EFFECTS OF PULSED LIGHT ON MICROBIAL DECONTAMINATION OF DRY FOOD POWDER AND FOOD PROCESSING AIDS

By

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To my family and friends
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TAC  Total Aerobic Count
TSA  Tryptic Soy Agar
USDA United States Department of Agriculture
UV   Ultraviolet
Abstract of Thesis Presented to the Graduate School of the University of Florida in Partial Fulfillment of the Requirements for the Degree of Master of Science

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Chair: Wade Yang
Major: Food Science and Human Nutrition

Food powders provide convenience and nutritional value for consumers while also providing manufacturers a manageable food form with a long shelf life and easy transportation. Food powders, however, carry food safety risks because of their high nutritional value, which has led to food outbreaks in the past. Due to the high nutritional and aroma requirements of food powders, thermal decontamination methods are not suitable. Furthermore, it is not easy to remove the chemical residue because of the special physical properties of dry food powder. Pulsed light (PL) technology effectively inactivate the spoilage microorganisms while maintaining the quality of the products. In the present study, the efficacy of PL inactivation on *Bacillus subtilis* and investigated the molds and yeast and total aerobic counts (TAC) has been evaluated on wheat flour and black pepper. Wheat flour and black pepper were inoculated with *B. subtilis* and treated with PL (3 pulses/s) at distances of 6 cm and 9 cm from the quartz window of the PL system. Wheat flour samples were treated for 15 s, 30 s, 60 s and 90 s. For black pepper, reduced times were viable and therefore the times were 5 s, 10 s, 15 s and 30 s. Natural flora were used in the molds and yeast counts and TAC. All the trials were conducted at the room temperatures (25 °C), and the temperature was monitored during
PL treatment. The quality parameters assessed were changes in color and water activity. Results reveal significant reduction (p<0.05) in microbial load after PL treatment. Maximum reduction attained in TAC of wheat flour is 2.5 ± 0.8 log CFU/g; while for black pepper, TAC can reach 3.2 ± 0.2 log CFU/g reduction. Furthermore, the maximum inactivation attained for *Bacillus subtilis* is as high as 4.1 ± 0.2 log CFU/g on wheat flour and 4.3 ± 0.1 log CFU/g on black pepper after 90 s and 30 s, respectively, with a distance from quartz window to sample at 6 cm. Likewise, temperature increased significantly (p<0.05) after the treatment, especially on black pepper. Meanwhile, a dramatic decrease in water activity, which enhanced the effects on further storage and decreased the potential microbial contamination. There were, however, no significant differences (p>0.05) observed in color. In conclusion, PL may be an effective and practical method for enhancing the safety of food powder and dry ingredients while preserving the food quality, which would have a positive impact on food safety and public health.
CHAPTER 1
INTRODUCTION

Food Powders

Food powders, such as dried milk powder, protein isolates, seasoning powders, and baking soda, have many benefits for both consumers and manufacturers. For consumers, food powders can provide a conveniently packaged product as well as nutritional value; for manufacturers, they offer an easy and manageable food form with a longer shelf life and can be easily transported. These food powders have been traditionally used in cakes, cookies, doughnuts, and crackers while newer applications involve use of food powders in spring roll wrappers, steamed breads, and instant noodles (Alan 2011).

Nutritional Values

Food powders contain nutrients, such as lactose, collagen, vitamins, and fiber, which are available in a condensed form and can be easily assimilated and absorbed by the gastrointestinal tract (Samonis and others 1990). For instance, powdered infant milk formula provides the main supplementary resource of nutrients (such as docosahexaenoic acid (DHA) and vitamins B1, B2 and B12) for infants and children (Camilla and others 2008). In bread making, wheat flour is commonly used as the major flour and provides essential nutrients, such as carbohydrates, proteins, vitamins, minerals, and fiber (Giannou and others 2003). Moreover, onion powder can provide one of the major sources for flavonoids and is used as a nutraceutical, foods that function both as medical and health foods (Debnath 2002).
Safety Concern

Despite their beneficial characteristics, however, several safety issues are associated with dry food powders. For instance, in the last two years there have been a few outbreaks related to contaminated food powders, particularly red pepper (*Capsicum annuum* L.), black pepper (*Piper nigrum* L.), and dry milk powder (CDC 2007; CDC 2010a; CDC 2010b). In April 2013, the Centers for Disease Control and Prevention (CDC) reported an outbreak related to the bread crust, frozen pasta products and pizza dough that infected 35 persons with *Escherichia coli* O121 (STEC O121) (CDC 2013). As a result, nine people were hospitalized, and two of them developed hemolytic uremic syndrome (HUS). In 2010, the CDC reported that approximately 250 individuals had contracted *Salmonellosis* attributed to meat contaminated by black and red pepper in at least 44 states and the District of Columbia (CDC 2010b). According to the Food and Drug Administration (FDA) the contamination of the meat led to over 15 related recalls (FDA 2010a). Such problems with food powder safety are not new. Goepfert and others (1972) pointed out that the high frequency poisoning in Hungary in the 1970s was due to the consumption of meat seasoned with black pepper and other spices. Based on their investigation, it is clear that spices need to be inactivated before consumption (Goepfert and others 1972).

Moreover, since food powder may be high in nutrients, such as protein and vitamins, it is a great medium for microbial growth, especially in products (such as paprika powder and seasoning) (Staak and others 2007). Cross-contamination is also possible because the processing of food powders in other foods or beverages may result in illness. For example, reconstituted infant foods are susceptible to *E. coli* O157:H7 when factors, such as water activity, pH, and temperature are not controlled.
Cross-contamination can also occur when hygiene is inadequate and can lead to contaminated foods in unexpected places, such as homes or day-care centers (Deng 1998). While low moisture and water activity can extend the shelf-life of dry food powders, most food powders are used as ingredients and will be rehydrated or combined with other ingredients that have high water activity, such as milk powder and spices.

This research focuses on wheat flour and black pepper. Wheat flour is traditionally sold in a pre-packaged form at retail and has a shelf life ranging from 6 to 8 weeks. Wheat flour is derived from wheat, which is one of the major crops produced in the world. In the United States, wheat products are the third most frequently consumed products, just behind soybean and corn production (USDA ERS 2012). Although the wheat industry faces growth challenges due to competition from other subsidized crops and cheaper foreign production, there remains a demand for wheat in the domestic market. In 2010, the United States Department of Agriculture (USDA) reported that U.S. production reached as high as 2,218.06 million bushels (USDA ERS 2012).

Several classifications of wheat exist, depending on the gluten content, protein content, and hardness (Giannou 2003). Regardless, the preparation for wheat flour is the same. As the wheat crop matures and reaches harvest, the kernel is removed from the stalks and the chaff. Next, the wheat kernel is ground to give the wheat flour and then is packaged for sale. The final product has a shelf life of approximately two months, after which it becomes susceptible to insect spoilage (Marathe and others 2002).
Decontamination Method

Several technologies have been employed in order to decontaminate food powders, including wheat flour. Technologies range from traditional heating methods to newer, non-thermal methods. These include the following: gamma radiation (Nagy and others 2002), ultraviolet (UV) radiation (Sharma and others 2003), steam processing (Schneider 1993), and infrared (IR) heating (Hamanaka and others 2000). Pulsed Light methods are the focus of this research.

Pulsed Light Technology

Pulsed light (PL) is an emerging technology and is made up of 54% UV light, 26% infrared light, and 20% visible light from the electromagnetic spectrum (Krishnamurthy 2007). The UV light can be emitted either as a continuous wave (continuous light) or in short duration pulses of 1 – 20 per second as in the case of pulsed light. Continuous UV illumination is a lower energy source, while pulsed light illumination functions at higher energies and is therefore more potent for microbial inactivation. By storing electrical energy in capacitors, that energy is released through arcing an inert gas, such as Xenon or Krypton, which intensifies the energy of the light and causes the light to have up to 20,000 times the energy of the sun ray at sea level (Kaack 2007).

Pulsed light has been proven to be effective in pathogen inactivation because it can provide intense momentary electromagnetic energy at wavelengths of 100 - 1000 nanometers (nm) (Gemma and others 2010). It has been shown that PL not only inactivates vegetative bacteria but also spores, which are hard to diminish (Nicorescu and others 2013; Smelt 1998; Warth 1978). In addition, PL has been used to modify compounds in foods and reduce allergens. For instance, PL was found effective in
reducing food allergens, such as soy (Yang and others 2010), peanut (Chung and others 2008), shrimp (Yang and others 2012), and almonds (Chung 2008).

**Inactivation Mechanisms**

Three mechanisms are responsible for the effect of PL on foods: photochemical, photothermal and photophysical (Robert and others 2000). The photothermal effect is the thermal component of PL and heats the surface of the material due to PL’s high energy. The photophysical effect is the vibration of the molecules within the material or bacterial cell. The photophysical effect can cause the rupture of the bacterial membrane, thereby releasing intra-cellular contents into the external environment. Lastly, there is the photochemical effect. It is suggested that the photochemical effect is the main mechanism of the PL treatment on microbial inactivation. In most microorganisms, such as bacteria, viruses and other pathogens, PL can cause structural changes in deoxyribonucleic acid (DNA). Thymine dimers form within the DNA that can inhibit DNA transcription and replication in the bacterial cell, which can lead to cell death, also known as apoptosis (Miller and others 1999; Wang and others 2005). And similar to UV light, oxygen in the air can generate ozone after PL exposure.

**Advantages and Disadvantages**

With respect to the beneficial advantages observed in other foods, the application of PL can provide a medium to decontaminate food powders, particularly wheat flour. Several associated risks are present in contaminated wheat flour that poses a foodborne danger. PL suggests an effective remedy for wheat flour and black pepper based on its effectiveness. Benefits would include a safer food, a longer shelf life for the product, and lower costs for both consumers and manufacturers, including health costs and processing costs when compared with gamma irradiation. The major disadvantages
of PL are still penetration and overheating after a longer exposure time unlike the conventional chemical methods.
CHAPTER 2
JUSTIFICATION OF STUDY

From a health perspective, contaminated food has resulted in approximately 1,000 reported diseases, 48 million illnesses, 128,000 hospitalizations, and 3,000 deaths annually in the United States (CDC 2010). It is estimated that there are approximately 76 million cases of foodborne illnesses each year in the United States alone, which presents both a major public health threat and economic burden (United States Department of Agriculture (USDA 2010). The pathogen *Salmonella* has resulted in an expenditure of $365 million dollars in direct medical costs annually (USDA 2010). For *E. coli* O157 H7, the societal cost of a single fatal case can be as high as $7 million (Frenzen and others 2005). Similarly, this amount is derived from direct and also non-direct medical costs. Direct medical costs include physician and hospital services, supplies, medications, and procedures required to alleviate the foodborne illness. Indirect medical costs include transportation to health care, relocation expenses, specialized education, and employment compensation.

Despite the potential health risk and economic costs of contamination, the application of decontamination techniques in the industry is a deterrent parameter due to its inconvenience. Initially, the industry adopted chemical methods as the primary method for decontaminate dry food powders, spices, and seeds. These methods used compounds, such as ethylene oxide (EO), ethylene glycol, and ethylene chlorohydrin (ECH) (Wesley 1965). In 1977, according to the American Spice Trade Association (ASTA), more than 80 million pounds (lbs) of spices were treated by EO (Gerhardt and Ladd 1982). The major problems with chemical treatments were uneven treatment and the absorption of residues (Thorén 1995). Thermal methods were also used, but led to
other issues regarding the quality of the food including aroma, flavor, and texture changes that were undesirable (Nazarowec-White 1997).

This study evaluated *B. subtilis* as our target bacteria. Not only has *B. subtilis* has been used as a Gram-positive model for more than a century, but also because *B. subtilis* is one of the commonly encountered spore-forming bacteria in spices (Barbe and others 2009). Based on a study in Nigeria, the natural contamination can reach as high as 8 log CFU/g (Antai 1988). The white and whole flour loaves contain up to $10^6$ CFU/g of *Bacillus* absent any preservation following two days of storage at modest summer temperatures of 25-30°C (Rosenkvist and Hansen 1995). Specifically, 70% of the contamination in the loaves arises from *B. subtilis*. Furthermore, it is reported that *B. subtilis* is a common bacterium in dough and flour products and cause rope spoilage of bread at $10^8$ CFU/g (and only $10^5$-$10^6$ CFU/g is needed for foodborne illness) (Kramer and Gilbert 1989). This indicates a relationship between *B. subtilis* and the consumption of ropy bread (Toddy 1982; Scokett 1991). Although *B. Subtilis* is usually non-pathogenic bacteria at low concentrations, it can cause special toxi-infections including severe vomiting, abdominal cramps and diarrhea at higher concentrations (Nicorescu and others 2013). Therefore, there is a need to control the growth and survival of *B. subtilis* in foods.

Previous studies have shown that PL can be effective in reducing and preventing the proliferation of both spoilage and pathogenic microorganisms (Wang and others 2005). This may play an essential role in decreasing the risk of foodborne illnesses in food powders, such as wheat flour. Consequently, this can bring about reduced
healthcare costs for consumers and provide manufacturers a potential processing method to decontaminate wheat flour.
CHAPTER 3
OBJECTIVES

The overall objective of this study was to evaluate the effectiveness of PL on the microbial decontamination of wheat flour and black pepper.

Specific Objectives: The following specific objectives were assessed in this study:

1. Evaluate the effects of PL treatment on the reduction of total aerobic microorganism counts, mold, and yeast counts in wheat flour and black pepper samples treated at different times and distances to the PL source.

2. Investigate the effects of PL treatment in disinfecting samples inoculated with *Bacillus subtilis*.

3. Measure the resultant color changes and water activity of samples after PL treatment for product quality purpose.

4. Monitor the temperature changes of the samples at different PL treatment times.
CHAPTER 4
REVIEW OF LITERATURE

Food Powders

Food powders, such as wheat flour, dairy, egg, gelatin, vitamins, starch, fruits and vegetables powders, spices, and some food aids like coloring or flavoring agents are commonly used in many food applications (Woo and others 2009). Generally, these food powders are obtained from raw agricultural materials processed by different methods, such as fragmentation, separation, drying, fermentation, milling and stabilization (Cuq and others 2011). In addition, food powders are easy to preserve, store, transport, weigh, and most importantly, they can have a relatively long shelf life without losing their nutritional values (Cuq and others 2011).

It is well known that the major aim of consuming food is to obtain nutrition, which can support the growth, development and productivity of animals (Brody and Lardy 1946). Nutritional value is one of the most important determinants and criteria in food and provides consumers an estimate as to whether the food can satisfy the customer’s nutritional needs. The higher the nutritional content of the food, the better it is. However, while nutritional food can provide growth, development, and productivity to animals, it also provides microorganisms a perfect media to grow at the same time (Bjornson 1982). Some powders, such as infant formulas and protein powders are at high risk for microbial contamination.

Moreover, in addition to nutritional value, other factors that enhance the value of the food are convenience and extended shelf life. Food powders provide these benefits by giving an easy, transportable, and convenient product and also by providing a longer shelf life. Food powders also preserve their nutritional value (Gonzales and others
2010). For example, infant formulas are a popular choice for young infants. Infant formulas have many purposes, from providing a balanced, nutritional source that is convenient, to giving a lactose free food for babies that are lactose-intolerant in the form of soy infant formula, and are the source of other nutrients, such as docosahexaenoic acid (DHA) and arachidonic acid (ARA), which are vital for proper visual and cognitive development (FDA 2012).

However, as stated earlier, the downside to all the nutritional gains is that a lot of food powders are vulnerable to microbial growth. In dry food powders, microorganisms can exist mainly as spores, which are not able to germinate when the water activity is low. However, when the seasonings are readjusted to higher moisture foods with a higher water activity, this can trigger the growth of microorganisms due to this rich media (Jaquette and Beuchat 1998). It is also the case that in some food powders, such as spices and seasonings, heating is avoided since this can cause the loss of flavor and aroma, and therefore no heating is applied. Most of the seasonings are delivered without decontamination and therefore the products may yield a high level of microorganisms and spores (Deng and others 1998; Staack and others 2008). For such reasons, decontamination methods are being developed in this area to avoid these shortcomings, and newer, novel methods are needed (Chee 2012).

Problems and Issues

In the last 30 years, there have been a few cases of severe incidents in the U.S., leading to more than 4 deaths from consuming microbial contaminated powder food (Padhye and Doyle 1992; CDC 2007; CDC 2009; CDC 2010a; CDC 2010b). In 2008, an outbreak of *Salmonella* Rissen infections was transmitted though white pepper (CDC 2010b). In 2009, an outbreak occurred in the District of Columbia and 44 states, of
which 272 cases were identified. The cause of the outbreak was black and red pepper contaminated with *Salmonella* Montevideo associated with salami products. In August 2010, 44 individuals were infected with *Salmonella* Chester in California, Georgia, and 16 other states (CDC 2010a). However, for spices, which are known to be harbors for fungi and bacteria, few reports have been recorded connecting spices with human illnesses (Vij and others 2006).

For infant formula, more than 40 infants were infected with *Enterobacter sakazakii* in the last 20 years because of contaminated infant formula (Drudy 2006). Once infants are infected with *Enterobacter sakazakii*, the outcome can be severe. Seizures, brain abscesses, hydrocephalus, developmental delay, and death rate can be as high as 80% and premature infants are at higher risk than mature infants and toddlers (Arseni 1987; Nazarowec-White 1997).

Part of the reason for the outbreaks is due to incomplete sterilization, which is determined by the special properties of the dry food ingredients (FDA 2012a; FDA 2012b). First, the difficulty with sterilization is correlated with the specific microflora present, such as vegetative cells and spores. The microflora can adapt to a low water activity ($a_w$) environment (Fine and Gervais 2005). Second, the heat resistance of the microorganism depends on the surrounding water activity within its environment. Murrell and Scoot (1966) reported that the thermal resistance of spores is more significant in dry media the liquid media. For example, the cells’ heat resistance in *Salmonella* will increase from 25% to 75%, as demonstrated by the D value when $a_w$ decreased (Goepfert and others 1970).
Additionally, companies order recalls whenever a product is found to be harmful or defective. The product is withdrawn from the market to avoid potential risks or to make corrections to the product, if possible (FDA 2010b). In order to protect the interest of most consumers and the reputation for the company, companies will recall their own products once problems are detected. Other times, FDA will raise concerns and the company will have to recall their products (FDA 2010b). From 2004 to 2012, 271 products related to food powder have been recalled, with the most recent recall occurring in May 2012. Over 13 tons of Select Roast Meat Type Flavor was recalled as a result of *Salmonella* contamination (FDA 2012).

**Decontamination Methods**

A variety of chemical, thermal, and non-thermal methods have been applied to dry food powders, including dry heating (Baron 2003), high temperature short time treatments (Faubion and Hoseney 1982), steam processing (Schneider 1993), microwave heating (Gerhardt and others 1985), and infrared (IR) heating (Hamanaka and others 2000).

Food powders that can be affected include natural spices, dry food ingredients, and vegetable powders. Natural spices, such as mace, sage, allspice, nutmeg, paprika, ginger, coriander, cinnamon, and cloves, are all susceptible to microbial contamination with spores of yeast, molds, and bacteria. Rice flour, soybean flour, wheat flour, and corn flour cocoa, all of which are grain powders and are used in comparatively larger amounts, are also at risk. Dry vegetable powders including asparagus powder, garlic powder, and onion powder also are liable to microbial contamination, as are their dry fruit equivalents, such as apples, raspberries, dates, figs, apricots, peaches, prunes, and raisins.

Traditionally, chemical methods were used to decrease the amount of microbes in dry food powders. Chemical methods involved fumigation with ethylene oxide (EO) or
other chemicals, but these chemicals are believed to cause chronic or carcinogenic effects (Farkas 1988). Treatment by heat application was also used as it could reduce the risk of contamination by modifying the DNA and protein profile of microorganisms. However, it would lead to a loss of nutritional quality at the same time, which is not desired. Meanwhile, since the subtle flavor and aroma elements of spices and herbs are sensitive to heat, traditional heat decontamination methods would affect the functional characteristics of the enzymes and the texturing agents found within the food powder. Gradually, non-thermal methods have been favored over thermal methods. Non-thermal methods give the advantage of preserving the quality of the food. In addition, since dry food ingredients have a higher heat resistance than moist food ingredients, it avoids extended thermal treatments (Murrell and Scott 1966). A survey of these methods is provided below and gives an overview of the different decontamination methods used to preserve and ensure safety in dry food powders.

**Chemical Methods**

Chemical methods were first adopted for spices decontamination (Gerhardt and Ladd 1982). Chemical methods have been proved can effectively inactivate microbes for decades. Ethylene Oxide is the method used first in industry which has been prohibited in many countries due to carcinogenicity (Fowles and others 2001).

**Ethylene Oxide and Derivatives**

In the 1980s, treatment with ethylene oxide (EO) was considered the most widely applied method for decontamination of dry ingredients. According to the American Spice Trade Association (ASTA), the U.S. had consumed over 800,000 lbs of EO in 1977 to treat 80,000,000 lbs of spices (Gerhardt and Ladd 1982). Ethylene chlorohydrin (ECH), which has a longer effect than EO, and ethylene bromohydrin (EBH) also were
considered as alternatives in the period during ethylene oxide fumigation (Wesley et al. 1965). EBH is a potential metabolite of EO and ethylene dibromide fumigation. However, both EO and ECH were later discovered to be mutagens and also were highly suspected of inducing other chronic or delayed toxic effects (Ehrenberg and Hussain 1981; Gerhardt and Ladd 1982; Kligerman and others 1983). EO would form toxic 2-hydroxyethyl compounds with lysine and cysteine and up to 30 parts per million (ppm) had been found in dried egg and milk powder (Gerhardt and Ladd 1982). Additionally, it had been found that there was 260 ppm of 2-chloroethanol in EO-sterilized flour and an average of 805 ppm was present in a spice mixture (Wesley and others 1965). In curry powder, 160 ppm of chloroethanol could be tested 182 days after fumigation (Scudamore and Heuser 1971). In wheat, levels of 20 ppm of EO were detected after wheat sterilization. However, according to the report, during the storage and milling procedures, any residues of EO were virtually removed (Pfeilsticker and Rasmussen 1968). In the U.S., the Environmental Protection Agency (EPA) (EPA 1977) had defined the tolerated level of propylene oxide in foodstuffs, such as cocoa and spices to 300 ppm. Thus, in the 1980s, EO fumigation was highly represented as a significant occupational health hazard and regulatory restrictions followed (Gerhardt and Ladd 1982). Subsequently, scientists acknowledged that in many experiments, EO caused harmful effects. It was shown that EO induced tumors in laboratory mice through via oral and inhalation routes formed adducts to proteins and DNA in humans and animals by direct genotoxic mechanisms (IARC 1994).

EO can significantly reduce microbial population, which has been proven by multiple researchers (De Boer and others 1985; Farkas 1985). However, other than
aroma and color changed a lot by using this chemical method, volatile compounds are lost because of the low pressure that is needed for sanitizing agent removing (Farkas and Andrassy 1988). Besides that, mutagen and carcinogen after inhalation is also a huge problem for this method to be more diversely apply (Steenland and others 2003).

**Ozone**

Ozonation, which is considered as an oxidation method, was developed in the 1990s to reduce the toxicity of aflatoxins in dry foods (Samarajeewa and others 1990). Ozone, or O$_3$, is a powerful oxidizing and disinfectant agent (McKenzie and others 1997). It reacts against the 8,9 double bond of the furan ring in aflatoxin via an electrophilic attack and causes the formation of primary ozonides, followed by its rearrangement into monozonide derivatives (Proctor and others 2004). In 2001, O$_3$ received approval in the U.S. for its use as an antimicrobial agent in food decontamination, especially for dried foods (such as pistachios, black pepper, grains, and cereals) (FDA 2001, Naitoh and others 1987, 1988, Zagon and others 1992, Kim and others 1999, Liangji 1999, Khadre and others 2001, Al-Haddad and others 2005, Akbas and Ozdemir 2006). Based on several studies, gaseous O$_3$ treatment methods gave 6 log CFU/g reductions of Micrococcus and *Bacillus* spp. counts in dried foods (Naitoh and others 1987, Naitoh and others 1988; Zhao and Cranston 1995; Khadre and Yousef 2001), which was more effective than hydrogen peroxide. Other studies showed that high ozone concentration (>18 ppm) with adequate exposure time (>8 h) could cause significant changes in food powders and bee pollen and also could decrease the pH value of Korean red ginseng powder (Byun and others 1997, 1998; Yook and others 1997).
Ozone is suitable for advanced oxidation processes (APOs) with appropriate inhibitors results in potential effectiveness on most resistant microorganisms. Meanwhile, ozone is able to apply on both liquid and solid food material with a quick decomposes leaving no residues (Khadre and others 2001). However, based on its strong oxidase ability, ozone removes the color and amour of spices a lot, which make the product less desirable for customers.

**Thermal Methods**

**Infrared heating**

Infrared heating has been applied in the inactivation of microorganisms. Infrared refers to wavelengths of light that lay between microwave and ultraviolet radiation in the electromagnetic spectrum. The wavelength ranges from 0.76 to 1000 μm and interacts with food components due to absorption, reflection, scattering, and transmission of the light (Staack and others 2008). The diffraction of radioactive energy as heat results in surface penetration and temperature depths specific to the products treated, depending on the food product, IR wavelength, water activity (a_w), and the thickness of sample.

Compared with other conventional heating methods, IR light heats the sample directly and therefore the samples will not be influenced by the airflow around the powder. Meanwhile, the treatment times are shorter (Ranjan and others 2002). Staack and others (2008) showed a chart depicting the number change in flora in order to assess the effectiveness of IR heating, which is shown below in Table 4-1.

To summarize briefly, the initial population was measured at 7.48 log10 CFU/g for *B. cereus* spores in the inoculated samples and 4.90 log10 CFU/g for the background flora in the non-inoculated samples.
Staack and others (2008) also concluded that IR could be used to lower the microbial numbers in paprika powder. However, a higher heat flux degraded the product quality on the surface of the powder bed. Using this method could reduce the treatment time significantly. However, the industrial implementation of this process should be designed in order to prevent overheating and over drying. Furthermore, it is possible that sometimes the thermal methods may not work that well because of the heat sensitivity of seasoning powders and herbs. Meanwhile, the higher temperatures influence the quality and sensory attributes of the food product.

**Microwave**

Microwaves are radio waves with wavelengths of 1 meter (m) to 1 millimeter (mm) that have frequencies that span from 300 MHz to 300 GHz (Pozar 2009). Omar and others (1995) used an electric microwave oven with a frequency of 2450 MHz (± 20 MHz) and treated samples of black pepper powder for 20 seconds (s), 40 s, and 75 s. They compared microwave exposure with gamma irradiation to measure the effectiveness of each treatment on microbial inactivation. The comparative results are shown below in Table 4-2.

Based on the results, Omar and others (1995) concluded that gamma rays are more effective than microwave treatments in reducing *Enterobacter* spp. and *Clostridium* spp. The concentration of the microbes decreased significantly after 75 s of microwave treatment whereas for the 40 s treatment, the results were not satisfactory.

Even though, microwave seems to be efficient in treating other samples, however, the efficiency of microwave treatment is based on the movement of polar molecular in food sample, thus water molecular as a polar molecular can influence the efficient of microbial inactivation. And it can be measured by quantified by water activity.
Instead of the explanation expressed by Omar and others (1995), in their study, they explain this phenomenon as oven’s frequency.

**High temperature short time process**

High temperature short time (HTST) treatment is based on a very high temperature (200-600 °C) treatment within a short duration (0.1-30s) followed by an instantaneous cooling procedure. As Fine and Gervais (2005) have reported that HTST can cause less chemical modifications in the product while maintain the same inactivation effect as relatively lower temperature and longer time.

The Fig 4-1 shows the schematic structure of the HTST machine, after the treatment, they reached a 5.2 log CFU/g reduction after 120s treatment, however, the log reduction is already 5 log CFU/g after 60s. And as some scientists mentioned, the decontamination effect is strongly influenced by water activity of the samples, the drier the sample is, the higher the resistance for the thermal treatment (Laroche and Gervais 2003).

In Almela and other’s study (2002), a temperature range of 130 to 170 °C temperature range was chosen, and the treatment time varies from 4 to 6 s respectively, all these combination were investigated in this study. Almela and others observed a 3-4 log CFU/g inactivation rate combined with up to 40% color loss (Almela and others 2002).

Some scientists have pointed out that process used for the decontamination of dried products, such as steam, microwaves, or Joule-effect treatments, can induce organoleptic degradation or achieve a relatively low destruction rate. Meanwhile, majority of the thermal methods can also lead to color modification, loss of essential oil,
flavor change and aroma changes (Almela and others 2002; Schweiggert and others 2005; Calucci and others 2003; Nicorescu and others 2013).

Non-Thermal Methods

Pulsed light

The electromagnetic spectrum is comprised of several wavelengths, including ultraviolet radiation, radio waves, microwaves, infrared radiation, visible light, X-ray and gamma radiation (Diffey 2002). One particular type of light, pulsed light, is an emerging technology and makes up 54% UV light, 26% visible light, and 20% infrared light (Krishnamurthy 2007). The UV wavelength is the largest component of PL and makes up the wavelength from 100 nm to roughly 400 nm, the parameter of UV light is shown in the Table 4-3.

Within UV light, there are further distinctions including UV-A, UV-B, UV-C, and Vacuum UV. Out of these, UV-C is considered to be the most efficient germicide against microbes, such as viruses, bacteria, protozoa, yeast, algae and molds (Bintsis 2000). Furthermore, UV treatment is considered as one of the most efficient methods for decontamination of the surface and has been found to be effective in several products, such as water and fruit juice (Guerrero-Beltrán and Barbosa-Cánovas 2004, 2005).

Moreover, since UV light has a low running cost and uses less energy than thermal pasteurizers and saves space and time, it has been widely applied in many areas. UV light applications will likely become more widely accepted in time, considering that UV is a simple, trustable and low-cost treatment. In addition, UV light is a clean technology that does not produce any residual irradiation, even at a high level exposure (Yaun and others 2003).
PL can be emitted either as a continuous wave or in short duration pulses of 1 – 20 per second. Continuous wave illumination is of a lower energy while pulsed light illumination is at a higher energy and is therefore used for microbial inactivation.

The mechanism for PL is that it releases electrical energy that is multiplied several folds by first storing the energy in a capacitor and then releasing it as a short duration intermittent pulse using a lamp filled with inert gases, such as Xenon or Krypton gas (Krishnamurthy 2006). This can cause the light to have 20,000 times the energy of the sun (Kaack 2007) as it comes into contact with the gas. The energy then is absorbed by the surface of the material, which can create changes within the material. Considerable research has been done to investigate the mechanism of PL technology. The changes in the material can be attributed due to the photochemical, photophysical, and photothermal effects of PL (Robert and others 2000).

The photothermal effect is the thermal component of pulsed light that can heat the surface of the material being treated due to PL’s high energy. It is a kind of heat damage caused to the bacterial cell because of the differences in the heating rates of the bacteria and the surrounding media. This results in a localized heating of the bacterial cell. Next, there is the photochemical effect. It has been suggested that the photochemical effect is the main mechanism of the PL treatment on microbial inactivation. It can cause structural changes in deoxyribonucleic acid (DNA) in most microorganisms, such as bacteria, viruses, and other pathogens. Thymine dimers can form within the DNA that can inhibit DNA transcription and replication in the bacterial cell, which can lead to cell death, also known as apoptosis (Wang and others 2005, Miller and others 1999). Lastly, there is the photophysical effect, which is the vibration of
the molecules within the material or bacterial cell that can cause the rupture of the bacterial membrane, therefore releasing intra-cellular contents into the external environment. However, the fatal action of pulsed light is mainly attributed to the photothermal and the photochemical effects of PL. It is possible that both mechanisms cooperate and the comparative importance of each one would depend on the fluency and target microorganism. However, most of the authors explain their results based on the photochemical effect. In practice, the application of PL on the decontamination of foods has three broad categories: (a) inhibition of microorganisms on the food surface; (b) destruction of microbes in the air; (c) and sterilization of liquids (Bintsis and others 2000).

As early as 1975, Bachman successfully applied UV rays to sterilization of air and water, which ended up giving almost no suspended subjects after treatment. Bachman also used this method for surface decontamination and found great success in this application. Furthermore, he demonstrated excellent potential in application of UV light on packaging materials (Bachman 1975).

UV light also has been applied in water decontamination for many years, since UV application will not influence color, pH, odor, or flavor (Bintsis and others 2000). As a result of these special properties of UV light, Wright and others (2000) decided to apply this technology into decontamination of apple cider. The results from the experiment showed a reduction of nearly 4 logs CFU/g of *Escherichia coli O157:H7* in unpasteurized apple cider. In another study on apple cider, a decrease of 2.67 logs CFU/g of the entire microbial population was seen (Harrington and Hills 1968).
Maricel and others (2002) applied UV radiation to inactivate the microorganisms in fruit juice. They concluded that UV radiation successfully decreased the microbiology load in various kinds of juices. The required dosage of UV light varies depending on the transparency of the juice. The clearer the juice is, the lower the dosage level needed. The reason a higher dosage is needed for less transparent juices is that the amount of suspended components, such as fruit cells and fiber in the fruit juice can protect the microorganisms against the UV illumination by preventing the passage of the light to the microbial surface. Another study showed that a 20 - 100 times higher dosage illumination solved the coverage and shading problem, giving sterilization of spores and other microorganisms (Wallhauser 1978).

Furthermore, Blalka and Demirci (2008) applied pulsed light to decontaminate E.coli and Salmonella populations in raspberries and strawberries. They found that most reduction of E.coli and Salmonella happened when they applied the PL treatment for 60 seconds (s). However, they did not evaluate the sensory aspects of the fruits following treatment. Such sensory evaluation would help determine whether PL could be applied within the industry.

Belgin and others (2011) used UV light to decontaminate cumin seeds. Since cumin seeds contain all the proteins and nutrients that a plant needs to grow, it happens to be a great habitat for microorganisms. From the experiments, Belgin and others discovered that a combination of UV-C and FIR application would decrease the microorganism count to a lower level without damaging the quality of the seed.

**Decontamination of food-contact surface.**

Since microbial contamination on food-contact surface, especially on food processing equipment surface is a major concern in food industry. Surface
contamination of equipment can cause severe cross-contamination of pathogenic microorganisms (Kusumaningrum and others 2003). Cause of the limitation of majority of contamination methods, it is better to use PL in order to achieve a better penetration. Rowan and others (1999) found that after 300 pulses on food contact surface, it can reach a 6 log CFU/g reduction.

**Allergen mitigation**

PL has been reported can significantly reduce peanut, peanut butter, soybean, milk, shrimp, egg and wheat allergens. Chung and others (2008) noted that for liquid peanut butter, after PL treatment, the Immunoglobulin E (Ig E) binding can reduced to approximately 20%. And PL also been noticed be able to reduce soybean protein, such as glycinin, and it shows a 50% reduction in Ig E binding with ELISA after 6 min PL treatment (Yang and others 2010).

**Irradiation**

Gamma ray, e-beam (electron beam) and x-ray all belong to irradiation. The most commonly used method is still gamma irradiation. Doses from 3-10 kGy proved to be reliable for improving microbial safety for spices (Reddy and others 2009). Lastly, despite the controversy of using gamma irradiation, this technology has been found to be effective in decontamination of foods while assuring food safety and improved shelf life (Reddy and others 2009, Bhat and others 2010). In a study mentioned earlier, Omar and others (1995) conducted a set of experiments to compare the effectiveness of gamma rays and microwave irradiation on black pepper. The results from the experiment showed a reduction in microbial count when the sample was exposed to average doses of 5.0 kilo Grays (kGy) and 10 kGy using Cobalt-60 at a dose rate of 1.2 Gy/s. Compared with other microwave decontamination methods, gamma irradiation,
when applied in shorter durations, could eliminate almost all of the microbial organisms. The effect of gamma irradiation on *T. foenum-graecum* seed has also been studied by Gupta and others, it shows a 2 log reduction in Total Aerobic Count (TAC), when the dosage is 2 kGy. (Gupta and others 2009)

Irradiation, has been proven to be reliable for improving the quality of spices product, but also herbs, vegetable seasonings with maximum 10kGy average dosage (Farkas 2006). Even though gamma irradiation has been proved as an efficient, environmentally friendly, energy-effective official approved method. However, the lack of application is mainly due to customer concern and high cost on equipment and maintain. Meanwhile, the flavor and aroma will be slightly alternated (Farag and others 1995). Other than the sensorial changes, the irradiation used on packaged food material, may results in the formation of small volatile particles or non-volatile radiolysis products from the packaging material (Goulas and others 2004).

**High hydrostatic pressure**

High hydrostatic pressure processing uses a pressure that varies from 100 to 1000 Mpa. Hite and others (1914) investigated the application of high pressure as a milk preserving methods, and later the study has been focus on providing shelf-stable fruit and vegetable products (Hite and others 1914). HHP applied on wheat flour as a method of inactivate vegetative cells and enzyme in wheat flour, the quality of wheat flour start to become a concern. Some scientists investigate the modification of structure and mixing properties after HHP treatment, based on their research, when pressure is higher 400 MPa, starch granule structure lost occur, also gluten protein will aggregate after the treatment (McCann and others 2013). However, the inactivation effect is mainly based on equilibrium relative humidity (ERH) of product. Dry food ingredients usually
show no reduction in microbial count when water activity lower than 0.66 (Butz and others 1994). Based on that respect, HHP can hardly make an efficient method for dry ingredients.
Table 4-1. Concentration of B. cereus SIK 340 spores and natural background flora (±SD) in different location in paprika powder with aw 0.5, 0.8, and 0.96 after exposure to IR heat flux from medium-IR of 5 kW/m² and near IR of 11kW/m² (Staack and others 2008; Bialka and Demirci 2008).

<table>
<thead>
<tr>
<th>Heat flux (kW/m²)</th>
<th>Location in powder</th>
<th>Bacillus cereus</th>
<th>Natural flora</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Total plate count</td>
<td>Spore plate count</td>
</tr>
<tr>
<td>$a_w$ 0.50</td>
<td>5 med-IR</td>
<td>Surface 7.12</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Overall</td>
<td>7.15(±0.04)</td>
<td>7.16(±0.08)</td>
</tr>
<tr>
<td></td>
<td>11 near-IR</td>
<td>Surface 6.78</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Overall</td>
<td>7.05(±0.06)</td>
<td>6.95(±0.05)</td>
</tr>
<tr>
<td>$a_w$ 0.80</td>
<td>5 med-IR</td>
<td>Surface 6.87</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Overall</td>
<td>7.17(±0.09)</td>
<td>7.01(±0.45)</td>
</tr>
<tr>
<td></td>
<td>11 near-IR</td>
<td>Surface 6.77</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Overall</td>
<td>6.34(±0.13)</td>
<td>6.23(±0.30)</td>
</tr>
<tr>
<td>$a_w$ 0.96</td>
<td>5 med-IR</td>
<td>Surface 7.02(±0.25)</td>
<td>6.98(±0.19)</td>
</tr>
<tr>
<td></td>
<td>Inside</td>
<td>2.21(±0.59)</td>
<td>2.11(±0.38)</td>
</tr>
<tr>
<td></td>
<td>Overall</td>
<td>6.19(±0.49)</td>
<td>6.05(±0.27)</td>
</tr>
<tr>
<td></td>
<td>11 near-IR</td>
<td>Surface 6.58(±0.21)</td>
<td>6.50(±0.42)  &lt;1</td>
</tr>
<tr>
<td></td>
<td>Inside</td>
<td>&lt;1</td>
<td>&lt;1</td>
</tr>
<tr>
<td></td>
<td>Overall</td>
<td>5.77(±0.24)</td>
<td>5.71(±0.17)</td>
</tr>
</tbody>
</table>
Table 4-2. Survival of natural microbial contaminations in black pepper powder following gamma radiation and microwave treatments. Legend: - No Growth; + Growth; ++ More Growth; +++ Heavy growth (Omar and others 1995).

<table>
<thead>
<tr>
<th>Contaminant</th>
<th>Gamma irradiation does (kGy)</th>
<th>Microwave exposure (s)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>5</td>
</tr>
<tr>
<td><strong>Bacterial flora</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Escherichia coli</em></td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td><em>Enterobacter sp.</em></td>
<td>++</td>
<td>-</td>
</tr>
<tr>
<td><em>Bacillus subtilis</em></td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td><em>B. megaterium</em></td>
<td>+++</td>
<td>++</td>
</tr>
<tr>
<td><em>Clostridium sp.</em></td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td><em>Pseudomonas sp.</em></td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td><em>Micrococcus sp.</em></td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td><strong>Fungal Flora</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Alternaria sp.</em></td>
<td>++</td>
<td>-</td>
</tr>
<tr>
<td><em>Aspergillus sp.</em></td>
<td>+++</td>
<td>-</td>
</tr>
<tr>
<td><em>Penicillium sp.</em></td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td><em>Cladosprium sp.</em></td>
<td>++</td>
<td>+</td>
</tr>
</tbody>
</table>
Table 4-3. Characterization of different UV components (Krishnamurthy 2006).

<table>
<thead>
<tr>
<th>Region</th>
<th>Wavelength (nm)</th>
<th>Frequency (Hz)</th>
<th>Photon Energy (eV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vacuum UV</td>
<td>100-200</td>
<td>$3 \times 10^{18} - 3 \times 10^{15}$</td>
<td>24-12.4</td>
</tr>
<tr>
<td>UV-C</td>
<td>200-280</td>
<td>$3 \times 10^{15} - 1.07 \times 10^{15}$</td>
<td>12.4-4.43</td>
</tr>
<tr>
<td>UV-B</td>
<td>280-315</td>
<td>$1.07 \times 10^{15} - 9.52 \times 10^{14}$</td>
<td>4.43-3.94</td>
</tr>
<tr>
<td>UV-A</td>
<td>315-400</td>
<td>$9.52 \times 10^{14} - 7.49 \times 10^{14}$</td>
<td>3.94-3.10</td>
</tr>
</tbody>
</table>
Figure 4-1. The HTST process procedure for seed and food powder decontamination
(Fine and Gervais 2005)
Figure 4-2. Schematic of pulsed light equipment (Bialka and Demici 2008).
Sample Preparation

Wheat flour and black pepper were purchased from a local grocery store (Gainesville, FL, U.S.A) and stored at 20°C at 55% relative humidity. Following storage, the wheat flour samples were aseptically transferred to sterile foil boxes for future inoculation and cultivation. While, for black pepper sample, was kept in controlled A_w chamber until inoculation.

To create optimal conditions for yeast and mold growth, the flour was moisturized to a water activity of 0.77 using 100 mL of distilled water in a sterilized aluminum plate combined with 1000 g of wheat flour. The moistened samples were incubated for 7 days at 35°C.

Growth Study

The growth curve of bacteria varies a lot, however, majority of them follow the same pattern of growth as: lag phase, logarithmic, stationary and death phase. The reason why growth curve study was performed in my research is to make sure that bacterial vegetative cells can maintain in stationary phase. In which, it can reach to its highest quantity and relatively bigger resistance to a range of stresses (e.g., thermal, oxidative, starvation) due to a large number of phonotypic and genotypic changes. Yoshikawa and Sueoka (1963) conducted a research on B. subtilis chromosome, they concluded that in the stationary phase, B. subtilis has the complete genetic sequence. It has been proved that bacterial are more resistance to extreme environment, such as temperature, pH. Besides that, the stationary phase is less likely to death regarding to the growing concentration of toxin generate by themselves.
After *B. subtilis* colonies were rehydrated in the original container with PW and the cells were transferred from bottle to TSB tubes for incubation. The strain grew at 30°C for 24 h with gentle agitation (180 rpm). Next, transferred the cultures in 10ml TSB-Rif (200 µg/L) tubes and incubated the tubes under 30°C for 6 h two times to achieve uniform vegetative cell. On the same day, the cultures were serially diluted (1:10) in peptone water (PW) (0.1%) and yielded cell populations of approximately $10^2$ CFU/ml. The sample were then transferred into 100 ml TSB flasks.

Bacterial growth was monitored at every 50 to 10 mins by direct and viable cell counts methods. The measuring time interval started with every 50 mins, after 300 mins, the time interval between two measurements reduced to every 10 mins. Viable cells counts were conducted simultaneously by a series of dilutions of the PW. Dilutions were pour-plate into TSA, incubated for 24 hours at 30°C. After enumeration, the counting of the plates was expressed as CFU/ml. A growth curve was obtained by creating scatter plots of Log of counts against time (min).

**Preparation of Inoculum**

The *B. subtilis* strain cells were obtained from the American Type Culture Collection (ATCC 6633) and maintained at -80°C on Columbia Agar (Franklin Lakes, NJ) (Arantxa and others 2011). The inoculum was prepared as described by Arantxa and others (2011) by streaking a loop-full of frozen culture onto Typtone agar and incubating anaerobically at 37°C for 24 h. After incubation, a 250 mL conical flask containing 100 mL brain-heat infusion (BHI) broth (Franklin Lakers, NJ) was inoculated with a single colony and incubated at 37°C with agitation (115 rpm). After the second incubation for 24 h, a new 250 mL conical flask containing 100mL of TSB was inoculated with 1mL of the pre-culture and incubated under the same conditions. Next, 2
mL of the cellular suspension were inoculated in a sporulation agar medium and incubated for 24 h at 37°C. The medium was autoclaved at 121 °C for 20 min. Then, spores and vegetative cells were collected by flooding the agar plates with sterile double-distilled water and scratching the surfaces with a glass spatula. After harvesting, cells were purified and washed three times with centrifugation at 4000 rpm for 20 min (Fine and Gervais 2005). The last step was to place the inoculum into the freeze-dryer for 24 hours and then dry the bacteria vegetative cells and spores.

**Inoculation**

Prior to the PL treatments, microbial cell and spores were prepared and stored at -5°C for less than 24 h. The dried inoculum and the samples were mixed in a mixing bowl and blended for 20 min at 25°C. The mixture was then stored at room temperature for 24 hours under a laminar flow hood to enhance the adherence (Akbas and Ozdemir 2008). Four different spot in the blender have been researched and the results shown that there is no significant differences among those spots.

**Pulsed Light Treatment**

Wheat flour and black pepper samples were weighed and placed on separate, aluminum weighing dishes (70 mL, Fisher Scientific, IL, U.S.A). The samples were then transferred to the Xenon continuous PL system (Model LH840-LMP-HSG, Xenon Corp., Wilmington, MA, U.S.A.), which is shown as a schematic diagram in Figure 3-1. The equipment can generate a spectrum wavelength ranging from 100 to 1100 nanometers with frequencies from 1 - 20 pulses per second. For this experiment, the settings were longer time pulses of 360 µs (three times/second) and spectrum wavelengths (100-1100 nm) where the white light is rich in UV (200-400 nm). The output source was the Xenon lamp. Different energies (PL fluence or PL dose) were adopted in this study. Based on a
previous study, the PL doses ranged from 16.2 J/cm$^2$ to 1.08×10$^2$ J/cm$^2$ and is conducted on the similar model showed in Figure 3-2 (Krishnamurthy 2006).

For the PL treatment, samples with natural flora and inoculated wheat flour were exposed under PL for different times and distances. The treatment parameters were 3 pulses per second for 15 s, 30 s, 60 s, and 90 s using a distance of about 6 cm, 9 cm from the lamp source, respectively for wheat flour samples. A different treatment time was used, however, for the black pepper samples, darker color increased the temperature and led to higher readings. Based on that fact, the treatment time was adjusted to 5 s, 10 s, 15 s and 30 s, respectively 6 cm, 9 cm. At the end of each treatment, following 5-10 s delay, the samples were removed from the PL chamber in order to record the surface temperatures.

**Temperature Profile Measurements**

The initial temperature of each sample was measured and recorded using a non-contact infrared thermometer (Omega OS423-LS, Omega Technologies, Stamford, CT, U.S.A.). This gave the surface temperature of each sample. After the PL treatment, the temperature was recorded again following a 5 – 10 s delay due to sample removal from the treatment chamber. Measurements for the natural flora and inoculated wheat flour samples were recorded at different treatment times and different distances.

**Microbial Recovery Procedure and Enumeration**

**Total Aerobic Count**

AOAC 966.23 C is cited as a reference procedure. From each PL treated wheat flour and black pepper sample, serial dilution (1/10 ml) was made up to 10$^{-4}$. Next, 1 ml of each diluted sample was placed into sterile petri dishes using a sterile pipette. A Molten Plate Count Agar (at 45°C) was poured into each of petri dishes containing the
1ml inoculum. The plates were allowed to solidify on a flat surface. Then, the plates were inverted and incubated at 35°C for 48 hours. After incubation, reduction of bacteria in tenfold was calculated for different treated wheat flour samples. This method was validated and approved as the official methods as prescribed by the Association of Official Analytical Chemists (AOAC).

**Yeast and Mold Counts**

According to AOAC Method 995.21, Yeast and Mold Counts (YMC) in foods, the powder needs to be thoroughly mixed by sterile spoon. The samples were diluted to 1:10 by aseptically weighing 1g of the samples into a sterile wide-mouth, screw-top bottle and then mixed with 9 ml peptone water (0.1%). Next, the bottle was shaken rapidly on a vortex for 25 s. Next, the homogenate was filtered using a vacuum source and placed on the surface of a pre-dried YM-11 plate. YM-11 agar is made from 20 g soy peptone mixed with 20 g tryptone, 5 g D-glucose, 5 g NaCl, 2.4 g anhydrous \( \text{K}_2\text{HPO}_4 \), 0.03 g trypanblue, 0.1 g chloramphenicol, 15 g of agar and 1 L distilled water. It was heated to boiling and stirred it before autoclave. The mix was autoclaved at 121°C for 15 min and then tempered to 45-50°C. Next, we aseptically poured larger than 3 mm layer of agar and let it solidify. Finally, the samples were cultured for 48 h at 26 °C.

**Bacillus subtilis Count**

For the *B. subtilis* analysis, agar Columbia agar was used for the detection (Fine and Gervais 2005; Levy and others 2011). Take out 10 g Columbia powder. Add further distilled water in a 1 L cylinder after suspension to ensure the accuracy. After the mixture was autoclave at 121 °C for 20 min, Treated samples and control groups were transferred to sterile bags. Each sample was hydrolyzed by 9 ml of sterile peptone
water (0.1%, w/v) in the sterile bags and homogenized with a vortex mixture at a medium speed for 2 min. Further decimal dilutions were prepared in peptone water solution and were plated out on the media for *B. subtilis* (Akbas and Ozdemir 2008). Viability was determined by counting “colony-forming units” (CFU). Experiments were performed no less than 3 times and mean values were calculated, as well as 95% confidence interval (CI) for the means. These CIs were found to be no more than 15% of the mean viability.

**Color Analysis**

The color of the wheat flour samples was measured using a color machine vision system (CMVS). This equipment consists of a Nikon D 200 digital color camera (Nikon Corp, Japan) housed in a light box [42.5 cm (W) x 61.0 cm (L) x 78.1(H)] (Wallat and others 2002). The camera (focal light, 35 mm; polarization, 18.44 mm), which was connected to a computer, was used to capture the images prior to color analysis. LensEye software (Engineering and Cybersolutions Inc., Gainesville, FL, USA) was used to analyze the wheat flour color based on the values of L (lightness), a* (redness) and b* (yellowness). The sample analyses are based on 3-dimensional color values with the following scale: L* value for whiteness (100 white, 0 black); a* value for red-green chromaticity (+60 red, −60 green); and b* value for yellowness (+60 yellow, −60 blue) (Labsphere, North Sutton, NH, USA). Color coordinates for with the untreated samples were L=81.22, a=2.75, and b=10.92. In this study, the color differences between the treated and untreated samples were assessed by three parameters shown as: total color differences ∆E, C* (chroma) and h° (hue angle), which is shown in Equation 1, 2 and 3 below. All the samples were aligned in the center of the light box before acquiring the images.
\[ \Delta E = \sqrt{\Delta L^*^2 + (\Delta a^*)^2 + (\Delta b^*)^2} \quad (1) \]

\[ c^* = \sqrt{a^2 + b^2} \quad (2) \]

\[ h^\circ = \tan^{-1}\left(\frac{b}{a}\right) \quad (3) \]

**Water Activity Measurements**

Water activity measurements were performed at various treatment times (t = 15, 30, 60, and 90s). During the treatment, changes in the water activity of the samples were monitored to maintain the low water activity to prevent any potential post-contamination (Laroche and others 2003). The water activities of the samples were checked using a dew-point osmometer (Decagon Devices, WA, U.S.A) before and after PL treatment. The control group wheat flour sample was placed in the water activity chamber to initiate water activity measurements and provided a baseline to compare against all the other treatment groups.

**Statistical Analysis**

Statistical analysis was carried out using JMP Version 9. Paired t-test was conducted to determine the different effectiveness among different distance. Multiple comparison of mean value were determined using the Turkey’s methods. The data was tabulated as the average of the 3 to 9 replicates± standard deviation and the level of significance was set at \( \alpha=0.05 \).
Figure 5-1. Schematic diagram of continuous PL system (Model LH840-LMP-HSG)
Figure 5-2. Energy dose (fluence) during PL treatment as measured by a radiometer (Adapted from Krishnamurthy 2006)
CHAPTER 6
RESULTS AND DISCUSSION

Growth curve study of *B. subtilis*

The purpose of the growth curve study is to monitor bacterial vegetative cells during the stationary phase, where the cells reach peak population and develop resistance to a range of stresses (e.g., thermal, oxidative, starvation) due to phenotypic and genotypic changes (Hall and Foster 1996). Yoshikawa and Sueoka (1963) conducted research on the *B. subtilis* chromosome and concluded that in the stationary phase, *B. subtilis* has the complete genetic sequence. In addition, the bacteria are less likely to die in the stationary phase due to the growing concentration of toxins that bacteria generate after stationary phase (Gardner and others 1998).

We employed the growth study curve on *B. subtilis* to determine the parameters for reaching a stationary phase prior to apply PL treatment. Figure 6-1 displays *B. subtilis* starting the stationary phase after approximately a 300 min incubation and the stationary phase lasts up to 600 min. Gao and others (2014) present similar results illustrating that *B. subtilis* reaches its stationary phase after 5 hours incubation and remains in the stationary phase until 14 hours. Based on the work of Gao and others (2014), the bacterial show a similar on the growth curve that after 5 h incubation, the flora entered the stationary phase. For this reason, the incubation time of *B. subtilis* inoculum for inactivation was optimized to 6±1 h.

**Efficiency of Microbial Decontamination**

**Evaluation of PL Efficiency in Inactivate *B. Subtilis* on Wheat Flour and Black Pepper**

As mentioned in the literature review, PL inactivation is based on three working mechanisms, which are photochemical, photophysical and photothermal and have
major effects in inactivating microbial cells. According to Wang and others (2005), for the inactivation study, wavelengths ranging from 200-300 nm align with photochemical effects and have the most lethal effect for bacterial vegetative cells. It has been well documented that formation thymine dimers on microbial DNA is the most lethal reaction, because it prevents the cell from replication by distorting the DNA helix, namely, causing the inability to replicate or clonogenic death (Bank and others 1990; Jay 1997). On bacterial spores, UVC results in forming 5-thyminyl-5, 6-dihydrothymine (spore photoproduct) and also forming single and double strand breaks and cyclobutance pyrimidine dimers (Slieman and Nichololson 2000). In addition, UV light leads to double bond cross-linking of aromatic amino acids, which results in membrane depolarization (Lado and Yousef 2002). Also, photochemical effects can generate ozone and peroxides after PL exposure. For example, hydroperoxide radicals can be generated by long wavelength UV treatment, which may react with membranes and lead to changes in permeability. Short wavelength UV can turn oxygen into ozone that has a strong oxidation potential which can be applied on inactivating bacteria (Nordhauser and Olson 1998).

There is also evidence provided by Hiramoto (1984) that rays absorbed into bacterial are expected to heat the bacteria, providing the same effect as thermal inactivation. Dunn and others (1989) explained that the light pulses heat a superficial layer of food product, and the heat will transfer to the center. For the photophysical effect, Wekho and others (2003) provided evidence that spores have an obvious deformation and rupture on top of their spores, which provides escape of an overheated
spore content. Later, Nicorescu and others (2013) showed an electron-microscope photograph that provided strong evidence for the theory in Figure 6-3.

In the first step, the inactivation effectiveness of PL was studied on *B. subtilis*, which was grown to a stationary phase, to make sure the resistance of *B. subtilis* is uniform. The results shown in Table 6-1 shows a significant difference from 0.1 log reduction to 4.1 log. As indicated by the results, the highest reduction achieved was at a distance of 6 cm for 90 s. The log reduction is 4.1 ±0.3 log on wheat flour with after 90 s treatment when 6 cm away from quartz window. As illustrated in Figure 6-4, survivor curves were characterized by a tail. Yaun and others (2003) explained several reasons for this phenomenon: running out of homogenous population, multi-hit phenomena, accumulation of suspended solids, and shading effects of Petri dish used in experiment. Accordingly, McDonald and others (2000) stated that decreasing population density reduces the likelihood of exposing the biological element under PL.

There are a few factors that can influence the efficiency of PL. The most crucial factor is fluence incident on the sample. Gómez-López and others (2007) stated that the energy emitted from the lamp is different from the energy incident on the food samples. The distance from the lamp to food sample can be a crucial factor, as shown in Table 6-1. A paired t-test was conducted to test the null hypothesis that there is no obvious difference between the inactivation effects of different distances from the sample to the lamp under the same exposure time. Based on the inactivation results (p<0.05), there are significant differences on inactivation effects between 6 cm and 9 cm. A tukey’s test was also conducted. Based on Table 6-1, bacterial counts dropped dramatically at 15 to 30 s (p<0.05) on wheat flour samples and lasted consistently for an
additional 60s as the fluence increases. The same phenomenon is also detected in black pepper samples. However, a tailing effect could be detected in this research. The results matches the finding in Hillegas and Demirci’s (2003) paper that a closer distance to the lamp would be more effective. The module used to describe this phenomenon was built by Sharma and Demirci (2003). In their research, the module was built based on the data they acquired from inactivating E. coli O157:H7 on inoculated alfalfa seed. The thickness of the sample is another factor can alter the results since it can affect penetrability. Overlapping opaque obstacle shield the surface from light exposure (Sharma and Demirci 2003). Lastly, another important factor for PL inactivation effect is the color of food sample. The details will be illustrated in the temperature profile analysis.

**Yeast Molds Inactivation and Total Aerobic Count**

For each treatment, six replicates were used on three days. The initial count of molds and yeast were measured as high as $6.8 \times 10^4$ CFU/ g. Total aerobic count contamination in the untreated sample was found to be about $10^7$ CFU/g. According to our observations, yeast and mold counts exhibited a 3.5 log reduction, which is higher than that reported in another study by Fine and Gervais (2004), who observed 0.7 log reduction in their wheat flour samples. However, in a study by Kazuko and others’, when they inoculate the yeast cells on agar, a 6 log CFU/ ml reduction can be achieved. The huge difference between the results may be attributable to the contamination surface. For agar, the surface is smooth and can barely influenced by the shadowing effect. In the food powder samples, the small particles shadowed each other, which could lead to uneven exposure. Another possible reason that could explain the difference in reduction may be the higher moisture content of agar, which makes the
heat transfer more rapidly, leading to the temperature being amassed at a higher level. Two distances of 6 cm and 9 cm were used as the distance from the sample to the quartz window of the pulsed light lamp. Starting at 45 pulses, reduction could be seen up to 270 pulses for wheat flour. When black pepper samples were analyzed, a strong overheating effect, accompanied with a darker color was observed. Therefore, the pulsed light dosages used to treat black pepper was reduced to no more than 90 pulses. In the study by Ito and Islam (1993), a 3 log CFU/g reduction has been achieved by using both gamma irradiation and electron-beam irradiation on the contaminated black pepper samples. When the dosage is higher than 7 kGy, it may achieve the 3 log reduction. An even higher dosage of (8 kGy) is required for the same reduction effect when the black pepper was treated with e-beam. The results achieved by Ito and Islam (1993) was quite close to the results for this study, however, according to Ito and Islam (1993), the dosage is still high, and penetration is the major limiting factor for those two irradiation methods.

Microbial distribution has always been treated as a critical parameter in PL applications in food industry (Fine and Gervais 2004). Microorganisms are not concentrated in the center and they can be present on the outer edges of the plates. In that case, the distribution of microorganism make it difficult for the PL to properly treat microorganisms disseminated at the edges. In addition to the challenge of uniform energy distribution of PL, the penetration of PL can vary depending on the vertical position of the microorganisms. Microbes that are closer to the lamp will experience more penetration than those which are beneath and those are shielded by microbes above. This allows microbes below to avoid being placed directly underneath the PL
treatment. Consequently, shielding may dramatically influence the reduction results (Dunn and others 1995)

Other reasons exist that may lead to the inefficiency of the decontamination process, one of the reasons is energy fluence (also called energy intensity). When the distance from the sample to the lamp was adjusted to 6cm, the intensity of energy for the 15 s treatment was 1.45 J/cm². Accordingly, as the treatment time extended to 90 s, the energy measurement increased to 28.8 J/cm². Despite the increase, the energy is still below the threshold for inactivation of microorganisms that exhibit a stronger resistance (Sharma and Demirci 2003; Kreitner and others 2001). Based on the data we acquired, it is obvious that there was a significant difference (P<0.05) between the effects when applied on different distances, which might mainly be caused by the differences among the fluence.

**Temperature Measurement**

Pulsed light has generally been considered as a non-thermal inactivation method when applied for short exposure times. As the treatment time is prolonged, more energy is absorbed by the food sample, resulting higher temperatures characteristic of a photothermal effect. As shown in Figure 6-2, the temperature varies for the treatment times at different distances on both wheat flour and black pepper samples.

Temperatures were measured by an infrared noncontact thermometer. The average temperature for control group wheat flour was 22.7±0.1 °C and for untreated black pepper, it was 22.6±0.1°C. Based on the results, wheat flour has up to a 25.1±1.7°C and 15.9±1.9°C temperature increase at 6 cm and 9 cm, respectively. While for black pepper, it has a larger temperature increase from 65.0±4.7 °C (6 cm) to 44.7±4.6 °C (9 cm). Comparison of the temperature rises in Table 6-2 between 6 cm
and 9 cm distance from quartz window illustrated that shorter distance generated greater photothermal impact during illumination given the same duration.

As illustrated in the literature, as the distance increases from the lamp source, temperature increase drops and the food samples receive a less intensive treatment (Krishnamurthy 2006). In Oms-Oliu and others’ (2010), the researchers pointed out that the temperature increase after PL treatment is strongly dependent on the color of the food products. Thus, the darker the product’s color, more energy will be absorbed and the product’s temperature will increase. According to Oms-Oliu and others (2010), the differences between fish’s colors can result in different temperature increases after the treatment. The darker the fish, the greater the temperature increase. Since black pepper has a generally darker color, it displays higher temperature increases. In Fine and Gervais’ work (2005), the researchers reached the same conclusion on wheat flour and black pepper temperature change. They found that black pepper has a more dominant temperature increase than wheat flour, which results in an overheating effect on black pepper.

In the statistical results, there was a significant difference among the samples treated at different distances based on the paired t-test for wheat flour. In addition, the Tukey HSD test showed that at the first 60 s, there were no dramatic temperature changes, but after 60 s, there is a 17°C temperature increase. For black pepper, a significant differences (P<0.05) in inactivation effect between 6 cm and 9 cm treatment distance can be observed through a paired t-test. This P value indicated that the different distances could lead to different temperature increases under the same treatment time. This phenomenon was also explained by Krishnamurthy and others.
(2008), i.e., temperature increase is proportional to treatment time, but it is inversely proportional to the sample’s distance from lamp.

**Water Activity Measurement**

Water activity has always been considered an important factor in microbial inactivation because water activity alone can nourish or not nourish microorganisms. Fine and Gervais (2005) pointed out in their study that the highest inactivation rate under the same conditions was achieved when water activity is 0.15, and the valley is found when water activity is 0.4 and goes up again. Moreover, Larchos and Gervin (2003) mentioned that heat resistance of dried vegetative cells is many times higher than the resistance of the same microbes in an aqueous environment. Thus, water activity is a crucial factor for the future quality regards to longer storage time. In the study, for 6 cm treatment on wheat flour, there was a dramatic decrease after 90 s of treatment on wheat flour. The highest water activity reduction was encountered in the samples at 6 cm distance to the quartz window, after 90 s treatment on wheat flour. The water activity was reduced to 0.14, which makes it almost impossible for microbial growth. While for the same sample, wheat flour, when the distance from the quartz window increased to 9 cm, the water activity was 0.42 after 90 s of exposure. The same phenomenon occurred in black pepper samples too, when the distance from the quartz window became shorter, the water activity decrease was more obvious. Given the same phenomenon observed in those two groups of samples, it was more probably due to higher instantaneous temperatures incurred on the samples.

In this study, there were significant differences (p<0.05) among water activities with different PL treatments. Meanwhile, an obvious decrease could be detected as the treatment time prolonged. Among all the treatments, the color parameters were
relatively consistent. However, there were significant differences (P<0.05) among the two distances. This provided assurance that PL treatment would positively influence the future storage of food powder and dry ingredients by decreasing the water activity.

**Color Analysis**

As mentioned previously, color is a major sensory characteristic associated with quality in the food industry and market, determining the acceptability of spices and wheat flour. The CIELAB system is defined by the Commission of International del'Eclairage (CIE) as a widely used color chromaticity system, using L*, a*, b* to describe a color. Additionally, hue and chrome also used in the color measurement.

Color parameters of wheat flour before and after PL treatments are summarized in Table 6-4. The L* value ranged from 62.77 ± 7.72 to 82.30 ± 3.22 for eggs exposed 90s at 6 cm and 60s at 9 cm from the PL lamp respectively, suggesting the lightness of wheat flour. The sudden drop of L* value indicated a strong over heating effect, which make wheat flour darker. a* value ranged from 11.04 ±1.03 to 11.79 ± 1.04 for wheat flour treated 60s at 6cm and 90s at 9 cm respectively, indicating the redness of samples. Lastly, b* value ranged from 17.83 ±1.49 to 20.98 ±3.04 in sample subjected to 15s and 9 cm and 90s at 6 cm, suggesting a yellow pattern on wheat flour surface. The L*, a*, b* values obtained suggested a relative consistent color after different PL treatment in general, except for the L* value after 90 s PL treatment with a 6 cm distance that was extremely low. Based on the observation, the overheating effect is believed to have a considerable influence on ΔE.

Hue and chrome is also used to determine the difference varies the color, however, based on the results, there is no significant differences (p>0.05) among different treatment. This might resulted from the hue angle is decided by real color of
food product, which can only be altered slightly. The similar results were shown by Ulu (2004).

Overall, the color results suggest that the PL treatment as a potential inactivation technique was capable of maintaining the original color of wheat flour and black pepper, if we set the treatment time less than 90 s with a 6 cm distance from quartz window.
<table>
<thead>
<tr>
<th>Treatment time (s)</th>
<th>Distance from quartz window (cm)</th>
<th>Log remaining (Log10 CFU/g [mean ± SD])&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Treatment time (s)</th>
<th>Distance from quartz window (cm)</th>
<th>Log remaining (Log10 CFU/g [mean ± SD])&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 (control)</td>
<td>6</td>
<td>6.3 ± 0.3</td>
<td>0 (Control)</td>
<td>6</td>
<td>5.9 ± 0.5</td>
</tr>
<tr>
<td>15</td>
<td>6</td>
<td>5.5 ± 0.4 A</td>
<td>5</td>
<td>6</td>
<td>5.4 ± 0.1 A</td>
</tr>
<tr>
<td>30</td>
<td>6</td>
<td>3.6 ± 0.3 B</td>
<td>10</td>
<td>6</td>
<td>4.6 ± 0.2 B</td>
</tr>
<tr>
<td>60</td>
<td>6</td>
<td>3.3 ± 0.2 C</td>
<td>15</td>
<td>6</td>
<td>3.7 ± 0.3 C</td>
</tr>
<tr>
<td>90</td>
<td>6</td>
<td>2.2 ± 0.2 D</td>
<td>30</td>
<td>6</td>
<td>1.7 ± 0.1 D</td>
</tr>
<tr>
<td>15</td>
<td>9</td>
<td>5.7 ± 0.1 A</td>
<td>5</td>
<td>9</td>
<td>5.8 ± 0.2 A</td>
</tr>
<tr>
<td>30</td>
<td>9</td>
<td>4.4 ± 0.3 B</td>
<td>10</td>
<td>9</td>
<td>5.2 ± 0.3 B C</td>
</tr>
<tr>
<td>60</td>
<td>9</td>
<td>3.8 ± 0.3 C</td>
<td>15</td>
<td>9</td>
<td>4.8 ± 0.2 C D</td>
</tr>
<tr>
<td>90</td>
<td>9</td>
<td>3.4 ± 0.2 D</td>
<td>30</td>
<td>9</td>
<td>4.3 ± 0.2 D</td>
</tr>
</tbody>
</table>

<sup>a</sup> Measured as log10 CFU per gram, where counts are average of 6 replicate trials. Value followed by sample letter do not differ at the P<0.05 level, whereas values followed by different letters differ at P<0.05 level.
Table 6-2. Temperature profile of two different distance (6 cm and 9 cm) at various exposure time

<table>
<thead>
<tr>
<th>Treatment time (s)</th>
<th>Distance from quartz window (cm)</th>
<th>Temperature (°C)</th>
<th>Treatment time (s)</th>
<th>Distance from quartz window (cm)</th>
<th>Temperature (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0(control)</td>
<td>22.7 ± 0.1 A</td>
<td>0 (Control)</td>
<td>22.6 ± 0.1 A</td>
<td></td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>6</td>
<td>26.9 ± 1.3 A</td>
<td>5</td>
<td>6</td>
<td>35.5 ± 2.1 B</td>
</tr>
<tr>
<td>30</td>
<td>6</td>
<td>31.6 ± 2.3 A B</td>
<td>10</td>
<td>6</td>
<td>50.7 ± 4.0 C</td>
</tr>
<tr>
<td>60</td>
<td>6</td>
<td>39.5 ± 2.1 B C</td>
<td>15</td>
<td>6</td>
<td>65.2 ± 4.7 D</td>
</tr>
<tr>
<td>90</td>
<td>6</td>
<td>48.8 ± 1.7 C</td>
<td>30</td>
<td>6</td>
<td>87.6 ± 3.7 E</td>
</tr>
<tr>
<td>15</td>
<td>9</td>
<td>26.7 ± 1.5 A</td>
<td>5</td>
<td>9</td>
<td>31.3 ± 0.8 B</td>
</tr>
<tr>
<td>30</td>
<td>9</td>
<td>29.4 ± 3.6 A B</td>
<td>10</td>
<td>9</td>
<td>44.3 ± 1.4 C</td>
</tr>
<tr>
<td>60</td>
<td>9</td>
<td>33.2 ± 2.7 B C</td>
<td>15</td>
<td>9</td>
<td>56.7 ± 2.7 D</td>
</tr>
<tr>
<td>90</td>
<td>9</td>
<td>38.6 ± 1.9 C</td>
<td>30</td>
<td>9</td>
<td>67.3 ± 4.6 E</td>
</tr>
</tbody>
</table>

* Temperature was recorded after taking out of chamber, where temperature are average of 3 replicate trials to avoid the error cause by different time delay. Value followed by sample letter do not differ at the P<0.05 level, whereas values followed by different letters differ at P<0.05 level.
Table 6-3. Effects on water activity of wheat flour and black pepper

<table>
<thead>
<tr>
<th>Treatment time (s)</th>
<th>Distance from quartz window (cm)</th>
<th>Water activity</th>
<th>Treatment time (s)</th>
<th>Distance from quartz window (cm)</th>
<th>Water activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>0(control)</td>
<td></td>
<td>0.68 ± 0.01 A</td>
<td>0 (Control)</td>
<td></td>
<td>0.67 ± 0.01 A</td>
</tr>
<tr>
<td>15</td>
<td>6</td>
<td>0.52 ± 0.01 B</td>
<td>5</td>
<td>6</td>
<td>0.57 ± 0.01 B</td>
</tr>
<tr>
<td>30</td>
<td>6</td>
<td>0.46 ± 0.02 C</td>
<td>10</td>
<td>6</td>
<td>0.53 ± 0.04 BC</td>
</tr>
<tr>
<td>60</td>
<td>6</td>
<td>0.32 ± 0.01 D</td>
<td>15</td>
<td>6</td>
<td>0.47 ± 0.01 C</td>
</tr>
<tr>
<td>90</td>
<td>6</td>
<td>0.14 ± 0.02 E</td>
<td>30</td>
<td>6</td>
<td>0.40 ± 0.02 C</td>
</tr>
<tr>
<td>15</td>
<td>9</td>
<td>0.63 ± 0.01 B</td>
<td>5</td>
<td>9</td>
<td>0.59 ± 0.01 B</td>
</tr>
<tr>
<td>30</td>
<td>9</td>
<td>0.61 ± 0.02 B</td>
<td>10</td>
<td>9</td>
<td>0.54 ± 0.01 BC</td>
</tr>
<tr>
<td>60</td>
<td>9</td>
<td>0.44 ± 0.01 C</td>
<td>15</td>
<td>9</td>
<td>0.50 ± 0.01 C</td>
</tr>
<tr>
<td>90</td>
<td>9</td>
<td>0.42 ± 0.01 C</td>
<td>30</td>
<td>9</td>
<td>0.43 ± 0.01 D</td>
</tr>
</tbody>
</table>

Water activity was recorded after taking out of chamber, where water activity are average of 3 replicate trials to avoid the error cause by different level of rehydration. Value followed by sample letter do not differ at the \( P<0.05 \) level, whereas values followed by different letters differ at \( P<0.05 \) level.
<table>
<thead>
<tr>
<th>Treatment time (s)</th>
<th>Distance from quartz window (cm)</th>
<th>L*</th>
<th>a*</th>
<th>b*</th>
<th>∆E</th>
<th>h°</th>
<th>C*</th>
</tr>
</thead>
<tbody>
<tr>
<td>0(control)</td>
<td>6</td>
<td>81.29±0.22A</td>
<td>10.92±1.26A</td>
<td>17.90±1.83A</td>
<td>58.7±0.4A</td>
<td>21.02±0.21A</td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>6</td>
<td>81.82±2.68A</td>
<td>11.15±1.11A</td>
<td>17.96±1.64A</td>
<td>2.09±0.06A</td>
<td>58.5±0.4A</td>
<td>20.90±0.22A</td>
</tr>
<tr>
<td>30</td>
<td>6</td>
<td>81.89±3.00A</td>
<td>11.06±1.12A</td>
<td>18.89±1.65A</td>
<td>1.97±0.97A</td>
<td>57.2±0.6A</td>
<td>20.07±0.18A</td>
</tr>
<tr>
<td>60</td>
<td>6</td>
<td>81.09±2.61A</td>
<td>11.04±1.03A</td>
<td>18.79±1.57A</td>
<td>3.27±0.95A</td>
<td>59.8±0.9A</td>
<td>22.22±0.19A</td>
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<tr>
<td>90</td>
<td>6</td>
<td>62.77±7.72B</td>
<td>11.59±1.58A</td>
<td>20.98±3.04A</td>
<td>22.95±0.94B</td>
<td>59.8±0.3A</td>
<td>20.12±0.17A</td>
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<tr>
<td>15</td>
<td>9</td>
<td>81.37±3.69A</td>
<td>11.14±1.08A</td>
<td>17.83±1.49A</td>
<td>1.01±0.87A</td>
<td>58.3±0.2A</td>
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<tr>
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<td>60</td>
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<td>82.77±2.97A</td>
<td>11.14±1.12A</td>
<td>19.11±1.61A</td>
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<td>1.99±0.10A</td>
<td>60.8±0.3A</td>
<td>22.12±0.17A</td>
</tr>
</tbody>
</table>

* Color parameters are average of 3 replicate trials to avoid the error cause by different. Value followed by sample letter do not differ at the P<0.05 level, whereas values followed by different letters differ at P<0.05 level.
Figure 6-1. Growth curve of *Bacillus subtilis* ATCC 6633 in TSB.
Figure 6-2. *Bacillus subtilis* (●) growth curve measured by spectrophotometer at 600 nm (Gao and others 2014)
Figure 6-3. *Bacillus subtilis* vegetative cells a). untreated samples; b) treated by PL at 3000 V, 1 Hz, 10 J cm$^2$ / flash (Nicorescu and others 2013)
Figure 6-4. Survivor curves for *B. subtilis* treated with PL at distances of 6 cm and 9 cm from the quartz window for wheat flour sample.
Figure 6-5. Survivor curves for *B. subtilis* treated with distance of 6 cm and 9 cm from the quartz window for black pepper sample.
Figure 6-6. Temperature profile of wheat flour under different distance (6 cm and 9 cm) and exposure times
Figure 6-7. Temperature profile of black pepper under different distance (6 cm and 9 cm) and exposure times.
Figure 6-8. Black pepper after under different treatment time at 6 cm (distance from sample to quartz window)
CHAPTER 7
CONCLUSIONS AND RECOMMENDATIONS

PL provides a viable method against thermal methods for the decontamination of dry food powders and food ingredients. Based on our results, pulsed light has an effect on the inactivation of yeast and molds in wheat flour and black pepper.

PL can significantly reduce the total number of \textit{B. subtilis} in both wheat flour and black pepper. The color does not show significant changes (p>0.05) after treatment. This non-thermal technology may be a good choice for the pasteurization of wheat and black pepper in the industry due to the short treatment time and effectiveness on microbial reduction on wheat flour and black pepper.

The PL treatment time and distance from the quartz window (also regarded as the PL fluence or intensity), number of pulses, thickness of the sample, and duration of illumination are considered as crucial factors for optimizing a PL system. In order to optimize the treatment for wheat flour, both inactivation effect and food quality should be taken into consideration. Based on those qualifiers, for wheat flour at 6 cm distance from the light source with 60 s treatment would be recommended. While for black pepper, a 30 s treatment with the distance from quartz window at 9 cm would be recommended to achieve the optimal inactivation effect while avoid the overheating.

There are, however, some other factors that deserve major consideration in the present and future studies. In this study, only non-pathogens were used, whereas the real concern in the food industry are real pathogens. It is recommended the inactivation of \textit{Bacillus cereus} should be studied. Although gamma irradiation and microwave can impair bacteria, some bacteria have the ability to recover after the treatment. Based on that, a longer term storage is recommended to ensure that the inactivate effect can last.
Meanwhile, different effects on the inactivation of *Bacillus subtilis* in wheat flour and black pepper have not been fully elucidated. Therefore, a single control factor experiment should be conducted in order to confirm the hypothesis that color is the major factor for different inactivation effects in the two products. Besides, the influence of PL treatment on nutritional values and composition also need to be studied.
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BIOGRAPHICAL SKETCH

Ying “Kelsey” Guo was born in 1989 in China. She graduated from Northwest University with a Bachelors of Science in environmental science. In her undergraduate studies, she became involved in laboratory research and interned with three beverage companies, where she eventually discovered an interest in food science.

Ying began her graduate studies in the Department of Food Science and Human Nutrition at the University of Florida in 2011. Outside academics, she partakes in several activities including volunteering for Graduate Information Day, Habitat for Humanity, American Cancer Society, and Gators for Heaven Hospice. During summer 2013, she interned with Paca Foods, Inc. as a technical intern under both the R&D Department and Quality Assurance Department.