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The Use of 405nm and 464nm Blue Light to Inhibit *Listeria monocytogenes* in Ready-to-Eat (RTE) Meat

Motts, Susan D[1], Guffey, J Stephen[2*], Payne, William C[3], Towery, Pam[4], Hobson, Todd[5], Harrell, Grafton[5], Meurer, Logan[6], Lancaster, Kristoffer[6]

[1] Assistant Professor, Arkansas State University,
Department of Physical Therapy, P.O. Box 910, State University, Arkansas 72467
smotts@astate.edu

[2]Professor, Arkansas State University,
Department of Physical Therapy, P.O. Box 910, State University, Arkansas 72467
jguffey@astate.edu

[3]Associate Professor, Arkansas State University,
Department of Clinical Laboratory Sciences, P.O. Box 910, State University, Arkansas 72467
wpayne@astate.edu

[4]Assistant Professor and Director, Arkansas State University,
Department of Dietetics, P.O. Box 910, State University, Arkansas 72467
ptowery@astate.edu

[5]Doctor of Physical Therapy Student, Arkansas State University,
Department of Physical Therapy, P.O. Box 910, State University, Arkansas 72467
grafton.harrell@smail.astate.edu
todd.hobson@astate.edu

[6]Bachelor of Science Student, Arkansas State University,
Department of Clinical Laboratory Sciences, P.O. Box 910, State University, Arkansas 72467
logan.meurer@smail.astate.edu
Kristoffer.lancaster@smail.astate.edu

Abstract: Foodborne illness resulting from contaminating organisms occurring in Ready-to-Eat (RTE) foods and vegetables is a serious health concern in the United States. Improved and cost-effective techniques for disinfection are needed. Visible light in the blue range (405nm and 464nm) was administered to an RTE meat product that had been inoculated with *Listeria monocytogenes*. One application of light energy, at doses of 10, 30, 60, 90, and 120 J/cm^2, was applied, *in vitro*, for each wavelength of 405nm and 464nm. After 20 hours of incubation, colony forming units were counted and compared to controls to determine whether the light energy inhibited growth of *L. monocytogenes*. Each of the dose / wavelength combinations used in the experiment resulted in a significant inhibition of *L. monocytogenes*. Kill rates ranging from 69.55 – 85.25% were obtained. Blue light, delivered in the wavelength / dose combinations used in this study, is an effective *in vitro* inhibitor of *L. monocytogenes*. Blue light should be considered as a potentially effective tool in the effort to secure (in terms of disinfection of microbes) the food supply.

Key words: LLLT, Photobiomodulation, Food Contamination, Foodborne Illness

1. Introduction

Forty-eight million foodborne illnesses occur annually in the United States, with an estimated 9.4 million foodborne illnesses occurring from known pathogens [1]. The Foodborne Disease Outbreak Surveillance System, the Center for Disease Control and Prevention's (CDC) foodborne disease tracker, defines foodborne outbreaks as the occurrence of two or more cases of a similar illness

Corresponding Author: J. Stephen Guffey, Department of Physical Therapy, Arkansas State University, jguffey@astate.edu

resulting from the ingestion of a common food [1]. Annually, approximately 128,000 of the 48 million foodborne illness result in hospitalization and 3,000 result in death [2].

In 2011, five specific pathogens were reported to contribute to the majority of deaths resulting from foodborne illness: *Salmonella, Toxoplasma gondii, Listeria monocytogenes*, Norovirus, and *Campylobacter spp* [3].

Not only is foodborne illness a dangerous public health problem, it is a costly one. In 2007, estimated worldwide costs related to foodborne illness reached 1.4 trillion dollars. As part of that S1.4 trillion price tag, treatment for illness related to *Listeria monocytogenes* infection accounted for $2.3 billion [4]. Food Safety News estimates the annual economic burden associated with foodborne illness in the United States for 2011 was $77.7 billion [5]. Some estimates range as high as $152 billion [6].

Foodborne illness is a preventable disease; however, the contemporary occurrences of outbreaks are well documented [7] [8]. Measures are taken to remove pathogens prior to and/or during food processing, but some pathogens are not effectively removed and outbreaks of foodborne illnesses occur [3] [9] [10] [11]. Sanitation, pasteurization, canning, and cooking methods have traditionally been employed to reduce the number of pathogens present on foods. Recently high-energy radiation has been adopted as another measure to prevent foodborne contamination [12] [13]. Each of these measures should eliminate pathogens, yet foodborne illnesses continue to occur. The Council for Agriculture, Science and Technology found that 70% of samples of uncooked meats obtained from seven different countries contained detectable levels of *L. monocytogenes* [14].

Recent outbreaks in the United States associated with *L. monocytogenes* have been reported in dairy products, deli meats, and frozen foods [7] [9] [10] [11]. Many persons exposed to the *L. monocytogenes* were hospitalized, with three deaths occurring in Kansas. The majority of persons diagnosed with listeriosis spontaneously clear the infection in approximately 7 days. Those patients with an increased risk for developing significant illness associated with *L. monocytogenes* (pregnant women, newborns, adults 65+, those immunocompromised) usually require IV antibiotic treatment to control the infection [15].

Given that foodborne pathogens continue to cause illness, a search for additional effective disinfection techniques that are applicable to the food industry is indicated. Blue light has been demonstrated inhibitive for various microbes including *Candida albicans* [16], *Staphylococcus aureus* [17] [18] [19] [20], *Mycobacterium smegmatis* [21], *Pseudomonas aeruginosa* (Guffey, Wilborn [17] [18], *Acinetobacter baumannii* [22], and *Klebseilla pneumoniae* [23]. The assumed mechanism for this inhibition is the production of reactive oxygen species [24]. Specifically considering *L. monocytogenes*, Murdoch, Maclean, Endarko, Macgregor, and Anderson [25] demonstrated effective inhibition *in vitro* in a liquid solution.

The purpose of this study is to extend the Murdoch et al [25] work to evaluate, *in vitro*, the effect of blue light (405nm and 464nm) on the growth of *L. monocytogenes* in a Ready-to-Eat meat product (packaged hot dogs).

2. Methods and Materials

The *Listeria monocytogenes* strain, American Type Culture Collection (ATCC®) 35152 (ATCC Manassas, VA), was used in this study. The organism was grown on BBL tryptic soy agar II supplemented with 5% sheep blood (Becton, Dickinson and Company, Sparks, MD). After an overnight incubation at 35-37° a sterile cotton-tipped swab was used to remove 3-4 well isolated colonies. The organism was then suspended in sterile deionized water and the turbidity adjusted to match a 0.5 McFarland standard, yielding an approximate cell density of 1.5 X 10^8 CFU/mL. Using an adjustable automatic pipette (to maintain accuracy and reproducibility) the cell suspension was diluted 1/20,000. This rendered the final culture density 7.5 X 10^3 CFU/ml. All dilutions were made immediately before administration of the light.

A Ready-to-Eat meat product (packaged hot dogs) was cut into small squares and placed into the bottom of a 60 X 15 mm sterile, polystyrene petri dish. The surface of the cut meat product was blotted with a sterile, absorbent cotton square to ensure the surface was not excessively moist and to remove any oily residue. An adjustable automatic pipette, designed to deliver 0.5-10 microliters, was used to instill a 10 µL aliquot of the diluted bacterial suspension onto the surface of the meat product. This preparation was then illuminated with light probes to deliver a pre-determined dose of 405nm and 464nm. After delivery of the appropriate dose of light, the surface of the meat product was pressed against the surface of tryptic soy agar (Becton, Dickinson and Company, Sparks, MD) in a 60 X 15 mm sterile, polystyrene petri dish. The meat product was removed and sterile, disposable, calibrated inoculating loops (10µL size) were used to spread the inoculum in a "star streak" pattern. After incubation at 35-37° C for a period of approximately 20 hours, the plates were examined for the presence of bacterial colonies and a colony count recorded.

For this experiment, we chose to illuminate the *L. monocytogenes* using supra-luminous diode (SLD) light probes capable of delivering primary wavelengths of 405nm and 464nm. The probes consisted of a 5 cm^2 illuminating surface area comprised of 34 supra-luminous diodes (SLDs) with a maximum power output of 1000 mW. Since output for the probes was held constant, adjustment in time of irradiation provided the doses used in the experiment (10, 30, 60, 90, and 120 J/cm^2). The probes were fixed in a frame so as to be very near (3 – 5mm) the surface of the inoculated meat product as the light energy was delivered.

3. Results

Ten separate light conditions were examined in this experiment to determine their respective ability to inhibit the growth of *L. monocytogenes*. All ten wavelength and dose conditions resulted in an effective inhibition

(dependent Student – t test; p = 0.000). Table 1 displays descriptive data associated with each condition.

Table 1. Descriptive Statistics by Condition (Colony Forming Units)

Condition	N	Mean	SEM	Standard Deviation
Control				
405nm* 10 J/cm^{2+}	5	26.0	3.40	7.62
405nm 30 J/cm^2	5	29.0	5.00	11.20
405nm 60 J/cm^2	5	12.6	2.42	5.41
405nm 90 J/cm^2	5	19.8	7.49	16.76
405nm 120 J/cm^2	5	14.8	2.20	4.91
465nm 10 J/cm^2	5	24.6	6.10	13.64
464nm 30 J/cm^2	5	18.2	3.73	8.34
464nm 60 J/cm^2	5	17.8	6.36	14.23
464nm 90 J/cm^2	5	13.0	2.04	4.58
464nm 120 J/cm^2	5	17.6	2.48	5.54

*nm = Nanometers
+J/cm^2 = Joules per square centimeters

To determine whether any of the wavelength / dose conditions were superior to the others, a 2 X 5 ANOVA was employed. One treatment condition (405nm at 60 J/cm^2) was more effective than the other wavelength / dose combinations (Using Least Significant Difference post hoc analysis; p = 0.032). This point is displayed graphically in Figures 1 and 2.

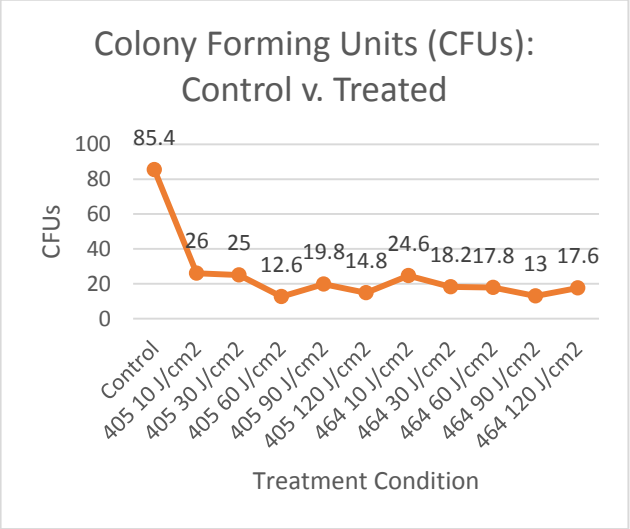

Figure1. Colony Forming Units: Control v. Treated (Wavelengths in nanometers).

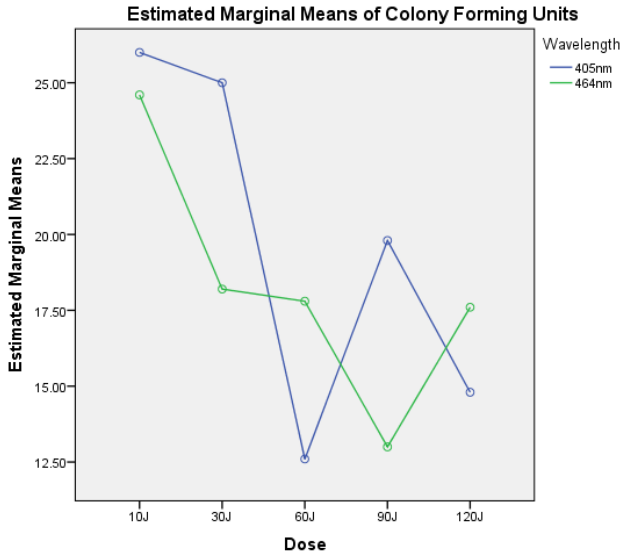

Figure 2. Comparison of Effects per Treatment Condition.

The actual kill rates associated with each wavelength / dose combination ranged from 69.55% - 85.25% (See Figure 3 for these details). Figures 4 provides a visual example of growth for control versus treated organisms.

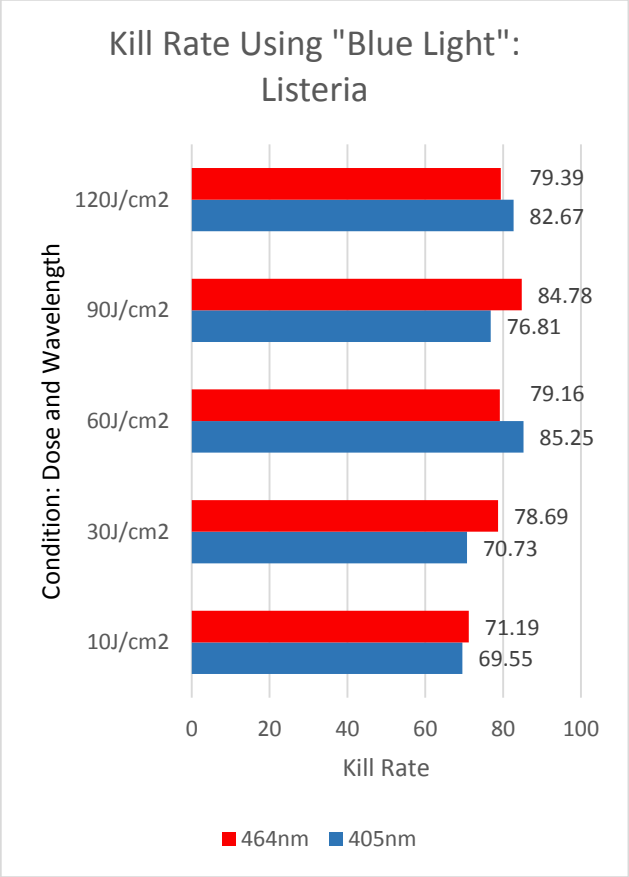

Figure 3. Kill Rate Using "Blue Light": *Listeria monocytogenes*.

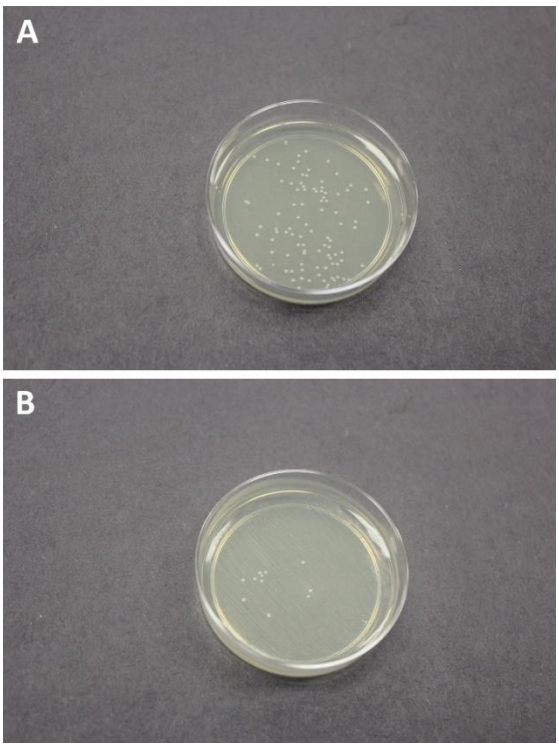

Figure 4. Colony Forming Units Example Outcomes
A – Control
B – 120 J/cm^2

4. Discussion

Security of the food supply is certainly a goal shared by health professionals and the public. Avoiding the use of dangerous or expensive processes to protect the food supply is also a goal all can share. The inclusion of visible light as a disinfecting process is safe, sterile and inexpensive. Given the degree of effectiveness demonstrated in this experiment, considering the use of blue light to disinfect food seems logical.

This experiment is admittedly limited to a single Ready-to-Eat meat product. Whether the technique we employed can be used across all such meats is yet to be determined. Additionally, only the surface of the meat was inoculated. It could be argued that light application (such as we delivered) might not be effective on organisms within the body of the meat, but it does seem possible that light application early in the processing of the product would be a potentially effective measure.

It is encouraging to note that the results of this experiment do correlate very well with work done by ourselves [16 – 19] [21 – 23] and others [20] [25]. The effectiveness of blue light in terms of disinfection has been established *in vitro*, and also *in vivo* [26], but not necessarily on Ready-to-Eat meat products. This study does demonstrate the potential for blue light as a disinfecting agent in a real world circumstance.

Ready-to-Eat meat products are not the only foods contaminated by microorganisms. Fruits, vegetables, and dairy products are also at risk. Future research should be directed toward these foodstuffs to evaluate the degree to which disinfection with blue light can be achieved.

5. Conclusion

Blue light is an effective inhibitor of *L. monocytogenes in vitro* for Ready-to-Eat meat products such as the one used in this experiment. While 405nm wavelength at 60 J/cm^2 proved the most effective wavelength / dose combination, both 405nm and 464nm at doses from 10 – 120 J/cm^2 were effective. There is an application of these findings to efforts to improve the safety of Ready-to-Eat foods.

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