ENVIRONMENTAL RELATED FACTORS THAT INFLUENCE PULSED LIGHT INACTIVATION OF BACTERIA

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Sheena T. Hilton

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ABSTRACT

Non-thermal food processing technologies are a growing area of research, due to the increasing consumer demand for minimally processed foods. Pulsed Light (PL), which consists of short pulses of high intensity broad-spectrum light ranging in wavelength from 200 to 1100 nm, is a promising non-thermal technology capable of improving food safety while maintaining many of the original nutritional and quality aspects of the food.

Many factors affect the effectiveness of PL treatments, including spectral distribution, the type of challenge microorganism, and the interaction of light with the substrate. The objective of this study was to investigate environmental related factors, namely temperature and reflectivity, on the bactericidal effectiveness of PL treatment.

The effect of temperature on PL microbial inactivation was investigated in the sub-lethal temperature range of 5 °C to 50 °C. Butterfield's phosphate buffer (BPB) was inoculated with either *Listeria innocua*, *Escherichia coli* ATCC 25922, or *Pseudomonas fluorescens*. A thin layer of the inoculated sample was equilibrated to 5 °C, 20 °C, 30 °C, 40 °C, or 50 °C and then treated with PL doses ranging from 1.02 to 12.29 J/cm^2. In the temperature range of 5 °C to 40°C, the average maximum reductions for *L. innocua*, *E. coli*, *P. fluorescens* were 6.27 ± 0.23 log CFU, 6.66 ± 0.36 log CFU, and 6.15 ± 0.19 log CFU, respectively. Temperature did not affect PL inactivation of *E. coli* or *P. fluorescens*, but a modest synergistic effect between PL and temperature was observed for *L. innocua* treated at 50 °C. At low temperatures and fluence levels, the gram-positive *L. innocua* was the most PL resistant of the three strains, followed by the gram-negative *E. coli* and *P. fluorescens*, respectively. Differences in sensitivities disappeared at higher temperatures and fluence levels. For all strains and treatment temperatures, the Weibull model was able to accurately describe PL inactivation kinetics.

The effect of UV-reflectivity on PL treatments was also investigated. A clear liquid substrate was inoculated with *L. innocua* and then exposed to PL in UV-reflective and non-UV-reflective sample containers, respectively. Liquid samples of equal thickness were exposed to a PL dose of 12.29 J/cm^2 (12 pulses). PL inactivation of *L. innocua* was significantly higher in the UV-reflective containers. The reductions achieved using UV-reflective and non-UV-reflective containers were 7.83 ± 0.41 log CFU and 6.41 ± 0.32 log CFU, respectively.

Overall, this study indicates that PL does not seem to be greatly affected by temperature within the range of 5 °C to 40 °C, although slight increases of PL effectiveness may be obtained in some cases by conducting the treatment at a higher temperature. Additionally, through the use of UV-reflective sample containers PL effectiveness can be enhanced by taking advantage of a UV fluence multiplication effect in liquids.

BIOGRAPHICAL SKETCH

Sheena T. Hilton was born on June 11, 1987 in Lawrenceville, Georgia. She is the only daughter of Catherine and Larry Hilton. Sheena has two brothers: Shaka, her older brother, and Sean, her younger brother. Sheena was raised in metro-Atlanta until she left home at age 14 to attend Phillips Academy in Andover, Massachusetts. She attended Phillips Academy as a boarding student and graduated in 2005.

Sheena attended Yale University in New Haven, Connecticut for her undergraduate education. She was an active member of Jonathan Edwards residential college. Sheena studied chemistry at Yale and conducted research on organometallic molecules used as catalysts in the lab of Dr. Robert Crabtree. Sheena graduated with a Bachelors of Science in chemistry in 2009.

Following graduation from Yale, Sheena began teaching chemistry at her alma mater, Phillips Academy, as a Teaching Fellow for the 2009 – 2010 school year. Upon completion of the Teaching Fellow program Sheena was hired by Greenwich Academy in Greenwich, Connecticut as a chemistry instructor. In addition to teaching chemistry Sheena also coached JV girls' volleyball and JV girls' basketball. Sheena taught at Greenwich Academy for one year before returning to Phillips Academy as a chemistry instructor in the fall of 2011, where she taught introductory and Advanced Placement chemistry, in addition to serving as a house counselor and the boys' and girls' JV volleyball coach.

During the summers between school years Sheena studied at Bucknell University in Lewisburg, Pennsylvania. She conducted research on the mass spectrometry of small peptide fragments under the guidance of her advisor, Dr. James Swan. Sheena graduated with a Master of Arts in chemistry from Bucknell in January of 2014.

After the conclusion of the 2013 – 2014 school year, Sheena took a leave of absence from Phillips Academy to pursue a Masters of Science degree in food science from Cornell University. She joined the lab of Dr. Carmen Moraru in the fall of 2014.

To my loved ones:

The Hiltons, the Johnsons, my Andover family, and my friends

John 6:68

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CHAPTER 1

PULSED LIGHT IN PROCESSING AND PRESERVATION OF FOODS: A REVIEW

ABSTRACT

Pulsed light (PL) treatment is a promising non-thermal technology for producing food safely, while maintaining their original nutritional and sensory properties. PL technology uses short duration pulses of high intensity broad-spectrum light ranging in wavelength from 200 nm to 1100 nm. It is generally accepted that PL inactivates microorganisms due to the absorption of UV light by microbial DNA, but other mechanisms may also contribute to inactivation. At a laboratory scale, PL has been successfully used for the disinfection of liquids, as well as for surface decontamination of produce, meats, food packaging, and food contact surfaces. Other uses of PL include inactivation of enzymes, toxins, and reduction of allergens in various food systems. Due to limitations related to penetration depth and microbial hiding, current commercial applications are limited to treatment of packaging materials.

INTRODUCTION

Pulsed light (PL) technology has emerged as a promising non-thermal technology, which has the capability of increasing food safety while maintaining many food quality aspects that are typically altered by thermal processes. PL has been proven effective against vegetative bacteria, spores, yeasts, and molds on food and food contact surfaces. In 1996, the FDA approved the use of PL technology on food and food contact surfaces, provided that a xenon flashlamp is used as

the light source, the pulse width does not exceed 2 milliseconds, and the total dose does not exceed 12 J/cm^2 (FDA Code 21CFR179.41).

General aspects of Pulsed Light technology

PL uses short duration pulses of high intensity broad-spectrum light, in the wavelength range from 200 nm to 1100 nm (**Fig. 1.1**).

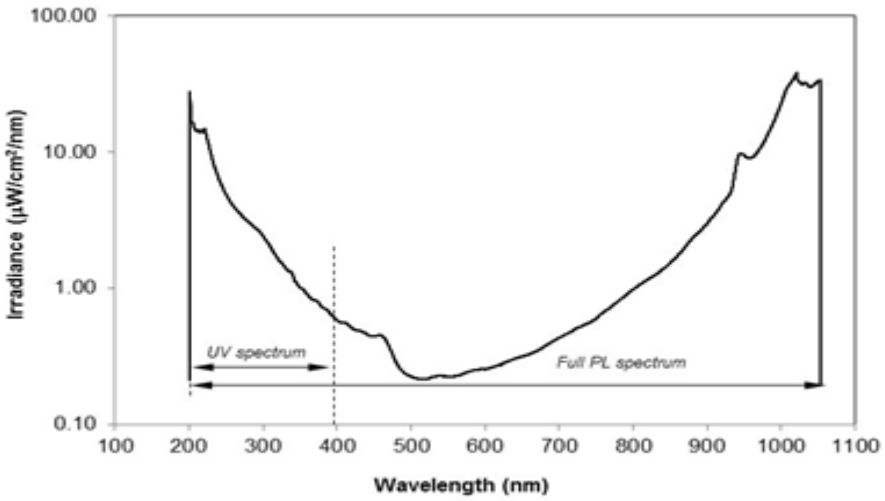

Figure 1.1. Spectral range of a Xenon pulsed lamp of a RS-3000C SteriPulse System (Xenon Corporation; Woburn, MA)

A PL unit is typically comprised of the following components (**Fig. 1.2**): a high voltage power supply (1), a storage capacitor (2), a pulse-forming network (3) that determines the pulse shape and spectrum characteristics, the gas discharge flash lamp (4) and a trigger (5) that initiates discharging of the electrical energy to the flash lamp. PL units also have cooling systems to reduce the heat buildup in the treatment area. Reflectors are sometimes incorporated in the treatment chamber in order to redirect the light to the sample.

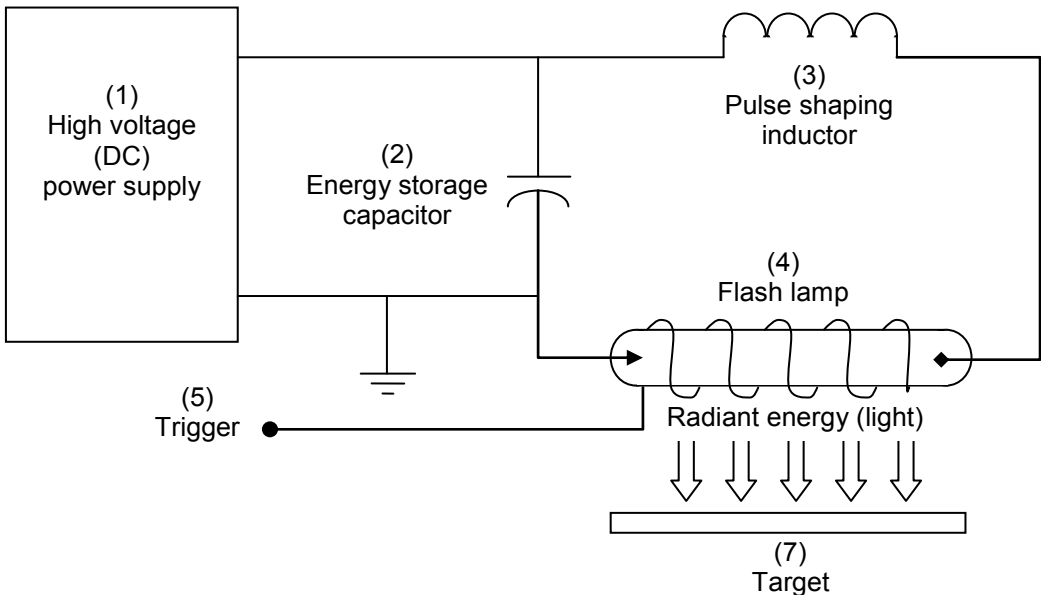

Figure 1.2. Functional diagram of a high intensity pulsed light system (Adapted from Xenon Corporation)

The high-voltage power supply delivers electrical energy to the storage capacitor. Energy is stored in the capacitor over relatively long periods of time (on the order of fractions of a second) and then released over a short period of time (on the order of thousandths of a second or shorter.) The electricity released by the capacitor ionizes the inert gas contained within the flashlamp. As the current passes through the gas in the flashlamp a short, intense burst of light is released. Xenon is typically used as the inert gas, although other gases have also been used (i.e., Krypton). In recent years, several new technologies have been explored as alternative sources of pulsed light to flash lamps, which include static discharge lamps, sparkers and light emitting diodes (LED). The latter are currently being explored as a more efficient way of delivering the light energy to a substrate.

Regardless of the source of PL, the treatment dose is called fluence and represents the energy delivered by the flashlamp at the substrate level. Fluence is commonly reported in units of joules per square centimeter (J/cm^2). While many studies report the number of pulses, pulse

characteristics (width, fluence per pulse), and distance from the lamp, ideally the total fluence should also be reported in order to allow direct comparisons of different PL treatments, regardless of the experimental setup used (Artíguez and de Marañón 2014, Hsu and Moraru 2011).

Comparison of Pulsed Light and continuous UV light

Continuous UV light treatments are typically performed using low pressure (LP) or medium pressure (MP) mercury lamps, which contain mercury at pressures of 100 – 200 Pa and greater than 100 kPa, respectively (McDonald 2000). LP lamps emit two lines in the UV region, at 185 nm and 254 nm, in addition to several other lines outside of the UV region. The germicidal region of the spectrum is considered to be between 200 nm and 280 nm, making LP mercury lamps a source of monochromatic radiation for UV disinfection purposes (Wang et al. 2005). MP lamps emit a more continuous spectrum than LP lamps, although the spectrum produced still has strong lines and is much less continuous in nature than the spectrum of a pulsed xenon flashlamp. MP mercury lamps are less electrically efficient than LP lamps, however they produce several emission lines in the germicidal range and require shorter treatment times than LP lamps to achieve the same level of inactivation (Wang et al. 2005).

PL is an attractive alternative to continuous UV treatment for several reasons. PL systems utilize xenon (or another inert) gas in the flashlamps as opposed to mercury, which is an environmental and health hazard. The use of mercury lamps in food disinfection could pose a risk to workers and consumers, should a lamp ever break. Even without lamp failure, the use of mercury lamps still requires special disposal procedures to prevent mercury contamination of the

environment. Furthermore, continuous UV light systems typically have poorer penetration depth and lower emission power than PL systems, leading to longer treatment times than PL.

MECHANISM OF MICROBIAL INACTIVATION IN PULSED LIGHT TREATMENT

Although there is some general consensus on the effect of the UV portion of the light spectrum in microbial inactivation by PL, the mechanism by which PL causes microbial inactivation is still not fully understood. A significant body of evidence supports the hypothesis that inactivation occurs as a result of the absorption of the UV portion of the PL spectrum by DNA, while others have presented evidence of non-UV related death mechanisms. These will be briefly reviewed in the section below.

Effect of spectral range on inactivation

Wang et al. (2005) investigated the wavelength dependence of the degree of *Escherichia coli* inactivation by PL. In that study, the light produced by the xenon flashlamp was directed into a monochromator, which allowed for the selection of particular wavelengths. Samples were treated at 14 different wavelengths within the range of 230 to 360 nm. Results showed that *E. coli* inactivation did depend on wavelength; the germicidal efficiency, expressed by the log reduction of *E. coli* per unit fluence, peaked at 270 nm, and no *E. coli* inactivation was observed at wavelengths higher than 300 nm. An earlier study (Takeshita et al. 2003) also found that no inactivation took place in PL experiments in which a filter removed UV light below 320 nm. Woodling and Moraru (2007) conducted a more in depth study of the effect of spectral range in microbial inactivation by PL using *Listeria innocua* as a challenge organism. Three filters were used to select different portions of PL: a cold mirror, a UV-A transmitting filter, and a UV

blocking filter. The cold mirror allowed partial transmission of wavelengths between 200 and 400 nm and near total transmission for wavelengths above 700 nm. The UV-A transmitting filter transmitted wavelengths between 300 and 400 nm, and the UV blocking filter transmitted wavelengths above 400 nm. A quartz filter that transmitted wavelengths between 200 and 2000 nm, similar to the range emitted by the xenon flashlamp, was used as a control. The absorption of light by the filters was accounted for by comparing treatments of similar fluence levels. Treatments with the quartz filter consistently resulted in slightly lower inactivation levels than treatments without a filter in place, indicating that the small portion of UV light excluded by the quartz filter played a significant role in inactivation. When the UV blocking filter was used, almost no inactivation occurred, confirming that the UV portion of the spectrum is responsible for the germicidal effects. The cold mirror and UV-A transmitting filter samples did show small levels of inactivation; the cold mirror was slightly more effective than the UV-A transmitting filter. Comparison of the cold mirror and UV-A transmitting filter results suggest that the lower wavelengths of the UV spectrum are more effective in microbial inactivation.

Effect of UV range on microbial inactivation by PL

Nucleic acids and proteins within microbial cells absorb UV light, with a maximum of absorption occurring in the UV-C range ($\lambda<280$nm). UV light absorbed by DNA causes crosslinking between the pyrimidine nucleoside bases (thymine and cytosine) of the same DNA strand. This crosslinking creates cyclobutane pyrimidine dimers (CPDs), which impair the formation of hydrogen bonds with the purine bases on the opposite strand. CPDs block DNA polymerases, preventing transcription and replication. Unless the cell can reverse or repair the

DNA damage, CPD formation hinders cellular function and eventually leads to cell death (Sinha and Hader 2002).

Dimerization of the pyrimidine nucleoside bases in microbial DNA can be reversed by several cellular repair mechanisms, the major three being photoreactivation, excision, and recombination repair (Sinha and Hader 2002, Witkin 1976). In photoreactivation, the enzyme photolyase binds to CPDs and reverses dimerization using light energy. Approximately 10 to 20 photolysases regularly scan DNA in the nucleus for lesions such as CPDs (Sinha and Hader 2002). Once a CPD is found, the photolyase binds to the CPD. Light energy is absorbed, and the photolyase facilitates the transfer of an electron to the CPD, causing the dimer to break apart. The DNA strand has now been returned to its original form and the enzyme is released. If the UV irradiated cells are kept in the dark after UV exposure, cells will be incapable of photoreactivation. Nonetheless, the other two major repair mechanisms can take place without light exposure. In excision repair, the affected strand of DNA is cut on either side of the CPD. The DNA section containing the CPD is removed and replaced with undamaged nucleotides by DNA polymerase. Finally, DNA ligase seals the newly added section to the rest of the original strand. Recombination repair, also referred to as post-replication repair, allows for replication to occur in the presence of the dimer. Replication of the damaged DNA creates two daughter strands, one complete daughter strand and one with a gap located across from the CPD. The cell now has completed one round of DNA replication, obtaining one intact daughter strand as a product (Witkin 1976).

Despite the various DNA repair mechanisms available to microbial cells, after a certain dose of UV light the cells' repair mechanisms are overwhelmed and are unable to undo the damage, leading to cell death. It has been reported that PL is more effective in microbial

inactivation than continuous UV (Grapperhaus et al. 2007). However, electrophoresis studies of DNA from continuous UV and PL treated cells show that DNA from cells treated with continuous UV exhibited greater amounts of damage (Cheigh et al. 2012, Takeshita et al. 2003). These results suggest that there might be additional inactivation mechanisms at work in PL as compared to continuous UV treatments. Some studies have claimed that while cells are able to repair DNA damage after continuous UV treatment, repair mechanisms can be inactivated by PL. For instance, Gómez-López et al. (2007) suggested that the broader spectrum might inactivate photolyase, which would make microbial DNA damage caused by PL irreversible.

Photothermal and photophysical effects of PL treatment

Direct comparison of PL and continuous UV treated cells revealed differences in the effects of the two treatments. Takeshita et al. (2003) measured protein elution of yeast cells treated by the two methods, on the premise that protein elution may indicate cell membrane damage. Protein elution from yeast cells treated by PL was higher than from yeast cells treated by continuous UV. This study also used transmission electron microscopy (TEM) to evaluate the structure of PL and continuous UV treated yeast cells. TEM images showed that the cell membranes of PL treated yeast cells were punctured in several places, while the cell membranes of the yeast cells treated with continuous UV remained intact and resembled those of untreated yeast cells (Takeshita et al. 2003).

Cheigh et al. (2012) compared TEM images of untreated, PL treated, and continuous UV treated *Listeria monocytogenes* and *E. coli* O157:H7 cells and found similar results for both microorganisms. The cell membranes of bacterial cells untreated or treated with continuous UV remained intact. In contrast, the cell membranes of the PL treated cells were significantly

disrupted, so much so that internal organization was lost and cellular components leaked out of the cells, leading to cell death. The study by Krishnamurthy et al. (2010) also reported that TEM images of *Staphylococcus aureus* cells treated with PL showed cell wall damage and leakage of intracellular components. An interesting finding of this study was that when monitoring temperature increases of the substrate using a type K thermocouple, the maximum increase in temperature observed was only 2 °C, suggesting that PL-induced structural damage might not be caused by heat as was previously hypothesized (Krishnamurthy et al. 2010). The authors rather suggested that cytoplasmic membrane damage could be caused by physical disturbances exerted on the microbial cell due to the intermittent high intensity pulses. Overall, such studies build a strong case for structural damage as a second major component in microbial inactivation by PL, but so far the cause of structural damage is not clearly and unequivocally understood. It should also be noted that most studies showing structural damage of microbial cells have used rather high doses of PL, well beyond the 12 J/cm^2 established by the FDA as the upper limit for PL treatment in the US.

Photothermal effects are still a possible explanation for the observed structural changes. Alexander Wekhof, an early pioneer of disinfection by PL, made a case for a photothermal effect (Wekhof 2000). As previously discussed, many studies have shown that the UV portion of the PL spectrum was found responsible for inactivation of several microorganisms. Wekhof argues that there must be an aspect of PL beyond the presence of UV light that inactivates UV-resistant microorganisms such as *Cryptosporidium*, *Aspergillus niger*, or *Bacillus subtilis*. He suggested that the high intensity light pulses delivered in PL treatments may cause rapid overheating in microorganisms due to the absorption of UV light. By estimating the size, absorption coefficient, and cooling properties of *E. coli*, Wekhof approximated that bacteria only lose 1 to 10% of the

heat gained during PL treatments to cooling. If over 90% of the energy received by the bacteria is retained as heat, the internal temperature in the cell could quickly rise to over 100°C during longer PL treatments, leading to the formation of steam microbubbles and subsequent cell rupture. Wekhof tested his hypothesis with *E. coli* cells. Results showed that little temperature increase occurred until a threshold fluence was reached, after which the temperature increased dramatically to over 120°C.

Other researchers have attempted to quantify the temperature changes associated with PL treatments. A study on *S. aureus* by Krishnamurthy et al. (2004) reported temperature increases for PL treatments longer than 5 s, corroborating Wekhof's findings. Negligible temperature increases were seen for PL treatments shorter than 5 s in a subsequent study on *S. aureus* (Krishnamurthy et al. 2010). While bulk heating of the sample was not observed in the latter study, it is still possible that localized heating of bacterial cells occurred, causing the structural damage observed by TEM. It is important to note that some *S. aureus* cells with intact cell walls were observed after PL treatment, indicating that both photochemical (formation of CPDs) and photothermal/photophysical (structural damage) effects may be at work in microbial inactivation by PL.

INACTIVATION KINETICS IN PULSED LIGHT TREATMENT

Survival curves for PL experiments are typically non-linear (Uesugi et al. 2007). At low fluence levels inactivation occurs quickly, but above a threshold fluence, which varies depending on the resistance of the treated microbial species and/or the substrate in which the treatment is performed, inactivation plateaus. This plateau, also known as the survivor curve tail, could be caused by inefficient exposure of some cells to PL (Marquenie et al. 2003).

This creates challenges in modelling the kinetics of PL and conducting process calculations. First order kinetics have typically been used to describe microbial inactivation processes, particularly heat treatments (Peleg and Cole 1998). However, survival curves from many different inactivation processes are non-linear, making the use of first order kinetics inappropriate for such processes. The Weibull model, an empirical model used in situations where a period of stress exposure causes failure (death in microorganisms or mechanical failure in engineering applications), has been suggested as an alternate model for microbial inactivation process calculations (van Boekel 2002). For PL treatments, the following form of the Weibull model was used (Uesugi et al. 2007):

$$log\frac{N}{N_0} = -\frac{1}{2.303}\left(\frac{F}{\alpha}\right)^{\beta} \quad (1)$$

where:

N = survivors after treatment

N_0 = initial number of microorganisms

α = scale parameter

β = shape parameter

F = pulsed light fluence (J/cm^2).

The parameter β indicates the shape of the survivor curve, with $\beta > 1$ indicating concave down survival curves, $\beta < 1$ concave up survival curves, and $\beta = 1$ linear survival curves. Predictions of *L. innocua* and *E. coli spp.* inactivation by PL in liquid and on solid substrates were made using the Weibull model and then compared to experimental results (Uesugi et al.

2007, Sauer and Moraru 2009). The Weibull model successfully predicted survivor ratios in clear liquids (**Fig. 1.3**).

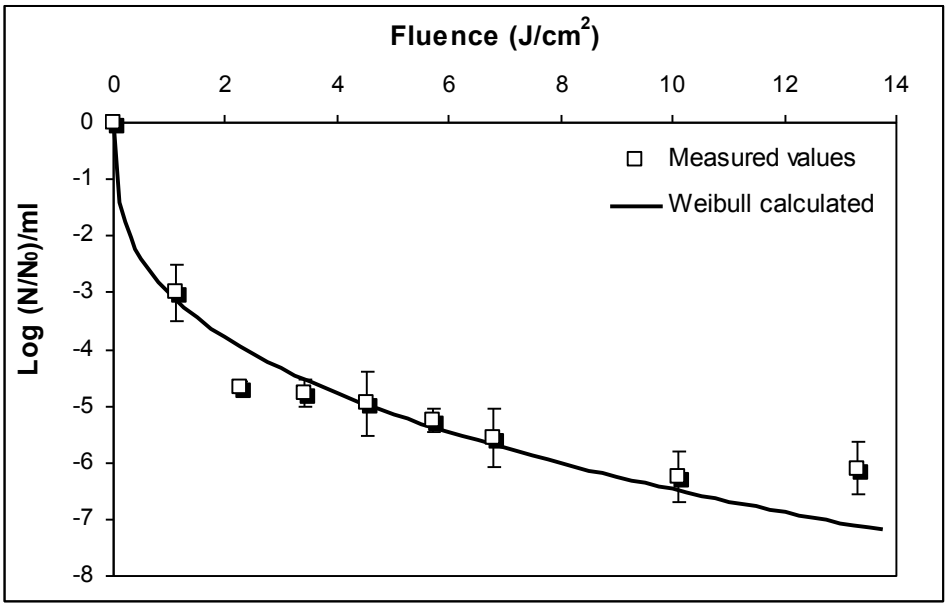

Figure 1.3. Experimental and Weibull-calculated survival ratios ($S = N/N_0$) for *L. innocua* treated with Pulsed Light in Butterfield's Phosphate Buffer (Uesugi et al. 2007)

Predictions were less accurate for turbid fluids however (Sauer and Moraru 2009), and the model failed to provide an accurate prediction of microbial inactivation on solid substrates, likely due to substrate related effects such as distribution of the microorganisms on the solid surface or surface reflectivity.

FACTORS AFFECTING THE EFFICIENCY OF PULSED LIGHT

Several factors affect the efficiency of PL treatments, including fluence, spectral distribution, the challenge microorganism, and the interaction of light with the substrate.

Hsu and Moraru (2011) studied in detail the dependence of fluence on the location of the sample in relation to the light source. Their results revealed that fluence does vary significantly in all directions within a PL chamber (**Fig. 1.4**). This has significant effects on the effectiveness and uniformity of the treatment. Therefore, PL treatments should be designed with this fact in mind, and direct exposure of the sample to the light source should be ensured. Additionally, the design of the treatment chamber will affect the treatment, and the strategic placement of light reflectors can be used to improve treatment effectiveness and efficiency.

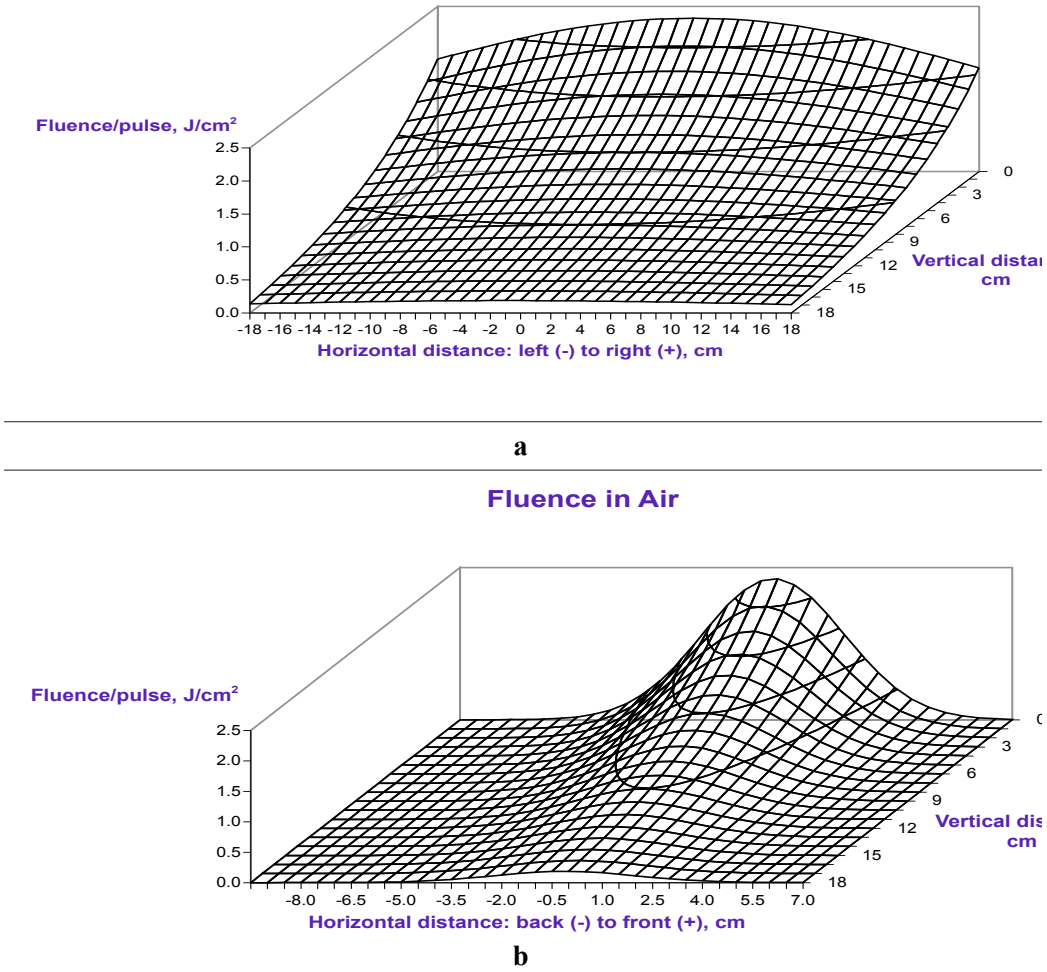

Figure 1.4. Mapping of fluence in a SteriPulse-XL 3000 PL chamber. **a)** Measurements in xy plane; **b)** Measurements in yz plane (Hsu et al. 2011)

Surface properties of the substrate play an important role in the effectiveness of PL surface treatments. In particular, microorganisms may lodge into surface irregularities that shield them from PL exposure. For instance, *E. coli* O157:H7 cells were found capable of penetrating up to 20 μm into cut lettuce leaves (Seo and Frank 1999). This means that using PL for surface decontamination of lettuce – or of any rough food surface for that matter - may not be very effective, given that PL has a low penetration depth. Nonetheless, a smoother surface does not necessarily guarantee a high level of inactivation. Woodling and Moraru (2005) have studied effectiveness of PL for the inactivation of *L. innocua* on solid substrates with different surface roughness, using stainless steel coupons with varying degrees of surface roughness and reflectivity as model surfaces. The authors expected the roughest surface to have the lowest level of inactivation, since surface roughness can provide locations that shield bacterial cells from PL. However, in this study the smoother surfaces actually had lower levels of inactivation. The authors attributed this to the hydrophobicity of the smoother coupons, which induced clumping and layering of cells, thus shielding some cells from direct exposure to PL. Surfaces of high reflectivity were also found to negatively impact inactivation levels by PL (Ringus and Moraru 2013). The nature of the substrate may also affect PL effectiveness. Some substrates contain UV absorbing components such as proteins or DNA. Absorption of UV light by substances other than the target microorganism will decrease inactivation.

The challenge organism also plays a role in the efficacy of PL treatments. Some studies report a difference between the susceptibility of gram-negative bacteria, gram-positive bacteria, and fungal spores to PL, with gram-negative being the most susceptible and fungal spores being the least susceptible out of the three groups (Anderson et al. 2000, Rowan et al. 1999). Other studies have found no susceptibility pattern among different types of microorganisms (Gómez-

López et al. 2005a), and another stated that susceptibility must be determined on an individual organism basis (Lasagabaster and de Marañón 2012). The way a microorganism is inoculated on a sample can also affect the success of PL treatments. Xu et al. (2013) spot inoculated and dip inoculated green onion leaves to model contamination of green onions by the unsanitary touch of workers or equipment during processing and contamination during washing, respectively. When using spot inoculated green onion leaves, a 5 J/cm^2 dose resulted in a 4.8 log CFU/g reduction in *E. coli* O157:H7. In contrast, the 5 J/cm^2 dose only yielded a 0.6 log CFU/g reduction in *E. coli* on dip inoculated green onion leaves. The optical properties of microbial cells can also affect the outcome of PL treatment. Waltham et al. (1994) measured light scattering and absorption from a population of *E. coli* in water and observed a significant decrease in these two properties from UV to VIS. Similar results were reported by Arakawa et al. (2003) for *Erwinia herbicola*. Tuminello et al. (1997) reported an increase in the transmission of *Bacillus* spores from UV to NIR. Such findings also support the idea that during PL treatment it is mostly the UV radiation that affects the microbial cells, since this component of the electromagnetic spectrum is absorbed the most. Montgomery and Banerjee (2015) reported reductions in *L. monocytogenes* and *E. coli* O157:H7 biofilms of 2.7 and 3.9 log CFU/mL, respectively (the total fluence received by the samples was not reported). The lower inactivation levels for these microorganisms when entrapped in a dense biofilm matrix as compared to situations when the same bacterial strains were treated in suspension show the importance of light-substrate interactions for PL inactivation.

An additional factor of influence in PL treatment is the level of contamination or the inoculum concentration (Uesugi et al. 2007). For clear liquid substrates, the initial inoculum level did not influence reduction by PL. For treatments conducted on stainless steel surfaces, a

higher level of inactivation was observed with the use of higher initial inoculum levels. This was attributed to the distribution of cells on the surface, since as the level of inoculum increases, the number of cells that are directly exposed to light also increases, leading to greater levels of inactivation (Uesugi et al. 2007).

Overall, there is a wide range of parameters that affect the efficacy of PL treatments. Therefore, consideration must be given to the substrate, target organism, sample location relative to the lamp, and the spectral output of the PL unit when designing PL treatments to ensure maximum efficiency.

APPLICATIONS OF PULSED LIGHT TREATMENTS IN FOOD AND FOOD SYSTEMS

Pulsed Light treatment of liquid foods

The efficacy of PL treatments in liquids depends on a number of factors, including fluence, the depth of the liquid sample, and the turbidity of the liquid. Innocente et al. (2014) used PL to treat raw milk. A fluence of 26.25 J/cm^2 produced a 3.2 log CFU/mL reduction in total microbial counts; however, this fluence is more than twice higher than the FDA approved limit. The penetration depth of UV light in milk was determined to be 0.17 mm, indicating that liquid samples should be treated in a thin layer to improve microbial inactivation. Another option for ensuring that the entire sample is uniformly exposed to UV light is to use turbulent or continuous flow treatments. Miller et al. (2012) performed static and turbulent treatments of 1 mL thin layer (1.3 mm thickness) samples of skim and 2% milk. Samples were inoculated with *E. coli* ATCC 25922 and then exposed to PL doses ranging from 2.14 to 14.85 J/cm^2. The maximum reduction of either milk type was 0.35 log CFU with static treatments. Microbial inactivation with turbulent treatments was significantly higher than in static mode; the maximum

reductions for skim and 2% milk were 3.36 and 2.89 log CFU, respectively. The authors hypothesized that inactivation in 2% milk was decreased due to scattering of light by milk fat. Similarly, Sauer and Moraru (2009) showed that PL applied under turbulence and in thin layer was able to effectively inactivate *E. coli* ATCC 25922 and *E. coli* O157:H7, in both filtered and unfiltered apple juice, beyond the FDA required 5-log reduction. Later, Pataro et al. (2011) used a continuous flow unit to inactivate *E. coli* and *L. innocua* in commercially available apple and orange juices. The juice samples flowed through thin tubes of 1 mm diameter. A fluence of 4 J/cm^2 resulted in *E. coli* reductions of 4.00 and 2.90 log CFU and *L. innocua* reductions of 2.98 and 0.93 log CFU in apple juice and orange juice, respectively. The transparency of the orange juice was significantly lower than that of the apple juice, which may explain the lower inactivation levels in orange juice. Palgan et al. (2011) also found that inactivation decreased with decreasing transparency of the liquid sample. The authors considered three beverages, listed here in order of decreasing transparency: apple juice, orange juice, and milk. The maximum reductions of *E. coli* were 4.7, 1, and less than 1 log CFU/mL for apple juice, orange juice, and milk, respectively.

While microbial inactivation is an essential factor in determining whether PL is an appropriate decontamination technology for foods, it is not the only factor that should be considered. From a practical perspective, the effect of PL on food quality is almost equally important. A small number of studies in the literature consider the effects of PL on food liquid quality. Elmnasser et al. (2008) explored the effect of PL treatments on the quality of milk and milk components. Milk proteins and lipids were evaluated for structural and chemical changes after PL exposure. Overall, PL treatments induced minimal changes in the properties of the milk components considered. Changes that were observed include the formation of disulfide bond

linked dimers in beta-lactoglobulin, the increased polarity of the tryptophan environment within milk proteins, and the destruction of Vitamin A.

Kasahara et al. (2015) inoculated pasteurized goat milk with *E. coli* and subsequently treated the inoculated goat milk with PL doses ranging from 1.3 to 10 J/cm^2. A 6 log CFU/mL reduction of *E. coli* was achieved with the 10 J/cm^2 dose. No statistically significant differences in the protein or lipid content of the goat milk were observed after PL treatments. Sensory panelists detected a difference in aroma between untreated and PL treated samples, but not in flavor.

Pulsed Light treatment of meat and fish products

PL treatments of meat and fish products are limited in their efficacy by the surface roughness of the product and the ability of the microorganisms to penetrate into the surface of the product. Ozer and Demirci (2005) examined the reduction of *L. monocytogenes* and *E. coli* O157:H7 on the muscle and skin side of raw salmon fillets using a SteriPulse-XL 3000. For *E. coli* O157:H7, a reduction of 0.30 and 1.09 log CFU/g was seen on the muscle and skin side, respectively, when treated at a distance of 80 mm with 180 pulses. For *L. monocytogenes*, on the salmon muscle and skin reductions of 0.74 and 1.02 log CFU/g, respectively, were reached after 180 pulses at 80 mm from the lamp. Surface temperatures increased after 60 s of pulsed light treatment from a distance of 80 mm by 28 and 51°C on the muscle and skin side, respectively. The increase in temperature may have contributed to the greater reduction observed on the skin side of the fish.

The inactivation of *L. innocua* on the surface of Vienna sausages was investigated by Uesugi and Moraru (2009). A reduction of 1.39 log CFU was observed after PL treatment at a

fluence of 9.4 J/cm^2. When PL was used in combination with a nisin dip, a reduction between 4 and 5 log CFU/sausage of *L. innocua* was observed. Hierro et al. (2012) observed modest inactivation of *L. monocytogenes* and *E. coli* O157:H7 on vacuum packed beef and tuna carpaccio samples over a fluence range of 0.7 to 11.9 J/cm^2. The maximum reduction achieved was a 1.2 log CFU/cm^2 reduction of *E. coli* on beef. The quality of the beef samples treated with higher fluences was deemed acceptable, but a sensory panel detected loss of freshness. Tuna samples treated with fluences greater than 4.2 J/cm^2 were found unacceptable because of a loss of color, possibly due to a change in myoglobin oxidation state. Wambura and Verghese (2011) also observed negative quality changes in sliced ham exposed to PL (no fluence values were reported).

Ganan et al. (2013) examined the ready-to-eat (RTE) cured meats salchichón (dry, fermented sausage) and dry, cured loin. A dose of 11.9 J/cm^2 yielded 1.5 to 1.8 log CFU/cm^2 reductions in *L. monocytogenes* and *Salmonella*, respectively. Sensory tests detected little practical difference between PL treated and untreated cured meats. Hierro et al. (2011) treated vacuum-packed ham and bologna that had been inoculated with *L. monocytogenes* before packaging. The samples received a maximum fluence of 8.4 J/cm^2, and reductions of 1.78 and 1.11 log CFU/cm^2 were obtained for ham and bologna, respectively. This treatment extended the shelf life of ham 30 days beyond vacuum-packed control samples and did not affect the sensory properties of the ham. In contrast, bologna samples treated with doses over 4.2 J/cm^2 exhibited negative changes in sensory properties including odor and flavor.

Keklik et al. (2010b) compared inactivation of *Salmonella* on unpackaged and vacuum-packed boneless chicken breast over a wide range of fluence values (2.9 to 67 J/cm^2). A 1.2 log CFU/cm^2 reduction of *Salmonella* was achieved on unpackaged chicken treated with a dose of

8.7 J/cm^2, and a 0.9 log CFU/cm^2 reduction was achieved on packaged chicken treated with a dose of 8.2 J/cm^2. These samples were treated for the same period of time at the same distance from the lamp, but the packaging absorbed a portion of the light, making the fluence available to the packaged chicken slightly smaller than the fluence received by the unpackaged chicken. The use of packaging did reduce the color change in the packaged chicken when compared to unpackaged chicken. The authors also tested for lipid oxidation using the TBARs test. Lipid oxidation of PL treated samples was not significantly different from that of untreated samples for treatments below the FDA limit of 12 J/cm^2. Paskeviciute et al. (2011) also found that PL treatments of unpackaged skinless chicken breast did not result in significant increases in lipid oxidation. Haughton et al. (2011) observed color changes in packaged chicken skin and chicken breast, but treatment doses were substantially larger than 12 J/cm^2. Similarly, Nicorescu et al. (2014) found that lipid oxidation in PL treated raw pork roast, roast pork, and raw salmon was only significant in samples treated with a dose of 30 J/cm^2. These studies of PL in meat product decontamination highlight the importance of determining the fluence that provides adequate levels of microbial inactivation while also maintaining product quality.

Pulsed Light treatment of fruits and vegetables

Decontamination with PL has been studied in a variety of produce. Surface irregularities in some fruits and vegetables lower inactivation levels, and both negative and positive effects on produce quality have been reported.

Gómez-López (2005b) treated naturally contaminated celeriac, green bell peppers, iceberg lettuce, radicchio, soybean sprouts, spinach, and white cabbage with up to 2,700 pulses with a xenon flash lamp with a pulse duration of 30 μs and an intensity of 7 J, at a distance of

128 mm from the lamp, and obtained microbial reductions of only 0.56 log to 2.04 log for the variety of produce examined. The processing (shredding, grating, chopped, or whole), shape or sample size did not produce any observable patterns in reduction. While the initial microbial load was reduced, shelf life was not increased compared to untreated controls. Kaack and Lyager (2007) treated carrot slices inoculated with *S. cerevisiae*. After 2 pulses of 0.7 J/cm^2 per pulse, a reduction of 3.07 log was obtained. After exposure to 24 pulses, the reduction increased to 4.93 log.

PL treatment of alfalfa seeds inoculated with 5 log CFU/g of *E. coli* O157:H7 resulted in a log reduction of 4.89 after applying 270 pulses of light, at a distance of 80 mm from the lamp, using a SteriPulse-XL 3000 system (Sharma and Demirci 2003). The same treatment performed at a distance of 130 mm resulted in a log reduction of only 1.42 CFU/g. Using the same PL system, Bialka and Demirci (2007) noted a reduction of 1.1 and 4.3 log CFU/g of *E. coli* O157:H7 with 1.9 and 22.6 J/cm^2, respectively, on blueberries treated at a distance of 80 mm from the lamp. Treatments at a distance of 30 mm resulted in slightly greater reductions, but also resulted in damaged fruit. For treatments of *Salmonella* on blueberries, the differences in fluence did not create significantly greater reductions at 30 and 80 mm from the lamp. However, at the 130 mm treatment distance the reduction of *Salmonella* increased significantly with fluence. Luksiene et al. (2013) observed reductions of 1.0 to 2.2 log CFU/g for several microorganisms after inoculated strawberries were treated with a PL dose of 3.9 J/cm^2. The PL treatment extended the shelf life of the strawberries by two days and did not cause any significant changes to the strawberries' color, firmness, or ascorbic acid, anthocyanin, and phenolic contents.

The treatment of red-ripe tomatoes by PL induced both positive and negative quality changes (Aguílo-Aguayo et al. 2013). Three days after treatment, PL treated tomatoes lost

firmness and developed a wrinkled exterior. However, lycopene became more bioavailable and carotenoid content increased slightly as a result of PL treatment. Charles et al. (2013) specifically focused on the quality effects of PL treatments using mangoes as a substrate. Fresh cut mangoes were exposed to a PL dose of 8 J/cm^2. After 3 days of storage, firmness, color, and carotenoid content were better preserved in treated mangoes than in control samples, and phenolic and ascorbic acid contents remained the same as those of controls. Mild treatments (0.1 to 1.8 J/cm^2) of pale fig fruit led to increased anthocyanin and phenolic content and to a darkening of the fruit during storage, which improved the marketability of the figs (Rodov et al. 2012).

The level of fluence determined whether negative quality effects were seen in mushrooms after PL treatments (Oms-Oliu et al. 2010). PL treatments of 4.8 J/cm^2 reduced the native yeast population (the limiting factor in shelf life) enough to extend shelf life by two to three days. Higher fluences changed the mushroom texture and promoted enzymatic browning. Ignat et al. (2014) found that PL treatments of 1.75 J/cm^2 on apple slices caused 2.7 and 3 log CFU/cm^2 reductions in *L. monocytogenes* and *Lactobacillus brevis*. Quality changes were similar to untreated cut slices at this fluence, but higher fluence levels caused dehydration and browning in the apple slices. Gómez et al. (2012) considered whether the negative effects of high fluence PL treatments on apple slices could be prevented. Apple slices were dipped in an anti-browning treatment (ascorbic acid in $CaCl_2$ solution) before PL treatments. The anti-browning pretreatment inhibited browning for PL doses up to 71.6 J/cm^2. Huang and Chen (2014) also creatively adapted the typical PL treatment setup to mitigate the negative effects of PL on fruits. Blueberries inoculated with *E. coli* O157:H7 were submerged in turbulent water and then treated with PL. Results were compared to dry treatments. The dry treatment resulted in a 3.8 log CFU/g reduction of *E. coli* with a fluence of 5.0 J/cm^2. While the dry treatment was effective in

inactivating *E. coli*, negative quality changes were observed on the blueberries. The water-assisted treatments resulted in a 4.5 log CFU/g reduction of *E. coli* at the same fluence of 5.0 J/cm^2 without inducing negative quality changes in the blueberries. In a 2015 study, Huang and Chen used the same water-assisted technique to treat strawberries and raspberries. The technique was less effective than on blueberries, likely due to the much higher degree of surface irregularities on raspberries and strawberries.

Pulsed Light treatment of cheese

A few recent studies examined the possibility of using PL for surface decontamination of cheeses. Can et al. (2014) studied the inactivation of *Penicillium roqueforti* and *L. monocytogenes* inoculated on packaged and unpackaged white American cheeses. A 10 J/cm^2 PL dose resulted in 0.81 to 1.0 log reductions of *P. roqueforti* on unpackaged and packaged cheeses, respectively. The 10 J/cm^2 treatments were more effective for *L. monocytogenes*; reductions of 2.3 and 2.6 log were achieved for packaged and unpackaged cheeses, respectively. The color and lipid oxidation in PL treated cheeses were not significantly different from those of controls. Fernández et al. (2014) examined the effects of PL on protein oxidation in processed cheese. While still within the normal range for cheese, the level of protein oxidation was significantly higher in samples treated with fluence values greater than 8.4 J/cm^2 than in control samples.

Proulx et al. (2015) examined the effectiveness of PL on *Pseudomonas fluorescens*, *E. coli* ATCC 25922, and *L. innocua* on the surface of commercial white cheddar and processed cheese slices, using PL doses ranging from 1.02 to 12.29 J/cm^2. Of the tested microorganisms, *L. innocua* was the least sensitive to PL treatment, with a maximum inactivation level of 3.37 log, followed by *P. fluorescens*, with a maximum inactivation of 3.74 log. *E. coli* was the most

sensitive to PL, with a maximum reduction of 5.41 log. All inactivation curves were non-linear, with plateaus typically achieved at PL doses above 3 J/cm^2. PL treatment through UV-transparent packaging and without packaging resulted in similar inactivation levels, indicating that PL has strong potential as a terminal treatment for decontamination of cheese surface through clear, UV transparent packaging.

Pulsed Light treatment of other foods

Macias-Rodriguez et al. (2014) reported a 5 log CFU reduction of *E. coli* K12 on hard-cooked peeled eggs without inducing any heat, color, or texture changes in the eggs. Fluence values were not provided for specific treatments in this study. Eggshells have also been decontaminated using PL; a fluence of 11 J/cm^2 yielded a 3.5 log reduction in *Salmonella* inoculated on eggshells (Keklik et al. 2010a).

Manzocco et al. (2014) inoculated egg pasta with *Salmonella*, both by adding it to the surface of the finished pasta and by incorporating it into the dough mixture before the pasta was formed. Significant differences were observed in the inactivation of the two types of samples. A dose of 3.50 J/cm^2 produced a 3.3 log reduction of *Salmonella* on surface inoculated pasta, but only a 1 log reduction on dough inoculated pasta. The rate of lipid oxidation was slower in PL treated samples of both types than in untreated samples, possibly due to the PL induced formation of non-enzymatic browning products with antioxidant properties.

Nicorescu et al. (2013) examined the efficacy of PL treatments on three spices: ground caraway, ground red pepper, and ground black pepper. The spices were inoculated with *B. subtilis* and exposed to 10 J/cm^2 of PL. Modest inactivation levels of 0.8 log (ground caraway and ground black pepper) and 1.0 log (ground red pepper) were obtained. Shadowing of

microorganisms by spice particles may have limited the efficacy of the PL treatment. Aron Maftei et al. (2013) treated both sides of wheat grain samples with PL in sterile polypropylene bags. The grains were packaged tightly to minimize rotation during the flipping of the bags. The highest energy treatment reduced the mold load in the wheat grains by approximately 4 log CFU/g.

Pulsed Light treatment of food packaging and contact surfaces

The use of PL in the disinfection of food packaging and contact surfaces has been deemed as one of the most promising applications of this technology in the Food Industry. Ringus and Moraru (2013) tested the effectiveness of PL on five different packaging materials inoculated with *L. innocua*: low density polyethylene (LDPE), high density polyethylene (HDPE), polyethylene-laminated ultra-metalized polyethylene terephthalate, polyethylene-coated paper-board, and polyethylene-coated aluminum foil paperboard laminate. The coupons were treated with a maximum PL fluence of 8 J/cm^2. Maximum reductions on the various packaging materials ranged from 3.5 to 7.2 log, and the highest levels of inactivation were found on LDPE and HDPE. According to the authors, high surface reflectivity and/or surface roughness lowered the efficiency of PL treatments on the other three packaging materials, which again demonstrates the complex PL-surface interactions. Haughton et al. (2011) inoculated a variety of packaging materials and contact surfaces common in poultry processing with *E. coli* ATCC 25922 and obtained a range of 1.50 to 4.17 log CFU/cm^2 reductions after a dose of 4.5 J/cm^2 was applied. Can et al. (2014) and Keklik et al. (2010b) found that the mechanical properties of polypropylene packaging material did not change after exposure to PL doses smaller than 27.5 J/cm^2 and 27.9 J/cm^2, respectively.

Rajkovic et al. (2010) examined the disinfection of a meat slicing knife by PL. The knife was used to slice meat products of varying protein and lipid contents or was dipped in meat extract, which was specifically chosen for its low protein and zero fat content. Following slicing or immersion in meat extract, the knife was inoculated on one side with approximately 6 log CFU/cm^2 of *L. monocytogenes* or *E. coli* O157:H7. The inoculated knife was then allowed to rest at room temperature for 1 to 60 minutes before PL treatment. Treatments consisted of 1 to 5 light pulses at 3 J/cm^2 per pulse. Interestingly, inactivation did not increase with increasing number of pulses, indicating that the tail or plateau of the survival curve was reached at the lowest dose applied (3 J/cm^2). The treatments were equally effective in inactivating *L. monocytogenes* or *E. coli*. Complete inactivation of 6 log CFU/side of knife was achieved in the samples treated with PL one minute after inoculation. As the time between inoculation and treatment increased, inactivation decreased, suggesting that the hiding of bacteria in crevices on the surface of the knife may be time dependent. The level of inactivation also depended on what substance the knife came in contact with before inoculation. As the lipid and protein contents in the meat products sliced increased, inactivation decreased. The highest levels of inactivation were seen in the knife samples dipped in the low protein, zero fat meat extract.

Combination of Pulsed Light with other disinfection technologies

As with other microbial inactivation technologies, researchers have explored the possibility of combining PL with other antimicrobial treatments that have complementary microbial inactivation mechanisms, in order to obtain additive or even synergistic effects. This could help minimize the dose of each of the treatments used, and thus better preserve the quality

of the treated foods. Not many of these studies are available yet for PL, and such studies are just beginning to be reported in the literature.

Ferrario et al. (2015) found that the combination of ultrasound and PL worked well for the inactivation of *Saccharomyces cerevisiae* cells suspended in commercial and fresh squeezed apple juices. Thermosonication before or after PL exposure improved the inactivation of *E. coli* in apple juice over thermosonication or PL treatments alone (Muñoz et al. 2012).

Most combination treatments involving PL have used antimicrobials for the combination treatments. For instance, Uesugi and Moraru (2009) combined PL with nisin, a natural antimicrobial, in the disinfection of ready-to-eat sausages inoculated with *L. innocua*. The combination of PL and nisin dip yielded a reduction of 4.03 log CFU, whereas PL and nisin dip treatments alone resulted in reductions of 1.37 and 2.35 log CFU, respectively. Ramos-Villarroel et al. (2015) compared the efficacy of PL, malic acid, and the combination of PL and malic acid treatments in the inactivation of *L. innocua* and *E. coli* on avocado, watermelon, and mushrooms. The malic acid treatment alone was the least effective, producing reductions of 0.96 to 1.08 log CFU/g in *E. coli* immediately after treatment. PL alone resulted in reductions of 2.58 to 2.97 log CFU/g. PL combined with malic acid treatment was the most effective, causing reductions of 3.14 to 3.48 log CFU/g.

One important consideration when using PL in conjunction with antimicrobial treatments is that the order of the individual treatments can be very important. If the antimicrobial used is able to absorb light in the microbicidal UV range, this might in fact diminish the effectiveness of PL instead of enhancing it.

Other applications of Pulsed Light in foods

PL has also been used in foods for other purposes besides microbial inactivation. Kalaras et al. (2012b) detected the formation of Vitamin D_2, pre-vitamin D_2, lumisterol$_2$, and tachysterol$_2$ from ergosterol in white button mushrooms. In a subsequent publication, Kalaras et al. (2012a) determined that a 2.4 J/cm^2 dose of PL increased the Vitamin D_2 content of mushrooms to greater than 100% the RDA per serving.

Janve et al. (2014) explored the possibility of using PL to deactivate soybean lipoxygenase (LOX). LOX oxidizes polyunsaturated fatty acids, producing free radicals and leading to the formation of off flavors and odors. Given that it is an enzyme, thermal treatments are one option for inactivation of LOX; however, thermal treatments may cause other undesirable changes in the food product. PL treatments of 16 seconds (fluence values not reported) resulted in 95 to 99.95% inactivation of LOX, depending on the distance from the sample to the lamp. Manzocco et al. (2013) studied the effect of PL treatments on another enzyme important in food spoilage, polyphenoloxidase (PPO). PPO causes browning in cut fruits and vegetables. PL treatments applied at fluences larger than 8.75 J/cm^2 resulted in complete inactivation of PPO.

PL technology may also be an interesting deactivation option for toxic substances that cannot be eliminated by heat treatment. Patulin, for example, is a toxin produced by many fungal species, and is known to contaminate apples. Since it is heat resistant at low pH values, standard pasteurization of apple juices does not eliminate significant amounts of patulin. Funes et al. (2013) found that PL is capable of reducing patulin levels and that the reduction increases with increasing dose. However, research on the mechanism of patulin decontamination by PL is still needed. Moreau et al. (2013) studied the deactivation of the mycotoxin aflatoxin B1 (AFB1) by

PL, finding that a PL dose of 8 J/cm^2 caused 92.7% AFB1 destruction. Analysis of PL treated AFB1 by LC-MS/MS revealed that PL induced fragmentation in the AFB1 structure. The Ames test (Ames et al. 1975) confirmed that the mutagenic activity had been destroyed in AFB1 samples treated with PL.

The reduction of food allergens is another potential application of PL technology. PL has been found to decrease the reactivity of soy allergens (Yang et al. 2010), the shrimp allergen tropomyosin (Shriver et al. 2011), and peanut allergens (Yang et al. 2012). However, in all three cases fluences over 100 J/cm^2 were used, and such high levels of treatment are expected to induce significant quality changes to treated foods. Nonetheless, this ability of PL to cause inactivation of undesirable or dangerous chemical components in foods is very intriguing and is worth further investigation.

CONCLUSION

The research data available in the scientific literature and discussed in this chapter clearly demonstrates the potential of PL technology to inactivate pathogenic and spoilage microorganisms in food products or on food contact materials. The fact that the effectiveness of the treatment is strongly influenced by substrate characteristics, particularly optical properties and topography, demonstrates that there are also significant limitations that need to be taken into consideration when using PL to treat complex substrates such as foods. Arguably, the best way to take advantage of the unique benefits of PL technology, such as fast inactivation and limited undesirable changes to the treated substrates, is to channel it towards applications where no other treatment can be used. An interesting prospect is the use of PL as a terminal, post-process treatment for surface decontamination of various products, including fresh cut fruits and

vegetables and ready to eat meats or cheeses. The treatment of packaging by PL, which is commercially used at the moment, is definitely a significant application of PL for food applications. Although PL is attracting increasing attention from scientists and the food industry, it remains one of the least studied emerging technologies, and much work still needs to be done in this area. In particular, more research is needed on determining the effect of PL treatments on the nutritional and sensory properties of the treated foods. The development of more energy efficient light sources in the future may also increase the utilization of this technology both for food, as well as non-food related applications.

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CHAPTER 2

THE EFFECT OF SUBLETHAL TEMPERATURES ON PULSED LIGHT INACTIVATION OF BACTERIA

ABSTRACT

Pulsed Light (PL) effectiveness is influenced by many substrate and environmental related factors. The objective of this study was to investigate the effect of temperature on the efficacy of PL treatment, using *Listeria innocua, Escherichia coli* ATCC 25922, and *Pseudomonas fluorescens* as challenge organisms. A thin layer of clear liquid phosphate buffer inoculated with one of the challenge organisms was equilibrated to 5 °C, 20 °C, 30 °C, 40 °C, or 50 °C and then treated with a PL dose of 1.02 to 12.29 J /cm^2. In the temperature range of 5 °C to 40 °C, the average maximum reductions for *L. innocua*, *E. coli*, *P. fluorescens* were 6.27 ± 0.23 log CFU, 6.66 ± 0.36 log CFU, and 6.15 ± 0.19 log CFU, respectively. Temperature did not affect PL inactivation of *E. coli* or *P. fluorescens*, but a modest synergistic effect between PL and temperature was observed for *L. innocua* treated at 50 °C.

INTRODUCTION

Pulsed light (PL) technology is a promising non-thermal microbial inactivation method that can improve food safety and shelf life, with minimal effects on the food's original nutritional and sensory attributes. PL has been shown to be effective in the inactivation of foodborne pathogens and their surrogates on foods and food contact surfaces (Proulx et al. 2015, Rajkovic et al. 2010).

PL consists of short duration pulses of high intensity broad-spectrum light ranging in wavelength from 200 to 1100 nm. The UV-C portion of the spectrum, 200 – 280 nm, is attributed with the germicidal effect of PL (Wang et al. 2005). The mechanism of inactivation by PL is still not fully understood, although it is generally accepted that PL treatment primarily inactivates microorganisms through the absorption of UV light by DNA. There is some evidence of non-UV related PL inactivation mechanisms. Specifically, some reports indicate that photothermal effects and subsequent physical disturbances exerted on the cells due to the high intensity of the light pulses may be additional mechanisms of PL microbial inactivation (Krishnamurthy et al. 2010, Wekhof 2000).

The efficiency of PL treatments is dependent on several factors, including fluence and spectral distribution of the light, the challenge microorganism, and the interaction of light with the substrate. Hsu and Moraru (2011) determined that fluence varies significantly within a PL chamber, making sample positioning relative to the lamp and lamp geometry crucial factors to consider when designing a treatment chamber. Surface properties of the substrate also affect PL treatment. If the substrate contains UV absorbing components such as proteins, pigments, or phenolic compounds, PL effectiveness can be reduced, as less UV light is available to reach target microorganisms. For solid substrates, microorganisms may lodge in surface irregularities or penetrate into the solid beyond PL's penetration depth, limiting the microorganisms' exposure to PL (Seo and Frank 1999). Properties of solid substrates such as reflectivity and roughness can also affect inactivation by PL. Smoother stainless steel coupons were found to have lower levels of inactivation than rougher coupons of the same material (Woodling and Moraru 2005), and high UV reflectivity was found to reduce inactivation on A1 substrates (Ringus and Moraru 2013).

No data currently exists in the literature on the effect of sublethal temperatures on PL efficacy. The effect of combining mild heat treatments and continuous UV treatments has been studied for *Escherichia coli* (Gayán et al. 2011), *Salmonella* (Gayán et al. 2012), *Staphylococcus aureus* (Gayán et al. 2014), and *Listeria monocytogenes* (Gayán et al. 2015). The challenge microorganisms were treated using a plug-flow reactor equipped with eight low-pressure UV lamps. Synergistic effects between heat and UV treatments were observed for each of the microorganisms studied within the 50 °C – 60 °C temperature range.

The objective of this study was to evaluate the effect of temperature on the PL inactivation of *Listeria innocua*, *E. coli* ATCC 25922, and *Pseudomonas fluorescens* in a liquid substrate. *L. innocua* and *E. coli* ATCC 25922 were determined to be non-pathogenic surrogates of *L. monocytogenes* and *E. coli* O157:H7 for PL treatments (Sauer and Moraru 2009, Uesugi and Moraru 2009). *Pseudomonas spp.* are commonly found spoilage bacteria in foods, particularly in dairy products (Ternström et al. 1993).

MATERIALS AND METHODS

Challenge organisms

L. innocua FSL C2-008 and *P. fluorescens* D3-266 were obtained from frozen stocks maintained by the Food Microbiology and Safety Laboratory at Cornell University (Ithaca, NY). *E. coli* ATCC 25922 was obtained from frozen stocks maintained by the Worobo Laboratory at Cornell University. The cultures were streaked onto tryptic soy agar (TSA) plates and incubated for 24 ± 2 h at 37 ± 2 °C for *L. innocua* and *E. coli* and at 30 ± 2 °C for *P. fluorescens*.

Optical properties of *P. fluorescens* samples

The absorbance spectra of *P. fluorescens* samples at 5 °C, 20 °C, 30 °C, and 40 °C were determined in the range of 200 to 1100 nm using a Fiber Optic Spectrometer HR 2000 + CG-UV-NIR (Ocean Optics, Inc.; Dunedin, FL).

Substrate preparation

A single colony of the challenge microorganism was isolated from TSA plates and transferred into tryptic soy broth (TSB). The broth was incubated with shaking at 210 rpm for 24 ± 2 h at 37 ± 2 °C for *L. innocua* and *E. coli* and at 30 ± 2 °C for *P. fluorescens*. Incubation times were selected to yield cells in stationary growth state. The culture was diluted 10 fold in Butterfield's Phosphate Buffer (BPB). The initial inoculum level was 10^8 CFU/mL for *L. innocua* and *E. coli* and 10^6 CFU/mL for *P. fluorescens*. One milliliter of the inoculated BPB was transferred into Nunc Lab-Tek II 1 well Chamber Slides (Thermo Fisher Scientific, Waltham, MA). The chamber slide and substrate were brought to the temperature of interest prior to PL exposure by placing the sample in an incubator for at least 15 minutes to ensure that the target temperature (± 0.5 °C) had been reached. Sublethal temperatures for the three challenge organisms were chosen. The temperatures studied were 5 °C, 20 °C, 30 °C, 40 °C, and 50 °C, although thermal inactivation of *E. coli* and *P. fluorescens* occurred at 50 °C. The temperature of each sample was measured immediately prior to PL treatment using a Type K temperature probe (ThermoWorks, Salt Lake City, UT).

PL treatments

PL treatments were performed using a Xenon RS-3000C SteriPulse System (Xenon Corp., Wilmington, MA). A xenon flashlamp within the unit emits broad spectrum light ranging in wavelength from 200 to 1100 nm, with a pulse width of 360 μs and a pulse frequency of 3 pulses per second. Chamber slides were centrally placed on an adjustable stainless steel shelf located 5.8 cm below the flashlamp housing. Samples were exposed to 1, 3, 6, 9, or 12 pulses of light, corresponding to fluence values of 1.02, 3.07, 6.14, 9.22, and 12.29 J/cm^2, respectively. A waiting period of at least 60 s between treatments was used to prevent overheating of the flashlamp.

Fluence measurements

A pyroelectric detector head (PE25BB-DIF) connected to a Nova II energy meter (Ophir Optronics, North Andover, MA) was used to quantify the fluence received by the samples. The detector was placed inside the PL unit treatment chamber with the diffusor opening of the detector centrally located 5.8 cm below the flashlamp housing. The detector cable and base were covered in aluminum foil, leaving only the diffuser opening exposed. Fluence measurements were conducted for 1, 3, 6, 9, and 12 pulses. Measurements were performed at least 60 s apart to prevent overheating of the flashlamp and pyroelectric detector. All measurements were performed in triplicate.

Recovery and enumeration of survivors

Following PL treatment the 1 mL sample was transferred from the chamber slide to a test tube containing 7 mL of TSB. The chamber slide was rinsed twice with 1 mL of TSB to yield a

final volume of 10 mL. Serial dilutions in BPB were performed and the appropriate dilutions were plated in duplicate on TSA plates. Plates were incubated for 24 ± 2 h at 37 ± 2 °C for *L. innocua* and *E. coli* and at 30 ± 2 °C for *P. fluorescens*. Survivors were enumerated using standard plate counting. When counts below 25 CFU/plate were expected the three-tube most probable number technique was used (Downes et al. 2001). Microbial reductions were calculated as $\log[N/N_0]$, where N is the number of survivors after PL treatment and N_0 is the initial inoculum level as determined by positive controls - samples exposed to temperature conditions, but not PL treatment.

Modeling of inactivation kinetics

The suitability of the Weibull model for predicting the survival curves of *L. innocua*, *E. coli*, and *P. fluorescens* across the temperature range of 5 °C to 50 °C was evaluated. The Weibull model (Eq. 1) was selected, since it has been shown to accurately predict PL inactivation of *L. innocua* in clear liquid substrates at room temperature (Uesugi et al. 2007):

$$\mathrm{Log}(N/N_0) = -b \times F^n \qquad (1)$$

where N is the number of survivors after PL treatment, N_0 is the initial inoculum level, b is the scale parameter, n is the shape parameter, and F is the fluence in J/cm^2. The shape parameter indicates the shape of the survivor curve, with $n > 1$ indicating concave down survival curves, $n < 1$ concave up survival curves, and $n = 1$ linear survival curves.

Statistical analysis

All experiments were performed in biological and technical triplicate. ANOVA and Tukey's HSD test were used to determine if differences in inactivation between treatments at different temperatures were significant ($p < 0.05$), using the statistical program RStudio (R Core Team 2014).

RESULTS AND DISCUSSION

Effect of PL and temperature on *L. innocua*

The inactivation of *L. innocua* as a function of fluence and sample temperature is shown in **Fig. 2.1**. The maximum reductions achieved were 6.06 ± 0.24 log CFU, 6.41 ± 0.57 log CFU, and 7.58 ± 0.53 log CFU at refrigeration temperature (5 °C), room temperature (20 °C), and 50 °C, respectively. The reductions achieved at 50 °C were significantly greater ($p < 0.05$) than the reductions achieved at room temperature for every fluence level, except 6.14 J/cm^2. Differences between inactivation levels at 5 °C, 20 °C, 30 °C, and 40 °C were not significant for the majority of the fluence levels tested.

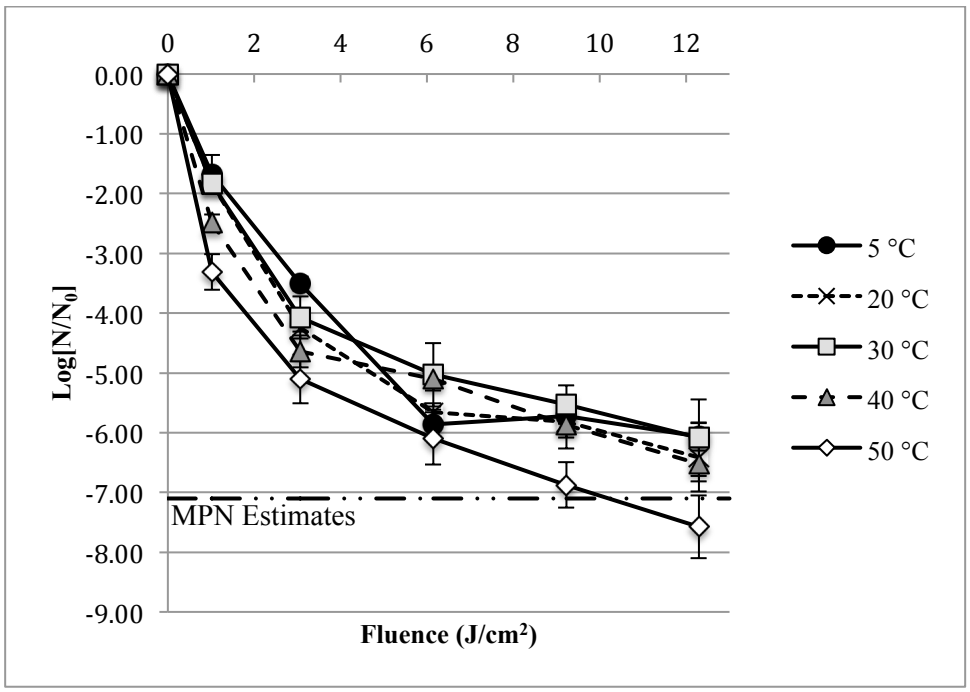

Figure 2.1. PL inactivation curves for *L. innocua* at different treatment temperatures

Synergism between temperature and PL treatment led to increased inactivation at 50 °C. A synergistic effect between continuous UV and temperature has been reported for *L. monocytogenes* UV treated at temperatures between 50 °C and 60 °C (Gayán et al. 2015). The authors suggested that this synergism is due to an increase in the fluidity of the cell membrane of the target microorganism, which may also explain the modest synergism in our study. Greater cell membrane fluidity may make the cell membrane more susceptible to breakdown (Jayaram and Castle 1992), which could lead to greater susceptibility to photothermal and/or photophysical damage that may be caused by PL treatments. In the present study, it is also possible that a slight increase in cell membrane fluidity resulted in a slight improvement of PL effectiveness at 50 °C.

The Weibull kinetic parameters for PL inactivation of *L. innocua* at 5 °C, 20 °C, 30 °C, and 40 °C, and 50 °C were also determined (**Table 1**).

Table 2.1. Weibull shape (n) and scale (b) parameters for the PL inactivation of *L. innocua* at different temperatures

Temperature, °C	Shape parameter (n)	Scale parameter (b)
5	0.54 ± 0.08^{a}	1.81 ± 0.32^{a}
20	0.50 ± 0.01^{a}	2.05 ± 0.05^{a}
30	0.47 ± 0.03^{ab}	2.02 ± 0.13^{a}
40	0.37 ± 0.00^{bc}	2.65 ± 0.15^{b}
50	0.33 ± 0.02^{c}	3.37 ± 0.30^{c}

*Different letters within the same column are significantly different ($p<0.05$)

A temperature dependence of the shape and scale Weibull parameters was observed (**Fig. 2.2**) and quantified (Eq. 2, 3):

$$n = -0.005T + 0.584 \quad (2)$$

$$b = 1.577e^{0.013T} \quad (3)$$

These equations can be used to calculate the shape and scale parameters at any temperature within the 5 °C to 50 °C range.

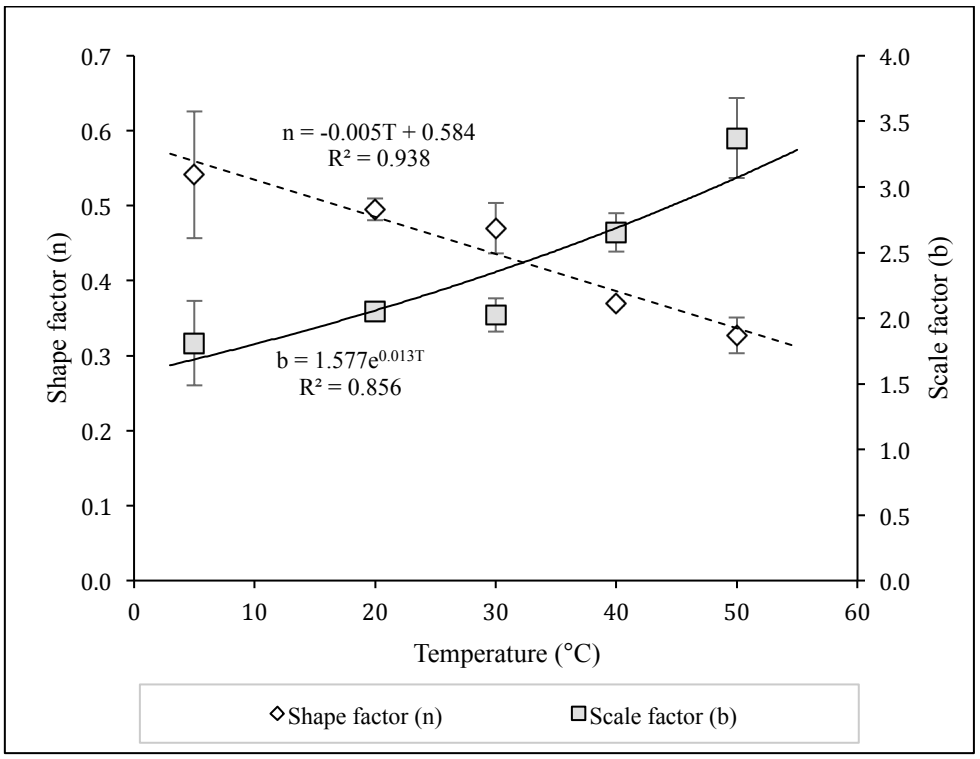

Figure 2.2. Weibull shape (n) and scale (b) parameters as a function of temperature (T), for the PL inactivation of *L. innocua*

A validation of the model was conducted for a PL treatment temperature of 45 °C. Specifically, the Weibull shape and scale parameters were calculated for this temperature and then substituted in Eq. 1 to predict the PL survival curve for *L. innocua* at 45 °C. Predicted values were compared to an experimental validation point at 10.2 J/cm^2 (**Fig. 2.3**).

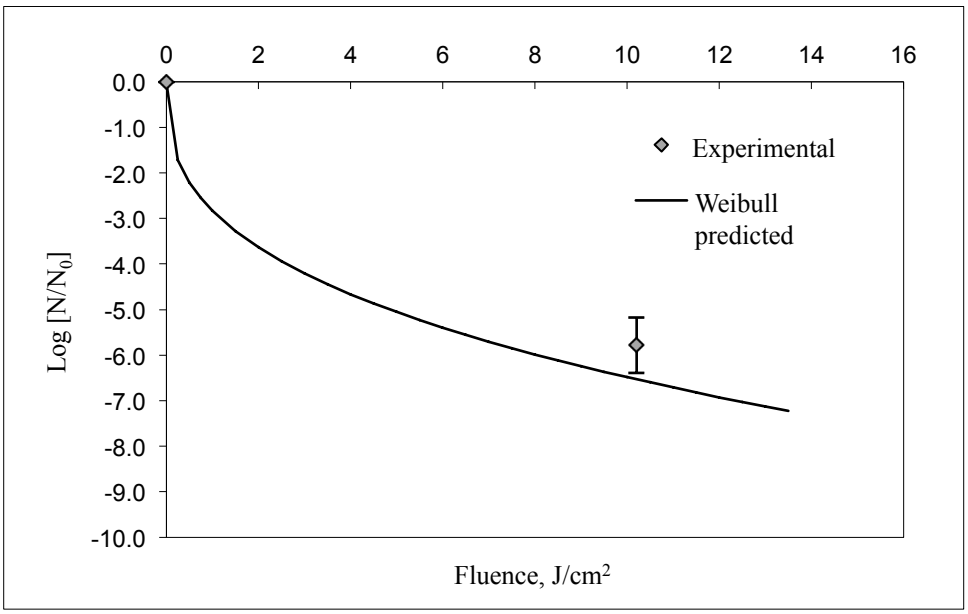

Figure 2.3. Weibull model predictions of PL inactivation compared to experimental PL inactivation of *L. innocua* at 45 °C and 10.2 J/cm^2

The Weibull model overestimated the inactivation of *L. innocua* under these conditions; the reductions were 6.52 log CFU and 5.78 ± 0.61 log CFU for the predicted and experimental values, respectively. The overestimation of the inactivation at 45 °C may be due to a slight model error caused by the synergism between PL and temperature, particularly at 50 °C.

Effect of PL and temperature on *E. coli*

Temperature did not have a significant effect in the inactivation of *E. coli* by PL in the temperature range of 5 °C – 40 °C (**Fig. 2.4**). The maximum temperature included in this analysis is 40 °C because thermal inactivation of *E. coli* occurred at 50 °C. The maximum PL reductions were 7.13 ± 0.87 log CFU, 6.66 ± 0.38 log CFU, and 6.25 ± 0.38 log CFU at 5 °C, 20 °C, and 40 °C, respectively.

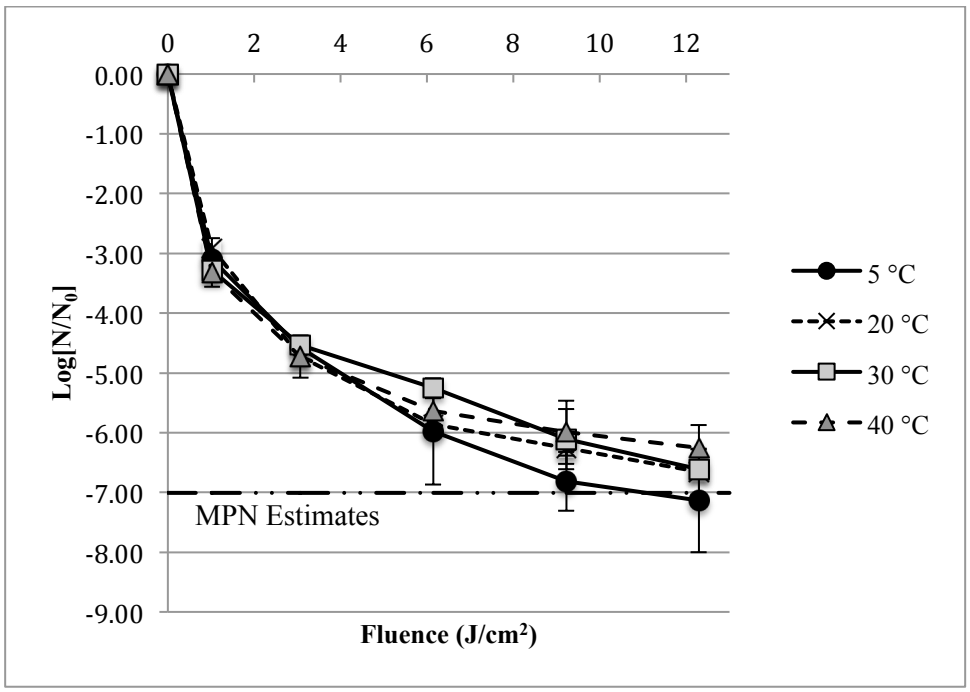

Figure 2.4. PL inactivation curves for *E. coli* at different treatment temperatures

Given that PL inactivation of *E. coli* was independent of temperature, average rather than temperature dependent Weibull parameters were determined and then used to predict the inactivation kinetics of *E. coli* within the temperature range of 5 °C to 40 °C. The average values for the shape (n) and the scale (b) parameters were 0.30 ± 0.05 and 3.21 ± 0.20, respectively. Therefore, PL inactivation of *E. coli* in a clear liquid substrate in the temperature range of 5 °C to 40 °C can be described by the following model:

$$\mathrm{Log}(N/N_0) = -3.21 \times F^{0.30} \qquad (4)$$

The resulting survival curve was compared to an experimental validation point of 35 °C and 10.2 J/cm^2 (**Fig. 2.5**). The experimental and predicted reductions were very close, at 6.67 ± 0.71 log CFU and 6.44 log CFU, respectively. This demonstrates that Eq. 4 can be used to accurately predict PL inactivation of *E. coli* in a clear liquid in the sublethal temperature range of 5 °C to 40 °C.

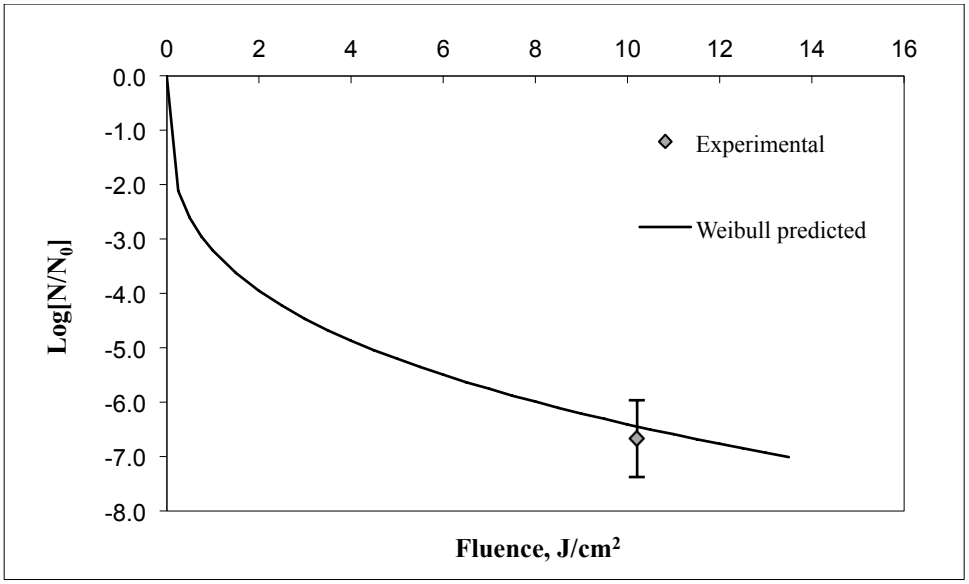

Figure 2.5. Weibull model predictions compared to experimental reduction for the PL inactivation of *E. coli* at 35 °C and 10.2 J/cm^2

Effect of PL and temperature on *P. fluorescens*

In general, temperature did not affect the PL inactivation of *P. fluorescens* in the temperature range of 5 °C to 40 °C. Thermal inactivation of *P. fluorescens* occurred at 50 °C, limiting the maximum temperature range considered to 40 °C. The maximum PL reductions achieved for *P. fluorescens* at 5 °C, 20 °C, and 40 °C were 6.41 ± 0.18 log CFU, 6.16 ± 0.21 log CFU, and 6.07 ± 0.16 log CFU, respectively (**Fig. 2.6**).

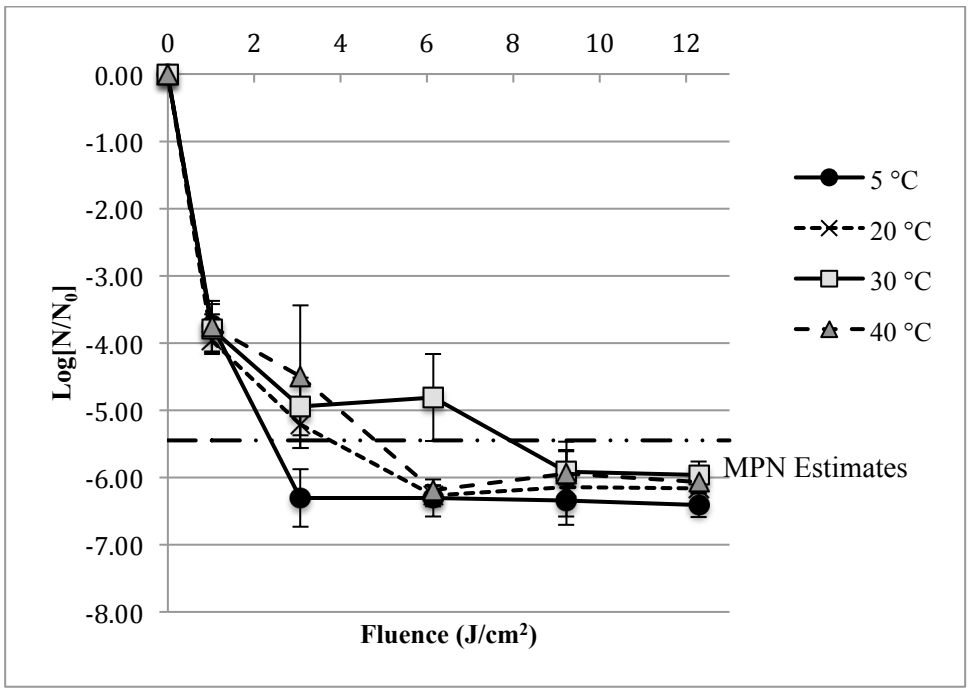

Figure 2.6. PL inactivation curves for *P. fluorescens* at different treatment temperatures

The reason for the 5 °C and 30 °C outliers at 3.07 J/cm^2 and 6.14 J/cm^2, respectively, is unclear. *Pseudomonas spp*. are known to produce extracellular polymeric substances (EPS), and the production of EPS can vary with temperature (Sandford 1979). One hypothesis was that EPS could potentially absorb UV light, changing the amount of UV light available to inactivate microorganisms. However, there was no difference in the absorbance measurements of BPB inoculated with *P. fluorescens* at the five temperatures studied. Other phenomena such as light scattering or cell clustering and subsequent shading of microorganisms from the microbicidal light may account for the variability of inactivation data observed at these two fluence levels.

Neither the shape (n) parameter nor the scale (b) parameter varied significantly ($p < 0.05$) with temperature for *P. fluorescens*. Thus, average shape and scale parameters were used to predict the PL inactivation kinetics for this microorganism. The predicted survival curve was compared to an experimental validation point at 35 °C and 10.2 J/cm^2 (**Fig. 2.7**).

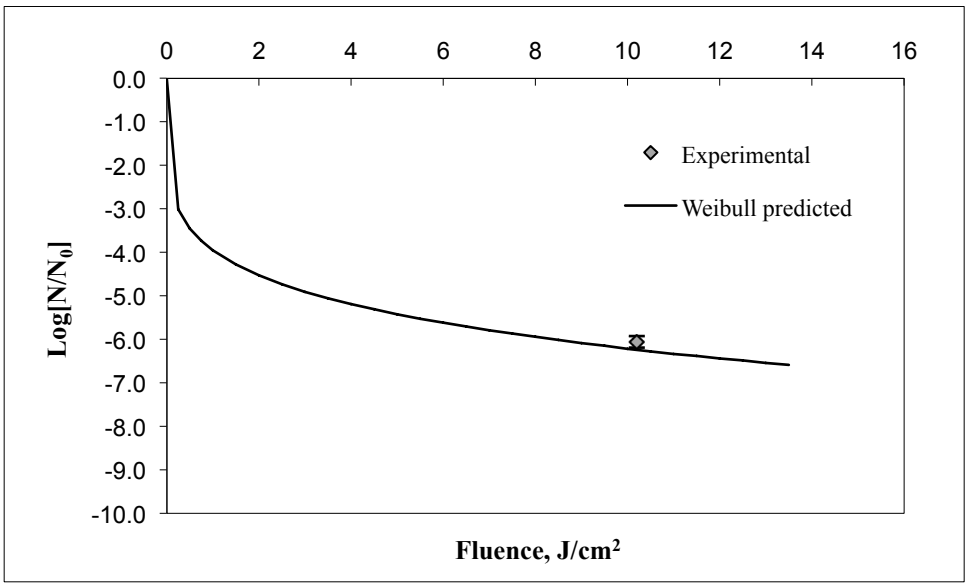

Figure 2.7. Weibull model predictions compared to experimental reduction for the PL inactivation of *P. fluorescens* at 35 °C and 10.2 J/cm^2

The Weibull model accurately predicted the inactivation of *P. fluorescens* by PL at 35 °C and 10.2 J/cm^2 in a clear liquid substrate. Therefore, PL inactivation of *P. fluorescens* can be accurately be predicted by the Weibull model with a shape factor (n) of 0.20 ± 0.03 and a scale factor (b) of 3.95 ± 0.34, in the temperature range of 5 °C to 40 °C.

Comparison of PL susceptibility of *L. innocua*, *E. coli*, and *P. fluorescens*

Comparison of PL inactivation at 5 °C, 20 °C, and 40 °C (**Fig. 2.8**) of the three microorganisms studied revealed that *L. innocua* is the most resistant and *P. fluorescens* is the least resistant to PL at low temperatures and low fluence levels, although the difference in the PL sensitivity of the three microorganisms decreases as temperature and PL dose increase. This finding is consistent with the results of other PL studies. Proulx et al. (2015) found that *L. innocua* was more resistant to PL than *E. coli* and *P. fluorescens* when treated on the surface of

cheese slices, and Jay et al. (2005) also found that gram-positive bacteria were less sensitive to UV treatments than gram-negative bacteria.

L. innocua also has higher heat resistance than *E. coli* and *P. fluorescens*, allowing for the detection of synergistic effects between PL and temperature at 50 °C. While synergism was not detected for *E. coli* and *P. fluorescens* in this study, synergism between continuous UV and heat treatments has been observed for the inactivation of *E. coli* in a liquid substrate (Gayán et al. 2011). It is possible that synergism between PL and temperature also occurs in the inactivation of *E. coli* and *P. fluorescens* at an intermediate temperature yet to be studied in this system.

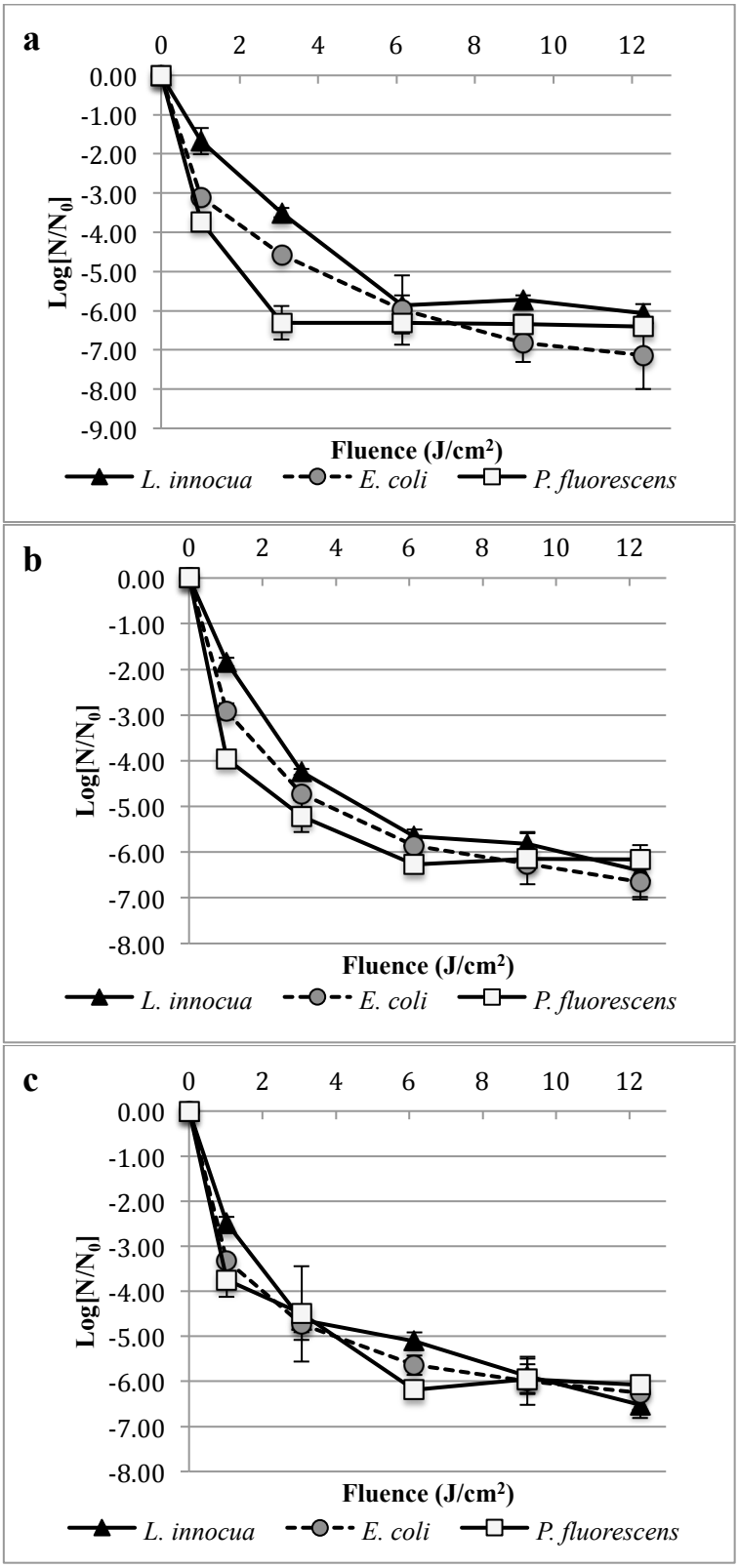

Figure 2.8. PL inactivation of *L. innocua*, *E. coli*, and *P. fluorescens* at 5 °C, 20 °C and 40 °C (**a**, **b**, **c**)

CONCLUSIONS

The results of this study indicate that the effects of PL treatment are independent of temperature for *E. coli* and *P. fluorescens* in clear liquid substrates within the temperature range of 5 °C to 40 °C, and the Weibull model accurately predicted the inactivation kinetics of these two microorganisms. A modest synergistic effect was observed for *L. innocua* at 50 °C. The lack of a temperature effect is a practical benefit for food processing. When predicting PL inactivation, the temperature of the substrate need not be considered within typical non-thermal processing temperatures of 5 °C to 40 °C.

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CHAPTER 3

THE EFFECT OF UV-REFLECTIVY ON PULSED LIGHT INACTIVATION OF BACTERIA

ABSTRACT

Pulsed Light (PL) is an antimicrobial light-based technology that consists of high intensity pulses of broad spectrum light ranging from 200 to 1100 nm. The effectiveness of PL treatment is highly dependent on light – substrate interactions, since light reflection, absorption and scattering by the treated substrate or its surroundings can affect the actual treatment dose that reaches the microbial cells. The objective of this study was to improve the effectiveness of PL treatment by inducing a fluence multiplication effect in the UV range, using UV reflective materials in the PL chamber. Specifically, the PL inactivation of *Listeria innocua* using either UV-reflective sample containers or UV-reflective coatings on low density polyethylene (LDPE) packaging was studied. Butterfield's phosphate buffer (BPB) was inoculated with *L. innocua* and treated with 12.29 J/cm^2 of PL in either UV-reflective or non-UV-reflective containers. PL inactivation of *L. innocua* was significantly higher ($p < 0.05$) in the UV-reflective containers. The PL reductions were 7.83 ± 0.41 log CFU and 6.41 ± 0.32 log CFU for the UV-reflective and non-UV-reflective containers, respectively. PL inactivation experiments using UV-reflective LDPE packaging have not been conducted because optical measurements revealed that the coatings did not increase the specular reflection of the packaging material in the UV range. The main conclusion of this study is that PL effectiveness in liquid substrates can be improved through the use of UV-reflective sample containers. Similar effects may be obtained by using truly UV-reflective packaging materials, but this will have to be tested in future experiments.

INTRODUCTION

Pulsed Light (PL) is a promising non-thermal technology for the surface decontamination of food and food packaging. PL has been shown to be effective in the inactivation of common foodborne pathogens and their surrogates in clear liquid substrates (Hsu and Moraru 2011), on food packaging (Ringus and Moraru 2013), and on the surface of foods such as cheese (Proulx et al. 2015). PL consists of short, high intensity pulses of broad spectrum light ranging in wavelength from 200 to 1100 nm. A xenon flashlamp is typically used to produce the light pulses. The FDA has approved the use of PL technology on food and food contact surfaces, provided that a xenon flashlamp is used as the light source, the pulse width does not exceed 2 milliseconds, and the total dose does not exceed 12 J/cm^2 (FDA Code 21CFR179.41).

While the mechanism of PL inactivation is not completely understood, it is generally accepted that PL inactivates microorganisms through the absorption of UV light by the microorganisms' DNA. DNA absorption of UV light causes crosslinking between pyrimidine nucleoside bases in the same DNA strand, inhibiting DNA replication and transcription (Sinha and Hader 2012). The cells are capable of repairing this DNA damage, but using an appropriate PL dose can still cause cell death.

The objective of this study was to improve the effectiveness of PL treatments on the inactivation of a non-pathogenic surrogate of *L. monocytogenes*, *L. innocua*, through the use of UV-reflective sample containers and LDPE packaging coated with a UV-reflective substance.

MATERIALS AND METHODS

Challenge organism

L. innocua FSL C2-008 was obtained from frozen stocks maintained by the Food Microbiology and Safety Laboratory at Cornell University (Ithaca, NY). The culture was streaked onto tryptic soy agar (TSA) plates and incubated for 24 ± 2 h at 37 ± 2 °C.

Substrate preparation

A single colony of the *L. innocua* was isolated from TSA plates and transferred into tryptic soy broth (TSB). The broth was incubated with shaking at 210 rpm for 24 ± 2 h at 37 ± 2 °C to obtain stationary growth state culture. The culture was diluted 10 fold in Butterfield's Phosphate Buffer (BPB), to give an initial inoculum level of 10^8 CFU/mL of *L. innocua*. For tests using non-UV-reflective sample containers, 3.5 mL of the inoculated BPB were transferred into Nunc Lab-Tek II 1 well Chamber Slides (Thermo Fisher Scientific, Waltham, MA) with black cardboard covering bottom exterior of the chamber. For tests using UV-reflective sample containers, 8 mL of inoculated BPB were transferred into Fisherbrand 42 mL aluminum weigh boats (Thermo Fisher Scientific, Waltham, MA). The different volumes for each type of sample container were chosen such that the substrate thickness in each chamber would be the same.

PL treatments

Pulsed Light treatments were performed using a Xenon RS-3000C SteriPulse System (Xenon Corp., Wilmington, MA). A xenon flashlamp within the unit emits broad spectrum light ranging in wavelength from 200 to 1100 nm, with a pulse width of 360 μs and a pulse frequency of 3 pulses per second. Sample containers were centrally placed on an adjustable stainless steel

shelf located 5.8 cm below the flashlamp housing. Samples were exposed to 12 pulses of light, corresponding to a fluence value of 12.29 J/cm^2. A waiting period of at least 60 seconds between treatments was used to prevent overheating of the flashlamp.

Fluence Measurements

A pyroelectric detector head (PE25BB-DIF) connected to a Nova II energy meter (Ophir Optronics; North Andover, MA) was used to quantify the fluence received by the samples. The detector was placed inside the PL unit treatment chamber with the diffusor opening of the detector centrally located 5.8 cm below the flashlamp housing. The detector cable and base were covered in aluminum foil, leaving only the diffuser opening exposed. Measurements were performed at least 60 seconds apart to prevent overheating of the flashlamp and pyroelectric detector. The measurements were performed in triplicate.

Recovery and enumeration of survivors

Following PL treatment the 1 mL sample was transferred from the chamber slide to a test tube containing 7 mL of TSB. The chamber slide was rinsed twice with 1 mL of TSB to yield a final volume of 10 mL. Serial dilutions in BPB were performed and the appropriate dilutions were plated on TSA plates. Plates were incubated for 24 ± 2 h at 37 ± 2 °C. Survivors were enumerated using standard plate counting. Log reductions were calculated as $\log[N/N_0]$, where N is the number of survivors after PL treatment and N_0 is the initial inoculum level.

Coated low density polyethylene packaging preparation

Clear low density polyethylene (LDPE) bags were coated with two to four coats, according to package instructions, of potentially UV reflective sprays. Rust-oleum Reflective Finish #214944, Rust-oleum Triple Thick Glaze #264985, Rust-oleum Clear Lacquer #1906830, and Krylon Fusion for Plastic: Clear Glass #2444 were purchased from a local hardware store. After spray application LDPE bags were left to dry for a minimum of 24 h. Two types of self-adhesive window strike bird deterrent, Ultraviolet Hawk Decals by Songbird Essentials and BirdTape by the American Bird Conversancy, were purchased from a local bird observatory supply store. The window strike deterrents were cut to the size of the LDPE bags and then applied to the surface of the bags.

Optical properties of sample containers and coated polyethylene bags

The specular reflection measurements of the two types of sample containers and coated and uncoated LDPE bags were collected using the Fiber Optic Spectrometer HR 2000 + CG-UV-NIR with a QR400-7-SR Reflection Probe and STAN-SSH standard (Ocean Optics, Inc.; Dunedin, FL). The probe was held at a 90° angle relative to the container surface using a CSH probe holder (Ocean Optics).

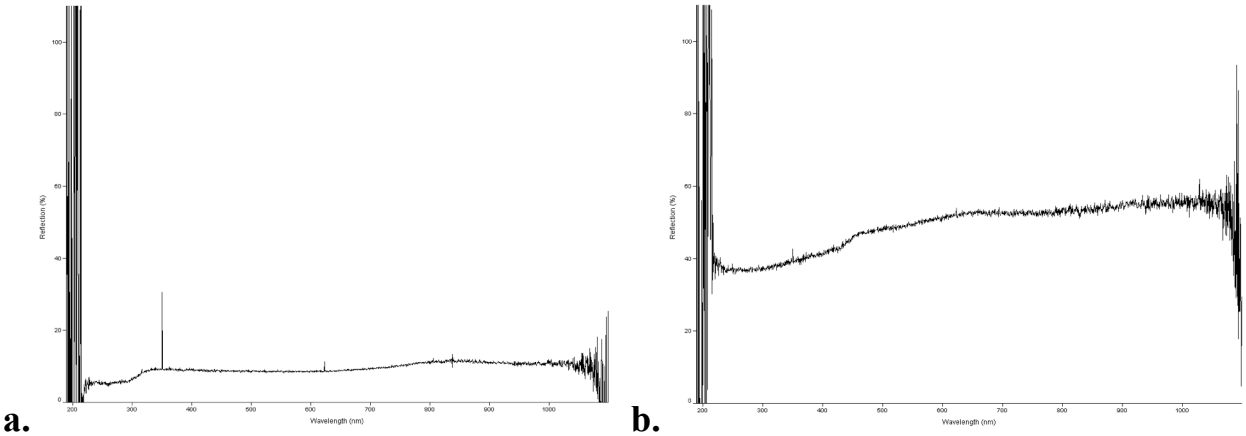
Statistical analysis

A Welch two sample t-test was used to determine if differences between the log reductions achieved using the two types of treatment chambers were significant ($p < 0.05$). The test was performed using the statistical program RStudio (R Core Team 2014).

RESULTS AND DISCUSSION

Reflectivity of sample containers

The specular reflectivity of the aluminum weigh boats and the chamber slides with black cardboard covering the bottom exterior were determined (**Fig. 3.1**).



a. **b.**

Figure 3.1. Specular reflectance of Nunc Lab-Tek II chamber slides with black cardboard on the bottom exterior (**a**) and Fisherbrand aluminum weigh boats (**b**) in the region 200 – 1100 nm

The aluminum weigh boats exhibited higher specular reflectivity across the entire range of light emitted by the xenon flashlamp, 200 to 1100 nm, and most importantly in the germicidal range of 200 to 280 nm. The chamber slides with black cardboard bottom exterior exhibited minimal reflection of UV light, whereas the aluminum weigh boats exhibited moderate reflection of UV light, making them suitable containers for the comparison of PL treatments using UV reflective and non-UV reflective containers.

PL inactivation of *L. innocua* in UV reflective and non-UV reflective containers

The reductions of *L. innocua* when treated with a PL dose of 12.29 J/cm^2 in UV reflective and non-UV reflective containers were 7.83 ± 0.41 log CFU and 6.41 ± 0.32 log CFU, respectively. The difference in inactivation between the two containers was statistically significant ($p < 0.05$), with more effective inactivation occurring in samples treated in UV reflective containers.

Reflectivity of low density polyethylene packaging materials

Given that UV-reflective treatment chambers increase the PL inactivation of *L. innocua*, UV-reflective packaging could also improve the efficacy of PL treatments. The coatings and window strike deterrents tested were selected for their potential to be UV-reflective, in order to find a UV-reflective coating for LDPE packaging that could be used in PL inactivation experiments. Unfortunately, none of the coatings tested were any more reflective in the germicidal range of 200 to 280 nm than uncoated LDPE bags. Select reflection spectra are shown in **Fig. 3.2**. No PL inactivation experiments with UV-reflective packaging were conducted due to the lack of a suitable coating.

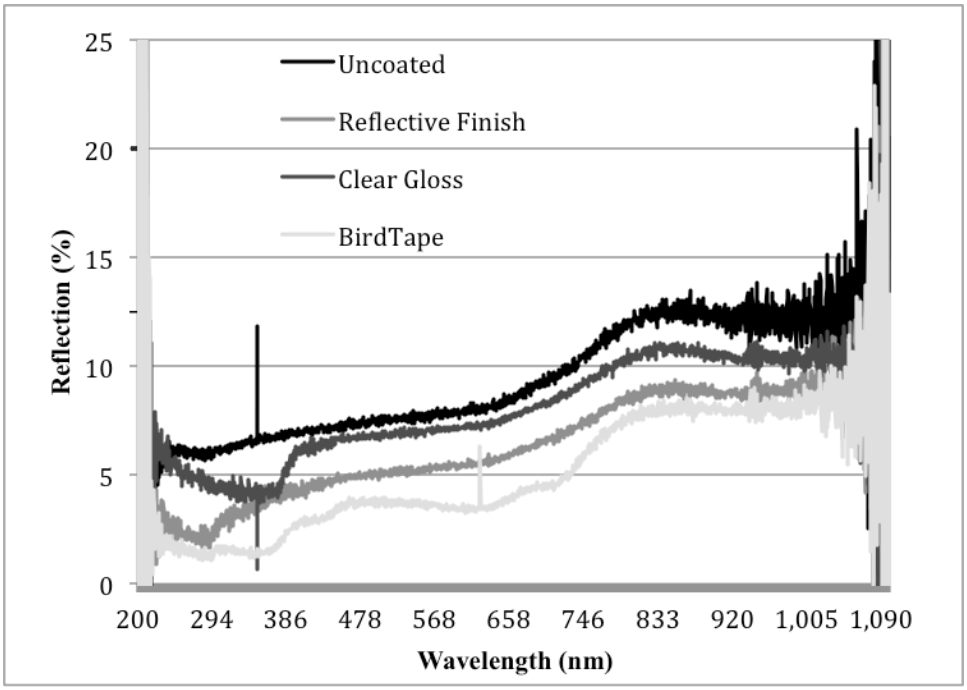


Figure 3.2. Specular reflection of uncoated and select coated LDPE packaging

CONCLUSIONS

This study shows that UV-reflective treatment chambers can improve the PL inactivation of *L. innocua* in clear liquid substrates at a dose of 12.29 J/cm^2. Future work should test for this effect at the additional fluence levels. The search for a UV-reflective coating for LDPE was unsuccessful. If an acceptable UV-reflective coating cannot be identified, other options, such as covering the PL treatment chamber in a UV-reflective material like aluminum, could be investigated to improve PL inactivation of packaged substrates.

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