations, and 3 deaths. The improper pasteurization of the milk used to make the cheese was known for 64 outbreaks, out of which 30 outbreaks (47%) involved cheese made from unpasteurized milk. The microorganisms caused the illness in unpasteurized cheeses were *Salmonella* (40%), *Campylobacter* (23%), *Brucella* (13%), *Listeria* (10%), *Shigella* (7%), and *E. coli* STEC (7%) while norovirus (52%), *Salmonella* (17%), *Listeria* (2.9%), and *Bacillus cereus* (4%), *Shigella* (4%), *Staphylococcus aureus* enterotoxin (4%), and another virus (4%) among the 23 pasteurized cheese outbreaks (Gould et al., 2011).

There has been recent outbreak reported by CDC is caused by *Listeria monocytogenes* in the result of consumption cheese product. Five people whose ages ranged between 31 to 67 years have been reported to have Listeriosis from the states of Illinois (1), Indiana (1), Minnesota (2), and Ohio (1). In addition to five hospitalizations, one death and one miscarriage have been reported (FDA, 2013).

FDA regulates legal standards in milk and milk products. Legal microbiologic standards for milk products without fruit, nuts, flavoring agents added after pasteurization are maximum 50,000 Standard Plate Count (SPC) (CFU/mL) and 10 coliform/mL (FDA, 1998). Systematic preventive approaches such as Hazard Analysis at Critical Control Points (HACCP), and Good Manufacturing Principles (GMP) should be implemented to meet these requirements and produce safe food.

2.3.1. Major Pathogenic Microorganisms Concerned for Cheeses

Contaminated cheese has been responsible for outbreaks of food poisoning. Several types of pathogen microorganisms have been reported to contaminate cheeses. Some of these pathogens can cause severe illness with long-term consequences and death while some of them cause mild illness. The major pathogens have been found in cheese are *Salmonella* spp., *Listeria monocytogenes, Campylobacter* spp., *Brucella* spp., *Bacillus cereus, Shigella* spp., Shiga Toxin-producing *Escherichia coli* (Gould et al., 2011).

2.3.1.1. *Salmonella* spp.

Salmonella is a motile, non-sporeforming, Gram- negative, rod-shaped bacterium in the family *Enterobacteriaceae* and the tribe *Salmonellae* (Hammack, 2012). It is widely dispersed in the nature. There are two species of *Salmonella* that can cause Salmonellosis infection in humans: *S. enterica* and *S. bongori* (Hammack, 2012).

Salmonella food poisoning results from the ingestion of foods containing appropriate strains of this genus in significant numbers (Jay, 2000). Every year approximately 42,000 cases of salmonellosis are reported in the United States and it is known that many milder cases are not diagnosed or reported, the actual number of infections may be twenty-nine or more times greater (CDC, 2012b). Eggs, poultry, meat, and meat products are the most common food vehicles of salmonellosis to humans (Jay, 2000). The mortality of Salmonellosis is generally less than 1%; however, *S. Enteritidis* has a 3.6% mortality rate in outbreaks (Hammock, 2012). The illness shows its effect in 6

to 72 hours after exposure and symptoms are usually nausea, vomiting, abdominal cramps, diarrhea, fever, headache (Hammock, 2012).

Prevention of Salmonellosis can be achieved by cooking thoroughly and not be eating or drinking foods containing raw eggs, or raw (unpasteurized) milk (CDC, 2013b). Cross contamination of foods should be avoided. Raw meats should be kept separate from produce, cooked foods, and ready-to-eat foods and also utensils are used should be washed thoroughly after touching (CDC, 2013b).

2.3.1.2. *Listeria monocytogenes*

Listeria monocytogenes is a Gram-positive, rod-shaped bacterium (Fig. 2.2). It is also a continuous concern for food producers. As a well-known psychrotroph, it is able to grow in refrigerated food products such as fermented raw and cooked poultry, raw meats and fish, dairy products, and raw vegetables (FDA/CFSAN, 2007).

The genus *Listeria* has six species: *L. monocytogenes, L. innocua, L. ivanovii, L. welshimeri, L. seeligeri,* and *L. grayi. L. ivanovii* has two subspecies: *L. ivanovii subsp. ivanovii* and *L. ivanovii subsp. Londoniensis* (Bhunia, 2008). *L. monocytogenes* is pathogenic to humans and animals, while *L. ivanovii* is pathogenic to animals and others are considered non-pathogenic (Bhunia, 2008).

L. monocytogenes is widely spread in nature and recognized as a cause of human disease since 1929 (Mazor et al., 1992). *L. monocytogenes* causes the disease listeriosis, a rare, but fatal disease primarily affecting immunocompromised individuals such as young, old, pregnant, and immunologically challenged people. About 2,500 people in the US contract invasive listeriosis each year (Bhunia, 2008). Mortality rates for this deadly

pathogen have been reported to be between 20 and 30%, though figures as high as 50% have also been cited (Bhunia, 2008). The US authorities maintain a zero- tolerance policy towards *L. monocytogenes*, while Canada and some European countries allow a limit of 100 CFU/25g (Bhunia, 2008).

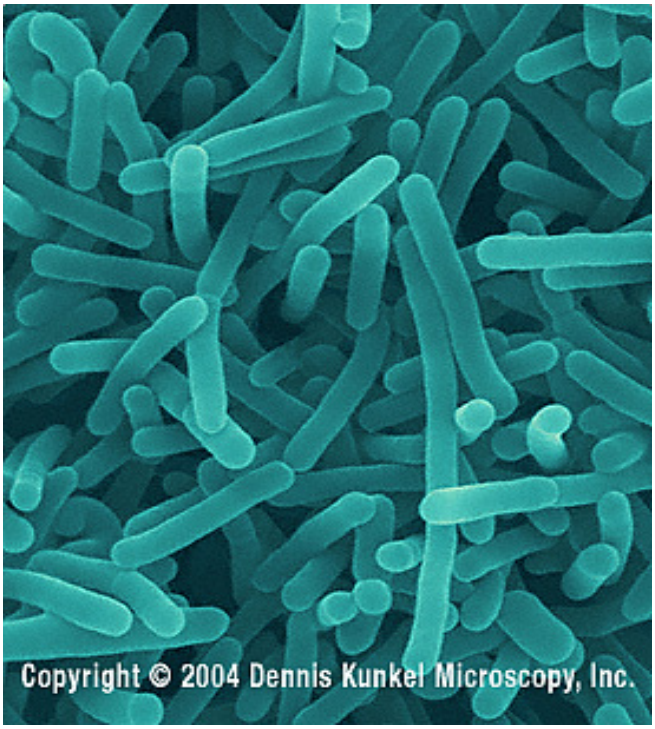

Figure 2.2. *Listeria monocytogenes* cells (With the permission of Dennis Kunkel Microscopy, Inc., Kailua, HI)

L. monocytogenes infection causes two forms of disease in humans: 1) non-invasive gastrointestinal illness, which generally resolves in otherwise healthy people. 2) the much more serious, invasive form of the illness, which may cause septicemia and meningitis (Chen, 2012). The symptoms of listeriosis are fever, muscle aches, nausea and vomiting, and, sometimes, diarrhea. When the more severe form of the infection develops and spreads to the nervous system, symptoms may include headache, stiff neck,

confusion, loss of balance, and convulsions (Chen, 2012). Listeriosis during pregnancy can lead to miscarriage, stillbirth, premature delivery, or life-threatening infection of the newborn (CDC, 2013c).

Compared to 1996-1998, listeriosis outbreaks had declined by about 37% by 2001 (CDC, 2013d). The largest listeriosis outbreak in U.S. history occurred in 2011, when 147 illnesses, 33 deaths, and 1 miscarriage occurred among residents of 28 states; the outbreak was associated with consumption of cantaloupe from Jensen Farm, Colorado. The most recent listeriosis outbreaks are from Frescolina Marte brand ricotta and Crave Brothers Farmstead cheese consumption at 2012 and 2013 respectively (CDC, 2013d). While 10 people were hospitalized because of the outbreak in 2012; 5 people were hospitalized from the states of Illinois, Indiana, Minnesota, and Ohio in the outbreak of 2013. One death was reported in Minnesota and one illness in a pregnant woman resulted in a miscarriage (CDC, 2013d).

FDA suggests some steps in order to prevent listeriosis include rinsing raw products well before eating, cutting, or cooking; scrubbing firm products, such as melons and cucumbers; drying the products with a clean cloth or paper towel; and separating uncooked meats and poultry from vegetables and cooked foods (CDC, 2013d).

2.3.1.3. *Campylobacter* spp.

Campylobacter is a non-sporeforming, microaerophilic, Gram-negative rod with a curved- to S-shaped morphology (Nayak and Foley, 2012). *Campylobacter* have been implicated in human disease called campylobacteriosis, with *C. jejuni* and *C. coli* the most common (Ryan and Ray, 2004).

The symptoms of campylobacteriosis are diarrhea, cramping, abdominal pain, and fever within two to five days after consuming the organism. The diarrhea may be bloody and can be accompanied by nausea and vomiting. The illness typically lasts about one week (CDC, 2013e). The common routes of transmission are ingestion of contaminated food or water, and the eating of raw meat and chicken and unpasteurized milk (Humphrey et al., 2007). *Campylobacter* is one of the most common causes of diarrheal illness in the United States (CDC, 2013e). In order to prevent Campylobacteriosis all poultry products should be cooked throughout with min 165°F, hands should be properly washed with soap before preparing food and unpasteurized milk should not be consumed (CDC, 2013e).

2.3.1.4. *Brucella* spp.

Brucella spp. are small, facultative, Gram-negative, short, non-sporeforming coccobacilli (Kase, 2012). *Brucella* is the cause of brucellosis, transmitted by ingesting contaminated food. Brucellosis is usually not transmitted from human to human; people become infected by contact with fluids from infected animals (sheep, cattle or pigs) or derived food products like unpasteurized milk and cheese (Atluri et al., 2011).

The symptoms of the brucellosis are initially fever, sweats, malaise, headache, muscle pain, and fatigue; while artritis, recurrent fevers, chronic fatigue, depression, swelling of the heart and liver (CDC, 2012c). Mortality rate is less than 2%. According to a recent estimate by the CDC, 839 cases of foodborne brucellosis occur each year in the United States (Kase, 2012). The best way of prevention of Brucellosis is to avoid consuming undercooked meat products and unpasteurized milk, cheese, ice cream

products. Also, people who handle animal tissues are under the risk of Brucellosis. They should use gloves, goggles, and gowns or aprons while handling animal tissues (CDC, 2012c).

2.3.1.5. *Bacillus cereus*

Bacillus cereus is a Gram-positive, facultative anaerobic, endospore-forming, large rod (Tallent et al., 2012). *B. cereus* is widespread in the environment and can be isolated from soil and vegetation. Some strains are harmful to humans and cause foodborne illness. There is two types of illness are caused by two distinct toxins of *B. cereus*: A diarrheal illness with an incubation time of approximately 10 to 16 hours, and an emetic (vomiting) illness with an incubation time of one to six hours (Tallent et. al., 2012).

B. cereus is estimated to be responsible for as much as 25% of foodborne intoxications in the US (Bennet and Tallent, 2012). Confirmed outbreaks reported to the CDC in 2005, 2006, and 2007 were 4, 3, and 6 and affected 69, 35, and 100 people, respectively.

The recent outbreak caused by *B. cereus* affected 103 people in 2011 in FL as a result of rice consumption and 100 people got sick from mac and cheese in CT in 2009. Since cooking temperatures less than or equal to 100°C (212°F) allows some *B. cereus* spores to survive, should be more attention given to proper cooking in order the avoid these kind of outbreaks.

2.3.1.6. *Shigella* spp.

Shigellae are Gram-negative, non-motile, non-spore-forming, rod-shaped bacteria. *Shigella* species includes *S. sonnei, S. boydii, S. flexneri,* and *S. dysenteriae.* Some strains are able to produce enterotoxins and Shiga toxins (Lampel, 2012). The organism is frequently found in water polluted with human feces. *Shigella* infection is typically via ingestion; depending on age and condition of the host, fewer than 100 bacterial cells can be enough to cause an infection (Lewinson, 2006).

Every year, about 14,000 cases of shigellosis are reported in the U.S. Because many milder cases are not diagnosed or reported, the actual number of infections may be twenty times greater (CDC, 2013f). Shigellosis usually shows itself with diarrhea which can be bloody with the fatality rate of 10-15% (Lampel, 2012). As a preventive action handwashing and basic hygiene rules should be followed. Especially, workers who work with raw consumed foods such as vegetables or fruits, should give more attention to hygiene rules (CDC, 2013f).

2.3.1.7 Shiga Toxin-producing *Escherichia coli* (STEC)

Shiga toxin-producing *E. coli* (STEC) may also be referred as verocy toxin-producing *E. coli* (VTEC) or enter hemorrhagic *E. coli* (EHEC) is a group of *E. coli* strains capable of causing significant human disease (Bower, 1999). The strain of Shiga toxin-producing *E. coli* O104:H4 that caused a large outbreak in Europe in 2011 was frequently referred to as EHEC. The most commonly identified STEC in North America is *E. coli* O157:H7 (CDC, 2013g). The symptoms of STEC often include severe stomach cramps, diarrhea (often bloody), and vomiting (CDC, 2013g).

Risky foods in the aspect of STEC are unpasteurized (raw) milk, water that has not been disinfected, unpasteurized apple cider, and soft cheeses made from raw milk (CDC, 2013g). The most recent STEC outbreak reported by CDC, infected a total of 35 persons (from 19 different states) with the strain of Shiga toxin-producing *E.coli* O121 (STEC O121) (CDC, 2013g).

2.3.2. Major Spoilage Microorganisms Concern for Cheeses

Food spoilage is a metabolic process that causes foods to be undesirable for human consumption due to changes in sensory characteristics (Doyle, 2007). Consumption of spoiled foods may be safe, because there may be no pathogens or toxins present, but changes in texture, smell, taste, or appearance cause them to be rejected (Doyle, 2007). The major spoilage microorganisms for cheese products are *Penicillium* spp., Coliforms, *Yarrowia lipolytica, Lactabacillus plantarum*, etc. (Ledenbach and Marshall, 2009).

2.3.2.1. *Penicillium* spp.

Penicillium is a genus of ascomycetous fungi of major importance in the natural environment as well as food and drug production. Some *Penicillium* strains play a main role in the production of various meat and cheese products such as Blue cheese, Roquefort, Camembert and Brie (Fox et al., 2004). Some strains are known as causative agent of unwanted visual and textural changes that affect the quality of food products (Pintado et al., 2010). Many species of *Penicillium* produce highly toxic mycotoxins such as isofumigaclavin C, penicillic acid, PR toxin, patulin, botryodiploidin and roquefortine (Siemens, 1993).

The ability of these *Penicillium* species to grow on seeds and other stored foods depends on their propensity to thrive in low humidity and to colonize rapidly by aerial dispersion, while the seeds are sufficiently moist (Pitt et al., 2000).

P. commune, P. chrysogenum, and *P. roqueforti* are some of the major concern for cheese products. *P. chrysogenum* is a fungus, common in temperate and subtropical regions and can be found on salted food products (Samson et al., 1977).
P. chrysogenum is used for penicillin production, however, it is also a common contaminant of grains, bread, and processed food (Garcia-Estrada et al., 2011).
P. commune, a mold frequently found on dry-cured meat products, is able to synthesize mycotoxin cyclopiazonic acid (Sosa et al., 2002). *P. commune* is widespread and frequently occurring spoilage fungi on cheese (Lund, 1995). Growth of *P. commune* on cheese results in discoloring of the surface and production of off flavors (Lund, 1995).

2.3.2.1.1. *Penicillium roqueforti*

Penicillium roqueforti is a common saprotrophic fungus, that is found widespread in nature and can be isolated from soil, decaying organic substances and plant parts (Fig 2.3). *P. roqueforti* is an essential component of the microflora of a number of cheeses such as Roquefort (France), Stilton (UK), Tulum (Turkey), Gorgonzola (Italy), Blauschimmelkase (Switzerland), and Danish Blue (Denmark) (Fox et al., 2004), because *P. roqueforti* produces a large number of enzymes that help a variety of flavor components (Gourama, 2013). Therefore, proteolytic activity of *P. roqueforti* plays a major role for the texture and flavor development in cheese ripening. However, *P. roqueforti* is also responsible for spoilage and damages a wide variety of food and feed

products owing to its survivability in harsh conditions. It is capable of growing in low-oxygen, high carbon dioxide environments, at low temperatures and after treatment with preservatives (Samson et al., 1977). *P. roqueforti* produces at least three toxins, PR toxin, roquefortine and patulin and some strains can also produce mycophenolic acid, penicillic acid, cyclopiazonic acid, penitrem A, isofumigaclavine A and B, festuclavine and chaetoglobosin A. However, PR toxin is not stable in cheese and breaks down to the less toxic PR imine (Siemens, 1993). A research has revealed that penicillic acid and patulin were found in 2 and 3.7% of cheddar cheese and 2.7 and 2.2% of Swiss cheese, respectively (Erdogan and Sert, 2003).

Figure 2.3. *Penicilium roqueforti* (With the permission of Dennis Kunkel Microscopy, Inc., Kailua, HI)

2.3.2.2. Coliforms

Coliforms are rod-shaped, Gram-negative, non-spore forming bacteria, which can ferment lactose with the production of acid and gas (APHA, 1995). Coliforms are a broad class of bacteria found in our environment, including the feces of mammals. The presence of coliform bacteria in drinking water may indicate a possible presence of harmful, disease-causing organisms. Coliforms, similar to psychrotrophs, can reduce the diacetyl content of buttermilk and sour cream (Wang and Frank, 1981), with producing a yogurt-like flavor. In cheese production, slow lactic acid production by starter cultures helps the production of gas by coliform bacteria. The pH increases during ripening of cheese, growth potential of coliform bacteria would increase (Ledenbach and Marshall, 2009).

2.3.2.3. *Yarrowia lipolytica*

Yarrowia is a fungal genus in the family *Dipodascaceae. Yarrowia lipolytica* strains are often isolated from dairy products such as cheeses (Camembert, Livarot, Roquefort, Blue), yoghurts and sausages (Fickers et al., 2005). This organism produces a range of important aroma compounds for blue cheese flavor when cultured outside of cheese (Gkatzionis et al., 2012). *Y. lipolytica* degrades all components of casein (Van den Tempel and Jakobsen, 2000), which is significant as this results in the production of amino acids and aroma compounds such as esters, free fatty acids, alcohols and ketones that are important in blue cheeses (Gkatzionis et al., 2012).

However, existence of *Y. lipolytica* is not always beneficial, since it can also help the spoilage of cheese product with causing visible growth of colonies on the surface of the

cheese, as unpleasant smell, odor and taste. There is limited study on yeast cheese spoilage in the literature probably due to the difficulties in determining when the presence of a particular yeast species is beneficial or associated with spoilage (Westall and Filtenborg, 1998).

2.3.2.4. *Lactobacillus plantarum*

L. plantarum is a gram positive bacterium that is found in a variety of niches. These niches include dairy, meat, and much vegetable fermentation, it is also found in the human gastrointestinal tract. It is a facultative hetero-fermentative lactic acid bacterium (De Vries et al., 2006; Zago, 2011). These microbes metabolize lactose, subsequently produce lactate, acetate, ethanol, and CO_2 (Hutkins, 2001). When the homo-fermentative lactic acid bacteria fail to metabolize all of the fermentable sugar in a cheese, the hetero-fermentative bacteria that are often present complete the fermentation, producing gas and off-flavors (Ledenbach and Marshall, 2009).

2.4. Cheese decontamination methods

In order to eliminate the pathogenic and spoilage microorganisms on contaminated cheese, there is a pronounced need for decontamination methods, which can certainly help to eliminate serious risk for public health and negatively affect cheese quality. A good decontamination process will not only deal with pathogens and spoilage, but will preserve desired product texture, taste, and olfactory profiles.

Various thermal and non-thermal processing methods have been evaluated for cheese decontamination. Thermal methods include heat-pasteurization by steam and

infrared heating. Non-thermal processing includes physical methods such as UV treatment, high hydrostatic pressure; chemical methods such as ozone; and biological methods such nisin. Sometimes they are combined to increase their effectiveness. However, each one of them has its own limitations and advantages.

2.4.1. Thermal Methods

2.4.1.1. Heat pasteurization by steam

Heat pasteurization has been most important method in food processing since it has a great capacity of destruction microorganisms, enzymes, insects and parasites. In addition to this, heat pasteurization has some main advantages: 1) relatively simple process control, 2) shelf stable products production 3) destruction of anti-nutritional factors such as trypsin inhibitor, 4) improvement in the availability of some nutrients like gelatinization (Fellows, 2000).

Hot water and steam are commonly used for heat pasteurization. Conventional steam pasteurization causes smaller loss of water-soluble components. Conventional hot water has lower capital cost and better energy efficiency, but higher cost for water use.

The two most common commercial methods of pasteurizing by steam involve passing food through an atmosphere of saturated steam or a bath of hot water. The simplest steam pasteurization system consists of a mesh conveyor belt that carries food through a steam atmosphere in a tunnel. Alternatively, food can enter and leave the system through rotary valves or hydrostatic seals to reduce steam losses (Fellows, 2000). As it mentioned above, heat treatment can cause some unwanted changes in the food

product. Thus, process calculation should be done and followed properly according to target microorganism's D-value.

For liquid products pasteurization, the steam is used should be culinary (potable) quality and contain no chemical additives. Mixing of steam with the liquid food can be accomplished by two methods (Hallstrom et al., 1988): a) Steam injection, by injecting steam into the fluid food through small holes, and b) Steam infusion, by mixing the steam with films and droplets of the liquid food, sprayed in a special vessel.

However, heat treatment has some disadvantages such as causing changes in food compounds, texture, color, and taste. Also, using hot water has a risk of contamination by thermphilic bacteria (Fellows, 2000).

2.4.1.2. Infrared heating

Infrared heating is a part of the electromagnetic spectrum with wavelengths between those of ultraviolet and of microwave radiation; range between 0.76 μm and 1 mm (Staack et al., 2008). Infrared energy is emitted by hot objects. When it is absorbed, the radiation gives up its energy to heat the surfaces (Fellows, 2000).

The International Commission on Illumination (CIE) recommends the division of infrared radiation into the following three bands (Henderson, 2007):

- IR-A (near IR): 700 nm – 1400 nm (0.7 μm – 1.4 μm, 215 THz – 430 THz)
- IR-B (medium IR): 1400 nm – 3000 nm (1.4 μm – 3 μm, 100 THz – 215 THz)
- IR-C (far IR): 3000 nm – 1 mm (3 μm – 1000 μm, 300 GHz – 100 THz)

Far-infrared (FIR) radiation has been used for treating food systems and inactivation of pathogens because of stronger absorption of microorganism and food components in the far-infrared wavelength range (3 to 1,000 μm) (Khrishnamurthy, 2006). Microbial inactivation by IR heating may include inactivation mechanism similar to that of ultraviolet light (DNA damage) and microwave heating (induction heating) in addition to thermal effect, as infrared is located between ultraviolet and microwave in the electromagnetic spectrum (Jun and Irudayaraj, 2009).

Infrared heating is a microbiologically effective method with high heat transfer rate and energy savings. It has been reported that IR heating is effective in the inactivation of highly heat-resistant microbial spores such as *Bacillus subtilis* and that direct application of IR heat to the bacterial spores resulted in an excellent killing efficiency (Hamanaka et al., 2011).

Rosenthal et al. (1996) studied the surface pasteurization of cottage cheese by infrared radiation. Surface heating of the cheese was done at a distance of 2.5 to 3 cm from the cheese surface. The initial microbial count before infrared pasteurization were <10 cells/g. After the treatment, cheeses were examined microbiologically during 8 weeks of storage at 4^{o}C. Even after 8 weeks storage at 4^{o}C, still less than 100 CFU/g were found, that is showed that infrared pasteurization was effective for surface sterilization (Krishnamurthy, 2006).

Infrared heating has several advantages such as, 1) higher heat transfer capacity, 2) instant heating due to direct heat penetration, 3) high energy efficiency, 4) shorter heat treatment time, 5) fast regulation response, 6) better process control, 7) no heating of

surrounding air, 8) equipment compactness, 9) uniform heating, 10) better quality of food (Khrishnamurthy, 2006).

IR heating has gained popularity recently due to its advantages. The main commercial application of infrared heating is in drying low-moisture foods such as breadcrumbs, cocoa, flours, grains, malt, pasta products and tea; also dried fruit and vegetable such as potatoes, kiwifruit (Fellows, 2000). It also has been applied in baking, roasting, blanching, pasteurization, and sterilization of food products (Krishnamurthy et al., 2002). As a limitation of IR method, being dependent on the penetration capacity of food product can be considered. Also, vitamin loss and some visual changes may be observed due to the increase of temperature of the food product.

2.4.2. Non- Thermal Methods

2.4.2.1. Nisin usage as a biological method

Nisin is a polycyclic antibacterial peptide with 34 amino acid residues used as a food preservative (Damodaran et al., 2007). Nisin is produced by fermentation using the *Lactococcus lactis,* commercially. It is obtained from the culturing of *L. lactis* on natural substrates such as milk or dextrose. While most bacteriosins generally inhibit only limited species, nisin is a rare example of a “broad-spectrum” bacteriosin effective against many bacteria (Jung and Sahl, 1991). Mode of action of nisin is that it acts on the cytoplasmic membrane forming transient pores which are dependent upon proton motive force and membrane lipid component, has been demonstrated by in vitro studies in lipsosomes (Driessen et al., 1995).

Nisin is currently used to prevent putrefication of processed cheese and cheese spreads. It is also used against bacterial spoilage of canned vegetables, fruit, meat, fish,

soups, and milk (Frazer et al., 1962) and to control lactic acid bacteria in beer production, and to control *Clostridium botulinum* type E in modified atmosphere packaged fresh fish (Osmanagaoglu and Beyatli, 2002). More recent applications of nisin include its use in high moisture, hot baked flour products (crumpets) and pasteurised liquid egg (Delves-Broughton et al., 1996).

In the UK, a cocktail of spores of *Clostridium* spp. at the level of 200 spores per gram applied to processed cheese samples. In this work spoilage prevented during storage at 37 °C by 6.25 μg/g nisin and partial control was achieved with 2.5 μg/g nisin (Delves-Broughton et al., 1996). Jung et al. (1992) showed that the presence of milk fat affected adversely the anti listerial property of nisin against *L. monocytogenes.* A concentration of nisin of 1.25 mg 1-1 was very effective in achieving a four to six log reduction in non-fat milk whereas in half and half cream less than one log reduction was achieved (Delves-Broughton et al., 1996). Zohri et al. (2013) investigated effectiveness of nisin usage on Feta cheese. To improve the instability of nisin, a hybrid of nisin at concentration of 450 IU/mL with chitosan/alginate nanoparticles was prepared and antibacterial strength of the hybrid was compared with free nisin against *Listeria monocytogenes* and *Staphylococcus aureus* in Feta cheese. Results indicated that the nisin-loaded nanoparticles were able to decrease the populations of *S. aureus* and *L. monocytogenes* up to five- and sevenfold on a logarithmic scale in comparison with free nisin, respectively (Zohri et al., 2013).

Addition levels of nisin to achieve effective preservation depend on the following factors: the microbial population present in the food, moisture content, pH, salt content, use of flavor additives, cooking process and the length and likely temperature of the shelf

life required (Osmanagaoglu and Beyatli, 2002). Nisin does not have a consistent effect on Gram-positive pathogen and spoilage microorganisms even at extreme levels. In order to increase effectiveness of nisin, it can be combined with chelating agents (EDTA, citric acid, Tween 20). For example, nisin alone is not enough to extent shelf life of meat products since nisin has very little effect on the Gram-negative microorganisms that are major concern at meat products (Lund et al., 1999).

Disadvantages of nisin are the limited effect against gram-positive bacteria and the costly waste management (Jones et al., 2005).

2.4.2.2. Ozone usage as a chemical method

Interest in ozone has expended recently due to customer's demand for greener food additives (O'Donnel et al., 2012). Ozone is a powerful antimicrobial substance due to its potential oxidizing capacity (Guzel-Seydim et al., 2004). The multifunctionality of ozone makes it a promising food processing agent. Excess ozone auto decomposes rapidly to produce oxygen and thus does not leave any residue in foods (O'Donnel et al., 2012). Ozone use may have some advantages in the food industry. There are suggested applications of ozone in the food industry such as food surface hygiene, sanitation of food plant equipment, reuse of waste water, lowering biological oxygen demand (BOD) and chemical oxygen demand (COD) of food plant waste (Guzel-Seydim, 1996).

The inactivation mechanism of ozone is the progressive oxidation of vital cellular components. The bacterial cell surface has been suggested as the primary target of ozonation. There are two major mechanisms that identified in ozone destruction of the microorganisms (Victorin, 1992): first mechanism is that ozone oxidizes sulfhydryl

groups and amino acids of enzymes, peptides and proteins to shorter peptides. The second mechanism is that ozone oxidizes polyunsaturated fatty acids to acid peroxides (Victorin, 1992).

Ozone has been used experimentally to control mold on Cheddar cheese surfaces and in cheese rooms. At high ozone concentrations ozone appeared to destroy molds (Guzel-Seydim, 1996). However, upon termination of ozonation, mold population flourished (Guzel-Seydim, 1996). The antimicrobial effectiveness of ozone depends on several factors including the amount of ozone applied, the residual ozone in the medium and various environmental factors such as medium pH, temperature, relative humidity, additives and the amount of organic matter surrounding the cells (O'Donnel et al., 2012). Restaino et al. (1995) investigated the antimicrobial effects of ozonated water against food related microorganisms and determined that ozone effectively inactivated Gram positive bacteria as *Listeria monocytogenes, Staphylococcus aureus, Bacillus cereus, Enterococcus faecalis,* and Gram-negative bacteria such as *Pseudomonas aeruginosa*, and *Yersinia enterocolitica*. Restaino et al. (1995) also determined that ozone was effective on the yeasts *Candida albicans* and *Zygosaccharomyces bacilli* and spores of *Aspergillus niger*. The destruction mechanism of ozone works by attacking on the bacterial membrane glycoproteins and/or glycolipids (Guzel-Seydim et al., 2004).

A major drawback of ozone is that due to its instability, ozone must be generated just before use and the equipment and operating costs can be quite high (Boglarski and Telikicherla, 1995). Also, workers' safety in the plant have to be paid attention closely, which may require extra cost in design and operation of the process.

2.4.2.3. High Pressure Processing as a physical method

High pressure processing (HPP) is a method that the food sample is subjected to very high hydrostatic pressure that causes microbial destruction. The atmospheric pressure is 0.1 MPa, while HPP operates between 100-800 MPa (Maroulis, 2003) (1000-8000 times more than atmospheric pressure).

The product is going to be processed is filled in a flexible pouch and sealed. Then, these pouches are filled in a pressure chamber filled with pressure transmitting fluid. Usually water is used as a fluid. This liquid is then pressurized with a pump and the product is held at the desired pressure for defined duration (Anantheswaran, 2013). Process pressure and time are defined according to target microorganism so that they change for each product.

The primary aim of treating foods with HPP in most cases is to reduce or eliminate the relevant foodborne microorganisms that may be present (Patterson et al., 2007). HPP allows the decontamination of foods with minimal impact on their nutritional and sensory features (Campus, 2010).

Destruction mechanism of HPP depends on breaking non-covalent bonds and thus can affect the secondary or tertiary structures of molecules. Also, some of the enzymes may denature, leading to cell death. HPP also changes the permeability of the cell wall. It is known that Gram positive are more tolerant than Gram negative (Adams and Moss, 2008).

HPP treated products continue to take place in the global market. Commercial food applications of HPP primarily focus on the ability of pressure to kill spoilage organisms and relevant foodborne pathogens and extend product shelf life (Patterson,

2007). In addition to success of HPP on microbiological safety and ability of extending shelf life, it provides superior quality products with original fresh taste, texture, and nutritional content. Some of the commercial products that treated with HPP are: Guacamole (US), orange juice (Europe), apple juice (Australia) (Anantheswaran, 2013).

Evrendilek et al. (2008) was investigated effectiveness of HPP on Turkish white cheese in terms of *Listeria monocytogenes,* total *Enterobacteriaceae,* total aerobic mesophilic bacteria, total molds and yeasts, total *Lactococcus* spp., and total *Lactobacillus* spp. reduction. Cheese samples made by raw and pasteurized milk, and *L. monocytogenes* inoculated after brining. Turkish white cheese subjected to HPP at 50 to 600 MPa for 5 and 10 min at 25^{o}C. The maximum reduction of the *L. monocytogenes* population was 4.9 log CFU/g at 600 MPa. The total molds and yeasts and total *Enterobacteriaceae* counts for the cheese samples produced from pasteurized milk were below the detection limit both before and after HPP due to the effective pasteurization. As a result, they concluded that HPP can be used effectively to decontaminate Turkish white cheese (Evrendilek et al., 2008).

Advantages of the HPP are in package processing, inactivation of enzymes, better tasting product and very low nutrient loss. However, HPP has some limitations such as being not very effective against barotolerant microbes and food enzymes, the dissolved oxygen caused by high pressure results in enzymatic and oxidative degradation of food components, and HPP processed food may require low temperature storage in order to retain sensory qualities. Most importantly, the capital cost is a significant issue for this technology.

2.4.2.4. Irradiation as a physical method

Food irradiation is a method used to treat various food products with ionizing radiation (IFST, 2006). Different food products can benefit from this method to protect them from spoilage microorganisms and to retain desirable qualities for a longer period. In principle, food irradiation works by expunging electrons at the atomic level. This is useful for decontamination purposes as it disrupts the ability of spoilage and pathogen organisms to survive and thrive in a food product (IFT, 1998). There are three principal ways of accomplishing this (IFT, 1998).

- Using ß–particle irradiation through radioactive decay or machine generation.
- Using X-Rays by impinging high-energy electrons at a suitable target.
- Using γ-radiation produced by isotopic decay.

These methods have been deemed effective to treat food products by the FDA and are routinely used to decontaminate meats and dairy. The main benefit associated with this method is that it allows for effective decontamination of the target product and significantly increases shelf-life (IFT, 1998).

Huo et al. (2013) evaluated the effectiveness of electron beam irradiation on the shelf life of mozzarella cheese. Shelf life tests were run at five different irradiation doses at 10°C. Microbial load was monitored on the consecutive days during storage. The experimental data were fitted through a modified version of the Gompertz equation and results showed significant increases in the shelf life of the mozzarella cheese. The irradiation dose of 2.0 kiloGray (kGy) was determined as the optimum dose, since it may inhibit the spoilage microorganisms with no change in sensorial characteristics of the product.

Some studies have shown between 99.9 and 99.999% of common pathogens such as *E. coli* and *Salmonella* are killed in the dose range of 1 to 3 kGy. This same range would also address all other Gram-negative bacteria (Dubey et al., 1986). Similarly, shelf life was increased by a factor of 4 - 6 in studies conducted by Lambert (1992) and Thayer et al. (1993).

Kim et al. (2010) were identified the efficacy of gamma and electron beam irradiation of *L. monocytogenes* and *S. aureus* as a food-borne pathogens in sliced and pizza cheeses. Treatment dose of 1 kGy irradiation for sliced cheese and 3 kGy for pizza cheese sufficiently reduced aerobic bacteria counts to below detectable levels. Gamma irradiation proved to be much more effective than electron beam irradiation at equal doses. D_{10} values of below 1 kGy effectively decontaminated the samples from *L. monocytogenes* and *S. aureus*. These findings imply that a low irradiation dose can have significant effects on microbial quality and greatly reduce the risk of contamination of sliced and pizza cheeses.

FDA establishes labeling requirements of irradiated foods. Labels must contain the words "Treated with Radiation" or "Treated by Irradiation" and display the irradiation logo, the Radura (EPA, 2012) since there is no test to detect irradiation. Disadvantages of irradiation are that can only be used on a very limited range of foods, consumer acceptance and be still a relatively expensive technology.

2.4.2.5. UV light treatment as a physical method

UV-light is a portion of electromagnetic spectrum ranging from wavelengths of 100 to 400 nm. It is categorized in three distinct wavelengths - the A, B and C bands, at 400 to 320 nm,

320 to 280 nm and 280 to 100 nm, respectively (Figure 2.3) (CCOHS, 2013).

UV-light sterilization provides a cost-effective alternative to the existing heat pasteurization techniques and preservation methods. Also, taste degradation of food material subjected to UV-light treatment is minimal, because UV-light treatment can be accomplished at an ambient temperature (Krishnamurthy et al., 2004).

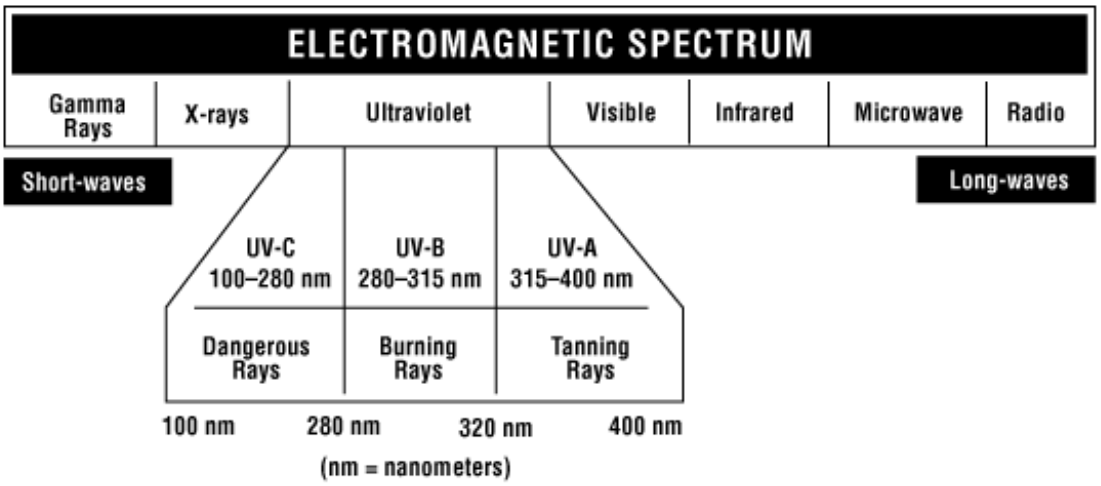

Figure 2.3. The electro-magnetic spectrum (CCOHS, 2013)

UV-light treatment can be accomplished in two modes; continuous or pulsed. Continuous UV-light has been used for surface decontamination, water treatment and treatment of liquid food products because of poor penetration depth and low dissipation power. Pulsed UV light systems offer high peak power that destroys microorganisms and provide a higher rate of sanitization than continuous UV light system (Xenon, 2013). Pulsed UV particularly offers faster processing, process flexibility, freedom from toxic lamp materials, penetration of plastic packages, and ease of meeting special lamp configuration requirements (Xenon, 2013). It is a relatively new method for food decontamination.

2.4.2.5.1. Continuous UV-light

Ultraviolet light (254 nm UV-C) is an U.S. Food and Drug Administration (FDA)-approved technology that can be used for decontamination of food and food contact surfaces (FDA, 2000). UV-C irradiation exerts its bactericidal effect by production of cyclobutane pyrimidine dimers and 6,4 photoproducts in the bacterial chromosome (Figure 2.4) either killing the bacteria or rendering them unable to reproduce (Reardon and Sancar, 2005)

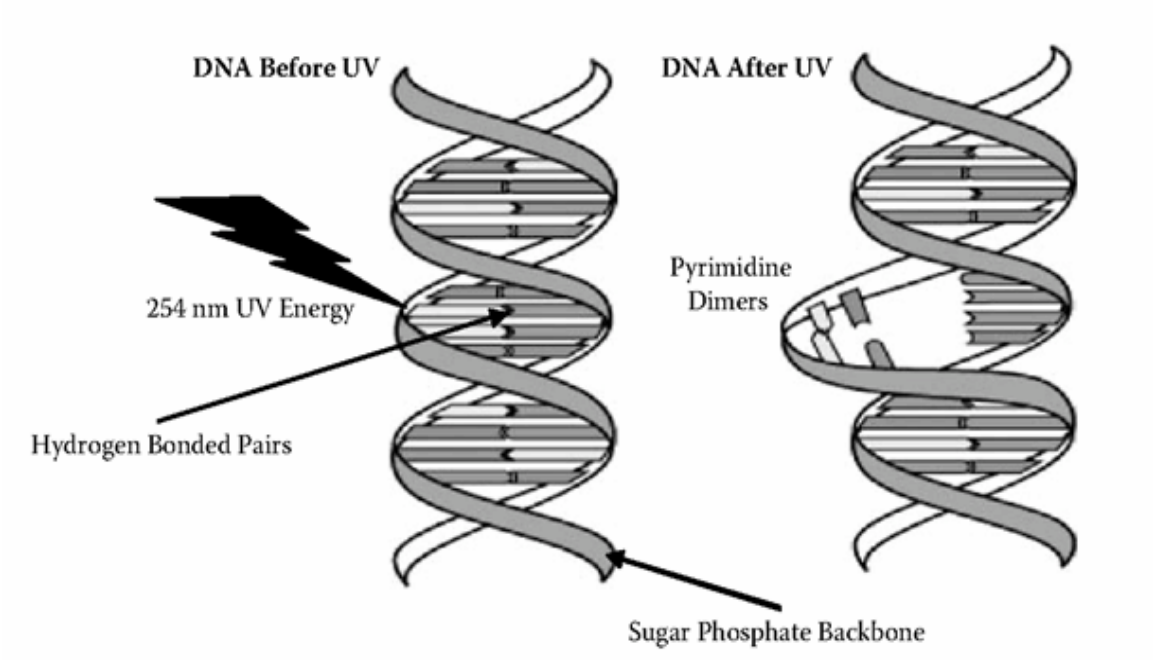

Figure 2.4. Structure of DNA before and after UV light treatment (Koutchma et al., 2009)

UV light can be used to inactivate many types of organisms, including viruses, but it is currently known that UV light works better on surfaces or clear liquids such as water. UV light radiation has been used for many years in pharmaceutical, electronic, and aquaculture industries as a disinfection medium (Anonymous, 2002). Very low intensity UV light can be used for food contact surface disinfection and moderately high doses

could be used for surface treatment of foods and 'cold pasteurization' of liquid foods (Anantheswaran, 2013). In 2000, the FDA approved UV-light as alternative treatment to thermal pasteurization of fresh juice products (FDA/CFSAN, 2000). The effectiveness of the UV light is defined by FDA for fruit and vegetable juice processing is a 5-$\log_{10}$ reduction in the number of the target pathogen of concern (FDA/CFSAN, 2000). Another use of UV light is to prolong shelf life of wrapped partially baked baguettes to minimize post contamination (Doulia et al., 2000).

Matak et al. (2005) investigated effectiveness of UV- light on goat milk, since goat milk is often used unpasteurized to make gourmet goat cheeses. They inoculated the goat milk with *Listeria monocytogenes* (L-2289) at 10^7 CFU/ml and treated with UV-light using the CiderSure 3500 apparatus (FPE Inc., Macedon, NY). They exposed the UV-light with the dose of range between 0 and 20 mJ/cm^2 and tried to determine the optimal UV dose. They achieved greater than 5-log reduction ($P < 0.0001$) when the milk received a cumulative UV dose of 15.8 ±1.6 mJ/cm^2. The results of this study indicated that UV light could be used for the inactivation of *L. monocytogenes* in goat's milk.

UV-C radiation has several advantages such as not producing chemical residues, by-products or radiation. Also, it is a simple dry and cold process (Bachmann, 1975) with low operating cost. In addition to these advantages, consumers' negative reaction on chemical preservatives added foods and intend to purchase healthier food, has been increased the interest in UV light treatment. The limitations of UV-light treatment are low penetration depth and color changes in the food product. Since it has lower energy output than pulsed UV-light, it requires longer treatment times.

There are some factors that affect effectiveness of UV treatment. These factors are: Intensity of UV light, duration of exposure (high durations ensure that there is no reactivation of injured microbial cells) and presence of UV absorbing compounds (Anatheswaran, 2013).

2.4.2.5.2. Pulsed UV-light

Pulsed UV-light is a non-thermal method for food preservation that involves the use of intense, short duration pulses of a board spectrum to ensure microbial decontamination on the surface of either foods or packaging materials (Elmnasser et al., 2007). The system consists of light pulses delivered by a xenon gas lamp. The pulses are created by compressing electrical energy into short pulses and using these pulses to energize an inert gas lamp. The energized lamp then emits intense light pulses in the continual broadband spectrum from deep UV to infrared with durations of a few hundred microseconds (Keklik et al., 2009).

Pulsed UV-light treatment offers inactivation of microorganisms on food surfaces in short times. This technology involves broad-spectrum light (100-1100 nm) and it is known that maximum inactivation is achieved at 254 nm (Koutchma et al., 2009). In pulsed UV-light treatment, the energy is stored in a high power capacitor and released constantly and in this way more power is supplied. Microbial inactivation by exposure to pulsed UV-light is attributed to the effect of the broad spectrum UV content and the energy density applied with the treatment, which in turn is related to the pulse width and the high peak power of the pulse (Gemma et al., 2008).

Since significant portion of the pulsed light spectrum includes UV light, it is accepted that UV plays a major role inactivation mechanism of pulsed UV-light. Antimicrobial effects of UV light on bacteria are attributed to the absorption of radiation by conjugated carbon-carbon double bonds in nucleic acid (Koutchma et al., 2009). Krishnamurthy et al. (2006) indicated three modes of inactivation mechanism in UV light: Photophysical, photochemical, and/or photothermal effect. In the photochemical mode, inactivation is mainly caused by chemical changes in the DNA and RNA. Thymine dimer formation is the major photochemical change that attributed to microbial inactivation. Photothermal effect leads to cell death due to the increase of temperature during long duration pulsed UV-light treatment. As the heating rate of the bacterial cell and surrounding media are different, localized heating of bacteria leads to cell death. In photophysical effect, structural damages in bacteria may occur due to constant disturbance caused by the high energy pulses. Thus, the effectiveness of pulsed UV-light treatment can be improved by optimizing the pulse width and number of pulses (Krishnamurthy, 2006).

Pulsed UV-light can be an alternative to the irradiation process. The wavelengths used in pulsed UV- light are much longer than those used in gamma irradiation. Therefore, unlike gamma irradiation, pulsed UV-light does not cause ionization of small molecules. Another advantage of pulsed UV-light technology is that no chemicals or chemical residues are involved. Moreover, pulsed UV-light is considered to be non-thermal for short treatment times, which makes this method applicable to minimally processed or raw foods such as fruits, vegetables, eggs, poultry, seafood, and meat. Pulsed UV-light treatment of food was approved by the FDA in 1999 with some

conditions such as treatment uses a xenon lamp with wavelength between 200-1000 nm, pulsed width not exceeding 2 ms, and the cumulative treatment level not exceeding 12 J/cm^2 (FDA, 1999).

There are some studies on the effectiveness of pulsed UV-light (PL) on dairy products. In the study of Miller et al. (2012), inactivation of *Escherichia coli* in milk and concentrated milk by PL evaluated. The study aimed to quantify the effectiveness of PL on inactivating *Escherichia coli* in cow milk and evaluate the effect of total solids and fat content on inactivation. Milk samples with total solids of 9.8, 25, and 45% and commercial cow milk with different fat contents (skim milk, 2% fat, and whole milk) were inoculated with nonpathogenic *E. coli*. PL was applied up to doses of 14.9 J/cm^2. Survivors were quantified after treatment. Concentrated milks (25 and 45% solids content) resulted in reductions of less than 1 log, whereas for the milk with 9.8% solids content 2.5 $\log_{10}$ CFU/mL were obtained after treatment with 8.4 J/cm^2. In the skim milk, a 3.4 $\log_{10}$ CFU/mL reduction at 14.9 J/cm^2 was obtained. Under the same conditions, inactivation levels of 2% and whole milk were greater than 2.5-$\log_{10}$ CFU/mL. These obtained data indicate that PL is effective for the inactivation of *E. coli* in milk, but has limited effectiveness in concentrated milk, due to the absorption of light by the milk solids. Milk fat also decreases the effectiveness of PL due to its light-scattering effects (Miller et al., 2012).

Krishnamurthy et al. (2007) investigated flow- through pulsed UV-light system effectiveness on *Staphylococcus aureus* inoculated milk. They evaluated the effect of sample distance from the UV-strobe, number of passes and flow rate of the milk. Milk was treated 5-, 8- and 11-cm distance from UV lamp at 20, 30, or 40 mL/min flow rate up

to 3 times by recirculating the milk. They achieved to maintain $\log_{10}$ reductions up to 7.26 CFU/mL (11 cm distance, 2 passes and 20 mL/min flow rate). Complete inactivation was observed after a single pass (28 cm distance, 20mL/min flow rate). Overall this work demonstrated that pulsed UV-light is an effective to eliminate milk pathogens.

The only study on effectiveness of pulsed UV-light treatment on cheese products in the literature has done by Dunn et al. (1991). They inoculated commercially dry cottage cheese with *Pseudomonas* spp. and treated with pulsed UV-light with an energy density of 16 J/cm^2 and pulse duration of 0.5 ms. As a result, the microbial population decreased by 1.5-log after 2 pulses (Dunn et al., 1991).

These studies indicate that pulsed UV-light is a powerful decontamination method with short treatment times for dairy products. However, heat generation during the treatment and low penetration rate of the UV-light are limitations and concerns (Keklik et al., 2009).

2.5. Effects of Decontamination Methods on Cheese Quality

Food quality is the major parameter for consumers in order to purchase. Consumers usually trust the safety of food product in the market while they are suspicious about the quality. All of the current decontamination methods have some effects on quality of the product more or less. Therefore, it is important to analyze quality changes of cheese product after treatment as well as microbial load. Quality changes are not necessarily visible, so some analytical and physical methods should be conducted in order to determine the extent of deterioration in the food product.

Quality parameters of cheese products determined as lipid peroxidation (thiobarbituric acid "TBA" value), and color changes. The reason of choosing these

parameters that cheese is lipid-rich product. It is known that the fatty acid radical is not a very stable molecule that reacts readily with molecular oxygen and cause oxidation, which gives the product oxidative taste (German, 1999). Color is also a very important parameter since it is the first thing that consumers look for have an idea of quality of the product.

2.5.1. Lipid oxidation

Lipid oxidation refers to the oxidative degradation of lipids. Lipid oxidation in foods occurs when unsaturated lipids come in contact with oxygen molecules (Keklik et al., 2009). The problem of lipid oxidation has greatest economic importance in the production of lipid-containing foods. Oxidation of unsaturated lipids not only produces offensive odors and flavors but can also decrease the nutritional quality and safety by the formation of secondary reaction products (Frankel, 1980).

Thiobarbituric acid reactive substrances (TBARS) test is the most common method is used to determine extent of lipid oxidation in cheese. Assay of TBARS measures malondialdehyde (MDA) present in the sample, as well as malondialdehyde generated from lipid hydroperoxides by the hydrolytic conditions of the reaction (Trevisan et al., 2001).

2.5.2. Color

Color plays a key role in food choice, often indicates the quality and freshness of a food product. During the decontamination, some chemical changes occur which can cause color changes. Chroma meters can be used to determine color parameter

quantitatively. CEILAB, A *Lab* color space, is a color scale with L*, a*, b* dimensions. L indicates the lightness (where L*=100 is light and L*=0 is dark). The –a, +a, -b, and +b values indicate green, red, blue and yellow color respectively (Bialka et al., 2008; Keklik et al., 2009).

2.5.3. Packaging material

Food packaging has a major role to protect food products from outside influences and damage. Food packaging can retard product deterioration, retain the beneficial effects of processing, extend shelf-life, and maintain or increase the quality and safety of food (Marsh and Bugusu, 2007). Thus, it is very important the quality of packaging materials of the foods. Pulsed UV-light can be applied packaged food products and it is essential to investigate the effects of it on the packaging material. The quality of thin layer of plastics, used in cheese slices packing, is evaluated by measuring tensile properties of the material (ASTM, 2002). Some of the important tensile properties are tensile strength at break, yield strength, elastic modulus, percent elongation at yield and break. These terms are defined in the ASTM D-882 Metod by ASTM (2002) (Keklik, 2009).

2.6. Summary of Literature Review

Cheese is the most consumed dairy product, which has a great impact on the U.S. agricultural economy. In spite of the fact that cheese was made from adequately pasteurized milk, it is often subsequently contaminated with pathogenic and spoilage microorganisms during processing and handling. Between the years of 1998-2008, CDC reported 68 outbreaks with the result of 1519 illnesses, 178 hospitalizations, and 3 deaths

due to the contaminated cheese consumption. Therefore, there is an urgent need to decontaminate cheese at the post-packaging stage in order to avoid illnesses and extend shelf life of cheese products.

Various thermal and non-thermal processing methods have been evaluated for cheese decontamination. Among those, pulsed UV-light is a non-thermal method for food preservation that involves the use of intense, short duration pulses of a board spectrum to ensure microbial decontamination on the surface of either foods or packaging materials. Pulsed UV-light treatment offers inactivation of microorganisms on food surfaces in short times. This technology involves pulsing broad-spectrum light (100-1100 nm). Current studies indicate that pulsed UV-light is a powerful decontamination method with short treatment times for food products. While being effective for decontamination, it may have a negative effect on the food quality. Therefore, this research aims to evaluate efficacy of pulsed UV-light treatment on cheese decontamination and quality parameters such as color, lipid oxidation and mechanical properties of packaging material.

CHAPTER 3

GOAL AND OBJECTIVES

3.1. Goal

This research aimed to inactivate pathogenic and spoilage microorganisms in packaged and unpackaged hard cheese via the application of pulsed UV-light without affecting the quality of cheese and packaging material.

3.2. Objectives

Objectives of this research are to:

1. Determine appropriate packaging materials for UV-light treatment.
2. Optimize pulsed UV-light treatment in terms of distance between cheese and UV strobe, and the number of pulses.
3. Determine quality changes on cheese after pulsed UV-light treatment.
4. Determine the effects of UV-light treatment on quality of plastic packaging.

CHAPTER 4

MATERIALS AND METHODS

4.1. Overview

This research includes determination of package material, pulsed UV-light treatment, and quality analysis of cheese product. First, appropriate package material was determined. In order to choose cheese-packaging material, plastic packages of nine different brands of American cheeses were evaluated for UV-transmittance. A plastic material with the highest UV-transmittance was chosen in order to efficiently transduce UV-light. Second, treatment time and distance between cheese and UV-light strobe were determined to inactivate *Listeria monocytogenes* and *Penicillium roqueforti* on packaged and unpackaged cheese samples. Finally, quality changes of pulsed UV treated cheeses and mechanical properties of packaging material evaluated. TBARS test conducted to evaluate extent of lipid oxidation in cheese samples. CEILAB color measurement was applied to determine color changes on cheese after the treatment. Also, percent elongation and change in tensile strength of packaging material was measured.

4.2. Microorganisms

Penicillium roqueforti (ATCC 10110) was obtained from the American Type Culture Collection (ATCC) (Manassas, VA). The stock cultures were stored in 20% of glycerol solution at -80°C. The working cultures were grown on Potato Dextrose Agar (PDA) (Difco, Sparks, MD) slants and incubated at 25°C for 5 days. Incubated working cultures were stored in refrigerator at 4°C. *Listeria monocytogenes (*ATCC 43256) was

also obtained from ATCC. To maintain working culture, loopful portion of de-frozen *Listeria* cells were spread to Tyriptic Soy Agar (TSA) (Difco, Sparks, MD) slants. TSA agar slants were incubated at 37°C for 24 h. After one day incubation, working cultures were stored in the refrigerator at 4°C. To maintain viability, sub-culturing was performed every 3 or 4 weeks.

4.3. Preparation of Inoculum

For each experiment*, P. roqueforti* was grown at 25°C for 5 days on PDA slants. Three PDA slants were rinsed with 5 mL of sterile 0.03% Tween 80 solution and resulting spore solution was filtered through 4 layers of sterile cheese clothes. The spore solution yielded approximately 7 $\log_{10}$ CFU/mL, which used as the inoculum. Similarly, *L. monocytogenes* culture was transferred from the agar slant with a loop into 50 mL of Tyriptic Soy Broth (TSB) (Difco, Sparks, MD), then incubated at 37°C for 24 h. Following incubation, the *Listeria* cells centrifuged for 30 min at 4,000 x *g* at 10°C. After centrifugation, cells were washed with 0.1% peptone water and re-centrifuged at same conditions. After discarding the supernatant, cells were re-suspended to a level of approximately 8 $\log_{10}$ CFU/ mL in 0.1% peptone water, which was used as the inoculum.

4.4. Plastic Packaging Selection

In order to determine the plastic material that was going to be used, nine different brands of American Cheeses purchased from the local market. Three plastic packages of each brand was removed from the cheese and washed with 70% ethanol. Washed package samples were sent to Xenon Corp in order to test for UV-transmittance. The

transmissions were observed at 270 nm since most of the microbial activation occurs at 250-270 nm wavelength range. A plastic material with the highest UV-transmittance was chosen in order to efficiently transduce UV-light.

4.5. Inoculation of Cheese Samples

Individually packaged White American Cheeses obtained from a local supermarket and stored in the refrigerator at 4°C until used. Just before the pulsed UV-light treatment, 0.1 mL of *L. monocytogenes* or 0.3 mL of *P. roqueforti* were used to inoculate the top surface of cheese samples and plastic packaging was closed properly for the packaged samples. For the treatment of unpackaged cheese samples, after opening the cheese slices packages, same procedure was followed as packaged cheese samples, but surface was not covered by the plastic packaging material.

4.6. Design of Experiment

Pulsed UV- light treatment on the packaged and unpackaged cheeses, inoculated with *P. roqueforti* and *L. monocytogenes,* was performed from 5, 8 and 13-cm distances. These distances were chosen based on the previous pulsed UV-light studies in the lab and the design limitation of pulsed UV light chamber. The plastic packaging material type is used for American cheese is polypropylene (0.03 mm thickness according the manufacturer specifications). In order to determine maximum treatment time, preliminary UV light treatments have been conducted until to the point cheese started melting. Treatment time was selected as maximum 40, 50, and 60 s from 5, 8, and 13-cm distances, based on the preliminary treatments (Table 4.1). For each treatment, three replications were performed. The same pulsed UV experiments were also performed for

unpackaged cheeses at the same conditions. Also, three replications of pulsed UV untreated cheeses were used as control for each experiment.

Table 4.1. Design of pulsed UV experiment.

Packaged Cheese Samples			**Unpackaged Cheese Samples**		
L. monocytogenes* or *P. roqueforti			***L. monocytogenes* or *P. roqueforti***		
5 cm	**8 cm**	**13 cm**	**5 cm**	**8 cm**	**13 cm**
5 s	5 s	5 s	5 s	5 s	5 s
10 s	10 s	10 s	10 s	10 s	10 s
15 s	15 s	15 s	15 s	15 s	15 s
20 s	20 s	20 s	20 s	20 s	20 s
30 s	30 s	30 s	30 s	30 s	30 s
40 s	40 s	40 s	40 s	40 s	40 s
-	50 s	50 s	-	50 s	50 s
-	--	60 s	-	-	60 s

4.7. Pulsed UV-light Treatment

Inoculated cheese slices were treated with the SteriPulse-XL®3000 Pulsed Light Sterilization System (Xenon Corporation, Wilmington, MA; Figure 4.1). The pulsed UV system provided 3 pulses per second. An input voltage of 3,800 V was used to generate 1.27 J/cm^2 per pulse of radiant energy on the strobe surface for a new lamp (Bialka et al., 2008). The pulsed characteristics of this system provide high peak power and deliver high penetration for the destruction of microorganisms at deeper levels. The system also includes a laboratory test chamber (0.64 x 0.15 x 0.19 m) and several shelf settings, which allow for adjustments. Cheese samples (packaged and unpackaged) were placed on

the shelf of the UV-light chamber at three different distances; 5, 8 and 13-cm from the quartz glass window. Also, there is 5.8-cm distance between the UV strobe and the quartz window. The pulsed UV-light treatment was performed at several time-distance combinations as shown in experimental design table (Table 4.1).

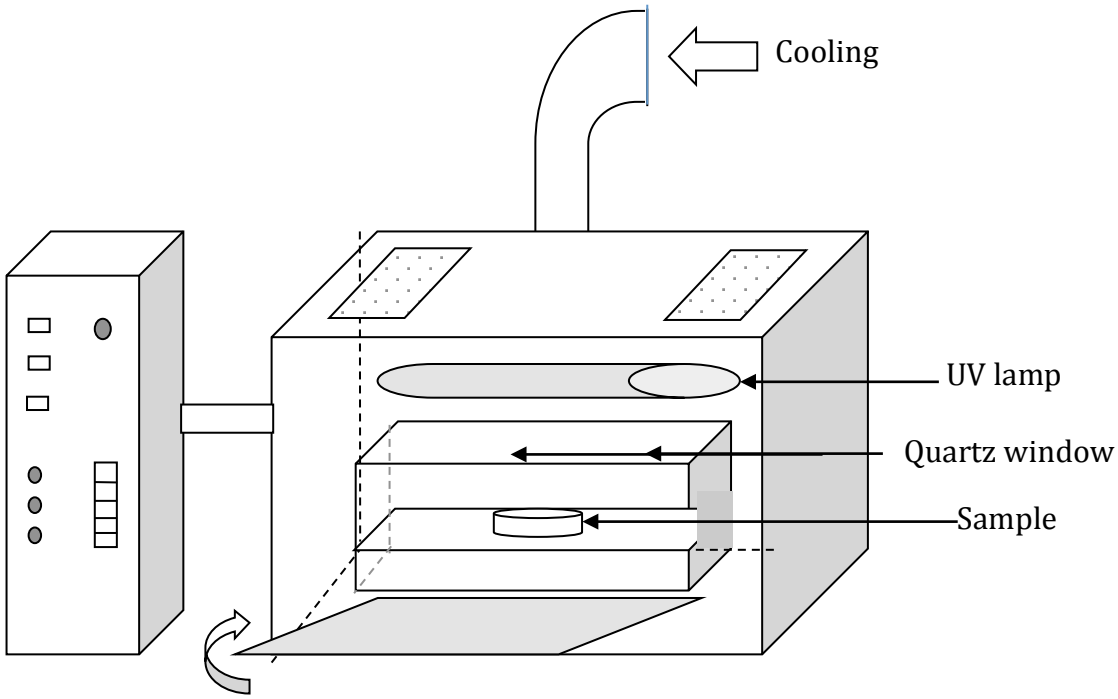

Figure 4.1. Schematic diagram of the pulsed UV light treatment system (Bialka et al., 2008).

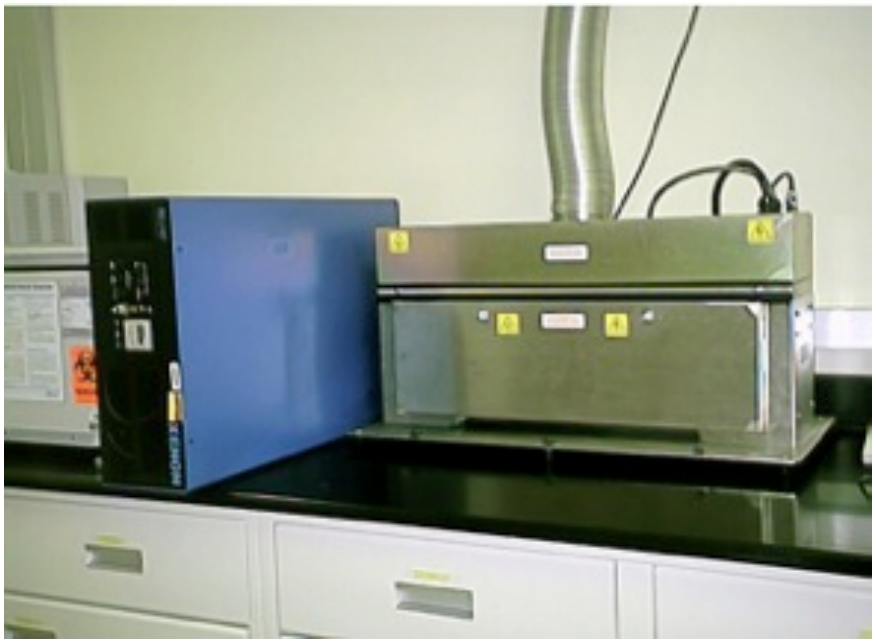

Figure 4.2. Picture of the pulsed UV light treatment system.

4.8. Sampling and Microbiological Analysis

After pulsed UV-light treatment, treated and untreated cheese samples were analyzed for tested microorganisms. Samples were put into a filtered stomacher bag (Gosselin, Borre, FR) containing 50 ml of 0.1% peptone water and stomached for 1 min at by a stomacher (model 400, Seward Ltd., Worthing, UK). The samples were then serially diluted with 0.1% peptone water followed by spiral plating on selective media by an autoplater (Autoplate 4000, Spiral Biotech, Norwood, MA). Palcam Agar (VWR, Gibbstown, NJ) was used for *L. monocytogenes,* while PDA was used for *P. roqueforti*. The plates were incubated at 37°C for 24 h for *L. monocytogenes* and at 25°C for 48 h for *P. roqueforti.* Then, plates were enumerated by an auto-counter (Q-Count, Version 2.1, Spiral Biotech).

4.9. Energy and Temperature monitoring

The amount of energy received at each three shelf height (5, 8, and 13cm) in the pulsed UV-light chamber was measured by a Nova Laser Power energy monitor (Ophir Optronics Ltd., Wilmington, MA) expressed in J/cm^2 /pulse. The energy level was averaged over 9 pulses (3 s). Also, temperature changes during the treatment were monitored using K thermocouple (Omegaette HH306; Omega Eng. Inc., Stamford, CT) up to 60 s by inserting the thermocouple in the middle point of the cheese sample.

4.10. Quality Measurements

To measure the quality changes of cheese samples after the pulsed UV treatment, lipid per-oxidation and color change were determined using the thiobarbituric acid-reactive substances (TBARS) method (Quattara et al., 2002) and CEILAB color method, respectively. For quality analysis untreated cheese samples as a control and treated at three levels at the mild (at 13 cm for 5 s), the moderate (at 8 cm for 20 s) and the extreme (at 5 cm for 40 s) were used. The procedure for the each test is described in details as follows.

4.10.1. TBARS test

TBARS method (Quattara et al., 2002) was used to determine the extent of lipid oxidation during pulsed UV light treatment. The principle of this test is the reaction of 1 molecule of malonaldehyde (a lipid metabolite in animal tissues) and 2 molecules of thiobarbituric acid (TBA) to form a red malonaldehyde-TBA complex that can be

analyzed spectrophotometrically (Keklik et al., 2009). Assay of TBARS measures malondialdehyde (MDA) present in the sample, as well as malondialdehyde generated from lipid hydroperoxides by the hydrolytic conditions of the reaction (Travisan et al., 2001).

The procedure used for this method is as follows: 10 g cheese samples before and after treatment with pulsed UV light at mild, moderate and extreme treatment conditions were homogenized for 1 min in a Waring blender (Waring Commercial, Torrington, CT) with 50 mL of distilled deionized water and 10 mL of 15% (wt/vol) trichloroacetic acid (VWR Intl., West Chester, PA). After the homogenate filtered was filtered through Whatman no. 4 filter paper (Whatman Inc., Florham Park, NJ), the filtrate was re-filtered through 0.45 μm syringe filter (Pall Life Sciences, East Hills, NY). The filtrate (8 mL) was then added to test tubes containing 2 mL of 0.06 *M* TBA (Merck, Whitehouse Station, NJ). After the test tubes incubated at 80°C for 90 min and cooled to room temperature before reading absorbencies at 520 nm using a spectrophotometer (DU series 500, Beckman, Fullerton, CA) (Keklik et al., 2009).

The malonaldehyde (MDA) content of the samples was calculated from a standard curve prepared by using serial dilutions of 1, 1, 3, 3- tetraethoxypropane (TEP) (TCI America, Portland, OR) (Lawlor et al., 2000; Keklik et al., 2009) and expressed as μm MDA/10 g cheese. First, $3x10^{-5}$ M solution was prepared by diluting $3x10^{-4}$ M stock solution of 1, 1, 3, 3-TEP. Then, aliquots of the solution (0, 0.33, 0.66, 1.0, 1.33, 1.66 and 2 mL) were diluted to 10 mL with deionized water. 5 mL of each dilution was added to test tubes containing 5 mL of 0.02 *M* TBA in 10% glacial acetic acid. Then, tubes were put into boiling water for 15 min. After cooling the tubes to the room temperature

absorbencies were read at 520 nm. Absorbance versus concentration of working solution was plotted to construct calibration curve (Keklik et al., 2009).

4.10.2. CIELAB color method

To assess the color changes of the cheese samples after treatment, a Minolta Chromo Meter CR 200 (Minolta Inc., Ramsey, NJ) colorimeter was used to measure *L**, *a**, and *b** color space (CIELAB). The CIELAB color spaces uses the following parameters: *L** indicates the lightness, and *a** and *b** are chromaticity coordinates*: -a** indicates green color, *+a* indicates red color, -b** indicates blue color while *+b** indicates yellow color (Bialka et al., 2008). Three randomly chosen spot on the untreated and treated samples were analyzed and then averaged to give the differences between *L**, *a** and *b**. Also the variation of color (ΔE) was calculated using ΔL*, Δa*, and Δb* values as shown in Equation 4.1 (Baur et al., 2005).

$$\Delta E = (\Delta L^{*2}+\Delta a^{*2}+\Delta b^{*2})^{0.5} \quad (5.2)$$

Figure 4.3. Minolta Chromo Meter CR 200 (Minolta Inc., Ramsey, NJ).

4.11. Plastic packaging material analysis

Plastic packages of cheese samples untreated and treated at mild, moderate and extreme conditions with pulsed UV light were evaluated for mechanical properties of plastics. Cheese packaging samples were cleaned by using 70% ethanol and cut to desired measurements (5 cm long and 1 cm width). The material of the plastic packages was polypropylene with the thickness of 0.03 mm according to the manufacturer's specifications. The samples were analyzed according to ASTM D 882 – 02 test methods using computer controlled Instron Model 3345 (Instron Corp., Canton, MA) (Figure 4.1) with BlueHill software. Samples (5 x 1 cm) gripped along-machine to test each condition. Three replications were performed for each condition. Based on the data, elastic modulus, yield strength, percentage of elongation at yield, maximum tensile strength, and percentage of elongation at break were determined.

Elastic modulus is the mathematical description of an object or material's to deform elastically when a force is applied (Askeland and Phule, 2006). Decrease in the elastic modulus value indicates that material loses stiffness (Keklik et al., 2009). The yield strength of a material is defined in engineering and materials science as the stress at which a material begins to deform plastically. Percent elongation at yield point indicates the amount of stretch materials at yield point (Keklik et al., 2009). Tensile strength measures the force required to pull material to the point where it breaks. Percent elongation at break point shows how the packaging material can stretch before it breaks.

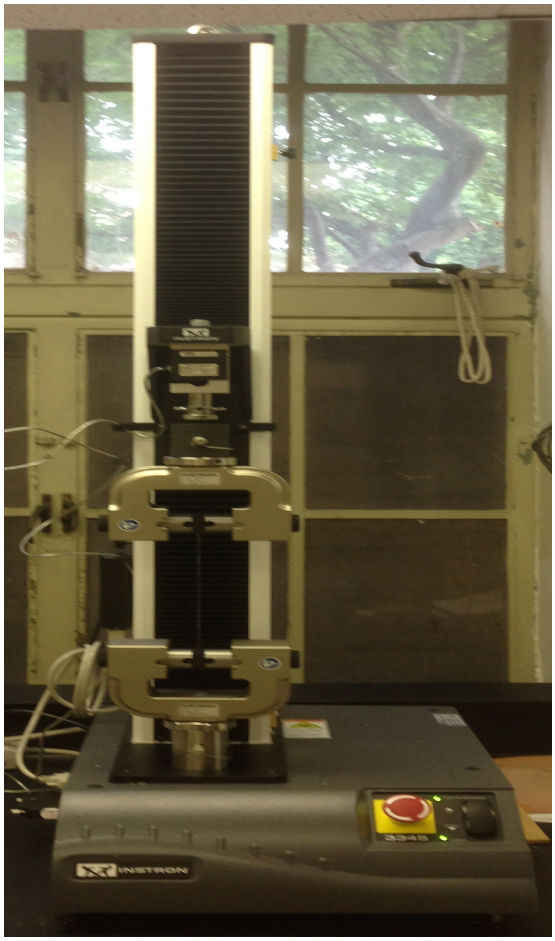

Figure 4.4. Instron machine (Model 3345, Instron Corp., Canton, MA) used for measurement of mechanical properties of plastic materials.

4.12. Statistical Analysis

Each treatment was triplicated. Minitab (Version 14, Minitab Inc., State College, PA) statistical program was used. The microbial reductions, MDA contents, color changes, energy levels and temperature changes, mechanical properties of the packaging material were analyzed by using ANOVA-General Linear Model. The significant differences in mean values determined using Tukey's method at 95% CI.

CHAPTER 5

RESULTS and DISCUSSION

In this study, packaging plastic material was selected based on the UV light transmission. Then, inactivation of *P. roqueforti* and *L. monocytogenes* on packaged and unpackaged cheeses by pulsed UV- light was studied. Finally, cheese quality and mechanical properties of plastic packaging were analyzed at mild, moderate, and extreme treatment conditions.

5.1. Selection of the Packaging Material

Nine different brands of packaged American Cheeses purchased from the local supermarket. Three plastic packages of each brand was removed from the cheeses and washed with 70% ethanol to clean. Then, plastic packaging samples were sent to Xenon Corp (Wilmington, MA) in order to measure their UV-transmittance characteristics. The transmissions were observed up to 270 nm since most of the microbial activation occurs between 250 and 270 nm (NESC, 2000). A plastic material with the highest UV-transmittance had to be chosen in order to efficiently transmit UV-light. Cheese sample #4 had the highest light transmittance with 45% at 270 nm. The manufacture of the sample #4 confirmed that this is polypropylene with 0.03 mm thickness. Results are shown in Figure 5.1. All readings were done at 270 nm because of the germicidal effect at that wavelength.

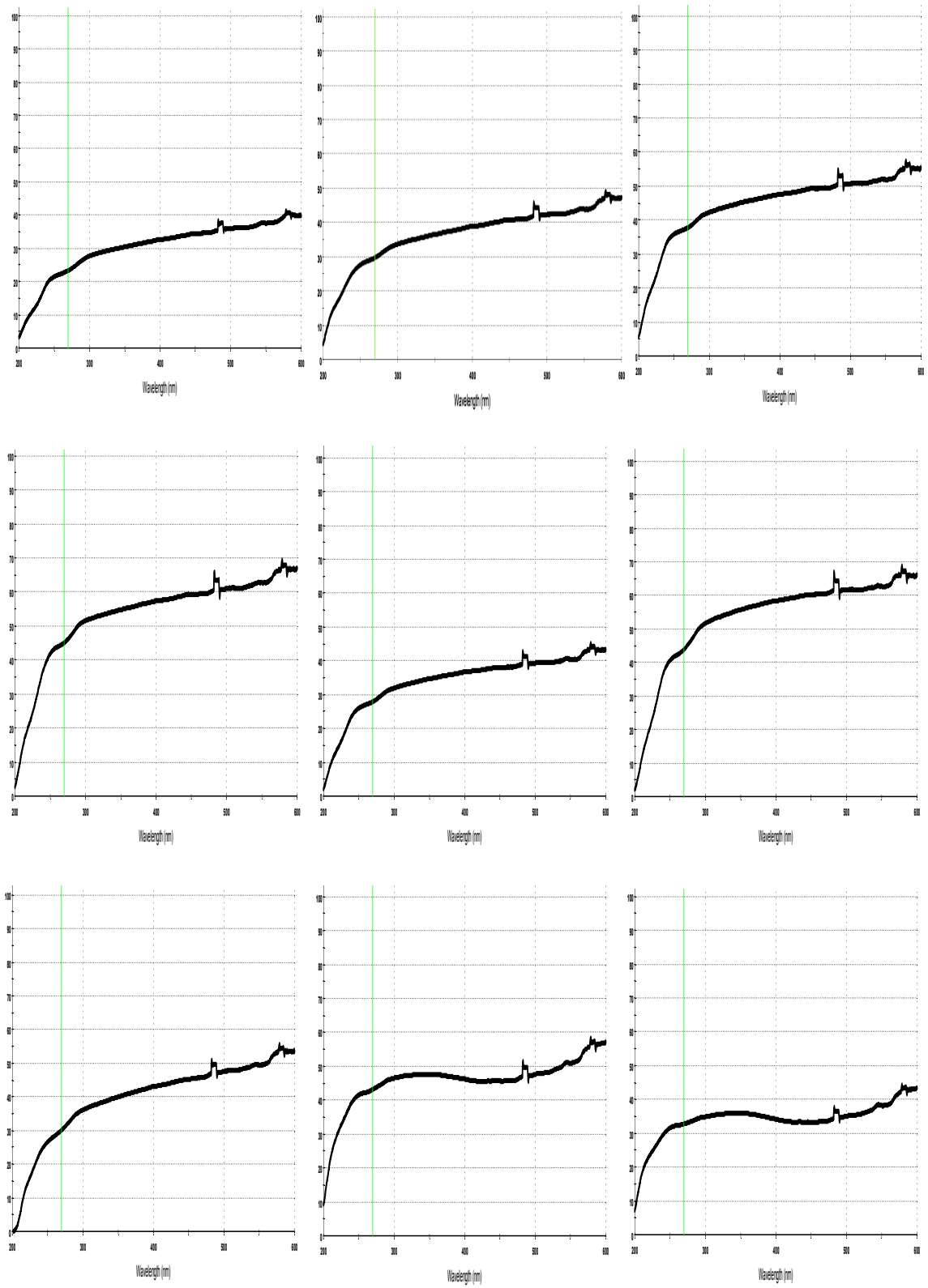

Figure 5.1. Percent light transmittance of plastic packages: A) Cheese Brand #1; B) CheeseBrand #2; C) Cheese Brand #3; D) Cheese Brand #4; E) Cheese Brand #5; F) CheeseBrand #6;G) Cheese Brand #7; H) Cheese Brand #8 I) Cheese Brand #9

5.2. Microbiological inactivation by Pulsed UV-light treatment

Artificially inoculated packaged and unpackaged cheese samples with *Listeria monocytogenes* and *Penicillium roqueforti* were microbiologically analyzed before and after pulsed UV-light treatments as shown as experimental design (Table 4.1). The $\log_{10}$ reductions determined.

5.2.1. *Penicillium roqueforti* inactivation

5.2.1.1. Packaged Cheese

The range of the microbial $\log_{10}$ reduction for *P.roqueforti* inoculated packaged cheese samples is 0.38 to 1.25 CFU/cm^2 for 5 – 40-s treatments at 13 and 5-cm distances, respectively (Table 5.1). The log reductions obtained after 20, 30 and 40-s treatments were not significantly different from each other ($p>0.05$), but significantly different from 15, 10 and 5-s treatments ($p<0.05$) for all distances. Treatments for 5 and 10-s were not significantly different from each other ($p>0.05$) and significantly different from all other treatments ($p<0.05$). At 5-cm distance from the quartz window, the log reductions were between 0.61-1.25 CFU/cm^2. While there was no significantly difference between the treatments of 5 and 10-s ($p>0.05$), they were significantly different from other treatments ($p<0.05$). 15-s treatment was significantly different from all other treatments at this distance ($p<0.05$). Also, there was no significant difference between the treatments at 20, 30 and 40-s ($p>0.05$). At 8-cm distance, the reductions ranged from 0.4 to 1.22 CFU/cm^2 until 50-s. However, visual appearance of cheese was changed after 30-s. The 5 and 10-s treatment was not significantly different ($p>0.05$) from each other, but significantly different from others ($p<0.05$). 15-s treatment was not significantly different from 20 and

30-s treatments ($p>0.05$), but different from 5, 10 and 40-s treatments ($p<0.05$). At 13-cm distance, reduction range is 0.38 to 1.24 log CFU/cm^2 till 60-s and the visual changes are observed after 40-s. The 5, 10 and 15-s treatments were not significantly different from each other ($p>0.05$), but significantly different from 20, 30 and 40-s treatments ($p<0.05$). Also, 20, 30 and 40-s treatments were not significantly different from each other ($p>0.05$). The optimum treatment condition was determined as 30-s at 5-cm distance and 40-s at 8-cm distance. Approximately 1.2 $\log_{10}$ reduction was obtained at the both conditions without observing any visible changes at cheese samples.

5.2.1.2. Unpackaged Cheese

For the decontamination of unpackaged cheese samples inoculated with *P. roqueforti,* the microbial reduction range is 0.42 to 1.32 CFU/cm^2 for 5 – 40-s treatments at 13 and 5-cm distances, respectively (Table 5.2). At the 5-cm distance, 5 and 10-s treatments were not again significantly different from each other ($p>0.05$), but they both were significantly different from15, 20, 30 and 40-s treatments ($p<0.05$). Treatment for 15-s was not significantly different from 20-s treatment ($p>0.05$), but significantly different from other treatments ($p<0.05$). At 8-cm distance, the microbial inactivation was achieved as high as 1.24 CFU/cm^2 at 50-s. The 5-s treatment was significantly different from others except 10-s treatment ($p<0.05$). The treatments of 15, 20 and 30-s were not significantly different from each other ($p>0.05$), but significantly different from 40-s treatment ($p<0.05$). At 13-cm distance, maximum $\log_{10}$ reduction was 1.26 CFU/cm^2 at 60-s. However, visual quality changes occurred after 40-s treatment. There was no significant difference between the treatments of 5, 10 and 15-s ($p>0.05$).

Table 5.1. Log_{10} reduction of *P.roqueforti* inoculated packaged cheese samples[1]

Distance[2] (cm)	**Treatment time (s)**	**Log_{10} reductions (CFU/cm^2)**
5	5	0.61±0.03a
	10	0.69±0.1a
	15	0.92±0.03b
	20	1.1±0.06bc
	30	1.18±0.08c
	40	1.25±0.02c
8	5	0.4±0.07a
	10	0.48±0.7a
	15	0.9±0.06b
	20	1.1±0.09bc
	30	1.12±0.09bc
	40	1.18±0.03c
	50	1.22±0.05c
13	5	0.38±0.02a
	10	0.4±0.13a
	15	0.62±0.02b
	20	0.97±0.07bc
	30	1.1±0.05bc
	40	1.15±0.13c
	50	1.19±0.03c
	60	1.24±0.03c

[1] At the same distance, values not followed by the same letter are significantly different ($p<0.05$).
[2] Distance from the quartz window, which is 5.8 cm away from UV-strobe.

The 15-s treatment was not significantly different from 10 and 20-s ($p>0.05$), but different from 5, 30 and 40-s treatments ($p<0.05$). There was also no significant difference between the 30 and 40-s treatments ($p>0.05$). As conclusion, the optimum treatment condition was determined as 20-s at 5-cm distance, since there was no remarkable difference between 20 and 30-s reduction values. At 8 and 13-cm distances, 40 and 50-s treatments were optimum with no observable sensory changes. Approximately, 1.2 $\log_{10}$ reduction was obtained at all three conditions at cheese samples.

Reduction of *P. roqueforti* was compared between distances from UV lamp for packaged and unpackaged samples. All distances were significantly different ($p<0.05$) from each other for both packaged and unpackaged samples and no interaction between time versus shelf distances. For the evaluation of package effect paired t-test ($\alpha=0.05$) was conducted between $\log_{10}$ reduction values of packaged and unpackaged samples, which suggested that there is no significant difference between packaged and unpackaged cheeses ($p\geq0.05$).

There is no published study for inactivation of *P. roqueforti* by pulsed UV-light on a food product. However, de Souza and Garica (2012) showed the resistance of different microorganisms to ultraviolet radiation at the approximate doses of UV at 254 nm (in µW.s.cm-2). They indicated there that inactivation of *P. roqueforti* for 1 $\log_{10}$ reduction 13.000, and 2 $\log_{10}$ reduction at 26.400 µW.s.cm^2 UV dose.

Jun et al. (2003) investigated the effectiveness of pulsed UV-light system for inactivating fungal spores of *Aspergillus niger* in corn meal. They evaluated three process parameters with treatment time (20–100 s), voltage input (2000–3800 V), and distance

Table 5.2. Log_{10} reduction of *P.roqueforti* inoculated unpackaged cheese samples[1]

Distance[2] (cm)	**Treatment time (s)**	**Log_{10} reductions (CFU/cm^2)**
5	5	0.56±0.05a
	10	0.7±0.01a
	15	0.95±0.06b
	20	1.1±0.06bc
	30	1.24±0.03cd
	40	1.32±0.02d
8	5	0.52±0.03a
	10	0.6±0.05a
	15	092±0.05b
	20	1.0±0.04bc
	30	1.13±0.08bc
	40	1.2±0.1bc
	50	1.24±0.17c
13	5	0.42±.002a
	10	0.51±0.07ab
	15	0.68±0.05ab
	20	0.81±0.01b
	30	1.15±0.08c
	40	1.18±0.18c
	50	1.25±0.11c
	60	1.26±0.08c

[1] At the same distance, values not followed by the same letter are significantly different ($p<0.05$).
[2] Distance from the quartz window, which is 5.8 cm away from UV-strobe.

from the UV strobe (3–13 cm). Optimization of the process was validated by a quadratic regression equation designed to fit the experimental $\log_{10}$ reduction of fungal spores. Model prediction for a 100-s treatment time, 3 cm of distance from the UV strobe, and with 3800 V input gave a 4.93$\log_{10}$ reduction of *A. niger* (Jun et al., 2003). Compared to our study log reductions they achieved are remarkably high since they treated the food sample closer and much longer. However, cheese was not available to use those conditions.

5.2.2. *Listeria monocytogenes* inactivation

5.2.2.1. Packaged Cheese

The range of the microbial $\log_{10}$ reduction for the *L. monocytogenes* inoculated packaged cheese samples is 1.13 to 2.98 CFU/cm^2 after 5 and 40-s treatments at 13 and 5-cm distances, respectively (Table 5.3). At 5-cm distance, 5 and 10-s treatments were not significantly different ($p>0.05$), whereas they were significantly different from other treatments ($p<0.05$). The 15 and 20-s treatments were significantly different from all other treatments ($p<0.05$). The 30 and 40-s treatments were not significantly different from each other's ($p>0.05$). Obtained microbial reduction was in the range of 1.41-2.98 CFU/cm^2 at 5-cm distance for 5 – 40-s treatments. Visual characteristics of cheese samples started changing after 20-s. At 8-cm distance, the microbial reduction range was 1.17-3.07 CFU/cm^2 for 5 – 50-s. Treatments of 20, 30, and 40-s were not significantly different from each other ($p>0.05$).). Also 30-s treatment was not significantly different from 15-s treatment ($p>0.05$), whereas they were significantly different from 10 and 5-s treatments ($p<0.05$). Visual characteristics and smell of cheese started changing after 30-s. At 13-cm distance, the microbial reduction range was 1.13-2.85 CFU/cm^2 for 5 – 60-s

treatments. Treatments of 20, 30 and 40-s were not significantly different from each other ($p>0.05$), but significantly different from the others ($p<0.05$). The 15 and 10-s treatments were not significantly different from each other ($p>0.05$), but significantly different from the other treatments ($p<0.05$). The 5-s treatment was significantly different from all other treatments ($p<0.05$). Visual characteristics and smell of cheese started changing after 40-s treatment. For the *L. monocytogenes* inoculated packaged cheese samples, optimum conditions for inactivation were 40-s at 5 and 8-cm, and 50-s at 13-cm. Approximately, 1.3 - 1.2 $\log_{10}$ reductions were observed at those conditions without any visible changes.

5.2.2.2. Unpackaged Cheese

For the decontamination of *L. monocytogenes* inoculated unpackaged cheese samples the inactivation range was 1.1 to 3.08 CFU/cm^2 for 5 – 40-s treatments at 13- and 8-cm distances, respectively (Table 5.4.). At 5-cm distance; 20, 30, 40-s treatments were not significantly different from each other ($p>0.05$). Treatments of 15, 20 and 30-s were not significantly different from each other ($p>0.05$), whereas they were significantly different from 5, 10 and 30-s treatments ($p<0.05$). The 5 and 10-s treatments were not significantly different from each other ($p>0.05$), but significantly different from the other treatments ($p<0.05$). At 8-cm distance; 20, 30, 40, and 50-s treatments were not significantly different ($p>0.05$) from each other, but 15-s treatment was significantly different from all other treatments ($p<0.05$). Treatments of 5 and 10-s were not significantly different from each other ($p>0.05$), but different from the others ($p<0.05$). The treatment at13-cm distance is same with 8-cm distance.

Table 5.3. Log_{10} reduction of *L. monocytogenes* inoculated packaged cheese samples[1].

Distance [2] (cm)	**Treatment time (s)**	**Log_{10} reductions (CFU/cm^2)**
5	5	1.41±0.15a
	10	1.63±0.10a
	15	2.11±0.03b
	20	2.64±0.01c
	30	2.95±0.05d
	40	2.98±0.16d
8	5	1.17±0.21a
	10	1.37±0.25a
	15	2.25±0.08b
	20	2.7±0.12bc
	30	2.91±0.03c
	40	2.93±0.04c
	50	3.07±0.04c
13	5	1.13±0.07a
	10	1.71±0.17b
	15	1.72±0.14b
	20	2.3±0.06c
	30	2.56±0.20c
	40	2.67±0.20c
	50	2.77±0.12c
	60	2.85±0.27c

[1] At the same distance, values not followed by the same letter are significantly different ($p<0.05$).
[2] Distance from the quartz window, which is 5.8 cm away from UV-strobe.

Table 5.4. Log_{10} reduction of *L.monocytogenes* inoculated unpackaged cheese samples[1]

Distance[2] (cm)	**Treatment time (s)**	**Log_{10} reductions (CFU/cm^2)**
5	5	1.28±0.14a
	10	1.7±0.04a
	15	2.41±0.17b
	20	2.7±0.23bc
	30	2.81±0.03bc
	40	3.08±0.06c
8	5	1.1±0.11a
	10	1.4±0.2a
	15	2.22±0.1b
	20	2.68±0.14c
	30	2.83±0.02c
	40	2.91±0.04c
	50	3.00±0.11c
13	5	1.33±0.1a
	10	1.59±0.09a
	15	2.17±0.07b
	20	2.62±0.09c
	30	2.71±0.24c
	40	2.79±0.03c
	50	2.87±0.2c
	60	3.06±0.02c

[1] At the same distance, values not followed by the same letter are significantly different ($p<0.05$).
[2] Distance from the quartz window, which is 5.8 cm away from UV-strobe

The optimum treatment conditions for *L. monocytogenes* inactivation on unpackaged cheeses was determined as 40, 50 and 50-s from the distances of 5, 8, and 13-cm, respectively. Approximately 3 $\log_{10}$ reduction was achieved at these conditions.

Log reduction of *L. monocytogenes* was compared between distances for packaged and unpackaged samples. All distances were significantly different from each other for packaged cheese samples ($p<0.05$), whereas 8 and 13-cm distances were not significantly different ($p>0.05$) for unpackaged cheese samples.
However, the evaluation of package effect paired t-test ($\alpha=0.05$) was conducted between $\log_{10}$ reduction values of packaged and unpackaged samples, which suggested that there is no significant difference between packaged and unpackaged cheeses (p-value$\geq$0.05).

Log reduction of *L. monocytogenes* was compared between distances for packaged and unpackaged samples. All distances were significantly different from each other for packaged cheese samples ($p<0.05$), whereas 8 and 13-cm distances were not significantly different ($p>0.05$) for unpackaged cheese samples. However, the evaluation of package effect paired t-test ($\alpha=0.05$) was conducted between $\log_{10}$ reduction values of packaged and unpackaged samples, which suggested that there is no significant difference between packaged and unpackaged cheeses (p-value$\geq$0.05).

Keklik et al. (2009) was evaluated of the effectiveness of pulsed UV-light on the inactivation of *L. monocytogenes* on unpackaged and vacuum-packaged chicken frankfurters. Samples were inoculated with *L. monocytogenes* Scott A on the top surfaces, and then treated with pulsed UV-light. Log reductions (CFU/cm^2) on unpackaged samples were between 0.3 and 1.9 after 5-s treatment at 13 cm and 60-s treatment at 5 cm, respectively. Log reductions on packaged samples ranged from 0.1 to

1.9 after 5-s treatment at 13 cm and 60-s treatment at 5 cm, respectively. In our study up to ~3 log10 reductions were observed. This difference may be caused because of the strain differences of *L. monocytogenes* and using different food products.

5.3. Energy and temperature measurements

Since the energy levels are same for samples and temperatures changes did not show difference between packaged and unpackaged cheeses after preliminary treatments, energy and temperature were measured only for unpackaged cheese samples. Energy levels and temperature changes at each treatment conditions are shown at the Table 5.5. Obtained total amount of energy values were between 3.08 and 64.4 J/cm^2. Energy levels were measured until 40, 50 and 60-s from 5, 8 and 13-cm distances, respectively. At 5-cm, the energy level varied from 8.05 to 64.4; while at 8 cm, 5.53 to 50.53; and at 13-cm distances, 3.08 to 36.96. Statistical analysis was conducted until 40-s for each distance. Energy levels obtained at 5, 8 and 13-cm were significantly different from each other ($p>0.05$). Energy levels increased significantly with treatment time at the each distance ($p<0.05$). Also, energy levels increased significantly at each treatment times ($p<0.05$).

The initial temperature of the cheese samples was 19.4°C. Temperature differences ranged from 2 to 38.6 from 5-s at 13-cm distance and 40-s at 5-cm distance, respectively. The temperature differences at 8 cm were not significantly different from those at 5 and 13 cm ($P>0.05$), while temperature differences at 5 cm were significantly higher than from those at 13 cm ($P<0.05$). At each distance, temperature changed significantly different with the time ($P<0.05$), except after 5 and 10-s at 13-cm distances.

Table 5.6. Energy levels and temperature changes for cheese samples [1]

Distance[2] (cm)	**Treatment time (s)**	**Total energy (J/cm^2)**	**Temperature change ($T-T_{inital}$) ($^{\circ}C$)**
5	5	6.67±0.00a	7.8±1.2a
	10	13.34±0.01b	18.2±1.2b
	15	20±0.01c	23.5±2.4c
	20	26.7±0.02d	28±1.8d
	30	40±0.03e	32.7±1.5e
	40	53.4±0.04f	38.6±1.1f
8	5	4.58±0.00a	3.9±0.9a
	10	9.16±0.01b	5.6±2.1b
	15	13.74±0.01c	7.6±2.4c
	20	18.3±0.02d	10.1±1.7d
	30	27.48±0.03e	14.2±1.8e
	40	36.6±0.04f	18.1±2.0f
	50	45.8±0.05g	20.6±3.9g
13	5	2.55±0.0a	2.0±0.8a
	10	5.11±0.01b	3.1±1a
	15	7.66±0.01c	3.7±1.5b
	20	10.2±0.02d	4.6±0.7c
	30	15.32±0.03e	6.9±1.3d
	40	20.42±0.04f	9.2±2.1e
	50	25.53±0.05g	11.5±2.0f
	60	30.63±0.06h	13.8±1.6g

[1] At the same distance, values not followed by the same letter are significantly different ($p<0.05$).
[2] Distance from the quartz window, which is 5.8 cm away from UV-strobe

The temperature changes and the energy levels at the optimum treatment conditions were 27.48 J/cm^2 and 14.2°C, respectively.

In the study by Keklik et al. (2009) effectiveness of pulsed UV-light on unpackaged and vacuum packaged chicken frankfurters was evaluated. They also measured energy and temperature changes in the frankfurters. The total amount of energy absorbed ranged from 2.7 to 60.2 J/cm^2 after 5-s treatment at 13-cm and after 60-s treatment at 5 cm, respectively, and temperature difference of packaged samples ranged from 2.2 to 43.6°C. The both energy and temperature measurement results are quite similar our results.

Bialka et al. (2008), inactivated *E. coli* O157:H7 and *Salmonella* on strawberries with pulsed UV-light. They observed maximum UV dose at 5-cm after 60-s treatment which was 64.8 J/cm^2, and approximately 50°C temperature difference, similarly our results.

Table 5.5. Broadband energy measurement during pulsed UV-light treatment[1]

Distance[2]	**Energy[3] (J/pulse)**	**Energy[4] (J/cm^2 per pulse)**
5	8.05	0.445
8	5.53	0.305
13	3.08	0.170

[1]Radiometer was calibrated at 254 nm and measurements have reported in the wavelength range of 100 to 1100 nm.
[2]The distance between the quartz window and the UV strobe was 5.8 cm.
[3]Energy was averaged over 9 pulses; three independent measurements were conducted.
[4]Surface area of the radiometer detector head was 18.096 cm^2.

5.4. Quality measurements

Quality of cheeses was measured for both packaged and unpackaged samples at the mild (13 cm; 5-s), moderate (8 cm; 30-s) and extreme (5 cm; 60-s) conditions. Cheese samples were analyzed in terms of lipid peroxidation and color. Also packaging material was analyzed for mechanical properties.

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5.4.1.TBARS test

The lipid oxidation extent of packaged and unpackaged cheese samples were measured using TBARS test (Table 5.7). The amount of MDA was expressed as micrograms of MDA/g cheese. For the packaged samples, the extent of lipid oxidation was between 12.6 and 30.7 μg MDA/g cheese after the 5-s treatment at 13-cm and the 40-s treatment at 5-cm, respectively. There was no significant difference between untreated and mild treated packaged and unpackaged cheese samples ($p>0.05$). For both the packaged and unpackaged cheeses, those were treated at moderate and extreme conditions were significantly different from each other and mild treated and untreated cheeses ($P<0.05$). For the unpackaged samples, the extent of lipid oxidation was between 13.0 and 34.8 μg MDA/g cheese. The MDA content was slightly higher in the unpackaged cheese samples then packaged cheese samples, although there was no significant difference between ($p>0.05$).

5.4.2. CIELAB color measurement

CIELAB color method was used to determine color changes at the treated packaged and unpackaged cheese samples. The samples were analyzed after the treatment conditions of mild (5-s at 13 cm), moderate (30-s at 8 cm), and extreme (60-s at 5 cm).

Table 5.7.TBARS test results for packaged and unpackaged cheese samples[1].

Sample type	Treatment conditions	µg MDA/g cheese
Packaged	Untreated	9.5a
	Mild (13 cm- 5s)	12.6a
	Moderate (8 cm-30s)	19.5b
	Extreme (5 cm-40s)	30.7c
Unpackaged	Mild (13 cm- 5s)	13.0a
	Moderate (8 cm-30s)	21.1b
	Extreme (5 cm-40s)	34.8c

[1] Within the same column, values not followed by the same letter are significantly different for packaged and unpackaged cheese samples ($p<0.05$).

Color differences between samples were expressed with using ΔL*, Δa*, and Δb* parameters (Table 5.8).

For each treatment, ΔL*, Δa*, and Δb* values of cheeses were measured before and after treatment. The maximum differences between L*, a* and b* values were -1.79, 0.65, and -2.04, respectively. Maximum ΔL* was observed at extreme (40-s at 5 cm) conditions on packaged cheese sample, while maximum Δa* was observed at moderate (30-s at 8 cm) on packaged cheese, and maximum Δb* was observed at unpackaged cheese samples at the extreme (40-s at 5 cm) conditions. There was no significant difference between ΔL* values for any treatment ($p>0.05$). However, Δb* value was significantly different ($p<0.05$) at mild conditions from those at moderate and extreme for the packaged cheeses. For the unpackaged cheese samples, there was no significant difference between the treatments for Δa*($p>0.05$). Also, there was no significant difference for Δb* values between mild and moderate conditions ($p>0.05$), but

they were significantly different from the extreme conditions for the unpackaged cheese samples ($p<0.05$). Also, paired t test was conducted to measure effect of plastic packaging to the change of color parameters ($\alpha=0.05$). ΔL^*, Δa^*, and Δb^* values for packaged and unpackaged cheeses were not statistically different. Overall, the largest variation of color (**ΔE**) observed at extreme conditions for packaged cheese. For **ΔE,** moderate and extreme treatments were significantly different from the mild treatment for packaged and unpackaged cheeses ($p<0.05$).

In the study by Keklik et al. (2009), the color changes caused by pulsed UV-light on chicken carcasses were evaluated. Chicken carcasses changed color at extreme (60-s at 5 cm) conditions statistically significant ($P<0.05$) while mild and moderate treatments did not cause significant changes ($P>0.05$) in the chicken color for both unpackaged and vacuum packaged samples.

Table 5.8. Color changes in packaged and unpackaged cheeses after pulsed UV-light treatments[1].

Sample type	**Treatment conditions**	**ΔL^*** ($L^*-L^*_{untreated}$)	**Δa^*** ($a^*-a^*_{untreated}$)	**Δb^*** ($b^*-b^*_{untreated}$)	**ΔE** $(\Delta L^{*2}+\Delta a^{*2}+\Delta b^{*2})^{0.5}$
Packaged	Mild	-0.36±0.29a	0.29±0.04a	-0.73±0.23a	0.86a
	Moderate	-1.33±0.62a	0.72±0.08a	-2.47±0.05b	2.90b
	Extreme	-1.38±0.18a	0.75±0.15b	-2.82±0.41b	3.23b
Unpackaged	Mild	-0.42±0.14a	0.31±0.07a	-0.64±0.21a	0.82a
	Moderate	-1.34±0.34a	0.9±0.06a	-2.56±0.07b	3.03b
	Extreme	-1.39±0.63a	0.99±0.17b	-2.31±0.19b	2.87b

[1] Within the same column, values not followed by the same letter are significantly different for packaged and unpackaged cheese samples ($p<0.05$).

5.5. Mechanical properties of packaging material

Mechanical properties of the plastic packaging material of cheese samples were evaluated after treatment at mild (5-s at 13 cm), moderate (30-s at 8 cm) and extreme (40-s at 13 cm) treatment conditions. Data for elastic modulus, yield strength, percent elongation at yield point, maximum tensile strength and percent elongation at break were obtained. Plastic material used for these tests was polypropylene with 5 cm length, 1 cm width and 0.003 cm thickness according to the manufacturer's specifications.

Elastic modulus values of the plastic package material are shown in Table 5.9. For the untreated sample the elastic modulus value was as high as 516.81 MPa. After the extreme pulsed UV-light treatment this value decreased to 397.64 MPa, which indicates that UV-light treatment, caused deformation on the plastic packaging. Even though there was a decreasing trend between elastic modulus data, there was no significant difference between untreated, mild and moderate treatments ($p>0.05$), while they all were significantly different ($p<0.05$) from those at extreme conditions.

Table 5.9. Elastic modulus of packaging material of cheese before and after UV-light treatments[1].

Treatment conditions	**Elastic modulus (MPa)**
Untreated	516.81±81.76a
Mild (13 cm- 5s)	496.16±22.64a
Moderate (8 cm-30s)	457±23.3a
Extreme (5 cm-40s)	397.64±45.55b

[1] Within the same column, values not followed by the same letter are significantly different ($p<0.05$).

As shown in Table 5.10, the yield strength of samples was between the range of 5.36 - 5.8 MPa. The yield strength of samples was not significantly different ($p>0.05$) at mild, moderate or extreme conditions. Percent elongation at yield point varied 0.0474 to 0.068. There was no significant difference ($p>0.05$) between the treatments.

Table 5.10. Yield strength and percent elongation of packaging material

Treatment conditions	Yield strength (MPa)	% Elongation at yield point
Untreated	5.8±1.3a	0.0634±0.003a
Mild (13 cm- 5s)	5.36±0.2a	0.0474±0.002a
Moderate (8 cm-30s)	5.63±0.25a	0.068±0.008a
Extreme (5 cm-40s)	5.39±0.2a	0.0522±0.000a
conditions	Max tensile strength (MPa)	% Elongation at break
Untreated	5.8±1.35a	524±57.90a
Mild (13 cm- 5s)	5.45±0.19a	477.3±14.72ab
Moderate (8 cm-30s)	5.96±0.25a	370±86.50ab
Extreme (5 cm-40s)	5.06±0.41a	295±81.10b

[1] Within the same column, values not followed by the same letter are significantly different ($p<0.05$).

Maximum tensile strength of the plastic material varied between 5.06 to 5.96 MPa (Table 5.10). For mild, moderate and extreme conditions, the maximum tensile strength did not differ significantly ($p>0.05$). Percent elongation at break was the between the range of 295 to 524%. A decreasing trend was observed at % elongation values with the increase of treatment time. Also, there was no significant difference ($p>0.05$) between the

untreated, mild and moderate treatments; while there was a significant difference ($p<0.05$) between untreated and the one treated extreme conditions.

In previous studies, yield strength and maximum tensile strength decreased after extreme pulsed UV light treatment similarly our results (Keklik et al., 2009: George et al., 2007) although it is not significantly ($p>0.05$).

5.6. Conclusions and suggestions for future research

The results obtained in this study demonstrated that pulsed UV- light can inactive both *P. roqueforti* and *L. monocytogenes* on cheese surface, it is relatively less effective on *P. roqueforti* than *L. monocytogenes,* since molds are more resistant than bacteria since *P. roqueforti* spores has darker color, which reduces the efficacy of pulsed UV light for the inactivation. Optimum pulsed UV-light treatment conditions for both microorganisms determined according to maximum $\log_{10}$ reductions with acceptable changes in evaluated characteristics of cheese. The optimum UV-light treatment for *P. roqueforti* inactivation for both packaged and unpacked cheeses were 8-cm distance for 30-s which caused ~1.1 $\log_{10}$ reduction. Reductions of *L. monocytogenes* at the same treatment conditions were about 2.9 and 2.8 packaged and unpackaged cheeses, respectively.

The lipid peroxidation and color changes of the cheeses were evaluated after mild (5-s at 13-cm), moderate (30-s at 8-cm) and extreme (40-s at 13-cm) treatment conditions. The color and chemical quality of cheeses did not show significant difference after mild treatments. However after moderate and treatment, a* and b* parameters have changed significantly ($p<0.05$), while L parameter did not significantly changed

(P>0.05). The plastic material (polypropylene) was evaluated in terms of mechanical properties after mild, moderate and extreme treatments, as well. There was a decreasing trend between elastic modulus data, however there was no significant difference between untreated, mild and moderate treatments (p>0.05). The other mechanical properties did not show significant difference after treatments, as well; except percent elongation at break after extreme treatment. In conclusion, this study clearly demonstrated pulsed UV has a potential to decontaminate packaged and unpackaged cheeses without causing quality changes in terms of evaluated parameters.

Further detailed study on the efficacy of the pulsed UV-light and caused quality changes should be compared to conventional UV-light. Sensory evaluation should be conducted to determine acceptable changes in the cheese quality. Also, detailed study of inactivation of other pathogenic microorganisms and spoilage molds by pulsed UV could be helpful in designing process equipment for decontamination of cheeses. Finally, development of mathematical models would be helpful to estimate processing parameters.

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