©2018

Jingwen Gao

ALL RIGHTS RESERVED

THE EFFECT OF THE PHOTOSENSITIZER CURCUMIN AND ASCORBIC ACID IN INACTIVATING *LISTERIA MONOCYTOGENES* AND *ESCHERICHIA COLI* O157:H7

By

JINGWEN GAO

A thesis submitted to the

School of Graduate Studies

Rutgers, the State University of New Jersey

In partial fulfillment of the requirements

For the degree of

Master of Science

Graduate Program in Food Science

Written under the direction of

Karl R. Matthews

And approved by

_____

_____

_____

New Brunswick, New Jersey

January, 2018

ABSTRACT OF THE THESIS

The Effect of the Photosensitizer Curcumin and Ascorbic Acid in Inactivating *Listeria monocytogenes* and *Escherichia coli* O157:H7

By JINGWEN GAO

Thesis Director:

Karl R. Matthews

Alternatives to the use of traditional chemical antimicrobials to improve food safety are needed. Curcumin is the principal curcuminoid of turmeric and approved for food use in Europe (E100) and the USA. Curcumin has been shown to have antimicrobial effects after activation by light and potential applications in treating cavities. The challenge of curcumin application is that curcumin will rapidly degrade in physiological and alkaline conditions, while it is more stable in slightly acidic conditions. Ascorbic acid (AA), which is also referred as vitamin C, is a GRAS substance commonly used in food. It can stabilize the curcumin by lowering pH and other unknown mechanisms. The purpose of this study was to evaluate the potential effect of ascorbic acid on the phototoxicity of photosensitizer curcumin in inactivating *Listeria monocytogenes* (gram-positive) and *Escherichia coli* O157:H7

(gram-negative) foodborne pathogens.

Curcumin was prepared by dissolving in 95% ethanol and diluting to desired concentrations with sterile distilled water (SDW). L-ascorbic acid was dissolved in SDW, filter sterilized, and diluted to the desired concentrations with SDW. The absorption spectrum of the curcumin with or without ascorbic acid was determined by UV/Vis spectrophotometer to ensure that no changes in absorption spectrum were found with different curcumin/AA combinations. The antimicrobial effects of the curcumin/AA were evaluated on both solid media using the modified Kirby Bauer method and liquid media. The effects of illumination time were studied in the liquid media (0, 1, 5, and 10 minutes). Overnight cultures of *Listeria monocytogenes* L008 and *Escherichia coli* O157:H7 86-24 were diluted to approximate 6.0 log CFU/mL. Curcumin, ascorbic acid, and cells were mixed and pre-incubated in the dark for 5 minutes. The mixture was then transferred to a 96-well plate for illumination treatment (450 - 455 nm, 0.13 W/cm^2). The suspensions were subjected to microbiological analysis by direct plating method.

A potential effect was observed for ascorbic acid on enhancing the phototoxicity of photosensitizer curcumin against both types of pathogen. Curcumin with or without AA did not show any antimicrobial effects without light exposure. Conversely, no *L. monocytogenes* were detected when exposed to 5 µM of curcumin and 25 µg/mL of AA, after one-minute illumination. *Escherichia coli* O157:H7 was not detected following treatment with 50 µM of curcumin and 25 µg/mL of AA after

10-minute light exposure. Overall, curcumin and AA combination showed higher bacterial reduction than curcumin only.

This study demonstrated that curcumin/AA combination has promising inhibitory effects against *Listeria monocytogenes* and *E. coli* O157:H7, indicating a potential application as a decontamination intervention on food surfaces including meat, poultry and fresh produce.

ACKNOWLEDGEMENT

I would like to express my sincere appreciation to my thesis advisor, Dr. Karl R. Matthews, for his patient guidance and endless support for my research. It is his creativity and rich experiences in food production that help me to shape the framework of my master thesis. He always inspired me with lots of new ideas, which were beneficial to addressing many problems in my research. Besides, he provided me with lots of information in food industry based on his experience, which played a key role in guiding my research to become more practical and applicable. He is also a fantastic tutor for my life. I appreciate his consideration and encouragement in joining various activities inside and outside campus.

I would also like to show my deepest appreciation to my committee member – Dr. Richard D. Ludescher and Dr. Donald W. Schaffner. Their knowledge in the field of photochemistry and food microbiology provided me with lots of valuable suggestions.

I am grateful to work with my lab mates – Yangjin Jung, Hyein Jang, Germaine Tsui, Mengqi Guo, Yi-Chun Lai, and Vi Dao. I can never finish my research without their guidance, encouragement and assistance, especially Yangjin and Hyein. I have learned a lot how to work in the lab when working with them. Besides Dr. Matthews, they were like my teachers who give me lots of useful guidance in the field of food microbiology. Meanwhile, they were like my elder sisters who have taken care of me all the time.

Last but not least, I want to send my warmest gratitude to my parents, Yanda Gao and Hongmei Zhen. They always try their best to send me out of China so that I can broaden my horizon. It is tough to live in a foreign country, but their selfless love motivates me to go through all the obstacles in my education and my life in the US.

DEDICATION

To my parents,

Yanda Gao and Hongmei Zhen

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

1. INTRODUCTION

Food safety is a global burden, leading to one-tenth of people experiencing foodborne illness annually (WHO, 2015). There are high production and consumption of meat and poultry all around the world. Although consumers usually cook their meat and poultry before consumption, inadequate cooking is a big issue accounting for foodborne illnesses. Several outbreaks associated with meat, poultry, and meat products were traced back to undercooked products. Additionally, most consumers (67 – 68%) tend to wash the chicken parts and whole chicken before cooking, which is not recommended by the Food and Drug Administration in the United States (FDA, 2016). It is because there are increased risks of cross-contamination when contaminated water is splattered to other surfaces. To mitigate the concerns with cross-contamination and inadequate cooking, reducing or eliminating the level of pathogenic microbes on the meat surfaces during processing becomes integral to improving the safety of meat and poultry products.

Several chemical intervention technologies have been applied in the production of meat and poultry. Chlorine and acids (e.g., lactic acid, citric acid, acetic acid, and ascorbic acid) are the two most commonly-used antimicrobial chemicals. However, the usage of chlorine and acids have brought up several concerns. Chlorine is inexpensive, but it could react with organic matter, leading to a decrease in the antimicrobial efficacy as well as the production of carcinogenic compounds like trihalomethanes (Pereira, 1983; Richardson, 2003). Additionally, chlorine may

promote the growth of “superbug,” which is resistant to multiple antimicrobial chemicals, so the chlorinated poultry is banned by the European Union and Australia. In terms of the acidic antimicrobial interventions, most result in equipment corrosion. Also, acid exposure may lead to the selection of acid-tolerance and the development of acid-adapted bacteria (Berry & Cutter, 2000; Samelis, Sofos, Kendall, & Smith, 2002). Acid tolerance and acid adaptation may enhance the virulence of pathogenic *Escherichia coli*, *Salmonella enterica*, and *Listeria monocytogenes* by increasing their survival under the gastrointestinal acidity (Diez-Gonzalez, Callaway, Kizoulis, & Russell, 1998; DuPont et al., 1971; O’Driscoll, Gahan, & Hill, 1996). Consequently, new types of antimicrobial interventions, both chemical and physical techniques, are needed.

Photodynamic therapy (PDT) was developed in the last century. It utilizes photosensitizer (PS), with the presence of light and oxygen, to treat specific diseases including cancer (Dolmans, Fukumura, & Jain, 2003), macular degeneration (Brown et al., 2009), and some noncancerous dermatological conditions. Eosin-Y, rose Bengal, curcumin, riboflavin (vitamin B12) and sodium chlorophyllin are some well-studied photosensitizer (Halili et al., 2016; Kreitner et al., 2001; Pileggi et al., 2013). PDT is a promising alternative to antibiotics to treat bacterial infections. The mode of inactivation by PDT is less specific than antibiotics, which is based on ROS production (Castano, Demidova, & Hamblin, 2004). The photodynamic products can cause the destruction of DNA, RNA, amino acids, fatty acids, and other cellular compounds, leading to the cell death (Böcking et al., 2000; Haeubl et al., 2009; Michaeli &

Feitelson, 1994). Several studies have shown that appropriate photosensitizer could efficiently inactivate methicillin-resistant *Staphylococcus aureus* (Ribeiro et al., 2013; Wainwright, Phoenix, Laycock, Wareing, & Wright, 1998) and vancomycin-resistant enterococci (Xing et al., 2011), which are the main resistant species of concern in the medical field. Recently, there were increased interests of using photosensitizer to inactivate foodborne pathogens on food surface, such as oysters, cucumbers, peppers, and chicken meat (Tortik, Spaeth, & Plaetzer, 2014; Wu et al., 2015; Wu et al., 2016).

Curcumin has been used as spice, colorants and topical medicines in southeast Asia, China, and India for a century. It can be naturally extracted from the turmeric root or *Curcuma longa* Linn (an East Indian plant) under the family of *Zingiberaceae*. Curcumin has been shown to have a variety of pharmacological activities. It has been approved for food use in baked goods, confectionery, and seasonings by both the European Union and the United States. Additionally, curcumin has the photodynamic property because of its chromophoric structure. However, curcumin will rapidly degrade under neutral and alkaline conditions. For a better stability, several studies used HCl to adjust the buffer solutions to pH 6.1 or used slightly acidic buffer to dissolve curcumin (Hegge, Bruzell, Kristensen, & Tonnesen, 2012; Hegge, Nielsen, Larsen, Bruzell, & Tonnesen, 2012; Winter, Tortik, Kubin, Krammer, & Plaetzer, 2013; Wu et al., 2016). Slightly acidic environment enhanced the photodynamic inactivation by improving curcumin stability, but the effects of organic acids in PS curcumin have not been investigated.

Consequently, this study was proposed to determine whether supplementation with ascorbic acid could enhance the antimicrobial effects of the photosensitizer curcumin (PS) in inactivating *Listeria monocytogenes* and *Escherichia coli* O157:H7.

2. HYPOTHESIS AND OBJECTIVES

The hypothesis driving this research is that there is a potential effect of ascorbic acid on the photodynamic inactivation of photosensitizer curcumin against the survival of *Listeria monocytogenes* and *Escherichia coli* O157:H7.

The specific objectives are:

1. To identify the light source with an appropriate wavelength that can efficiently activate the photodynamic properties of the photosensitizer curcumin.
2. To determine the pH value of the designed photosensitizer after the addition of ascorbic acid.
3. To determine the antimicrobial effects of photosensitizer curcumin in a solid-media system after defined illumination period.
4. To optimize the illumination time of photosensitizer curcumin in inhibiting *Listeria monocytogenes* and *Escherichia coli* O157:H7 in a planktonic system, respectively.
5. To determine the antimicrobial effects of curcumin/ascorbic acid combinations in a planktonic system under the optimized illumination time.

3. LITERATURE REVIEW

3.1 Meat and poultry industry

3.1.1 Microbial safety issues in meat and poultry industry

Food Safety has become a global burden, leading to one-tenth of people becoming ill the result of foodborne pathogens annually (WHO, 2015). In the United States, it is estimated by the Centers for Disease Control and Prevention (CDC) that approximate 48 million people (1/6) suffer from foodborne illnesses every year (CDC, 2016). Historically, there are high production and consumption of red meat and poultry globally. According to Economic Research Service (ERS, United States Department of Agriculture), more than 97 billion pounds of red meat and poultry were produced in the United States in 2016, and the total consumption of red meat and poultry was estimated to be over 200 pounds per capita (USDA, 2017a). With such a high production and consumption, unsafe production of meat and poultry would create a great threat to the public health. From 1998 to 2008, meat and poultry (22%) accounted for fewer cases of illnesses than produce (46%). However, more cases of death were associated with meat and poultry (29%), which was 6% higher than produce (Painter et al., 2013). As a result, the safety of meat and poultry is a global concern on public health.

Microbial contamination of meat and poultry could potentially lead to a significant economic loss. According to the report from USDA ERS, at least $6.9 billion was lost due to the foodborne illnesses (Hoffmann & Anekwe, 2013). In 2016, a total

47,709,328 pounds of meat and poultry were recalled because of bacterial contamination. In particularly, *Listeria monocytogenes* contributed to more than 99% of the recalls (USDA, 2017b). If meat and meat products were sold at $4/pound, the recalled foods would cause over $200 million economic loss to the industry as well as retailers. In a worse case, industries and retailers may face legal action by consumers becoming ill through consumption of contaminated products.

3.1.2 Current usage of chemical interventions and their associated problems

Currently, chemical treatments commonly used to disinfect the surface of meat and poultry carcasses include but are not limited to chlorine, inorganic and organic acids (e.g., acetic acid, lactic acid, peracetic acid, citric acid, etc.). However, several problems are related to the usage of these antimicrobial compounds.

Chlorine is inexpensive, so it is widely used in the food industry to wash produce, meat, and poultry. However, the interaction between chlorine and organic matter not only reduces the antimicrobial effects, but it also generates carcinogenic compounds like halomethanes (Richardson, 2003). Additionally, the usage of chlorine could likely promote the growth of highly resistant “superbugs,” increasing the challenges to decontaminate the resistant bacteria on food. Typically, a higher concentration of chlorine is more effective in inactivating microorganisms. However, it would lead to discoloration on the surface as well as the generation of off-flavors, which will affect the food quality. Based on the concerns mentioned, chicken exposed to chlorinated washes or rinses during processing is prohibited by the European Union and Australia,

although chlorine is allowed in the poultry wash for up to 50 ppm in the United States (Sohaib, Anjum, Arshad, & Rahman, 2016). Some other common types of antimicrobial treatments that are allowed to be used on poultry by USDA but are prohibited by EU include acidified sodium chlorite, trisodium phosphate, peroxy acid, etc. (Johnson, 2015). Even though chlorine has been used in the US, the efficacy of chlorine wash/rinse is questioning. No significant reductions in aerobic bacteria, *Escherichia coli*, *Salmonella*, or *Campylobacter* were found up to 50 ppm chlorine (Northcutt, Smith, Musgrove, Ingram, & Hinton, 2005). As a result, more efficient alternatives have aroused great interests.

Inorganic and organic acids (1 – 3%) are commonly-used antimicrobial chemicals that are rinsed or sprayed on the surface of carcasses. Increasing acid concentrations could enhance antimicrobial activity (Cutter & Siragusa, 1994; Dickson & Anderson, 1992), and most of the acidic interventions used in food industry have pH value below or slightly above three (Cutter & Siragusa, 1994). High acidity could cause corrosion problems as well as bleaching effects on the surface of carcasses, particularly for red meat (Sohaib et al., 2016). Additionally, acids may result in the selection of acid-tolerant as well as the development of acid-adapted microbes; including both commensal and pathogenic bacteria (Berry & Cutter, 2000; Samelis et al., 2002). Acid-tolerant and acid-adapted bacteria show higher resistance to acid washed, so higher acid concentration is needed to inactivate the targeted pathogens. Finally, inappropriate disposal of acidic antimicrobial chemicals could cause environmental pollutions.

In conclusion, practical and efficient approaches to inactivate foodborne pathogens on meat and poultry are needed globally, for the concerns about public health and economic loss.

3.2 *Listeria monocytogenes*

3.2.1 Listeriosis

Listeriosis is one of the most severe bacterial infections caused by pathogenic *Listeria monocytogenes*. It is estimated that 1,600 people suffer from listeriosis annually, leading to around 260 cases of death (CDC, 2017). Listeriosis symptoms vary from case to case, depending on the physiological status of an individual as well as the infected parts of a body (Farber & Peterkin, 1991). For those who have a healthy immune system, they may suffer from fever and diarrhea – similar symptoms as other bacterial infections caused by common foodborne pathogens. However, they may have an acute but self-limited febrile gastroenteritis after ingesting a high dose of *Listeria* cells. By contrast, listeriosis is remarkably deadly for pregnant women, the neonate, the elderly, and other immunocompromised individuals through invasive infections. The mortality rate of listeriosis is over two-times higher among immunocompromised individuals than healthy people, making listeriosis become one of the most severe bacterial infections (Farber & Peterkin, 1991).

Listeria monocytogenes is dangerous because it has multiple virulence factors. For example, it can induce parasite-directed endocytosis, allowing it to invade the host cells, to be transported to the base of the cells, and be exocytosed into the

subepithelial tissues (McGee, Gorby, Wyrick, Hodinka, & Hoffman, 1988). In other words, *L. monocytogenes* is not only able to cause local diseases, but it can also result in severe disseminated diseases like meningitis by entering the blood stream. Also, *Listeria monocytogenes* is capable to secret hemolysin, which plays a crucial role in protecting *Listeria* cells from phagosomes and promoting the intracellular growth of bacteria within the host cells (Vázquez-Boland et al., 2001). The hemolytic activity controlled by gene hly is considered as the marker gene to differentiate avirulent and virulent *Listeria* species.

3.2.2 Prevalence and outbreaks

Listeria monocytogenes is ubiquitous in the environment, so foods can be contaminated by multiple sources from the farm to the table, such as soil, plant, animal (e.g., cattle, sheep, goats, and poultry), and water (Gray & Killinger, 1966; Weis & Seeliger, 1975). Outbreaks associated with listeriosis have achieved greater attentions since the California Jalisco Cheese outbreak in 1985. There were total 142 cases, among which 48 deaths were reported (Linnan et al., 1988). Although lots of *Listeria* outbreaks were traced back to dairy products, *Listeria* contamination has been found in meat, poultry, and fresh produce. High prevalence of L. monocytogenes (12 – 60%) was found in poultry, highlighting the importance of developing antimicrobial approaches to control *L. monocytogenes* in poultry (Elmali, Can, & Yaman, 2015; Farber & Peterkin, 1991).

3.2.3 Challenges of controlling listeriosis in foods

L. monocytogenes is tolerant to multiple stress factors, making it harder to be inactivated during the preparation of foods. For instance, *Listeria monocytogenes* can tolerate up to 10% NaCl by accumulating K^+, betaine, glutamate, and other solutes to maintain the intracellular osmolality (Liu, Lawrence, Ainsworth, & Austin, 2005; Patchett, Kelly, & Kroll, 1992; Vasseur, Rigaud, Hébraud, & Labadie, 2001). Capability of responding to osmotic pressure enables *L. monocytogenes* to survive in various cheese and meat products with relatively high salt concentration. *Listeria monocytogenes* can also tolerate a broad range of pH (2.0 – 9.5). Dykes and Moorhead (2000) evaluated the acid-tolerant ability of *L. monocytogenes*, both meat and clinical isolates, by exposing the cell culture to pH 2.5 for 2 hours. Only two out of thirty strains showed significant reductions in the population, indicating a high acid tolerance. *Listeria monocytogenes* also has a high tolerance to low temperature. It can survive and multiple under refrigerated temperature. Depending on the type of meat, the population of *L. monocytogenes* either maintained a constant population or increased by 10^3 ~ 10^5 log in vacuum packaged meat products after being stored at 4.4 °C for six weeks (Glass & Doyle, 1989). Potential approaches to address the issue of controlling *L. monocytogenes* during refrigerated storage would be an antimicrobial that shows residual effects during the shelf-life or can be re-activated at a point before consumption.

3.3 *Escherichia coli* O157:H7

3.3.1 Health concerns

Escherichia coli is a normal resident in animal and humans intestinal track. Most of *Escherichia coli* is harmless or even beneficial to human intestinal health known as probiotics. However, some are pathogenic and cause diarrhea or illness outside of the intestinal tract. Pathogenic *E. coli* has multiple serotypes, but *E. coli* O157:H7 is one of the most prevalent foodborne pathotypes. In severe cases, *E. coli* O157:H7 can lead to life-threatening symptoms, such as hemolytic uremic syndrome (HUS). Individuals with HUS have less frequency of urination and other severe symptoms due to the potentially unfunctional kidneys.

3.3.2 Prevalence and outbreaks

A variety of foods have been found to be the contaminant source of *Escherichia coli* O157:H7 illness. For example, meat (e.g., ground beef), fresh produce (e.g., spinach, lettuce, sprouts, etc.), dairy products, hazelnut, and peanut butter. However, ground beef is the most commonly reported among all the potential transmission routes. From 1982–2002, total 41% of outbreaks were related to ground beef (Rangel, Sparling, Crowe, Griffin, & Swerdlow, 2005). In 1993, a multi-state *E. coli* O157:H7 outbreak was linked to undercooked ground beef, leading to over 500 reported cases of infection (Bell et al., 1994; CDC, 1993). Typically, the hides of the beef carcass are the primary source of contamination in beef processing, and higher prevalence of foodborne pathogens is found on the hides than the feces, such as *E. coli* O157:H7, *Salmonella enterica* and non-O157 Shiga toxin-producing *E. coli* (Barkocy-Gallagher et

al., 2003). As a result, decontamination of the beef carcass surface plays a critical role in the hazard analysis and critical control point plans in the beef processing facilities (Bosilevac, Nou, Osborn, Allen, & Koohmaraie, 2004; USDA, 1996). In addition to the beef industry, *Escherichia coli* O157:H7 is also considered as a high-risk pathogen in the poultry industry, particularly in the temperature-abused and extended shelf-life of ready-to-eat and/or poultry-meat products (Mataragas, Skandamis, & Drosinos, 2008).

3.3.3 Challenges of controlling *E. coli* O157:H7 in foods

E. coli O157:H7 has relatively high probability to cause diseases with low number of cells (Lim, Yoon, & Hovde, 2010). This highlights the significance of implementing approaches that can significantly reduce or eliminate *E. coli* O157:H7 on foods. In addition, control of *E. coli* O157:H7 is a necessity for raw food products (e.g., ground beef) as well as ready-to-eat foods that would likely being undercooked or never be cooked by consumers. Livestock is the natural reservoir for *E. coli* O157:H7, imposing greater challenges to control the cell number. Very few conventional chemicals were able to cause over 3-log reduction against *E. coli* O157:H7 on beef carcasses, which is considered as the threshold to be considered as an effective antimicrobial treatment by American Society for Microbiology (Cutter & Siragusa, 1996; Dorsa, Cutter, & Siragusa, 1998; Harris, Miller, Loneragan, & Brashears, 2006; Laury et al., 2009; Ransom, Belk, Sofos, Scanga, & Smith, 2003). The low efficacy of conventional antimicrobials may be related to the fact that pre-chilled beef surfaces make *E. coli* O157:H7 harder to be detached from the carcass (Hardin, Acuff, Lucia, Oman, &

Savell, 1995). Also, the moderate acid exposure before washing may select the acid-tolerant and/or induces the acid-adapted *E. coli* O157:H7, resulting in the decrease on the efficacy of acid washes (Cutter & Siragusa, 1994). In addition to *Escherichia coli* O157:H7, other Shiga toxin-producing *E. coli* (STEC) have attracted more and more attention because increasing number of outbreaks related to STEC have been reported. However, limited number of studies were conducted on the chemical interventions in inactivating STEC on the surface of meat and poultry carcass.

3.4 Photosensitizer

3.4.1 History of photosensitizer

Light has been used for therapeutic purposes for thousands of years, but photodynamic therapy (PDT) was developed until the last century. PDT utilizes photosensitizer with the presence of light and oxygen to treat specific diseases, such as cancer (Dolmans et al., 2003), macular degeneration (Brown et al., 2009), and some non-cancerous dermatological conditions. It was approved for specific indications in the regulations by several countries (Dougherty et al., 1998). Antibiotic resistance is one of the biggest challenges in the 21st century. The efficacy of current antibiotics used to treat bacterial infections decreases due to increasing antibiotic resistance and higher prevalence of antibiotic-resistant microorganisms. In recent years, PDT becomes more and more popular in the field of medicine, and it is considered as a promising approach to treat bacterial infections as an alternative to antibiotics. Instead of having specific targeting sites like antibiotics, PDT cause

non-specific disruption on cellular structure. PDT could efficiently inactivate methicillin-resistant *Staphylococcus aureus* (Ribeiro et al., 2013; Wainwright et al., 1998) and vancomycin-resistant enterococci (Xing et al., 2011), which are the main resistant species of concern. In addition to therapeutic applications, photosensitizers have been evaluated to improve food safety of oysters, cucumbers, peppers, and chicken meat (Tortik et al., 2014; Wu et al., 2015; Wu et al., 2016).

3.4.2 Mode of inactivation

Compound that shows photodynamic change is called photosensitizer (PS). The photodynamic inactivation ability of the PS is based on the phototoxicity. The photophysical and photochemical processes of the photodynamic inactivation are demonstrated in Figure 3.1. Castano et al. (2004) published a comprehensive review paper about the photochemical mechanism of PS from the molecular perspective. The chromophore structure not only contributes to the color of the PS, but it also allows PS in the ground state to be excited by a particular range of wavelength of light (λ_{abs}) and to undergo a transition to an excited singlet state, which is a short-lived species. These short-lived species rapidly lose their energy in nanoseconds by emitting fluorescence, internal conversion into heat, or through intersystem crossing (ISC). A relatively long-lived species (microseconds to seconds) is formed in the process of intersystem crossing. The molecules will then lose energy when moving from the triplet-state to the singlet-state by emitting phosphorescence or by releasing energy as heat.

The PS in the excited triplet state can undergo two types of reactions – Type 1 and Type 2. In Type 1 reaction, the PS directly reacts with substances in the environment, like oxygen, by transferring protons or electrons. Products can further generate reactive oxygen species (ROS), including superoxide radicals, hydrogen peroxide, and hydroxyl radicals. In Type 2 reaction, the excited PS transfer the energy to the oxygen, leading to the activation of ground-state molecular oxygen to excited-state singlet oxygen. These two types of reactions can occur simultaneously, depending on the reaction conditions.

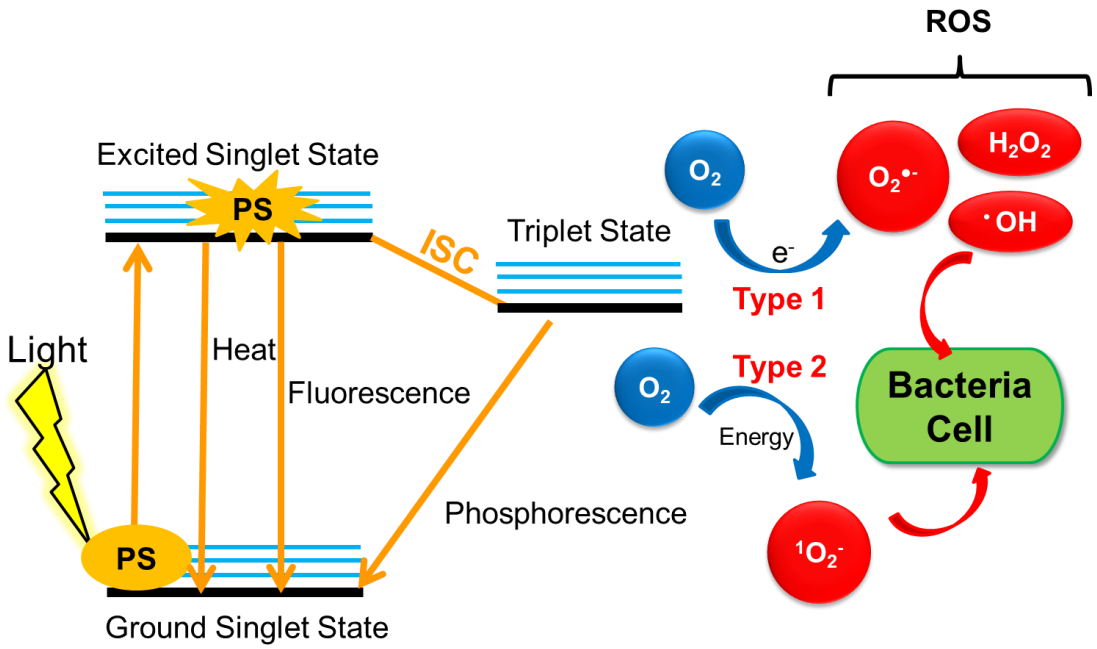


Figure 3.1. Diagram of the photodynamic inactivation process of photosensitizer. PS = photosensitizer; ROS = reactive oxygen species; ISC = intersystem crossing.

The phototoxicity of PS is strictly related to the photoproducts generated in the excited triplet state. These highly reactive products are capable to oxidize a variety of cellular components (e.g., amino acids, nucleic acids and fatty acids). The oxidation

process will lead to the destruction of cell membrane, proteins, DNA and RNA, resulting in cell death (Böcking et al., 2000; Haeubl et al., 2009; Michaeli & Feitelson, 1994).

3.4.3 Factors affect the effectiveness of inactivation

The effectiveness of photoinactivation is affected by a number of factors, such as PS concentration, availability of oxygen, the amount of light energy, types of photosensitizer solvent, etc. For example, the photosensitizer methylene blue dissolved in a mixture of glycerol, ethanol, and water was shown to have better antimicrobial effects than using water as the only solvent (George & Kishen, 2008). The differences in the antimicrobial effects may be related to the production and stability of ROS in the solvent, which are the active compounds against bacteria (Chignell et al., 1994).

Although a number of factors that can affect the phototoxicity of PS, the key criteria is to localize within a radius of 20 nm from the cells because singlet oxygen has short half-life in microseconds (Moan & Berg, 1991).

3.5 Curcumin

3.5.1 Historical use and production of curcumin

Curcumin has broad applications in Southeast Asia, China, and India as flavors, dyes, topical medicines and cosmetics. Curcumin becomes more and more popular in medical field because studies have shown that curcumin has a variety of pharmacological activities, including anti-inflammatory (Srimal & Dhawan, 1973),

anticancer (Aggarwal, Kumar, & Bharti, 2003), and antioxidant effects (Sharma, 1976). Also, turmeric has been shown to protect skin from sunburns and diseases like leprosy, psoriasis, and cancer (Huang, Liu, Xie, Newmark, & Ho, 2003; Kurd et al., 2008).

Curcumin is approved to be used in foods as colorant or flavor by both the European Union and the United States. In the European Union, curcumin is known as E 100 categorized as a food colorant in beverages, baked goods, desserts, soft drinks, sauces, and seasonings, as well as confectionery. Owning an E number indicates that curcumin have passed a series of safety assessments by the European Food Safety Authority (EFSA). In the United States, curcumin has the status of Generally Recognized as Safe (GRAS) and can be used as a flavor, flavor enhancer, or an ingredient in baked goods, soups, snack foods, imitation dairy products, and seasoning. The maximum level permitted to use in foods is 20 milligrams per serving in the United States. Srimal and Dhawan (1973) showed that curcumin has a relatively high LD_{50} value, up to 2.0 $g \cdot kg^{-1}$. Besides, a three-month human clinical trial suggested that humans had high tolerance towards curcumin, which could be up to 8 g/day (Cheng et al., 2001).

Curcumin is the principal polyphenolic compound of the root turmeric or *Curcuma longa* Linn (an East Indian plant) under the family of *Zingiberaceae* (2 - 8% by weight) (Priyadarsini, 2009). Commercial curcumin, as a food additive, is extracted from turmeric using solvents. A variety of solvents can be used to extract curcumin,

such as acetone, carbon dioxide, ethyl acetate, methanol, ethanol, and hexane (Stankovic, 2004). With the assistance of microwave, extraction using acetone from water pre-soaked crude powder can produce more than 90% yield of curcumin (Wakte et al., 2011). The "pure curcumin" products on the market usually contain two other active compounds – demethoxycurcumin and bisdemethoxycurcumin. Demethoxycurcumin, bisdemethoxycurcumin, and curcumin are generally known as curcuminoids (Figure 3.2), among which curcumin has the highest portion. A relatively simple and high-yield extraction processing accounts for a low price of 95% curcuminoid on the market ($1 – 20/kg).

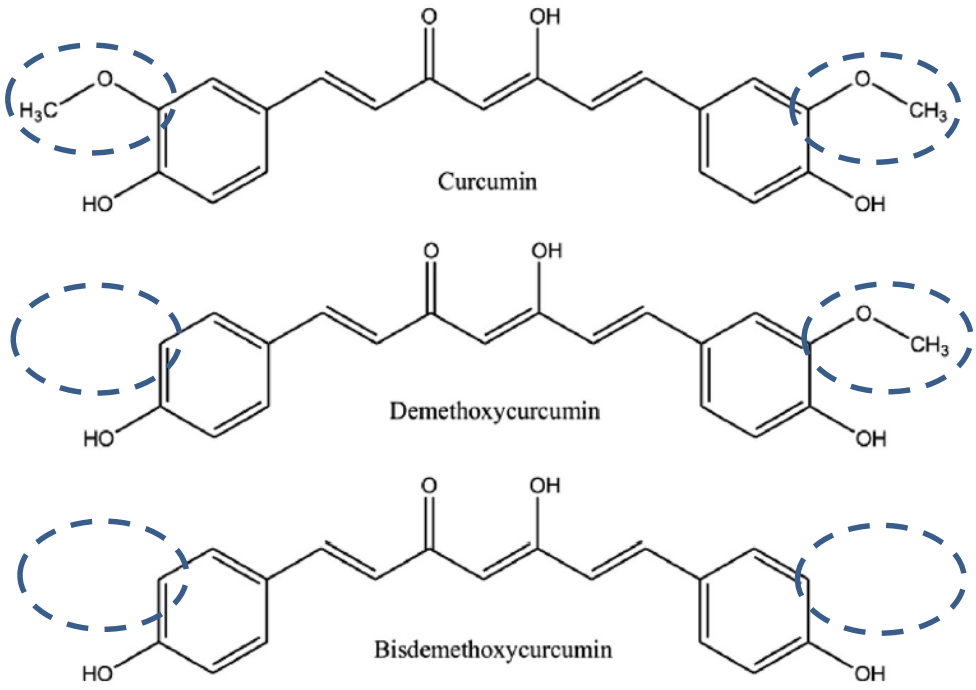


Figure 3.2. Components of curcuminoids in commercial curcumin product: curcumin ($C_{21}H_{20}O_6$; formula weight = 368), demethoxycurcumin ($C_{20}H_{18}O_5$; formula weight = 338), and bisdemethoxycurcumin ($C_{19}H_{16}O_4$; formula weight = 308) (Monton, Charoenchai, Suksaeree, & Sueree, 2016).

3.5.2 Chemical structure and properties of curcumin

Until 1910, curcumin was identified as diferuloylmethane (Aggarwal et al., 2003). According to International Union of Pure and Applied Chemistry (IUPAC), the full name of curcumin is (1E, 6E)-1,7-bis(4-hydroxy-3-methoxy phenyl)-1,6-heptadiene-3,5-dione. The key characteristic structure of curcumin is diferuloylmethane, consisting of two aromatic rings connected by two α,β–unsaturated carbonyl groups. The chromophore structure of curcumin contributes to the yellow color as well as the photodynamic inactivation ability of curcumin. Another characteristic structure would be the β-diketone structure, which plays a key role in the keto–enol tautomeric equilibrium of curcumin in solutions. Keto–enol tautomeric equilibrium is closely related to the physical and chemical properties (Figure 3.3). The β-diketone moiety allows for the hydrogen transfer intramolecularly, contributing to different tautomeric conformations (Lee et al., 2013). Also, enolic and keto forms can exist in both cis and trans forms, resulting in more diverse configurations and properties. Different confirmations of curcumin show different abilities of intramolecular hydrogen bonding, which subsequently influences the stability and photophysical properties of curcumin in solutions. It is predicted that cis-enolic tautomer would be more energetically stable due to its strong intramolecular hydrogen bonding (Khopde, Priyadarsini, Palit, & Mukherjee, 2000). As a result, curcumin tends to exist in an enolic form, particularly at room temperature (Markov, 1984; Nikolov & Markov, 1981). In addition to temperature, the relative contribution of keto and enolic form is affected by pH, hydrogen-bonding

capacity of the solvent, etc. (Arnaut & Formosinho, 1992, 1993; Markov, 1984; Nikolov & Markov, 1981; Wright, 2002).

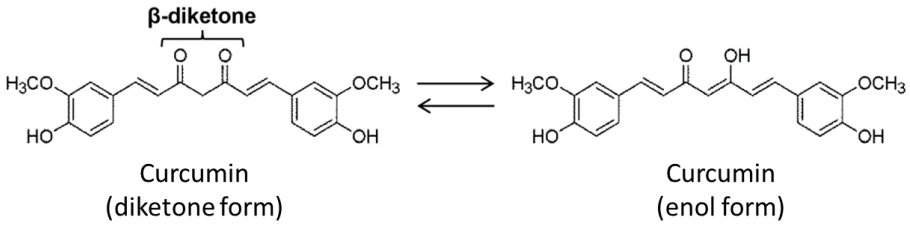


Figure 3.3. Keto-enol tautomeric equilibrium of curcumin and different isomers of keto/enolic tautomer (Modified from Priyadarsini, 2009).

Curcumin has poor water solubility at acidic and neutral pH. Under neutral condition, only 1.34 µg/mL of curcumin can dissolve in 1 L of distill water; meanwhile, over higher solubility was found in 99.5% EtOH (8895.89 µg/mL) (Carvalho, Takeuchi, Geraldine, Moura, & Torres, 2015). Solubility may affect the photodynamic inactivation of PS, because precipitated PS cannot interact with the cells. Therefore, several approaches have been proposed to increase curcumin solubility. For example, incorporating dimethyl sulfoxide (DMSO) as a solubility enhancer (Haukvik, Bruzell, Kristensen, & Tønnesen, 2009), surfactants (Hegge, Bruzell, et al., 2012), and hydrocolloids as encapsulation materials (Tønnesen, Másson, & Loftsson, 2002). Although DMSO is a common solubility enhancer, it is not promising for food use, because the safety of consuming DMSO is still controversial. On one hand, DMSO was shown to be able to reduce pain and inflammation (Hollebeeck et al., 2011; Zuurmond et al., 1996). On the other hand, it could penetrate into phospholipid

membranes, leading to tissue damage or even systemic effects (Rowe, Sheskey, & Weller, 2006). Incorporation of cyclodextrin has been demonstrated to improve both the stability and solubility of curcumin by a thousand-fold (Bruzell, Morisbak, & Tonnesen, 2005; Tønnesen et al., 2002). However, less free curcumin molecules are available to interact with the cells because of their higher affinity to the cyclodextrin cavity than the outer membrane. Therefore, the cyclodextrin-capsulated curcumin showed less phototoxicity than the non-encapsulated curcumin (Haukvik et al., 2009). Similarly, the incorporation of surfactant polyethylene glycol (PEG 400) decreased the phototoxicity of PS curcumin on *E. coli* (ATCC 25922) by 0.25 – 1.4 log, depending on the curcumin concentration (Haukvik, Bruzell, Kristensen, & Tønnesen, 2010). Consequently, incorporation of DMSO, hydrocolloids or surfactants to enhance curcumin solubility, but higher curcumin concentration is needed to produce a supersaturation solution so that more free curcumin is available for cell interactions.

Curcumin has higher solubility in alkaline than the neutral or acidic solvent, probably due to the ionization of phenolic/enolic groups, but it will rapidly go through hydrolytic degradations (Wang et al., 1997). A large number of hydroxyl ions in the alkaline environment could attack the carbonyl carbon, leading to the degradation products like vallinin, ferulic acid, and feruloylmethane (Tønnesen & Karlsen, 1985). In phosphate buffer, nearly all curcumin was degraded at pH 8.0 within 2 min after preparation, while around 55% of curcumin was stilled remained after 120 min (Wang et al., 1997). It is assumed that the conjugated diene structure at acidic pH contributes to the better stability. In alkaline environment, the

conjugated structure is destroyed by the ionization of the phenolic groups. As a result, acids are commonly added to adjust the pH to around pH 6 for a better stability. A study showed that curcumin supplemented with ascorbic acid showed higher antioxidant as well as antifungal activity, which was assumed to be due to the increasing stability of curcumin and other unknown mechanisms (Khalil et al., 2012).

In summary, physiological pH of the solution influences both stability and solubility of curcumin, which further affect the bactericidal effects. It is critical to tune the pH value to maximize the phytotoxicity of curcumin against bacteria.

3.5.3 Current research on curcumin as a photosensitizer

Due to the poor solubility in water and the degradation in alkaline conditions, researchers mainly focused on examining the effects of different formulations in improving the improving photodynamic inactivation of curcumin against microorganisms by enhancing its solubility and stability. The most commonly-used approaches include nanotechnology and adjusting the pH value (~pH 6). Table 3.1 summarized several studies associated with photosensitizer curcumin. Most of the experiment designs are media-based, but limited studies examined the photodynamic inactivation of curcumin in a food system. Therefore, more research is needed to demonstrate the potential application of curcumin on food preparation to control microbial growth and improve the food safety without sacrifices of quality.

Table 3.1. Summary of literature review on photodynamic inactivation on bacteria in the planktonic system by photosensitizer curcumin.

Microorganisms	Formula of PS	PS Concentration (μM)	Wavelength (nm)	Light Intensity (mW/cm^2)	Illumination Time (min)	Log Reduction	Reference
Bacteria (Gram Positive)							
Staphylococcus aureus	Polyvinylpyrrolidone	5 - 50	435	9.4	60	> 6	Winter el al., 2013
	Polyvinylpyrrolidone	50 & 100	435	9.4	60	2.6 (Cucumber); 2.5 (Pepper); 1.7 (Chicken)	Tortik et al., 2014‡
Staphylococcus epidermidis	20 % polyethylene glycol (PEG) 400; 0.5% HPγCD	10	400 – 500 (Emission Max. at 430)	15.8	5	BDL (10^{7-8})†	Hegge et al., 2012
Streptococcus mutans	10% dimethyl sulfoxide (DMSO)	0.75	400 – 440	95	4	BDL (10^6)	Paschoal et al., 2015
	Distill water	2000, 4000 & 8000	400 -500 (Max. at 450)	240.1	1.6 – 5	1.5 – 5.3	Paschoal et al., 2013
Enterococcus faecalis	0.025% DMSO	5	450 – 500	450	4	5.5	Pileggi et al., 2013

† BDL: Below Detection Limit. The initial level of bacteria (CFU/mL) is indicated in the parentheses.

‡ Research conducted in the food system.

Table 3.1. (continued): summary of literature review on photodynamic inactivation on bacteria in the planktonic system by photosensitizer curcumin.

Microorganisms	Formula of PS	PS Concentration (μM)	Wavelength (nm)	Light Intensity (mW/cm^2)	Illumination Time (min)	Log Reduction	Reference
Bacteria (Gram Negative)							
Escherichia coli	Polyvinylpyrrolidone (preincubate with $CaCl_2$)	50	435	9.4	60	3	Winter el al., 2013
	Supersaturation (0.01% methylated β-cyclodextrin)	10	400 – 500 (Emission Max. at 430)	16.1	30	4.16	Hegge et al., 2012
	2.5% PEG 400	25	300 – 500 (Emission Max. at 430)	17.3	30	2.8	Haukvik et al., 2010
Vibrio parahaemolyticus	Ethanol	10	470	60	1	5 (Oyster)	Wu et al., 2016‡

‡ Research conducted in the food system.

Table 3.1. (continued): summary of literature review on photodynamic inactivation on bacteria in the planktonic system by photosensitizer curcumin.

Microorganisms	Formula of PS	PS Concentration (μM)	Wavelength (nm)	Light Intensity (mW/cm^2)	Illumination Time (min)	Log Reduction	Reference
Yeast							
Candida albicans	10% DMSO	20	440 – 460 (Emission Max. at 455)	22	4	BDL (~10^6) †	Dovigo et al., 2011
Viruses							
Murine Norovirus	Ethanol	20	470	60	1	1.15 (Oyster)	Wu et al., 2015‡

† BDL: Below Detection Limit. The initial level of bacteria (CFU/mL) is indicated in the parentheses.

‡ Research conducted in the food system.

4. MATERIALS AND METHODS

4.1 Preparation of bacterial culture and inoculum

Three strains of *Listeria monocytogenes,* four strains of *Escherichia coli* O157:H7 and three strains of *Salmonella enterica* were used in this study. The origin and the antibiotic resistance of all the strains are listed in Table 4.1. Nalidixic acid (Alfa Aesar™, England), streptomycin (streptomycin sulfate salt; MP Biomedicals™, Aurora, OH), or ampicillin (ampicillin sodium salt; Alfa Aesar™, Ward Hill, MA) were added to the media according to the antibiotic resistance ability of each strain. The nalidixic acid stock was prepared by dissolving in 0.3 N of NaOH (VWR, Solon, OH); streptomycin and ampicillin stock were prepared with sterile distilled water (SDW). Both stocks were filtered through 0.2 µm filter (Ascrodisc® Syringe Filters, Pall Corporation, Ann Arbor, MI) and stored at 4 °C.

Stock cultures of each strain were prepared by overnight incubation at 37 °C in brain heart infusion (BHI, Difco, Becton Dickinson, Sparks, MD) for *L. monocytogenes* or tryptic soy broth (TSB; Difco, Becton Dickinson, Sparks, MD) for *E. coli* O157:H7 and *S. enterica*. The nutritious broth was supplemented with the appropriate antibiotic listed in Table 4.1. The stock cultures were stored in 20% glycerol at -80 °C. To prepare the working culture, frozen stock was re-cultured in BHI or TSB with appropriate antibiotics and incubated at 37 °C for 20 h, followed by streaking on tryptic soy agar (TSA; Difco, Becton Dickinson, Sparks, MD). Isolated colonies were obtained after 18 - 20 h of incubation. Before each experiment, one isolated colony

was transferred into 10 mL of BHI or TSB with the appropriate antibiotics and incubated at 37 °C for 20 h. Cells of each strain were harvested by centrifugation at 2880 x g for 15 min (Allegra^{TM} 21R, Beckman Coulter with S4180 rotor) at 4°C and washing with 10 mL of 0.1% peptone water (SPW; Difco, Becton Dickinson, Sparks, MD) and 10 mL of SDW sequentially. The re-suspended cultures were used as working cultures (~ 9.0 log CFU/mL).

Table 4.1. Information about the strains used in this research.

	Strains	Origin	Antibiotic Resistance
Listeria monocytogenes	L008	1981 Canadian cole slaw/ cabbage outbreak, serotype 4b	100 µg/mL of nalidixic acid
	L2624	2011 cantaloupe outbreak, serotype 1/2b	100 µg/mL of nalidixic acid
	L2625	2011 cantaloupe outbreak, serotype 1/2a	100 µg/mL of nalidixic acid
***Escherichia coli* O157:H7**	7386	2008 Washington State Bagged Lettuce Outbreak	100 µg/mL of nalidixic acid
	86-24	1986 Walla Walla ground beef outbreak, Washington State	100 µg/mL of streptomycin + 30µg/mL of nalidixic acid
	ATCC® 43895™	Hamburger outbreak associated with raw meat	100 µg/mL of ampicillin
	Sakai	1996 Radish Sprouts in Sakai City	100 µg/mL of nalidixic acid
***Salmonella enterica* Montevideo**	G4639	1993 raw tomato outbreak	100 µg/mL of nalidixic acid
***Salmonella enterica* Stanley**	H0558	1995 sprout outbreak	100 µg/mL of nalidixic acid
***Salmonella enterica* Newport**	H1275	Sprout Outbreak	100 µg/mL of nalidixic acid

4.2 Preparation of photosensitizer curcumin and ascorbic acid

A stock solution of curcumin with (95% curcuminoid content) (Alfa Aesar™, Ward Hill, MA) was dissolved in 95% ethanol in amber tubes (VWR International, LLC Randor, PA) to achieve a final concentration of 40 mM pure curcumin (Mw = 368.38). SDW was used to dissolve L-ascorbic acid (99%, ACROS Organics™) to prepare a 10 mg/mL stock solution. Both stocks were diluted with SDW to desired concentrations right before each usage. The pH of the photosensitizer was determined by pH meter (accumet™ AB15, Fisher Scientific). The absorption spectrum of the photosensitizers was obtained by UV/Vis spectrophotometer (Agilent Cary 60 Spectrophotometer, Agilent Technology).

4.3 Light apparatus set up and the analysis of its spectral output

The light device used in this research consisted of eighty light-emitting diodes in total (LED; 450 – 455 nm; 5mm blue LEDs; Chanzon), which were assembled on the lid of a 96-well plate (Figure 4.1a & b). The power density of the light apparatus (W/cm^2) was measured using a spectroradiometer (Apogee Instruments model PS - 300) at a distance of 16 cm (P_0). Figure 4.1c shows the light apparatus and the spectroradiometer detector. The following equation was used to determine the power density:

$$\text{Power Desnity} = \frac{(16\ cm)^2}{(2\ cm)^2} \times P_0\ (W/cm^2) \qquad \text{Eq. 1}$$

When conducting antimicrobial assays, a distance of 2 cm was used. Since, the power density is affected by the distance, the relationship between the power density used

in the antimicrobial assays and P_0 can be demonstrated as Eq. 1, based on the inverse-square law (the light power density is inversely proportional to the square of the distance between the light source and the subject).

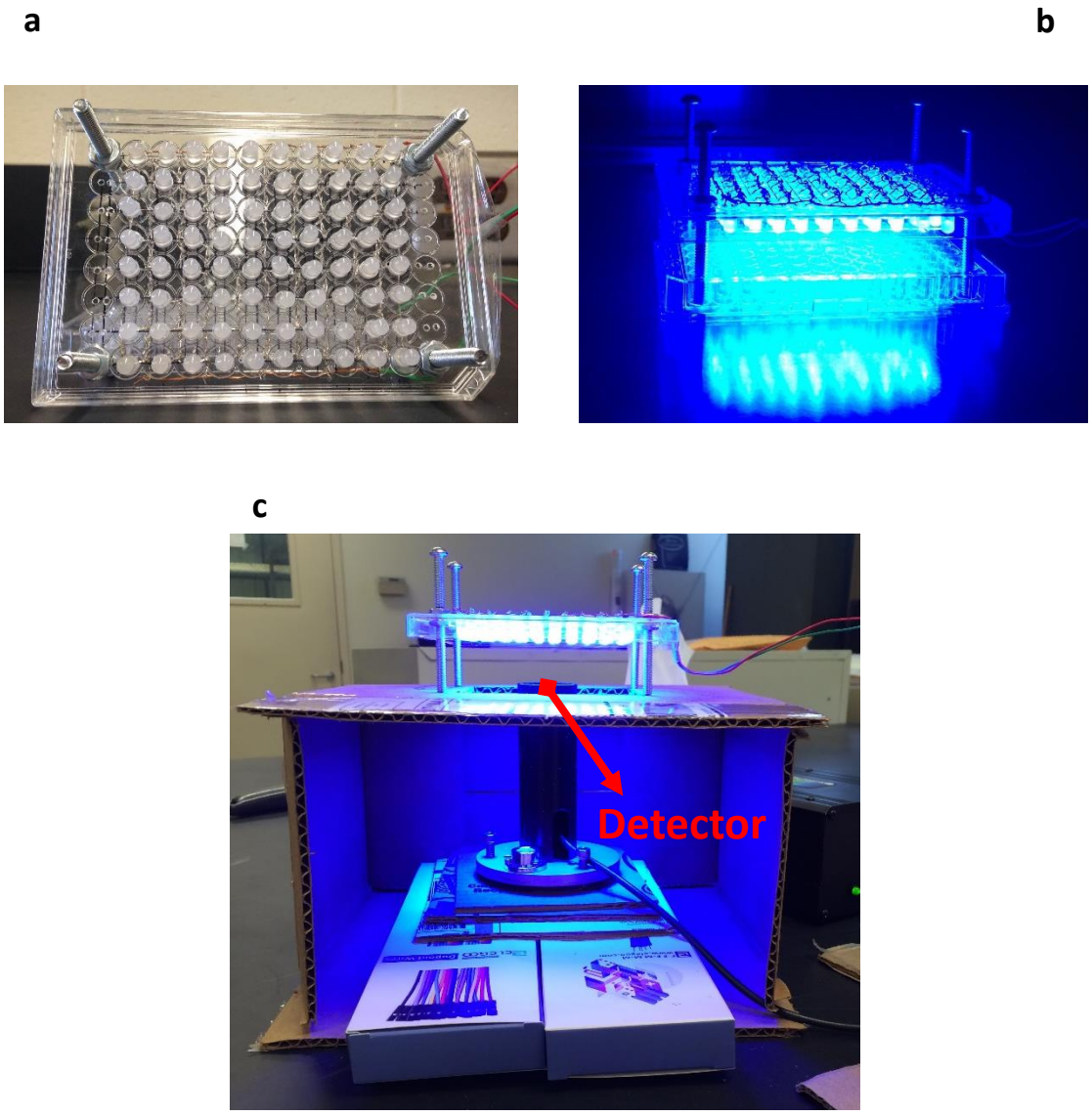


Figure 4.1. Setting of light apparatus (a & b) and the measurement setting for the analysis of the spectra output of the light apparatus (c). The LED light array with 80 LEDs is used to activate the photosensitizer in this research to deliver a uniform radiation, unilluminated (a) and illuminated (b).

4.4 Evaluation of the antimicrobial effects of curcumin on a solid system

The antimicrobial effects of the photosensitizing solutions on a solid system were evaluated by the modified Kirby Bauer Method. The working cultures were diluted with TSB (*E. coli* O157:H7 and *S. enterica*) or BHI (*L. monocytogenes*) to achieve the value of OD_{600} in the range of 0.08 to 0.1, resulting in the bacterial level of approximate 8.0 log CFU/mL. The inoculum was used within 15 min after adjusting the bacterial concentration. The culture was streaked evenly on the surface of TSA by using a sterile cotton swab. After 5 min of drying, a 20 µL of the testing solution (Ethanol Control, AA, Cur, or Cur/AA) was dropped onto the surface of TSA. After 5 min incubation in the dark, plates were subjected to 10 min illumination. The distance between the LEDs and the agar surface was 2 cm. Dark controls were performed in parallel with the illuminated samples. Both illuminated and dark control plates were incubated at 37 °C for 18 h. The presence/absence of inhibition zone was recorded.

4.5 Optimization of the illumination time in the planktonic system

The working cultures were diluted with SDW to approximate 6.0 log CFU/mL of *L. monocytogenes* L008 or *E. coli* O157:H7 86-24 as the inoculum. A 500 µL aliquot of the inoculum was incubated with 500 µL of the photosensitizers in amber tubes in the dark for 5 min. Photosensitizers with 1 and 10 µM of curcumin plus 250 µg/mL of ascorbic acid were tested in inactivating *L. monocytogenes*, while 100 µM of curcumin plus 250 and 500 ug/mL of ascorbic acid were tested for *E. coli* O157:H7. An aliquot (100 µL) was transferred to a sterile flat-bottom 96-well plate (Greiner

Bio-One GmbH, Frickenhausen, Germany) in duplicate. The 96-well plate was then put under the light for 0, 1, 5, and 10 min. The distance between the light device and the surface of the mixtures was 2 cm. The illuminated samples were serially diluted and plated on duplicate TSA to determine the microbial level after treatments. Dark controls were performed in parallel. Experiment was conducted triplicate.

4.6 Evaluation of the antimicrobial effects of curcumin in a planktonic system

The inoculum was prepared as described above. A 500 µL aliquot of the inoculum was incubated with 500 µL of different concentrations of the photosensitizers (curcumin, ascorbic acid, and curcumin/ascorbic acid) in the amber tubes in the dark for 5 min. A 100 µL of the aliquot was transferred to 96-well plates in duplicate. The illuminated samples were irradiated for 1 and 10 min for *L. monocytogenes* and *E. coli* O157:H7, respectively. The distance between the light and suspension was maintained in 2 cm. The illuminated samples were subjected to microbiological analysis on TSA. Dark controls were performed in parallel. Experiment was repeated for three times.

4.7 Statistical analysis

The experiments were replicated for three times. The average populations of bacteria after treatment were analyzed by SAS University Edition for analysis of variances (ANOVA) test followed by Waller-Duncan t test as post hoc test. A p value < 0.05 was considered as significant difference.

5. RESULTS

5.1 The color and pH value of the photosensitizer curcumin

The color of curcumin (Cur), ascorbic acid (AA), and curcumin/ascorbic acid (Cur/AA) is shown in Figure 5.1 a - b. Solutions containing only AA were colorless, regardless of the concentrations of AA (Figure 5.1 a). With increasing concentration of curcumin, the yellowish color was more apparent (Figure 5.1 b). It was difficult to distinguish the yellowish color when the curcumin concentration was lower than 10 µM. The addition of ascorbic acid into curcumin did not cause any apparent differences (Figure 5.1 c - d) compared to the solutions with curcumin only. Also, the concentration of ascorbic acid did not have visible impacts on the degree of yellowish color.

The pH values of different curcumin and ascorbic acid combinations are listed in Table 5.1. With the same concentration of ascorbic acid, there were no apparent differences among the samples. Expectedly, increasing concentration of ascorbic acid lowered the pH value. With curcumin solutions plus 25 µg/mL of ascorbic acid, the pH was around 6.00, which was close to neutral. When the concentration of ascorbic acid was increased to 250 µg/mL, the pH value decreased but remained close to 4.00.

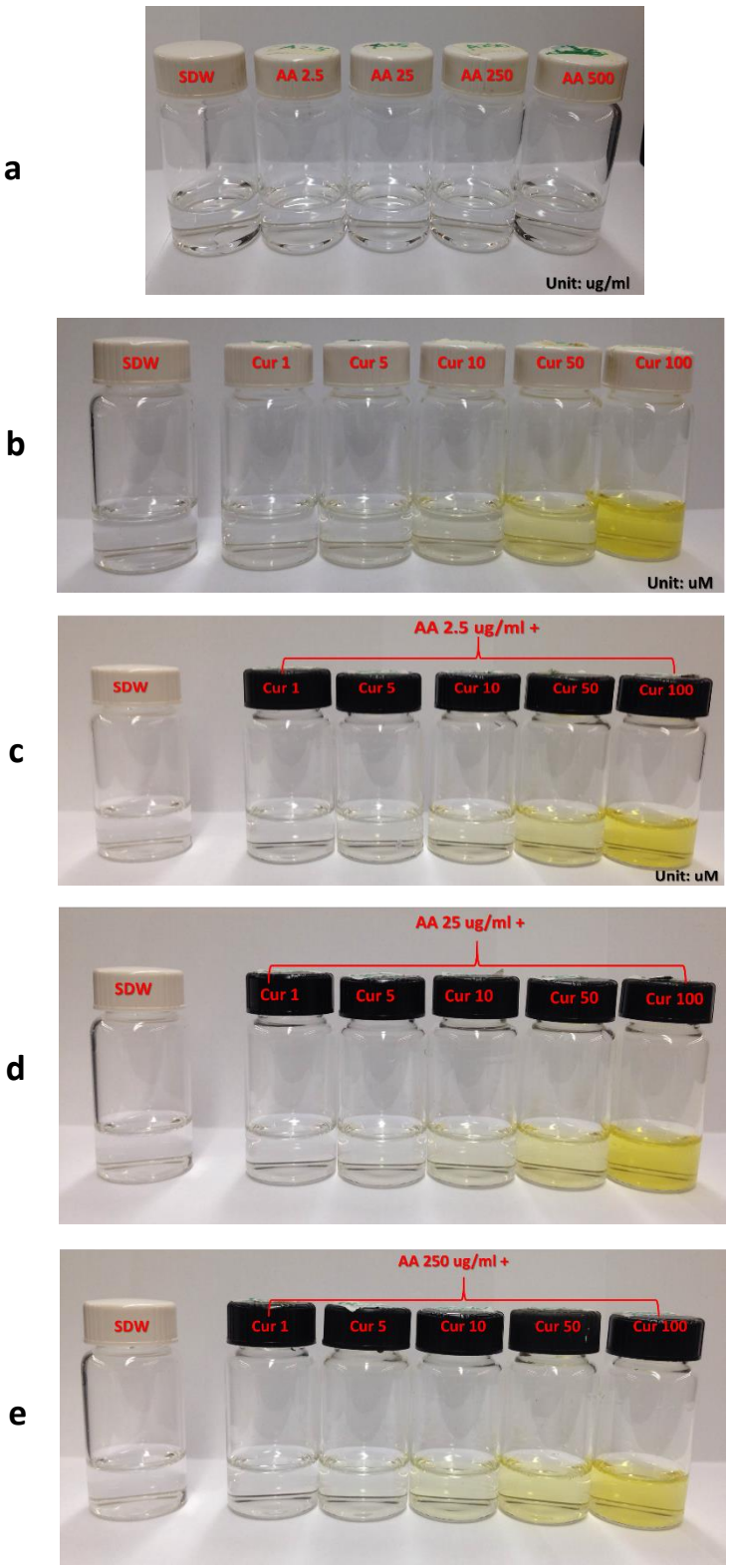


Figure 5.1. Photos of ascorbic acid solutions (a), curcumin solutions (b), and 2.5, 25, and 250 µg/mL of ascorbic acid plus a series of concentration of curcumin solutions (c, d & e). SDW was used as a diluent and a control for comparison.

Table 5.1. The pH value of the Cur, AA, and Cur/AA solutions.

pH		Ascorbic Acid (μg/mL)		
		0	25	250
Curcumin (μM)	0	6.82 ± 0.16	4.65 ± 0.08	3.76 ± 0.05
	1	6.46 ± 0.30	5.31 ± 0.36	3.82 ± 0.01
	5	6.59 ± 0.22	4.97 ± 0.13	3.82 ± 0.02
	10	6.47 ± 0.33	5.07 ± 0.10	3.78 ± 0.07
	50	6.48 ± 0.21	4.80 ± 0.24	3.77 ± 0.04
	100	6.50 ± 0.18	4.92 ± 0.40	3.77 ± 0.01

5.2 Absorption spectrum of the photosensitizer curcumin

According to the absorption spectrum (Figure 5.2), the solution with solely 250 µg/mL of ascorbic acid was very close to the base line. Samples containing the photosensitizer curcumin had a relatively broad absorption spectrum, running from 350 to 550 nm. The maximum absorbance increased with increasing curcumin concentration. However, the peak wavelength did not have an apparent change with different concentrations of curcumin. There was a slight red shift after the addition of the ascorbic acid, but it was not significantly different than the samples with curcumin at the same curcumin concentration. The wavelength of the light apparatus (450 – 455 nm) was chosen based on the absorption spectrum of the photosensitizer curcumin. It was within the absorption range and not far from the maximum. More importantly, it was farther away from the UV range. Therefore, it had fewer safety concerns with respect to human exposure during industrial application.

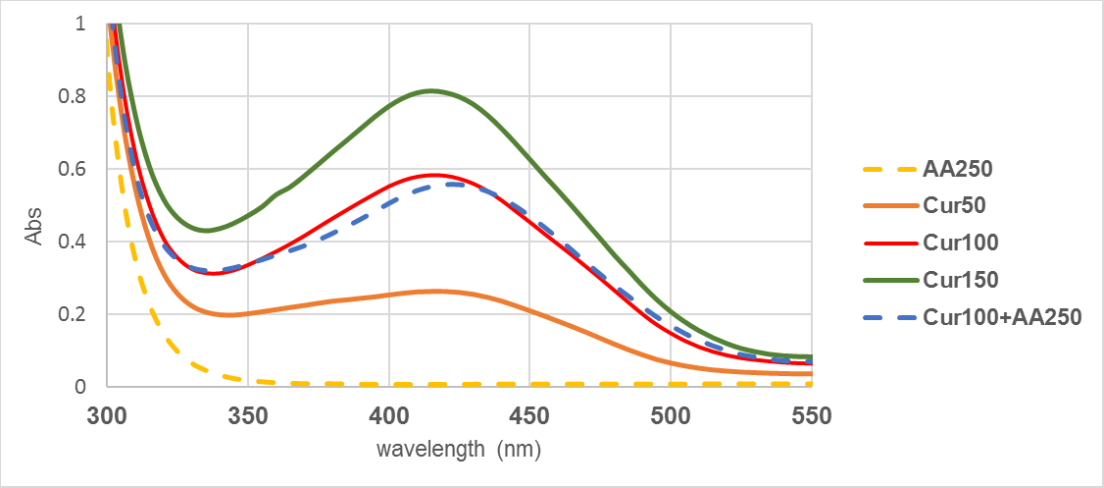


Figure 5.2. Absortion spectrum of ascorbic acid (AA), curcumin (Cur), and the combination. The number after AA indicates the cocentration of AA (µg/mL), while the number after Cur indicates the concentration of Cur (µM). No absorption peak was observed for all the samples above 550 nm (data not shown). SDW was used as a diluent.

5.3 Spectra output (power density) of the light apparatus

From the spectroradiometer, data points were obtained as W/m^2/nm in every 0.5 nm change in the wavelength, running from 226 to 999.5 nm. Data was plotted with the wavelength (nm) as the x-axis and W/m^2/nm as the y-axis (Figure 5.3). The powder density (P_0) between the light and the spectroradiometer detector can be calculated by integration, which was 0.00208 W/m^2. According to the Eq. 1, the powder density used in the antimicrobial assays was 0.13 W/cm^2.

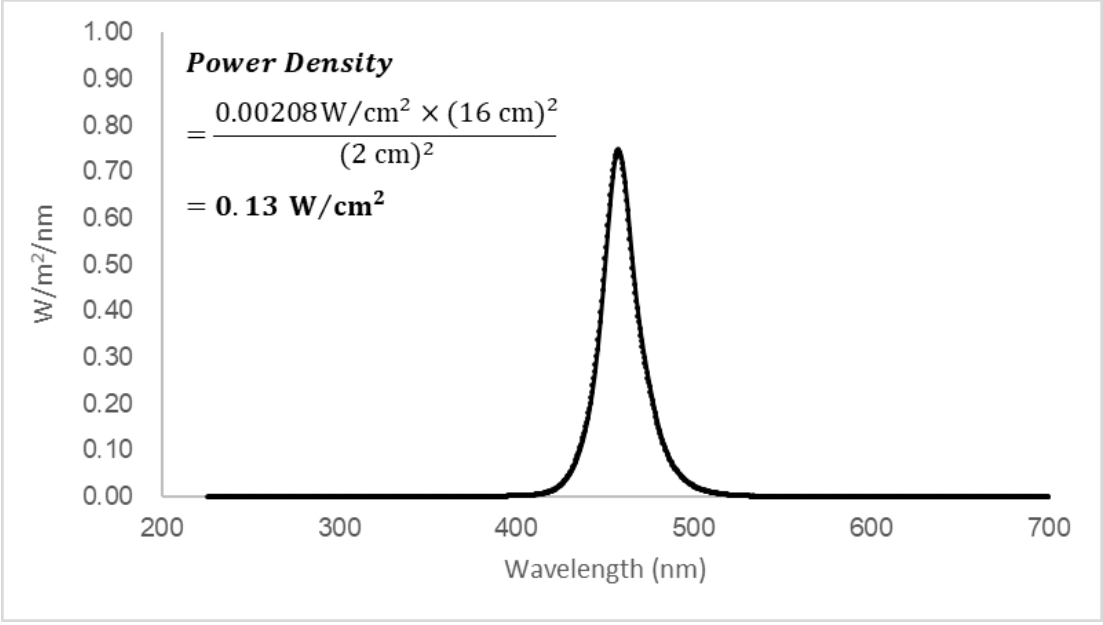


Figure 5.3. Spectra output of the light apparatus from the spectroradiometer.

5.4 Antimicrobial effects of the photosensitizer curcumin on solid media

The antimicrobial effects of photosensitizer curcumin were tested in inactivating three common types of foodborne pathogens – *L. monocytogenes*, *E. coli* O157:H7, and *S. enterica*. There were no inhibition zones on all the dark control samples for all the strains tested (data not shown). Two strains of *E. coli* O157:H7 (ATCC® 43895™ and Sakai) and all the three strains of *S. enteric* could not be inactivated by curcumin up to 1000 μM supplemented with 250 μg/mL of ascorbic acid (data not shown).

As shown in Figure 5.4 a - c, neither the residual ethanol in the PS solution coming from the stock nor the 250 μg/mL of ascorbic acid alone resulted in any antimicrobial activity against *L. monocytogenes*. Different strains of *L. monocytogenes* had different susceptibility to the photosensitizer. *Listeria monocytogenes* L008 was inactivated by 10 μM of curcumin, while *L. monocytogenes* L2625 was not. However, all the three strains of *L. monocytogenes* could not grow under the conditions with the combination of 10 μM curcumin and 250 μg/mL of ascorbic acid. The results are summarized in Table 5.2. Overall, the photosensitizer curcumin showed better effects in inactivating *L. monocytogenes* after the addition of 250 μg/mL of ascorbic acid.

Photosensitizer containing 100 μM of curcumin and 250 μg/mL of ascorbic acid showed the best effects in inactivating both strains of *E. coli* O157:H7 (Figure 5.4 d - e & Table 5.2), which was better than 1000 uM of curcumin with or without ascorbic acid. However, few colonies were found within the clear zone on the plates.

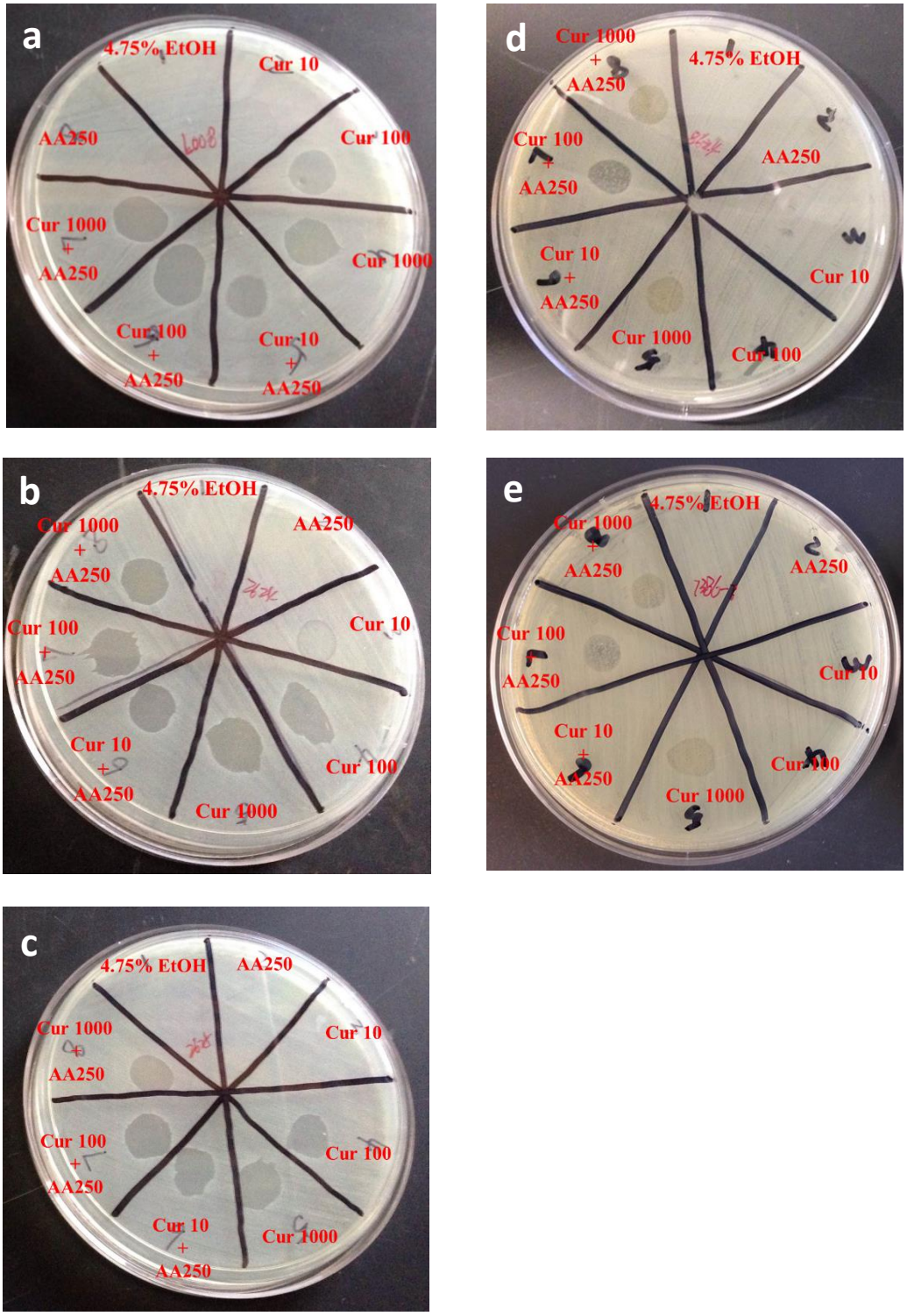


Figure 5.4. Presence/absence of inhibition zone on the TSA plates after the treatment. *L. monocytogenes* L008 (a), L2624 (b), and L2625 (c); *E. coli* O157:H7 86-24 (e) and 7386 (f). The number after AA indicates the concentration of ascorbic acid (µg/mL), while the number after Cur indicates the concentration of curcumin (µM).

Table 5.2. Summary of the results of the modified Kirby Bauer Method.

		4.75% EtOH	AA 250*	Cur 10*	Cur 100	Cur 1000	Cur 10 AA250	Cur100 AA250	Cur1000 AA250
Listeria monocytogenes	L008	-	-	++**	++	++	++	++	++
	L2624	-	-	+	++	++	++	++	++
	L2625	-	-	-	++	++	++	++	++
E. coli O157:H7	7386	-	-	-	-	+	-	++	-
	86-24	-	-	-	-	+	-	++	-

* The numbers after AA indicates the cocentrations of ascobic acid (µg/mL), and those after Cur indicate the concentration of curcumin (µM).

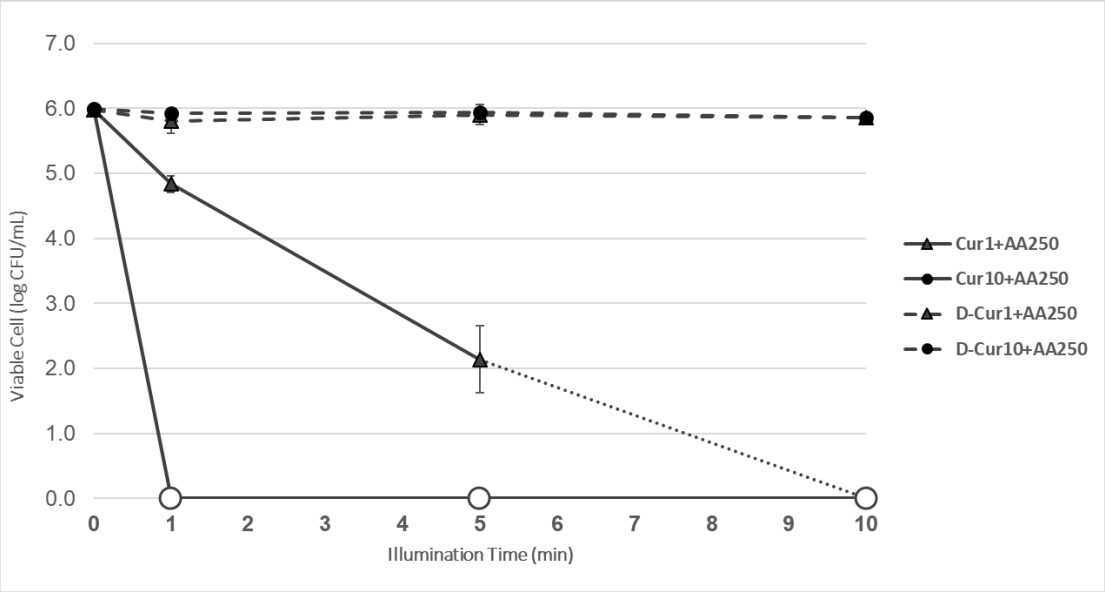
** The clearest zone for each strain was marked as “++”; less clear or some resistant bacteria growth within a clear zone was marked as “+”.

5.5 Effects of illumination time for photosensitizer in inactivating bacteria

The initial level of bacteria per well was 6.0 and 5.8 log CFU/mL per well for *L. monocytogenes* and *E. coli* O157:H7, respectively. Without light activation, the photosensitizing solutions did not show any antimicrobial effects, regardless of the type of bacteria (Figure 5.5 a & b). Two concentrations of curcumin, 1 and 10 μM, were tested in inactivating *L. monocytogenes*. The number of viable cells dropped dramatically to non-detectable level before enrichment, treating with 10 μM of curcumin and illuminating for 1 min (60 s $\times$ 0.13 W/cm^2 = 7.8 J/cm^2) (Figure 5.5 a). One min was chosen as the illumination time in the subsequent study.

Two concentrations of ascorbic acid were tested for inactivating *E. coli* O157:H7. No significant difference was observed between the two treatments (Figure 5.5 b). After 10 min of illumination (78 J/cm^2), no bacteria were detected. As a result, 10 min was chosen in the subsequent study. Higher concentration of curcumin may shorten the illumination time to achieve complete inactivation. However, more concentrated curcumin has more yellow color, which may potentially affect the food quality.

a



b

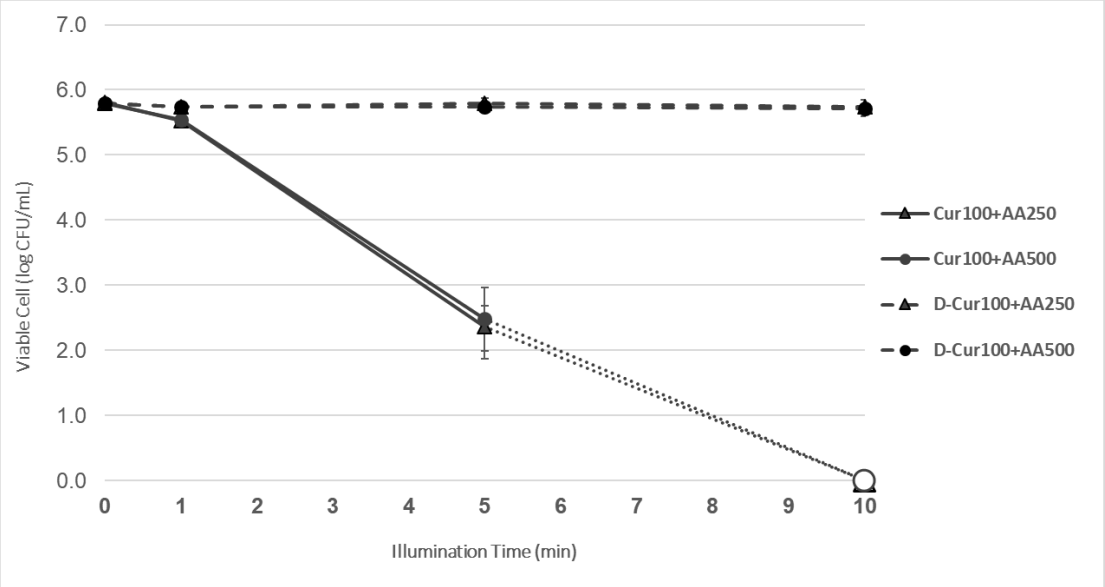


Figure 5.5. Effects of illumination time of the photosensitizer in inactivating *L. monocytogenes* (a) and *E. coli* O157:H7 (b). The dash lines represent the dark control samples, while the solid lines indicate illuminated samples. The initial levels of *L. monocytogenes* and *E. coli* O157:H7 were 6.0 and 5.8 log CFU/mL, respectively. The number after AA indicates the cocentration of ascorbic aicd (μg/mL), while the number after Cur indicates the concentration of curcumin (μM). Empty symbol indicates that growth was only detected after enrichment.

5.6 Potential effect of ascorbic acid on the phototoxicity of curcumin

Figure 5.6 shows the antimicrobial effects of curcumin (Cur), ascorbic acid (AA), and curcumin/ascorbic acid (Cur + AA) in inactivating *L. monocytogenes* after 1 min of illumination. Ethanol was used to prepare the curcumin stock, followed by diluting with SDW to the desired concentration. The highest residual concentration of ethanol (0.05%) in the curcumin solutions was used as the control to evaluate whether the residual ethanol could contribute to the bacterial reduction. No matter whether illumination was applied or not, the number of the bacteria after ethanol treatment was remained at the level of 6.0 log CFU/mL, indicating that the residual ethanol did not have any inhibitory effects. Similarly, nearly zero reductions were found in the samples containing solely ascorbic acid, even up to 250 μg/mL. Interestingly, a higher concentration of AA showed lower effects in inactivating the bacteria.

Regardless of the ascorbic acid concentration, *L. monocytogenes* was not detected after treatment with 10 μM of Cur. Regarding to the solutions containing 5 μM of Cur, no cells were detected for the samples with the addition of 25 or 250 μg/mL of AA, resulting in an approximate 6-log reduction in viable cells. Similar log reductions were achieved by 5 μM of Cur with or without 2.5 μg/mL of AA, causing 3.3 and 3.1 log reduction, respectively. The differences between Cur/AA and Cur were more evident when Cur concentration was 1 μM. After treatment with the combination of 1 μM Cur and 250 μg/mL of AA, a 1.5 log reduction in the cells was observed, which was 1.2 log higher than treating with 1 μM Cur alone.

The initial level of the *E. coli* O157:H7 per well was 5.9 log CFU/mL. Similar to *L. monocytogenes*, all the treatments did not show any inhibitory effects against *E. coli* O157:H7 without illumination (Figure 5.7). The residual ethanol (0.24%) in the final photosensitizer solution was also too low to kill *E. coli* O157:H7. Also, no reductions were found in reducing the number of *E. coli* O157:H7 after treatment with ascorbic acid only. Although the photosensitizers containing solely 50 or 100 μM of Cur caused 1.6 and 2.3 log reduction in the bacterial cells, respectively, Cur/AA demonstrated higher log reductions.

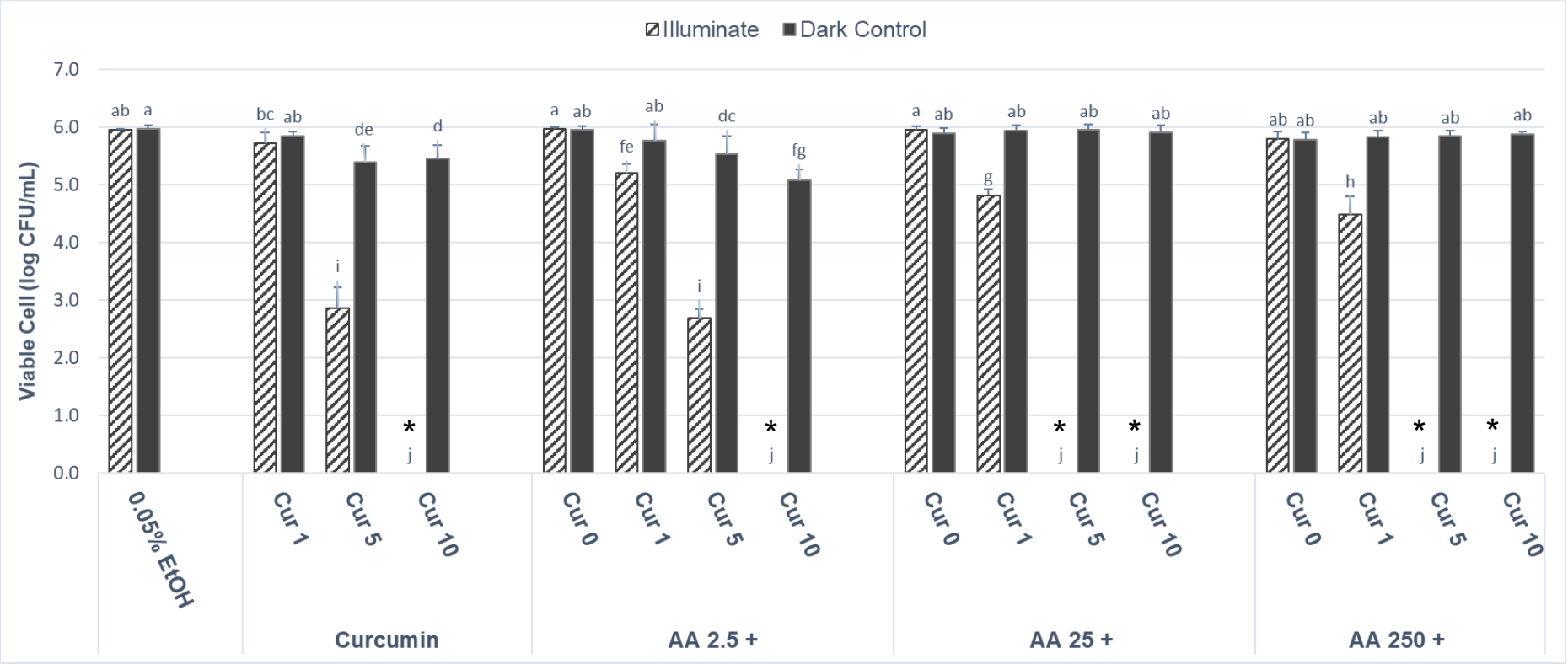


Figure 5.6 Antimicrobial effects of Cur, AA, and Cur/AA in inactivating *L. monocytogenes* after 1 min illumination.

* Growth was only detected after enrichment.

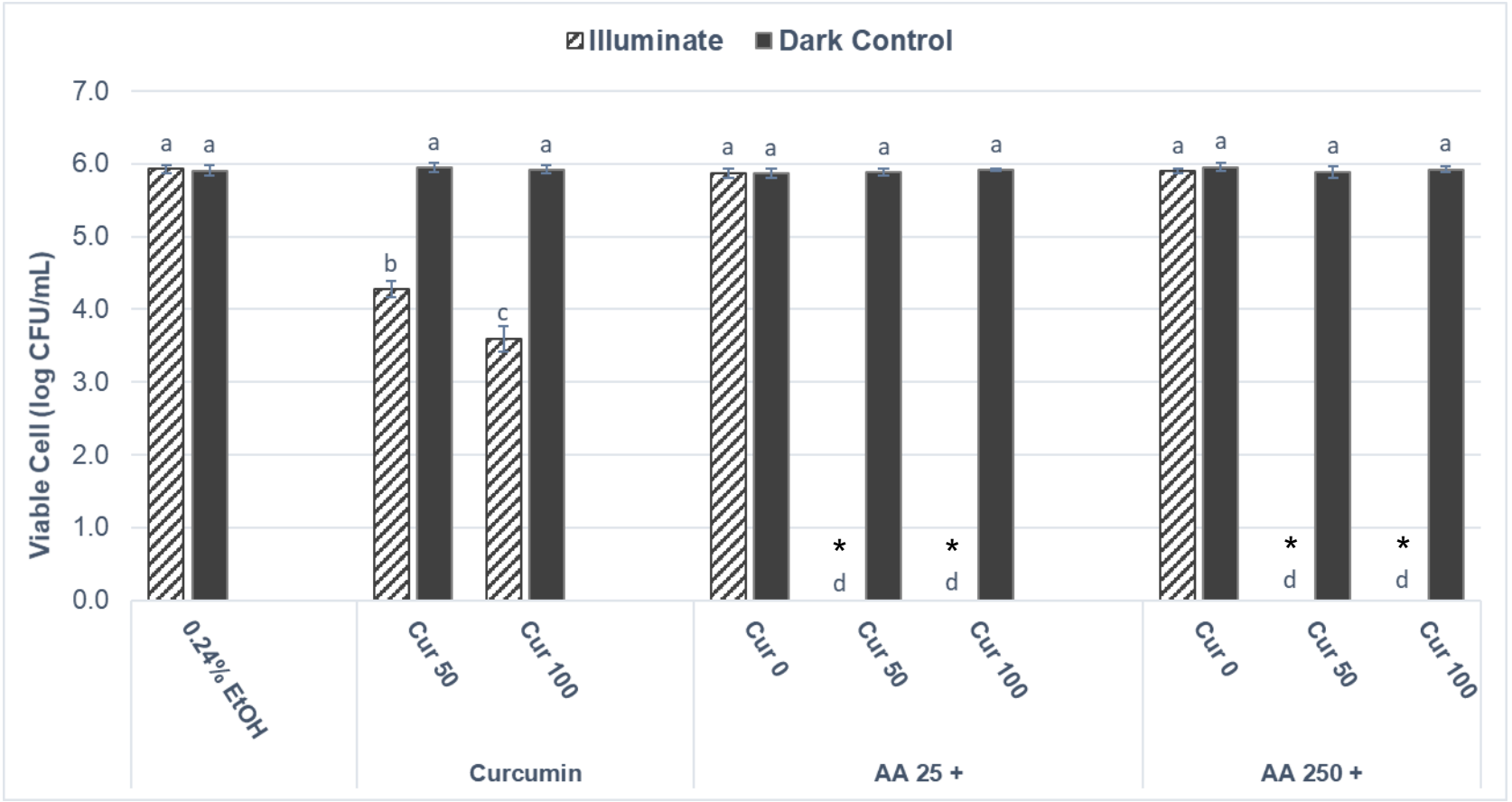


Figure 5.7. Antimicrobial effects of Cur, AA, and Cur/AA in inactivating *E. coli* O157:H7 after 10 min illumination.

* Growth was only detected after enrichment.

6. DISCUSSION

The absorption maximum of curcumin (λ_{max}) varies from 408 to 463 nm, depending on the type of solvent (Barik, Goel, Priyadarsini, & Mohan, 2004; Chignell et al., 1994). More specifically, curcumin in ethanol has a broad absorption spectrum with the absorption maximum ranging from 427 to 430 nm (Nardo et al., 2008), which was similar to the spectrum obtained in this study (Figure 5.2). The λ_{max} at approximately 420 nm probably result from the π-π^* transition in the enolic form (Shen & Ji, 2007). The λ_{max} was found to be highly affected by the polarity of the solvent. Ethanol is a polar solvent due to its hydroxyl group (-OH) with high hydrogen bonding capacity. In polar solvents, the keto-enol tautomeric equilibrium of curcumin tends to shift towards the enolic conformer, resulting in a loss of vibrational fine structure and the red shift (Patra & Barakat, 2011). In other words, red shifted absorption maximum was observed on the more polar solvents. However, the polarity and the hydrogen bonding capacity of solvent could typically result in slight changes in the λ_{max}, but usually less than 10 nm differences (Priyadarsini, 2009). Instead, the absorption spectrum of curcumin was shown to change remarkably between acidic and base conditions because of the protonation/deprotonation of the phenolic groups (Shen & Ji, 2007; Zsila, Bikádi, & Simonyi, 2003). Although absorption spectrum is pH-dependent, the spectrum did not change significantly after the addition of ascorbic acid (Figure 5.2). It is probably because the pH of curcumin alone (~ pH 6.7) and all the curcumin/ascorbic acid mixture are all below the pK_a values of curcumin (pH 7.8, 8.5, and 9.0), making no significant changes in

the protonation of the structure (Table 5.1). Therefore, no noticeable differences were observed in the absorption spectrum. Regarding the maximum absorbance (Abs_{max}), the results met the expectation that the Abs_{max} is proportional to the curcumin concentration, which obeyed the Lambert-Beer law in the μM – mM concentration range in organic solvents (Chignell et al., 1994).

Higher log reductions on *L. monocytogenes* and *E. coli* O157:H7 were achieved with a longer time of illumination (Figure 5.5) or higher light dose. Light dose (J/cm^2) or energy can be calculated by multiplying the power density with the illumination time. Therefore, this study showed that the antimicrobial effects of the photosensitizer increased with the power dose. Similarly, Paschoal et al. (2013) found that applying 24 J/cm^2 of light induced 1.88 log reduction in *Streptococcus mutans*, which was less than 72 J/cm^2. The same dose-dependent effect was found in inactivating *Staphylococcus aureus* (gram-positive), *E. coli* (gram-negative) and *Candida albicans* (yeast) (Demidova & Hamblin, 2005). The photodynamic inactivation against bacterial cells is based upon the activation of ground-state to excited-state PS, resulting in the production of the ROS. Ergaieg, Chevanne, Cillard, and Seux (2008) found a linear relationship between the production of $^1O_2$ and the illumination time up to 15 min. Therefore, it is assumed that longer illumination time generates more excited PS curcumin, so more ROS are produced to inactivate the bacteria.

Curcumin was shown to have phototoxicity after being activated by light

(Figure 5.6 & Figure 5.7). The phototoxicity of curcumin was based upon the products such as singlet oxygen and superoxide radicals (Radeglia & Arrieta, 1998), which could cause oxidization of membrane protein, and degradation of DNA and RNA (Wu et al., 2016). Previous studies have shown that curcumin without light activation could have inhibitory effects on a variety of microorganisms, such as *L. monocytogenes*, *E. coli* O157:H7, *Salmonella typhimurium*, and methicillin-resistant *Staphylococcus aureus* (Kim et al., 2005; Niamsa & Sittiwet, 2009). The antimicrobial effects of curcumin were found to be associated with the regulation of redox status of bacteria by changing the NADH and NADPH concentrations (Shlar, Droby, & Rodov, 2017). The minimum inhibitory concentration (MIC) of curcumin was varied by different types of strains, but it was usually above 1000 ppm without blue light activation (Kim et al., 2005; Negi, Jayaprakasha, Jagan Mohan Rao, & Sakariah, 1999). Without lighting activation, the concentrations required to show practical effects were much higher than the maximum level in this study (approximate 100 ppm). In other words, a lower concentration of curcumin can be used if blue LEDs are applied, which could both reduce the cost of producing the photosensitizing solution as well as the adverse impacts of the yellow color on food quality. Also, a higher concentration of curcumin did not represent greater inhibitory effects. In Figure 5.4 d & e, the inhibition zone was clearer after treated with 100 μM of curcumin with ascorbic acid than treated with 1000 μM curcumin with acid. It is probably because SDW was used as the diluent in this study, and 1000 μM curcumin is well above the solubility limit of curcumin. The curcumin will thus aggregate and precipitate, leading

to a decrease in the phototoxicity. The aggregation and precipitation may be in a concentration-dependent manner. In other words, they happen faster at high concentration, so more curcumin molecules are precipitated at the concentration of 1000 μM than 100 μM.

Listeria monocytogenes (gram-positive) was shown to be more susceptible to photodynamic inactivation than *E. coli* O157:H7 (gram-negative), because higher concentration of curcumin (Table 5.2) and longer illumination time (Figure 5.5) were required to completely inactivate *E. coli* O157:H7, which is consistent with previous studies (Banfi et al., 2006; Valduga, Bertoloni, Reddi, & Jori, 1993; Winter et al., 2013). The differences in the susceptibility between gram-positive and gram-negative could be explained by the differences in their cell wall structure. Gram-positive bacteria have relatively porous cell walls, which are composed of peptidoglycan and lipoteichoic acid. As the production of highly reactive photo-products is short, the porous structure facilitates the photosensitizer to go inside the cell, resulting in possible reactions occurring both outside and inside the cell. By contrast, gram-negative species have both outer and inner cell membrane, which are separated by the peptidoglycan-containing periplasm. The outer membrane is a very effective barrier to prevent many photosensitizers from both binding and penetration, leading to a reduction in the antimicrobial effects (Dai, Huang, & Hamblin, 2009; Minnock et al., 2000). Unlike water-soluble photosensitizers such as sodium chlorophyllin, curcumin was found to effectively inactivate gram-negative bacteria in this study (Figure 5.7). Several hypotheses were proposed to explain the

killing effects of the photosensitizer curcumin against gram-negative bacteria. Firstly, the lipophilic property of curcumin makes curcumin bind preferentially to the phospholipid membrane as well as some cell proteins (Priyadarsini, 2009). Secondly, the diketone structure allows curcumin to act as a chelator (Began, Sudharshan, Udaya Sankar, & Appu Rao, 1999). The chelating ability of curcumin was confirmed by the formation of mixed hydroxo species by the reaction of curcumin as a bidentate chelating ligand (Borsari, Ferrari, Grandi, & Saladini, 2002). The outer membrane of gram-negative bacteria is negatively charged, producing a dense barrier efficiently against antimicrobial compounds. However, chelators can chelate the divalent cations like Mg^{2+} and Ca^{2+} that connect the lipopolysaccharide, leading to the disruption of the outer membrane and the facilitation of PS uptake. Curcumin itself has fluorescence in a specific wavelength range, so the uptake and absorption of PS were observed by the fluorescence microscopy equipped with an excitation filter 420 – 480 nm after washing the surface-binding curcumin through centrifugation (Haukvik et al., 2009). Finally, the potential hydrogen bonding interaction helps to keep the PS firmly bound to the outer membrane, increasing the phototoxicity of curcumin. The hydroxyl groups in the curcumin molecule allow its oxygen atoms to form hydrogen bonds with the bacterial outer membrane, which acts as a hydrogen acceptor (Haukvik et al., 2009).

Organic acids, such as propionic acid, lactic acid, citric acid, acetic acid and benzoic acid, have been used to inactivate surface associated microbes. The mode of inactivation by organic acids includes cytoplasmic acidification and accumulation of

free acid anions (González-Fandos & Herrera, 2013), leading to energy imbalance, enzyme inactivation (Morey et al., 2014), failure to neutralize the excessive acid by decreasing proton motive force (Drosinos, Mataragas, Kampani, Kritikos, & Metaxopoulos, 2006), and etc. As a commonly-used reducing agent, ascorbic acid has antimicrobial and antiviral activity by the formation of free radicals during autoxidation of the acid (Salo & Cliver, 1978; Tajkarimi & Ibrahim, 2011). However, in this study, ascorbic acid alone did not show any inhibitory effects against both *L. monocytogenes* (Figure 5.6) and *E. coli* O157:H7 (Figure 5.7), probably because the acid concentrations are over 100 times lower than the concentrations used in the previous study.

With the addition of ascorbic acid, greater reductions in viable cell numbers were achieved than curcumin treatment alone (Figure 5.6 & Figure 5.7), indicating a potential effect of ascorbic acid on enhancing the phototoxicity of PS curcumin. Similar results were observed by Khalil et al. (2012), who found that curcumin/ascorbic acid showed higher reduction on the growth of *Candida* spp than curcumin only, even though no light was used in their study. It was hypothesized by Khalil et al. (2012) that ascorbic acid improve the stability of curcumin, making more active curcumin molecules available for killing the microbes. Curcumin undergoes rapid degradation under alkaline and neutral conditions, leading to the production of vanillin, ferulic acid and feruloylmethane (Tønnesen & Karlsen, 1985; Wang et al., 1997). Oetari, Sudibyo, Commandeur, Samhoedi, and Vermeulen (1996) found that there was a significant increase of stability of curcumin in pH 6.5 than in pH 7.4

phosphate buffer up to 30 min after preparation. In another study, Hegge, Nielsen, et al. (2012) found that curcumin dissolved in 10% ethanol in PBS (pH 6.1) decreased to half of its initial concentration, even after 3-day storage. These results indicate that slightly acidic solvent helped to improve the stability of curcumin in aqueous solution, which is probably related to the conjugated diene structure of curcumin. Under alkaline and neutral conditions, the nucleophilic OH^- ions attack the carbonyl carbon in the keto-enol moiety, leading to the destruction of the conjugated structure and destabilization of the compound (Tomren, Masson, Loftsson, & Tønnesen, 2007; Wang et al., 1997). In addition to lowering the pH, the stability of curcumin can be improved by adding thiol and non-thiol containing antioxidants, such as ascorbic acid (25 μM), glutathione (1 mM), and N-acetyl L-cysteine (50 μM) (Oetari et al., 1996). It is probably because antioxidants may play a role in retarding the autoxidative degradation of curcumin before photodynamic inactivation takes place (Griesser et al., 2011; Schneider, Gordon, Edwards, & Luis, 2015). However, no studies have been conducted to provide direct evidence about how antioxidants stabilize curcumin.

Photosensitizer curcumin supplemented with ascorbic acid was shown to have promising inhibitory effects against both *L. monocytogenes* and *E. coli* O157:H7 in the media system. However, similar reductions are difficult to be achieved in the food system, because light reflecting properties of complex food surfaces may reduce the light intensity. Several approaches may be used to further enhance the phototoxicity of PS curcumin. For example, adding some types of membrane permeability enhancement compounds (e.g., $CaCl_2$, ethylenediaminetetraacetic acid,

or polycationic agent polymyxin B nonapeptide) may facilitate greater uptake of curcumin by disorganizing the outer membrane (Nitzan, Gutterman, Malik, & Ehrenberg, 1992). In a previous study, pre-incubation with $CaCl_2$ for 15 min induced over a 3-log reduction in *E. coli* higher than no $CaCl_2$ added (Winter et al., 2013). Also, lactic acid would also be a good substitute for ascorbic acid to improve both stability and solubility of curcumin, which are two important factors influencing the phototoxicity of curcumin (Rao & Rao, 2011).

7. CONCLUSION

This study showed a potential effect of ascorbic acid on enhancing the photodynamic inactivation ability of PS curcumin against both gram-positive *Listeria monocytogenes* and gram-negative *Escherichia coli* O157:H7, probably by improving the stability of curcumin. Light was essential to activate the photosensitizer curcumin to show antimicrobial effects in low curcumin concentrations (< 100 μM). Solutions with 5 μM of curcumin and 25 mg/mL of ascorbic acid completely resulted in a nearly 6-log reduction on *L. monocytogenes*. Although *Escherichia coli* O157:H7 was generally considered as more resistant to photodynamic inactivation than gram-positive bacteria, it was not detected after treatment with 50 μM of curcumin and 25 mg/mL of ascorbic acid. The developed photosensitizing solution has relatively low acidity, which has a potential application as an antimicrobial treatment on the surface of meat, poultry, and produce.

8. References

Aggarwal, B. B., Kumar, A., & Bharti, A. C. (2003). Anticancer potential of curcumin: preclinical and clinical studies. *Anticancer Research, 23*, 363-398.

Arnaut, L. G., & Formosinho, S. J. (1992). Excited-state proton transfer reactions II. Intramolecular reactions. *Journal of Photochemistry and Photobiology A: Chemistry, 75*, 21-48.

Arnaut, L. G., & Formosinho, S. J. (1993). Excited-state proton transfer reactions I. Fundamentals and intermolecular reactions. *Journal of Photochemistry and Photobiology A: Chemistry, 75*(1), 1-20. doi:10.1016/1010-6030(93)80157-5

Banfi, S., Caruso, E., Buccafurni, L., Battini, V., Zazzaron, S., Barbieri, P., & Orlandi, V. (2006). Antibacterial activity of tetraaryl-porphyrin photosensitizers: an in vitro study on Gram negative and Gram positive bacteria. *Journal of Photochemistry and Photobiology. B: Biology, 85*(1), 28-38. doi:10.1016/j.jphotobiol.2006.04.003

Barik, A., Goel, N. K., Priyadarsini, K. I., & Mohan, H. (2004). Effect of deuterated solvents on the excited state photophysical properties of curcumin. *Journal of Photoscience, 11*(3), 95-99.

Barkocy-Gallagher, G. A., Arthur, T. M., Rivera-Betancourt, M., Nou, X., Shackelford, S. D., Wheeler, T. L., & Koohmaraie, M. (2003). Seasonal prevalence of shiga toxin–producing *Escherichia coli*, including O157:H7 and Non-O157 serotypes, and *Salmonella* in commercial beef processing plants. *Journal of Food Protection, 66*(11), 1978–1986.

Began, G., Sudharshan, E., Udaya Sankar, K., & Appu Rao, A. G. (1999). Interaction of curcumin with phosphatidylcholine: a spectrofluorometric study. *Journal of Agricultural and Food Chemistry, 47*, 4992−4997.

Bell, B. P., Goldoft, M., Griffin, P. M., Davis, M. A., Gordon, D. C., Tarr, P. I., . . . Wells, J. G. (1994). A multistate outbreak of *Escherichia coli* O157: H7—associated bloody diarrhea and hemolytic uremic syndrome from hamburgers: the washington experience. *JAMA, 272*(17), 1349-1353.

Berry, E. D., & Cutter, C. N. (2000). Effects of acid adaptation of *Escherichia coli* O157:H7 on efficacy of acetic acid spray washes to decontaminate beef carcass tissue. *Applied Environmental Microbiology, 66*(4), 1493–1498.

Borsari, M., Ferrari, E., Grandi, R., & Saladini, M. (2002). Curcuminoids as potential new iron-chelating agents: spectroscopic, polarographic and potentiometric study on their Fe (III) complexing ability. *Inorganica Chimica Acta, 328*(1), 61-68.

Bosilevac, J. M., Nou, X., Osborn, M. S., Allen, D. M., & Koohmaraie, M. (2004).

Development and evaluation of an on-line hide decontamination procedure for use in a commercial beef processing plant. *Journal of Food Protection, 68*(2), 265–272.

Brown, D. M., Michels, M., Kaiser, P. K., Heier, J. S., Sy, J. P., Ianchulev, T., & Group, A. S. (2009). Ranibizumab versus verteporfin photodynamic therapy for neovascular age-related macular degeneration: Two-year results of the ANCHOR study. *Ophthalmology, 116*(1), 57-65 e55. doi:10.1016/j.ophtha.2008.10.018

Bruzell, E. M., Morisbak, E., & Tonnesen, H. H. (2005). Studies on curcumin and curcuminoids. XXIX. Photoinduced cytotoxicity of curcumin in selected aqueous preparations. *Photochemical & Photobiological Sciences, 4*(7), 523-530. doi:10.1039/b503397g

Böcking, T., Barrow, K. D., Netting, A. G., Chilcott, T. C., Coster, H. G. L., & Höfer, M. (2000). Effects of singlet oxygen on membrane sterols in the yeast *Saccharomyces cerevisiae*. *European Journal of Biochemistry, 267*, 1607-1618.

Carvalho, D. d. M., Takeuchi, K. P., Geraldine, R. M., Moura, C. J. d., & Torres, M. C. L. (2015). Production, solubility and antioxidant activity of curcumin nanosuspension. *Food Science and Technology (Campinas), 35*(1), 115-119.

Castano, A. P., Demidova, T. N., & Hamblin, M. R. (2004). Mechanisms in photodynamic therapy: part one—photosensitizers, photochemistry and cellular localization. *Photodiagnosis and Photodynamic Therapy, 1*(4), 279-293. doi:10.1016/s1572-1000(05)00007-4

CDC. (1993). Update: multistate outbreak of Escherichia coli O157: H7 infections from hamburgers--western United States, 1992-1993. *MMWR. Morbidity and mortality weekly report, 42*(14), 258.

CDC. (2016, July 15, 2016). Burden of Foodborne Illness: Findings. Retrieved from https://www.cdc.gov/foodborneburden/2011-foodborne-estimates.html

CDC. (2017, June 29, 2017). *Listeria* (Listeriosis). Retrieved from https://www.cdc.gov/listeria/index.html

Cheng, A.-L., Hsu, C.-H., Lin, J.-K., Hsu, M.-M., Ho, Y.-F., Shen, T.-S., . . . Ming-Shiang, W. (2001). Phase I clinical trial of curcumin, a chemopreventive agent, in patients with high-risk or pre-malignant lesions. *Anticancer Research, 21*(4B), 2895-2900.

Chignell, C. F., Bilskj, P., Reszka, K. J., Motten, A. G., Sik, R. H., & Dahl, T. A. (1994). Spectral and photochemical properties of curcumin. *Photochemistry and Photobiology, 59*(3), 295-302.

Cutter, C. N., & Siragusa, G. R. (1994). Efficacy of organic acids against *Escherichia coli* O157:H7 attached to beef carcass tissue using a pilot scale model carcass washer. *Journal of Food Protection, 57*(2), 97-103

Cutter, C. N., & Siragusa, G. R. (1996). Reduction of *Brochothrix thermosphacta* on beef surfaces following immobilization of nisin in calcium alginate gels. *Letters in Applied Microbiology, 23*, 9-12.

Dai, T., Huang, Y. Y., & Hamblin, M. R. (2009). Photodynamic therapy for localized infections--state of the art. *Photodiagnosis and Photodynamic Therapy, 6*(3-4), 170-188. doi:10.1016/j.pdpdt.2009.10.008

Demidova, T. N., & Hamblin, M. R. (2005). Effect of cell-photosensitizer binding and cell density on microbial photoinactivation. *Antimicrobial Agents & Chemotherapy, 49*(6), 2329-2335. doi:10.1128/AAC.49.6.2329-2335.2005

Dickson, J. S., & Anderson, M. E. (1992). Microbiological decontamination of food animal carcasses by washing and sanitizing systems: A Review. *Journal of Food Protection, 55*(2), 133-140.

Diez-Gonzalez, F., Callaway, T. R., Kizoulis, M. G., & Russell, J. B. (1998). Grain feeding and the dessemination of acid-resistant *Escherichia coli* from cattle. *Science, 281*(5383), 1666-1668.

Dolmans, D. E. J. G. J., Fukumura, D., & Jain, R. K. (2003). Photodynamic therapy for cancer. *Nature Reviews Cancer, 3*(5), 380-387. doi:10.1038/nrc1070

Dorsa, W. J., Cutter, C. N., & Siragusa, G. R. (1998). Bacterial profile of ground beef made from carcass tissue experimentally contaminated with pathogenic and spoilage bacteria before being washed with hot water, alkaline solution, or organic acid and then stored at 4 or 12°C. *Journal of Food Protection, 61*(9), 1109-1118.

Dougherty, T. J., Gomer, C. J., Henderson, B. W., Jori, G., Kessel, D., Korbelik, M., . . . Peng, Q. (1998). Photodynamic therapy. *JNCI: Journal of the National Cancer Institute, 90*(12), 889-905.

Dovigo, L. N., Pavarina, A. C., Ribeiro, A. P., Brunetti, I. L., Costa, C. A., Jacomassi, D. P., . . . Kurachi, C. (2011). Investigation of the photodynamic effects of curcumin against *Candida albicans*. *Photochemistry and Photobiology, 87*(4), 895-903. doi:10.1111/j.1751-1097.2011.00937.x

Drosinos, E. H., Mataragas, M., Kampani, A., Kritikos, D., & Metaxopoulos, I. (2006). Inhibitory effect of organic acid salts on spoilage flora in culture medium and cured cooked meat products under commercial manufacturing conditions. *Meat Science, 73*(1), 75-81. doi:10.1016/j.meatsci.2005.11.003

DuPont , H. L., Formal , S. B., Hornick , R. B., Snyder , M. J., Libonati , J. P., Sheahan , D. G., . . . Kalas , J. P. (1971). Pathogenesis of *Escherichia coli* Diarrhea. *New England Journal of Medicine, 285*(1), 1-9. doi:10.1056/nejm197107012850101

Dykes, G. A., & Moorhead, S. M. (2000). Survival of osmotic and acid stress by *Listeria monocytogenes* strains of clinical or meat origin. *International Journal of Food*

Microbiology, 56, 161–166.

Elmali, M., Can, H. Y., & Yaman, H. (2015). Prevalence of *Listeria monocytogenes* in poultry meat. *Food Science and Technology (Campinas), 35*(4), 672-675. doi:10.1590/1678-457x.6808

Ergaieg, K., Chevanne, M., Cillard, J., & Seux, R. (2008). Involvement of both Type I and Type II mechanisms in Gram-positive and Gram-negative bacteria photosensitization by a meso-substituted cationic porphyrin. *Solar Energy, 82*(12), 1107-1117. doi:10.1016/j.solener.2008.05.008

Farber, J. M., & Peterkin, P. I. (1991). *Listeria monocytogenes*, a food-borne pathogen. *Microbiology and Molecular Biology Reviews, 55*(3), 476-511.

FDA. (2016). 2016 Food Safety Survey Report. Retrieved from https://www.fda.gov/food/foodscienceresearch/consumerbehaviorresearch/ucm529431.htm

George, S., & Kishen, A. (2008). Influence of photosensitizer solvent on the mechanisms of photoactivated killing of *Enterococcus faecalis*. *Photochemistry and Photobiology, 84*, 734–740.

Glass, K. A., & Doyle, M. P. (1989). Fate of *Listeria monocytogenes* in processed meat products during refrigerated storage. *Applied and Environment Microbiology, 55*(6), 1565-1569.

González-Fandos, E., & Herrera, B. (2013). Efficacy of propionic acid against *Listeria monocytogenes* attached to poultry skin during refrigerated storage. *Food Control, 34*(2), 601-606. doi:10.1016/j.foodcont.2013.05.034

Gray, M. L., & Killinger, A. H. (1966). *Listeria monocytogenes* and listeric infections. *Bacteriological Reviews, 30*(2), 309-382.

Griesser, M., Pistis, V., Suzuki, T., Tejera, N., Pratt, D. A., & Schneider, C. (2011). Autoxidative and cyclooxygenase-2 catalyzed transformation of the dietary chemopreventive agent curcumin. *Journal of Biological Chemistry, 286*(2), 1114-1124. doi:10.1074/jbc.M110.178806

Haeubl, M., Reith, L. M., Gruber, B., Karner, U., Muller, N., Knor, G., & Schoefberger, W. (2009). DNA interactions and photocatalytic strand cleavage by artificial nucleases based on water-soluble gold(III) porphyrins. *Journal of Biological Inorganic Chemistry, 14*(7), 1037-1052. doi:10.1007/s00775-009-0547-z

Halili, F., Arboleda, A., Durkee, H., Taneja, M., Miller, D., Alawa, K. A., . . . Parel, J. M. (2016). Rose bengal- and riboflavin-mediated photodynamic therapy to inhibit methicillin-resistant *Staphylococcus aureus* keratitis isolates. *American Journal of Ophthalmology, 166*, 194-202. doi:10.1016/j.ajo.2016.03.014

Hardin, M. D., Acuff, G. R., Lucia, L. M., Oman, J. S., & Savell, J. W. (1995). Comparison of methods for decontamination from beef carcass surfaces. *Journal of Food*

Protection, 58(4), 368-374. doi:10.4315/0362-028x-58.4.368

Harris, K., Miller, M. F., Loneragan, G. H., & Brashears, M. M. (2006). Validation of the use of organic acids and acidified sodium chlorite to reduce *Escherichia coli* O157 and *Salmonella Typhimurium* in beef trim and ground beef in a simulated processing environment. *Journal of Food Protection, 69*(8), 1802–1807.

Haukvik, T., Bruzell, E., Kristensen, S., & Tønnesen, H. (2009). Photokilling of bacteria by curcumin in different aqueous preparations. Studies on curcumin and curcuminoids XXXVII. *Die Pharmazie-An International Journal of Pharmaceutical Sciences, 64*(10), 666-673.

Haukvik, T., Bruzell, E., Kristensen, S., & Tønnesen, H. (2010). Photokilling of bacteria by curcumin in selected polyethylene glycol 400 (PEG 400) preparations. Studies on curcumin and curcuminoids, XLI. *Die Pharmazie-An International Journal of Pharmaceutical Sciences, 65*(8), 600-606.

Hegge, A. B., Bruzell, E., Kristensen, S., & Tonnesen, H. H. (2012). Photoinactivation of *Staphylococcus epidermidis* biofilms and suspensions by the hydrophobic photosensitizer curcumin--effect of selected nanocarrier: studies on curcumin and curcuminoides XLVII. *European Journal of Pharmaceutical Sciences, 47*(1), 65-74. doi:10.1016/j.ejps.2012.05.002

Hegge, A. B., Nielsen, T. T., Larsen, K. L., Bruzell, E., & Tonnesen, H. H. (2012). Impact of curcumin supersaturation in antibacterial photodynamic therapy--effect of cyclodextrin type and amount: studies on curcumin and curcuminoides XLV. *Journal of Pharmaceutical Sciences, 101*(4), 1524-1537. doi:10.1002/jps.23046

Hoffmann, S. A., & Anekwe, T. D. (2013). *Making sense of recent cost-of-foodborne-illness estimates*: United States Department of Agriculture, Economic Research Service.

Hollebeeck, S., Raas, T., Piront, N., Schneider, Y. J., Toussaint, O., Larondelle, Y., & During, A. (2011). Dimethyl sulfoxide (DMSO) attenuates the inflammatory response in the in vitro intestinal Caco-2 cell model. *Toxicology Letters, 206*(3), 268-275. doi:10.1016/j.toxlet.2011.08.010

Huang, M.-T., Liu, Y., Xie, J.-G., Newmark, H. H., & Ho, C.-T. (2003). Protective effect of dibenzoylmethane on chemically-and uv light-induced skin, inflammation, sunburn lesions, and skin carcinogenesis in mice. In: ACS Publications.

Johnson, R. (2015). U.S.-EU poultry dispute on the use of pathogen reduction treatments (PRTs). *Congressional Research Service Report*.

Khalil, O. A. K., de Faria Oliveira, O. M. M., Vellosa, J. C. R., de Quadros, A. U., Dalposso, L. M., Karam, T. K., . . . Khalil, N. M. (2012). Curcumin antifungal and antioxidant activities are increased in the presence of ascorbic acid. *Food*

Chemistry, 133(3), 1001-1005. doi:10.1016/j.foodchem.2012.02.009

Khopde, S. M., Priyadarsini, K. I., Palit, D. K., & Mukherjee, T. (2000). Effect of solvent on the excited-state photophysical properties of curcumin. *Photochemistry and Photobiology, 72*(5). doi:10.1562/0031-8655(2000)072<0625:eosote>2.0.co;2

Kim, K. J., Yu, H. H., Cha, J. D., Seo, S. J., Choi, N. Y., & You, Y. O. (2005). Antibacterial activity of *Curcuma longa* L. against methicillin-resistant *Staphylococcus aureus*. *Phytotherapy Research, 19*(7), 599-604. doi:10.1002/ptr.1660

Kreitner, M., Wagner, K.-H., Alth, G., Ebermann, R., Foiβy, H., & Elmadfa, I. (2001). Haematoporphyrin- and sodium chlorophyllin-induced phototoxicity towards bacteria and yeasts - a new approach for safe foods. *Food Control, 12*, 529-533.

Kurd, S. K., Smith, N., VanVoorhees, A., Troxel, A. B., Badmaev, V., Seykora, J. T., & Gelfand, J. M. (2008). Oral curcumin in the treatment of moderate to severe psoriasis vulgaris: A prospective clinical trial. *Journal of the American Academy of Dermatology, 58*(4), 625-631. doi:10.1016/j.jaad.2007.12.035

Laury, A. M., Alvarado, M. V., Nace, G., Alvarado, C. Z., Brooks, J. C., Echeverry, A., & Brashears, M. M. (2009). Validation of a lactic acid– and citric acid–based antimicrobial product for the reduction of *Escherichia coli* O157:H7 and *Salmonella* on beef tips and whole chicken carcasses. *Journal of Food Protection, 72*(10), 2208–2211.

Lee, W.-H., Loo, C.-Y., Bebawy, M., Luk, F., Mason, R. S., & Rohanizadeh, R. (2013). Curcumin and its derivatives: their application in neuropharmacology and neuroscience in the 21st century. *Current Neuropharmacology, 11*(4), 338-378.

Lim, J. Y., Yoon, J. W., & Hovde, C. J. (2010). A brief overview of *Escherichia coli* O157:H7 and its plasmid O157. *Journal of Microbiology and Biotechnology, 20*(1), 5–14.

Linnan, M. J., Mascola, L., Lou, X. D., Goulet, V., May, S., Salminen, C., . . . Broome, C. V. (1988). Epidemic listeriosis associated with Mexican-style cheese. *New England Journal of Medicine, 319*(13), 823-828.

Liu, D., Lawrence, M. L., Ainsworth, A. J., & Austin, F. W. (2005). Comparative assessment of acid, alkali and salt tolerance in *Listeria monocytogenes* virulent and avirulent strains. *FEMS Microbiology Letters, 243*(2), 373-378. doi:10.1016/j.femsle.2004.12.025

Markov, P. (1984). Light-induced tautomerism of β-dicarbonyl compounds. *Chemical Society Reviews, 13*(1), 69-96.

Mataragas, M., Skandamis, P. N., & Drosinos, E. H. (2008). Risk profiles of pork and poultry meat and risk ratings of various pathogen/product combinations.

International Journal of Food Microbiology, 126(1-2), 1-12. doi:10.1016/j.ijfoodmicro.2008.05.014

McGee, Z. A., Gorby, G. L., Wyrick, P. B., Hodinka, R., & Hoffman, L. H. (1988). Parasite-directed endocytosis. *Reviews of Infectious Diseases, 10*(2), S311-S316.

Michaeli, A., & Feitelson, J. (1994). Reactivity of singlet oxygen toward amino acids and peptides. *Photochemistry and Photobiology, 59*(3), 284-289.

Minnock, A., Vernon, D. I., Schofield, J., Griffiths, J., Parish, J. H., & Brown, S. B. (2000). Mechanism of uptake of a cationic water-soluble pyridinium zinc phthalocyanine across the outer membrane of *Escherichia coli*. *Antimicrobial Agents and Chemotherapy, 44*(3), 522-527.

Moan, J., & Berg, K. (1991). The photodegradation of porphyrins in cells can be used to estimate the lifetime of singlet oxygen. *Photochemistry and Photobiology, 53*(4), 549-553.

Monton, C., Charoenchai, L., Suksaeree, J., & Sueree, L. (2016). Quantitation of curcuminoid contents, dissolution profile, and volatile oil content of turmeric capsules produced at some secondary government hospitals. *Journal of Food and Drug Analysis, 24*(3), 493-499.

Morey, A., Bowers, J. W., Bauermeister, L. J., Singh, M., Huang, T. S., & McKee, S. R. (2014). Effect of salts of organic acids on *Listeria monocytogenes*, shelf life, meat quality, and consumer acceptability of beef frankfurters. *Journal of Food Science, 79*(1), M54-60. doi:10.1111/1750-3841.12220

Nardo, L., Paderno, R., Andreoni, A., Másson, M., Haukvik, T., & TØnnesen, H. H. (2008). Role of H-bond formation in the photoreactivity of curcumin. *Spectroscopy, 22*(2-3), 187-198.

Negi, P., Jayaprakasha, G., Jagan Mohan Rao, L., & Sakariah, K. (1999). Antibacterial activity of turmeric oil: a byproduct from curcumin manufacture. *Journal of Agricultural and Food Chemistry, 47*(10), 4297-4300.

Niamsa, N., & Sittiwet, C. (2009). Antimicrobial activity of *Curcuma longa* aqueous extract. *Journal of Pharmacology and Toxicology, 4*(4), 173-177.

Nikolov, G. S., & Markov, P. (1981). Photochemical hydrogen abstraction as a radiationless transition in the photoketonization of β-dicarbonyl compounds. *Journal of Photochemistry, 16*(2), 93-104.

Nitzan, Y., Gutterman, M., Malik, Z., & Ehrenberg, B. (1992). Inactivation of gram‐negative bacteria by photosensitized porphyrins. *Photochemistry and Photobiology, 55*(1), 89-96.

Northcutt, J. K., Smith, D. P., Musgrove, M. T., Ingram, K. D., & Hinton, A. (2005). Microbiological impact of spray washing broiler carcasses using different

chlorine concentrations and water temperatures. *Poultry Science, 84*, 1648–1652.

O'Driscoll, B., Gahan, C. G. M., & Hill, C. (1996). Adaptive acid tolerance response in *Listeria monocytogenes*: isolation of an acid-tolerant mutant which demonstrates increased virulence. *Applied and Environmental Microbiology, 62*(5), 1693–1698.

Oetari, S., Sudibyo, M., Commandeur, J. N., Samhoedi, R., & Vermeulen, N. P. (1996). Effects of curcumin on cytochrome P450 and glutathione S-transferase activities in rat liver. *Biochemical Pharmacology, 51*(1), 39-45.

Painter, J. A., Hoekstra, R. M., Ayers, T., Tauxe, R. V., Braden, C. R., Angulo, F. J., & Griffin, P. M. (2013). Attribution of foodborne illnesses, hospitalizations, and deaths to food commodities by using outbreak data, United States, 1998–2008. *Emerging Infectious Diseases, 19*(3), 407.

Paschoal, M. A., Lin, M., Santos-Pinto, L., & Duarte, S. (2015). Photodynamic antimicrobial chemotherapy on *Streptococcus mutans* using curcumin and toluidine blue activated by a novel LED device. *Lasers in Medical Science, 30*(2), 885-890. doi:10.1007/s10103-013-1492-1

Paschoal, M. A., Tonon, C. C., Spolidorio, D. M., Bagnato, V. S., Giusti, J. S., & Santos-Pinto, L. (2013). Photodynamic potential of curcumin and blue LED against *Streptococcus mutans* in a planktonic culture. *Photodiagnosis and Photodynamic Therapy, 10*(3), 313-319. doi:10.1016/j.pdpdt.2013.02.002

Patchett, R. A., Kelly, A. F., & Kroll, R. G. (1992). Effect of sodium chloride on the intracellular solute pools of *Listeria monocytogenes*. *Applied and Environment Microbiology, 58*(12), 3959-3963.

Patra, D., & Barakat, C. (2011). Synchronous fluorescence spectroscopic study of solvatochromic curcumin dye. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy, 79*(5), 1034-1041.

Pereira, M. (1983). Carcinogenicity of chlorination by-products: Trihalomethanes. *Water Chlorination: Environmental Impact and Health Effects, 4*.

Pileggi, G., Wataha, J. C., Girard, M., Grad, I., Schrenzel, J., Lange, N., & Bouillaguet, S. (2013). Blue light-mediated inactivation of *Enterococcus faecalis* in vitro. *Photodiagnosis and Photodynamic Therapy, 10*(2), 134-140. doi:10.1016/j.pdpdt.2012.11.002

Priyadarsini, K. I. (2009). Photophysics, photochemistry and photobiology of curcumin: Studies from organic solutions, bio-mimetics and living cells. *Journal of Photochemistry and Photobiology C: Photochemistry Reviews, 10*(2), 81-95. doi:10.1016/j.jphotochemrev.2009.05.001

Radeglia, R., & Arrieta, A. (1998). 1H-und 13C-NMR-Untersuchungen an ausgewählten Vinylanaloga des Dibenzoylmethans (Curcuminanaloga).

Pharmazie, 53(1), 28-32.

Rangel, J. M., Sparling, P. H., Crowe, C., Griffin, P. M., & Swerdlow, D. L. (2005). Epidemiology of *Escherichia coli* O157:H7 Outbreaks, United States, 1982–2002. *Emerging Infectious Diseases, 11*(4), 603-609.

Ransom, J. R., Belk, K. E., Sofos, J. N., Scanga, J. A., & Smith, G. C. (2003). Comparison of intervention technologies for reducing *Escherichia coli* O157:H7 On fresh beef carcass adipose tissue. *Journal of Food Protection, 23*(1), 23-34.

Rao, J. V., & Rao, M. (2011). Increased solubility and stability of curcumin in lactic acid. *International Journal of Pharma and Bio Sciences, 1*(1), 50-53.

Ribeiro, A. P., Pavarina, A. C., Dovigo, L. N., Brunetti, I. L., Bagnato, V. S., Vergani, C. E., & Costa, C. A. (2013). Phototoxic effect of curcumin on methicillin-resistant *Staphylococcus aureus* and L929 fibroblasts. *Lasers in Medical Science, 28*(2), 391-398. doi:10.1007/s10103-012-1064-9

Richardson, S. D. (2003). Disinfection by-products and other emerging contaminants in drinking water. *European Journal of Cancer, 22*(10), 666-684. doi:10.1016/j.ejca.2003.09.021

Rowe, R. C., Sheskey, P. J., & Weller, P. J. (2006). *Handbook of pharmaceutical excipients* (Vol. 6): Pharmaceutical press London.

Salo, R., & Cliver, D. (1978). Inactivation of enteroviruses by ascorbic acid and sodium bisulfite. *Applied and Environmental Microbiology, 36*(1), 68-75.

Samelis, J., Sofos, J. N., Kendall, P. A., & Smith, G. C. (2002). Effect of acid adaptation on survival of *Escherichia coli* O157:H7 in meat decontamination washing fluids and potential effects of organic acid interventions on the microbial ecology of the meat plant environment. *Journal of Food Protection, 65*(1), 33-40.

Schneider, C., Gordon, O. N., Edwards, R. L., & Luis, P. B. (2015). Degradation of curcumin: from mechanism to biological implications. *Journal of Agricultural and Food Chemistry, 63*(35), 7606-7614. doi:10.1021/acs.jafc.5b00244

Sharma, O. P. (1976). Antioxidant activity of curcumin and related compounds. *Biochemical Pharmacology, 25*, 1811-1812.

Shen, L., & Ji, H. F. (2007). Theoretical study on physicochemical properties of curcumin. *Spectrochimica Acta. Part A: Molecular and Biomolecular Spectroscopy, 67*(3-4), 619-623. doi:10.1016/j.saa.2006.08.018

Shlar, I., Droby, S., & Rodov, V. (2017). Modes of antibacterial action of curcumin under dark and light conditions: A toxicoproteomics approach. *Journal of Proteomics, 160*, 8-20. doi:10.1016/j.jprot.2017.03.008

Sohaib, M., Anjum, F. M., Arshad, M. S., & Rahman, U. U. (2016). Postharvest intervention technologies for safety enhancement of meat and meat based

products; a critical review. *Journal of Food Science and Technology, 53*(1), 19-30. doi:10.1007/s13197-015-1985-y

Srimal, R. C., & Dhawan, B. N. (1973). Pharmacology of diferuloyl methane (curcumin), a non-steroidal anti-inflammatory agent. *Journal of Pharmacy and Pharmacology, 25*, 447-452.

Stankovic, I. (2004). Curcumin: Chemical and Technical Assessment (CTA). *JECFA, Rome, 8*.

Tajkarimi, M., & Ibrahim, S. A. (2011). Antimicrobial activity of ascorbic acid alone or in combination with lactic acid on *Escherichia coli* O157:H7 in laboratory medium and carrot juice. *Food Control, 22*(6), 801-804. doi:10.1016/j.foodcont.2010.11.030

Tomren, M., Masson, M., Loftsson, T., & Tønnesen, H. H. (2007). Studies on curcumin and curcuminoids: XXXI. Symmetric and asymmetric curcuminoids: stability, activity and complexation with cyclodextrin. *International Journal of Pharmaceutics, 338*(1), 27-34.

Tønnesen, H. H., & Karlsen, J. (1985). Studies on curcumin and curcuminoids. *Zeitschrift für Lebensmittel-Untersuchung und Forschung, 180*(5), 402-404.

Tønnesen, H. H., Másson, M., & Loftsson, T. (2002). Studies of curcumin and curcuminoids. XXVII. Cyclodextrin complexation: solubility, chemical and photochemical stability. *International Journal of Pharmaceutics, 244*(1-2), 127-135. doi:10.1016/s0378-5173(02)00323-x

Tortik, N., Spaeth, A., & Plaetzer, K. (2014). Photodynamic decontamination of foodstuff from *Staphylococcus aureus* based on novel formulations of curcumin. *Photochemical & Photobiological Sciences, 13*(10), 1402-1409. doi:10.1039/c4pp00123k

USDA. (1996). Pathogen Reduction; Hazard Analysis and Critical Control Point (HACCP) Systems; Final Rule. *Federal Register, 61*(144), 38805-38989.

USDA. (2017a, July 27, 2017). Livestock & Meat Domestic Data. Retrieved from https://www.ers.usda.gov/data-products/livestock-meat-domestic-data/livestock-meat-domestic-data/#Total red meat and poultry

USDA. (2017b, April 17, 2017). Summary of Recall Cases in Calendar Year. Retrieved from https://www.fsis.usda.gov/wps/portal/fsis/topics/recalls-and-public-health-alerts/recall-summaries

Valduga, G., Bertoloni, G., Reddi, E., & Jori, G. (1993). Effect of extracellularly generated singlet oxygen on Gram-positive and Gram-negative bacteria. *Journal of Photochemistry and Photobiology B: Biology, 21*(1), 81-86.

Vasseur, C., Rigaud, N., Hébraud, M., & Labadie, J. (2001). Combined effects of NaCl,

NaOH, and biocides (monolaurin or lauric acid) on inactivation of *Listeria monocytogenes* and *Pseudomonas* spp. *Journal of Food Protection, 64*(9), 1442-1445. doi:10.4315/0362-028x-64.9.1442

Vázquez-Boland, J. A., Kuhn, M., Berche, P., Chakraborty, T., Dominguez-Bernal, G., Goebel, W., . . . Kreft, J. (2001). *Listeria* pathogenesis and molecular virulence determinants. *Clinical Microbiology Reviews, 14*(3), 584-640. doi:10.1128/CMR.14.3.584-640.2001

Wainwright, M., Phoenix, D. A., Laycock, S. L., Wareing, D. R. A., & Wright, P. A. (1998). Photobactericidal activity of phenothiazinium dyes against methicillin-resistant strains of *Staphylococcus aureus*. *FEMS Microbiology Letters, 160*, 177-181.

Wakte, P., Sachin, B., Patil, A., Mohato, D., Band, T., & Shinde, D. (2011). Optimization of microwave, ultra-sonic and supercritical carbon dioxide assisted extraction techniques for curcumin from *Curcuma longa*. *Separation and Purification Technology, 79*(1), 50-55.

Wang, Y. J., Pan, M. H., Cheng, A. L., Lin, L. I., Ho, Y. S., Hsieh, C. Y., & Lin, J. K. (1997). Stability of curcumin in buffer solutions and characterization of its degradation products. *Journal of Pharmaceutical and Biomedical Analysis, 15*(12), 1867-1876.

Weis, J., & Seeliger, H. P. R. (1975). Incidence of *Listeria monocytogenes* in Nature. *Applied Microbiology, 30*(1), 29-32.

WHO. (2015). *WHO estimates of the global burden of foodborne diseases: foodborne disease burden epidemiology reference group 2007-2015*: World Health Organization.

Winter, S., Tortik, N., Kubin, A., Krammer, B., & Plaetzer, K. (2013). Back to the roots: photodynamic inactivation of bacteria based on water-soluble curcumin bound to polyvinylpyrrolidone as a photosensitizer. *Photochemical & Photobiological Sciences, 12*(10), 1795-1802. doi:10.1039/c3pp50095k

Wright, J. S. (2002). Predicting the antioxidant activity of curcumin and curcuminoids. *Journal of Molecular Structure: THEOCHEM, 591*(1), 207-217.

Wu, J., Hou, W., Cao, B., Zuo, T., Xue, C., Leung, A. W., . . . Tang, Q. J. (2015). Virucidal efficacy of treatment with photodynamically activated curcumin on murine norovirus bio-accumulated in oysters. *Photodiagnosis and Photodynamic Therapy, 12*(3), 385-392. doi:10.1016/j.pdpdt.2015.06.005

Wu, J., Mou, H., Xue, C., Leung, A. W., Xu, C., & Tang, Q. J. (2016). Photodynamic effect of curcumin on *Vibrio parahaemolyticus*. *Photodiagnosis and Photodynamic Therapy, 15*, 34-39. doi:10.1016/j.pdpdt.2016.05.004

Xing, B., Jiang, T., Bi, W., Yang, Y., Li, L., Ma, M., . . . Yeow, E. K. (2011). Multifunctional divalent vancomycin: the fluorescent imaging and photodynamic

antimicrobial properties for drug resistant bacteria. *Chemical Communications (Cambridge, England), 47*(5), 1601-1603. doi:10.1039/c0cc04434b

Zsila, F., Bikádi, Z., & Simonyi, M. (2003). Molecular basis of the Cotton effects induced by the binding of curcumin to human serum albumin. *Tetrahedron: Asymmetry, 14*(16), 2433-2444. doi:10.1016/s0957-4166(03)00486-5

Zuurmond, W., Langendijk, P., Bezemer, P., Brink, H., Lange, J. d., & Loenen, A. v. (1996). Treatment of acute reflex sympathetic dystrophy with DMSO 50% in a fatty cream. *Acta Anaesthesiologica Scandinavica, 40*(3), 364-367.