

**PULSED LIGHT INACTIVATION OF *SALMONELLA* ON THE SURFACE OF
ALMONDS AND WHOLE BLACK PEPPERCORN**

by

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fulfillment of the requirements for the degree of Master of Science in Food Science

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ABSTRACT

A shaker-assisted Pulsed Light (PL) process was developed to investigate the effectiveness of PL technology on the inactivation of *Salmonella* on the surface of almonds and whole black peppercorn as well as to examine the quality of treated products by weight and color parameters, and fatty acid analysis. Almonds and black peppercorn inoculated with a cocktail of four nalidixic-acid-resistant strains of *Salmonella enterica* were treated with PL directly or covered with a quartz plate during PL processing for 2-30 min. Weight before and after treatment was recorded and color measured using a colorimeter with traditional L, a, and b values. When dry almonds were exposed to ultraviolet and pulsed light at 29 mW/cm² for 30 min and 0.26J/cm² for 3 min respectively, obvious surface discoloration were observed. Dipping the almonds in water for 1 min once or twice prior to light exposure resulted in a reduction in *Salmonella* by 5 log reduction at medium intensity. The medium and low intensity better preserved surface color but required longer time for inactivation on *Salmonella*. In addition, steam-assisted pulsed light treatments were carried by covering stainless pans with a quartz plate. This PL treatment achieved 4.78 log reduction in *Salmonella* when the light intensity was 0.25 J/cm² for 10 min on small-scale study. Since the treatment resulted in a temperature over 100°C that caused some negative effect on almond quality, temperature cycling was performed to achieve over 5 log reduction of *Salmonella* between 80°C and 90°C after 10 min PL treatment with favorable color and weight parameters for small-scale almond. For whole black peppercorn, controlling the temperature (55-80°C) at medium intensity in the steam-assisted system was the most desirable combination since the quality of black peppercorn was significantly better than high intensity treatment.

Thus, steam-assisted PL at medium intensity in combination with temperature cycling could lower the risk of *Salmonella* contamination during low-moisture food processing. This treatment is a non-chemical means of pasteurization that is effective and more environmentally friendly than dry roasting, blanching, steam and propylene oxide (PPO) for almond products, or steam, chemical treatment, gamma irradiation, chemical and microwave sterilization and pressure sterilization for peppercorn.

Chapter 1

INTRODUCTION

The demand for nuts grows fast in USA since they are widely used in both healthy foods and candies. Particularly, almonds and almond products are a source of vitamin E, high-quality protein, magnesium and they also contain unsaturated fatty acids, dietary fiber and bioactive molecules, which are helpful to prevent cardiovascular disease and lower cholesterol levels and cancer risk (Nordqvist, 2017). California produces over 75% of the world's supply of almonds, making the USA the single largest market for almonds grown in California (Sumner et al., 2014). In recent years, the consumption of almonds has increased rapidly. In 2018, California's almond industry produced a record crop of 2.3 billion pounds that was 1.3% above the 2017 production of 2.27 billion pounds (Almond Board of California, 2018). Also, non-dairy milk sales have increased a lot over the past five years, and almond milk had a 64% market share in 2018 (Food and Drink News, 2018).

Pepper is one of the most important spice besides salt in the world. It is widely used in many recipes to add flavor to foods and there are also other commercial products derived from pepper such as pepper oil, tea, candy and preservatives (Gottardi et al., 2016). The FDA has reported that 12% of spices imported into U.S. were contaminated and they focused on this issue with pepper and sesame seeds (CIDRAP News, 2013). Black peppercorn is harvested and then laid on the soil for drying where it is susceptible to contamination with *Salmonella* from birds and other animals (Erdogan et al., 2004).

There have been several large outbreaks associated with almonds that have raised concern about the food safety of this product. In 2004, five patients were infected with *Salmonella* Enteritidis (SE) in Oregon and 29 patients in 12 states and Canada were also identified that year. Raw almonds sold in the United States and the whole world are the source of SE infections (Isaacs et al., 2005). Additionally, two *Salmonella* outbreaks in 2001 and 2004 in California caused many cases of food poisoning from the consumption of raw almonds. In addition, there was a *Salmonella* outbreak that caused at least 252 people to become sick in over 40 states from 2009 to 2010 because some salami products were processed with contaminated pepper (Drew, 2010).

Traditional pasteurization techniques are not able to reduce the potential pathogen contamination efficiently and almonds and peppercorn products may lose nutrients and other quality attributes in the process (Waje et al., 2008). Propylene oxide treatments can lead to undesirable chemical residues (Danyluk et al., 2005). Therefore, development of a food processing technology to decontaminate almonds and peppercorn product is very important.

Non-thermal processing technologies have received great interests as commercial alternatives to traditional thermal processing methods in the food industry. Not only can they effectively inactivate undesirable microorganisms in foods, but increase shelf-life, improve taste and maintain the quality and nutritional value of product (Pereira et al., 2010).

Pulsed light (PL) is a nonthermal decontamination technique with significant benefit. It has been approved by the FDA for rapidly inactivating microorganisms in foods without toxicity (Bhavya et al., 2017). PL is an effective treatment for surfaces

and packaging of products Compared to ultraviolet light treatment, PL is more effective in damaging cells and inactivating bacteria (Cheigh et al., 2012).

The overall goal of this project was to develop an effective non-thermal processing method to inactivate *Salmonella* on the surface of raw almonds and black peppercorn prior to market distribution. The quality degradation due to the PL treatment was also evaluated. Finally, time and temperature combinations that reach large log-CFU/g reduction with minimal effect to quality were determined.

Chapter 2

LITERATURE REVIEW

2.1 Almond and black pepper production

Almonds have been one of the most popular nuts in USA in recent years since they are rich in fiber, protein, magnesium and vitamin E . and have been studied extensively for their health benefits such as heart health, diabetes and weight management (Szalay, 2017). In the United States, production of almonds is concentrated in California where almond is the sixth leading agricultural product and top agricultural export (New World Encyclopedia-Almonds). Once the nuts are harvested and collected from the field, they are dumped into a kit and then a vibrating screen helps remove orchard debris (Lowman et al., 1992). A destoner and detwigger can remove things such as stones, sticks and leaves. Precleaned almonds are transferred to storage area for further processing. A hulling cylinder is use to crack the almond hulls and then they pass through screens to get some products such as in-shell almonds, almond meat and trash. (Varzakas et al., 2010). In-shell almonds may undergo further processing into various final products and the almond meat is sold as a raw product or further processed by slicing, roasting or smoking (Du et al., 2007). Several pasteurization methods including oil roasting, steaming, blanching, and propylene oxide (PPO) are used to increase the safety of the products while maintaining taste, texture and nutrients (Mercola, 2018).

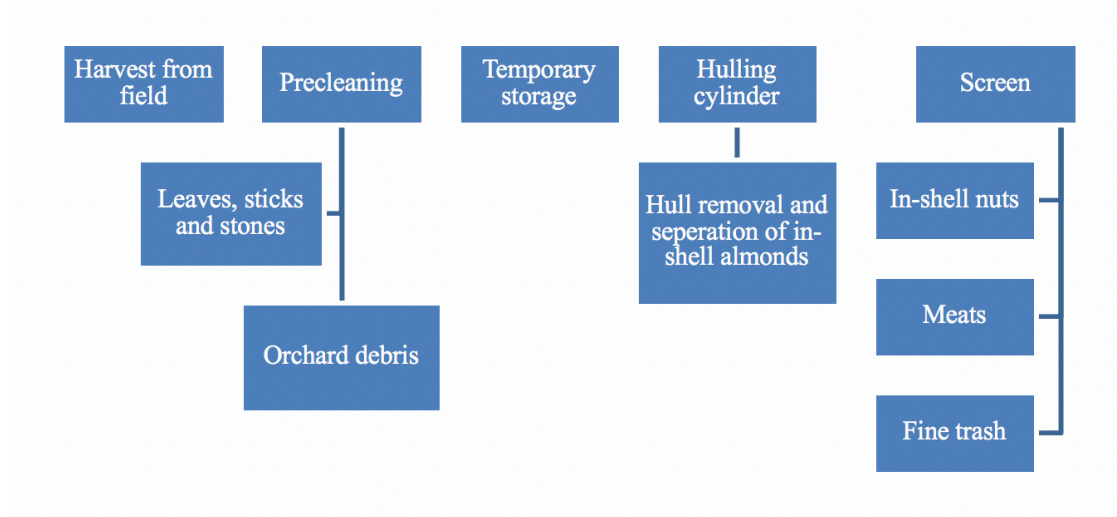


Figure 2.1. Fresh almonds processing flow chart (Priya Varshney, 2017)

Almond meats can be used to produce almond milk and oils for different purposes (Jaffa and Myer, 1908). The hulls are rich in nutrition and cheaper than grain, and therefore they are used to feed animals (Huber, 2018).

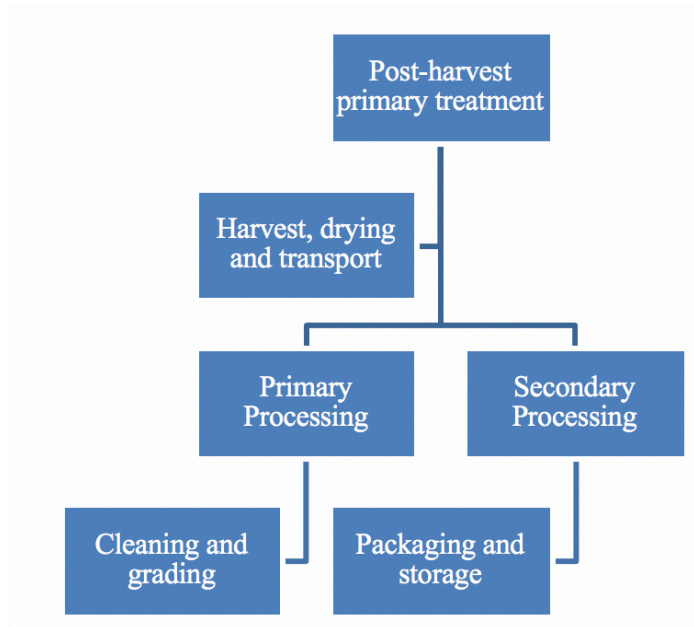


Figure 2.2. Black pepper processing flow chart (Food and Agriculture Organization of the United Nations, 2007).

Nowadays, there are two types of pepper sold in market: black and white pepper. Black peppercorn is the end point of seed maturation (Zachariah et al., 2008). Not only is it used as spice, but it has medical usage such as carminative (Sandhu et al., 2005). The processing of black pepper consists of three parts. Post-harvest primary treatment is able to make integrity of grains before processing steps. Harvesting and drying are susceptible to contamination. Primary processing aims to remove fractions and clean pepper polluted grains. Finally, the cleaned pepper is treated in the secondary processing, which is responsible for making it more valuable (Food and Agriculture Organization of the United Nations, 2007).

2.2 Bacterial pathogens associated with almonds and peppercorn

People usually think that low-moisture food is safe to eat, and the consumption has grown rapidly (Rawat and Seema, 2015). These food products are considered less susceptible to microbial spoilage and the growth of foodborne pathogens; however, some pathogens can survive in dry environments and outbreaks linked to low-moisture foods have increased (Sánchez et al., 2018). Particularly, the shelf-life of nuts can be over one year, and consumers may store nuts for additional time. Microbial populations on drying foods remain unchanged at ambient temperatures and *Salmonella* cross-contamination of nuts has been traced to factors such as poor control and sanitation process, where *Salmonella* can survive for long periods and their heat resistance increases with reduced moisture (Gao et al., 2011).

There are various ways that almond and pepper could get contaminated. *Salmonella* contamination usually happens when fecal matter is transferred to almonds or peppercorn through water and soil during ripening, especially when the fields are sprayed with contaminated water for irrigation (Allende et al., 2015).

Also, low-moisture foods can be contaminated during transportation from farm to factory or from processing product to consumers when the truck is not cleaned after loading animals or animal products (Gorris and Leon, 2005). In processing and handling, they might be contaminated by poor sanitary conditions such as dirty water and contaminated facilities (Centers for Disease Control and Prevention, 2017). Typically, spice farming is different from traditional field farming because many farmers grow their crop on a small scale on hills where the plants are surrounded by dirt, dust and animals, making foodborne pathogen contamination possible (Tainter et al., 2001).

There were two *Salmonella* outbreaks traced to California almonds in 2001 and 2004, one in Sweden in 2006, and one *E. coli* O157:H7 outbreak occurred in 2013 (Danyluk et al., 2007). Besides, there has been an outbreak due to *Salmonella* Enteritidis (SE) in Canada (Isaacs et al., 2005).

Store-brand organic black peppercorns were recalled from all grocery stores after regular testing by the USDA in Arizona due to potential *Salmonella* contamination (News Desk, 2014). *Salmonella* Montevideo was associated with black pepper in 2010 (Centers for Disease Control and Prevention, 2010).

2.2.1 *Salmonella*

Salmonella is one of the most common causes of food poisoning in the United States, and it can cause serious illness such as diarrhea and fever (Pope et al., 1998). It ranks as the second most common intestinal infection in the United States (Santos et al., 2001). Though *Salmonella* outbreaks are associated with eggs, meat and poultry, *Salmonella* can survive in dry environment, especially in high-protein and high-fat food for several years (Shachar et al., 2018). It was reported that *Salmonella* outbreaks even came from tree nuts, peanuts, and sesame seeds (Moussavi et al., 2009-2013).

2.2.2 *E. coli* O157:H7

E. coli O157:H7 is a foodborne pathogen that has been a critical public health issue in the United States (Tauxe and Robert, 1997). It was first recognized as a pathogen in 1982, and it is the most common cause of hemolytic uremic syndrome (HUS) and the leading cause of kidney failure among children in the United States (Hilborn et al., 2000). In addition to fresh produce, low moisture foods have been involved in *E. coli* O157:H7 outbreaks in recent years. In 2001, Canadian announced

13 illnesses from *E. coli* O157:H7 due to the consumption of shelled walnuts. Later between 2010 and 2011, there were eight *E. coli* O157:H7 infections in three U.S. States associated with hazelnuts from California (Berry et al., 2011).

2.3 Interventions to control foodborne pathogens in almond and peppercorn

2.3.1 Decontamination methods for almonds

Contamination of the nuts may happen at any point in the harvesting and packaging process (Podolak et al., 2010). In the United States, almonds are required to go through a minimum 4-log CFU/g reduction in *Salmonella* and 5-log reduction is required for pasteurization purpose (Pan et al., 2012).

Some processing technologies have been evaluated and approved by the FDA and are used to reduce pathogens on almonds, which include oil roasting, dry roasting and blanching, steam processing, and propylene oxide treatment (Almond Board of California, 2007). USDA developed a new effective strategy of almond pasteurization, a combination of hot air roasting and infrared heat. This method has been used for commercial-scale production, eliminating *Salmonella* over 4-log or 99.99%. However, the costs and time investments are huge even though it is more environmentally friendly without any use of chemicals (Food Safety News, 2012).

2.3.1.1 Chemical Decontamination

Currently there are two main methods to pasteurize raw almonds. One is using steam to sanitize the nuts and the other is using propylene oxide. The industry has been aware that PPO has to be discontinued with the constant rising public criticism while it is effective in reducing *Salmonella* population by over 5-log on raw almonds several days after treatment (Danyluk et al., 2005). Propylene oxide is an organic toxic

chemical compound that is usually used in furniture, insulation and other plastics product (ICIS Chemical Business, Rihian 2017). It is also used in decontamination of food products and plastic medical stuff, and it can cause some eye and respiratory disease (U.S. IRIS, 1999).

Other chemical treatments that have been used include acidic solution sprayed onto the surface. An estimated 5-log reductions on raw almonds can be reached after three continuous sprays with 10% citric acid (Pao et al., 2006). Chemical pasteurization is fast and effective for bacteria elimination though people may have concerns that those chemical residues could cause disease.

Most almonds in the USA have been sterilized with PPO and both steamed, and PPO treated almonds are marked as raw (Alliance for natural health, 2012).

Although the mandatory minimum criterion is 4-log reduction of *Salmonella* on almonds, this organic chemical is able to achieve 5- log pathogen reduction using Standard Operating Procedure while following PPO pasteurization parameters (Almond Board of California, 2008). This organic chemical is so cheap that industry prefer to use chemical treatment for saving time and money. It is effective at killing pathogens and does not change the nutritional and sensory quality of almonds. However, the total process takes over 5 days and the residual limit of PPO is 300 ppm (Isikber et al., 2004).

2.3.1.2 Steam Decontamination

Steam pasteurization is very effective for reducing pathogens in foods, and it has significant reduction efficacy in *Salmonella* on raw almonds. It is called high temperature short time (HTST) treatment. A superheated steam is used to pasteurize almonds which are exposed to hot air for several seconds around 100 °C then they are

transferred to a dehydration device where the air temperature is around 200°C to remove moisture (Almond Lane, 2018). Heating almonds at 90°C for 10-15 min and holding at 80°C for over 22 min can reach over 5-log reductions and over 4-log reductions of *Salmonella*, respectively (Bingol et al., 2011). Additionally, it was shown that *Salmonella* Enteritidis are totally eliminated when almonds were treated with 115°C steam followed by 70 s infrared heating (Bari et al., 2010).

The last step of steam pasteurization is drying to lower the moisture level of almonds. People use a controlled condensation pasteurization (CCP) system to remove water from almonds. The high temperature above 70 °C causes loss of nutrients as well as sensory characteristics and visual quality degradation. However, it is much safer than and more effective than in reducing pathogenic bacteria in foods, and it is important that steam treatment will not leave residues on food products.

2.3.1.3 Non-thermal Sterilization

The goal of non-thermal pasteurization process is to only minimally affect the quality of food products and achieving good killing effect. Irradiation itself is unlikely to eliminate *Salmonella* on raw almond (Prakash et al., 2010). Lactic acid spray along with near-infrared radiation (NIR-LA) heating for inactivating *Salmonella enterica* serovar Enteritidis was also investigated on almond. Cold air plasma treatment achieved a more than 5-log reduction, which is required by U.S. FDA for an alternative inactivation technology for food products although it is hard to be applied to commercial treatment levels (Hertwig et al., 2017). Although the temperature of the almonds is not increased significantly by this treatment, it is not known if the nutritional and sensory quality is maintained.

2.3.2 Decontamination methods for peppercorn

In the spice industry, there are three methods used for bacterial reduction: steam, ethylene oxide and irradiation (Leistritz, 1997). Compared with chemical sterilization, steam is a more acceptable method which is so effective and safe that consumers are willing to accept it. Although the popularity of irradiation is increasing, people still have big concerns about whether it is safe or not (Leistritz, 1997).

2.3.2.1 Steam Decontamination

Steam pasteurization is getting popular because it satisfies most customers' desire for its safety. This method is widely applied to white and black pepper in the US (Gurtler et al., 2014). Black peppercorn is usually placed on a rapid flow of high-pressure steam between 100 and 200 °C for a very short time followed by drying with hot air and rapid cooling because the hot steam condensing on the surface of peppercorn can kill microorganisms (Joanna et al., 2014). A continuous steam sterilization has been proved useful for decontamination of spices. This method can achieve 5-log reduction of *Salmonella* without changing flavor and appearance attributes of raw product and this process is easy to control and monitor.

Therefore, this technique is adopted by industry for reduction of pathogens with many benefits such as rapid cycle time, no chemical residue, easy to operate and low maintenance cost. On the other hand, there are disadvantages. For example, oil cannot be sterilized by steam and there might be unfavorable color change of chlorophyll since some substances might be damaged by exposure to hot steam (Gorgani et al., 2017).

2.3.2.2 Chemical Decontamination

It is very likely for pepper to get contaminated with insects during growth and people disinfect them by fumigation with ethylene oxide. It was reported that 40% to 85% of the spices in US are decontaminated with ethylene oxide each year (Suerfoodly, 2017). Ethylene oxide fumigation is widely used for microbial reduction. Advantage is that it does not alter the flavor and appearance of black pepper significantly. The main issue with this process is that the fumigant is toxic and harmful for humans, also, it impairs the content of pepper and leaves some chemical residue which is deleterious for environment (McKee, 1995).

2.3.2.3 Gamma irradiation

FDA has approved this method for microbial disinfestation of spices (Thayer et al., 1996). Gamma irradiation (10 kGy) has been used for some types of spice pasteurization (SádECKá, 2007). Black peppercorn is usually prepackaged to prevent other contamination and maintain the content and flavor (Schweiggert et al., 2007). In this case, irradiation has the advantage that the product can be treated in its package and the process is flexible with good killing effect; however, this treatment does have adverse effects on aroma, color and other sensory characteristics of spices and also the packaging materials, leading to bad product quality. The FDA has issued a maximum dose of 30 kGy for the irradiation of spices (FDA, 2008).

2.4 Pulsed light technology

Pulsed light or pulsed UV-light process can overcome some limitations of thermal processing techniques, gaining more popularity in food processing. Pulsed light refers to non-thermal technologies with high-intensity light pulses of short durations on surfaces of foods and high-transmission liquids to achieve sterilization

and decontamination (Bhavya et al., 2017). Generally, most energy from pulsed light comes from the UV portion of the spectrum. During the late 1970s in Japan, intense and short pulses of UV light were supplied by inert-gas flash lamps for microbial inactivation (Singh et al., 2001). After FDA approved this technology in foods, food industry adopted it in 1996 (Rowan and Neil, 2019). Electromagnetic energy is stored in a capacitor during one second fraction, and then it is release as light during a very short time (millisecond). Pulsed light covers wavelengths from 200-1100 nm and pulses used in food industry can emit 1-20 flashes per second on surfaces with energy in the range of 0.01-50 J/cm² (Elmnasser, et al., 2007).

Pulsed light is effective against multiple microorganisms and spores in various food products. It could reach higher microbial inactivation than continuous UV light (Palmieri et al., 2005). Pulsed light inactivation of pathogens such as *L. monocytogenes*, *E. coli*, *Salmonella* Enteritidis, *Pseudomonas aeruginosa*, *Bacillus cereus* etc. in fresh produce, meat, fish and other foods has been well-studied, and it requires 5-log reduction process in fresh produce by the U.S. FDA (US FDA 2004). Additionally, pulsed light is also effective in inactivating pathogens in low-moisture foods, such as almonds and black pepper. An obvious benefit of pulsed light over UV is that energy is delivered very quickly, and the operation costs is much lower (Sauer et al., 2009).

Although pulsed light inactivation of bacteria pathogens has been well-documented, studies on pulsed light inactivation of *Salmonella* in almond and black peppercorn is still limited. Currently main issue we have to deal with is that the pulsed light application causes heating which might damage the quality of samples. In

addition, samples exposed to pulsed light cannot be treated evenly (Huang and Chen, 2014).

2.5 Research objectives

The overall objective of this research was to develop an effective pulsed light processing method to inactivate *Salmonella* on almonds and black peppercorns and in their products. Additionally, impact of this method on product quality was evaluated.

REFERENCES

- Allende, Ana, and James Monaghan. "Irrigation water quality for leafy crops: a perspective of risks and potential solutions." *International journal of environmental research and public health* 12.7 (2015): 7457-7477.
- Alliance for natural health, 2012. Available at: <https://anh-usa.org/usda-protect-us-from-raw-almonds/>
- Almond Board of California, 2007. Guidelines for validation of dry roasting processes. Available at: <http://www.almondboard.com/Handlers/Documents/Dry-Roast-Validation-Guidelines.pdf>.
- Almond Board of California, 2008. Guidelines for Validation of Propylene Oxide Pasteurization Available at: <https://www.almonds.com/sites/default/files/content/attachments/ppo-validation-guidelines.pdf>
- Almond Lane, 2018. Available at: <https://www.almondlane.us/our-process>
- Bari, Latiful, et al. "Effectiveness of superheated steam and gas catalytic infrared heat treatments to inactivate *Salmonella* on raw almonds." *Foodborne pathogens and disease* 7.7 (2010): 845-850.
- Berry, Elaine D., and James E. Wells. "Escherichia coli O157: H7: recent advances in research on occurrence, transmission, and control in cattle and the production environment." *Advances in food and nutrition research*. Vol. 60. Academic Press, 2010. 67-117.
- Bhavaya, M. L., and H. Umesh Hebbar. "Pulsed light processing of foods for microbial safety." *Food Quality and Safety* 1.3 (2017): 187-202.
- Bingol, Gokhan, et al. "Infrared pasteurization of raw almonds." *Journal of Food Engineering* 104.3 (2011): 387-393.
- Black pepper. "Food and Agriculture organization of the United Nations." *Spices Processing Toolkit*. 2007.
- Centers for Disease Control and Prevention, 2010. Available at: <https://www.cdc.gov/Salmonella/2010/montevideo-5-4-2010.html>
- Centers for Disease Control and Prevention, 2017. Available at: https://www.cdc.gov/healthywater/global/wash_statistics.html

- Danyluk, Michelle D., Aaron R. Uesugi, and Linda J. Harris. "Survival of *Salmonella* Enteritidis PT 30 on inoculated almonds after commercial fumigation with propylene oxide." *Journal of food protection* 68.8 (2005): 1613-1622.
- Danyluk, Michelle D., et al. "Prevalence and amounts of *Salmonella* found on raw California almonds." *Journal of food protection* 70.4 (2007): 820-827.
- Du, Wen-Xian, Michelle D. Danyluk, and Linda J. Harris. "Evaluation of cleaning treatments for almond-contact surfaces in hulling and shelling facilities." *Food protection trends* 27.9 (2007): 678-683.
- Elmnasser, N., et al. "Pulsed-light system as a novel food decontamination technology: a review." *Canadian journal of microbiology* 53.7 (2007): 813-821.
- Ethylene Oxide Sterilization: Are ETO Treated Spices Safe? Available at: <https://www.superfoodly.com/ethylene-oxide-sterilization-non-eto-spices/>
- Food and Drug Administration, 2008. FDA Regulatory Report on Irradiation of Food Packaging Materials. Available at: <https://www.fda.gov/food/ingredients-additives-gras-packaging-guidance-documents-regulatory-information/regulatory-report-irradiation-food-packaging-materials>
- Food Safety News, 2012. Available at: <https://www.foodsafetynews.com/2012/02/usda-develops-new-more-efficient-method-of-almond-pasteurization/>
- Food technology, 2017 Available at: <https://discoverfoodtech.com/hulled-shelled-almond-processing/>
- Gao, M., et al. "Pasteurization process development for controlling *Salmonella* in in-shell almonds using radio frequency energy." *Journal of Food Engineering* 104.2 (2011): 299-306.
- Gorgani, Leila, et al. "Piperine—the bioactive compound of black pepper: from isolation to medicinal formulations." *Comprehensive Reviews in Food Science and Food Safety* 16.1 (2017): 124-140.
- Gorris, Leon GM. "Food safety objective: an integral part of food chain management." *Food Control* 16.9 (2005): 801-809.
- Gurtler, Joshua B., Michael P. Doyle, and Jeffrey L. Kornacki, eds. *The microbiological safety of low water activity foods and spices*. New York: Springer, 2014.

- Hertwig, Christian, et al. "Inactivation of *Salmonella* Enteritidis PT30 on the surface of unpeeled almonds by cold plasma." *Innovative food science & emerging technologies* 44 (2017): 242-248.
- Hilborn, E. D., et al. "An outbreak of *Escherichia coli* O157 [ratio] H7 infections and haemolytic uraemic syndrome associated with consumption of unpasteurized apple cider." *Epidemiology & Infection* 124.1 (2000): 31-36.
- Huang, Yaixin, and Haiqiang Chen. "A novel water-assisted pulsed light processing for decontamination of blueberries." *Food microbiology* 40 (2014): 1-8.
- Huber, J. Tal. *Upgrading Residues and By-products for Animals*: 0. CRC Press, 2018.
- Huber, J. Tal. *Upgrading Residues and By-products for Animals*: 0. CRC Press, 2018.
- ICIS Chemical Business, Rihian 2017. Available at: <https://www.icis.com/subscriber/icb/2017/07/06/10122038/the-propylene-oxide-problem/>
- Isaacs, S., et al. "An international outbreak of salmonellosis associated with raw almonds contaminated with a rare phage type of *Salmonella* Enteritidis." *Journal of food protection* 68.1 (2005): 191-198.
- Isikber, A. A., et al. "Propylene oxide: a fumigant for quarantine purposes as a potential alternative to methyl bromide." *Compte Rendu de la Réunion* 27 (2004): 277.
- Jaffa, Myer Edward. *Nuts and their uses as food*. No. 332. US Dept. of Agriculture, 1908.
- Joanna Brodowska, Agnieszka, et al. "The impact of ozone treatment on changes in biologically active substances of cardamom seeds." *Journal of food science* 79.9 (2014): C1649-C1655.
- Kapoor, I. P. S., et al. "Chemistry and in vitro antioxidant activity of volatile oil and oleoresins of black pepper (*Piper nigrum*)." *Journal of agricultural and food chemistry* 57.12 (2009): 5358-5364.
- Leistritz, W. "Methods of bacterial reduction in spices." (1997).
- Live Science Contributor, 2017. Available at: <https://www.livescience.com/51627-almonds-nutrition.html>
- Lowman, Ben. *Bareroot nursery equipment catalog*. No. 9224. USDA Forest Service, Missoula Technology and Development Center, 1992.

- McKee, L. H. "Microbial contamination of spices and herbs: a review." *LWT-Food Science and Technology* 28.1 (1995): 1-11.
- Mercola, Food Facts, 2018 Available at: <https://products.mercola.com/raw-snacks/>
- Moussavi, Mahta, et al. "Growth of *Salmonella* and Other Foodborne Pathogens on Inoculated Inshell Pistachios during Simulated Delays between Hulling and Drying." *Journal of food protection* 82.5 (2019): 815-825.
- New World Encyclopedia-Almonds, 2018. Available at: <https://www.newworldencyclopedia.org/entry/Almond>
- News Desk, "Organic Black Peppercorns Recalled for Possible *Salmonella* Contamination." *Food Safety News*, 2014
- Palmieri, Luigi, and Domenico Cacace. "High intensity pulsed light technology." *Emerging technologies for food processing*. Academic Press, 2005. 279-306.
- Pan, Zhongli, et al. "Review of current technologies for reduction of *Salmonella* populations on almonds." *Food and bioprocess technology* 5.6 (2012): 2046-2057.
- Pao, Steven, Aref Kalantari, and Guangwei Huang. "Utilizing acidic sprays for eliminating *Salmonella enterica* on raw almonds." *Journal of food science* 71.1 (2006): M14-M19.
- Podolak, Richard, et al. "Sources and risk factors for contamination, survival, persistence, and heat resistance of *Salmonella* in low-moisture foods." *Journal of food protection* 73.10 (2010): 1919-1936.
- Poppe, Cornelius, et al. "*Salmonella typhimurium* DT104: a virulent and drug-resistant pathogen." *The Canadian Veterinary Journal* 39.9 (1998): 559.
- Prakash, A., et al. "The effects of ionizing irradiation on *Salmonella* inoculated on almonds and changes in sensory properties." *Radiation Physics and Chemistry* 79.4 (2010): 502-506.
- Rawat, Seema. "Food Spoilage: Microorganisms and their prevention." *Asian Journal of Plant Science and Research* 5.4 (2015): 47-56.
- Rowan, Neil J. "Pulsed light as an emerging technology to cause disruption for food and adjacent industries—Quo vadis?." *Trends in Food Science & Technology* (2019).

- SádEČká, Jana. "Irradiation of spices—a review." *Czech J. Food Sci* 25.5 (2007): 231-242.
- Sánchez-Maldonado, Alma Fernanda, Alvin Lee, and Jeffrey M. Farber. "Methods for the control of foodborne pathogens in low-moisture foods." *Annual review of food science and technology* 9 (2018): 177-208.
- Sandhu, Davinder S., and Michael Heinrich. "The use of health foods, spices and other botanicals in the Sikh community in London." *Phytotherapy Research: An International Journal Devoted to Pharmacological and Toxicological Evaluation of Natural Product Derivatives* 19.7 (2005): 633-642.
- Santos, Renato L., et al. "Animal models of *Salmonella* infections: enteritis versus typhoid fever." *Microbes and infection* 3.14-15 (2001): 1335-1344.
- Sauer, Anne, and Carmen I. Moraru. "Inactivation of *Escherichia coli* ATCC 25922 and *Escherichia coli* O157: H7 in apple juice and apple cider, using pulsed light treatment." *Journal of Food Protection* 72.5 (2009): 937-944.
- Schweiggert, Ute, Reinhold Carle, and Andreas Schieber. "Conventional and alternative processes for spice production—a review." *Trends in Food Science & Technology* 18.5 (2007): 260-268.
- Shachar, Dina, and Sima Yaron. "Heat tolerance of *Salmonella* enterica serovars Agona, Enteritidis, and Typhimurium in peanut butter." *Journal of food protection* 69.11 (2006): 2687-2691.
- Singh, R. Paul, and Dennis R. Heldman. *Introduction to food engineering*. Gulf Professional Publishing, 2001.
- Tainter, Donna R., and Anthony T. Grenis. *Spices and seasonings: a food technology handbook*. John Wiley & Sons, 2001.
- Tauxe, Robert V. "Emerging foodborne diseases: an evolving public health challenge." *Emerging infectious diseases* 3.4 (1997): 425.
- Thayer, Donald W., et al. "Radiation pasteurization of food." *Council for agricultural science and technology* 7.1 (1996).
- U.S. Environmental Protection Agency. *Integrated Risk Information System (IRIS) on Propylene Oxide*. National Center for Environmental Assessment, Office of Research and Development, Washington, DC. 1999.

US Food and Drug Administration. (2004). FDA Guidance to Industry, 2004: Recommendations to Processors of Apple Juice or Cider on the Use of Ozone for Pathogen Reduction purposes.

Varzakas, T., G. Zakyntinos, and I. Arvanitoyannis. "Application of failure mode and effect analysis (FMEA) and cause and effect analysis in conjunction with ISO22000 to an almond processing plant." *Options Méditerranéennes* 94 (2010): 289-97.

Zachariah, T. John, and V. A. Parthasarathy. "Black pepper." *Chemistry of spices* 196 (2008): 21.

Chapter 3

PASTEURIZATION OF RAW ALMONDS USING PULSED LIGHT ALONG WITH PRIOR WATER DIPPING OF ALMONDS

3.1 Abstract

For the purpose of microbial inactivation, pulsed-light treatments of low intensity, medium intensity and high intensity were conducted to evaluate decontamination efficacy of *Salmonella* on wet raw almonds for both small scale and large-scale experimental setups. Raw almonds were dip inoculated with *Salmonella* and treated with pulsed light. Inoculated almonds after drying were immersed in tap water for 1 min followed by pulsed light treatments. Over 5-log reduction on *Salmonella* was achieved for both small and large-scale samples. According to the inactivation efficacy and product quality, the distance between lamp and sample was set at 19 cm. To investigate the effect of times of dipping almonds in water, inoculated almonds (300 g) were immersed in tap water for 0.5 min followed by PL for some time and immersed again for another 0.5 min followed by PL for the same time interval. As for larger amount almond (500 g), single continuous treatment achieved over 5-log reduction of *Salmonella* after 18 min, while two-time water dipping only got 4.5-log reduction for 22 min PL treatment. In conclusion. this study suggested a good pasteurization method for wet raw almonds in future applications.

3.2 Introduction

Almonds are rich in various nutrients such as magnesium, vitamin E, monounsaturated fatty acids and protein. They are also claimed to have health benefits such as a reduction in the low-density lipoprotein cholesterol level (Chen et al., 2006). The almond market in US has been increasing continuously in the past decades.

Almonds are commercially produced in California and US is the largest and dominant supplier of almonds around the world (Almond Board of California, 2017). The majority of almonds (approximately 70%) in the US are exported in shelled form and include roasted, blanched, sliced and chopped versions (Sumner et al., 2014). The shells and hulls are used in the livestock industry as feeding materials.

Traditionally, food with a low water activity has been considered as low risk for microbial safety, but it is now known that *Salmonella* can survive on low water activity ($a_w \leq 0.60$) foods and such foods had to be recalled due to *Salmonella* contamination (Brockmann et al. 2004). In 2003 to 2004, a worldwide recall of raw almonds was issued due to *Salmonella* contamination (Centers for Disease Control and Prevention, 2004). From 2000 to 2001, a *Salmonella* Enteritidis outbreak related to raw almond occurred in Canada and the USA and caused 168 people to become sick (CDPH, 2002). In 2004, 47 people got sick due to *Salmonella* Enteritidis contamination of California raw almonds in Canada and the USA (CDPH, 2004). Also, from 2005 to 2006, there were 15 cases of *Salmonella* Enteritidis infections due to raw almond in Sweden (MullerF et al., 2007). In 2012, raw almond were found to be contaminated with *Salmonella* Typhimurium in Australia (FSANZ, 2012), and in 2014, almonds and peanut butter were affected by *Salmonella* Braenderup in the USA (CDC, 2014b). In 2015, *Salmonella* Paratyphi B contaminated sprouted almond and cashew almond spreads in USA (CDC, 2016a).

After two main *Salmonella* outbreaks associated with California raw almonds, a mandatory minimum 4-log reduction of *Salmonella* was required in 2007 by the Almond Board of California (Federal Register, 2007). However, for pasteurization

purpose, 5-log reduction of *Salmonella* must be achieved as required by the Food and Drug Administration (Almond Board of California, 2007).

To decontaminate almonds, researchers have also explored traditional thermal processing methods and some novel technologies such as infrared heating, radio frequency heating, electron beam irradiation, hydrostatic pressure and cold plasma (Pan et al., 2012). It was found that steam pasteurization is an effective method to inactivate *Salmonella*. Enteritidis inoculated on raw almonds, eventually providing an over 5-log reduction of *Salmonella* Enteritidis after 25 s of steam treatment without visual quality degradation (Chang et al., 2010). Chemical treatments have also been applied (Pan et al., 2012). Propylene oxide is widely used on raw almonds and other tree nuts in the USA, and desirable inactivation of *Salmonella* attached to raw almonds by a 4-h treatment and 5 days of storage was achieved (Danyluk et al., 2005). In addition, high pressure and irradiation were explored. High pressure at 60,000 psi and 50°C for 5 min reduced S, Enteritidis on raw almonds to one log reduction (Goodridge et al., 2006). A 4-log CFU/g reduction of *Salmonella* Enteritidis PT30 on raw almonds was reached after 5.0 kGy irradiation, but that this intensity level induced significant sensory changes as indicated by a consumer panel, therefore, irradiation is not an ideal method to eliminate *Salmonella* on raw almonds (Prakash, A., et al. 2010). Irradiation can also make almonds lose protein content since they contain high unsaturated fatty acids which is easy to be damaged by protein radiolysis from irradiation treatment (Park et al., 1993; Farkas et al., 2011; Farkas et al., 1998). Other researchers pointed out that air plasma, O₂ plasma, N₂ plasma could reduce *Salmonella* Enteritidis PT30 by over 5-log, 4.8-log and 2.0-log, respectively after 15 min treatment, but the

treatments resulted in unfavorable color changes to the almonds' surfaces (Hertwig et al., 2017).

Pulsed-light (PL) is a microbial decontamination technology that has been proved to work effectively against various bacteria, fungi and viruses on agar plates or in culture liquids (Elmnasser et al., 2007). In contrast to conventional UV light, PL can produce a broad spectrum of light delivered in short bursts (Roberts et al., 2003), but the broad spectrum of UV light and its energy density contribute most to microbial inactivation (Palmieri et al., 2005). It is more flexible and rapid than conventional decontamination methods, does not produce residual compounds, has a low energy cost and is efficient in inactivating spoilage microorganisms in solid and liquid foods (Oms-Oliu et al., 2010); however, due to its high energy-density, intense PL-treatments can cause damage to sample surfaces and induce surface heating (Oms-Oliu et al., 2010). Although PL has shown great ability of inactivating microorganism, PL studies of low-moisture food is scarce.

Jun et al. (2003) used a pulsed light UV-system to inactivate fungal spores of *Aspergillus niger* in corn meal and a 4.93-log reduction was achieved by this treatment. PL treatment reduced *E. coli* O157:H7 on blueberries by over 4.9-log (Bialka et al., 2007). For a 6.25-mm thick layer of alfalfa seeds, a 4.89-log reduction of *E. coli* O157:H7 could be reached after exposure for 90s at a 8-cm distance ($5.6\text{J}/\text{cm}^2$) from the lamp (Sharma et Al., 2003). A dose of $58\text{J}/\text{cm}^2$ was required to reduce *Saccharomyces cerevisiae* by 7-log (Fine and Gervais, 2004). Pulsed UV light was also used as a nonthermal technology to treat peanut and soybean extracts (Yang et al., 2010).

In this study, we investigated the efficacy of PL-treatments on inactivating *Salmonella* attached to dry and wet raw almonds. The goal was to achieve an over 5-log reduction of *Salmonella* with minimum visual and chemical changes.

3.3 Materials and Methods

3.3.1 Bacterial strains and inoculum preparation

Four nalidixic-acid-resistant strains of *Salmonella enterica* (*S. Montevideo* 51, *S. Newport* H1073, *S. Typhimurium* 14028, and *S. Heidelberg* 4595J) were used in this study. The working cultures were maintained on tryptic soy agar (Difco Laboratories, Sparks, MD., U.S.A.) supplemented with 0.6% yeast extract and 50 µg/mL of nalidixic acid (Difco; TSAYE-N) at 4°C. Individual cultures were grown in tryptic soy broth (Fisher Scientific) supplemented with 0.6% yeast extract and 50 µg/mL nalidixic acid (TSBYE-N) overnight at 35 °C and transferred into a new tube of TSBYE-N for another 24 h at 35°C. The cultures was mixed to form a 4-strain cocktail of *Salmonella*. Bacterial cells were harvested by centrifugation at 4000 RPM for 10 min. The pellet was resuspended in 0.1% peptone water to yield a final concentration of $\sim 10^9$ CFU/ml.

3.3.2 Inoculation of almonds

Packaged raw almonds were purchased from a local store. For the small-scale study, 50 g of almonds were immersed in 400 ml of the *Salmonella* cocktail for 6 min. For the large-scale study, 300 or 500g of almonds were immersed in 800 ml of the *Salmonella* cocktail for 6 min. Inoculated samples were then dried in a biological safety hood for 4 h at room temperature before being stored at 4°C for 24 h to

facilitate bacterial attachment. The initial *Salmonella* level on almonds was $\sim 10^5$ CFU/ml.

3.3.3 PL and UV units

A modified commercial PL unit (Xenon Steripulse-XL RS-3000, Xenon Corp., Wilmington, MA) was used for this study. A PL lamp was mounted at the top of a home-built enclosed stainless-steel chamber (inner size 60 cm (L) \times 45 cm (W) \times 70 cm (H)) connected to a high-flow ozone destruction unit (Ozone Solutions Inc, Hull, IA). A height-adjustable shelf was placed inside the PL chamber to alter the distance between the almond sample and the PL lamp so that different PL intensity could be achieved. Pulses at wavelength of 180-1100 nm were generated at 3 pulses/s with a pulse width of 360 μ s. The intensity of PL was measured with a Vega laser power meter (Ophir Optronics, Wilmington, MA) coupled with a pyroelectric energy sensor (PE-50C, Ophir Optronics, Wilmington, MA). The wavelength setting was 300 nm with pulse width of 500 mm.

A home-built UV system with a stainless-steel chamber (size: 120 cm (L) \times 40 cm (W) \times 60 cm (H)) had four 90-cm long amalgam UVC lamps (265 W power/lamp, Heraeus Noblelight, Buford, GA) mounted on the top of the UV chamber. A UV radiometer (ILT77, International Light Technologies, Peabody, MA) was used to determine the UV intensity.

3.3.4 Pasteurization of raw almonds inoculated with *Salmonella*

3.3.4.1 UV and PL treatments of dry raw almonds

Dry almond samples (50 g) inoculated with *Salmonella* were placed in a small stainless-steel pan (size: 10 cm (L) \times 10 cm (W) \times 9 cm (H)), which was secured on a mechanical shaker (Orbi-shaker Jr, Benchmark Scientific, Sayreville, NJ) using duct tape. During PL or UV treatment, the shaker was turned on to agitate the samples inside the pan to achieve better uniform light exposure. A general system setup used for the whole study is shown in Figure 1. The almond samples were treated with UV for 30 min or PL for 3 min. The distance between UV lamps and almond samples was 20 cm and the UV intensity ~ 0.029 W/cm². The distance between the PL lamp and almond samples was 19 cm and the PL intensity was 0.75 W/cm².

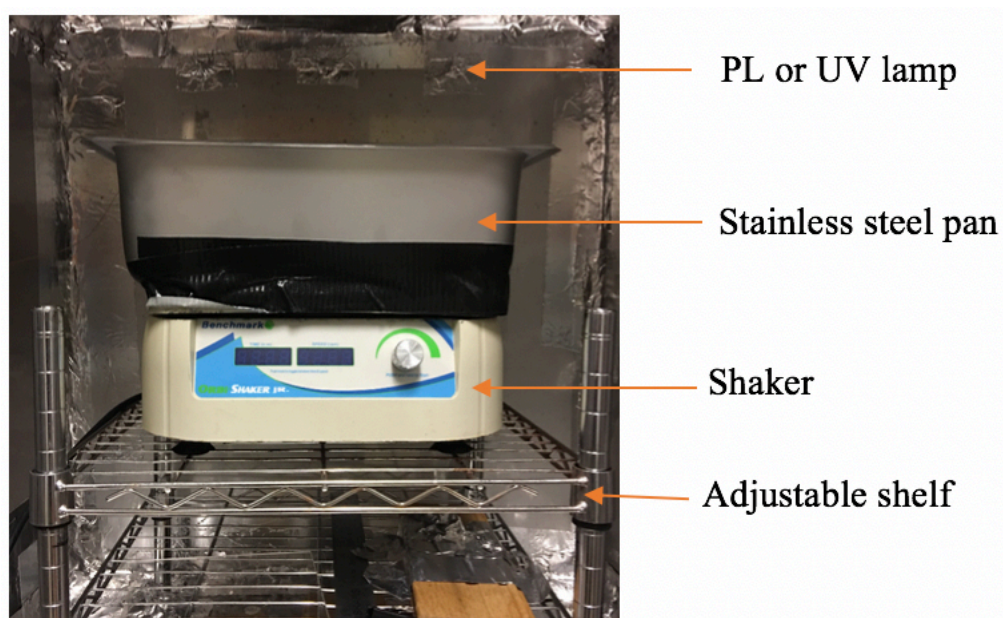


Figure 3.1. System setup. Dry or wet almonds in an open stainless-steel container were exposed to PL or UV while being shaken by a mechanical shaker.

3.3.4.2 PL treatments of wet raw almonds

Inoculated almonds were immersed in tap water for 1 min and then placed in the small stainless-steel pan (for the 50 g almond experiment) or a large stainless-steel pan (size: 30 cm (L) × 27 cm (W) × 9 cm (H)) (for the 300 g almond experiment) mounted on the mechanical shaker. The distance between the almond sample and the PL lamp was adjusted to vary between 16 and 27 cm to determine the effect of PL intensity on *Salmonella* inactivation. The PL intensities were 0.96, 0.75, 0.54, 0.45 and 0.39 W/cm² at 16, 19, 22, 25, and 27 cm, respectively.

To determine whether the number of times almonds were dipped in water would affect *Salmonella* inactivation, inoculated almonds (300 g or 500 g) were subjected to one of these two treatments: a) One-time water dipping: Almonds were immersed in tap water for 1 min followed by exposed to PL as described above and b) Two-time water dipping: Almonds were immersed in tap water for 0.5 min, treated by PL for 5 - 9 min while being shaken in the large stainless steel pan, immersed in tap water for another 0.5 min, and treated by PL for the same time interval used in the first PL treatment while being shaken. The distance between the almond sample and the PL lamp was set at 19 cm for this part of the study.

3.3.5 Microbial analysis

After PL and UV treatments, each 50-g untreated and PL-treated or UV-treated sample was transferred into a sterile filter bag containing 200 ml 0.1% peptone water and then pummeled in a laboratory stomacher for 2 min at 260 rpm. The homogenate was serially diluted in sterile 0.1% peptone water and surface plated on TSAYE-N followed by incubation at 35°C for 72 h. Presumptive colonies of *Salmonella* formed on the plates were counted.

3.3.6 Color and weight measurement

Uninoculated raw almonds (500 g) were treated using the two selected PL treatments. Raw almonds were immersed in tap water for 1 min and then treated by PL for 10 - 18 min while being shaken in the large stainless-steel pan. Alternatively, almonds were immersed in tap water for 0.5 min, treated by PL for 5 – 9 min while being shaken in the pan, immersed in tap water again for another 0.5 min, and treated by PL for the same time interval used in the first PL treatment while being shaken. The distance between the almond sample and the PL lamp was set at 19 cm for this part of the study. A colorimeter (Konica Minolta, Inc, Japan) was used to measure the color of almond samples. Randomly selected untreated and PL-treated almonds were measured in this study. Three spots on three individual almonds were recorded for color measurements. Color parameters were quantified in the Hunter L, a, b color space where L refers to lightness, ranging from 0 (blackness) to 100 (whiteness), positive a means red and negative a green, and positive b means yellow and negative b blue. The weight of almonds before water dipping and after PL treatment was measured.

3.3.7 Statistical analysis

Three replicates were conducted for all experiments. Colony counts were converted to log CFU/g or log CFU/mL. Means and standard deviations were calculated. Statistical analyses were conducted using JMP (SAS Cary, NC, USA). One-way analysis of variance (ANOVA) and Tukey's one-way multiple comparisons were used to determine significant differences between treatments at the 95% confidence level ($P < 0.05$).

3.4 Results

3.4.1 Effect of UV and PL treatments on the inactivation of *Salmonella* on dry almonds

Treatment with UV light for 30min with shaking achieved a 1.58-mean-log reduction of *Salmonella*. The long treatment time caused drying of the almonds, resulting in shriveling. PL for 3 min caused a 0.23-log reduction. Extending the treatment time over 3 min caused burning of almonds due to the large amount of heat generated during the PL treatment.

3.4.2 Effect of PL treatments on the inactivation of *Salmonella* on wet almonds – One-time water dipping

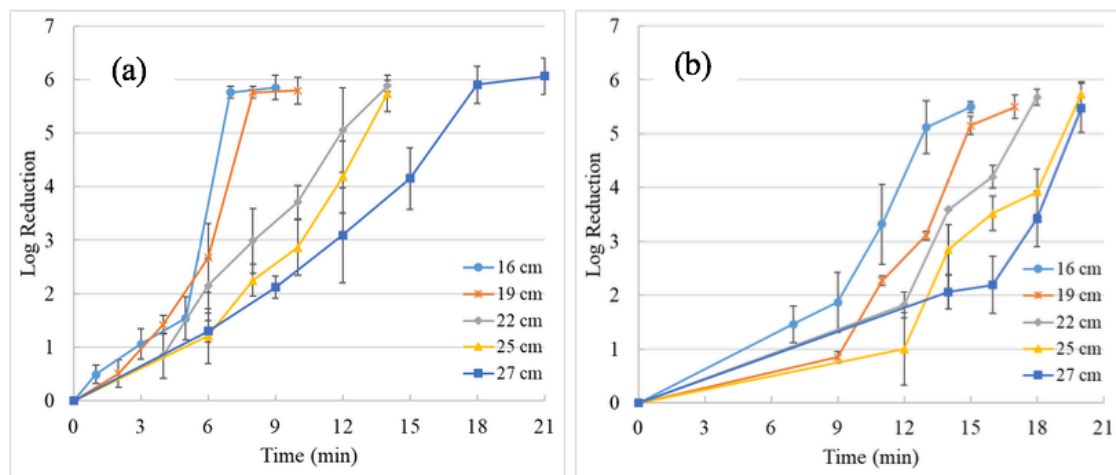


Figure 3.2. PL inactivation of *Salmonella* on wet almond. Raw almonds inoculated with *Salmonella* were immersed in tap water for 1 min before being exposed to PL at distances of 16 – 27 cm. Data represent mean of three replicates \pm standard deviation. (a) Small-scale study (50 g of almonds) (b) Large-scale study (300 g of almonds)

As one would expect, the log reductions of *Salmonella* increased with dosage and with exposure time. The distance of 19 cm was selected since it had a good killing effect and quality degradation of almonds after 10 min was minimal.

3.4.3 Effect of PL treatments on the inactivation of *Salmonella* on wet almonds and color and weight – comparison of one-time and two-time water dipping

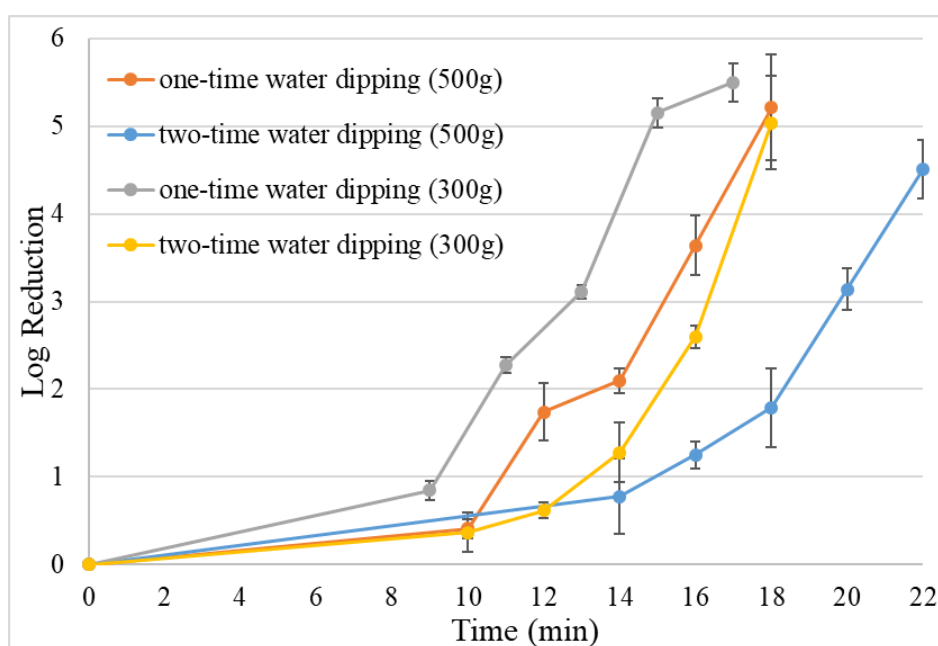


Figure 3.3. PL inactivation of *Salmonella* on wet almond – effect of two-time of water dipping. Raw almonds (300 g or 500 g) were inoculated with *Salmonella* and then subjected to one of these two treatments: a) One-time water dipping: Almonds were immersed in tap water for 1 min before being exposed to PL and b) Two-time water dipping: Almonds were immersed in tap water for 0.5 min, treated by PL for 5 - 9 min, immersed in tap water for another 0.5 min, and treated by PL for the same time interval used in the first PL treatment. The distance between the PL lamp and the almond samples was 19 cm for all the treatments. Data represent mean of three replicates \pm standard deviation.

For the same PL intensity at a distance of 19 cm, continuous treatment caused higher inactivation of *Salmonella*. The bacteria in the 500-g samples were more difficult to inactivate than those in the smaller samples (Figure 3.3), and one-time dipping treatment was more effective than the two-time dipping (Figure 3.3).

Table 3.1-Comparison of one and double water dipping on almond color and weight. Raw almonds (500 g) without *Salmonella* inoculation were treated by PL with the lamp placed at a distance of 19 cm.

Time (min)	Color: % decrease						% weight decrease
	L		a		b		
	Surface	Inside	Surface	Inside	Surface	Inside	
One-time water dipping							
10	5.80±0.12	1.10±0.10	2.30±0.02	2.40±0.15	0.45±0.05	0.10±0.06	0.30±0.04
12	4.50±0.32	2.40±0.19	3.60±0.04	3.60±0.10	5.6±0.15	4.0±0.15	0.80±0.06
14	11.90±0.14	2.90±0.30	3.90±0.04	3.20±0.12	6.20±0.24	5.70±0.17	0.90±0.04
16	12.10±0.22	3.30±0.10	3.70±0.04	4.34±0.12	10.30±0.15	9.40±0.24	1.00±0.14
18	11.60±0.13	3.50±0.19	5.10±0.05	4.50±0.14	11.20±0.12	10.50±0.16	1.23±0.11
Two-time water dipping							
14	4.33±0.28	1.91±0.23	5.22±0.22	3.35±0.24	6.62±0.17	4.62±0.26	0.72±0.04
16	9.62±0.18	5.37±0.21	7.67±0.24	5.22±0.25	13.85±0.16	5.89±0.28	0.88±0.04
18	11.64±0.14	7.04±0.16	8.24±0.23	6.61±0.33	14.87±0.30	10.64±0.29	1.12±0.45
20	13.78±0.12	8.25±0.15	17.21±0.54	7.26±0.16	22.14±0.18	11.00±0.20	1.75±0.14
22	17.58±0.28	12.37±0.25	34.89±0.34	8.68±0.13	30.72±0.15	13.70±0.09	1.57±0.10

The 18 min one-time water dipping resulted in much better surface and interior product quality than the 22-min double water treatment (Table 3.1). In addition, for a treatment time of 18 min, the two treatments resulted in similar color and weight parameters; however, one-time water dipping resulted in an over 5-log reduction of *Salmonella* while dipping the almonds in water twice achieved less than 2 log. Although high temperature and water evaporation during treatment cause approximate 1% weight loss, this loss is acceptable.

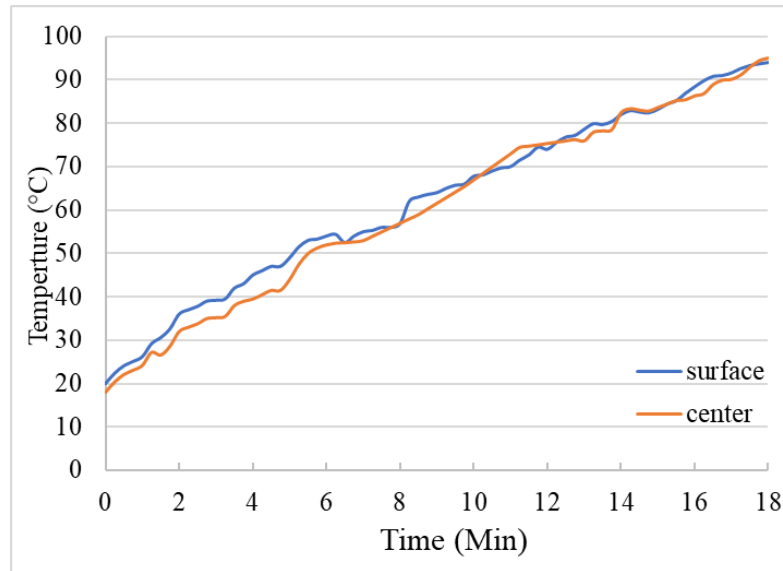


Figure 3.4. Surface and center temperature of almond during 18 min PL treatment using the one-time water dipping method and lamp – sample distance of 19 cm.

During this process, surface and center temperature of almonds was recorded, and it went up to over 90°C at the end. The temperature of surface is higher than center since the skin was exposed to pulsed light directly, after 10min treatment, the temperature inside almond was almost same with surface till the end of treatment. Therefore, around 5-log reduction might be achieved if central temperature is between 80°C and 90°C.



Figure 3.5. Pictures of untreated and 18 min PL-treated almonds (one-time water dipping method and lamp – sample distance of 19 cm). (a) Untreated control, (b) Untreated control showing the inside almond meats, (c) PL-treated almonds, and (d) PL-treated almonds showing the inside almond meats.

The pictures show that skin and meat appearance for control and treated almonds, there is slight visual color change after treatment although meat was not significantly affected by heat. Actually, the meat under skin still looked good as showed in Figure 3.5.

3.5 Discussion

Low water activity foods ($a_w < 0.70$) were once considered to be microbiologically safe from foodborne pathogen contamination (Gurtler et al., 2014). However, in recent years, *Salmonella* is a great concern in low-moisture food products

like nuts, flour, peanut butter and spice. This microorganism is born with the ability to persist for long periods in dry environment within raw materials, and unsanitary facilities (Finn et al., 2014). Therefore, the inactivation of *Salmonella* in low-moisture food is getting increased attention. Some technologies have been applied on inactivation of pathogenic microorganisms such as heat treatments, irradiation, high pressure, and chemical treatment. In this study, we investigated the efficacy of UV and PL treatment on almond, and those two methods are evaluated on dry and wet almonds.

Prior water dipping PL treatment was more effective than dry PL and UV treatment for decontamination of almonds. Typically, water activity and moisture content are both important predictors of heat resistance which determined the inactivation effect on *Salmonella* (GARCES-VEGA et al., 2019). During short time UV and PL treatment, sample surface was exposed to UV and PL directly all the time and both the interior and surface of almond experienced high temperature due to too much heat was generated in this process. Intense heat accelerated drying rate and decreased moisture in food to cause the damage of the almonds quality (Bari et al., 2010), it has also been found that low water activity limits the effectiveness of non-thermal processing technologies to improve the safety of nuts (Prakash, 2013). Dry-heat processes is less lethal than wet-heat for microbial disinfection, and it usually requires much longer time and higher temperature to obtain same lethality as wet-heat treatment (Doyle et al., 2012). Most importantly, dry PL treatment caused significant quality degradation of almonds and it could be clearly noticed that the skins of almonds were shriveled due to high temperature.

The decontamination efficacy of wet PL treatment was influenced by many factors such as sample size, distance from the light, and times of prior water dipping. It is well recognized that wet heat is more effective than dry heat for microbial inactivation and able to achieve better product quality (Scott et al., 2009). This is also supported by some other studies. Early in 1970, Goepfert and others found that thermal resistance of some selected microorganisms increased as the water activity decreased in low-moisture foods (Goepfert et al., 1970). He et al. (2013) also found that increased water activity reduced the thermal resistance of *Salmonella enterica* in peanut butter. In a study by Laroche et al. (2005), water activity affects heat resistance of microorganisms in food powder. Apparently, *Salmonella* in low-moisture environment is more tolerant to heat. Both high temperature and high water activity of almonds during PL treatment is able to achieve desired microbial inactivation result and little damage in quality. It is reported that PL intensity could affect the decontamination effect of wet treatment and higher *Salmonella* log reduction was achieved under higher PL intensity.

PL process produces short duration flashes of broad spectrum to kill pathogenic bacteria including microbial spores, viruses and other pathogens (DUNN, 1996). PL intensity significantly affect *Salmonella* inactivation on almond, the greater the intensity, the higher lethality (Oner et al., 2017). In other words, the inactivation efficacy is higher if almonds are closer to lamp (Ozer et al., 2006). Obviously, some unfavorable changes in almonds can be detected by weight and color parameters, when designing experiments, many distances from sample to PL lamp were tried to minimize the thermal damage on surface. In this study, it was found that 19cm (0.75 W/cm²) was the best choice among 16-27cm for both small and large-scale sample.

It was reported that treatment time could affect the decontamination effect of wet PL treatment (Huang et al., 2014). In our study, higher *Salmonella* log reduction was achieved after longer time treatment on dip inoculated almonds, and there was significant difference between short and long time in all treatments. It is likely that when the light treatment duration is high, the temperature would increase greatly, improving the effectiveness of PL treatments. This is also confirmed in our study and by Hillegas (Hillegas et al., 2003), who found the surface temperature of honey increase from 20 to 100 °C when exposed to PL for much longer time.

The scale of almonds may also influence the decontamination effect of PL treatment. Guo (2017) reported that *Salmonella* inoculated on lettuce was more difficult to be killed in large-scale experiments than small-scale ones. Uesugi (2007) also found that the efficiency of PL was influenced by inoculum size for surface treatment. It is likely that PL is able to work better on central and upper layers' almonds which will shadow the rest ones and protect them from exposure to pulsed light. In this study, significant difference was found in *Salmonella* reductions between small-scale (50g) and large-scale (300g-500g) ones. This may due to the huge difference in sample size that would affect the decontamination effect of dry PL treatments.

One-time dipping or two-time dipping can also affect PL decontamination. One-time dipping means dry almonds were dipped in tap water for 1 min before PL treatment. As both the moisture content and surface temperature will affect the inactivation efficacy, the advantage of this continuous PL treatment is that the temperature is always increasing and the whole process is rapid and efficient. But the texture, color, flavor and fatty acid of almonds were damaged due to long heat

treatment (Kumar et al., 2009). Moreover, continuous treatment might even cause surface burning with a deterioration in quality. In contrast, two-time dipping means dry sample was dipped in tap water for 30s and treated for some time, after cooling down to room temperature, our sample was dipped again for another 30s followed by same time treatment. It takes much longer time to complete this experiment where almonds will undergo temperature change. In this study, there is no significant product quality difference between these two treatments indicated by color and weight values, while the inactivation effect on *Salmonella* of continuous system is better than two-time dipping for both 300g and 500g almonds.

Quality of almonds varied from different treatments. The efficacy of PL treatment is usually limited by its low penetration, it can only destroy surficial pathogenic and spoilage microorganisms or in some media such as water (Elmnasser et al., 2007). Color and weight are two main indicators in this study, surface change of almonds was proved by decrease of L, a, b values, due to the high temperature during treatment, weight loss is a big issue that could also be found. Although the surface of almonds turned into dark after long time treatment, the nutritional quality of almond meat might not be affected a lot. In this study, fatty acid analysis was conducted to check the product quality.

3.6 Conclusion

The efficacy of wet PL treatment in inactivating *Salmonella* dip-inoculated on almonds was more effective than dry UV and PL treatments. As a rapid disinfection method with high microbial reduction efficacy for the low-moisture food industry, continuous wet PL treatment could be potentially used as an alternative to traditional

technologies to pasteurize nuts and other foods. For industrial scale needs, the applicability of PL treatment needs to be optimized to achieve good killing effect.

REFERENCES

- Almond Board of California, 2008. CALIFORNIA ALMOND INDUSTRY FACTS
- Almond Board of California, 2008. Guidelines for Validation of Oil Roasting Processes. Available at: <https://www.almonds.com/sites/default/files/oil-roast-validation-guidelines.pdf>
- Almonds grown in California; outgoing quality control requirements. *Federal Register*, 72(61), 15021–15036. Available at: <https://www.federalregister.gov/documents/2007/03/30/07-1557/almonds-grown-in-california-outgoing-quality-control-requirements>
- Available at:
https://www.almonds.com/sites/default/files/2016_almond_industry_factsheet.pdf
- Bari, Latiful, et al. "Effectiveness of superheated steam and gas catalytic infrared heat treatments to inactivate *Salmonella* on raw almonds." *Foodborne pathogens and disease* 7.7 (2010): 845-850.
- Bialka, Katherine L., and Ali Demirci. "Decontamination of Escherichia coli O157: H7 and *Salmonella* enterica on blueberries using ozone and pulsed UV-light." *Journal of Food Science* 72.9 (2007): M391-M396.
- Brockmann, Stefan O., Isolde Piechotowski, and Peter Kimmig. "*Salmonella* in sesame seed products." *Journal of food protection* 67.1 (2004): 178-180.
- California Department of Public Health [CDPH]. 2002. Environmental investigation of *Salmonella* Enteritidis Phage Type 30 outbreak associated with consumption of raw almonds.
- California Department of Public Health [CDPH]. 2004. Environmental investigation of *Salmonella* Enteritidis Phage Type 9c outbreak associated with consumption of raw almonds.
- Centers for Disease Control and Prevention (CDC). "Outbreak of *Salmonella* serotype Enteritidis infections associated with raw almonds--United States and Canada, 2003-2004." *MMWR. Morbidity and mortality weekly report* 53.22 (2004): 484.

- Centers for Disease Control and Prevention [CDC]. 2014b. Multistate outbreak of *Salmonella* Braenderup infections linked to nut butter manufactured by nSpired Natural Foods, Inc. (final update). Available at: <http://www.cdc.gov/Salmonella/braenderup-08-14/index.html>.
- Centers for Disease Control and Prevention [CDC]. 2016a. Multistate outbreak of *Salmonella* Paratyphi B variant L(+) tartrate(+) infections linked to JEM Raw brand sprouted nut butter spreads (final update). Available at: <http://www.cdc.gov/Salmonella/paratyphi-b-12-15/index.html>.
- Chang, S-S., et al. "Evaluation of steam pasteurization in controlling *Salmonella* serotype Enteritidis on raw almond surfaces." *Letters in applied microbiology* 50.4 (2010): 393-398.
- Chen, Chung-Yen, Karen Lapsley, and Jeffrey Blumberg. "A nutrition and health perspective on almonds." *Journal of the Science of Food and Agriculture* 86.14 (2006): 2245-2250.
- Danyluk, Michelle D., Aaron R. Uesugi, and Linda J. Harris. "Survival of *Salmonella* Enteritidis PT 30 on inoculated almonds after commercial fumigation with propylene oxide." *Journal of food protection* 68.8 (2005): 1613-1622.
- Doyle, Michael P., and Robert L. Buchanan, eds. *Food microbiology: fundamentals and frontiers*. American Society for Microbiology Press, 2012.
- DUNN, JOSEPH. "Pulsed light and pulsed electric field for foods and eggs." *Poultry Science* 75.9 (1996): 1133-1136.
- Elmnasser, N., et al. "Bacterial inactivation using pulsed light." *Acta alimentaria* 36.3 (2007): 373-380.
- Elmnasser, N., et al. "Pulsed-light system as a novel food decontamination technology: a review." *Canadian journal of microbiology* 53.7 (2007): 813-821.
- Farkas, József, and Csilla Mohácsi-Farkas. "History and future of food irradiation." *Trends in Food Science & Technology* 22.2-3 (2011): 121-126.
- Farkas, József. "Irradiation as a method for decontaminating food: a review." *International journal of food microbiology* 44.3 (1998): 189-204.
- Fine, F., and P. Gervais. "Efficiency of pulsed UV light for microbial decontamination of food powders." *Journal of food protection* 67.4 (2004): 787-792.

- Finn, Sarah, et al. "Transcriptomic Responses of *Salmonella* Species to Desiccation and Low-Moisture Environments: Extending Our Knowledge of How Bacteria Cope with Low-Moisture Stress." *The microbiological safety of low water activity foods and spices*. Springer, New York, NY, 2014. 49-66.
- Food Standards Australia New Zealand [FSANZ]. 2012. Warning and advice on raw almonds and possible *Salmonella* contamination. Available at: <http://www.foodstandards.gov.au/media/pages/mediareleases/mediareleases2012/23october2012warning5699.aspx>.
- GARCES-VEGA, FRANCISCO J., ELLIOT T. RYSER, and BRADLEY P. MARKS. "Relationships of Water Activity and Moisture Content to the Thermal Inactivation Kinetics of *Salmonella* in Low-Moisture Foods." *Journal of food protection* 82.6 (2019): 963-970.
- Goepfert, J. M., I. K. Iskander, and C. H. Amundson. "Relation of the heat resistance of *Salmonellae* to the water activity of the environment." *Appl. Environ. Microbiol.* 19.3 (1970): 429-433.
- Goodridge, L. D., John Willford, and N. Kalchayanand. "Destruction of *Salmonella* Enteritidis inoculated onto raw almonds by high hydrostatic pressure." *Food Research International* 39.4 (2006): 408-412.
- GUERRERO-BELTRÁN, JOSÉ A., GUSTAVO V. BARBOSA-CÁNOVAS, and BARRY G. SWANSON. "High hydrostatic pressure processing of peach puree with and without antibrowning agents." *Journal of food processing and preservation* 28.1 (2004): 69-85.
- Guo, Shuanghuan, Runze Huang, and Haiqiang Chen. "Application of water-assisted ultraviolet light in combination of chlorine and hydrogen peroxide to inactivate *Salmonella* on fresh produce." *International journal of food microbiology* 257 (2017): 101-109.
- Gurtler, Joshua B., Michael P. Doyle, and Jeffrey L. Kornacki. "The microbiological safety of spices and low-water activity foods: Correcting historic misassumptions." *The microbiological safety of low water activity foods and spices*. Springer, New York, NY, 2014. 3-13.
- He, Yingshu, et al. "Increased water activity reduces the thermal resistance of *Salmonella enterica* in peanut butter." *Appl. Environ. Microbiol.* 79.15 (2013): 4763-4767.

- Hertwig, Christian, et al. "Inactivation of *Salmonella* Enteritidis PT30 on the surface of unpeeled almonds by cold plasma." *Innovative food science & emerging technologies* 44 (2017): 242-248.
- Hillegas, Sara L., and Ali Demirci. "Inactivation of *Clostridium sporogenes* in clover honey by pulsed UV-light treatment." 2003 ASAE Annual Meeting. American Society of Agricultural and Biological Engineers, 2003.
- Huang, Yaixin, and Haiqiang Chen. "A novel water-assisted pulsed light processing for decontamination of blueberries." *Food microbiology* 40 (2014): 1-8.
- Jun, Soojin, et al. "Pulsed UV-light treatment of corn meal for inactivation of *Aspergillus niger* spores." *International Journal of Food Science & Technology* 38.8 (2003): 883-888.
- Kumar, Sanjeet, Sukumar Debnath, and Umesh H. Hebbar. "Pulsed infrared roasting of groundnuts and its quality." *International journal of food engineering* 5.4 (2009).
- Laroche, C., F. Fine, and P. Gervais. "Water activity affects heat resistance of microorganisms in food powders." *International Journal of Food Microbiology* 97.3 (2005): 307-315.
- López-Malo, A., et al. "Polyphenoloxidase activity and color changes during storage of high hydrostatic pressure treated avocado puree." *Food Research International* 31.8 (1998): 549-556.
- Muller, L. Ledet, et al. "Cluster of *Salmonella* Enteritidis in Sweden 2005-2006—suspected source: almonds." *Eurosurveillance* 12.6 (2007): 9-10.
- Oms-Oliu, Gemma, Olga Martín-Belloso, and Robert Soliva-Fortuny. "Pulsed light treatments for food preservation. A review." *Food and Bioprocess Technology* 3.1 (2010): 13.
- Oner, Manolya Eser. "Inactivation of *Salmonella* Enteritidis on Almonds by Pulsed Light Treatment." *Academic Food Journal/Akademik GIDA* 15.3 (2017).
- Ozer, Nil P., and Ali Demirci. "Inactivation of *Escherichia coli* O157: H7 and *Listeria monocytogenes* inoculated on raw salmon fillets by pulsed UV-light treatment." *International journal of food science & technology* 41.4 (2006): 354-360.
- Palmieri, Luigi, and Domenico Cacace. "High intensity pulsed light technology." *Emerging technologies for food processing*. Academic Press, 2005. 279-306.

- Pan, Zhongli, et al. "Review of current technologies for reduction of *Salmonella* populations on almonds." *Food and bioprocess technology* 5.6 (2012): 2046-2057.
- Park, J., et al. "High-protein texturized products of defatted soy flour, corn starch and beef: Shelf-life, physical and sensory properties." *Journal of food science* 58.1 (1993): 21-27.
- Prakash, A. "Non-thermal processing technologies to improve the safety of nuts." *Improving the Safety and Quality of Nuts*. Woodhead Publishing, 2013. 35-55.
- Prakash, A., et al. "The effects of ionizing irradiation on *Salmonella* inoculated on almonds and changes in sensory properties." *Radiation Physics and Chemistry* 79.4 (2010): 502-506.
- Roberts, Peter, and Andrew Hope. "Virus inactivation by high intensity broad spectrum pulsed light." *Journal of virological methods* 110.1 (2003): 61-65.
- Scott, Virginia N., et al. "Control of *Salmonella* in low-moisture foods I: Minimizing entry of *Salmonella* into a processing facility." *Food Protection Trends* 29.6 (2009): 342-353.
- Sharma, R. R., and A. Demirci. "Inactivation of *Escherichia coli* O157: H7 on inoculated alfalfa seeds with pulsed ultraviolet light and response surface modeling." *Journal of Food Science* 68.4 (2003): 1448-1453.
- Sumner, Daniel A., et al. "The economic impacts of the California almond industry." A report prepared for the Almond Board of California. Online publication. [http://aic.ucdavis.edu/almonds/Economic% 20Impacts% 20of% 20California% 20Almond% 20Industry_Full% 20Report_FinalPDF_v2.pdf](http://aic.ucdavis.edu/almonds/Economic%20Impacts%20of%20California%20Almond%20Industry_Full%20Report_FinalPDF_v2.pdf)[Google Scholar] (2014).
- Uesugi, Aaron R., Sarah E. Woodling, and Carmen I. Moraru. "Inactivation kinetics and factors of variability in the pulsed light treatment of *Listeria innocua* cells." *Journal of Food Protection* 70.11 (2007): 2518-2525.
- Yang, Weihua Wade, et al. "Use of pulsed ultraviolet light to reduce the allergenic potency of soybean extracts." *International Journal of Food Engineering* 6.3 (2010).

Chapter 4

STEAM-ASSISTED PULSED LIGHT PASTEURIZATION OF RAW ALMONDS

4.1 Abstract

To investigate the impacts of steam-assisted pulsed light inactivation on raw almonds, a medium intensity 0.25 J/cm^2 of pulsed light treatments were applied to inoculated raw almonds. Evaluations of using continuous PL-treatments and continuous treatments followed by holding samples for some time showed that 4.78 and 5.70-log reduction of *Salmonella* were achieved respectively for small-scale samples (50g). The quality values were also recorded for comparison purpose. For temperature concerns, the PL-treatment achieved 5.28-log reduction for 20min and 5.08-log reduction for 10min against dip-inoculated raw almonds (50g) in 70-80°C and 80-90°C respectively. For large scale (300g), the PL-treatment also worked effectively against dip inoculated *Salmonella* and achieve 5.32-log reduction right after treatment for 32min while the temperature was between 70°C and 80°C. Finally, 5.07-log reduction of *Salmonella* was reached after 18 min PL-treatment between 80°C and 90°C. Due to much higher temperature, the antimicrobial effect of 18 min was almost same as 32 min for large sample size, however, the quality parameters of shorter time were better than longer treatment. Therefore, PL-treated 18min with temperature between 80°C and 90°C was selected for fatty acid analysis. In short, this method could be used in future raw almonds pasteurization industries.

4.2 Introduction

People are getting more concerned about human health due to the prevalence of outbreaks associated with human pathogens like *E. coli* and *Salmonella*. In addition

to the health benefits of cholesterol reduction, almonds play an important role in body weight control, glucose regulation and diabetes risks since they are rich in monounsaturated fat, minerals and fiber (Kamil et al., 2012). Consumption of almonds has become increasingly popular, showing great market share in these few decades. USA is the largest producer of almonds in the world with total 80% of world's crop, and California is the largest producer in U.S. (Almonds, Agricultural Marketing Resource Center, 2018). However, almonds are susceptible to bacterial infection during mature seasons with different storage conditions in transportation, and handling steps while processing (Lambertini et al., 2012). There has been two Salmonellosis outbreaks of almonds in 2001 and 2004, making minimum 4-log reduction of *Salmonella* mandatory for almonds sold in North America (Lambertini et al., 2012).

As USDA reported that almonds must be processed to achieve a minimum 4-log reduction of *Salmonella* using inactivation methods before sold into market. In response to this regulation, steam has been approved by FDA and widely used in almond pasteurization industry (U.S. Department of Agriculture, 2007). In this study, residual heat after PL treatment was utilized to stimulate steam process to kill *Salmonella*, however it will increase the disinfection time compared with steam treatment.

When the pulsed light intensity is high or treatment time is long, the surface temperature will go up fast and lead to surface burning (Oner, 2017). Similar results were also observed by other researchers. In Danyluk's (2005) study, when PL treatment duration increased, the surface temperature of almonds also increased. Surface temperature of almonds increased from 25 to 50°C during 60s PL treatment

with 3800V, shorter distanced and longer treatment time increased the inactivation of *Salmonella* Enteritidis PT30 on almonds (Oner, 2017). In the present study, both small and large-scale almonds were treated homogeneously by using shaker and there was visible change in almonds.

As have been mentioned in last chapter, pulsed light is a non-thermal antimicrobial method that can eliminate foodborne pathogens effectively. The results of this study show that PL-treatments can significantly kill *Salmonella* on surface of almonds. Also, in previous study, 19cm was selected with good product quality and in order to achieve over 5-log reduction of *Salmonella*, there might be some surface heating caused by PL-treatments. To further investigate the influence of pulsed light on almonds quality attributes, in this study, we evaluated the steam-assisted PL-treatments and temperature-controlled treatments on almonds to look for application methods of PL in almond pasteurization industries.

4.3 Materials and Methods

4.3.1 Bacterial strains and inoculum preparation

Four nalidixic-acid-resistant strains of *Salmonella* enterica (*S.* Montevideo 51, *S.* Newport H1073, *S.* Typhimurium 14028, and *S.* Heidelberg 4595J) were used in this study. The working cultures were maintained on tryptic soy agar (Difco Laboratories, Sparks, MD., U.S.A.) supplemented with 0.6% yeast extract and 50 µg/mL nalidixic acid (Difco; TSAYE-N) at 4°C prior to the experiment, individual cultures were grown in tryptic soy broth (Fisher Scientific) supplemented with 0.6% yeast extract and 50µg/mL nalidixic acid (TSBYE-N) overnight at 35 °C and transferred into a new tube of TSBYE-N for another 24h incubation at 35°C. Then

each culture was mixed to form a 4-strain cocktail of *Salmonella*. Bacterial cells were harvested by centrifugation at 4000 for 10min. Then, the supernatant was discarded, and the pellet was resuspended in 0.1% peptone water to yield a final concentration of $\sim 10^9$ CFU/ml.

4.3.2 Inoculation of almond

Packaged raw almonds were purchased from a local store. For the small-scale study, 50g of almonds were immersed in 400ml of the *Salmonella* cocktail prepared above for 6 min. For the large-scale study, 300g of almonds were immersed in 800ml of the *Salmonella* cocktail for 6min. Inoculated samples were then dried in a biological safety hood for 4h at room temperature before stored at 4°C for 24 h to facilitate bacterial attachment. The initial *Salmonella* level on almonds was $\sim 10^5$ CFU/ml.

4.3.3 Pulsed light and UV units

A modified commercial PL unit (Xenon Steripulse-XL RS-3000, Xenon Corp., Wilmington, MA) was used for this study. A PL lamp was mounted at the top of a home-built enclosed stainless-steel chamber (inner size 60 cm (L) \times 45 cm (W) \times 70 cm (H)) connected with a high flow ozone destruction unit (Ozone Solutions Inc, Hull, IA). A height-adjustable shelf was placed inside the PL chamber to alter the distance between the almond sample and the PL lamp so that different PL intensity could be achieved. Pulses at wavelength of 180-1100 nm were generated at 3 pulses/s with a pulse width of 360 μ s. Hsu and Moraru (2011) reported that 40% of PL energy generated was within the UV spectrum. The intensity of PL was measured with a Vega laser power meter (Ophir Optronics, Wilmington, MA) coupled with a pyroelectric

energy sensor (PE-50C, Ophir Optonics, Wilmington, MA). The wavelength setting was 300 nm with pulse width of 500 mm.

4.3.4 Steam-assisted PL pasteurization of raw almonds

4.3.4.1 Continuous PL processing

Inoculated almonds (50 g) were immersed in tap water for 1 min and then placed in a stainless-steel pan (size: 10 cm (L) \times 10 cm (W) \times 9 cm (H)) which was wrapped with a layer of insulation material to preserve heat inside the container. A quartz plate (15.2 cm \times 15.2 cm) was used to cover the container. The plate allowed PL to pass through and at the same time kept the steam generated by PL inside the container. The container was then mounted on a mechanical shaker. The system setup was shown in Figure 1. The distance between the almond sample and the PL lamp was adjusted to 19 cm. The PL intensity was 0.75 W/cm² at this distance. The almond sample was then treated by PL for up to 10 min.

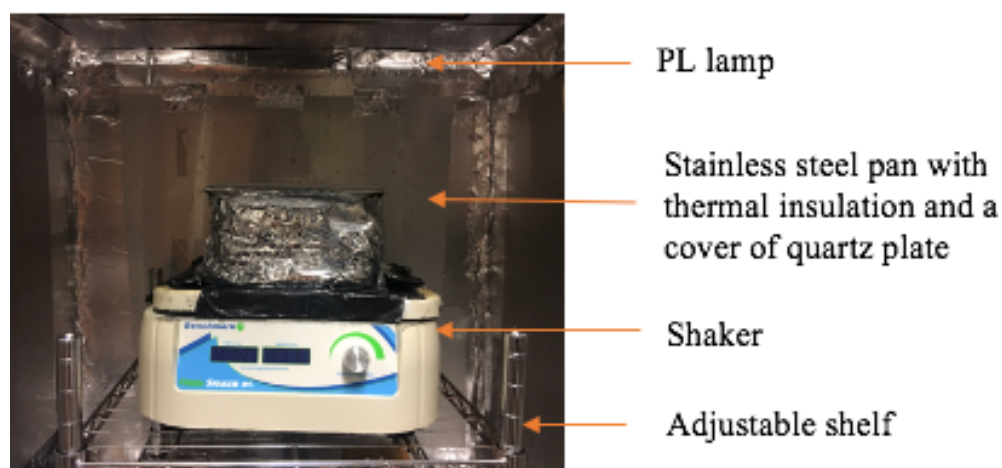


Figure 4.1. System setup. Wet almonds in a stainless-steel container were exposed to PL while being shaken by a mechanical shaker.

4.3.4.2 PL processing followed by holding almonds for certain periods of time

Inoculated almonds (50 g) were prepared and PL-processed as described above. After PL treatments, the almonds were held inside the insulated container covered with the quartz plate for certain periods of time before being removed for microbial analysis.

4.3.4.3 Temperature cycling by turning PL on and off

Inoculated almonds (50 g) were prepared as described above. A K-type thermocouples connected to a thermometer (HH506RA, Omega engineering, Stamford, CT) was used to monitor the temperature profiles of an almond's center. The center temperature was controlled between 70°C and 80°C or between 80°C and 90°C during the PL treatment by turning the PL unit off when the temperature reached the upper limit and on when it decreased to the lower limit. A larger scale study (300 g of almond) using this on-off PL processing was also conducted. A large stainless steel pan (size: 30 cm (L) × 27 cm (W) × 9 cm (H)) was used to hold the 300 g almonds.

4.3.4.4 Microbial analysis

After PL treatments, each 50g untreated and PL-treated was transferred into a sterile filter bag containing 200ml 0.1% peptone water and then pummeled in a laboratory stomacher for 2 min at 260 rpm. The homogenate was then serially diluted in sterile 0.1% peptone water and surface plated on TSAYE-N followed by incubation at 35°C for 72 h. Presumptive colonies of *Salmonella* formed on the plates were counted.

4.3.5 Color and weight measurement

Un-inoculated raw almonds (300 g) were immersed in tap water for 1 min and then treated using the on-off PL processing as described above. The untreated and PL-treated almonds were analyzed for color and weight loss.

4.3.5.1 Color and weight measurement

A colorimeter (KONICA MINOLTA, INC, JAPAN) was used to measure the color of almond samples. Three spots on three individual almonds respectively were randomly selected for color measurements. Color parameters were quantified in the Hunter L, a, b color space where L refers to lightness, ranging from 0 (blackness) to 100 (whiteness), positive a means red and negative a green, and positive b means yellow and negative b blue. The weight of almonds before water dipping and after PL treatment was measured.

4.3.6 Statistical analysis

Three replicates were conducted for all experiments. Colony counts were converted to log CFU/g or log CFU/mL. Means and standard deviations were calculated. Statistical analyses were conducted using JMP (SAS Cary, NC, USA). One-way analysis of variance (ANOVA) and Tukey's one-way multiple comparisons were used to determine significant differences between treatments at the 95% confidence level ($P < 0.05$).

4.4 Results

4.4.1 Continuous PL processing and continuous PL processing followed by holding almonds in the thermal-insulated pan

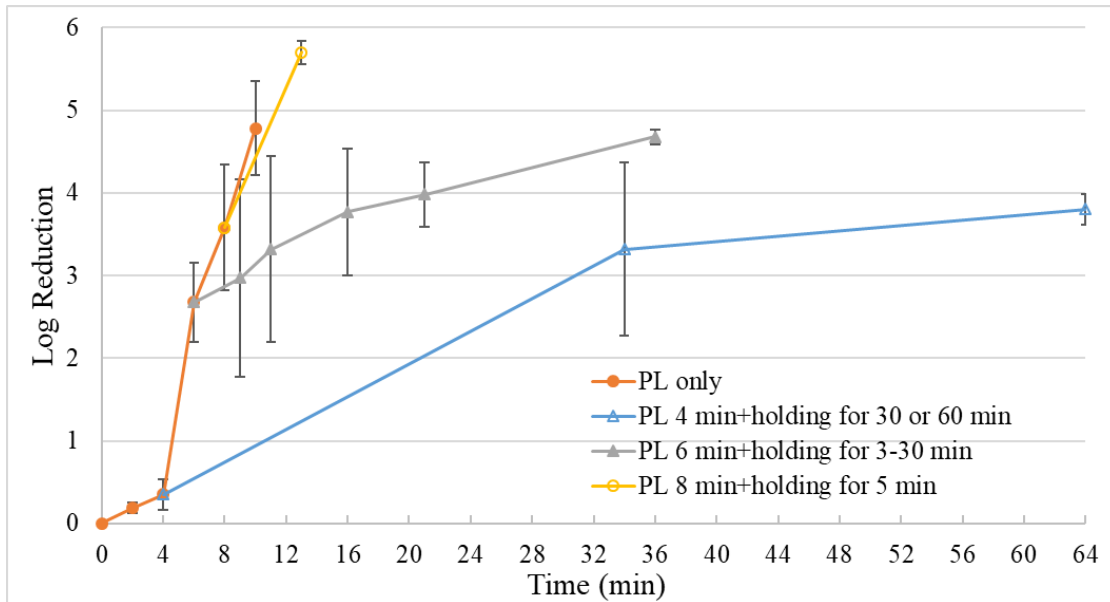


Figure 4.2. Effect of PL processing and holding on inactivation of *Salmonella* inoculated on almonds. Inoculated almonds (50 g) were immersed in tap water for 1 min and then placed in a stainless-steel container wrapped with a layer of insulation material and covered with a quartz plate. The almond sample was treated by PL for up to 10 min or treated by PL for 4, 6, or 8 min followed by holding the almonds in the pan for different time intervals.

The effect of continuous PL processing with/without holding the almond samples inside the thermal-insulated pan on *Salmonella* inactivation is shown in Figure 4.2. For the continuous PL processing without the holding period, the inactivation effect was small within the 4-min treatment time. Rapid *Salmonella*

inactivation was observed between 4 and 10 min. However, more than 5-log reduction of *Salmonella* could not be achieved after 10 min treatment, at which point appearance of almonds was adversely affected as shown in Figure 3. Compared with the control, the PL-treated almonds became darker and skin of some of the almonds cracked. Holding almonds inside the thermal-insulated pan after PL treatment could further inactivated *Salmonella* by using the residual heat within the pan. The inactivation effect during the holding period depended on the prior PL treatment time; higher *Salmonella* rate was achieved with longer prior PL treatment time. PL treatment for 4 min and holding the almond sample inside the pan for 60 min could only achieved 3.8 log reduction of *Salmonella*. However, PL treatment for 8 min and holding the almond sample inside the pan for 5 min achieved 5.7 log reduction of *Salmonella*. Unfortunately, this combined treatment negatively affected the visual appearance of almonds. They became much darker and skin of some of the almonds cracked (Figure 4.3.).

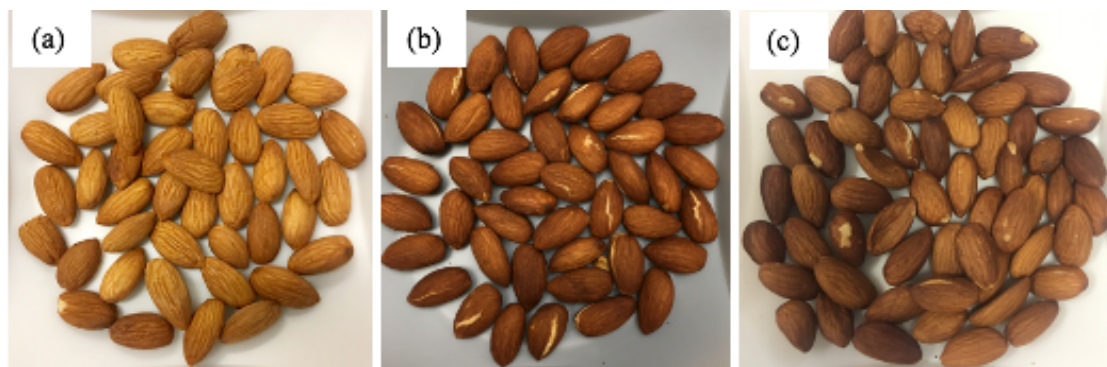


Figure 4.3. Almond pictures. a) Control, b) continuous PL processing for 10 min, c) PL processing for 8 min followed by holding for 5 min in the insulated pan

4.4.2 Temperature cycling by turning PL on and off

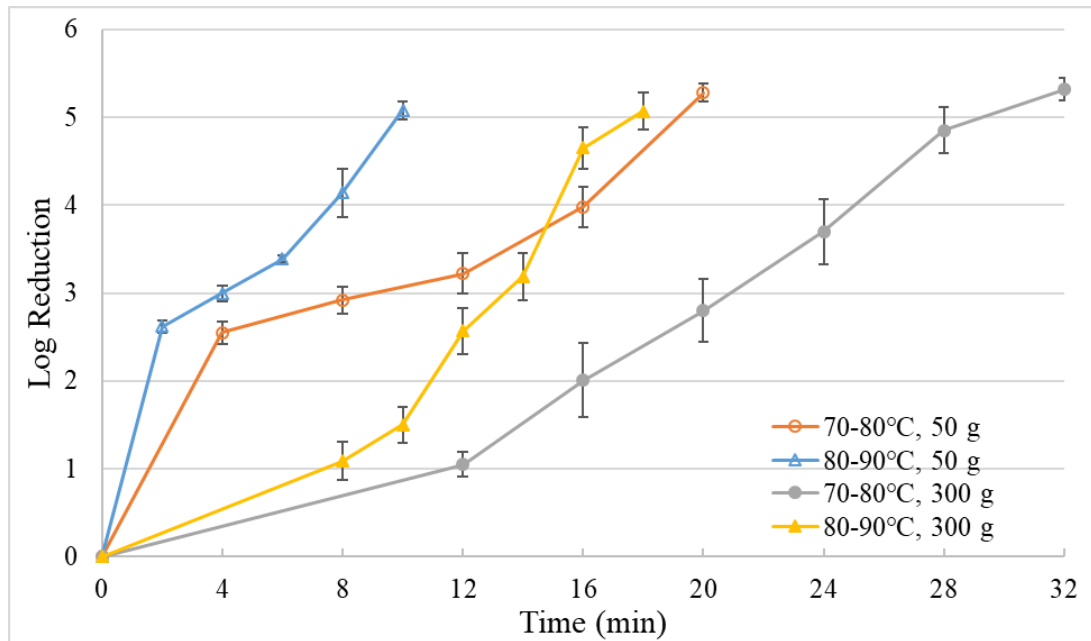


Figure 4.4. Effect of temperature cycling on inactivation of *Salmonella* inoculated on almonds. Inoculated almonds (50 g or 300 g) were immersed in tap water for 1 min and then placed in a small (for 50 g) or large (for 300 g) stainless-steel pan covered with a quartz plate. The almonds were treated by PL while being shaken. The center temperature of almond was controlled between 70°C and 80°C or between 80°C and 90°C during the PL treatment.

From the figure above, the combination of 10min treatment and 80-90°C temperature range achieved good killing effect that was as over 5-log reduction as 70-80°C for 20 min, and there was significant difference between different temperature-controlled systems. However, we cannot find great difference for the product quality between the two treatments mentioned above. Large scale requires much longer time

than small scale to achieve over 5-log reduction of *Salmonella*. Also, take 10 min as an example, 50g almonds significantly improved inactivation efficacy. For same inactivation value, 80-90°C for small scale required minimum treatment time.

Table 4.1-Effect of temperature cycling on almond color and weight.

Raw almonds (300 g) without *Salmonella* inoculation were dipped in water for 1 min and treated by PL. The center temperature of almond was controlled between 70°C and 80°C or between 80°C and 90°C during the PL treatment.

Time (min)	Color: % decrease						% weight decrease
	L		a		b		
	Surface	Inside	Surface	Inside	Surface	Inside	
70-80°C							
12	2.44±0.10	0.40±0.09	7.18±0.17	3.77±0.21	0.71±0.11	1.22±0.17	0.17±0.05
16	4.68±0.08	1.05±0.20	8.72±0.24	5.66±0.20	1.89±0.11	2.44±0.12	0.47±0.07
20	6.92±0.12	1.58±0.08	15.90±0.11	11.32±0.16	3.89±0.17	5.28±0.16	0.57±0.05
24	8.96±0.30	2.37±0.28	17.44±0.32	15.09±0.12	6.36±0.13	8.13±0.56	0.43±0.12
28	12.02±0.13	2.77±0.11	22.05±0.12	18.87±0.41	10.95±0.51	10.16±0.19	0.77±0.20
32	16.50±0.18	4.61±0.11	27.69±0.72	32.08±0.07	18.73±0.25	15.45±0.32	1.59±0.11
80-90°C							
8	4.07±0.13	0.40±0.09	11.28±0.12	1.89±0.13	1.77±0.21	0.41±0.25	0.07±0.01
10	5.91±0.13	0.66±0.09	14.87±0.20	3.77±0.36	6.01±0.10	2.03±0.41	0.50±0.10
12	7.13±0.06	1.45±0.12	17.44±0.21	11.32±0.11	9.89±0.22	3.25±0.15	0.83±0.06
14	10.18±0.11	2.37±0.09	21.54±0.22	13.21±0.61	12.01±0.17	5.28±0.49	0.90±0.13
16	11.20±0.17	3.69±0.12	23.59±0.20	16.98±0.43	12.72±0.17	6.50±0.35	1.23±0.13
18	12.63±0.10	3.82±0.11	26.67±0.45	20.75±0.49	14.84±0.47	8.13±0.30	1.07±0.12

Surface darkening is an unfavorable attribute for almonds. According to L value, which indicates surface brightness that is the most important parameter for color change. In previous work of this study, the side effects of high intensity of PL treatments on almonds surface has also been mentioned. For that reason, this is also

why wet treatment was designed to protect almonds from direct exposure to pulsed light.

Higher temperature induced more weight loss and discoloration in both almond surface and meat. “a” value of sample minus control represents the difference in red and green, it was most influenced by PL treatment. It was likely that untreated almonds meat was much brighter than treated ones. Quality degradation might impede PL application in almond pasteurization industry.

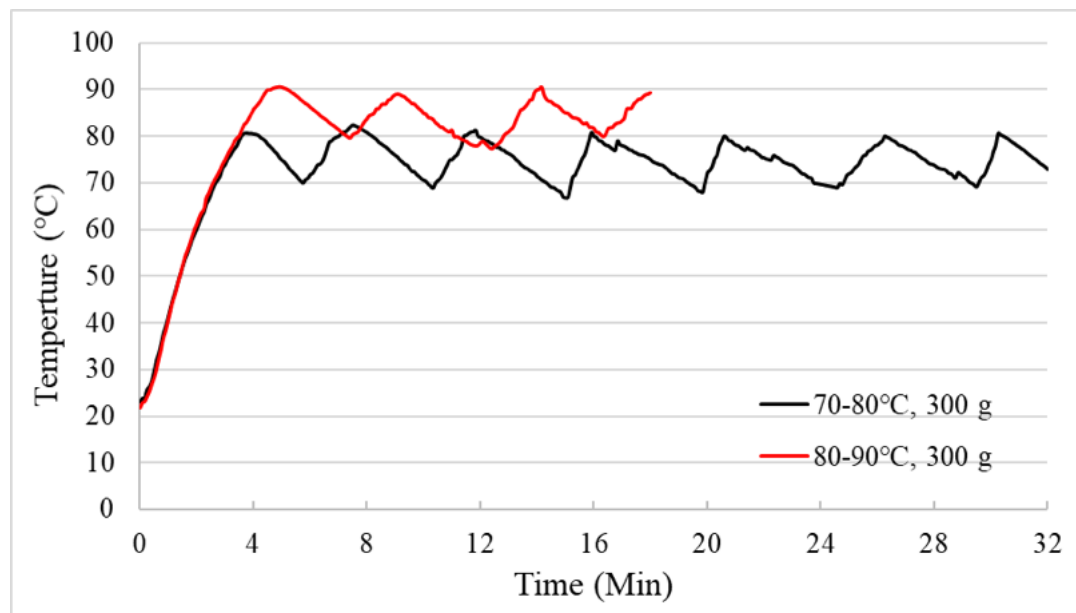


Figure 4.5. Temperature profile of almonds. Almonds (300 g) were immersed in tap water for 1 min and then placed in a large stainless-steel pan covered with a quartz plate. The almonds were treated by PL while being shaken. The center temperature of almond was controlled between 70°C and 80°C or between 80°C and 90°C during the PL treatment.

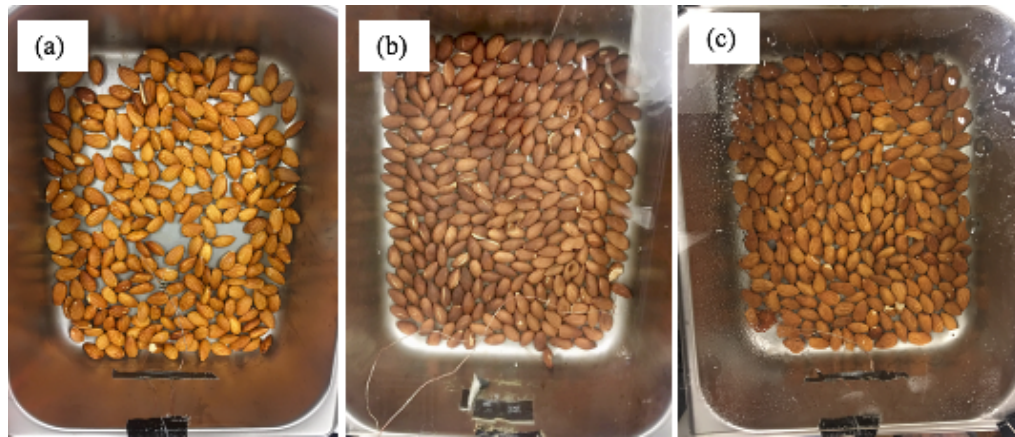


Figure 4.6. Almond pictures. a) Control, b) PL for 32 min with almond center temperature controlled at 70 - 80°C, c) PL for 18 min with almond center temperature controlled at 80 - 90°C.

Form the visual observations, the skin of almonds was damaged due to overheat. Quartz plate helped accumulate so much heat that water drop can be observed beneath quartz plates because it also played an important role in retaining heat and moisture that cannot escape much from the system. It can be found that almonds in picture b were much drier than a, because 32 min PL treatment induced a lot of heat that made water evaporate fast. For that reason, pulsed light could achieve good killing effect on *Salmonella*. From color parameters, it can be found that both surface and meat of almonds underwent discoloration shown by decrease rate of L, a and b values. After long time treatment, weight loss was in acceptable range for both 70-80°C and 80-90°C PL treatments.

In order to reach our goal, treatment time must be extended much longer for 300g almonds. Product quality was not good after 32 min. However, much higher temperature and shorter time actually has good killing effect of *Salmonella*, over 5-log reduction was achieved on almonds.

4.5 Discussion

In order to function better, the process system should be efficient and energy-saving. In low-moisture foods, water activity is related to temperature and useful for inactivation of *Salmonella* Enteritidis PT30 since the thermal resistance was lower with the increased relative humidity according to Liu's study (2018). Quartz plate is used in this study for retaining heat in the pan, as well as increasing the humidity to effectively decrease *Salmonella* inoculated on raw almonds. At 25°C, the thermal conductivity of quartz is 3W/(m K), which is higher than common materials (ToolBox, E. 2003). Pulsed light can go through quartz plate that could make it difficult for vapor to go outside. However, the major disadvantage of quartz plate covered treatment is the loss of visual quality since the temperature increased rapidly as the treatment time was longer. As shown in Figure 4.3, both detachment and wrinkle of almond skin were visible after 10 min PL treatment.

Superheated steam (SHS) followed by catalytic infrared (IR) heat treatment has been previously used in almond surface decontamination, and 70 seconds SHS followed by IR heat treatment for another 70 seconds could reduce over 5 log CFU/g *Salmonella* population, the overall quality parameters were not significantly altered (Bari et al., 2010). The large amount of heat can increase the surface temperature when they arrive the targeted foods (James et al., 2000). For that reason, increasing steam treatment time should improve the killing effect. Similar results were observed by others. 25 seconds of steam pasteurization is sufficient to reach over 5-log reduction of *Salmonella* population inoculated on raw almonds without significant quality degradation (Chang et al., 2010). Lee et al. (2006) treated *Salmonella* on raw almonds by steam for 65 seconds, reducing 5.7 log *Salmonella* population for a specific cultivars (Nonpareil) of raw almonds (Lee et al., 2006). In the present study,

Salmonella on almonds was decreased to detection limit 5.7 log CFU/g inactivated after 8 min PL treatment followed by 5 min residual steam. Although increasing treatment time of PL treatment did increase the efficacy of inactivation, this combination did not prevent quality deterioration for almonds. Obviously, the inactivation rate of *Salmonella* gradually decreased after pulsed light stopped working, thus the combination of 6 min PL treatment and 30 min steam applied achieved 4.68 log reduction.

Temperature also significantly affected the effectiveness of PL treatment, and this is due to different heat applied to almonds' surface. In this study, continuous treatment with or without holding in PL chamber for some period were compared to investigate the inactivation effect of residual heat. Within first 4 min treatment, *Salmonella* inactivation rate was slower than 4 min to 10 min, since rapid *Salmonella* inactivation is more due to the thermal effect when temperature increased faster. Furthermore, PL treatment was more effective in *Salmonella* inactivation than residual heat. It was observed that longer prior PL treatment time caused higher *Salmonella* inactivation rate. It was likely that longer PL treatment time resulted in higher almond temperature and the residual heat caused *Salmonella* destruction. Additionally, on-off PL treatment was used to control temperature within a range. As the product is drier and moisture level is lower, *Salmonella* is more resistant to heat treatment (Annear et al., 1965; Boziaris et al., 1998) which means it is critical to treat almonds with temperature control system. In that case, both higher inactivation efficacy and good quality could be achieved. Water evaporation occurred faster during 80-90°C treatment than 70-80°C, in that higher humidity and quartz-covered situation, there was much more vapor condensed on the surface of almonds to get better killing effect.

Similar observation was also explored in Jeong's (2009) study, almonds were treated in temperature and humidity in a moist-air oven to determine the survival of *Salmonella*, a higher log reduction (4.27 log reduction) was achieved in a shorter time during high-humidity process than during the low-humidity process (1.74 log reduction). In industry, the classical pasteurization is usually between 50 to 90 that aims to inactivate pathogenic and spoilage microorganisms (Da Silva and Gibbs, 2009). Almond Board of California recommended that the balancing process of 1.6 to 2 min requires minimum 88 °C at the cold spot to achieve 4 to 5 log reduction of *Salmonella* Enteritidis PT30 respectively (Almond Board of California, 2007). Therefore, this temperature-controlled system to control foodborne pathogen on almonds can obtain more than 5-log reduction in long time treatment and protect against much quality degradation.

The pulsed light decontamination of *Salmonella* on almonds depends on a variety of factors, sample size significantly influenced the microbial killing efficacy. From Figure 4.7, it is clear that 50g almonds showed higher bacterial reduction than 300g for both 70-80°C and 80-90°C temperature range. Also, for the treated temperature between 80 and 90, 4.14 and 1.09 log reduction of *Salmonella* were achieved by 8min PL treatments for dip inoculated almonds respectively.

Discoloration by steam-assisted pasteurization was observed in this study. The similar result was also found by Kispeter (Kispeter et al., 2003), there was much color change in spice by saturated steam treatment. Bari (2010) also pointed out sensory analysis indicated treated almonds were significant different from untreated ones by odor, taste and texture. In this study, quartz plate covered treatment without controlling temperature indicated much change in the overall visual quality in PL

treated almonds, because the color turned from brown to dark brown (Figure 2). For temperature-controlled experiments, the quality parameters suggested that PL treatment for 18 min between 80 and 90 is efficient in eliminating the population of *Salmonella* without significant affecting the overall quality. However, for 32min PL treatment, the prolonged treatment time made almonds lose much weight and color.

4.6 Conclusion

This part of study can suggest the decontamination efficacy of quartz plate covered PL system. As have stated above, 80-90°C center temperature after 18 min PL treatment achieved over 5-log reduction of *Salmonella*. Though the tested parameters showed some quality damage in almonds, the industrial almond pasteurization process should control treatment temperature in order to heat the almonds to desired temperature rapidly, thus reducing the treatment time to achieve good killing effect.

REFERENCES

- Almond Board of California. (2007a). Considerations for proprietary processes for almond pasteurization and treatment. v1.0, April 13, 2007. Available at: <http://www.almond-board.com/Handlers/FoodQualitySafety/Pasteurization/PasteurizationProgram/ValidationGuidelines/>. Accessed Nov 1, 2010.
- Almonds, Agricultural Marketing Resource Center, 2018.
- Annear, D. I., and G. A. Bottomley. "Survival of bacteria in desiccates at 100 C in dry atmospheres." *Nature* 206.4991 (1965): 1373.
- Available at: <https://www.agmrc.org/commodities-products/nuts/almonds>
- Bari, Latiful, et al. "Effectiveness of superheated steam and gas catalytic infrared heat treatments to inactivate *Salmonella* on raw almonds." *Foodborne pathogens and disease* 7.7 (2010): 845-850.
- Bari, Latiful, et al. "Effectiveness of superheated steam and gas catalytic infrared heat treatments to inactivate *Salmonella* on raw almonds." *Foodborne pathogens and disease* 7.7 (2010): 845-850.
- Boziaris, I. S., L. Humpheson, and M. R. Adams. "Effect of nisin on heat injury and inactivation of *Salmonella* enteritidis PT4." *International journal of food microbiology* 43.1-2 (1998): 7-13.
- Chang, S-S., et al. "Evaluation of steam pasteurization in controlling *Salmonella* serotype Enteritidis on raw almond surfaces." *Letters in applied microbiology* 50.4 (2010): 393-398.
- Da Silva, F. V. M., & Gibbs, P. A. (2009). Principles of thermal processing: pasteurization. In Simpson (Ed.), *Engineering aspects of thermal food processing* (pp. 13–49). Boca Raton: Taylor & Francis.
- Danyluk, M.D., Uesugi, A.R., Harris, L.J., 2005. Survival of *Salmonella* Enteritidis PT 30 on inoculated almonds after commercial fumigation with propylene oxide. *Journal of Food Protection* 68: 1613–1622.
- James, C., et al. "Surface pasteurisation of poultry meat using steam at atmospheric pressure." *Journal of Food Engineering* 45.2 (2000): 111-117.

- Jeong, Sanghyup, Bradley P. Marks, and Alicia Orta-Ramirez. "Thermal inactivation kinetics for *Salmonella* Enteritidis PT30 on almonds subjected to moist-air convection heating." *Journal of food protection* 72.8 (2009): 1602-1609.
- Jeong, Sanghyup, Bradley P. Marks, and Michael K. James. "Comparing thermal process validation methods for *Salmonella* inactivation on almond kernels." *Journal of food protection* 80.1 (2016): 169-176.
- Kamil, Alison, and C-Y. Oliver Chen. "Health benefits of almonds beyond cholesterol reduction." *Journal of agricultural and food chemistry* 60.27 (2012): 6694-6702.
- Kispéter, J., et al. "Changes induced in spice paprika powder by treatment with ionizing radiation and saturated steam." *Radiation Physics and Chemistry* 68.5 (2003): 893-900.
- Kispéter, J., et al. "Changes induced in spice paprika powder by treatment with ionizing radiation and saturated steam." *Radiation Physics and Chemistry* 68.5 (2003): 893-900.
- Lambertini, Elisabetta, et al. "Risk of salmonellosis from consumption of almonds in the North American market." *Food Research International* 45.2 (2012): 1166-1174.
- Lee, Sun-Young, et al. "Reduction of *Salmonella* enterica serovar Enteritidis on the surface of raw shelled almonds by exposure to steam." *Journal of food protection* 69.3 (2006): 591-595.
- Liu, Shuxiang, et al. "Exponentially increased thermal resistance of *Salmonella* spp. and *Enterococcus faecium* at reduced water activity." *Appl. Environ. Microbiol.* 84.8 (2018): e02742-17.
- Oner, Manolya Eser. "Inactivation of *Salmonella* Enteritidis on Almonds by Pulsed Light Treatment." *Academic Food Journal/Akademik GIDA* 15.3 (2017).
- Ozer, N.P., Demirci, A. 2006. Inactivation of *Escherichia coli* O157:H7 and *Listeria monocytogenes* inoculated on raw salmon fillets by pulsed UV-light treatment. *International Journal of Food Science and Technology* 40: 1-7.
- ToolBox, E. "Thermal Conductivity of common Materials and Gases." Tillgänglig: https://www.engineeringtoolbox.com/thermal-conductivity-d_429.html [Hämtad 2018-03-06](2003).

U.S. Department of Agriculture, 2007. Almonds grown in California; outgoing quality control requirements. Fed. Regist. 72, 15021–15036.

Chapter 5

PASTEURIZATION OF WET BLACK PEPPERCORNS USING PULSED LIGHT

5.1 Abstract

The aim of this project was to develop a quartz-covered and temperature-controlled pulsed light system to decontaminate black peppercorns for industry. The effect of pulsed light on the inactivation of *Salmonella* was evaluated. In this study, increasing pulsed light intensity or treatment time could significantly increase the *Salmonella* log reduction. Pulsed light treatments with distance at 19cm from sample to lamp and intensity at 0.25 J/cm² for small scale (50g) was investigated. Black peppercorns were dip-inoculated with *Salmonella*. This intensity was the most effective for both good quality and higher *Salmonella* inactivation. In order not to lose too much heat during PL-treatments, we still use quartz plate in previous studies for this project. 4.84-log reduction after 8min PL-treatment (0.25 J/cm²) was achieved with quartz covered. Then the effectiveness of PL treatment was completed on larger black peppercorns at three distances 16cm, 19cm, and 22cm without controlling temperature which was over 100°C. 4.69, 4.94 and 4.47-log reduction of *Salmonella* dip-inoculated on black peppercorns were achieved respectively. The quality measurements showed different degree of degradation and the inactivation of *Salmonella* was significantly influenced by temperature. Based on those results, temperature cycling system was used to control the temperature between 55°C and 80°C to make sample quality better.

5.2 Introduction

Dry black peppercorn is a dry product which is one of the most common spices in the world (Ravindran et al., 2012). Some chemical contents play an important role in inhibiting microorganisms (Zaika and Laura L, 1988) and other aromatic compounds from essential oil are also important to red wine industries (Siebert et al., 2008). Therefore, black peppercorns can be used as preservatives to extend shelf-life, as well as applied to medical treatments and cosmetic industry due to its high antioxidant properties (Balasubramanian et al., 2016).

Generally speaking, black peppercorns can be contaminated with human pathogens like *Bacillus cereus* and *Salmonella* (De Boer et al., 1985). They are susceptible to be contaminated in growing environment, which has raised food safety concerns and spoilage risks. However, people usually think low-moisture food has a lower water activity and bacteria cannot survive in such a dry environment. However, some types of *Salmonella* and bacterial spores can still survive. In 2010, there was a nationwide outbreak of *Salmonella* Montevideo infections associated with contaminated imported black and red pepper, causing 272 cases in 44 states with illness (Van et al., 2013).

Due to unsanitary storage and transportation conditions, from 1973 to 2010, there were also 14 reported illness outbreaks induced by contaminated spices like black pepper, and thus pasteurization process of spices is getting attention of researchers (Oner and Manolya, 2017; Van et al., 2013). Currently, thermal and nonthermal technologies are both used in processing black peppercorns. Some traditional methods such as steam, chemical, irradiation treatments are widely used in decontamination for herbs and spices (Demirci et al., 2012; Duncan et al., 2017).

Steam blanching has immediate inactivation effect but causes deterioration of quality attributes that was determined in low moisture food like paprika and chill powder (Schweiggert et al., 2015; Hadidi et al., 2019). Some novel mechanical and thermal combination methods were also used to retain good quality on black pepper, a modification of the vacuum-steam-vacuum system could get 3 log CUF/g at 120 °C for 20s treatment (Lilie et al., 2007). Fumigation with ethylene oxide (EtO) process can significantly reduce microbial populations on spices (Leistritz, 1997). However, the food product lost aroma and color appearance, as well as nutrient contents (Farkas and Andrassy, 1988). Additionally, this treatment is expensive and energy-wasting (Vajdi, M et al, 1973) and consumers might worry about the side effects of toxic chemical residuals remained in processed products. In contrast, irradiation is time saving and energy-efficient for decontaminating pathogens on spices (Schweiggert et al., 2007). Farkas and Andrassy (1988) found the sanitation method of irradiation could be an alternative to ethylene oxide treatment for maintaining quality and sensory characteristics of ground black pepper. However, intense dose has been proved to alter some attributes of food products in 2006 by Suhaj and others (Suhaj et al., 2006; Al-Bachir, 2004). On the other hand, gamma irradiation is neither a common or acceptable pasteurization method for food safety by most consumers (Hackwood, 1991; Ronteltap et al., 2007). Nonthermal method like radio-frequency (RF) was investigated by Kim (2011) who found RF heating for 50s could achieve 2.80 to 4.29 log CFU/g reduction of *S. Typhimurium* and *E. coli* O157:H7 in black peppers.

Pulsed light is a novel technology which has been approved by the U.S. Food and Drug Administration. Unlike chemical methods, pulsed light will not produce any undesirable by-product and it is effective and simple to use without any chemical

residues. Basically, it uses very short time and intense pulses of a broad spectrum rich in UV-C to inactivate microorganisms on the surface of foods (Elmnasser et al., 2007). As has been explained in Chapter 3, PL treatment has positive effects in low-moisture food decontamination. For that reason, its application in spice and aromatic herb industry is what we are exploring in this chapter. Also, in previous work, PL treatments were performed on wet almonds to achieve better inactivation result as well as to minimum quality damage by dry PL treatments (Huang et al., 2015). It has also been demonstrated that PL is effective on disinfection against various bacteria, yeasts, as well as fungal spores in vitro (MacGregor et al., 1998). In Nicorescu's study (2013), PL treatment was developed on ground black pepper that was inoculated with *B.subtilis* , and he found that PL treatment reduced the bacterial population to 1 log reduction and there was serious damage on microorganisms' structure. Some similar results about the potential inactivation effect on spice were also observed by other researchers. Pulsed light over 6 pulses treatments were carries out on *B.subtilis* contaminated black peppercorns, 2.7 log reduction of *B.subtilis* spores was achieved on surface of sample (Moreaua et al., 2011). These years many studies show decontamination efficacy of food powders by some novel nonthermal technologies but research about pulsed light treatment on spice and other low-moisture food is still limited.

In this study, we further investigated the efficacy of wet PL treatments on black peppercorns, and quality measurements including color change and weight loss, and fatty acid analysis. Temperature profiles were also recorded to look for proper treatment temperature range. The overall objective was to investigate the antimicrobial

effect of pulsed light on black peppercorns to provide applications of this method in spice industries.

5.3 Materials and Methods

5.3.1 Bacterial strains and inoculum preparation

Four nalidixic-acid-resistant strains of *Salmonella enterica* (S. Montevideo 51, S. Newport H1073, S. Typhimurium 14028, and S. Heidelberg 4595J) were used in this study. The working cultures were maintained on tryptic soy agar (Difco Laboratories, Sparks, MD., U.S.A.) supplemented with 0.6% yeast extract and 50 µg/mL nalidixic acid (Difco; TSAYE-N) at 4°C prior to the experiment, individual cultures were grown in tryptic soy broth (Fisher Scientific) supplemented with 0.6% yeast extract and 50µg/mL nalidixic acid (TSBYE-N) overnight at 35 °C and transferred into a new tube of TSBYE-N for another 24h incubation at 35°C. Then each culture was mixed to form a 4-strain cocktail of *Salmonella*. Bacterial cells were harvested by centrifugation at 4000 for 10min. Then, the supernatant was discarded and the pellet was resuspended in 0.1% peptone water to yield a final concentration of ~10⁹ CFU/ml.

5.3.2 Inoculation of black peppercorns

Packaged black peppercorns were purchased from a local store. For the small-scale study, 50g of black peppercorns were immersed in 400ml of the *Salmonella* cocktail prepared above for 6 min. For the large-scale study, 300g of black peppercorns were immersed in 800ml of the *Salmonella* cocktail for 6min. Inoculated samples were then dried in a biological safety hood for 6h at room temperature before

stored at 4°C for 24 h to facilitate bacterial attachment. The initial Salmonella level on peppercorns was $\sim 10^5$ CFU/ml.

5.3.3 Pulsed light unit

A modified commercial PL unit (Xenon Steripulse-XL RS-3000, Xenon Corp., Wilmington, MA) was used for this study. A PL lamp was mounted at the top of a home-built enclosed stainless steel chamber (inner size 60 cm (L) \times 45 cm (W) \times 70 cm (H)) connected with a high flow ozone destruct unit (Ozone Solutions Inc, Hull, IA). Pulses at wavelength of 180-1100 nm were generated at 3 pulses/s with a pulse width of 360 μ s. Hsu and Moraru (2011) reported that 40% of PL energy generated was within the UV spectrum. The intensity of PL was measured with a Vega laser power meter (Ophir Optronics, Wilmington, MA) coupled with a pyroelectric energy sensor (PE-50C, Ophir Optronics, Wilmington, MA). The wavelength setting was 300 nm with pulse width of 500 mm.

5.3.4 PL pasteurization of black peppercorns

5.3.4.1 Comparison of pan with/without a quartz plate cover

Inoculated black peppercorns (50 g) were immersed in tap water for 1 min and then placed in a small stainless-steel pan (size: 10cm (L) \times 10 cm (W) \times 9 cm (H)) mounted on a mechanical shaker (Fig. 1). The distance between the almond sample and the PL lamp was adjusted to 19 cm. The PL intensity was 0.75 W/cm² at that distance. Two different setups were used: one setup with the pan covered with a quartz plate (15.2 cm x 15.2 cm) and one without. The inoculated black peppercorns in the pan with/without cover were treated by PL for up to 10 min while being shaken by the shaker. A K-type thermocouple connected to a thermometer (HH506RA, Omega

engineering, Stamford, CT) was attached to the bottom of the pan and used to monitor the temperature profile of peppercorns during PL treatments. After PL treatments, the un-treated and PL-treated almonds were analyzed for *Salmonella* populations.

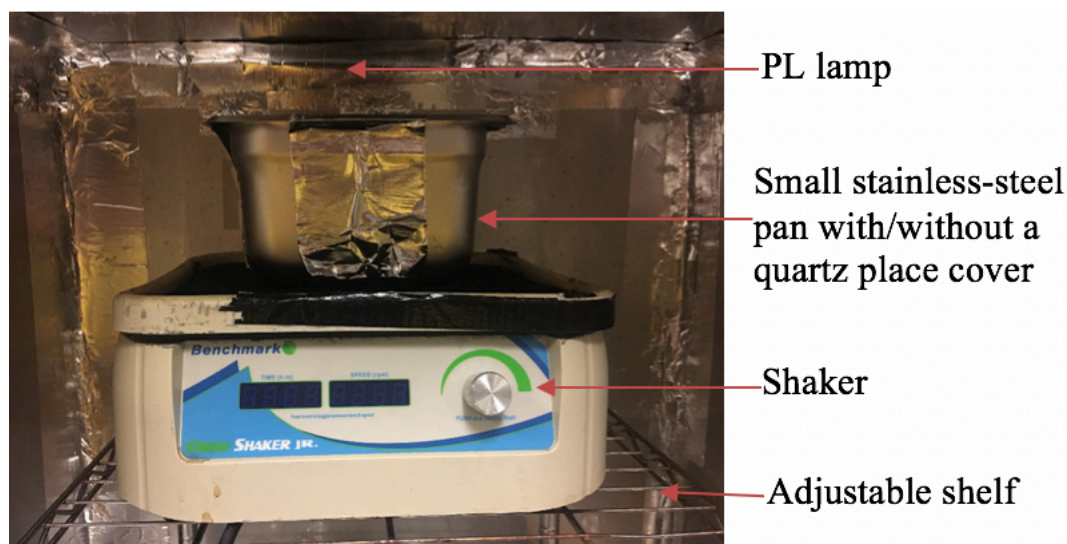


Figure 5.1. System setup. Wet almonds in a small stainless-steel pan with/without a quartz place cover were exposed to PL while being shaken by a mechanical shaker.

5.3.4.2 Effect of temperature cycling and PL intensities

Inoculated black peppercorns (300 g) were immersed in tap water for 1 min and then placed in a large stainless steel pan (size: 30 cm (L) × 27 cm (W) × 9 cm (H)) mounted on the mechanical shaker. A quartz plate (30.5 cm x 30.5 cm) was used to cover the pan. The system setup is the same as the one shown in Fig 1 except that the large pan and large quartz plate were used. Effect of temperature cycling on *Salmonella* inactivation was determined. The temperature was controlled between 55-80°C by turning the PL unit off when the temperature reached 80°C and on when it

decreased to 55°C. To determine the effect of PL intensity on *Salmonella* inactivation, the distance between the almond sample and the PL lamp was adjusted to 16, 19 and 22 cm. The PL intensities were 0.96, 0.75, and 0.54 W/cm² at 16, 19, 22cm, respectively. Temperature cycling was not used for the PL intensity study.

5.3.5 Microbial analysis

After pulsed light treatment, each 50g untreated and PL-treated sample was transferred into a sterile filter bag containing 200ml 0.1% peptone water and then pummeled in a laboratory stomacher for 2 min at 200 rpm to remove *Salmonella* from black peppercorns. For large scale 300g sample, only 50g was transferred into the bag. The homogenate was then serially diluted in sterile 0.1% peptone water and surface plated on TSAYE-N followed by incubation at 35°C for 72 h. Presumptive colonies of *Salmonella* formed on the plates were counted.

5.3.6 Color and weight measurement

Un-inoculated peppercorns (50g or 300 g) were used for studying the effect of PL on color and weight of almonds. They were immersed in tap water for 1 min and then placed in the small (for 50 g almonds) or (for 300 g almonds) large stainless-steel pan with/without the quartz plate cover mounted on the mechanical shaker. The peppercorn samples were then treated using those PL treatments for the *Salmonella* study. The untreated and PL-treated almonds were analyzed for color and weight loss. A colorimeter (KONICA MINOLTA, INC, JAPAN) was used to measure the color of black peppercorn samples. Three spots on three locations were randomly selected for color measurements. Color parameters were quantified in the Hunter L, a, b color space where L refers to lightness, ranging from 0 (blackness) to 100 (whiteness),

positive a means red and negative a green, and positive b means yellow and negative b blue. The weight of peppercorn before water dipping and after PL treatment was measured.

5.3.7 Statistical analysis

Three replicates were conducted for all experiments. Colony counts were converted to log CFU/g or log CFU/mL. Means and standard deviations were calculated. Statistical analyses were conducted using JMP (SAS Cary, NC, USA). One-way analysis of variance (ANOVA) and Tukey's one-way multiple comparisons were used to determine significant differences between treatments at the 95% confidence level ($P < 0.05$).

5.4 Results

5.4.1 Effect of PL treatments on the inactivation of *Salmonella* on wet black peppercorns--comparison of pan with/without a quartz plate cover

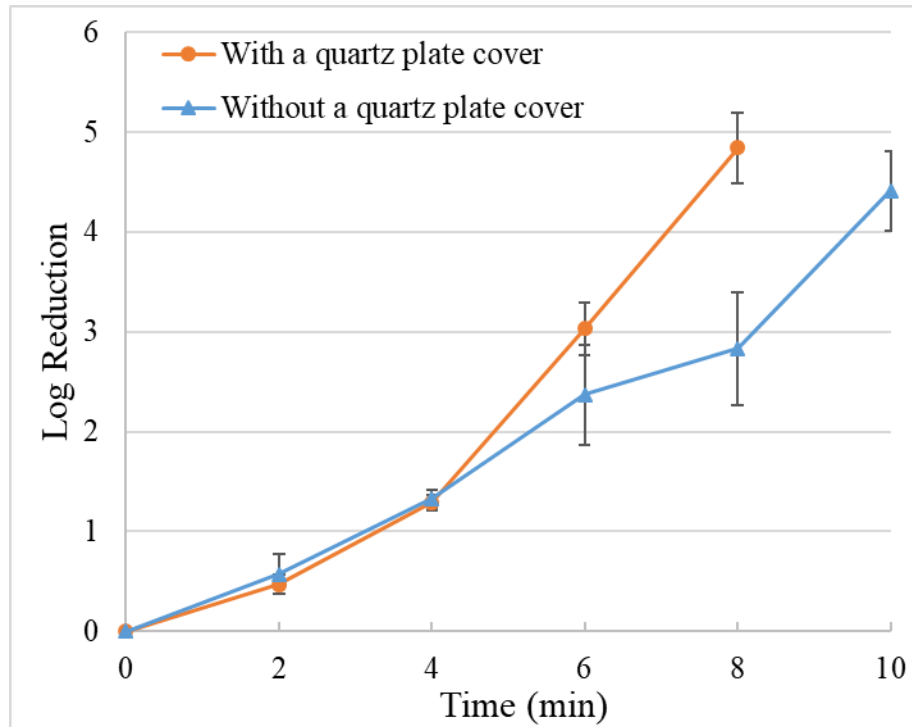


Figure 5.2. PL inactivation of *Salmonella* on wet black peppercorns (50 g). Black peppercorns inoculated with *Salmonella* were immersed in tap water for 1 min before being exposed to PL at a distance of 19 cm. Data represent mean of three replicates \pm standard deviation.

Within the first 4 min, there was no significant disinfection difference between two PL treatments, less than 1.5 log-reduction of *Salmonella* was achieved. It was likely that the temperature during this stage did not increase a lot, not making the inactivation effect improve. However, as the treatment time was longer, the difference of inactivation efficacy was enlarged. For both 8 min PL treatment, experiments with quartz plate covered could reduce *Salmonella* by 4.84 log CFU/g, and that was only 2.83 log CFU/g for open system. As has mentioned in previous chapter, quartz plate is helpful in accumulating moisture and heat, resulting in effective inactivation on pathogens.

Table 5.1-Comparison of pan with/without a quartz plate cover on sample color and weight. Black peppercorns (50 g) were immersed in tap water for 1 min and then exposed to PL at distance of 19 cm with/without a quartz plate cover. Data represent mean of three replicates \pm standard deviation.

Time (min)	% Color decrease			% weight change
	L	a	b	
Pan with a quartz plate cover				
2	10.56±1.05	0.81±0.34	5.34±0.48	8.35±1.11
4	12.89±0.73	1.21±0.16	11.23±0.72	7.78±0.37
6	13.25±1.23	6.23±0.27	24.67±0.34	5.16±0.16
8	15.00±0.60	16.80±0.99	52.40±0.52	1.00±0.30
Pan without a quartz plate cover				
2	11.71±9.39	0.84±0.55	6.76±7.14	11.31±1.5
4	13.05±5.65	1.49±1.34	11.51±1.35	4.57±1.0
6	13.44±9.38	7.39±4.96	16.98±5.05	1.53±0.2
8	16.86±3.97	19.04±6.39	49.54±7.10	-0.80±0.3
10	19.16±3.59	21.61±8.23	50.31±5.68	-2.79±1.0

For pan with quartz plate treatment, surface color parameter L measured the surface brightness or darkness of black peppercorns. The decrease rate of L indicating that black peppercorns were getting darker with longer treatment time, which is the most important indicator for this sample. a and b values showed that black peppercorns were becoming green and blue, typically, PL treatment had more significant effect on greenness than blueness. Additionally, it could be found that the darkening degree of pan without quartz plate treatment was larger than another. Weight loss is a critical standard for quality degradation, in this experiment from 2 to 6 min, weight of black peppercorns showed an obvious increase trend, which is positive for further study. That is likely that 1 min dipping in water before treatment increased the total weight of black peppercorns, and they were still wet within 6 min

treatment. However, treatment with quartz plate caused a little more weight loss than without cover.

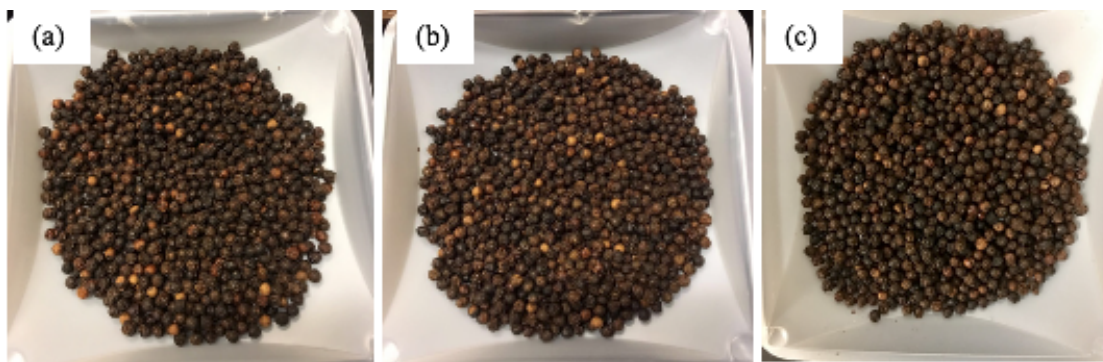


Figure 5.3. Appearance of black peppercorns. a) control; b) PL-treatment for 8 min (with a quartz plate cover); c) PL-treatment for 10 min (without a quartz plate cover)

From color and weight table, both of the two parameters showed unfavorable quality damage for black peppercorns. The pictures showed the surface color was turning darker as treatment time was longer, black peppercorns did lose brightness after 8 and 10 min PL treatment compared with control group. In addition, they were much drier. However, we cannot tell much difference between 8 min treatment with and without quartz plate cover visually. Overall, visible characteristics are desirable for us.

Consequently, due to the high temperature during PL-treatments, water evaporated fast, causing much moisture accumulated beneath the quartz plate, indicating this system was helpful in microbial inactivation. Although the quartz-covered treatment resulted in much color degradation, it played an important role in

black peppercorns pasteurization, and thus in the following studies, all treatments were completed using quartz plate.

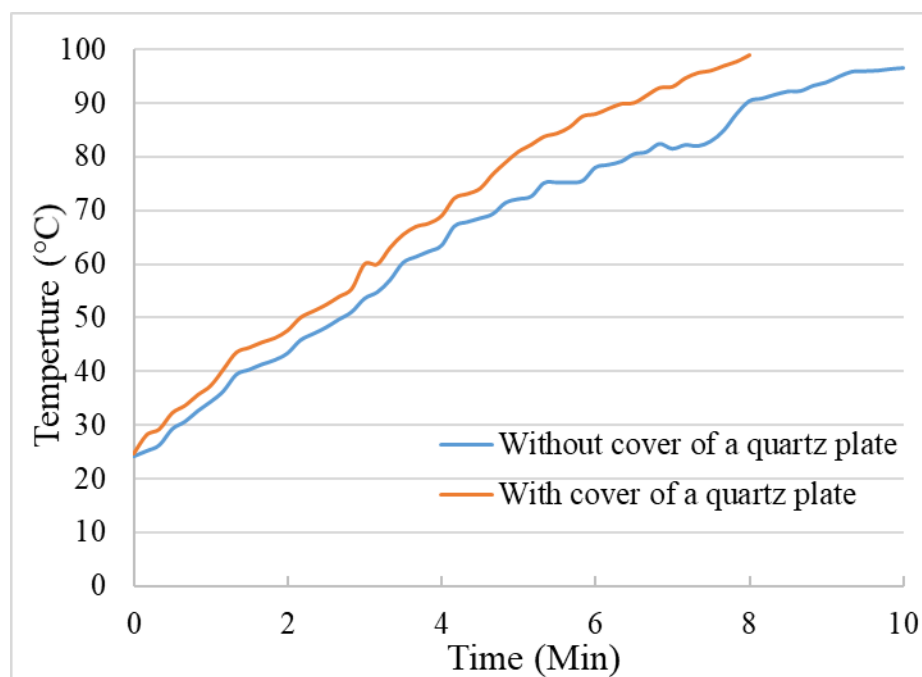


Figure 5.4. Temperature profile with/without quartz-covered PL treatment for 8 and 10 min respectively.

The temperature depends on surface of samples, it went up over 50°C within 3 min. In order to achieve desirable disinfection effect, the temperature was very close to 100°C. This period for quartz-covered treatment was completed much faster than that for without covered, due to the quartz helped remain heat inside the pan.

5.4.2 Effect of temperature cycling between 55-80°C

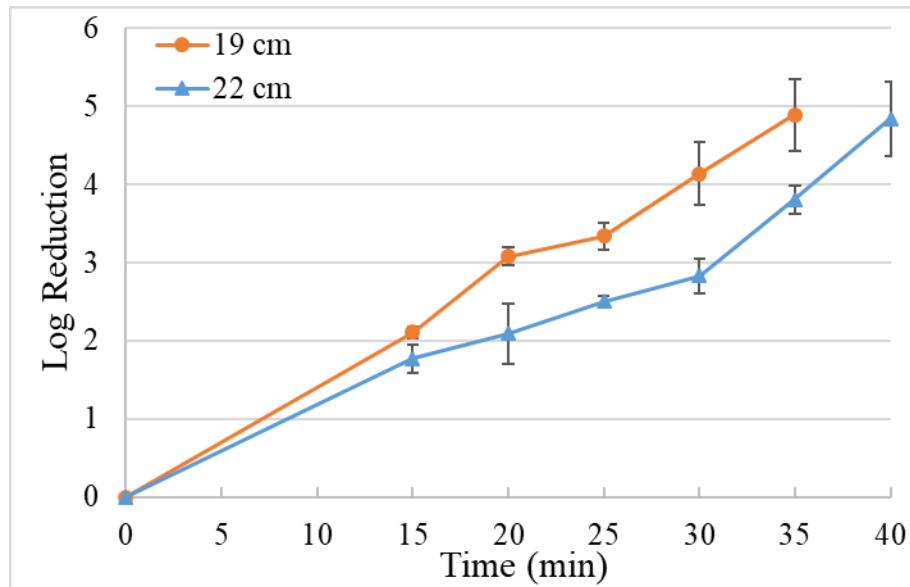


Figure 5.5. PL inactivation of *Salmonella* on black peppercorns with temperature cycling. Black peppercorns (300g) inoculated with *Salmonella* were immersed in tap water for 1 min placed in the large stainless steel pan with a quartz plate cover, and then exposed to PL at distances of 19 and 22 cm. The temperature was control between ~55-80°C during the PL treatment. Data represent mean of three replicates \pm standard deviation.

In order not to damage product quality, temperature should be controlled in a lower range with longer treatment time. From Figure 5, it could be found that 19cm treatment with temperature between 55 and 80 °C for 35 min PL treatment could reduce *Salmonella* population to approximate 5 log CFU/g, and that need additional 5 minute for 22 cm treatment.

Table 5.2-Effect of temperature cycling on black peppercorns' color and weight.

Black peppercorns (300g) were immersed in tap water for 1 min, placed in the large stainless-steel pan with a quartz plate cover, and then exposed to PL at distances of 19 and 22 cm. The temperature was control between ~55-80°C during the PL treatment. Data represent mean of three replicates \pm standard deviation.

Time (min)	% Color decrease			% weight change
	L	a	b	
19cm				
15	16.43±0.20	12.21±0.52	18.54±0.51	7.20 ±0.15
20	17.65±0.29	13.66±0.47	20.68±0.27	2.60±0.42
25	19.70±0.29	17.14±0.46	26.89±0.28	-1.00±0.07
30	22.58±0.16	30.00±0.18	30.13±0.11	-2.20±0.14
35	28.37±0.65	36.02±0.32	42.36±0.21	-3.80±0.31
22cm				
15	10.50±1.05	5.55±0.33%	9.30±0.22	9.80±0.17
20	12.30±0.73	24.30±0.28	19.00±0.29	4.60±0.23
25	18.74±1.23	23.77±0.29	23.85±0.16	1.00±0.27
30	20.02±0.17	34.87±0.17	35.28±0.53	-1.00±0.06
35	25.69±0.33	35.27±0.23	39.57±0.23	-2.20±0.12
40	35.65±0.84	58.37±0.18	41.56±0.30	-5.20±0.11

Also, for the same inactivation efficacy, color and weight parameters for 19 cm with 35 min treatment led to much lower quality deterioration than that for 22cm.

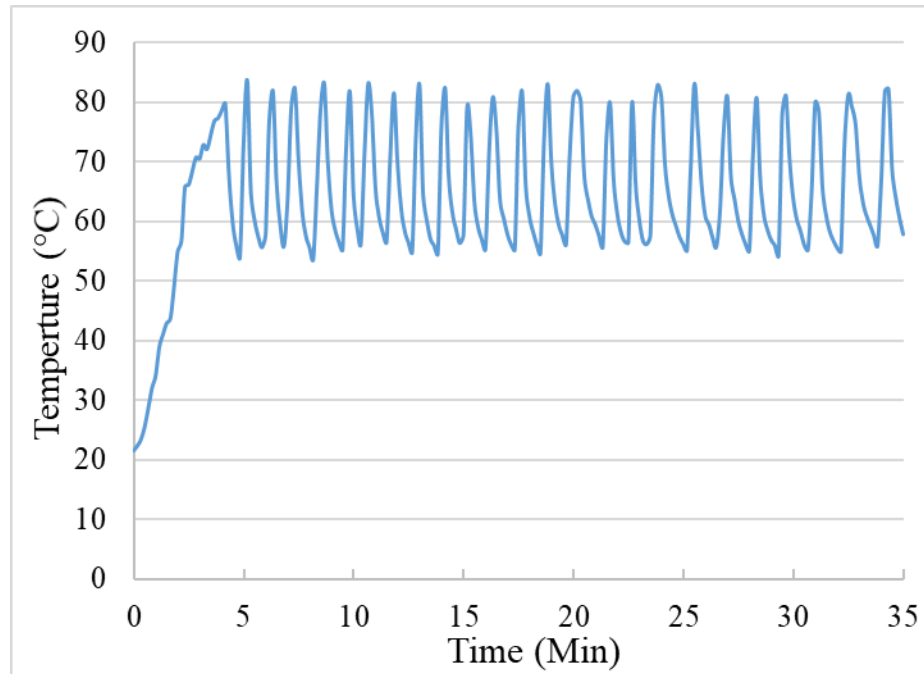


Figure 5.6. Temperature profile for the 19 cm PL-treatment with temperature cycling.

Temperature was rapidly rising up to 80°C and changed very fast by manually turning on and off the pulsed light machine.

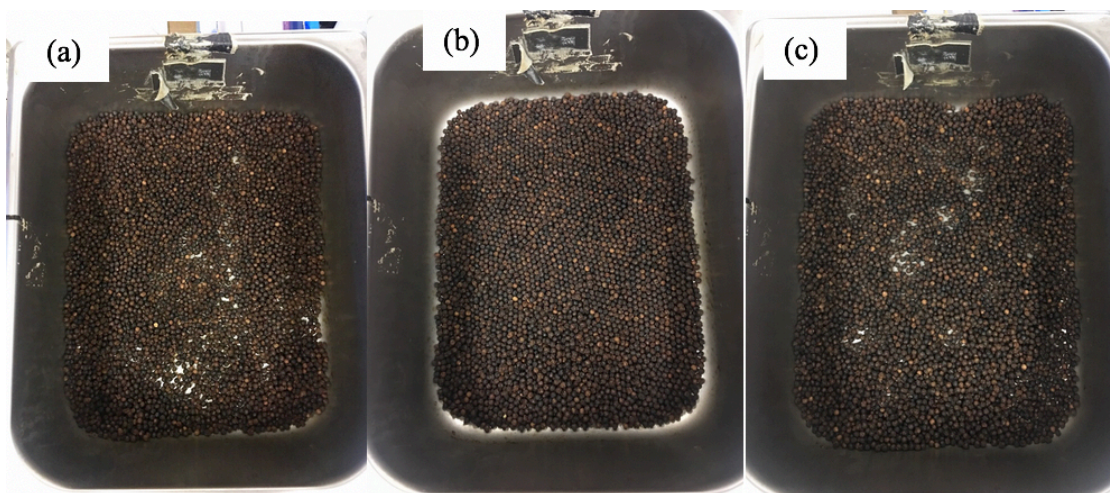


Figure 5.7. Appearance of black peppercorns after PL-treatment for two distances with temperature control a) control-untreated sample; b) 19cm from lamp after 35min treatment; c) 22cm from lamp after 40min treatment

Visually, the treated black peppercorns were darkening compared with control group since heat produced by PL treatment caused discoloration. It can be found that black peppercorns in picture b were much brighter than c, that was also proved by L value in Table 3, the decrease rate for 35 min is smaller than 40 min PL treatment.

5.4.3 Effect of PL intensities: With a quartz plate cover, but without temperature cycling

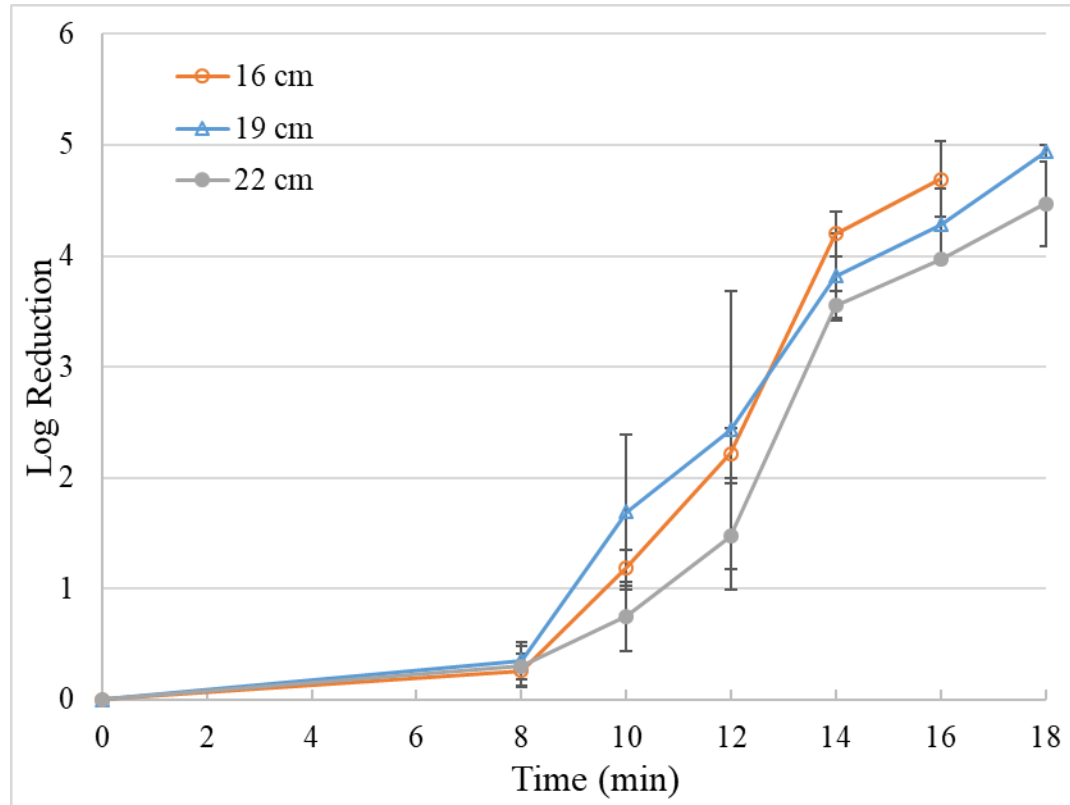


Figure 5.8. PL inactivation of *Salmonella* on black peppercorns without temperature cycling. Black peppercorns (300g) inoculated with *Salmonella* were immersed in tap water for 1 min, placed in the large stainless-steel pan with a quartz plate cover, and then exposed to PL at distances of 16, 19, and 22 cm. Data represent mean of three replicates \pm standard deviation.

As have mentioned in previous chapters, the closer to pulsed light lamp, the higher the intensity as well better inactivation effect. Sample size also influenced the result, it took much longer time to reach over 5-log reduction of *Salmonella* for large-scale black peppercorns than small-scale.

Table 5.3-Effect of PL intensity on black peppercorns' color and weight. Black peppercorns (300g) were immersed in tap water for 1 min, placed in the large stainless-steel pan with a quartz plate cover, and then exposed to PL at distances of 16, 19, and 22 cm. Data represent mean of three replicates \pm standard deviation.

Time (min)	% Color decrease			% weight change
	L	a	b	
16cm				
8	12.93±0.84	16.78±0.38	41.23±1.27	2.59±0.35
10	16.45±0.38	21.92±0.51	44.85±0.16	0.99±0.24
12	18.02±1.39	25.06±0.23	47.31±0.17	-0.20±0.06
14	25.54±0.55	28.91±0.25	53.92±0.09	-2.58±0.37
16	29.06±1.34	31.25±0.65	56.34±0.40	-5.78±0.15
19cm				
8	10.30%±0.23	14.30±0.46	39.60±0.51	9.00±0.14
10	10.78±0.22	15.32±0.46	41.56±0.81	7.16±0.21
12	11.02±0.48	15.98±0.19	43.23±0.84	3.19±0.23
14	11.50±0.32	18.34±0.38	45.90±1.24	-1.00±0.18
16	12.10±0.83	24.78±0.40	46.12±0.49	-2.39±0.18
18	12.40±0.37	28.30±0.78	49.70±0.46	-2.79±0.14
22cm				
8	5.21±0.09	13.85±0.34	38.45±0.44	7.00±0.20
10	7.93±0.11	14.56±0.30	39.72±0.31	3.58±0.30
12	8.43±0.12	15.33±0.30	40.07±0.59	1.20±0.09
14	8.95±0.15	17.94±0.24	41.06±0.84	-1.99±0.18
16	9.04±0.14	24.21±0.40	41.33±0.94	-2.02±0.11
18	9.34±0.19	24.50±1.24	41.67±0.79	-2.43±0.24

To be treated conveniently, black peppercorns were placed on an adjustable shelf inside the PL chamber. For each intensity, shorter time treatment increased sample weight without much damage on quality. 16 cm treatment required 16 min to get desirable log-reduction value; however, the black peppercorns underwent maximum color degradation and weight loss. There was slight color difference between 19 and 22cm treatment, but log reduction of *Salmonella* for 19 cm was higher

than that for 22 cm. Overall, the L, a, and b values showed that lower intensity was helpful for good product quality.

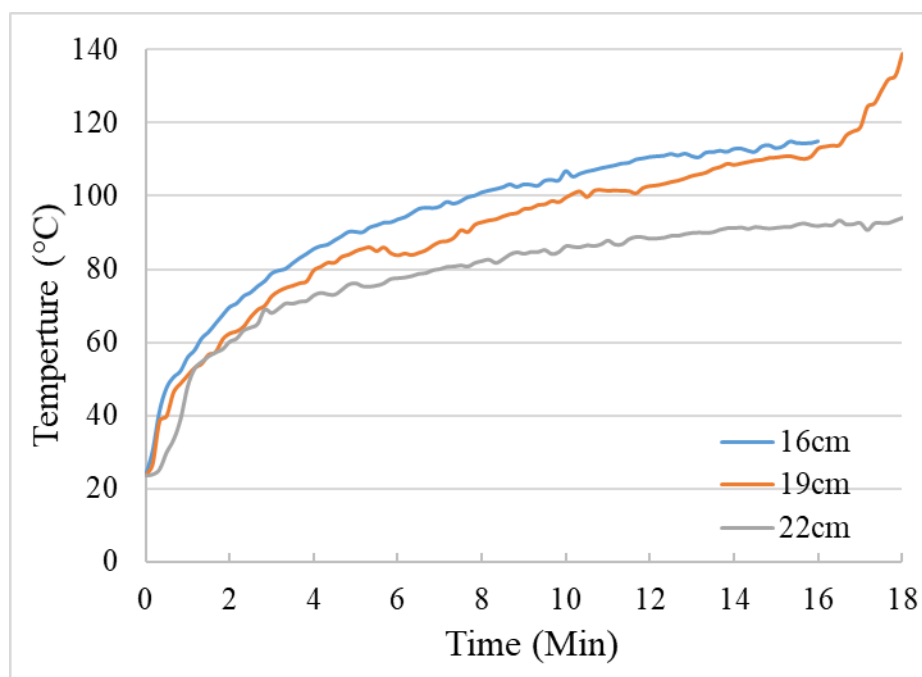


Figure 5.9. Temperature profiles for all three distances.

Temperature increased rapidly and kept going up with PL treatment. Three intensities showed similar temperature change and all temperature ended over 90°C, which caused surface burning. For that reason, black peppercorns lost much color and weight.

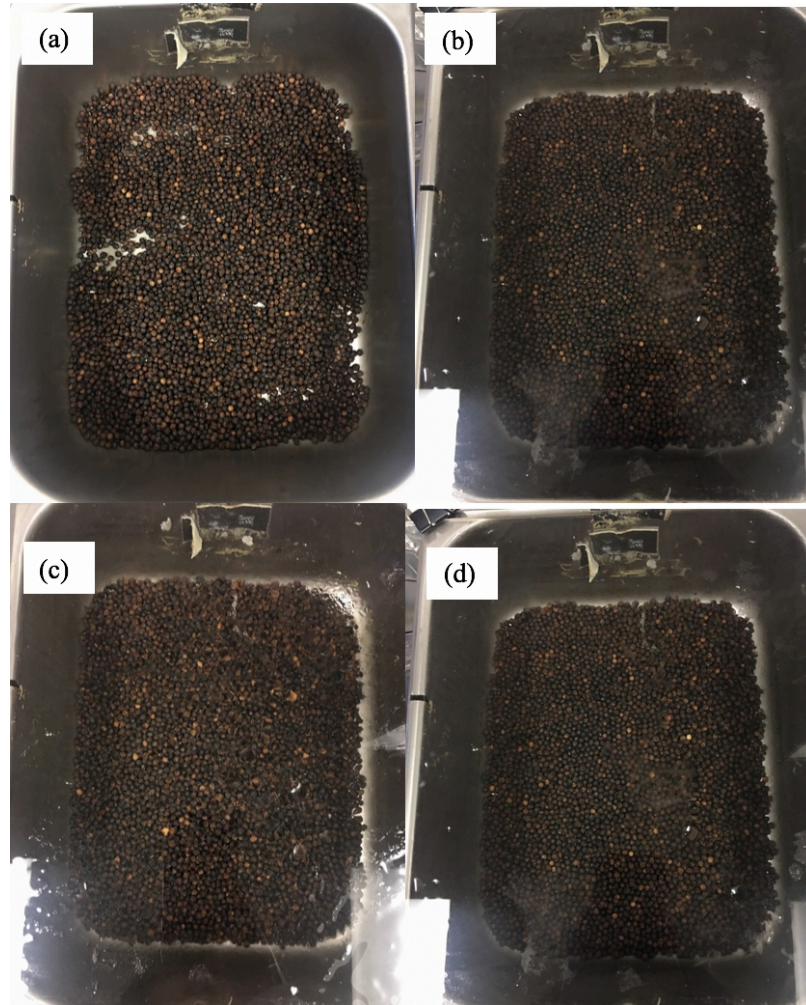


Figure 5.10. Appearance of black peppercorns. a) Control; b) PL for 16 min at a distance of 16 cm; c) PL for 18 min at a distance of 19 cm; d) PL for 18 min at a distance of 22 cm

Although color and weight parameters showed much change on black peppercorns quality, three intensities produce good visible quality attributes. People cannot tell much difference among them visually, and there was much water under quartz plate due to evaporation during heat treatment, but black peppercorns were pretty dry after 18 min treatment.

5.5 Discussion

Quartz glass has good optical transmittance (Ueda et al. 2003) and it is transparent to accept wide wavelength from near infrared region to ultraviolet region of lights (Ikuta et al., 2003). It is so stable that has high resistance to heat and irradiation (Ohga et al. 1995). Therefore, quartz glass was selected as the coverage for metal pan to let PL pass through and treat samples. Based on the result of this study, quartz plate significantly increased inactivation rate and caused slight weight loss compared with treatment without quartz cover. This finding can be combined with other pasteurization methods to efficiently decontaminate black peppercorns and other low-moisture foods.

PL intensity on inactivation efficacy has been widely investigated by many researchers, in this study, three distances for 50g black peppercorns were selected to test the efficacy of different treatments for further experiments. For high intensity treatments (16cm), although the *Salmonella* inhibition effect was the highest, much more weight loss and color degradation on sample were also observed. Cao (2017) also found similar result for pulsed light treatments on decontamination of strawberries and blueberries. Therefore, high intensity was not selected for further investigation, medium (19cm) and low (22cm) intensities were used for large-scale study while controlling treatment temperature.

Temperature is one of the most important factors for reducing spoilage and pathogenic microorganisms in food. In this study, pulsed light treatment caused the increase of temperature which was recorded for both small and large-scale sample. When identical treatment time was conducted on black peppercorns for open and quartz covered unit, the temperature of closed system was higher and trendlines were almost parallel for those two systems. Comparison between treatment with and

without temperature controlled showed that product quality differed in higher temperature and 55-80°C. Lower temperature in combination with medium intensity maximum retain food quality and achieved desirable killing effect.

Black peppercorns had both extra weight increase and loss due to 1 min immersed in water where large amount of water attached to black peppercorns. Black peppercorns treated for longer time showed higher weight loss than those treated for shorter time, nutrients and chemical compounds of product usually lose with heat treatment (Choi et al., 2006). Other researchers also found chemical composition of nutmeg oil changed when heated up to 180°C (Tomaino et al., 2005). In our study, 3.8% weight decrease for 19cm after 35 min PL treatment is highly unfavorable for consumers. Similarly, high temperature during quartz plate covered system resulted in darkening surface of black peppercorns. According to Jeong's (2007) study, decrease of color level resulted in worse quality of spice. Last but not least, there was no significant visible difference between any control group and treated one in this chapter although color and weight parameters showed some side effects of PL treatments in tables.

5.6 Conclusion

Quartz plate covered system significantly worked better in inactivating *Salmonella* than an open system inside PL chamber. What's more, medium intensity of PL treatment was a good selection for killing microorganisms or food surface. Not temperature-controlled system with quartz cover required much shorter time than temperature between 55-80°C with quartz plate treatment under medium intensity (19 cm) is desirable for us. Based on this study, high temperature with short time PL treatment is recommended for antimicrobial processing.

REFERENCES

- Al-Bachir, M. "Effect of gamma irradiation on fungal load, chemical and sensory characteristics of walnuts (*Juglans regia* L.)." *Journal of Stored Products Research* 40.4 (2004): 355-362.
- Balasubramanian, S., et al. "Postharvest processing and benefits of black pepper, coriander, cinnamon, fenugreek, and turmeric spices." *Critical reviews in food science and nutrition* 56.10 (2016): 1585-1607.
- Cao, Xinang. Effects of pulsed-light treatments on shelf-life and decontamination of strawberries and blueberries. Diss. University of Delaware, 2017.
- Choi, Y., et al. "Influence of heat treatment on the antioxidant activities and polyphenolic compounds of Shiitake (*Lentinus edodes*) mushroom." *Food Chemistry* 99.2 (2006): 381-387.
- De Boer, E., W. M. Spiegelberg, and F. W. Janssen. "Microbiology of spices and herbs." *Antonie van Leeuwenhoek* 51.4 (1985): 435-438.
- Demirci, Ali, and Michael O. Ngadi, eds. *Microbial decontamination in the food industry: Novel methods and applications*. Elsevier, 2012.
- Duncan, Susan E., et al. "Processes to preserve spice and herb quality and sensory integrity during pathogen inactivation." *Journal of food science* 82.5 (2017): 1208-1215.
- Farkas, J., and E. Andrassy. "Comparative analysis of spices decontaminated by ethylene oxide or gamma radiation." *Acta Alimentaria* 17.1 (1988): 77-94.
- González, I., et al. "Thermal inactivation of *Bacillus cereus* spores formed at different temperatures." *International Journal of Food Microbiology* 51.1 (1999): 81-84.
- Hackwood, S. "An introduction to the irradiation processing of foods." *Food irradiation*. 1991.
- Hadidi, Milad, et al. "Optimisation of steam blanching on enzymatic activity, color and protein degradation of alfalfa (*Medicago sativa*) to improve some quality characteristics of its edible protein." *Food chemistry* 276 (2019): 591-598.
- Huang, Yaixin, et al. "Application of water-assisted pulsed light treatment to decontaminate raspberries and blueberries from *Salmonella*." *International journal of food microbiology* 208 (2015): 43-50.

- Ikuta, Yoshiaki, et al. "Synthetic quartz glass and method for preparing the same." U.S. Patent No. 6,576,578. 10 Jun. 2003.
- Jeong, Seul-Gi, and Dong-Hyun Kang. "Influence of moisture content on inactivation of *Escherichia coli* O157: H7 and *Salmonella enterica* serovar Typhimurium in powdered red and black pepper spices by radio-frequency heating." *International journal of food microbiology* 176 (2014): 15-22.
- Leistritz, W. "Methods of bacterial reduction in spices." (1997).
- Lilie, Markus, et al. "Decontamination of spices by combining mechanical and thermal effects—an alternative approach for quality retention." *International journal of food science & technology* 42.2 (2007): 190-193.
- MacGregor, S. J., et al. "Light inactivation of food-related pathogenic bacteria using a pulsed power source." *Letters in Applied Microbiology* 27.2 (1998): 67-70.
- Moreaua, M., et al. "Decontamination of spices by using a pulsed light treatment." *Food Process Engineering in a Changing World* (2011): 22-26.
- Nicorescu, I., et al. "Pulsed light inactivation of *Bacillus subtilis* vegetative cells in suspensions and spices." *Food Control* 31.1 (2013): 151-157.
- Ohga, Yuichi, et al. "UV light-permeable glass and article comprising the same." U.S. Patent No. 5,474,589. 12 Dec. 1995.
- Oner, Manolya E. "Minimally Processed Herbs, Spices, Medicinal and Aromatic Plants." *Minimally Processed Refrigerated Fruits and Vegetables*. Springer, Boston, MA, 2017. 385-401.
- Ravindran, P. N., and Johny A. Kallapurackal. "Black pepper." *Handbook of herbs and spices*. Woodhead Publishing, 2012. 86-115.
- Ronteltap, Amber, et al. "Consumer acceptance of technology-based food innovations: lessons for the future of nutrigenomics." *Appetite* 49.1 (2007): 1-17.
- Schweiggert, Ute, Andreas Schieber, and Reinhold Carle. "Inactivation of peroxidase, polyphenoloxidase, and lipoxygenase in paprika and chili powder after immediate thermal treatment of the plant material." *Innovative Food Science & Emerging Technologies* 6.4 (2005): 403-411.
- Schweiggert, Ute, Reinhold Carle, and Andreas Schieber. "Conventional and alternative processes for spice production—a review." *Trends in Food Science & Technology* 18.5 (2007): 260-268.

- Siebert, Tracey E., et al. "Determination of rotundone, the pepper aroma impact compound, in grapes and wine." *Journal of agricultural and food chemistry* 56.10 (2008): 3745-3748.
- Suhaj, Milan, et al. "Effect of γ -irradiation on antioxidant activity of black pepper (*Piper nigrum* L.)." *Food Chemistry* 97.4 (2006): 696-704.
- Tomaino, A., et al. "Influence of heating on antioxidant activity and the chemical composition of some spice essential oils." *Food chemistry* 89.4 (2005): 549-554.
- Ueda, Tetsuji, Akira Fujinoki, and Hiroyuki Nishimura. "Synthetic quartz glass for optical use, heat treatment method and heat treatment apparatus for the same." U.S. Patent No. 6,578,382. 17 Jun. 2003.
- Vajdi, M., and R. R. Pereira. "Comparative effects of ethylene oxide, gamma irradiation and microwave treatments on selected spices." *Journal of Food Science* 38.5 (1973): 893-895.
- Van Doren, Jane M., et al. "Foodborne illness outbreaks from microbial contaminants in spices, 1973–2010." *Food microbiology* 36.2 (2013): 456-464.
- Zaika, Laura L. "SPICES AND HERBS: THEIR ANTIMICROBIAL ACTIVITY AND ITS DETERMINATION 1." *Journal of Food Safety* 9.2 (1988): 97-118.