High Intensity Pulsed Light Emitting Diode (LED) Treatment for Simultaneous *Salmonella* Inactivation and Drying of Wheat Flour and Pet Food

by

Samir Subedi

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Department of Agricultural, Food and Nutritional Science
University of Alberta

Abstract

According to the world health organization, almost 600 million people, or 1 in 10 people suffer from foodborne illnesses globally. Food industry uses several intervention methods to produce safe food products however, cases of food recalls and outbreaks due to microbial pathogens keep increasing every year. High intensity light pulses, emitted from Light Emitting Diode (LED) have the potential to reduce microbial pathogens and dry food products. The overall research objective of this thesis was to develop a LED reactor and determine the efficacy of high intensity light pulses with specific wavelengths to inactivate *Salmonella* and dry food products simultaneously, inside a newly built LED reactor. The first part of this research used light pulses with 275, 365, 395, and 455 nm wavelengths, emitted from LEDs to inactivate a 5 strains cocktail of *Salmonella* in wheat flour at 40%, 75% and 90% environmental relative humidity (RH) conditions. The 60 min treatment of the wheat flour using 275, 365, 395, and 455 nm light pulses at 25°C and 75% RH resulted in 1.07, 2.42, 3.67, and 2.64 log reductions in *Salmonella*, respectively. For the same energy dosage of 1199 J/cm^2, treatments using 365, 395, and 455 nm light pulses resulted in 2.22, 2.48, and 1.61 log reductions in *Salmonella* in wheat flour, respectively. Environmental RH did not have significant influence ($p \geq 0.05$) on *Salmonella* inactivation in wheat flour during LED treatments. Significant temperature increase resulted, decrease in water activity and drying of wheat flour during LED treatments with 275, 365, 395, and 455 nm light pulses.

In the second part of this reserch, an LED reactor using 395 nm light pulses with a vibratory platform and mild hot air fluidization was developed to achieve simultaneous decontamination and drying of food products. Pet food pellets were inoculated with two strain cocktail of *Salmonella* and treated in three modes: 1. using LED treatment alone, 2. vibration + mild hot air (50 ºC)

fluidization, 3. inside LED reactor (LED treatment + vibrations + mild hot air (50 ºC) fluidization). The highest average reduction of 2.26 log was observed after 30 min treatment of pet food pellets using the LED reactor. The water content of pet food pellets decreased from 0.27 to 0.06 kg water/kg dry solids, while the water activity decreased from 0.9 to 0.44 after 30 min treatment using mode 3, showing the fast drying efficacy of the LED reactor. Page and Weibull models were fit to describe the pet food drying kinetics while log-linear and Weibull models were used to fit the *Salmonella* inactivation kinetics. Significant lipid oxidation in pet food pellets was observed during LED treatment. The results suggest that the LED treatment is a promising method for achieving simultaneous *Salmonella* inactivation and drying of food products. An LED-based process can be developed in the future at the industrial level to achieve drying and decontamination of food products in a single processing step, with the integration of approaches to reduce product oxidation.

Preface

This thesis is an original work done by Samir Subedi at the Food Safety and Sustainability Engineering lab of the University of Alberta under the supervision of Dr. Roopesh Mohandas Syamaladevi. The LED reactor described in Chapter 3 was designed and assembled by me under the supervision of Dr. Roopesh Mohandas Syamaladevi. The tray and box for the LED system were built at the fabrication workshop in the biological science building of the University of Alberta.

The thesis consists of five Chapters: Chapter 1 gives the general background information of the work, thesis rationale, hypotheses and objectives; Chapter 2 is the literature review of the work conducted in the field of studies mentioned in Chapter 3 and 4; Chapter 3 focused on determining the potential of four different wavelength light pulses for *Salmonella* inactivation and simultaneous drying of wheat flour at three relative humidity conditions; Chapter 4 described the development of an LED reactor using 395 nm light pulses with hot air fluidization for fast drying and *Salmonella* inactivation on pet food pellets; Chapter 5 includes overall conclusions of the research and future recommendations. The selection of the 395 nm wavelength light pulses for the development of the LED reactor (Chapter 4) was made based on the results of Chapter 3.

Manuscripts based on the study described in Chapter 3 has been submitted to a peer-reviewed journal. Technical research abstract based on results from Chapter 3 was peer-reviewed, and a research poster was presented at the Institute of Food Technologists annual meeting, held at New Orleans, USA from June 2-5, 2019. Research work described in Chapter 4 has been submitted to a peer-reviewed journal for publication.

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My sincere thanks to Dr. Thava Vasanthan for being my thesis defense examination committee member and for his valuable feedback. Thanks to Dr. Michael Gänzle for his advice and support during the two years of my master's program. I am thankful to Jeffrey Johnston from the fabrication workshop at biological science, who helped me to improve the design and build the tray, tray base and the reactor box. Also, I would like to thank Amritha Prasad for helping with the LED treatment protocol development. Thanks to Alvita Mathias, Abdullahi Adam, Barun Yadav, Bina Gautam, Dr. Basheer Iqdiam, Dr. Lihui Du, Ehsan Feizollahi, Harleen Kaur Dhaliwal and Shreyak Chaplot, for their support and encouragements.

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I would like to thank my parents, brother and my sister for their continuous love and support, which helped me to achieve success in academics. Finally, thanks to my wife who drives me to give my best always.

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Abbreviations

AlGaN	Aluminium gallium nitride
CDC	Centre for Disease Control
CPDs	Cyclobutane pyrimidine dimers
CSPI	Center for science in the public interest
R^2	Coefficient of determination
DNA	Deoxyribose Nucleic Acid
ERH	Equilibrium relative humidity
FDA	Food and Drug Administration
FSA	Food Standard Agency
GaAs	Gallium arsenide
GaN	Gallium nitride
GHG	Greenhouse gases
GOC	Government of Canada
LMF	Low-moisture foods
LED	Light Emitting Diode
MDA	Malonaldehyde
MR	Moisture ratio
PMMA	Polymethyl methacrylate
RH	Relative humidity
ROS	Reactive oxygen species
RMSE	Root mean square error
TBA	Thiobarbituric acid
TBARS	Thiobarbituric acid reactive substances
TSA	Tryptic soy agar
TSB	Tryptic soy broth
UV	Ultraviolet
a_w	Water activity

WHO	World health organization
YE	Yeast extract

1. Introduction and objectives

1.1 Introduction

Foodborne illnesses are caused by the consumption of food and water contaminated with microorganisms or harmful chemical substances (WHO, 2019). Every year 10% of the global population suffers from foodborne illnesses (WHO, 2019). Annually, 48 million foodborne illnesses and 3000 mortality cases are reported in the USA by the Centre for Disease Control (CDC, 2019). Government of Canada (GOC, 2016) reported that around 12.5% of the Canadian population encounters foodborne illnesses yearly.

Food products with water activity (a_w) of 0.85 or less are considered less prone to microbial growth and spoilage (Sánchez-Maldonado et al., 2018). However, recent recalls and outbreaks related to *Salmonella* in low-moisture foods (LMF) signified the importance of LMF decontamination. More than 250 different types of foodborne illnesses have been reported, and *Salmonella* is one of the major microorganisms responsible for these illnesses (CDC, 2019). Among the LMFs, wheat flour and pet foods were recently associated with several foodborne outbreaks and recalls caused by *Salmonella* (McCallum et al., 2013; Rose et al., 2012). *Salmonella* Senftenberg and *Salmonella* Typhimurium were among the major *Salmonella* serotypes associated with pet food contamination (Li et al., 2012). Several microbial intervention technologies are being used in the food industry to address this problem. At low-moisture conditions, *Salmonella* exhibits high resistance to microbial intervention technologies. Therefore, the challenge to inactivate *Salmonella* in LMF has drawn significant interest from researchers and the food industry.

Heat treatment is conventionally used for microbial inactivation in the food processing industry but excessive heat treatment can result in oxidation, degradation in sensory properties, loss in nutritional attributes and sometimes development of the unwanted chemicals and flavors (Arnoldi,

2001; Corradini & Peleg, 2004). This leads to the development of several new emerging technologies including high intensity pulsed light, pulsed electric field, high-pressure processing, microwave and radiofrequency heating, ultrasound processing to reduce microbial pathogens in food (Roohinejad et al., 2018). But, most of these technologies are not effective in reducing pathogenic microorganisms in LMFs. High intensity pulsed light has shown the potential to reduce microbial pathogens such as *Cronobacter sakazakii* in LMFs such as non-fat dry milk, wheat flour, and egg white powder (Chen et al., 2019, 2018)

Light Emitting Diode (LED) technology is a novel approach to generate light pulses and it has several applications in the agriculture and food industry. Treatment of food products with high intensity light pulses, emitted from LEDs is a novel microbial intervention approach. LEDs use semiconductor materials to emit light, which makes the system non-hazardous, compared to conventional mercury lamps, which use mercury. The robust design and long lifetime make LEDs ideal for manufacturing and processing facilities. The light pulses generated from LEDs are monochromatic and available in the market for a range of wavelengths.

The light pulses with wavelengths of 275 nm (UV-C), 365 nm (UV-B), 395 nm (UV-A) and 455 nm (Blue) were used for comparative microbial inactivation analysis in this research. These wavelengths have been reported to have antimicrobial effects. Mechanisms of bacterial inactivation by these light pulses were mostly due to the initiation of lethal reactions within bacterial cells. The UV-C light absorbed by deoxyribonucleic acid (DNA) of bacterial cells generate mutagenic pyrimidine 6-4 pyrimidone and cyclobutane pyrimidine dimers (CPDs) within the bacterial cells, resulting in the inhibition of DNA replication and transcription (Friedberg, 2003; Lin et al., 2018). UV-A exposure to bacterial DNA results in the photooxidation of guanine base due to the generation of reactive oxygen species (ROS) within the cells with or without the

help of photosensitizers (Nelson et al., 2018; Jiang et al., 2009). Blue light exposure results in photooxidation within bacterial cells by the generation of ROS with the help of photosensitizers like nucleic acids, flavins, vitamins, and porphyrins (Ashkenazi et al., 2003; Lipovsky et al., 2009). These studies and results proved the lethal effect of UV-C, UV-A and blue light. But, comparative studies on understanding the antimicrobial efficacy of light pulses with these wavelengths for microbial inactivation in foods are rare.

Long exposure to high intensity light can result in significant heat generation (Fine & Gervais, 2004). This heat generation can lead to drying by the removal of water during the LED treatment of food products. Food products like grains, dried fruits, and pet foods are dried before packaging. Effectiveness of light pulses emitted from LEDs for drying and decontamination of food products has never been studied previously.

Process parameters like dosage or light energy per unit area, irradiance, relative humidity of the environment, water activity of the food sample, wavelength of light pulses, and temperature can have significant effects on microbial inactivation during LED treatment. Previous research reported that microbial resistance to intervention processes is higher at lower water activity values of food products (Archer et al.,1998; Kataoka et al., 2014; Syamaladevi, et al., 2016a; Villa-Rojas et al., 2013). The wavelength of the emitted light has an effect on the mechanism of bacterial cell death (Nelson et al., 2018). However, more research is required to understand the effect of important process and product parameters on microbial inactivation during LED treatment.

1.2 Hypotheses

Light pulses with certain wavelengths, emitted from LEDs can inactivate microorganisms significantly, depending on the important product (e.g., water activity) and process (e.g., the

wavelength of light pulses, dosage, relative humidity of the environment etc.) parameters. As the microbial inactivation mechanisms are dependent on the wavelength and energy of light pulses, it was hypothesized that the wavelength and energy of light pulses will determine the microbial inactivation rates during LED treatments (Chapter 3). The inactivation rate of *Salmonella* can be enhanced at high water activity conditions by microbial intervention methods. It was hypothesized that higher *Salmonella* inactivation can be achieved by the incorporation of moisture during LED treatments at high humidity conditions (Chapter 3). In addition, the heat generated during the high intensity LED treatments can result in water removal, leading to drying of food products (Chapter 3).

Treatment by light pulses from LEDs is essentially a surface decontamination method. Hence, better exposure of microbial pathogens to light pulses can enhance their inactivation during LED treatments. It was hypothesized that the addition of fluidization by hot air and vibration will enhance the *Salmonella* inactivation efficacy and drying of high intensity pulse LED treatments (Chapter 4).

1.3 Objectives

The overall objective of the research conducted in this thesis research was to develop an LED reactor, which can decontaminate and dry food products simultaneously. The specific objectives of this research project were to:

i) compare *Salmonella* inactivation efficacy of light pulses with wavelengths of 275, 365, 395 and 455 nm emitted from LEDs in wheat flour (Chapter 3);

ii) compare the drying potential of light pulses with wavelengths of 275, 365, 395 and 455 nm in wheat flour (chapter 3);

iii) compare the effect of the environmental RH and product water activity on the *Salmonella* inactivation efficacy and drying potential pulsed LED light treatments (chapter 3);

iv) design and build an LED reactor to achieve simultaneous drying and decontamination of the food products (Chapter 4) and;

v) evaluate the *Salmonella* inactivation efficacy and fast drying potential of the new LED reactor (chapter 4).

Salmonella enterica was selected as the model microorganism in this thesis research as it was associated with several low-moisture food recalls and outbreaks, as described in chapter 2. Wheat flour is a low-moisture food and has been previously associated with recalls. Wheat flour was selected to understand the *Salmonella* inactivation efficacy of light pulses with different wavelengths and energy, emitted from LEDs (chapter 3). Based on the results from chapter 3, the 395 nm light pulse was selected to use inside the LED reactor (chapter 4). Pet food pellets were selected in chapter 4 to evaluate the drying and decontamination efficacy of the new LED reactor because of the following reasons. Pet food has been associated with *Salmonella* related recalls previously. Pet food pellets are generally dried before packaging to a target water activity and were ideal to study the drying efficacy of the LED reactor. Also, pet food pellets are small particulate foods, ideal for air fluidization, which was used inside the LED reactor.

Chapter 2. Literature review

2.1 Foodborne illnesses and recalls

One in ten people globally (WHO, 2019), one in eight in Canada (GOC, 2016), and 1 in 6 people in the USA (CDC, 2018), suffers from foodborne illnesses every year. Government of Canada (GOC, 2016) reported that 4 million Canadians suffer from foodborne illnesses annually, resulting in 11,600 hospitalizations and 238 cases of death. Consumption of food contaminated with *Salmonella* is responsible for 5% of cases of estimated illnesses, 24% of hospitalizations, and 16% of cases of mortality in Canada. Similarly, the food standard agency (FSA, 2012) reported, around 1 million population in the United Kingdom, to suffer from foodborne illnesses every year, which accounts for annual financial loss of £1.5 billion. In 2010, 1.4 million incidents of salmonellosis were recorded in the USA resulting in $2.71 billion in financial loss (Andino & Hanning, 2015).

2.2 Low-moisture food safety

LMFs include a variety of food products including dried fruits and vegetables, herbs, egg powder, wheat flour, milk powders, peanut butter, honey, dried pet food pellets, peanuts, grains, spices, cereals, hydrolyzed vegetable protein powder, etc. (Beuchat et al., 2013; Gautam, 2019). LMFs are generally considered as safe from microbial proliferation however, several incidents of foodborne outbreaks and recalls are associated with LMFs (Podolak et al., 2010). *Salmonella* is one of the common microorganisms associated with LMF recalls and outbreaks (Sánchez-Maldonado et al., 2018), including wheat flour (Mccallum et al., 2013), almonds (Müller et al., 2007), chocolate (Werber et al., 2005), dried pet foods (FDA,2019), infant formula (Angulo et al., 2008) etc.

2.2.1 *Salmonella* spp.

Salmonella genus belongs to the Enterobacteriaceae family and consists of two species *S. bongori* and *S. enterica* which includes more than 2500 serovars of *Salmonella* spp., identified to date (Lamas et al., 2018). *Salmonella enterica* strains are responsible for more than 99% of human salmonellosis and associated with foodborne outbreaks and recall (Chousalkar et al, 2018; Lamas et al., 2018; Wilson et al., 2018). *Salmonella enterica* strains are facultatively anaerobic, rod-shaped gram-negative bacteria and when transmitted to humans through foods, that will result in gastroenteritis(Andino & Hanning, 2015). Every year, 93.8 million population suffers from gastroenteritis globally, resulting in 155,000 cases of mortality due to salmonellosis (Chousalkar et al., 2018). *S. enterica* serovar Typhimurium is responsible for 64.1% of the total reported cases of human illnesses among all the cases associated with non-typhoidal strains of *Salmonella* (Wilson et al., 2018).

2.2.2 Wheat flour and *Salmonella* associated food safety risk

Wheat flour and wheat flour-based food products are vastly consumed throughout the world. Wheat flour has been associated with several outbreaks associated with *Salmonella*. FDA (2019) has recently reported that the wheat flour recalls of Pillsbury brand due to potential *Salmonella* contamination. In wheat flour, *Salmonella* can survive for several months at room temperature (Forghani et al., 2019). In 2008, sixty-seven cases of illness were confirmed during an outbreak associated with the consumption of unbaked wheat flour-based cookie-mix in New Zealand (McCallum et al., 2013). In 1993, around 1.3% of wheat flour samples tested for *Salmonella* were positive out of more than 3000 samples (Rose et al., 2012). In research conducted during 2003-2005, six out of 4358 samples of wheat flour were tested positive for *Salmonella* (Mccallum et al., 2013). These results suggest the need for significant measures to decontaminate *Salmonella* from

wheat flour. Between 2004 to 2013 in the US, bakery products are involved in 142 food-related outbreaks and 2822 individuals were reported as ill due to consumption of contaminated baked products made from wheat flour (CSPI, 2015).

Wheat flour needs to be properly baked before eating but the wrong eating habit of any individual can increase the food safety risk associated with its consumption. Country-wide survey about eating habits of consumers conducted in the United States among 1032 consumers in the year 2010 reported that 67% of the population had licked batter before baking and 58% had eaten the unbaked cookie dough purchased from a store. Raw pie, pizza, and biscuits dough were tasted by 22%, 11%, and 24% individuals in the survey, respectively nationwide (ConAgra Mills,2011).

2.2.3 Pet foods and *Salmonella* associated food safety risk

FDA's reportable food registry 5th annual report suggested that 49 out of 343 cases of the *Salmonella* associated recalls and outbreaks in food products between Sept. 2009 to Sept. 2015 were associated with pet foods (FDA, 2016) . Li et al. (2012) reported that 257 out of 2058 pet food samples tested were positive to *Salmonella* strains. Li et al. (2012) isolated 45 different serotypes of *Salmonella* strains including *Salmonella* Senftenberg (8.9%) and *Salmonella* Typhimurium (5.4%) from contaminated pet foods. The *Salmonella* strains in pet foods not only just harm the pet but the people living around as well. Several incidents of human salmonellosis transmitted through pets were observed and reported in the past (Hoelzer et al., 2011; Woodward et., 1997). There was 32 pet food recalls associated with *Salmonella* contamination and outbreaks in the USA between February 8, 2018, to November 15, 2019 (Table 2.1).

Table 2.1 Recalls associated with *Salmonella* contamination in pet foods from February 8, 2018 to November 15, 2019 (FDA, 2019).

Date	Brand Name	Description of product	Company Name
11/14/2019	Quest	Beef Cat Food	Go Raw, LLC
09/24/2019	TDBBS	Pig ear pet treat	TDBBS LLC
09/03/2019	Berkley & Jensen	Pig Ears	Dog Goods USA LLC
08/27/2019	Brutus & Barnaby	Pig ears	Brutus & Barnaby
08/16/2019	Chef Toby	Pig ears	Dog Goods USA LLC
08/15/2019	Texas Tripe	Raw frozen pet food	Texas Tripe Inc.
07/30/2019	Lennox	Pet treat-Pig ears	Lennox Intl
07/26/2019	Lennox	Pig ears	Lennox Intl
07/03/2019	PSP	Pig ears	Pet Supplies Plus
06/26/2019	Aqueon	Aqueon Betta Food	Central Aquatics
12/21/2018	Columbia River Natural Pet Foods	Dog and Cat fresh frozen meats	Columbia River Natural Pet Foods
09/12/2018	Performance Dog	frozen raw pet food	Bravo Packing, Inc.
09/10/2018	Steve's Real Food, Quest	Pet food	Steve's Real Food
04/18/2018	TRUDOG	Pet food-freezed dried raw beef topper	TruPet, LLC
04/17/2018	Vital Essentials®	Dog/pet food Freeze-Dried Beef Toppers and Frozen Beef Chub	Carnivore Meat Company
04/16/2018	Sunseed Vita Prima	Sugar Glider Formula	Sun Seed Inc
03/26/2018	Blue Ridge Beef	Complete raw pet food	Blue Ridge Beef
03/26/2018	Natural Selections and ZooLogics	Fresh raw meals for dogs	Darwin’s Natural Pet Products
03/07/2018	Redbarn	Dog Chews	Redbarn Pet Products
03/07/2018	Redbarn, Chewy Louie, Dentley's, and Good Lovin'	Bully Stick dog food	Redbarn Pet Products, LLC
03/06/2018	Tucker's	5lb Pork-Bison Box	Raw Basics, LLC
03/02/2018	Blue Ridge Beef	Raw pet food for cats	Name Blue Ridge Beef
03/02/2018	Steve’s Real Foods	Raw frozen dog food turkey canine recipe	Steve’s Real Foods
02/26/2018	Vital Essentials	Freeze-dried beef nibblets for dogs	Carnivore Meat Company
02/23/2018	TruDog	Dog food	TruPet, LLC

02/16/2018	Smokehouse	Dog treats	Smokehouse Pet Products, Inc.
02/15/2018	Smokehouse	Dog treats	Smokehouse Pet Products, Inc.
02/15/2018	ZooLogics and more	ZooLogics Duck with Vegetable Meals for Dogs, ZooLogics Chicken with Vegetable Meals for Dogs and more	Arrow Reliance Inc. dba Darwin's Natural
02/09/2018	Raws for Paws	Ground turkey pet food	Raws for Paws
02/08/2018	Barnsdale Farms®, HoundsTooth® and Mac's Choice®	Dog treat- Pig Ears	EuroCan Manufacturing
02/08/2018	Smallbatch	Dog and cat food	Smallbatch Pets Inc.
02/08/2018	Loving Pets, Whole Hearted	Dog treats	Loving Pets

2.2.4 Effect of water activity on survival of *Salmonella* in low-moisture foods

Water activity (a_w) of the food is defined as the ratio of the partial water vapor pressure of food (P) to the partial pressure of the pure water (Po).

$$a_w = P/Po \quad (2.1)$$

The a_w of the food characterized as the chemical potential of the water present in the food and is equal to available partial molar energy of water for a reaction (Al-Muhtaseb et al., 2002, Syamaladevi et al., 2016). Water movement inside and outside of the food product is a function of the a_w of that food. The equilibrium relative humidity (ERH) of the food product can be expressed as: (Troller and Christian 1978).

$$ERH = a_w \times 100\ \% \quad (2.2)$$

A list of the foods with their approximate water activity is presented in Table 2.2.

Table 2.2 Water activity values of different food products

Food product	Water Activity (a_w)	Reference
Apples	0.988-0.975	Schmidt & Fontana, (2008)
Cheese spread	0.95	FDA, (2014)
Red bean pasta	0.93	FDA, (2014)
Butter	0.894	Schmidt & Fontana, (2008)
Canned moist cat food	0.88	Schmidt & Fontana, (2008)
Fudge sauce	0.83	FDA, (2014)
Soft moist pet food	0.83	FDA, (2014)
Soy Sauce	0.8	FDA, (2014)
Peanut butter (15% moisture)	0.7	FDA, (2014)
Dry milk (8% moisture)	0.7	FDA, (2014)
Milk chocolate	0.6	Schmidt & Fontana, (2008)
Almond sliced	0.476	Schmidt & Fontana, (2008)
Walnuts	0.427	Schmidt & Fontana, (2008)
Wheat flour	0.4-5	Carter et al., (2015)
Dry cat chow, Purina	0.236	Schmidt & Fontana, (2008)

Food products with a_w below 0.6 are generally safe from microbial growth (Labuza et al., 1970). Food recalls and outbreaks related to *Salmonella* in low a_w foods show that these microorganisms can survive in desiccated condition even though they cannot grow. Forghani et al., (2019) reported that *Salmonella* strains survived for more than one year in inoculated wheat flour with 0.33 a_w at 23 ^oC. Similarly, Beuchat & Mann (2015) showed that *Salmonella* survived in cookie's filling with 0.33 a_w for more than 182 days. Rayman et al. (1979) observed the survival of *S. infantis* and *S.* Typhimurium in pasta for more than a year. Brar et al. (2015) reported that the *Salmonella* can survive longer than one year in peanuts and pecan kernels at 22 ^oC and the survival rate is even higher at lower temperatures (4 and -24 ^oC). Kataoka et al. (2014) reported that *Salmonella* survived for more than one year at 0.6 and 0.3 a_w in peanut paste. The *Salmonella* strains capability to survive for longer period in dry condition increases the food safety risk associated with the LMFs.

2.2.5 Effect of the water activity on *Salmonella* inactivation

The ability of *Salmonella* survival at low a_w for a long time has developed the need for their inactivation in low-moisture foods by additional antimicrobial interventions. The inactivation efficacy of any of these additional interventions (e.g., heat treatment) reduces significantly with decreased a_w (Table 2.3) which adds further challenge in food decontamination.

Table 2.3 Effect of the a_w of food products on the microbial inactivation efficacy of heat treatment

Food sample	Strain	Temperature (°C)	Time (min)	a_w	Log reduction	Reference
Wheat flour	*Salmonella* Weltevreden	60-62	875	0.4	1	Archer et al., (1998)
Wheat flour	*Salmonella* Weltevreden	60-62	≈110	≈0.56	1	Archer et al., (1998)
Wheat flour	*Salmonella* Weltevreden	66-68	≈75	≈0.59	1	Archer et al., (1998)
Wheat flour	*Salmonella* Weltevreden	66-68	≈800	≈0.21	1	Archer et al., (1998)
Sucrose Sol.	*Salmonella* Typhimurium	57.2	1.1	0.99	1	Goepfert et al., (1970)
Sucrose Sol.	*Salmonella* Typhimurium	57.2	46.7	0.9	1	Goepfert et al., (1970)
Sucrose Sol.	*Salmonella* Senftenberg 775W	57.2	14.5	0.99	1	Goepfert et al., (1970)
Sucrose Sol.	*Salmonella* Senftenberg 775W	57.2	62	0.9	1	Goepfert et al., (1970)
Sucrose Sol.	*Salmonella* Typhimurium	65	6.95	0.95	1	Corry, (1974)
Sucrose Sol.	*Salmonella* Typhimurium	65	31.5	0.85	1	Corry, (1974)
Pet food pellets	Mixed *Salmonella enterica* strains	75	23	0.33	1.85	Gautam, (2019)

Pet food pellets	Mixed *Salmonella enterica* strains	75	23	0.54	3.16	Gautam, (2019)
Pet food pellets	Mixed *Salmonella enterica* strains	75	5	0.75	1.8	Gautam, (2019)

Studies were conducted to understand the survival mechanism and improved resistance of the *Salmonella* in low water activity conditions, still, research is required to understand the greater resistance mechanisms to microbial interventions (Forghani et al., 2019). Finn et al. (2013) described that biological activity responsible for restricting the loss of water through osmoprotectants solute accumulation, rRNA degradation for nutrients requirement fulfillment, living in the viable but non-culturable state, porins expression, filament and biofilm formation are some of the mechanisms that help *Salmonella* to survive at desiccated conditions. Maserati et al. (2017) reported that osmV gene, which regulates the osmoprotectants, induced by 2.91-fold under the desiccated condition and mentioned the influence of the sseD and sopD virulence genes for the survival of *S. enterica* serovar Typhimurium at low a_w conditions.

Heat treatments can be used to reduce *Salmonella* populations in low-moisture foods effectively (Syamaladevi et al., 2016a). However, as described above, *Salmonella* exhibit extremely high resistance to heat treatments at low a_w. Hence, long treatment times are required to achieve the required level of inactivation in the *Salmonella* population in low-moisture foods by heat treatments (Syamaladevi et al., 2016b). This can lead to a significant quality loss in low-moisture food products. Hence, the potential of alternative microbial intervention technologies should be explored to improve low-moisture food safety. Treatment of low-moisture foods by high intensity light pulses emitted from Light Emitting Diode (LED) is one such novel approach.

2.3 LED working mechanism

LEDs are solid-state optoelectronic devices, which emit light upon the application of an electric field. Industrial manufacturing of LED semiconductor chips responsible for light emission, consists of three layers, top layer comprises of concentrated holes (lack of electrons) while the lower layer has concentrated electrons separated by multiple thin layering of materials, which forms a recombination region for these electrons and holes (Pimputkar et al., 2009).

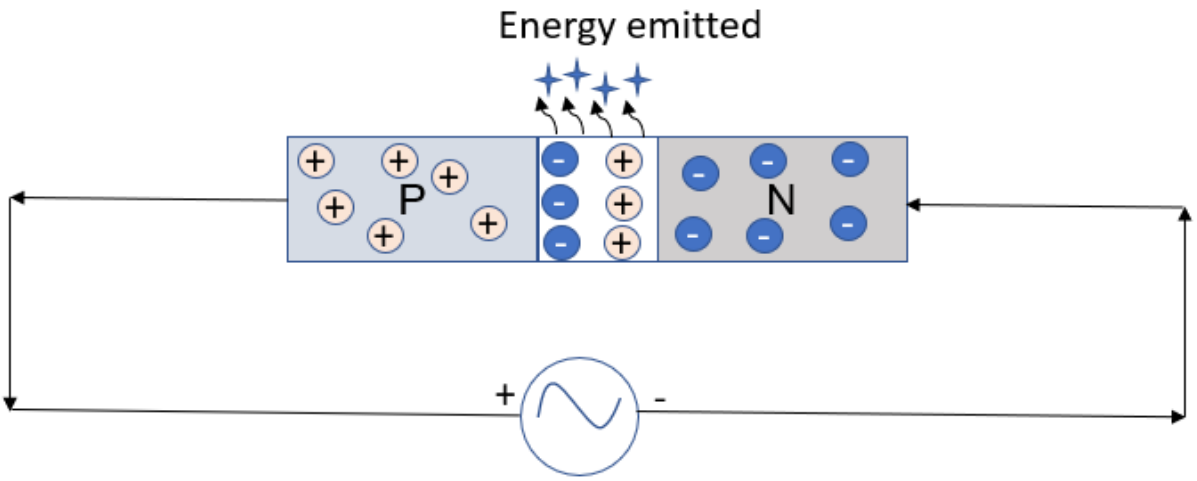


Figure 2.1 LED light emission mechanism

The process of luminescence used to emit light using LEDs is termed as low-field or injection electroluminescence, where excitation ensued by the injection of minority carriers in diodes (DenBaars, 1993). The radiative recombination of electrons and holes emits energy when the free electrons from the conduction band come to the lower energy level of the valence band (Figure 2.2)

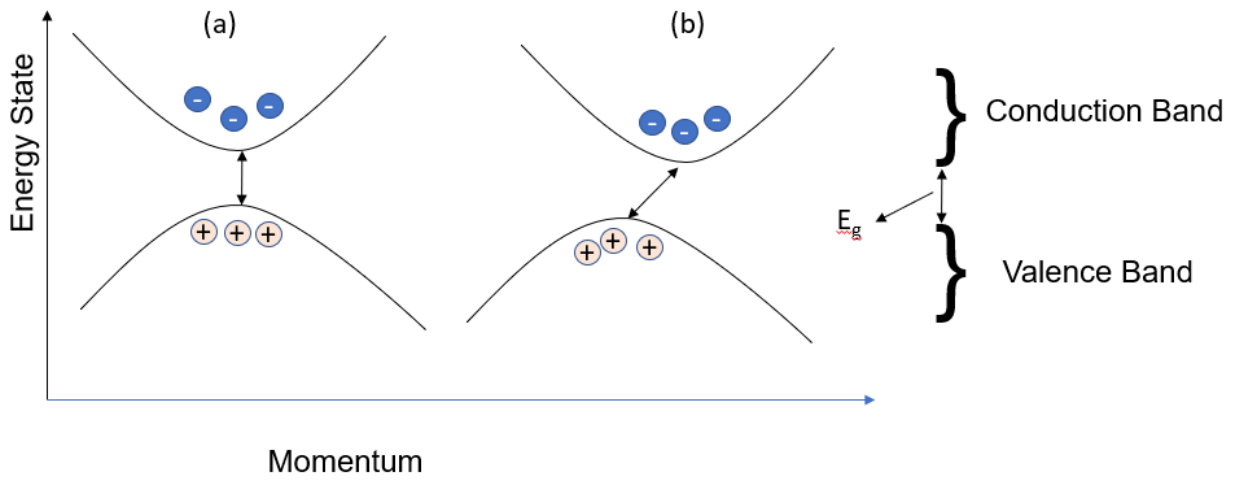


Figure 2.2. Energy state of electrons and holes in semiconductors: a) direct bandgap, b) indirect bandgap

The bandgap having the lowest energy level of the conduction band just above the highest energy level of the valence band possessed direct bandgap, and most of the energy is converted to light energy (Figure 2.2). In the case of the indirect bandgap (Figure 2.2), momentum is not conserved and the causing difficulty on radiative emission, which can be improved by the addition of iso-electronic traps (DenBaars, 1993; Schubert, 2006; Winkler et al., 2014).

The energy released after the recombination of electrons and hole is in the form of photons and thermal energy. Schubert (2006) considered that the heat energy produced during light emission was much lower than the light energy and explained the equation of the photon of the light emitted as given in equation

$$h \times \nu = E_c - E_v \approx E_g \qquad (2.3)$$

where E_c is the energy of electrons in conduction band while E_v is the energy of holes in the valence band and E_g is the bandgap of diodes, signifies the total energy generated during the

electroluminescence and $h \times \nu$ collectively describes the energy of a photon emitted; h is the Plank's constant and ν is the frequency of the photon of light.

The wavelength of light produced using LEDs can be estimated by using equation (Schubert, 2006; Winkler et al., 2014)

$$\lambda = (h \times c) / E_g \quad (2.4)$$

where λ is the wavelength of the light emitted, c is the speed of the light. GaAs have energy between conduction and valence band of 1.42 eV so the light emitted using GaAs semiconductor will have a wavelength of 870 nm (Schubert, 2006).

The equation shows the negative correlation between the wavelength of the light emitted and the bandgap energy of the semiconductors, which means lower bandgap energy emits higher wavelength light and vice versa. The bandgap $Al_{0.1}Ga_{0.9}N$ (3.781eV) has a larger bandgap than GaN (3.42eV) and $In_{0.05}Ga_{0.95}N$, so it is preferred over others to produce lower wavelength light generation (Chang et al.,2002). The use of AlGaN by varying aluminum and gallium doped concentration to increase the bandgap is necessary for the UV and deep UV light (200-280 nm) emission (Bao et al.,2015). However, the light emission efficiency of the UV-LED decreases for shorter wavelengths (Guo et al., 2018; Kim et al., 2015).

2.4 Drying of food products

Drying is an energy-intensive process used in the food industry and energy consumption for drying accounts from 12 to 20% of the total energy use of the industries (Raghavan et al., 2005). Drying of a food product usually needs electrical, coal, natural gas, or liquid oil as a source of energy. Low greenhouse gas (GHG) emitting technologies are required due to the refocus in industry and

government visions to reduce GHG emissions. A drying technology using an electrical source can be potentially efficient in reducing the GHG gas.

Along with bacterial inactivation, the use of the light pulses from LEDs can dry food products. The light pulses from the LED sources can generate a significant increase in product temperature (Du et al., 2020). A positive correlation exists between the drying rate and temperature increase (Mohapatra & Rao, 2005; Xie et al., 2018). Other mechanisms could also be responsible for the quick drying of food products by UV and blue light pulses emitted from LEDs. Further studies on drying mechanisms should be conducted to understand the drying phenomenon caused by UV and blue light pulses.

Several drying models have been proposed to describe drying kinetics of a food product. Moisture ratio (MR) of a food sample can be used to describe the extent of water removed by a drying method. MR of a food sample can be predicted for any drying time using the drying models (Table 2.4) and by regression analysis (Erbay & Icier, 2010; Jian & Jayas, 2018; Midilli et al., 2002; Mohapatra & Rao, 2005)

Table 2.4. Common drying models for describing drying kinetics

Model	Equation
Lewis (Newton)	$MR= \exp(-kt)$
Weibull	$MR= \exp(-(t/\alpha)^{\beta})$
Page	$MR= \exp(-kt^{n})$
Henderson and Pabis	$MR= A \times \exp(-kt)$
Logarithmic	$MR= A \times \exp(-kt) + C$

where MR is the moisture ratio of the food products and t is the total drying time. The value of the constants in the equation can be calculated by regression analysis. The best fit model can be

selected based on the lowest root mean square error (RMSE) value and the highest coefficient of regression (R^2) to predict the MR as the function of the drying time.

2.5 Lipid oxidation in pet foods

Light pulses used for the bacterial inactivation and drying can result in light oxidation of food components such as lipids. Lower wavelength light, especially the UV light has a higher effect on oxidations of the fat and the oils present in food products (Bekbölet, 1990). Lin et al. (1998) suggested that the use of thiobarbituric acid reactive substances (TBARS) method could help to understand the extent of the lipid oxidation in dried pet foods. TBARS assay to determine the extent of the lipid oxidation in food has several advantages since the thiobarbituric acid (TBA) reacts not just with aldehydes produced after oxidation but also with ketones, acids, sugar, vitamins, and amides produced or deteriorated during oxidation (Guillén-Sans et al., 1998).

2.6 Application of UV and blue light for microbial inactivation

2.6.1 Bacterial inactivation mechanisms

Ultraviolet (UV) light can be classified as UV-C (200-280nm), UV-B (280-320nm), UV-A (320-400 nm) based on the wavelength (Surjadinata et al., 2017). The antimicrobial efficacy of UV-C, UV-A, and blue light has been reported before (Wang et al., 2017). UV-C is absorbed by the DNA of the bacteria, resulting in photo products like pyrimidine 6-4 pyrimidone and cyclobutane pyrimidine dimers (CPDs) formation (Figure 2.3) and bacterial cell death (Friedberg, 2003; Lin et al., 2018; Sinha & Häder, 2002; Witkin, 1976).

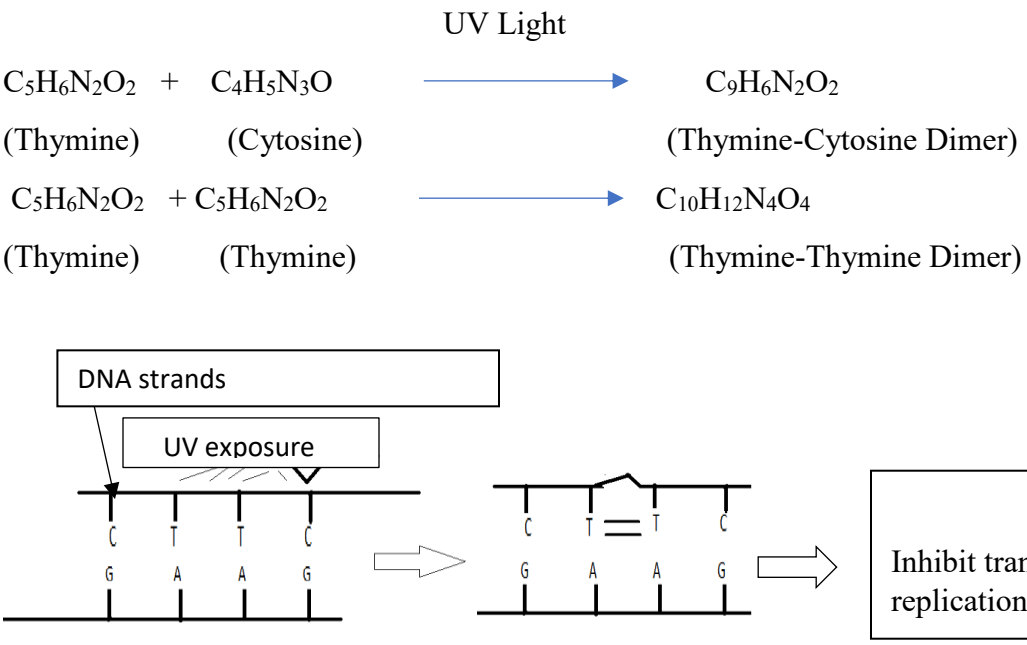


Figure 2.3 Effect of the UV light exposure on the bacterial DNA

DNA consists of purine and pyrimidine nitrogenous bases, and the purine base consists of adenine (A) and guanine (G) bases while pyrimidine consists of thymine (T) and cytosine (C) bases. Figure 2.3 illustrated the bond formation between pyrimidine base-pairs induced by UV light, causing the inhibition of DNA replication and transcription and ultimately the bacterial cell death.

UV-B inactivates the microorganisms by the formation of photoproduct dimers of nucleotides base pair and reactive oxygen species (ROS) inside the bacterial cell (Nelson et al., 2018; Ravanat et al., 2001). UV-A exposure can result in bacterial cell death by the formation of abasic sites and CPDs within DNA (Jiang et al., 2009). UV-A exposure to bacteria causes guanine base photo-oxidation and ROS generation inside bacterial cells causing the formation of irreversible oxidative reactions with or without the help of the endogenous sensitizers (Nelson et al., 2018). Endogenous photosensitizers are flavins (riboflavin), nucleic acids, vitamins, nicotinamide adenine dinucleotides and porphyrins available inside bacterial cells (Nelson et al., 2018). These

photosensitizers can absorb light of broad wavelength and result in the production of lethal photo products like ROS, singlet oxygen, hydroxyl radical etc. causing bacterial death (Lipovsky et al., 2009; Nelson et al., 2018).

2.6.2 Studies on antimicrobial efficacy of UV-C light treatment

UV-C light has been used as a germicidal agent in water treatment, air purification, etc. Syamaladevi et al. (2014) reported the exposure of the 1.7 kJ/m^2 of UV-C radiation of 254 nm resulted in 2.8 log CFU/ml reduction of *Penicillium expansum* on pear surface, but browning of the fruits after storage for 4 weeks was observed and the fruits were less preferred by sensory panelists. Liao et al. (2017) showed that UV-C could penetrate 0.5 cm thick ice to inactivate the bacteria like *E. coli*, *Salmonella* and *L. monocytogenes.* Energy dosage of 780 mJ/cm^2 of UV-C on frozen raspberries inoculated with *L. monocytogenes* resulted in 1.5 logs CFU/g bacterial reduction (Liao et al 2017). Bacterial inactivation in flour could be affected by the shadowing effect of the food products during UV treatment, since the layer of flour powder over bacteria limits the light exposure (Condón-Abanto et al., 2016). Fluidization of food products could be a possible solution to reduce the shadowing effect of the food during UV treatment.

The inactivation efficacy of UV-C LED light with 266, 270, 275 and 279 nm wavelength was compared by Kim et al. (2016) on inoculated sliced cheese showed that at lower energy dosage (1 and 2 mJ/cm^2), no significant effect of increasing wavelength was observed but for higher dosage (3 mJ/cm^2), decontamination efficacies of 266 and 270 nm light treatments were better than those of 275 and 279 nm light treatments. On the contrary, the use of 275 nm UV LED light showed higher *E.coli* inactivation compared to 255 nm light emitted by LEDs (Bowker et al., 2011). Effective energy required for inactivation was better in the case of 280 nm compared to 265 nm light treatment (Zou et al., 2019). These inconsistent results could be due to the lower irradiance

for the deep UV light since the improvement in the intensity of the UV-C light is still work in progress. In addition to that, Gayán et al., (2012) reported that the use of the heat and UV-C could have a synergetic effect on the microbial inactivation of the *Salmonella enterica*.

2.6.3 Studies on antimicrobial efficacy of UV-A light treatment

Li et al., (2010) demonstrated that microbial count in biofilms of the *Candida albicans* and *E. coli* can be significantly reduced by the use of UV-A light emitted from LEDs. The generation of lethal hydroxyl radicals during photoreactions and its effect on the microbial inactivation was studied and proved (Hamamoto et al., 2007; Li et al., 2010). Use of 395 nm LED treatment for *Salmonella enterica* inactivation in wheat flour powder showed the significant lethal effect of the UV-A light and the treatment resulted in product surface temperature increase (Du et al., 2020). A positive correlation between the amount of the radiant energy dosage supplied and *Salmonella* reduction was observed in wheat flour. Both pathogenic and non-pathogenic bacteria can be inactivated using the UV-A light of 365 nm wavelength (Hamamoto et al 2007). Zhao et al. (2009) demonstrated the use of high intensity 350 nm light emitted from LEDs for the inactivation of *Bacillus cereus* spores on a dry surface. Inactivation of microorganisms could be compromised in dense or colored liquid foods since the penetration of the UV-A is affected by increased opacity of the liquid foods. Lian et al. (2010) demonstrated the increase in the color concentration of liquid foods inversely affect the inactivation efficacy of the UV-A light. The light treatment is a surface treatment method and the shadowing effect on solid foods could significantly affect the decontamination process efficiency of light treatment (Gómez-López et al., 2007).

2.6.4 Studies on antimicrobial efficacy of blue light treatment

Blue light compromises the light wavelength between (400-470 nm) and has shown promising microbial inactivation efficacy (Wang et al., 2017). Fang et al. (2015) reported that the use of the blue light of 470 nm inactivated *P. aeruginosa* by 92.4% in 80 min by using irradiance of 100 mW/cm^2. The energy consumed for inactivation was comparatively much higher however, the potential antibacterial effect of blue light cannot be denied. Liang et al. (2015) studied the inactivation efficacy of the 462 nm and reported that blue light treatment could significantly inactivate microorganisms and validated the fact that the addition of the external photosensitizers could enhance its inactivation efficacy. The use of the blue light for the inactivation of *S. aureus* was improved by supplying excessive oxygen during inactivation (Maclean et al., 2008). The sufficient supply of oxygen during blue light inactivation reduces the required energy dosage by 3.5 fold for the same level of inactivation. Ashkenazi et al. (2003) showed that blue light could be instrumental to decontaminate the bacteria including *P. acnes*, demonstrating the significance of the blue light treatment in the medical field. The inactivation of *S. aureus* by blue light was induced by photoexcitation of porphyrins and oxidative reactions (Maclean et al., 2008). Kim et al. (2017) reported that the use of 405 ± 5 nm LED light (1.7 kJ/cm^2) resulted in a 1.2 log reduction of *Salmonella* without the use of photosensitizers. This mechanism proved that, not just with exogenous sensitizers, but blue light could have sufficient interaction with endogenous photosensitizers for the bacterial inactivation.

2.7 Potential of LED treatment for microbial inactivation in low-moisture foods

Recently, the application of pulsed light produced by xenon lamps to inactivate microbial pathogens in powdered solid foods is being studied (MacGregor et al., 1998; Rowan et al., 1999). The use of the xenon lamp induced pulsed light was able to inactivate microorganisms in powdered

food like wheat flour, egg white powder and milk powder (Chen et al., 2018, 2019). However, xenon lamps produce a broad spectrum of the light with peak spectral emission in visible light range (Rowan et al., 1999) while LED generates narrow bandwidths with highly efficient emission of the monochromatic light (D'Souza et al., 2015). LEDs have several advantages compared to conventional mercury lamps, producing UV light. These include compact size, long operational lifetime, requires no warm-up time, no mercury use, and radiate consistent light energy throughout the operation (Kim et al., 2016; Bowker et al., 2011). LEDs possessed the potential of high photoelectric efficiency and irradiance and very low heat loss during light emission (D'Souza et al., 2015). The LED system is easy to install, no need for chemical addition and LEDs do not generate any harmful by-products after food treatments (Hamamoto et al., 2007). The inactivation efficacy of light pulses emitted from LEDs varies with pulse length for the pulsed irradiation. Li et al. (2010) compared the range of the pulse frequency from 1-1000 Hz and proposed the use of pulsed UV-LEDs at 100 Hz is most efficient for the inactivation of biofilms. Pulsed LED light was reported to have higher microbial inactivation for same energy dosage compared to continuous light (Fine & Gervais, 2004; Li et al., 2010; Lin et al., 2018).

Light treatment using LEDs is essentially a surface decontamination process and there may be shadowing effect in powder and solid foods during light treatment, which could reduce its inactivation efficacy. Fine & Gervais (2004) demonstrated that air fluidization had a positive impact on microbial inactivation in powdered foods. Solid food products like grains, pet food pellets, seeds can have contamination on any portion of their surfaces. Continuous light exposure throughout the surfaces of solid foods, whether they are powdered (e.g., wheat flour) or small particulate (e.g., pet food pellets) is important to ensure microbial food safety after light treatments using LEDs.

Significant research is required to understand the inactivation efficacy of light with a broad range of wavelengths (UV-C, UV-A and blue light), emitted by LEDs to compare their antimicrobial efficacy. Previous studies mentioned food drying but did not report drying kinetics and efficacy of LED treatments to produce low-moisture foods. This research gap is addressed in Chapter 3, which focuses on a comparative study of 275 nm, 365 nm, 395 nm and 455 nm light pulses emitted by LEDs for *Salmonella* inactivation and drying in wheat flour. Chapter 3 also compared the effect of the initial a_w on *Salmonella inactivation* efficacy. Development of LED reactor, which can dehydrate foods to desired water activity and inactivate the microorganisms in a single step was developed as part of this thesis research (Chapter 4). This addressed the research gap of understanding the continuous exposure on microbial inactivation efficacy of light pulses from LEDs by combining the light treatment with air fluidization and vibration.

Chapter 3

Inactivation of *Salmonella* and drying in wheat flour after pulsed light emitting diode (LED) treatments

3.1 Introduction

The Centre for Disease Control estimated (CDC, 2018) that one out of six people in the United States (US), and the Government of Canada estimated (GOC, 2016) that one out of eight Canadians, suffer from food-borne illnesses every year. In low a_w foods, including wheat flour, spices, dry nuts, pet foods, infant foods, and chocolates, *Salmonella* and other pathogenic bacteria can survive at viable but not culturable state for long periods of time with increasing resistance to antimicrobial interventions such as heat treatment (Archer et al., 1998; Beuchat et al., 2013; Smith et al., 2016; Forghani et al., 2019; Syamaladevi, et al., 2016 a,b). Thus there is a need to develop novel processing technologies to reduce pathogenic bacteria in low a_w foods.

LEDs are solid-state devices that emit light by electroluminescence. The high intensity light pulses produced by LEDs can be used to reduce pathogenic bacteria in low a_w foods. LEDs are rigid and robust in design; they are easy to install and have a long lifetime (D'Souza et al., 2015; Song et al., 2018). Unlike conventional lamps that produce light pulses, LEDs do not use harmful mercury. LEDs are semiconductors in which free electrons from a high energy conduction band recombine with holes in a valence band, releasing energy in the form of photons, and emitting monochromatic light (Huang et al., 1997). Light wavelengths in the ranges of 200-280 nm (ultraviolet-C, UV-C), 280-320 nm (ultraviolet-B, UV-B), 320-400 nm (ultraviolet-A, UV-A), and 400-470 nm (blue light) are being explored for their ability to inactivate microbes (Surjadinata et al., 2017; Wang et al., 2017). LEDs can be easily installed in an industrial food process line. However, it is necessary to understand the microbial inactivation process and the product parameters for the commercial utilization of LED technology.

Different inactivation mechanisms have been reported in microbes treated at different light wavelengths. Absorption of UV-C by bacterial DNA resulted in bond formation among adjacent

pyrimidine bases, and photo-products like pyrimidine 6-4 pyrimidone and cyclobutane pyrimidine dimers (CPDs) were induced (Gayán et al., 2012). Such mutagenic products inhibited the transcription and replication of DNA, resulting in the clonogenic death of the cell (Friedberg, 2003; Zou et al., 2019; Sinha & Häder, 2002; Witkin, 1976). Light exposure at 365 and 395 nm resulted in the photooxidation of bacterial DNA guanine bases and the generation of reactive oxygen species (ROS). The singlet oxygen produced caused the formation of irreversible oxidative reactions(Nelson et al., 2018; Ravanat et al., 2001; Zhao et al., 2009). Light energy at UV-A range caused the formation of abasic sites and CPDs within bacterial DNA, without the help of a photosensitizer (Jiang et al., 2009). Absorption of blue light by endogenous photosensitizers such as flavins, nucleic acids, vitamins, nicotinamide adenine dinucleotides, and porphyrins induces the synthesis of ROS, e.g., singlet oxygen, hydroxyl, and superoxide radicals (Ashkenazi et al., 2003; Lipovsky et al., 2009; Nelson et al., 2018; Soukos et al., 2005). The generation of such photoproducts in a bacterial cell causes its death. Exposure to light also induces a photothermal effect that can inactivate microorganisms (Fine & Gervais, 2004).

Studies of the LED potential to reduce the microbial presence in foods are limited. Research using LED applications to achieve microbial inactivation in low a_w foods has not been reported. This study explored the inactivation of thermally resistant *Salmonella* strains cocktail in wheat flour using high intensity pulsed LED treatments at selected wavelengths. Wheat flour was selected for this study because it is a low a_w food and is currently associated with recalls and outbreaks related to *Salmonella* (Myoda et al., 2019).

3.2 Materials and methods

3.2.1 Inoculum preparation

Five strains of *Salmonella enterica* spp.—*Salmonella enterica* serovar Typhimurium ATCC13311, *S. enterica* serovar Senftenberg ATCC43845 from American Type Culture Collection, and three wastewater isolates of *Salmonella enterica* spp. with high thermal resistance: FUA1946, FUA1934, and FUA1955—were used to prepare *Salmonella* cocktail (Du et al., 2020). Tryptic soy agar (TSA) (Becton, Dickinson and Company, NJ, USA) enriched with 0.6% yeast extract (YE) (Fisher Bioreagents, MD, USA) was used to revive and grow the bacteria in a frozen stock of tryptic soy broth and 70% (v/v) glycerol at -80 ^{o}C (Du et al., 2020). A loop of a single isolated colony was transferred to a 15 ml Falcon tube containing 5 ml of sterile tryptic soy broth (Becton, Dickinson and Company, MD, USA) enriched with 0.6% yeast extract (TSB-YE) and the bacteria were incubated at 37 °C for 24 h inside a shaking incubator, then 100 µl of inoculated broth was transferred to 5 ml of TSB-YE and incubated for 20 h.

TSAYE plates were individually inoculated with 100 µl of one of the five strains of *Salmonella* and incubated at 37 °C for 24 h. Each bacterial lawn was washed with 1.5 ml of sterile 0.1% peptone water (Fischer Bioreagents, NJ, USA) and collected in an Eppendorf tube to a concentration of 10^{12} CFU/ml. The bacterial suspensions were centrifuged at 10,000 rpm for 5 minutes, followed by the removal of the supernatant and the addition of sterile peptone water to a volume of 1 ml. A cocktail of *Salmonella* strains was prepared by mixing an equal volume of each strain to make 5 ml of inoculum.

3.2.2 Sample preparation for LED treatments

Unbleached wheat flour (Royal Flour Farine Royal, P&H Milling Group, Lethbridge, AB, Canada) with an a_w of 0.22 at 20 ºC was inoculated with *Salmonella* strains cocktail. Inoculum, 1.25 ml, was well mixed with 10 g of wheat flour in order to avoid lump formation. The inoculated samples were later mixed using a stomacher (Seward; stomacher 400 circulator, FL, USA) for 5 min at 230 rpm. The a_w of the sample before equilibration was around 0.42. Petri dishes containing the inoculated samples were placed inside a humidity chamber (BTL-433, ESPEC North America Inc., MI, USA) for 4 h at 75% relative humidity (RH) at 25 ºC. After reaching an a_w of 0.65 $\pm$ 0.05, the samples were transferred to an equilibration cabinet containing saturated sodium chloride solution and equilibrated for three days to obtain an a_w of 0.75 (Syamaladevi et al., 2010). The two step equilibration were performed to obtain final a_w consistently within three days. The bacterial concentrations were 6.3×10^9 and 1.3×10^9 CFU/g, respectively, before and after three days of equilibration.

3.2.3 Experimental set-up

The LED head (JL3 series, Clearstone Technologies Inc., MN, USA) was connected to the power controller (CF3000, Clearstone Technologies Inc., MN, USA) during experiments (Figure 3.1(i)). Emission spectra of LEDs with wavelengths of 275 nm (JL3-275S-40), 365 nm (JL3-365G2-6), 395 nm (JL3-395G2-6), and 455 nm (JL3-455F-90) were obtained using a spectrometer (StellarNet Inc Black-comet C-25) connected to an optical fiber (StellarNet Inc F600-UV-SR) (Figure 3.1(i)). The wavelength of the emitted light was plotted versus irradiance using Origin 2019b analysis and graphing software (OriginLab Corporation, MA, USA) as shown in Figure 3.1(iii). Radiant power per unit surface area (irradiance; W/cm^2) for each diode was determined using a photodiode sensor connected to a laser power/energy meter (7Z01580, Starbright, Ophir Photonics, USA).

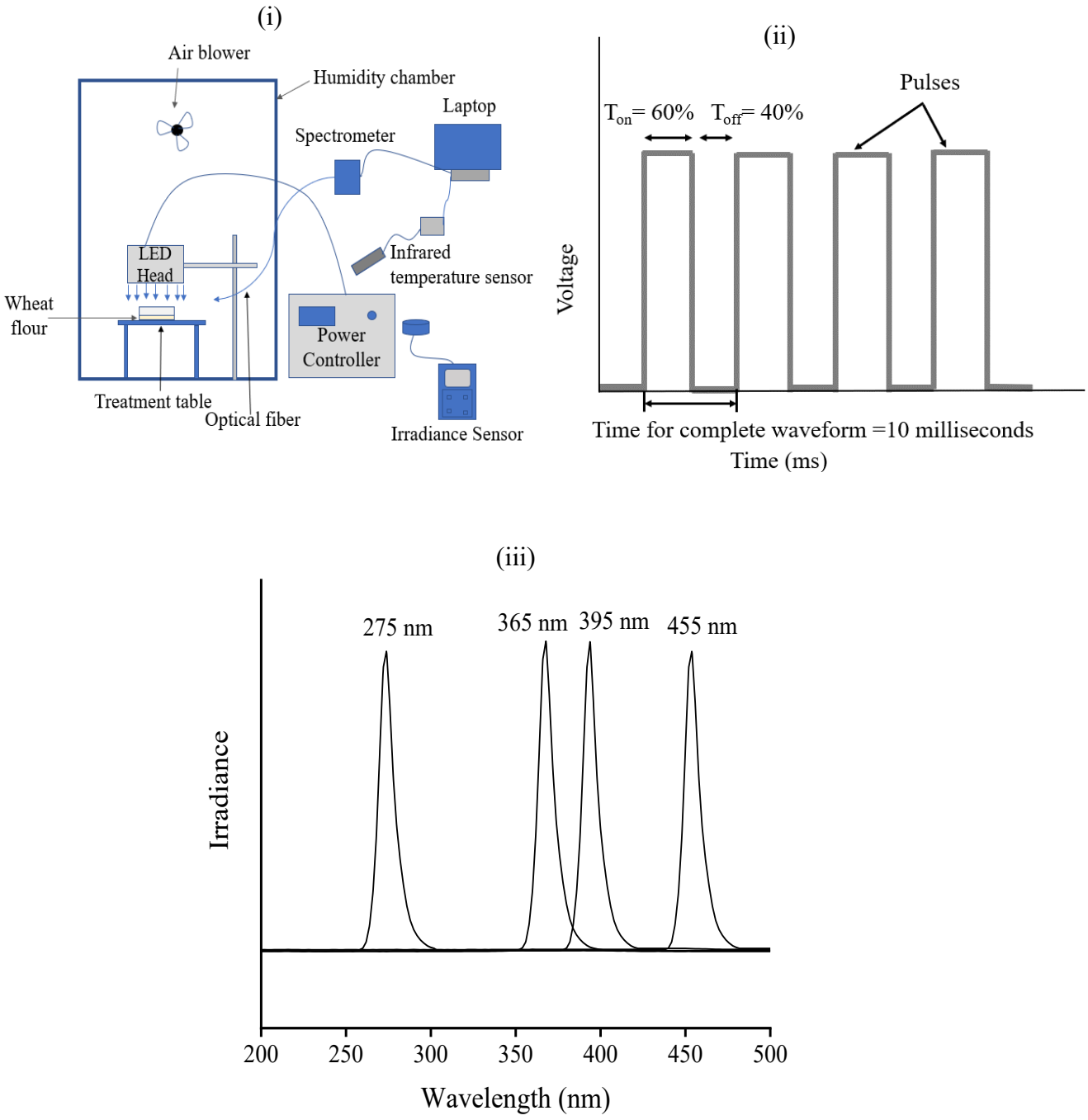


Figure 3.1 Schematic view of experimental set-up (i), light pulse signal (ii), emission spectra of 275 nm, 365 nm, 395 nm, and 455 nm (iii)

The irradiance value of a 275 nm pulsed LED was measured using an intensity sensor connected to a radiometer (ILT2400; MA, USA). The sample cup was placed 2 cm below the LED head (Figure 3.1(i)). The irradiance was calibrated to zero for the treatment environment, solely the

irradiance from light source was calculated. The operating frequency of each LED was 100 Hz. The duty cycle of one complete waveform was equal to the ratio of the pulse width to the period (Zou et al., 2019) as explained in equations 3.1 and 2. For instance, when the pulsed LED light operated at 60% duty cycle and 100 Hz frequency, the pulse width was 0.006 s and the period for one complete waveform was 0.01 s, (Figure 3.1(ii)).

$$\text{Duty Cycle \%} = (\text{Pulse width}/\text{Period of one waveform}) \times 100\% \quad (3.1)$$

$$\text{Pulsed light frequency} = \frac{1}{\text{Period of one waveform}} \quad (3.2)$$

3.2.4 Treatment time and radiant energy calculation

Irradiance varies with the wavelength of the light. The irradiances of 275, 365, 395, and 455 nm LED treatments were 16.7, 418, 666, and 488 mW/cm^2, respectively, when the gap between the sample and the LED head was 2 cm at a 60% duty cycle. The radiant energy exposure (the energy dosage), of a sample to light at a constant height and exposure time, is equal to the irradiance times the exposure time (Almeida et al., 2011), as expressed in equation

$$\text{Radiant energy dosage per unit area} = \text{irradiance} \times \text{exposure time} \quad (3.3)$$

The radiant energy dosages were 60.1, 1479.6, 2397.6, and 1756.8 J/cm^2 for 60 min sample exposures to 275, 365, 395, and 455 nm, respectively. To compare the efficacy of different LED wavelengths (365, 395, and 455 nm light pulses) on microbial inactivation and quality changes in wheat flour samples, wheat flour samples inoculated with *Salmonella* were exposed to an equal radiant energy dosage of 1199 J/cm^2, achieved by varying the treatment times. The sample of wheat flour inoculated with *Salmonella* was treated for 47.8, 30, and 41 min using 365, 395, and

455 nm, respectively to achieve 1199 J/cm^2 radiant energy dosage. The microbial inactivation efficacy of the light emitted by high intensity pulsed LED light from a height of 2 cm was studied by providing equal dosages of irradiance and energy. To achieve equal irradiance in all samples, the power level of the LED controller was set to 60%, 40%, and 53% for 365, 395, 455 nm LED lights, respectively. The irradiance was 0.42 W/cm^2 for all three LEDs and the total energy dosage was 504 J/cm^2. The LED emitting light at 275 nm could not be compared in this case because of its very low irradiance. The efficacy of *Salmonella* inactivation and the quality changes in the wheat were compared with respect to the environmental relative RH and the treatment time of the four LEDs.

3.2.5 Water activity, water content, drying rate, and temperature change during LED treatments

The initial water content of the flour before inoculation with *Salmonella* was 0.07 kg water/kg dry solids, and the water activity (a_w) of the sample was around 0.22. After equilibration of wheat flour sample to achieve 0.75 a_w, water content was 0.202 kg water/kg dry solid. The water of the equilibrated wheat flour was calculated after drying using a convection oven (Heratherm OGS60, Thermo Scientific, MA, USA) at 105°C for 8 h. (Rabha et al., 2017). The change in water content of the sample after LED treatment was determined from the initial and final weights of the sample.

$$\text{Moisture Ratio (MR)} = \frac{(M_f - M_e)}{(M_i - M_e)}\ , \qquad (3.4)$$

where M_f is the water content of the flour sample after LED treatment and M_e is equilibrium water content, and M_i is the initial water content of the wheat flour sample.

The a_w of the flour sample was measured using a water activity meter (4TE, Aqualab, USA). When the humidity chamber was set to 75% RH and 25 °C, changes in the a_w of the sample during LED treatments were measured after 2, 5, 7, 10, 12, 20, 30, and 60 min.

The continuous localized heating of the air during LED treatments created an uncontrolled variation in the RH of the air inside the humidity chamber. When the drying air RH condition is not constant, the moisture ratio of the product after LED treatment, expressed in equation 3.4, can be further simplified as the ratio of the water content of the wheat flour sample after treatment and the initial water content of the flour sample as in equation 3.5 (Midilli et al., 2002).

$$MR = \frac{M_f}{M_i} \tag{3.5}$$

The loss in the mass of the flour sample after 2, 5, 7, 10, 30, and 60 min LED treatments were recorded to develop drying rate curves for the LED treatments. The temperature of samples during LED treatments was monitored using an infrared sensor with an integrated controller at 2, 4, 6, 8, 10, 12, 30, and 60 min (Micro-epsilon; Hoskin scientific limited, BY, Germany).

3.2.6 Desorption isotherm calculation

A desorption isotherm of the wheat flour sample was developed at 24°C (ambient temperature) and at 80°C (~ surface temperature of the wheat flour during LED treatment). First, the wheat flour sample was equilibrated to 0.9 a_w inside the forced air circulated humidity chamber. Samples of the equilibrated flour were transferred into Petri dishes and equilibrated inside different chambers, each with a different saturated salt solution: i.e., LiCl, CH_3COOK, $MgCl_2$, K_2CO_3, $MgNO_3$, $NaNO_2$, and NaCl (Fisher Scientific, Houston, TX) at a_w values of 0.113, 0.225, 0.328, 0.432, 0.529, 0.658, 0.75, respectively (Syamaladevi et al., 2010). Syamaladevi et al. (2016a) developed a high temperature a_w meter to determine the a_w at 80°C. Wheat flour samples sealed inside an a_w meter were placed in a water bath at 80°C and the a_w was values were recorded after consistent a_w

reading (Syamaladevi et al., 2016a). The Guggenheim, Anderson, de Boer (GAB) model was used to predict the wheat flour desorption isotherm (Al-Muhtaseb et al., 2002; Moreira et al., 2010; Syamaladevi et al., 2016a). The GAB equation is expressed as:

$$x_{aw} = \frac{S_b C_f a_w M_L}{(1-C_f a_w)(1-C_f a_w+S_b C_f a_w)}, \quad (3.6)$$

where x_{aw} is the water content of the flour (dry basis) at a certain a_w, S_b is water-binding strength of the flour sample to primary binding sites, M_L is the water content of the monolayer, C_f is a correction factor for the GAB equation. The factors S_b, M_L, and C_f in the GAB equation can be expressed as,

$$S_b = S_o \exp(\frac{\Delta H_s}{RT}), \quad (3.7)$$

$$C_f = C_o \exp(\frac{\Delta H_c}{RT}), \quad (3.8)$$

$$M_L = M_{mo} \exp(\frac{\Delta H_x}{RT}), \quad (3.9)$$

where ΔH_s is the difference between monolayer sorption enthalpy and multilayer sorption enthalpy, S_o and C_o are entropic accommodation factors, ΔH_c expresses the difference in the enthalpy of condensation of the water and the multilayer sorption enthalpy, M_{mo} and ΔH_x are temperature-dependent constants that express monolayer moisture corresponding to the temperature of the treatment, R is the universal gas constant, and T stands for temperature. The water content values corresponding to the a_w values were determined by fitting the experimental water content values using EXCEL© solver.

3.2.7 LED treatment of *Salmonella* inoculated wheat flour

Wheat flour samples inoculated with *Salmonella*, each with a weight of 0.3 g, were spread on the AquaLab decagon water activity cup (Meter group Inc., WA, USA). The LED head was placed 2 cm above the surface of the treatment table containing a cup with the sample (Figure 3.1(i)). The

humidity chamber accommodated three RH conditions: 40, 75, and 90% humidity at 25ºC. The inoculated wheat flour samples were treated with the LED for 10, 30, and 60 min to evaluate the microbial inactivation efficacy at the selected RH conditions. To determine the influence of a_w on microbial inactivation kinetics at different light wavelengths, inoculated wheat flour was equilibrated to 0.5 and 0.75 a_w before LED treatment. In order to make a uniform initial irradiance in three of the LEDs power levels were changed as mentioned in section 3.2.4. Samples at 0.5 and 0.75 a_w were treated with the LED at 50% and 75% RH, respectively at 25ºC. The inoculated wheat flour sample was transferred in 100 mL of 0.1% peptone water in a stomacher bag for 15 min, then mixed in the stomacher at 230 rpm for 5 min. Inoculated wheat flour samples were serially diluted using 0.1% peptone water and plated on TSAYE plates. Microbial counts were compared in terms of the logarithm of colony-forming unit per gram of wheat flour sample (log CFU/g) before and after LED treatment.

3.2.8 Statistical analysis

Triplicates data were analyzed using a two-way analysis of variance (ANOVA). The results were statistically analyzed using SAS university edition 9.1 (SAS Institute, Cary, USA).

3.3 Results and discussion

3.3.1 Changes in surface temperature and water activity after LED light treatments

LED treatments of wheat flour took place inside a humidity chamber at 25°C and 75% RH. Sixty minutes LED treatments at 275, 365, 395, and 455 nm of the sample resulted in increases in the surface temperature of the wheat flour from 25 °C to 31.3, 81.4, 86.1, and 81 °C, respectively (Figure 3.2(i)). Chen et al., (2018) also reported the increase in temperature of the powdered food (non-fat dry milk) when subjected to pulsed light treatments. Krishnamurthy et al. (2010) and Fine & Gervais, (2004) had observed the potential photothermal effect of the pulsed light as well. It

was observed that the surface temperature of the wheat flour significantly increased within the first 5-10 min of the LED treatment, and later became stable when the photothermal energy supplied by the system and heat energy losses due to conduction, convection, and radiation from the sample attains equilibrium state.

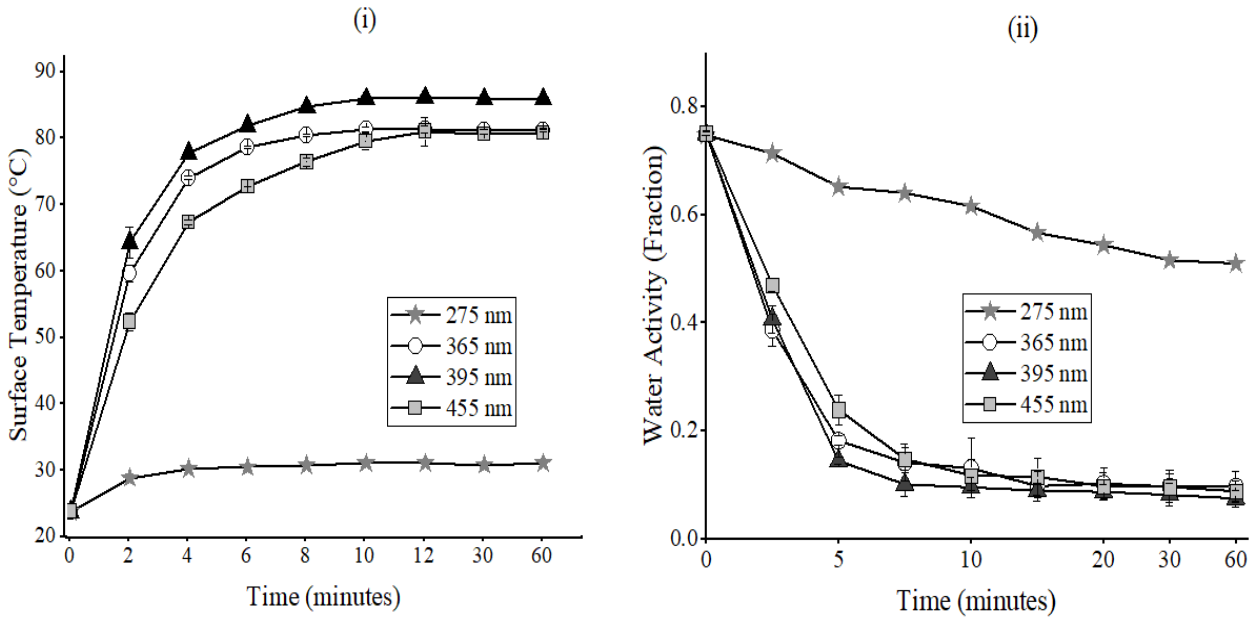


Figure 3.2 Surface temperature (i) and water activity (ii) change in wheat flour during LED treatments

*The error bars represent value's mean ± SD

The a_w of the wheat flour was 0.75 before LED light treatment. Within the first 5-10 min (Figure 3.2(ii)) of LED treatments using different wavelength light pulses, a significant decrease in the wheat flour a_w was observed. The 275 nm UV-C LED treatment produced a relatively smaller decrease in the wheat flour a_w compared to other wavelengths, likely because of the smaller increase in temperature during UV-C LED treatments and with the lowest irradiance. After LED light treatments with 275, 365, 395, and 455 nm, the final a_w of the wheat flour were 0.51, 0.10 0.08 and 0.09 respectively, after 60 min. When the wheat flour was subjected to heat treatment, a

rapid drop in a_w within the first 10 min was also reported in Archer et al. (1998). The treatment of the powdered food using high intensity pulsed light had been reported to result in the lowering water activity of the sample (Chen et al., 2018; Du et al., 2020).

3.3.2 Desorption isotherm of wheat flour

The initial water content of the wheat flour after equilibration was 0.202 kg water/kg dry solids. The desorption isotherms (Figure 3.3) of wheat flour at ambient temperature (24°C) and the approximate surface temperature (80°C) were developed and fitted using the GAB model (Equation 3.6). Desorption isotherms of wheat flour are used to map and predict the a_w and water content of wheat flour during LED treatments.

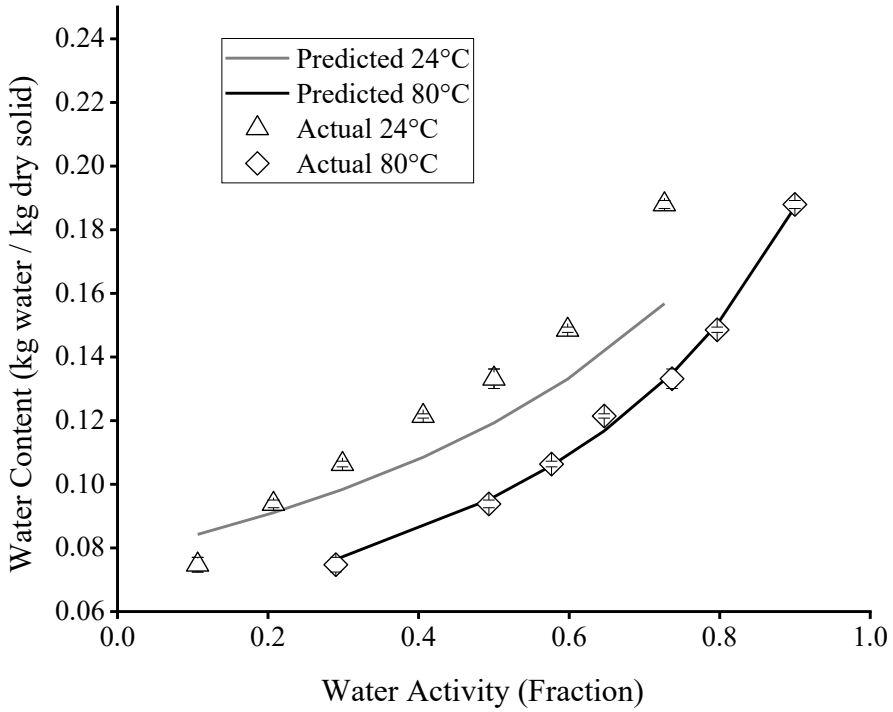


Figure 3.3. Predicted and experimental desorption isotherm of wheat flour at 24 ºC and 80 ºC

However, the desorption isotherm of the wheat flour in our experiment showed that, at 80ºC, the a_w of the wheat flour was higher than the a_w of the wheat flour at 24°C for the same water content which can be due to the increase in intermolecular distance and reduction in binding energy between molecules resulted by the exposure of high temperature, this is in agreement to result by Syamaladevi et al. (2016a) and in Moreira et al. (2010).

3.3.3 Drying effect of the pulse LED lights

Moisture removal due to drying of the sample is indicated if the moisture ratio (MR) of the sample after treatment is smaller than the moisture ratio before treatment. Significant effects ($p < 0.05$) of the RH and the treatment time on the MR were observed for 275, 365, 395, and 455 nm LED treatments.

During the 365 nm LED treatment, the moisture ratio decreased at 40% RH and 75% RH. A slight increase in the moisture ratio was observed after 60 min of treatment compared to 30 min of treatment at 90% RH condition for 365 nm which could be due to lower irradiance of the unit compared to 395 and 455 nm. The 275 nm LED treatment resulted in a higher MR compared to 365, 395, and 455 nm LED treatments. A higher moisture ratio indicated less water removal from the sample during treatment under similar environmental conditions. Significantly lower sample drying was observed after the 275 nm LED treatment compared to the 365, 395, and 455 nm LED treatment. This indicates the photothermal effect of the LED light has a significant contribution to the drying of the food sample. Big error bars in Figure 3.4 was observed which could be due to slightest of variation in positioning and spreading of sample. Du et al. (2020) reported the intense pulsed LED light of 395 nm treated wheat flour lost 1.88% of weight of the wheat flour sample due to drying after 60 min treatment.

The significant decrease in MR was found with an increase in treatment time from 10 to 60 min in the 40% RH environment compared to 75% and 90% RH for all four wavelengths. In the 455 nm, 395 nm, and 365 nm LED treatments, the MR reached below 0.5 within the first 10 min of treatment (Figure 3.4). The MR after 10 min treatment with the 395 nm LED was not significantly different at 40%, 75%, and 90% RH conditions. When the wheat flour was treated for a longer time using the 395 nm LED, a significant decrease in the MR was observed at 40% and at 75% RH, but no significant change in the MR was observed at 90% RH.

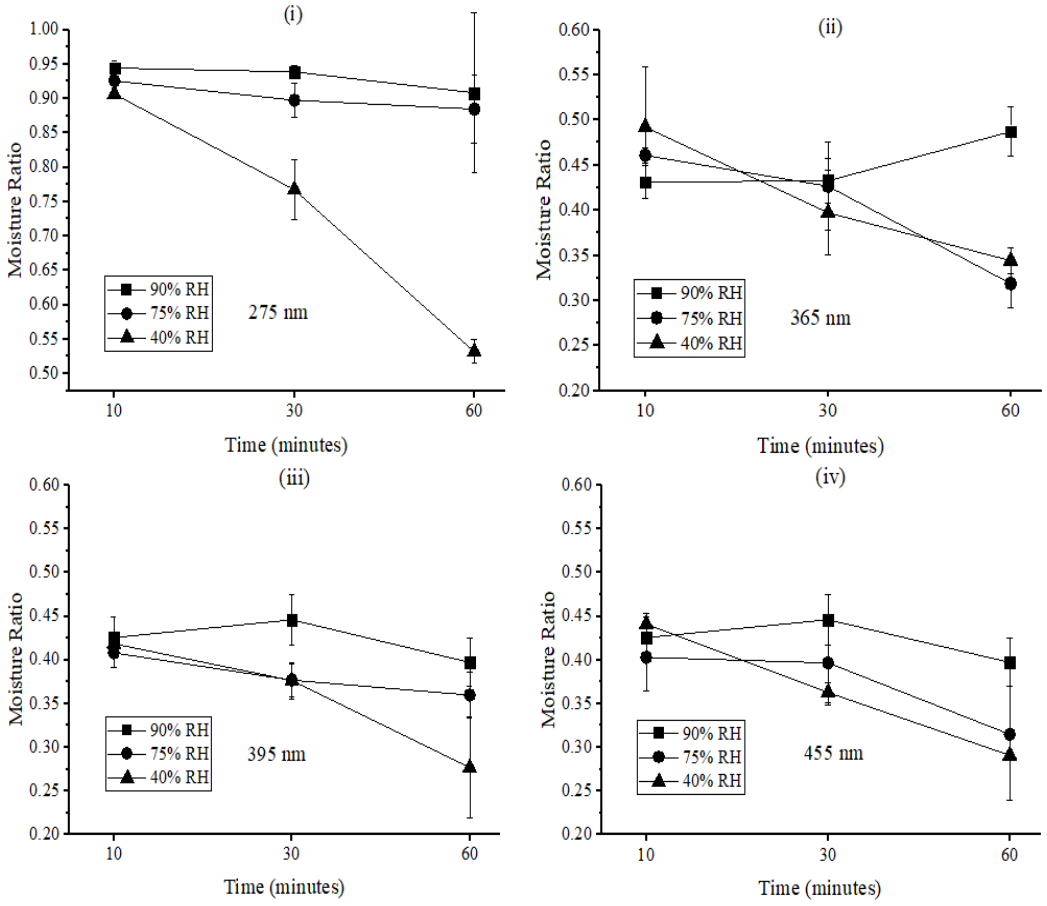


Figure 3.4 Moisture ratio of the LED light treated wheat flour at selected RH conditions and treatment times for different wavelengths: (i) 275 nm, (ii) 365 nm, (iii) 395 nm, (iv) 455 nm

*The error bars represent value's mean ± SD.

Significant drying of the wheat flour occurred in the first 10 min of the heat treatments, but small change or no change in the MR was observed as the irradiation progressed.

The drying rates of 0.3 g wheat flour samples spread over an area of 10.7 cm^2 and subjected to 365 nm, 395 nm, and 455 nm LEDs from a height of 2 cm were determined. During 365, 395, and 455 nm LED treatments predominantly falling drying rate was only observed for wheat flour samples. (Figure 3.5).

The initial drying rate was the highest for the 365 nm LED treatment followed by lower drying rates in the 395 nm and the 455 nm LED treatments, respectively. The drying rate in wheat flour during LED treatments decreased with a reduction in water content (Figure 3.5). The rise in the surface temperature of the sample during LED exposure would be expected to result in the evaporation of water.

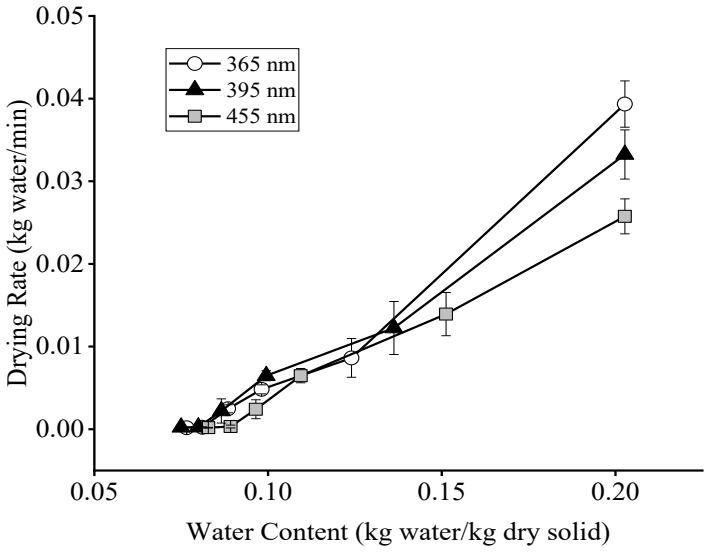


Figure 3.5 Rate of drying of wheat flour sample against water content (dry basis) at 75% RH and 25 ºC inside humidity chamber during LED treatments with 365nm, 455nm and 395nm wavelength light pulses.

*The error bars represent value's mean ± SD.

3.3.4 Effect of LED treatments on *Salmonella* inactivation in wheat flour

LED treatments at 275, 365, 395, and 455 nm reduced *Salmonella* strains cocktail count in wheat flour significantly, as illustrated in Figure 3.6 (i-iv). A significant interaction ($p < 0.05$) between the treatment time and the RH on the microbial inactivation was observed for 275 and 365 nm LED treatments, while no interaction between those parameters was observed to affect 395 and 455 nm LED treatments.

Significant effects of the treatment time were observed in all four LED treatments. An increase in treatment time increased the total energy dosage of each treatment and resulted in a significant increase in microbial inactivation (Gayán et al., 2012; Quintero-Ramos et al., 2004). The 395 nm LED treatment was more energy-intensive than the other wavelengths for the same treatment time and resulted in greater *Salmonella* inactivation (Figure 3.6). For instance, the total microbial count reductions were 3.50, 3.67, and 3.63 log CFU/g, and 2.36, 2.47, and 2.18 log CFU/g after 395 nm LED treatments for 60 min (2398 J/cm^2 energy dosage) and for 30 min (1199 J/cm^2 energy dosage) at 40%, 75%, and 90% RH conditions, respectively. Microbial reductions after 455 nm LED treatments with 1764 J/cm^2 energy dosage for 60 min were 2.58, 2.60, and 2.21 log CFU/g at 40%, 75%, 90% RH, respectively. LED treatments at 365 nm with 1476 J/cm^2 energy dosage for 60 min resulted in 2.82, 2.42, and 2.58 log reductions in *Salmonella* cell count at 40%, 75%, and 90% RH, respectively. In some cases, the RH affected the LED-driven *Salmonella* reduction in wheat flour, however, the difference was not biologically relevant. We hypothesize that the maintenance of a constant high humidity inside the humidity chamber helped to achieve a higher LED-driven *Salmonella* inactivation in wheat flour. At high-moisture conditions, thermal treatments result in the breakage of sulfide and hydrogen bonds in bacterial proteins, causing bacterial death due to the vibration of water molecules in food samples (Hiramatsu et al., 2005). However, localized

heating of samples caused drying of the sample within a controlled humidity chamber making it dry treatment conditions for LED treatments due to significant drying of the sample. Wheat flour particles have opaque surface with shodowing effect, photothermal energy could have significant effect during inactivation of microorganisms during opaque food sample treatments using pulsed UV light (Krishnamurthy et al., 2010).

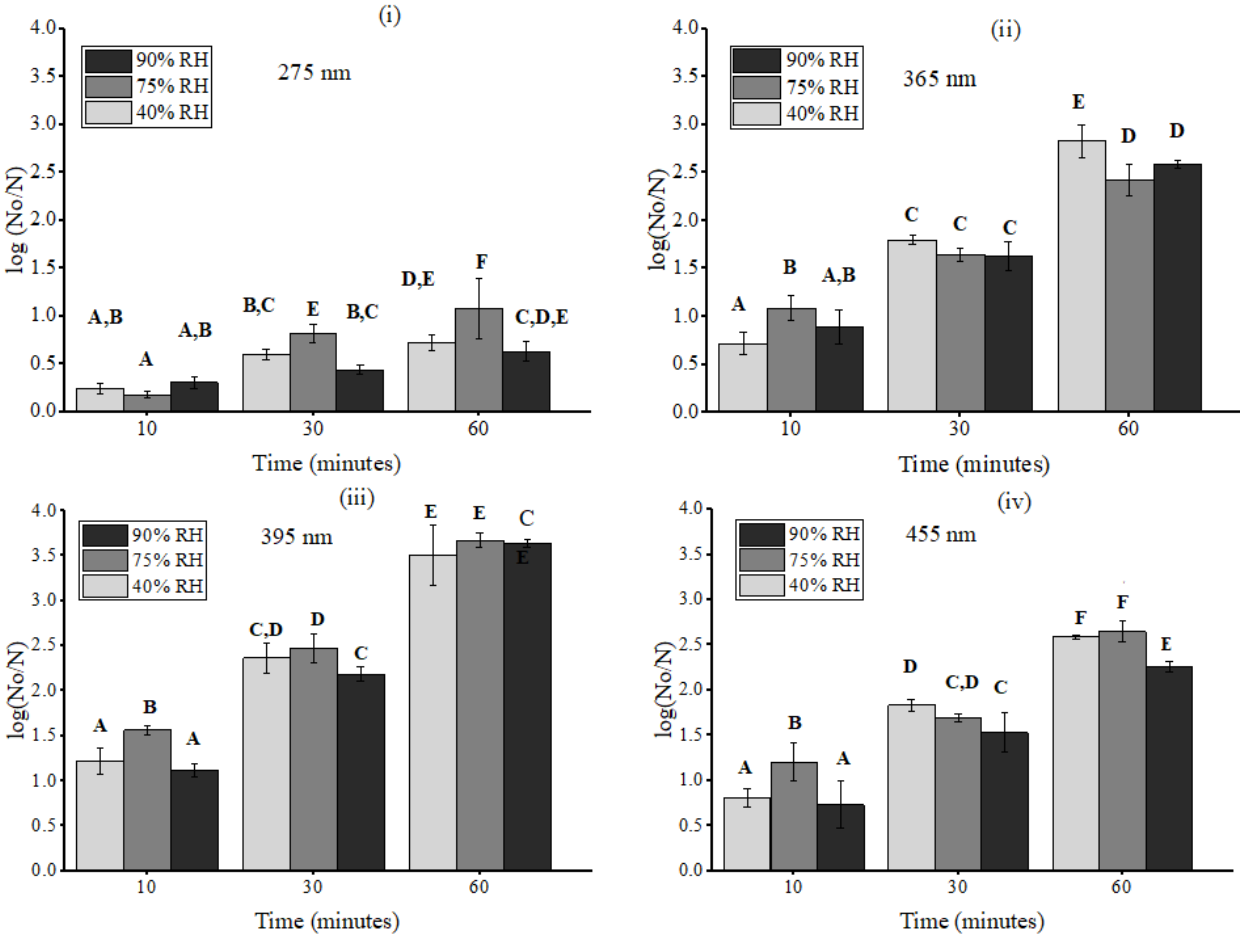


Figure 3.6 (i) 275 nm, (ii) 365 nm, (iii) 395 nm and (iv) 455 nm, Comparative rate of inactivation of *Salmonella* cocktail after LED treatments with selected wavelength light pulses at selected RH and treatment times inside the humidity chamber (T=25 °C).

*The error bars represent value's mean ± SD. Bar having different letter are significantly different ($p < 0.05$)

At an equal radiant energy dosage of 1199 J/cm^2, the 395 (2.48 log reduction) and 365 nm (2.22 log reduction) LED treatments were more effective than the 455 nm (1.61 log reduction) LED treatment in reducing the number of *Salmonella* strains in the wheat flour (Figure 3.7).

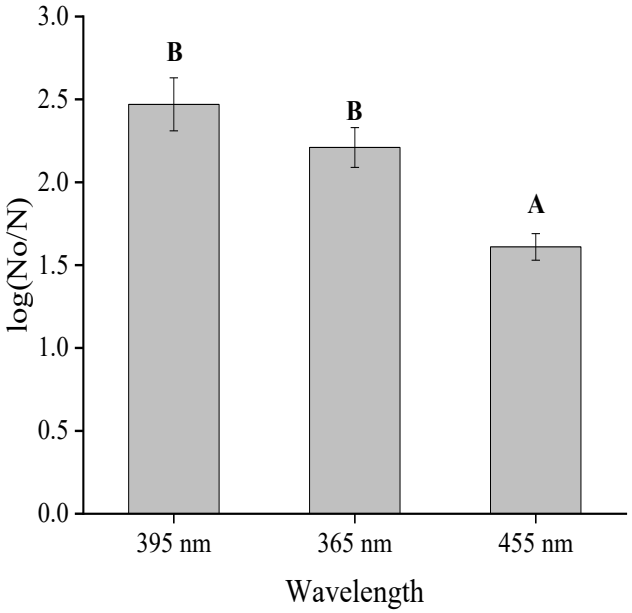


Figure 3.7. Comparative study of the *Salmonella* inactivation rates of 365 nm, 395 nm and 455 nm wavelength LED treatments with 1199 J/cm^2 energy dosage (RH = 75%, T = 25 °C)

*The error bars represent value's mean ± SD. Bar having different letter are significantly different ($p < 0.05$)

The inactivation levels of *Salmonella* after 365 nm and 395 nm LED treatments were similar. The 365 and 395 nm LED treatments in the presence of oxygen generated ROS, such as singlet oxygen, which caused the formation of abasic sites and cyclobutane pyrimidine dimers (CPDs), improving the UV-A bacterial inactivation efficacy (Jiang et al., 2009).

Table 3.1 Effect of initial water activity of the wheat flour on the inactivation of *Salmonella* after LED treatments with irradiance of 0.42 W/cm^2 and energy dosage of 504 J/cm^2

Water activity	Wavelength	Log reduction
0.5	365 nm	-1.31±0.07$^{A, B}$
0.5	395 nm	-1.36±0.04^{B}
0.5	455 nm	-1.14±0.16$^{A, B}$
0.75	365 nm	-1.82±0.01^{C}
0.75	395 nm	-1.85±0.07^{C}
0.75	455 nm	-1.08±0.12^{A}

The value mentioned are mean ± SD. Values having different letter are significantly different ($p < 0.05$)

To determine the effect of the initial a_w on *Salmonella* inactivation, wheat flour samples with a_w of 0.5 and 0.75 were irradiated with LEDs at 0.42 W/cm^2 and an energy dosage of 504 J/cm^2. *Salmonella* reductions in the 0.5 a_w wheat flour sample after 365, 395, and 455 nm LED treatments were not significantly different (Table 3.1). However, in the 0.75 a_w wheat flour sample, *Salmonella* reductions were significantly higher after 365 and 395 nm LED treatments than after the 455 nm LED treatment (Table 3.1). The wheat flour with initial a_w of 0.5 had lower bacterial inactivation compared to a_w of 0.75 for 365 and 395 nm LED treatments. Previous research has also reported that at a lower a_w, *Salmonella* strains exhibited higher resistance to antimicrobial LED treatments at 365 and 395 nm (Archer et al., 1998; Smith et al., 2016; Syamaladevi, et al., 2016a). At low a_w conditions, bacteria are under desiccation stress, and mechanisms such as biofilm formation, filamentation, and osmoregulation inside the bacterial cells might increase the thermal resistance of *Salmonella* spp. (Finn et al., 2013).

3.4 Conclusions

The 275 nm, 365 nm, 395 nm, and 455 nm LED treatments showed promising *Salmonella* strains cocktail inactivation efficacy in wheat flour. The efficacy of *Salmonella* inactivation with 365 nm and 395 nm LED treatments were significantly greater than the efficacy of *Salmonella* inactivation with the 455 nm LED at an equal energy dosage. The high intensity light pulses from LEDs produced a photothermal effect, increasing the surface temperature of the wheat flour resulting wheat flour water content loss and reduction in the a_w. The RH of the environment did not influence the *Salmonella* inactivation efficacy of LED treatments, probably because of localized heating. Further work with equal irradiance for UV-C, UV-A**,** and blue light can be done in the future once highly efficient UV-C LED light becomes available in the market. LED treatments for *Salmonella* inactivation in LMFs are at an early stage of research and development.

3.5 Acknowledgments

We acknowledge funding (Grant number 2018F040R) from Alberta Agriculture and Forestry. We thank the P&H Milling Group (Lethbridge, AB, Canada) for supplying the unbleached wheat flour for this study.

Chapter 4

Simultaneous drying of pet food pellets *and Salmonella* inactivation by 395 nm light pulses in an LED reactor

4.1 Introduction

The pet food market is growing. Globally, the pet food industry was valued at $53, $58.6, and $75 billion in 2005, 2011, and 2017, respectively (Aldrich, 2006; Aldrich and Koppel, 2015; Watt Global Media, 2018). Pet foods are sometimes associated with *Salmonella* related recalls (FDA, 2019). The Government of Canada (GOC, 2016) reported that, annually, 5% of illnesses, 24% of hospitalizations, and 16% of deaths are caused by the consumption of foods contaminated with *Salmonella*. The Food and Drug Administration (FDA, 2016) reported that *Salmonella* was one of the major contributors to 14.3% of the cases associated with pet food recalls from 2009 to 2014. In 2018, the FDA (FDA, 2019) reported 22 pet food recalls due to potential contamination with *Salmonella*. As exposure to contaminated pet food can be a source of microbial infection in humans (Cavallo et al., 2017), the production of safe pet foods is important to human health. Hale et al., 2012, estimated that 11% of the illnesses associated with animal contact with humans was caused by non-typhoidal *Salmonella* and reported that salmonellosis was the major reason for human hospitalization caused by pet contact.

Low-moisture foods with are conventionally considered microbiologically safe. However, recent illness outbreaks and food recalls related to low-moisture foods, including pet foods, have raised concerns about pet food safety. Most of the outbreaks and food recalls related to *Salmonella* were linked to poor hygiene or handling after processing (Cavallo et al., 2017). *Salmonella* strains were observed to be resistant to desiccation and antimicrobial interventions (Santillana et al., 2013; Syamaladevi et al., 2016 a,b&c), and *Salmonella* strains can survive in viable but nonculturable states in low-moisture foods. Hence, there is a need to decontaminate low-moisture food products after their manufacture. *Salmonella* contamination commonly exists on the surface of low-moisture foods, including pet foods. In the final stage of production, extruded pet food pellets are

submitted to a lengthy, high-temperature, air drying procedure to achieve the required moisture content and a_w. To replace the long, high-temperature air drying process, it is desirable to find a relatively fast, low-temperature treatment that can decontaminate and adequately dry pet foods without degrading their quality.

Pulsed UV treatment is a novel technology for food product surface decontamination (Gómez-López et al., 2007; Li et al., 2010; Mahendran et al., 2019). As the effects of UV light differ with wavelength, UV treatments are defined with respect to wavelength range, where UV-C is 200-280 nm, UV-B is 280-320 nm, and UV-A is 320-400 nm (Surjadinata et al., 2017). UV-A light exposure causes bacterial cell death by generating reactive oxygen species (ROS) within the cell (Hua et al., 2009). UV-A photons are absorbed by the photosensitizers within the microbial cells, resulting in impairment at different sites due to photooxidation reactions that lead to cell death (Nelson et al., 2018; Ravanat et al., 2001). The antimicrobial potential of UV-C light has been studied extensively in different food systems (Adhikari et al., 2015; Liao et al., 2017; Syamaladevi et al., 2013, 2014, & 2015). The long lifetimes and compact and robust designs of light-emitting diodes (LEDs) are ideal for food processing facilities, compared to the hazardous mercury UV lamps that have been traditionally employed (Kim et al., 2016; Song et al., 2018).

High intensity light pulses emitted from LEDs can reduce surface contamination in low-moisture foods, including pet foods. Our recent studies (Prasad et al. 2019; Du et al., 2020) used high intensity light (395 nm) pulses to inactivate *Salmonella* in wheat flour and pet foods. We also demonstrated the significant drying potential of UV light and it ability to lower the water activity (a_w) in wheat flour (Du et al., 2020). The significant photothermal and drying effects can be controlled by a continuous supply of low-temperature air. Mild hot air fluidization of pet food pellets can increase the surface area of exposure, which can improve microbial decontamination

(Nicorescu et al., 2013). The photothermal effect of LED treatment along with air fluidization can help to replace the long hot air drying step in pet food processing, providing fast drying and decontamination simultaneously. In this study, we tested the potential of 395 nm light pulses to simultaneously inactivate *Salmonella* and dry pet food pellets using an LED reactor.

4.2 Materials and methods

4.2.1 Materials

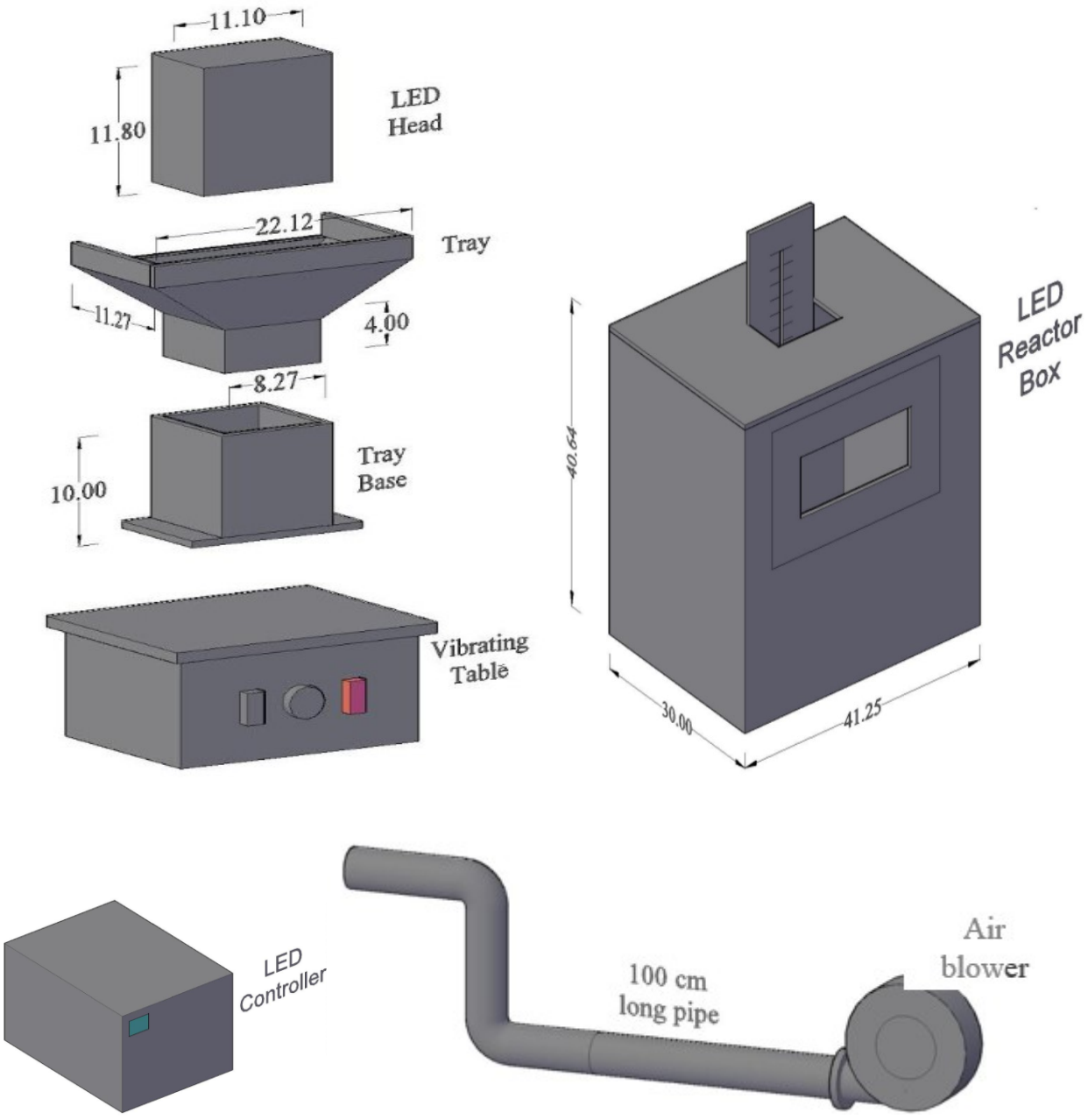
Pet food pellets were received from a private company. The chemical composition denoted in the product label was a minimum of 44% protein, 15% fat, and a maximum of 10% moisture. The initial a_w in the pet food pellets was $\approx 0.53 \pm 0.05$.

4.2.2 LED reactor development

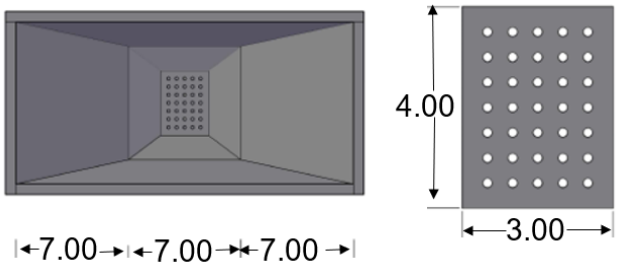
The LED light reactor (Figure 4.1) consisted of 5 major components: (1) an LED head and controller, (2) a variable temperature air blower and an air pipe, (3) a sample tray and a tray base, (4) a vibrating table, and (5) a reactor box.

The LED head (JL3-395G2-6, Clearstone Technologies Inc., USA) consisting of 6 LEDs that generated 395 nm light pulses, was connected to the controller (CF3000, Clearstone Technologies Inc., Hopkins, MN, USA). The light pulse frequency was 100 Hz at a power level of 60% (Du et al., 2020). The LEDs produced an irradiance of 0.66 W/cm^2 at the surface of the sample. A variable temperature air blower (VT-750C, Master appliance corp., WI, USA) and an air pipe served as a heating/cooling system. The operating temperature range of the hot air blower was 21 ^{o}C to 538 ^{o}C, and was generated with a ceramic-based heater

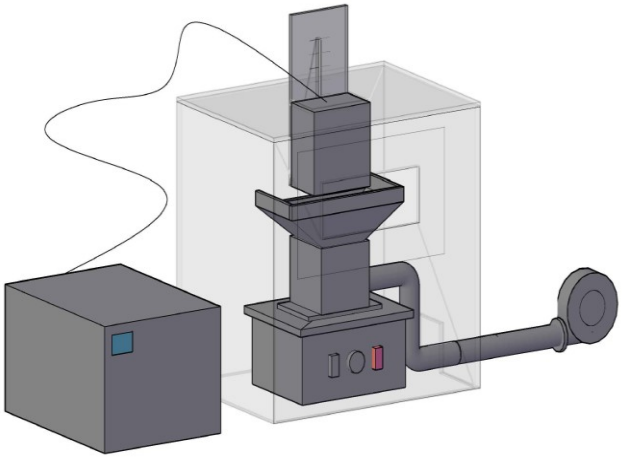
(i)


11.10
11.80
LED
Head
22.12
Tray
11.27
4.00
8.27
Tray
Base
10.00
Vibrating
Table
40.64
LED
Reactor
Box
30.00
41.25
LED
Controller
100 cm
long pipe
Air
blower

(ii)



(iii) (iv)



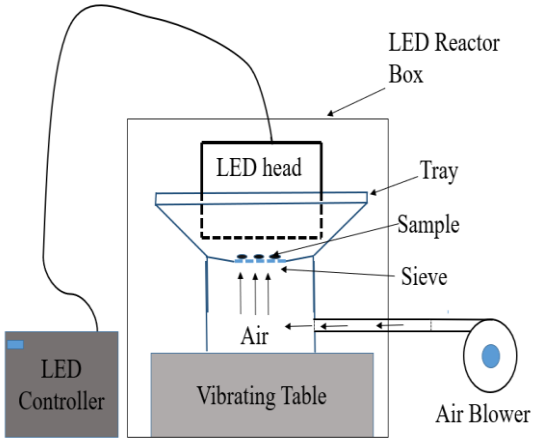


Figure 4.1 Different components of new LED reactor (unit in cm), Exploded image view of the reactor (i), Sample tray with sieve and sieve top view (ii), 3D image of assembled LED reactor (iii) and 2D image of the LED reactor (iv)

The flow rate of the air supplied by the blower at its outlet was 0.011 m^3/s. The hose outlet diameter for the air blower was 3.18 cm and the nozzle length of the blower was 11.43 cm. A black polyethylene pipe of 100 cm was used to connect the sample tray base and hot air blower. The temperature of the hot air blower was set to achieve 50 ^oC at the sieve of the sample tray. The sample tray and tray base (14.6 cm by 9.54 cm) were connected and made airtight during experiments. The tray base unit was connected to the vibrating table (JT51, TJDent, GD, China) and the air pipe was sealed with a gasket. The sample tray and tray base were made of 0.64 cm thick polymethyl methacrylate (PMMA); its dimensions (cm) are shown in Figure 4.1. Pet food samples were placed on the sample tray during experiments. The tray base contained a sieve (3 cm by 4 cm) with holes 2 mm in diameter to generate air fluidization to the products (Figure 4.1(ii)). The vibrating table had a maximum load capacity of 3 kg, and operated at a frequency of 60 + 10% Hz, which aided the air fluidization of the sample. The tray and tray base (≈ 0.9 kg) was fixed to the vibrating platform. The vibrating table was placed over a foam sheet to reduce the noise produced by the platform vibration. The displacement near the sieve of the tray due to vibration was 0.047 ± 0.005 cm peak to peak displacement and the peak velocity was 8.9 ± 1cm/s. A vibration meter (Balmac Inc., OH, USA) was used to measure the vibration of the unit. The surfaces of pet food pellets were completely exposed to LED treatment using the vibratory platform and the hot air fluidization. The LED reactor box (41.25 cm × 30 cm × 40.64 cm) (Figure 4.1(i)) was made of 0.64 cm thick PMMA. The reactor box enclosed the LED head, the sample tray, and the vibratory platform, and helped to avoid harmful UV-A exposure to laboratory personnel. The door on the reactor box was used to adjust the LED head setting and for sample transfer (Figure 4.1(i)).

The units were assembled as shown in Figure 4.1(iii) and the sample treatment was conducted as shown in Figure 4.1(iv). Three treatment modes were used to compare the microbial inactivation efficacy and the resulting quality changes in the pet food: the sample was exposed only to high intensity (395 nm) LED light pulses (mode 1), the sample was exposed only to hot air (50 ºC) fluidization plus vibration of the sample platform (mode 2), the sample was exposed to high intensity (395 nm) LED light pulses simultaneously with hot air (50 ºC) fluidization and platform vibration (mode 3). The combination of LED light pulses, hot air fluidization, and vibration (mode 3) depicts the LED reactor conditions used throughout the manuscript. The dosage values during LED treatments corresponding to each treatment time were determined using a laser energy meter (7Z01580, Starbright, Ophir Photonics, USA). The total light energy dosage per cm^2 of food surface was calculated by multiplying the irradiance (W/cm^2) of the given wavelength emitted by the LED by the time of exposure (Almeida et al., 2011).

4.2.3 Temperature, water content and water activity of pet food pellets.

The air temperature (50 ºC) was measured using a thermocouple placed at the outlet of the tray above the sieve (Figure 4.1). The air temperature was controlled with a temperature controller in all experiments. The surface temperature of the pet food samples was measured using an infrared sensor with an integrated controller (Micro-epsilon; Hoskin scientific limited, BY, Germany).

The weight of each pet food sample before and after treatment was determined with an analytical balance to measure the moisture loss during the treatment. The initial moisture of the pet food sample was calculated using the gravimetric method. Samples were weighed, then placed in a convection oven (Heratherm OGS60, Thermo Scientific, MA, USA) at 105 °C for 8 h. The final sample weight was recorded and calculated to obtain the dry basis water content (M_i; kg water/kg dry solid).

$$M_i = \frac{(W_1 - W_2)}{(W_2 - W_3)} \times 100\%, \quad (4.1)$$

where W_1 is the weight of the sample before drying, including the sample tray, W_2 is the weight of the sample after drying, including the sample tray, and W_3 is the tray weight. The water content (dry basis) of the treated sample was calculated using the initial water content of the untreated sample (control) and the weight of the treated and untreated samples.

$$M_f = \frac{Y_1 - (Z_1 - M_i \times Z_1)}{Z_1 - M_i \times Z_1} \times 100\%, \quad (4.2)$$

where M_f is the moisture content (dry basis) of the treated sample (Table 4.1), Z_1 is the untreated sample weight, and Y_1 is the sample weight after treatment. The moisture ratio (MR) of the sample for an uncontrolled relative humidity of the drying air was determined as the ratio of the final water content to the initial water content of the sample (Midilli et al., 2002).

$$MR = \frac{M_f}{M_i}. \quad (4.3)$$

The a_w in the sample was measured before and after treatment using an a_w meter (4TE, Aqualab, USA).

4.2.4 Modeling of drying kinetics of pet food pellets

The moisture ratio (MR) values versus treatment times for all three modes were fitted with different empirical and semi-empirical models for drying (Jian & Jayas, 2018; Xie et al., 2018). Experimental and predicted values of MR were used to determine the root mean square error (RMSE) and the regression coefficients (R^2). The regression analysis was performed using Solver in Microsoft EXCEL©, and the correlations between predicted and observed values were

evaluated by comparing the RMSE and R^2 (Wang, 2002). Page and Weibull drying models were used to fit the MR kinetics based on the lowest RMSE and the highest R^2 values obtained.

Equation 4.4 and equation 4.5 represent the Page and Weibull procedurals, respectively, that were used to predict the moisture ratio of the pet food pellets.

$$MR = \exp(-kt^n), \quad (4.4)$$

where k and n are empirical drying parameters (Page, 1949).

$$MR = \exp\left(-\left(\frac{t}{\alpha}\right)^{\beta}\right), \quad (4.5)$$

where α is a scale parameter and β is a shape parameter for the drying model (Dai et al., 2015).

4.2.5 Microbial inoculation, enumeration and inactivation modeling

Two *Salmonella enterica* spp. strains, *S. enterica* serovar Typhimurium ATCC13311 and *S. enterica* serovar Senftenberg ATCC43845, stored as frozen stock at -80 ºC were prepared in tryptic soy broth and 70% (v/v) glycerol (Du et al., 2020). Tryptic soy agar (TSA, Becton, Dickinson and Company, USA) supplemented with 0.6% yeast extract (YE, Fisher Bioreagents, USA) was used as the media to recover and grow the *Salmonella* strains. A loop of frozen stock was streaked onto a plate of TSAYE media and incubated for 24 h. After incubation, a single isolated colony was transferred (using a loop) into 5 ml of sterile tryptic soy broth (TSB, Becton, Dickinson and Company, USA) supplemented with 0.6% yeast extract and incubated for 24 h at 37 °C inside an incubator shaker. 100 µl of the inoculated broth was transferred to 5 ml of TSBYE media and incubated for 20 h. Inoculated broth (100 µl) was spread over the TSAYE plate, the bacterial lawn was retrieved using peptone water followed by centrifugation and a bacterial suspension of 1 ml for each *Salmonella enterica* strain was prepared using peptone water (Du et al., 2020). Both

Salmonella strains were mixed and 20 µl of mixed inoculum was added to the top and bottom flat surfaces of the pet food pellets. Inoculated samples were kept inside a biosafety cabinet for 2 h. Samples were then equilibrated in a humidity chamber (BTL-433, ESPEC North America Inc., MI, USA) for 16 h at 90% relative humidity (RH) to achieve an a_w of 0.9, then transferred into sealed containers before treatment. Inoculated pet food pellets were treated using the three modes described in section 4.2.2 for 5, 10, 15, 20, and 30 min to determine the *Salmonella* inactivation efficacy. The untreated sample was considered as control. The average log reductions of three replicates were reported for each treatment.

Modeling of *Salmonella* inactivation kinetics was performed using log-linear (equation 4.6) and Weibull models (equation 4.7) (Peleg, 2006).

$$\log (N/N_o) = -t / D, \quad (4.6)$$

where N is the *Salmonella* population after treatment, N_o is the initial number of *Salmonella*, t is the treatment time, and D is the time required in min for a 1 log (90%) reduction in microbial population at a specific LED treatment condition, described as decimal reduction time or thermal death time (D value). The 5D value, which is the time required for a 5 log microbial reduction (99.999%) was also determined from the obtained D values. Non-linear trends of microbial inactivation kinetics were fitted using the Weibull (Peleg, 2006, Van Boekel 2002) (equation 4.7).

$$2.303 \log_{10}(N/N_o) = - (\frac{t}{\alpha})^{\beta}, \quad (4.7)$$

where α and β are scale and shape parameters; α determines the steepness of the microbial inactivation kinetics curve, and β describes the shape of the curve and the bacterial response to the inactivation mechanisms. $\beta > 1$ indicates a concave downward curve, $\beta < 1$ indicates a concave upward curve, and $\beta = 1$ predicts a linear trend for the Weibull model (Villa-Rojas et al., 2013).

The time required for 5 log reductions in a microbial population was predicted using equation 4.8 based on the Weibull model (Van Boekel, 2002).

$$D_t = \alpha\,(-\ln(10^{-d})^{1/\beta}), \quad (8)$$

where d is the desired log reduction and D_t is the time (min) required to achieve a "d" log reduction in a microbial population. D values and 5D values were determined for the *Salmonella* cocktail after treatments in each of the three modes.

4.2.6 TBARS for lipid oxidation estimation

The extent of lipid oxidation in the pet food was calculated using the thiobarbituric acid (TBA) method revised by Bedinghaus and Ockerman (1995). Lin et al. (1998) suggested the use of the thiobarbituric acid-reactive substances (TBARS) to determine the extent of lipid oxidation in pet foods TBARS are expressed in terms of mg (MDA) equivalents/kg of pet food sample. Briefly, 5 ml of 20% trichloroacetic acid (TCA, Acros organics, Jansesen Pharmaceuticalaan, Belgium) containing 1.6% phosphoric acid (Aldrich chemistry, Sigma-Aldrich, USA) was used to extract malonaldehyde (MDA) from 0.5 gm of the ground pet food sample. The mixture was homogenized using a homogenizer (Polytron, Kinematic AG, Switzerland) for 1 min at 15 ×1000 rpm, then centrifuged (Allegra 25R Centrifuge, NRW, Germany) at 5311g for 5 min at 4 ºC (Yadav et al., 2019). One ml of supernatant was transferred to a 15 ml Falcon tube containing an equal volume of 0.02 M TBA (Sigma-Aldrich, USA). Samples were then vortexed, incubated in a water bath for 35 min at 95 ºC, then cooled inside an icebox for 5 min. Two sets of 200 µl aliquots from each sample were transferred to 96 well plates (Costar, Corning Incorporated, NY, USA), and the fluorescence intensity of the samples was recorded using a spectrophotometer (Variskon flash, Thermo Electron Corporation, Canada) at an excitation wavelength of 532 nm and an emission

wavelength of 553 nm. The slope of the TBARS calculation was obtained by plotting the curve for the fluorescence readings for pure water, and for 0.25, 0.5, 1, 2, and 3 μM 1,1,3,3-tetramethoxypropane (TMP) (Acros Organics, NJ, USA).

4.2.7 Statistical analysis

Each experiment was performed three times to complete a three replicates of data. The interaction effect between treatment time and procedure was studied using a two way analysis of variance (ANOVA), while the significance of the treatment was studied using a one way ANOVA. The average means were compared using Tukey's honestly significant difference (HSD) at a significance level of 95% ($p < 0.05$). The results were statistically analyzed using IBM SPSS statistics (version 21.0, IBM, NY, USA).

4.3 Results and Discussion

4.3.1 Surface temperature of pet food pellets

The surface temperature of the pet food pellets increased during LED light treatment, especially after long treatment times (Table 4.1). The temperature of the untreated sample (the control) was 25 ºC after equilibration and before treatment. The highest recorded surface temperatures after 30 min treatments were 76.7, 48.6, and 66.3 ºC for LED treatment alone (mode 1), hot air fluidization with vibration (mode 2), and LED treatment with hot air fluidization and vibration (mode 3), respectively. A significant interaction between treatment time and modes used on the product surface temperature ($p < 0.05$) was observed. Using LED treatment alone (mode 1), the effect of treatment time on product surface temperature was not significantly different for all the treatment times except for the 5 min treatment ($p < 0.05$). Similar observations were reported by Du et al., 2020, where no significant difference in sample surface temperature was observed between 10 min

and 30 min LED treatments. The significantly higher surface temperatures measured on pet food pellets during LED treatment alone (mode 1) indicate that LED treatment for long times is not a non-thermal treatment method. Except for the 5 min treatment, after all the other treatment times using mode 1 (LED treatment alone), significantly higher temperatures resulted compared to mode 2 (hot air fluidization with vibration) and mode 3 (LED treatment with hot air fluidization and vibration). Recently, Prasad et al., 2019, reported temperature increase from 25 °C to 57–62 °C after 10 min (~329 J/cm^2) and to 66–67 °C after 20 min of 395 nm LED treatment (~658 J/cm^2).

In mode 2, where food pellets were treated with hot air fluidization and vibration, no significant change in food pellet surface temperature was observed for all five treatment times. The sample treated with hot air (50 ºC) fluidization and vibration showed significantly lower temperature increase than the samples treated with the LED + vibration + mild hot air (50 ºC) fluidization (mode 3) ($p < 0.05$). In mode 2, the temperature of the pet food pellets did not achieve the hot air fluidization temperature of 50 ºC, even after the longest treatment time of 30 min.

The high intensity LED light pulses generated much higher temperatures than the 50 ºC air fluidization treatments. Hence, the 50 ºC air fluidization essentially cooled the samples when combined with LED treatment and vibration in mode 3. With the hot air fluidization, the surface temperatures of the pet food pellets were reduced in comparison with the LED treatment alone (mode 1) for all the treatment times (Table 4.1). A similar cooling effect of air fluidization during pulsed UV light treatment was reported in Fine and Gervis (2004).

Table 4.1 Change in water content, surface temperature, and lipid oxidation in pet food pellets after treatments (mean ± SD) with three treatment modes. Mean value having different letters are significantly different (Tukey's HSD, $p < 0.05$)

System	Time (min)	Water Content (kg water/kg dry sample)	Temperature (°C)	TBARS (mg MDA/kg)
Untreated (control)	0	0.27 ± 0.01^{A}	25.00 ± 0.00^{A}	0.44 ± 0.15^{A}
LED only	5	0.20 ± 0.02^{B}	$59.6 \pm 3.91^{D,E}$	$0.85 \pm 0.34^{A,B,C,D}$
Vibration and hot air	5	0.20 ± 0.01^{B}	39.1 ± 4.01^{B}	$0.58 \pm 0.02^{A,B}$
LED reactor	5	$0.18 \pm 0.01^{B,C}$	$52.2 \pm 3.83^{C,D}$	$0.63 \pm 0.36^{A,B,C}$
LED only	10	$0.17 \pm 0.02^{B,C}$	$71.1 \pm 1.63^{F,G}$	$1.16 \pm 0.29^{B,C,D,E}$
Vibration and hot air	10	$0.18 \pm 0.002^{B,C}$	41.7 ± 1.55^{B}	0.46 ± 0.08^{A}
LED reactor	10	$0.14 \pm 0.01^{C,D}$	$58.4 \pm 2.57^{D,E}$	$0.98 \pm 0.31^{A,B,C,D,E}$
LED only	15	$0.14 \pm 0.01^{C,D}$	74.5 ± 2.84^{G}	$1.25 \pm 0.06^{C,D,E}$
Vibration and hot air	15	$0.15 \pm 0.02^{C,D}$	43.1 ± 2.20^{B}	0.46 ± 0.10^{A}
LED reactor	15	$0.14 \pm 0.01^{C,D,E}$	$58.7 \pm 2.90^{D,E}$	$1.00 \pm 0.34^{A,B,C,D,E}$
LED only	20	$0.09 \pm 0.02^{F,G}$	$75.5 \pm .58^{G}$	$1.42 \pm 0.18^{D,E}$
Vibration and hot air	20	$0.13 \pm 0.02^{C,D,E,F}$	$45.7 \pm 2.56^{B,C}$	0.49 ± 0.11^{A}
LED reactor	20	$0.10 \pm 0.01^{E,F,G}$	63.1 ± 1.75^{E}	$1.26 \pm 0.14^{D,E}$
LED only	30	0.04 ± 0.02^{H}	75.7 ± 1.03^{G}	1.50 ± 0.17^{E}
Vibration and hot air	30	$0.11 \pm 0.02^{D,E,F}$	44.6 ± 2.10^{B}	0.50 ± 0.08^{A}
LED reactor	30	$0.06 \pm 0.01^{G,H}$	$64.4 \pm 2.33^{E,F}$	1.51 ± 0.08^{E}

*Temperature of the supplied hot air was 50 °C and flow rate was 0.011 m^3/s. The peak velocity of vibrating platform was 8.9 ± 1 cm/s. TBARS is thiobarbituric acid-reactive substances and MDA stands for malonaldehyde.

4.3.2 Drying of pet food pellets

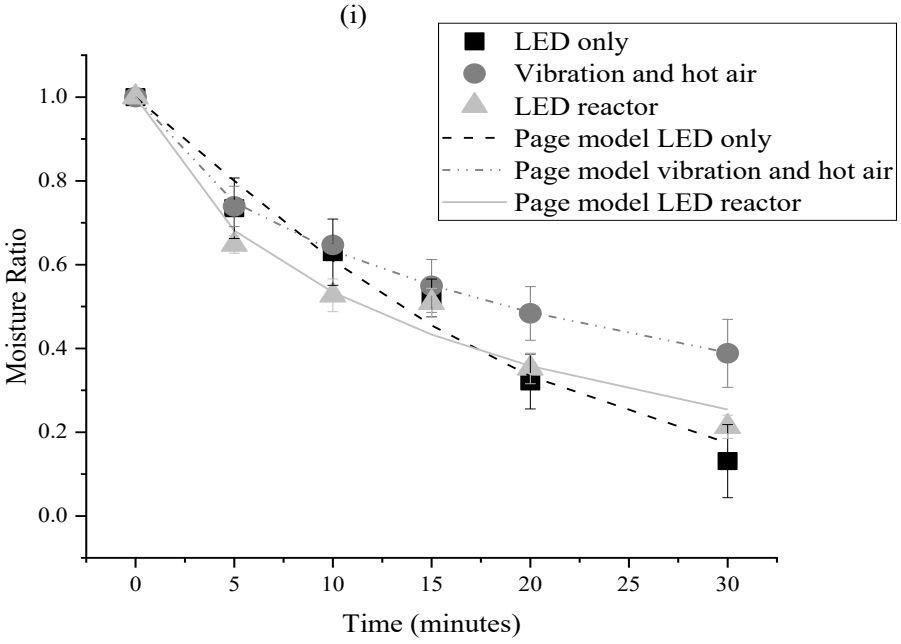
The average initial water content of the equilibrated (a_w = 0.9) pet food pellets was 0.27 kg water/kg dry solids. A continuous decrease in food pellet water content with time was observed

during all the three modes. The water content of pet food pellets was reduced from 0.27 kg water/kg dry sample to 0.04, 0.11, and 0.06 kg water/kg dry sample after 30 min treatments with the LED only (mode 1), with vibration and hot air (mode 2, and with the LED + vibration + mild hot air (mode 3), respectively.

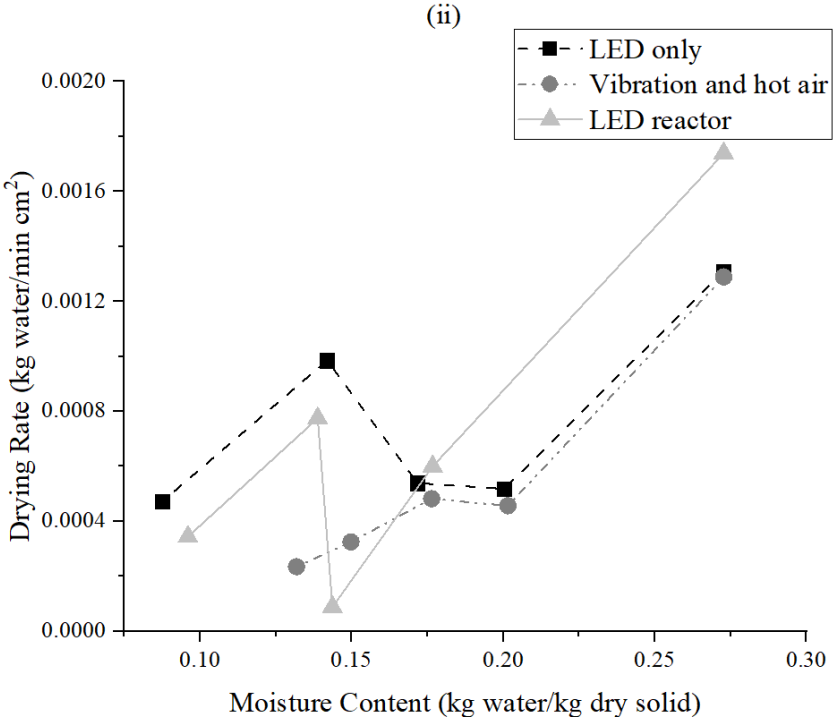
Significantly lower water content was observed after longer treatments of pet food pellets in all three modes (Table 4.1). Treatments of 20 and 30 min with the LED only and with the LED + vibration + mild hot air resulted in significantly lower water content compared to treatment with vibration and hot air fluidization (Table 4.1). Pet food pellet temperatures were 75.7 and 64.4 ^{o}C after 30 min treatments with the LED alone (mode 1) and with the LED + vibration + mild hot air (mode 3). The average surface temperature of the pet food pellets was 44.6 ^{o}C after a 30 min treatment with vibration plus hot air fluidization (mode 2). The greater surface temperatures of pet food pellets during exposure to high intensity light pulses from the LED resulted in higher water vapor pressure differences, which probably increased the diffusion of moisture from the pet food pellets and increased the drying rates.

The moisture ratio (MR), i.e., the ratio of final water content to initial water content of the food pellets, is plotted against the treatment time in Figure 4.2(i) for all three systems. A lower MR value represents a higher moisture loss and vice-versa. The rate of food pellet drying (kg water/min.cm^2) versus water content (kg water/kg dry solid) represents the drying rate in each system (Figure 4.2(ii)). In mode 1, MR values of the pet food pellets after LED treatments for 5 min and 10 min were not significantly different, but were significantly higher in samples treated for 15, 20, and 30 min. The highest moisture loss was observed after the 30 min treatment. The loss of moisture in the food pellets was directly proportional to the treatment time when heat energy was applied (Wankhade et al.,2012).

(i)


LED only
Vibration and hot air
LED reactor
Page model LED only
Page model vibration and hot air
Page model LED reactor
Moisture Ratio
1.0
0.8
0.6
0.4
0.2
0.0
0
5
10
15
20
25
30
Time (minutes)

(ii)


LED only
Vibration and hot air
LED reactor
Drying Rate (kg water/min cm2)
0.0020
0.0016
0.0012
0.0008
0.0004
0.0000
0.10
0.15
0.20
0.25
0.30
Moisture Content (kg water/kg dry solid)

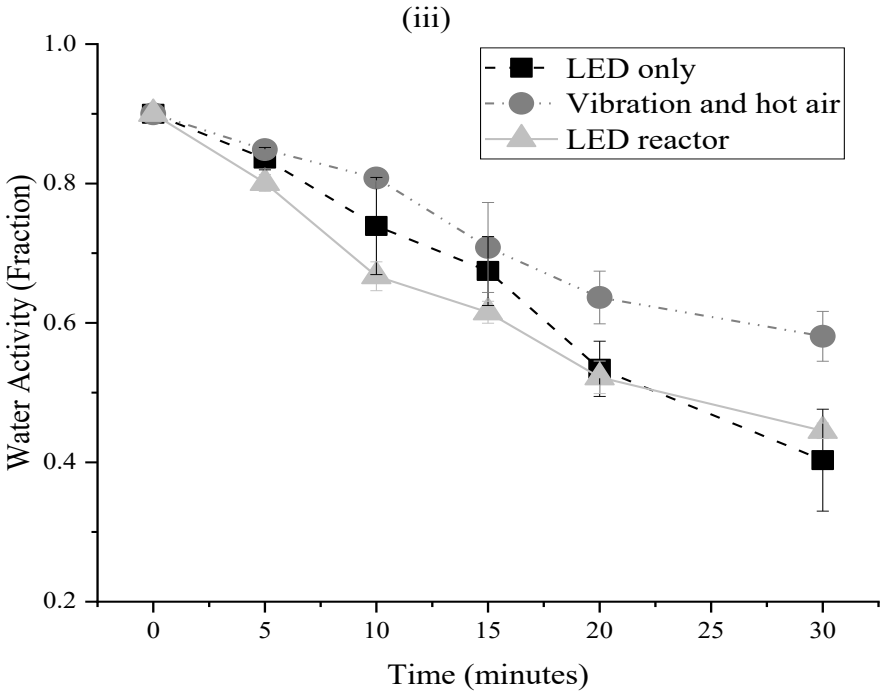


Figure 4.2 Drying kinetics and water activity changes in pet food using three different modes. Change in predicted (Page model) and actual moisture ratio of the pet food pellets with treatment time (i), Change in drying rate with water content during treatments (ii), Pet food pellet water activity change with treatment time (iii)

*The error bars represent value's mean ± SD.

The MR decreased with an increase in treatment time (Figure 4.2(i)). Higher MR values of pet food pellets were observed with mode 2 (vibration and hot air fluidization at 50 ^{o}C) after 5 min treatments compared to 15, 20, and 30 min treatments, but were not significantly different from the MR value after the 10 min treatment ($p < 0.05$). In mode 2, the 50 ^{o}C air fluidization had a significant drying effect on the pet food pellets, which underwent a maximum moisture loss after 30 min of treatment.

In mode 3 (LED + vibration + mild hot air), the pet food pellet MR after 5 min of treatment was significantly higher ($p < 0.05$) than the MRs after 10, 15, 20, and 30 min treatments (Figure 4.2(i)). The MR values were small in pet foods treated for long times with the LED alone and with the LED + vibration + mild hot air, a fact that was attributed to an increase in surrounding temperatures during the treatment (Wankhade et al., 2012). Mode 3 (LED + vibration + mild hot air) produced a significantly lower operating temperature than mode 1, but a similar drying effect was observed in mode 3 compared to mode 1 (LED treatment alone), and this was attributed to the forced air circulation due to the hot air fluidization, which would be expected to result in an increase in moisture transfer, and thus in pet food pellet drying.

Table 4.2 Model parameters, R^2, and RMSE for the drying kinetics of pet food pellets

System	Model	Model's parameters		R^2	RMSE
LED only	Weibull	$\alpha = 18.4$	$\beta = 1.15$	0.977	0.073
LED only	Page	$k = 0.035$	$n = 1.15$	0.977	0.073
Vibration and hot air	Weibull	$\alpha = 32.8$	$\beta = 0.658$	0.999	0.047
Vibration and hot air	Page	$k = 0.100$	$n = 0.658$	0.999	0.047
LED reactor	Weibull	$\alpha = 19.2$	$\beta = 0.711$	0.976	0.048
LED reactor	Page	$k = 0.122$	$n = 0.711$	0.976	0.048

* R^2 is coefficient of determination and RMSE is root mean square error value

The MR values of the pet food pellets were fitted with five empirical and semi-empirical models to select the best fit models Page and Weibull models were later selected (Table 4.2). Although all five models fitted well, Page and Weibull models were selected as the best models for mode 3 (LED + vibration + mild hot air), as they had consistently higher R^2 and lower RMSE values. Dai et al. (2015) described the Weibull model as an easy and good fit model for drying biological materials and nutrients in non-isothermal conditions. Wang (2002) reported the Page and Weibull models to be the most consistent and best fit model for the radiative drying of thin layer foods.

The values of the model parameters in Table 4.2 were used in equations 4 and 5 to predict the MR of pet food pellets in all three treatment modes for any specific treatment time.

The observed surface area of each pet food pellets (approximately ellipsoid shape) was 1.4 cm^2 and three pellets were used during drying. Initial drying rates of pet food pellets for treatments with the LED alone, the vibration with hot air fluidization, and the LED + vibration + mild hot air were 1.3 $\times 10^{-3}$, 1.3 $\times 10^{-3}$, and 1.7 $\times 10^{-3}$ kg water/min.cm^2 (Figure 4.2(ii)). In the first 5 min of treatment, the LED + vibration + mild hot air treatment resulted in the highest drying rate of the three modes. This could be attributed to the combined heating effect of the LED and the hot air. Reyes et al., 2002 reported that the drying air velocity was positively correlated to the drying rate of the sample. The addition of vibration to hot air fluidization increased the drying rate in the system by exposing the entire surface of the pet food pellets to LED light, and created a uniform temperature distribution within the product (Das et al., 2004; Nindo et al., 1995). The temperature increase due to the LED created a water vapor pressure gradient between the pet food pellets and the surrounding environment, resulting in a fast removal of water in the case of modes 1 and 3. In addition, the forced air circulation in mode 3 created a dynamic environment that increased the transfer of water from the pet food pellets to the surrounding environment. Falling rate of drying was observed initially in all three treatment modes (Figure 4.2(ii)). With decreasing water content, the slight decrease and then increase in drying rate observed in modes 1 and 3 in our study, could be attributed to a decrease in free water due to immediate surface drying followed by the removal of available bound water present in the pet food pellets (Baysal et al., 2003). Since, these pellets are high in protein, they would be expected to contain a significant quantity of protein-bound water. At lower levels of water content, the drying rates in the LED treatment alone and the LED

+ vibration + mild hot air treatment were higher compared to the vibration and hot air fluidization treatment.

The a_w of the pet food pellets after equilibration but before treatment was 0.9 ± 0.02. A decrease in the a_w value with treatment time was observed after all three treatment modes (Figure 4.2(iii)). The a_w values of pet food pellets after 30 min of treatment with the LED alone, with vibration and hot air fluidization, and with the LED + vibration + mild hot air, were 0.340, 0.541, and 0.438, respectively. The decrease in the a_w during all treatments can be attributed to the heat energy produced by high intensity light pulses and hot air fluidization, which would cause an increase in pet food surface temperature and a decrease in the a_w (Du et al., 2020). No significant change in the a_w was observed after 5 min treatments compared to the untreated sample (control) ($p \geq 0.05$); 10 min treatments led to significantly lower a_w values when the LED was used alone ($p < 0.05$), but a_w values for 5 min and 10 min treatments were not significantly different ($p \geq 0.05$). After 15, 20, and 30 min treatments, the a_w values were significantly lower compared to 5 min treatments ($p < 0.05$). A gradual decrease in the mean value of a_w was observed with an increase in treatment time (Figure 4.2(ii)). Our previous study reported a significant temperature increase and a reduction in the a_w value in wheat flour after LED treatment (Du et al., 2020)

Sample drying was due to the hot air, and the drying efficacy in mode 2 was probably enhanced by the vibration and air fluidization, which exposed a larger maximum surface area of pet food pellets than LED treatment alone (Nindo et al., 1995). However, after 30 min of exposure to mode 2 (vibration-assisted hot air fluidization), pet food pellets had higher a_w values than samples treated by modes 1 and 3; this result probably reflected the fact that samples exposed to mode 2 had the lowest product surface temperatures.

In mode 3 (LED treatment + vibration + mild hot air (50 °C) fluidization), the a_w values in pet food pellets after each of 5 treatment times were significantly different from each other ($p < 0.05$) (Figure 4.2(iii). Therefore, there was a positive correlation between treatment time and water activity decrease when LED treatment was augmented by vibration and hot air fluidization.

The a_w values of pet food pellets treated for 30 min by the LED alone and treated by the LED + vibration + mild hot air fluidization were significantly lower compared to treatment with vibration and hot air fluidization ($p < 0.05$). No significant difference ($p \geq 0.05$) in a_w values was observed in pet food pellets after 30 min of treatment using the LED alone (mode 1) and after 30 min of treatment using the LED + vibration + mild hot air fluidization (mode 3). The lower a_w values in the cases of LED only and LED treatment in addition to hot air fluidization were likely due to the LED light pulses treatment, which caused a higher product temperature.

4.3.3 Microbial inactivation efficacy

Previous research (Jiang et al., 2009; Nelson et al., 2018; Du et al., 2020) reported the microbial inactivation efficacy of UV-A light treatment, which made lethal changes in bacterial DNA (Elmnasser et al., 2008; Nicorescu et al., 2013). In our study the longer treatments with 395 nm light pulses increased the reductions in *Salmonella* cells in modes 1 and 3, due to higher total energy dosages (Du et al., 2020). In mode 1, in pet food pellets treated with the LED alone, the average *Salmonella* reduction achieved after a 30 min treatment was 1.2 log, and was not significantly different ($p \geq 0.05$) from the *Salmonella* reductions obtained after other treatment times. This might be attributed to a shadowing effect, as explained below. The pet food pellets were inoculated with *Salmonella* on the top and the bottom of their flat surfaces. As the pet food pellets were opaque, the light pulses could not pass through them; therefore, the inoculated bacteria on the bottom surface of the pet food pellets were not exposed 395 nm light pulses. In mode 2, the

Salmonella reductions for pet food pellets exposed to hot air fluidization and vibration were not significantly different after 5,10, 15, 20, and 30 min. The maximum *Salmonella* inactivation after 30 min of treatment with mode 2 was 0.55 log, and this small inactivation level could be attributed to the relatively low air temperature. *Salmonella* inactivation after vibration and hot air fluidization treatment for 30 minutes was significantly smaller than *Salmonella* inactivation after 30 min of LED treatment alone and *Salmonella* inactivation after 30 min of treatment with LED + vibration + mild hot air (50 ºC) fluidization ($p < 0.05$). The higher log reductions in *Salmonella* after LED treatment alone compared to treatment with vibration and hot air fluidization could be due to an greater increase in surface temperature of pet food pellets during mode 1 treatment condition.

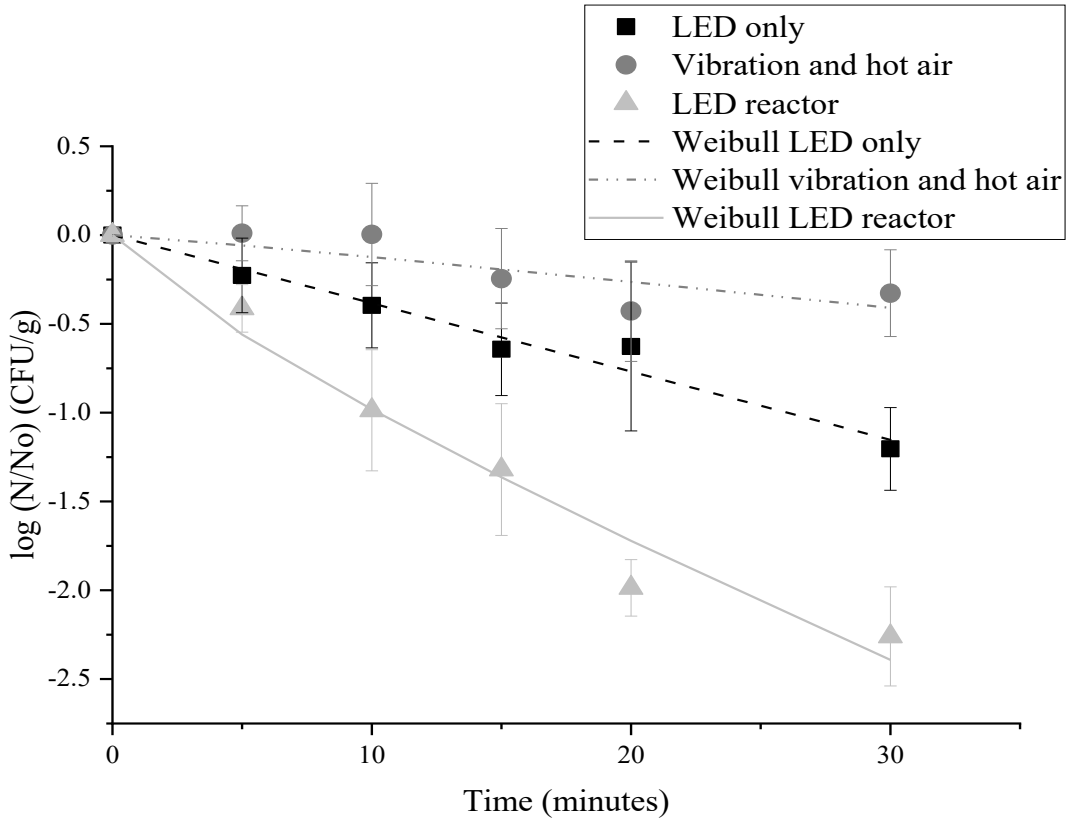


Figure 4.3 Comparison of the inactivation kinetics of *Salmonella* in three treatment modes, predicted by Weibull equation

*The error bars represent value's mean ± SD.

The highest *Salmonella* reduction on pet food pellets was 2.26 log, which was achieved in mode 3 (LED + vibration + mild hot air (50 ºC) fluidization) after 30 min. Mode 3 produced higher log reductions in *Salmonella* than mode 1 (LED treatment alone) and mode 2 (hot air fluidization and vibration). This could be attributed to the combined effects of high energy 395 nm light pulses, vibration, and mild hot air (50 ºC) fluidization, because vibration and air fluidization could increase the exposure of *Salmonella* to light pulses and heat. *Salmonella* inactivation on pet food pellets treated for 10 and 15 min using LED + vibration + mild hot air (50 ºC) fluidization (mode 3) was significantly greater than *Salmonella* inactivation using vibration and hot air fluidization (mode 2), but was similar to *Salmonella* inactivation using LED light pulses alone (mode 1). This could be due to the higher standard deviation due to an inconsistent flipping of pet food pellets. The fluidization effect created by supplied air and vibration created the tossing effect of the pet food pellets. Three *Salmonella* inoculated pellets were treated at each time, which increased the irregularity of exposure to 395 nm light pulses, and might have contributed to a higher standard deviation for *Salmonella* inactivation using mode 3 (LED + vibration + mild hot air (50 ºC) fluidization). There was no significant difference in *Salmonella* decontamination when food pellets were treated with mode 3 for shorter times (5, 10, and 15 min), but for 20 min and 30 min treatments, the inactivation efficacy of LED + vibration + mild hot air (50 ºC) fluidization was significantly higher than the inactivation efficacy of treatment with the LED only.

In mode 3, where vibration and hot air fluidization was added to LED light pulses, the least exposed surface of the pet food would receive a maximum of 50% LED exposure (both surfaces of a pet food pellets were inoculated with *Salmonella*). The log reductions in *Salmonella* depended on the amount of light energy supplied to the bacterial cells. The light energy dosage values were 198, 396, 594, 792, and 1,188 J/cm^2 after 5, 10, 15, 20, and 30 min treatments with 395 nm light

pulses, respectively. The increase in microbial decontamination efficacy with treatment time (Figure 4.3) clearly signified a positive correlation between the energy supplied and the inactivation rate of *Salmonella* on pet food pellets, as the energy delivered by light pulses from LEDs at constant irradiance is directly proportional to irradiation time (Almeida et al., 2011). Du et al. (2020) reported that 1620 J/cm^2 light energy dosage of 395 nm light pulses was required to achieve a 2.42 log reduction of *Salmonella* in wheat flour. Fine & Gervais (2004) used a 16.5 J/cm^2 energy dosage assisted with fluidization to inactivate bacteria in glass beads, but significant decontamination was not achieved. The food pellets in the current study were treated with significantly higher energy.

4.3.4 Modeling of *Salmonella* inactivation kinetics

In the current study, log-linear and Weibull models fitted well (> 0.95) with the *Salmonella* inactivation kinetics in pet food pellets for all treatment modes except vibration and hot air fluidization (mode 2), in which no significant microbial inactivation on pet food pellets was detected. The Weibull model predicted the *Salmonella* inactivation kinetics with same or higher R^2 value for all the modes and was used in Figure 4.3 to fit the experimental data.

For modes 1 and 2, the *Salmonella* inactivation kinetics exhibited a linear trend with a shape factor value, β, close to 1. For mode 3, the *Salmonella* inactivation kinetics exhibited a non-linear trend in which the Weibull model fitted well with the inactivation kinetics. D and 5D values, representing the treatment times required for 1 and 5 log reductions in the *Salmonella* population, respectively, were calculated for all three modes (Table 4.3) by log-linear and Weibull models and compared. For mode 1 (LED treatment alone) the D and 5D values calculated from log-linear and Weibull models were similar, due to the linearity of the inactivation kinetics; the Weibull parameter β, which defines the concavity of the curve was close to 1, making the equation

approximately linear (Villa-Rojas et al., 2013). The high temperatures produced in mode 1 inactivated *Salmonella* cells without recovery. The top and bottom surfaces of the pet food pellets were inoculated with *Salmonella,* but only top surface was exposed to LED treatment in mode 1. Inactivation of *Salmonella* cells on the bottom surface of the food pellets could be due to the heat generated during the LED treatment. The higher D and 5D values for *Salmonella* inactivation observed during vibration and hot air fluidization (mode 2) represented a higher resistance of *Salmonella* (Table 4.3). In mode 3 (LED + vibration + mild hot air (50 ºC) fluidization), the 5D values of *Salmonella* predicted using the Weibull were greater than the 5D values of *Salmonella* predicted in the log-linear model which included a non-linear trend of inactivation kinetics, showing the recovery of *Salmonella* cells. The higher 5D value for *Salmonella* predicted by the Weibull model should be considered, as it includes the non-linearity of the inactivation kinetics, which improves the accuracy of the prediction (Rachon et al., 2016). The D and 5D values of *Salmonella* during pet food treatment using the LED + vibration + mild hot air (50 ºC) fluidization (mode 3) were smaller than those during LED treatment alone (mode 1). The D and 5D values were reduced considerably by incorporating vibration and hot air fluidization to the LED treatment (mode 3) (Table 4.3). For instance, the D and 5D values calculated using the Weibull model were 10.2 min (light energy dosage of 404 J/cm^2) and 74.5 min (light energy dosage of 2950 J/cm^2), respectively, in mode 3, compared to 26 (light energy dosage of 1030 J/cm^2) and 130 min (light energy dosage of 5148 J/cm^2), respectively, in mode 1. The addition of vibration and hot air fluidization added a cost of energy, but it significantly reduced the treatment time to inactivate *Salmonella*. The hot air supplied at 50 ºC reduced overheating by controlling the temperature of the pet food pellets. Drying is an energy intensive process, and the high energy dosages of LED treatments can be justified by achieving significant drying and microbial decontamination of food

products. The predicted final water activity values of the pet food pellets using the Weibull model ($R^2 > 0.97$) corresponding to 5D values (predicted using the Weibull model in table 4.3) for all LED only, vibration and hot air and LED reactor were 0.003, 0.003 and 0.17 respectively.

Table 4.3 Model parameters, calculated D- and 5D-values, R^2, and RMSE values for the *Salmonella* inactivation kinetics

System	Model	Model parameters		D (min)	5D (min)	R^2	RMSE
LED only	Weibull	α = 11.3	β = 1.00	26	130	0.967	0.299
LED only	Log-linear	D = 26.0		26	130.0`	0.967	0.282
Vibration and hot air	Weibull	α = 31.7	β = 1.09	68.3	300.7	0.703	0.268
Vibration and hot air	Log-linear	D = 75.1		75.1	375.5	0.707	0.268
LED reactor	Weibull	α = 3.65	β = 0.810	10.2	74.5	0.972	0.302
LED reactor	Log-linear	D = 11.9		11.9	59.5	0.957	0.325

* R^2 is coefficient of determination, RMSE is root mean square error value, α and β are scale and shape parmeters in Weibull model, D and 5D are time required for 1 log and 5 log reduction of the *Salmonella* strains

Previous research of LED treatments of solid foods with low a_w values is limited. Microorganisms are extremely resistant to antimicrobial treatments at low a_w conditions. For instance, the time required for a 1 log inactivation of *S. typhimurium* at an a_w of 0.85 in sucrose solution was 31.5 min at 65 ºC (Corry, 1974). At a comparable temperature and an a_w of 0.9, mode 3 (395 nm LED + vibration + mild hot air (50 ºC) fluidization) achieved much higher reductions in *Salmonella* on pet food pellets in 30 min. Goepfert et al., 1970, showed that heat treatment at 57.2 ºC of sucrose solution inoculated with *S. Senftenberg* 775W had a D value of 62 min at an a_w of 0.9. Du et al. (2020) reported 2.91 log reductions in a 5 strain cocktail of *Salmonella* in wheat flour with an initial a_w of 0.75 after 60 min of 395 nm LED treatment. However, an absolute comparison of different studies is not possible due to the different food matrices, the microorganisms used, and

the procedures followed. This study suggests that 395 nm LED + vibration + mild hot air (50 ºC) fluidization were effective in achieving fast drying and decontamination of small particulate foods such as pet food pellets. Future studies may test the drying and decontamination efficacy of these conditions in powdered foods.

4.3.5 Lipid oxidation in pet food pellets

TBARS values increased with an increase in pet food pellet treatment time for 395 nm LED treatment alone and for 395 nm LED + vibration + mild hot air (50 ºC) fluidization (Table 4.1). The TBARS value for pet food pellets treated with the LED alone for 10, 15, 20, and 30 min was significantly higher compared to the untreated sample ($p < 0.05$), illustrating significant oxidation during the treatments. Samples treated for 30 min with the LED alone resulted in no significant change in TBARS values compared to 10, 15, and 20 min treatments ($p \geq 0.05$). In mode 2 (vibration and hot air fluidization), no significant increase in TBARS values with treatment times was observed for pet food pellets. Hence, no significant lipid oxidation of food occurred during drying of the pet food pellets using hot air fluidization and vibration.

In mode 3 (395 nm LED treatment + vibration + mild hot air (50 ºC) fluidization), no significant increase in the concentration of TBARS was observed compared to the untreated samples for 5,10, and 15 min treatments, but a significant increase in TBARS values was observed in samples treated for 20 and 30 min ($p < 0.05$). However, no significant increase in the TBARS value was observed for pet food pellets treated for 30 min compared to samples treated for 10, 15, and 20 min inside the LED reactor.

TBARS values for the pet food pellets treated with the LED alone and with LED + vibration + mild hot air (50 ºC) fluidization, were not significantly different for all treatment times. The

considerable temperature increase in pet food pellets during treatments with the LED alone and with LED + vibration + mild hot air (50 ºC) fluidization might account for the higher TBARS values. Sullivan et al., 2011, reported a positive correlation between temperature increase and peroxide value increase in oil, which could lead to an increase in the TBARS values in pet food pellets during LED treatments. Also, the increase in TBARS with time in modes 1 and 3 could be due to the high intensity light pulses and the exposure of pet food pellets to light for a long time. Lin et al., 1998, demonstrated that the change in TBARS values in the pet food was directly proportional to time. Air fluidization at different temperatures and flow rates could be further studied to see if the effect of the operational temperature of the LED was related to the lipid oxidation in pet food pellets. Approaches to reduce lipid oxidation or other quality changes in the food product should be developed when scaling-up the LED technology in the future. The use of low or ambient temperature air fluidization to reduce the heating involved in LED treatments could exemplify such an approach.

4.4 Conclusions

The use of 395 nm LED light pulses with hot air fluidization and vibration inside the reactor reduced the *Salmonella* population effectively while achieving the fast drying of pet food pellets. The average water content in the pet food pellets was reduced from 0.27 to 0.06 kg water/kg dry solids, and the water activity decreased from 0.9 to 0.44 after 30 min of 395 nm LED light pulses + vibration + mild hot air (50 ºC) fluidization (mode 3). The drying efficacy of mode 3 was similar to that of LED treatment alone, but mode 3 had a higher bacterial inactivation rate than mode 1 (LED treatment alone), and this was achieved with a comparatively lower operational temperature. Page and Weibull models were fit to describe the drying kinetics data of pet food pellets, while Weibull and log-linear models were used to fit the *Salmonella* inactivation kinetics. The treatment

time and light energy required for a 5 log reduction in *Salmonella* on pet food pellets, predicted by Weibull model, were lower for LED treatment + vibration + mild hot air (50 ºC) fluidization (mode 3) than for treatment with the LED alone (mode 1). Considerable lipid oxidation of the pet food pellets was observed when mode 3 and mode 1 were used for long treatment times. Future studies are required to understand the potential of LED treatment with vibration and low-temperature air fluidization, which could be added as a processing step to achieve drying and decontamination of a variety of foods, while approaches to reduce product oxidation should be explored.

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Chapter 5: Conclusions and recommendations

5.1 Summary of key findings

The high intensity UV and blue light pulses emitted by LEDs can inactivate *Salmonella enterica* in wheat flour and pet food pellets. In the past, application of the UV light for microbial inactivation was considered as a non-thermal approach, but in this research, we observed a significant increase in surface temperature of wheat flour and pet food pellets after using light pulses with 365, 395 and 455 nm wavelengths, emitted from LEDs. During high intensity LED treatments using 275, 365, 395 and 455 nm wavelength light pulses, the wheat flour attained the maximum temperature of 31.3, 81.4, 86.1, and 81°C within the first 10 min of the treatment and that temperature was almost stable for rest of the treatment time. UV-C LED light (275 nm) demonstrated temperature increase, but lower than the temperature increase compared to the treatments using 365, 395 and 455 nm light pulses, attributed to the lower energy dosages.

Significant drying of wheat flour and reductions in water activity values were observed during treatments using 275, 365, 395, and 455 nm light pulses along with *Salmonella* inactivation. The *Salmonella* inactivation efficacy of LED treatments using 365 and 395 nm light pulses was influenced by the initial water content of wheat flour. For an equal energy dosage, the *Salmonella* inactivation efficacies of the 365 and 395 nm LED treatment were better than that of 455 LED treatment. The 395 nm LED treatment resulted in the greatest *Salmonella* inactivation in wheat flour compared to 275, 365, 455 nm LED treatments for 60 min, attributed to the highest energy dosage of 395 nm light pulses from LEDs.

The relative humidity conditions of 40%, 75% and 90% had no significant effect on the inactivation of the *Salmonella* in wheat flour during LED treatments. At a lower RH of 40%, drying of wheat flour during 275 nm LED treatments was enhanced compared to 75% and 90% RH

conditions, due to the synergistic effect of surrounding dry air and LED treatment. However, high RH conditions of 75 and 90% did not control the drying of wheat flour during high intensity LED light pulses exposure. The LED emitting light pulses of 275 nm used for drying had lowest drying rate in wheat flour. Most of the drying in wheat flour during LED treatment with 365, 395 and 455 nm occurred during the first 10 min.

In chapter 4, a new LED reactor was designed and built-in Food Safety and Sustainability Engineering laboratory at the University of Alberta to achieve simultaneous microbial inactivation and drying of food product, with consideration of industrial scale-up in mind. For initial testing of the reactor, the 395 nm wavelength was selected as it exhibited higher *Salmonella* inactivation efficacy for certain time based on chapter 3 research. For better light exposure to samples, a vibratory platform and air fluidization were added to the reactor. This can be operated using LEDs emitting light pulses with variable wavelengths at selected temperature conditions.

The use of 395 nm LED treatment alone resulted in the highest increase in the surface temperature of the pet food pellets compared to LED treatment with vibration and 50^{o}C air fluidization. The air fluidization and vibration essentially cooled the pet food pellets during LED treatment. *Salmonella* was resistant to treatments with hot air (50 ^{o}C) fluidization and vibration while LED treatment alone was able to inactivate them significantly on pet food pellets. The newly built LED reactor using 395 nm light treatment was the most efficient to inactivate *Salmonella* on pet food pellets. The D-values of *Salmonella* were lower when treated inside the LED reactor with 395 nm light pulses, compared to 395 nm LED treatment alone. Weibull and log-linear model fit to describe the microbial inactivation during LED treatments with or without air fluidization.

LED treatment with or without hot air fluidization resulted in considerable drying of pet food pellets. Page and Weibull models were fit to describe pet food pellet drying kinetics using the LED

reactor. The oxidation of the pet food pellets was observed after the LED treatment using 395 nm light pulses. The microbial inactivation and drying efficacy of the LED reactor can be improved by changing the airflow rate, the temperature of air fluidization, and using light pulses with different wavelengths. The pet food industry may explore this technology to achieve the target a_w of 0.4 to 0.5 of pet food pellets within 30 min treatment using the LED reactor along with significant microbial inactivation. Further, exposure to light pulses emitted from LEDs along with air fluidization on a vibratory platform can be explored to develop other low-moisture foods (e.g., wheat flour, nuts, skim milk powder, seeds etc.) and used as a novel drying and decontamination technology by the low-moisture food industry.

5.2 Recommendations for future work

Based on the summary of the key findings of this thesis research, the following recommendations can be the scope for future investigations:

- Comparative study of 275, 365, 395 and 455 nm LED treatment along with hot air fluidization inside the LED reactor for microbial inactivation in wheat flour sample or other powdered foods associated with recalls (e.g., skim milk powder);
- Use of the 275 nm with higher irradiance could give a better comparison with other wavelengths for microbial inactivation and drying;
- Use of a higher airflow rate could help to control the product and process temperatures, so microbial inactivation on pet food pellets using higher airflow rate and reduced air pipe length could be studied;
- The TBARS value of the pet food was higher for the LED treatment, which could be due to the temperature increase. Effect of the different temperatures of air supplied during LED treatment on lipid oxidation can be studied;

- Study the microbial inactivation efficacy of LED reactor and the inactivation mechanisms using other potential pathogenic microorganisms associated with low-moisture foods (bacteria including *E. coli* or *Listeria*, bacterial spores, harmful fungi, etc.);
- The LED reactor could be used for other small particulate foods including food pellets, grains or seeds, to understand the effect of LED treatment along with air fluidization on drying and inactivation efficacy of the system;
- To reduce the oxidation of products, a low oxygen environment or fluidization with CO_2 or nitrogen can be tested to achieve microbial inactivation and drying of food products. The antimicrobial synergy of high-pressure CO_2 along with LED treatments can be explored and a new reactor based on this concept can be designed to achieve fast drying and significant microbial inactivation in foods.
- The new LED reactor designed in this research can be scaled-up to industrial level after microbial inactivation and drying optimization studies.
- The effect of the treatments of the food products using LED reactor on product quality characteristics need to be established.

Bibliography

Adhikari, A., Syamaladevi, R. M., Killinger, K., & Sablani, S. S. (2015). Ultraviolet-C light inactivation of *Escherichia coli* O157: H7 and *Listeria monocytogenes* on organic fruit surfaces. *International Journal of Food Microbiology*, *210*, 136–142. https://doi.org/10.1016/j.ijfoodmicro.2015.06.018

Al-Muhtaseb, A. H., McMinn, W. A. M., & Magee, T. R. A. (2002). Moisture Sorption Isotherm Characteristics of Food Products: A Review. *Food and Bioproducts Processing*, *80*(2), 118–128. https://doi.org/10.1205/09603080252938753

Aldrich, G. C., & Koppel, K. (2015). Pet Food Palatability Evaluation: A Review of Standard Assay Techniques and Interpretation of Results with a Primary Focus on Limitations. *Animals*, *5*, 43–55. https://doi.org/10.3390/ani5010043

Almeida, A., Cunha, A., Faustino, M. A. F., Tomé, A. C., & Neves, M. G. P. M. S. (2011). Chapter 5. Porphyrins as Antimicrobial Photosensitizing Agents (pp. 83–160). https://doi.org/10.1039/9781849733083-00083

Andino, A., & Hanning, I. (2015). *Salmonella enterica*: Survival, colonization, and virulence differences among serovars. *Scientific World Journal*, *2015*(Table 3). https://doi.org/10.1155/2015/520179

Angulo, F. J., Cahill, S. M., Wachsmuth, I. K., Costarrica, M. d. L., & Embarek, P. K. B. (2008). Powdered Infant Formula as a Source of *Salmonella* Infection in Infants. *Clinical Infectious Diseases*, *46*(2), 268–273. https://doi.org/10.1086/524737

Archer, J, Jervis, E. T., Bird, J., & Gaze, J. E. (1998). Heat resistance of *Salmonella weltevreden* in low-moisture environments. *Journal of Food Protection*, *61*(8), 969–973. https://doi.org/10.4315/0362-028X-61.8.969

Arnoldi, A. (2001). Thermal processing and food quality: analysis and control. In *Thermal Technologies in Food Processing* (pp. 138–159). https://doi.org/10.1533/9781855736610.2.138

Ashkenazi, H., Malik, Z., Harth, Y., & Nitzan, Y. (2003). Eradication of Propionibacterium acnes by its endogenic porphyrins after illumination with high intensity blue light. *FEMS Immunology and Medical Microbiology*, *35*(1), 17–24. https://doi.org/10.1016/S0928-8244(02)00423-6

Bao, X., Sun, P., Liu, S., Ye, C., Li, S., & Kang, J. (2015). Performance improvements for AlGaN-based deep ultraviolet light-emitting diodes with the p-type and thickened last quantum barrier. *IEEE Photonics Journal*, *7*(1). https://doi.org/10.1109/JPHOT.2014.2387253

Baysal, T., Icier, F., Ersus, S., & Yildiz, H. (2003). Effects of microwave and infrared drying on the quality of carrot and garlic. *European Food Research and Technology*, *218*(1), 68–73. https://doi.org/10.1007/s00217-003-0791-3

Bedinghaus, A. J., & Ockerman, H. W. (1995). Antioxidative Maillard Reaction Products from Reducing Sugars and Free Amino Acids in Cooked Ground Pork Patties, *60*(5).

Bekbölet, M. (1990). Light effects on food. *Journal of Food Protection*, *53*(5), 430–440. https://doi.org/10.4315/0362-028X-53.5.430

Beuchat, L. R., Komitopoulou, E., Beckers, H., Betts, R. P., Bourdichon, F., Fanning, S., Joosten H. M., & Ter kuile, B. H. (2013). Low–Water Activity Foods: Increased Concern as Vehicles of Foodborne Pathogens. *Journal of Food Protection*, *76*(1), 150–172. https://doi.org/10.4315/0362-028x.jfp-12-211

Beuchat, L. R., & Mann, D. A. (2015). Survival of *Salmonella* in cookie and cracker sandwiches containing inoculated, low-water activity fillings. *Journal of Food Protection*, *78*(10), 1828–1834. https://doi.org/10.4315/0362-028X.JFP-15-142

Bowker, C., Sain, A., Shatalov, M., & Ducoste, J. (2011). Microbial UV fluence-response assessment using a novel UV-LED collimated beam system. *Water Research*, *45*(5), 2011–2019. https://doi.org/10.1016/j.watres.2010.12.005

Brar, P. K., Proano, L. G., Friedrich, L. M., Harris, L. J., & Danyluk, M. D. (2015). Survival of S*almonella*, *Escherichia coli* O157:H7, and *Listeria monocytogenes* on raw peanut and pecan kernels stored at 224, 4, and 22uC. *Journal of Food Protection*, *78*(2), 323–332. https://doi.org/10.4315/0362-028X.JFP-14-327

Carter, B. P., Galloway, M. T., Morris, C. F., Weaver, G. L., & Carter, A. H. (2015). The case for water activity as a specification for wheat tempering and flour production. *Cereal Foods World*, *60*(4), 166–170. https://doi.org/10.1094/CFW-60-4-0166

Cavallo, S. J., Daly, E. R., Seiferth, J., Nadeau, A. M., Mahoney, J., Finnigan, J., Wikoff, P., Kiebler, C. A., & Simmons, L. (2017). Human Outbreak of *Salmonella* Typhimurium Associated with Exposure to Locally Made Chicken Jerky Pet Treats, New Hampshire, 2013. Physiology & Behavior, 176(3), 139–148. https://doi.org/10.1016/j.physbeh.2017.03.040

CDC (2018). https://www.cdc.gov/foodsafety/cdc-and-food-safety.html accessed on 12-04-2019

CDC (2019). https://www.cdc.gov/foodsafety/foodborne-germs.html accessed on 11-11-2019

Chen, D., Cheng, Y., Peng, P., Liu, J., Wang, Y., Ma, Y., Anderson, E., Chen, C., Chen, P., & Ruan, R. (2019). Effects of intense pulsed light on *Cronobacter sakazakii* and *Salmonella* surrogate Enterococcus faecium inoculated in different powdered foods. *Food Chemistry*, *296*(February), 23–28. https://doi.org/10.1016/j.foodchem.2019.05.180

Chen, D., Wiertzema, J., Peng, P., Cheng, Y., Liu, J., Mao, Q., Ma, Y., Anderson, E., Chen, P., Baumler, D.J., Chen, C., Vickers, Z., Feirtag, J., Lee, L., & Ruan, R. (2018). Effects of intense pulsed light on *Cronobacter sakazakii* inoculated in non-fat dry milk. Journal of Food Engineering, 238(June), 178–187. https://doi.org/10.1016/j.jfoodeng.2018.06.022

Chousalkar, K., Gast, R., Martelli, F., & Pande, V. (2018). Review of egg-related salmonellosis and reduction strategies in United States, Australia, United Kingdom and New Zealand. *Critical Reviews in Microbiology*, *44*(3), 290–303. https://doi.org/10.1080/1040841X.2017.1368998

ConAgra Mills (2011) The raw truth about consumer eating habits. Published online at www.conagramills.com/media/Food%20Habits%20of%20American%20Consumers%20Fin

al.pdf. accessed on 04-02-2019

Condón-Abanto, S., Condón, S., Raso, J., Lyng, J. G., & Álvarez, I. (2016). Inactivation of *Salmonella typhimurium* and *Lactobacillus plantarum* by UV-C light in flour powder. *Innovative Food Science and Emerging Technologies*, *35*, 1–8. https://doi.org/10.1016/j.ifset.2016.03.008

Corradini, M. G., & Peleg, M. (2004). A model of non-isothermal degradation of nutrients, pigments and enzymes. *Journal of the Science of Food and Agriculture*, *84*(3), 217–226. https://doi.org/10.1002/jsfa.1647

Corry, J. E. L. (1974). The Effect of Sugars and Polyols on the Heat Resistance of *Salmonellae*. *Journal of Applied Bacteriology*, *37*(1), 31–43. https://doi.org/10.1111/j.1365-2672.1974.tb00412.x

CSPI (2015) https://cspinet.org/sites/default/files/attachment/outbreak-alert-2015.pdf accessed on 03-05-2019

D'Souza, C., Yuk, H. G., Khoo, G. H., & Zhou, W. (2015). Application of Light-Emitting Diodes in Food Production, Postharvest Preservation, and Microbiological Food Safety. *Comprehensive Reviews in Food Science and Food Safety*, *14*(6), 719–740. https://doi.org/10.1111/1541-4337.12155

Dai, J. W., Rao, J. Q., Wang, D., Xie, L., Xiao, H. W., Liu, Y. H., & Gao, Z. J. (2015). Process-Based Drying Temperature and Humidity Integration Control Enhances Drying Kinetics of Apricot Halves. *Drying Technology*, *33*(3), 365–376. https://doi.org/10.1080/07373937.2014.954667

Das, I., Das, S. K., & Bal, S. (2004). Drying performance of a batch type vibration aided infrared dryer. *Journal of Food Engineering*, *64*(1), 129–133. https://doi.org/10.1016/j.jfoodeng.2003.09.020

DenBaars, S. P. (1993). Light emitting diodes: materials growth and properties. *Solid State Luminescence*, 263–291. https://doi.org/10.1007/978-94-011-1522-3_8

Du, L., Prasad, A. J., Gänzle, M., & Roopesh, M. S. (2020). Inactivation of *Salmonella* spp . in wheat fl our by 395 nm pulsed light emitting diode (LED) treatment and the related functional and structural changes of gluten. *Food Research International*, *127*(September 2019), 108716. https://doi.org/10.1016/j.foodres.2019.108716

Elmnasser, N., Leroi, F., Orange, N., Bakhrouf, A., Guillou, S., & Federighi, M. (2008). Pulsed-light system as a novel food decontamination technology: a review. *Canadian Journal of Microbiology*, *53*(7), 813–821. https://doi.org/10.1139/w07-042

Erbay, Z., & Icier, F. (2010). A review of thin layer drying of foods: Theory, modeling, and experimental results. *Critical Reviews in Food Science and Nutrition*, *50*(5), 441–464. https://doi.org/10.1080/10408390802437063

Fang, J., Xing, J., Gao, L., Shen, B., Kang, H., Jie, L., & Peng, C. (2015). LED array designing and its bactericidal effect researching on Pseudomonas aeruginosa in vitro. *AOPC 2015: Advanced Display Technology; and Micro/Nano Optical Imaging Technologies and Applications*, *9672*(October 2015), 96720C. https://doi.org/10.1117/12.2197305

FDA (2014). https://www.fda.gov/inspections-compliance-enforcement-and-criminal-investigations/inspection-technical-guides/water-activity-aw-foods accessed on 03-03-2019

FDA (2016). https://www.fda.gov/food/reportable-food-registry-industry/reportable-food-registry-annual-report accessed on 15-09-2019

FDA (2019). https://www.fda.gov/safety/recalls-market-withdrawals-safety-alerts accessed on 07-11-2019

Fine, F., & Gervais, P. (2004). Efficiency of Pulsed UV Light for Microbial Decontamination of Food Powders. Journal of Food Protection, 67(4), 787–792. https://doi.org/10.4315/0362-028X-67.4.787

Finn, S., Condell, O., Mcclure, P., Amézquita, A., & Fanning, S. (2013). Mechanisms of survival , responses , and sources of *Salmonella* in low-moisture environments. *Frontiers in Microbiology*, *4*(November), 1–15. https://doi.org/10.3389/fmicb.2013.00331

Forghani, F., den Bakker, M., Liao, J.-Y., Payton, A. S., Futral, A. N., & Diez-Gonzalez, F. (2019). *Salmonella* and Enterohemorrhagic *Escherichia coli* Serogroups O45, O121, O145 in Wheat Flour: Effects of Long-Term Storage and Thermal Treatments. *Frontiers in Microbiology*, *10*(February), 1–12. https://doi.org/10.3389/fmicb.2019.00323

Friedberg, E. C. (2003). DNA damage and repair. *Nature*, *421*(6921), 436–440. https://doi.org/10.1038/nature01408

FSA (2012). https://www.food.gov.uk/sites/default/files/media/document/711-1-1393_IID2_FINAL_REPORT.pdf accessed on 21-03-2019

Gautam, B. (2019). *Influence of water activity on thermal resistance of Salmonella enterica and quality changes in low-moisture foods*. https://doi.org/10.7939/R3-A8GY-MS37

Gayán, E., Serrano, M. J., Raso, J., Álvarez, I., & Condón, S. (2012). Inactivation of *Salmonella* enterica by UV-C light Alone and in combination with mild temperatures. *Applied and Environmental Microbiology*, *78*(23), 8353–8361. https://doi.org/10.1128/AEM.02010-12

Goepfert, J. M., Iskander, I. K., & Amundson, C. H. (1970). Relation of the heat resistance of *Salmonellae* to the water activity of the environment. *Applied Microbiology*, *19*(3), 429–433.

Gómez-López, V. M., Ragaert, P., Debevere, J., & Devlieghere, F. (2007). Pulsed light for food decontamination: a review. *Trends in Food Science and Technology*, *18*(9), 464–473. https://doi.org/10.1016/j.tifs.2007.03.010

GOC (2016). https://www.canada.ca/en/public-health/services/food-borne-illness-canada/yearly-food-borne-illness-estimates-canada.html accessed on 21-02-2019

Guillén-Sans, R., & M. Guzmán-Chozas. (1998). The Thiobarbituric Acid (TBA) Reaction in Foods : A Review. *Critical Reviews in Food Science and Nutrition*, *38*(4), 315–350. https://doi.org/10.1080/10408699891274228

Guo, Y., Yan, J., Zhang, Y., Wang, J., & Li, J. (2018). Enhancing the light extraction of AlGaN-based ultraviolet light-emitting diodes in the nanoscale. *Journal of Nanophotonics*, *12*(04),

1. https://doi.org/10.1117/1.jnp.12.043510

Hale, C. R., Scallan, E., Cronquist, A. B., Dunn, J., Smith, K., Robinson, T., Lathrop, S.,Tobin-D'Angelo, M., & Clogher, P. (2012). Estimates of enteric illness attributable to contact with animals and their environments in the United States. Clinical Infectious Diseases, 54(SUPPL.5), 472–479. https://doi.org/10.1093/cid/cis051

Hamamoto, A., Mori, M., Takahashi, A., Nakano, M., Wakikawa, N., Akutagawa, M., Ikehara, T., Nakaya, & Y., Kinouchi, Y. (2007). New water disinfection system using UVA light-emitting diodes. Journal of Applied Microbiology, 103(6), 2291–2298. https://doi.org/10.1111/j.1365-2672.2007.03464.x

Hiramatsu, R., Matsumoto, M., Sakae, K., & Miyazaki, Y., (2005). Ability of Shiga toxin-producing Escherichia coli and *Salmonella* spp. to survive in a desiccation model system and in dry foods. *Applied and Environmental Microbiology*, *71*(11), 6657–6663. https://doi.org/10.1128/AEM.71.11.6657

Hoelzer, K., Isabel, A., Switt, M., & Wiedmann, M. (2011). Hoelzer 2011 animal contact as a source of human non-typhoidal salmonellosis. Veterinary Research, 42(34), 1–28.

Huang, J., Yang, Y., Xue, S., Yang, B., Liu, S., & Shen, J. (1997). Photoluminescence and electroluminescence of ZnS:Cu nanocrystals in polymeric networks. *Applied Physics Letters*, *70*(18), 2335–2337. https://doi.org/10.1063/1.118866

Jian, F., & Jayas, D. S. (2018). Characterization of isotherms and thin-layer drying of red kidney beans, part i: Choosing appropriate empirical and semitheoretical models. *Drying Technology*, *36*(14), 1696–1706. https://doi.org/10.1080/07373937.2017.1422515

Jiang, Y., Rabbi, M., Kim, M., Ke, C., Lee, W., Clark, R. L., Mieczkowski, P. A., &Marszalek, P. E. (2009). UVA generates pyrimidine dimers in DNA directly. *Biophysical Journal*, *96*(3), 1151–1158. https://doi.org/10.1016/j.bpj.2008.10.030

Kataoka, A., Enache, E., Black, D. G., Elliott, P. H., Napier, C. D., Podolak, R., & Hayman, M. M. (2014). Survival of *Salmonella* Tennessee, *Salmonella* Typhimurium DT104, and Enterococcus faecium in peanut paste formulations at two different levels of water activity and fat. *Journal of Food Protection*, *77*(8), 1252–1259. https://doi.org/10.4315/0362-028X.JFP-13-553

Kim, D. Y., Park, J. H., Lee, J. W., Hwang, S., Oh, S. J., Kim, J., Sone, C., Schubert, E. F., & Kim, J. K. (2015). Overcoming the fundamental light-extraction efficiency limitations of deep ultraviolet light-emitting diodes by utilizing transverse-magnetic-dominant emission. Light: Science and Applications, 4(January), 1–8. https://doi.org/10.1038/lsa.2015.36

Kim, M. J., Tang, C. H., Bang, W. S., & Yuk, H. G. (2017). Antibacterial effect of 405 ± 5 nm light emitting diode illumination against *Escherichia coli* O157:H7, *Listeria monocytogenes*, and *Salmonella* on the surface of fresh-cut mango and its influence on fruit quality. *International Journal of Food Microbiology*, *244*, 82–89. https://doi.org/10.1016/j.ijfoodmicro.2016.12.023

Kim, S. J., Kim, D. K., & Kang, D. H. (2016). Using UVC light-emitting diodes at wavelengths of 266 to 279 nanometers to inactivate foodborne pathogens and pasteurize sliced cheese.

Applied and Environmental Microbiology, *82*(1), 11–17. https://doi.org/10.1128/AEM.02092-15

Krishnamurthy, K., Tewari, J. C., Irudayaraj, J., & Demirci, A. (2010). Microscopic and spectroscopic evaluation of inactivation of staphylococcus aureus by pulsed UV light and infrared heating. *Food and Bioprocess Technology*, *3*(1), 93–104. https://doi.org/10.1007/s11947-008-0084-8

Lamas, A., Miranda, J. M., Regal, P., Vázquez, B., Franco, C. M., & Cepeda, A. (2018). A comprehensive review of non-enterica subspecies of *Salmonella enterica*. *Microbiological Research*, *206*(September 2017), 60–73. https://doi.org/10.1016/j.micres.2017.09.010

Labuza, T.P., Tannenbaum, S.R., & Karel, M. (1970). Water content and stability of low-moisture & intermediate-moisture foods

Li, J., Hirota, K., Yumoto, H., Matsuo, T., Miyake, Y., & Ichikawa, T. (2010). Enhanced germicidal effects of pulsed UV-LED irradiation on biofilms. *Journal of Applied Microbiology*, *109*(6), 2183–2190. https://doi.org/10.1111/j.1365-2672.2010.04850.x

Li, X., Bethune, L. A., Jia, Y., Lovell, R. A., Proescholdt, T. A., Benz, S. A., Schell, T. C., Kaplan, G., & McChesney, D. G. (2012). Surveillance of *Salmonella* prevalence in animal feeds and characterization of the *Salmonella* isolates by serotyping and antimicrobial susceptibility. Foodborne Pathogens and Disease, 9(8), 692–698. https://doi.org/10.1089/fpd.2011.1083

Lian, X., Tetsutani, K., Katayama, M., Nakano, M., Mawatari, K., Harada, N., Hamamoto, A.,Yamato, M., Akutagawa, M., Kinouchi, Y.,Nakaya, Y., & Takahashi, A. (2010). A new colored beverage disinfection system using UV-A light-emitting diodes. Biocontrol Science. https://doi.org/10.4265/bio.15.33

Liang, J. Y., Cheng, C. W., Yu, C. H., & Chen, L. Y. (2015). Investigations of blue light-induced reactive oxygen species from flavin mononucleotide on inactivation of E. coli. *Journal of Photochemistry and Photobiology B: Biology*, *143*, 82–88. https://doi.org/10.1016/j.jphotobiol.2015.01.005

Liao, Y. Te, Syamaladevi, R. M., Zhang, H., Killinger, K., & Sablani, S. (2017). Inactivation of listeria monocytogenes on frozen red raspberries by using uv-c light. *Journal of Food Protection*, *80*(4), 545–550. https://doi.org/10.4315/0362-028X.JFP-16-245

Lin, S., Hsieh, F., & Huff, H. E. (1998). Effects of lipids and processing conditions on lipid oxidation of extruded dry pet food during storage. *Animal Feed Science and Technology*, *71*(12590), 283–294.

Lin, Y.-L., Tang, Y.-L., Gao, N.-Y., Gao, Z.-C., Cao, T.-C., Xu, B., Pan, Y., & Zou, X.-Y. (2018). Enhanced inactivation of E. coli by pulsed UV-LED irradiation during water disinfection. *Science of The Total Environment*, *650*, 210–215. https://doi.org/10.1016/j.scitotenv.2018.08.367

Lipovsky, A., Nitzan, Y., Friedmann, H., & Lubart, R. (2009). Sensitivity of Staphylococcus aureus strains to broadband visible light. *Photochemistry and Photobiology*, *85*(1), 255–260. https://doi.org/10.1111/j.1751-1097.2008.00429.x

MacGregor, S. J., Rowan, N. J., McIlvaney, L., Anderson, J. G., Fouracre, R. A., & Farish, O. (1998). Light inactivation of food-related pathogenic bacteria using a pulsed power source. *Letters in Applied Microbiology*, *27*(2), 67–70. https://doi.org/10.1046/j.1472-765X.1998.00399.x

Maclean, M., MacGregor, S. J., Anderson, J. G., & Woolsey, G. A. (2008). The role of oxygen in the visible-light inactivation of Staphylococcus aureus. *Journal of Photochemistry and Photobiology B: Biology*, *92*(3), 180–184. https://doi.org/10.1016/j.jphotobiol.2008.06.006

Mahendran, R., Ramanan, K. R., Barba, F. J., Lorenzo, J. M., López-Fernández, O., Munekata, P. E. S., Roohinejad, S., Sant'Ana, A.S., & Tiwari, B. K. (2019). Recent advances in the application of pulsed light processing for improving food safety and increasing shelf life. Trends in Food Science & Technology, 88(March), 67–79. https://doi.org/10.1016/j.tifs.2019.03.010

Maserati, A., Fink, R. C., Lourenco, A., Julius, M. L., & Diez-Gonzalez, F. (2017). General response of *Salmonella enterica* serovar Typhimurium to desiccation: A new role for the virulence factors sopD and sseD in survival. *PLoS ONE*, *12*(11), 1–23. https://doi.org/10.1371/journal.pone.0187692

McCallum, L., Paine, S., Sexton, K., Dufour, M., Dyet, K., Wilson, M., Campbell, D., Bandaranayake, D., & Hope, V. (2013). An outbreak of *Salmonella typhimurium* phage type 42 associated with the consumption of raw flour. Foodborne Pathogens and Disease, 10(2), 159–164. https://doi.org/10.1089/fpd.2012.1282

Midilli, A., Kucuk, H., & Yapar, Z. (2002). A new model for single-layer drying. *Drying Technology*, *20*(7), 1503–1513. https://doi.org/10.1081/DRT-120005864

Mohapatra, D., & Rao, P. S. (2005). A thin layer drying model of parboiled wheat. *Journal of Food Engineering*, *66*(4), 513–518. https://doi.org/10.1016/j.jfoodeng.2004.04.023

Moreira, R., Chenlo, F., Torres, M. D., & Prieto, D. M. (2010). Water adsorption and desorption isotherms of chestnut and wheat flours. *Industrial Crops and Products*, *32*(3), 252–257. https://doi.org/10.1016/j.indcrop.2010.04.021

Müller, L. L., Hjertqvist, M., Payne, L., Pettersson, H., Olsson, A., Forshell, L. P., & Andersson, Y. (2007). Cluster of *Salmonella* Enteritidis in Sweden 2005-2006 suspected source: almonds. *Eurosurveillance*, *12*(3–6), 153–155.

Myoda, S. P., Gilbreth, S., Akins-lewenthal, D., & Davidson, S. K. (2019). Occurrence and Levels of *Salmonella* , Enterohemorrhagic *Escherichia coli* , and *Listeria* in Raw Wheat, *82*(6), 1022–1027.

Nelson, K. L., Boehm, A. B., Davies-Colley, R. J., Dodd, M. C., Kohn, T., Linden, K. G., Liu, Y., Maraccini, P. A., McNeill, K., Mitch, W. A., Nguyen, T. H., Parker, K. M., Rodriguez, R. A., Sassoubre, L. M., Silverman, A. I., Wigginton, K. R., & Zepp, R. G. (2018). Sunlight-mediated inactivation of health-relevant microorganisms in water: a review of mechanisms and modeling approaches. Environmental Science: Processes and Impacts, 20(8), 1089–1122. https://doi.org/10.1039/c8em00047f

Nicorescu, I., Nguyen, B., Moreau-ferret, M., Agoulon, A., Chevalier, S., & Orange, N. (2013).

Pulsed light inactivation of Bacillus subtilis vegetative cells in suspensions and spices. *Food Control*, *31*(1), 151–157. https://doi.org/10.1016/j.foodcont.2012.09.047

Nindo, C. I., Kudo, Y., & Bekki, E. (1995). Test model for studying sun drying of rough rice using far-infrared radiation. *Drying Technology*, *13*(1–2), 225–238. https://doi.org/10.1080/07373939508916951

Page, G. E. (1949). Factors Influencing the Maximum Rates of Air Drying Shelled Corn in Thin Layers. MSc Thesis, Purdue University, West Lafayette, IN.

Peleg, M. (2006). Isothermal microbial heat inactivation. Advanced quantitative microbiology for foods and biosystems: Models for predicting growth and inactivation (1st ed., pp. 1-48). Boca Raton, FL: CRC Press.

Pimputkar, S., Speck, J. S., Denbaars, S. P., & Nakamura, S. (2009). Prospects for LED lighting, *3*(April), 2–4.

Podolak, R., Enache, E., Stone, W., Black, D. G., & Elliott, P. H. (2010). Sources and Risk Factors for Contamination, Survival, Persistence, and Heat Resistance of *Salmonella* in Low-Moisture Foods. *Journal of Food Protection*, *73*(10), 1919–1936. https://doi.org/10.4315/0362-028X-73.10.1919

Prasad, A., Gänzle, M., & Roopesh, M. S. (2019). Inactivation of Escherichia Coli and *Salmonella* using 365 and 395 nm high intensity pulsed light emitting diodes. Foods, 8(12), 679. https://doi.org/10.3390/foods8120679

Quintero-Ramos, A., Churey, J. J., Hartman, P., Barnard, J., & Worobo, R. W. (2004). Modeling of *Escherichia coli* Inactivation by UV Irradiation at Different pH Values in Apple Cider. *Journal of Food Protection*, *67*(6), 1153–1156. https://doi.org/10.4315/0362-028x-67.6.1153

Rabha, D. K., Muthukumar, P., & Somayaji, C. (2017). Experimental investigation of thin layer drying kinetics of ghost chilli pepper (Capsicum Chinense Jacq.) dried in a forced convection solar tunnel dryer. *Renewable Energy*, *105*, 583–589. https://doi.org/10.1016/j.renene.2016.12.091

Rachon, G., Peñaloza, W., & Gibbs, P. A. (2016). Inactivation of *Salmonella*, *Listeria monocytogenes* and *Enterococcus faecium* NRRL B-2354 in a selection of low moisture foods. *International Journal of Food Microbiology*, *231*, 16–25. https://doi.org/10.1016/j.ijfoodmicro.2016.04.022

Raghavan, G. S. V., Rennie, T. J., Sunjka, P. S., Orsat, V., Phaphuangwittayakul, W., & Terdtoon, P. (2005). Overview of new techniques for drying biological materials with emphasis on energy aspects. *Brazilian Journal of Chemical Engineering*, *22*(2), 195–201. https://doi.org/10.1590/S0104-66322005000200005

Ravanat, J. L., Douki, T., & Cadet, J. (2001). Direct and indirect effects of UV radiation on DNA and its components. *Journal of Photochemistry and Photobiology B: Biology*, *63*(1–3), 88–102. https://doi.org/10.1016/S1011-1344(01)00206-8

Rayman, M. K., D'aoust, J.-Y., Aris, B., Maishment, C., & Wasik, R. (1979). Survival of Microorganisms in Stored Pasta. *Journal of Food Protection*, *42*(4), 330–334.

https://doi.org/10.4315/0362-028x-42.4.330

Reyes, A., Alvarez, P. I., & Marquardt, F. H. (2002). Drying of carrots in a fluidized bed. I. Effects of drying conditions and prasa. Drying Technology, 20(7), 1463–1483. https://doi.org/10.1081/DRT-120005862

Roohinejad, S., Koubaa, M., Sant'Ana, A. S., & Greiner, R. (2018). Mechanisms of microbial inactivation by emerging technologies. In *Innovative technologies for food preservation: Inactivation of spoilage and pathogenic microorganisms* (pp. 111–132). https://doi.org/10.1016/B978-0-12-811031-7.00004-2

Rose, D. J., Bianchini, A., Martinez, B., & Flores, R. A. (2012). Methods for reducing microbial contamination of wheat flour and effects on functionality. *Cereal Foods World*, *57*(3), 104–109. https://doi.org/10.1094/CFW-57-3-0104

Rowan, N. J., MacGregor, S. J., Anderson, J. G., Fouracre, R. A., McIlvaney, L., & Farish, O. (1999). Pulsed-light inactivation of food-related microorganisms. *Applied and Environmental Microbiology*, *65*(3), 1312–1315.

Sánchez-Maldonado, A. F., Lee, A., & Farber, J. M. (2018). Methods for the Control of Foodborne Pathogens in Low-Moisture Foods. *Annual Review of Food Science and Technology*, *9*(1), 177–208. https://doi.org/10.1146/annurev-food-030117-012304

Santillana Farakos, S. M., Frank, J. F., & Schaffner, D. W. (2013). Modeling the influence of temperature, water activity and water mobility on the persistence of *Salmonella* in low-moisture foods. *International Journal of Food Microbiology*, *166*(2), 280–293. https://doi.org/10.1016/j.ijfoodmicro.2013.07.007

Schmidt, S. J., & Fontana, A. J. (2008). Appendix E: Water Activity Values of Select Food Ingredients and Products. *Water Activity in Foods*, *2*, 407–420. https://doi.org/10.1002/9780470376454.app5

Schubert, E. F. (2006). LED basics: Optical properties. In *Light-Emitting Diodes* (pp. 86–100). Cambridge: Cambridge University Press. https://doi.org/10.1017/CBO9780511790546.006

Sinha, R. P., & Häder, D.-P. (2002). UV-induced DNA damage and repair: a review. *Photochemical & Photobiological Sciences*, *1*(4), 225–236. https://doi.org/10.1039/b201230h

Smith, D. F., Hildebrandt, I. M., Casulli, K., Dolan, K. D., & Marks, B. P. (2016). Modeling the Effect of Temperature and Water Activity on the Thermal Resistance of *Salmonella* Enteritidis PT 30 in Wheat Flour. *Journal of Food Protection*, *79*(12), 2058–2065. https://doi.org/10.4315/0362-028x.jfp-16-155

Song, K., Taghipour, F., & Mohseni, M. (2018). Microorganisms inactivation by continuous and pulsed irradiation of ultraviolet light-emitting diodes (UV-LEDs). *Chemical Engineering Journal*, *343*(February), 362–370. https://doi.org/10.1016/j.cej.2018.03.020

Soukos, N. S., Som, S., Abernethy, A. D., Ruggiero, K., Dunham, J., Lee, C., Doukas, A. G., &

Goodson, J. M. (2005). Phototargeting oral black-pigmented bacteria. *Antimicrobial Agents and Chemotherapy*, *49*(4), 1391–1396. https://doi.org/10.1128/AAC.49.4.1391-1396.2005

Sullivan, J. C., Budge, S. M., & St-Onge, M. (2011). Modeling the primary oxidation in commercial fish oil preparations. *Lipids*, *46*(1), 87–93. https://doi.org/10.1007/s11745-010-3500-6

Surjadinata, B. B., Jacobo-Velázquez, D. A., & Cisneros-Zevallos, L. (2017). UVA, UVB and UVC light enhances the biosynthesis of phenolic antioxidants in fresh-cut carrot through a synergistic effect with wounding. *Molecules*, *22*(4), 1–13. https://doi.org/10.3390/molecules22040668

Syamaladevi, R. M., Lu, X., Sablani, S. S., Insan, S. K., Adhikari, A., Killinger, K., Rasco, B.,Dhingra, A., Bandyopadhyay, A., & Annapure, U. (2013). Inactivation of Escherichia coli Population on Fruit Surfaces Using Ultraviolet-C Light: Influence of Fruit Surface Characteristics. Food and Bioprocess Technology, 6(11), 2959–2973. https://doi.org/10.1007/s11947-012-0989-0

Syamaladevi, R. M., Lupien, S. L., Bhunia, K., Sablani, S. S., Dugan, F., Rasco, B., Killinger, K., Dhingra, A., & Ross, C. (2014). UV-C light inactivation kinetics of Penicillium expansum on pear surfaces: Influence on physicochemical and sensory quality during storage. *Postharvest Biology and Technology*, *87*, 27–32. https://doi.org/10.1016/j.postharvbio.2013.08.005

Syamaladevi, R. M., Sablani, S. S., Tang, J., Powers, J., & Swanson, B. G. (2010). Water sorption and glass transition temperatures in red raspberry (Rubus idaeus). *Thermochimica Acta*, *503–504*(1), 90–96. https://doi.org/10.1016/j.tca.2010.03.013

Syamaladevi, R. M., Tadapaneni, R. K., Xu, J., Villa-Rojas, R., Tang, J., Carter, B., Sablani, S., & Marks, B. (2016a). Water activity change at elevated temperatures and thermal resistance of *Salmonella* in all purpose wheat flour and peanut butter. *Food Research International*, *81*, 163–170. https://doi.org/10.1016/j.foodres.2016.01.008

Syamaladevi, R. M., Tang, J., Villa-Rojas, R., Sablani, S., Carter, B., & Campbell, G. (2016b). Influence of Water Activity on Thermal Resistance of Microorganisms in Low-Moisture Foods: A Review. *Comprehensive Reviews in Food Science and Food Safety*, *15*(2), 353–370. https://doi.org/10.1111/1541-4337.12190

Syamaladevi, R. M., Tang, J., & Zhong, Q. P. (2016c). Water Diffusion from a Bacterial Cell in Low-Moisture Foods. *Journal of Food Science*, *81*(9), R2129–R2134. https://doi.org/10.1111/1750-3841.1341

Troller, J.A. & Christian, J.H.B. 1978. “Water Activity and Foods.” Academic Press, Inc., New York. San Francisco and London.

Van Boekel, M. A. J. S. (2002). On the use of the Weibull model to describe thermal inactivation of microbial vegetative cells. International Journal of Food Microbiology, 74(1–2), 139–159. https://doi.org/10.1016/S0168-1605(01)00742-5

Villa-Rojas, R., Tang, J., Wang, S., Gao, M., Kang, D. H., Mah, J. H., Gray, P., Sosa-Morales, M., & ElenaPez-Malo, A. L. (2013). Thermal inactivation of *Salmonella enteritidis* PT 30

in almond kernels as influenced by water activity. Journal of Food Protection, 76(1), 26–32. https://doi.org/10.4315/0362-028X.JFP-11-509

Wang, J. (2002). A single-layer model for far-infrared radiation dying of onion slices. Drying Technology, 20(10), 1941–1953. https://doi.org/10.1081/DRT-120015577

Wang, Y., Wang, Y., Wang, Y., Murray, C. K., Hamblin, M. R., Hooper, D. C., & Dai, T. (2017). Antimicrobial blue light inactivation of pathogenic microbes: State of the art. *Drug Resistance Updates*, *33–35*(April), 1–22. https://doi.org/10.1016/j.drup.2017.10.002

Wankhade, P. K., Sapkal, R. S., & Sapkal, V. S. (2012). Drying characteristics of okra slices using different drying methods by comparative evaluation. *Lecture Notes in Engineering and Computer Science*, *2*, 757–760.

Watt Global Media. (2018). Update : Global pet food sales , production grew in 2017. *Pet Food Industry*, *March*, 2018–2019. https://doi.org/00316245

Werber, D., Dreesman, J., Feil, F., van Treeck, U., Fell, G., Ethelberg, S., Hauri, A. M.,Roggentin, P., Prager, R., Fisher, I. S.T., Behnke, S. C., Bartelt, E., Weise, E., Ellis, A.,Siitonen, A., Andersson, Y., Tschäpe, H., Kramer, M. H., & Ammon, A. (2005). International outbreak of *Salmonella* Oranienburg due to German chocolate. *BMC Infectious Diseases*, 5, 1–10. https://doi.org/10.1186/1471-2334-5-7

WHO (2019). https://www.who.int/news-room/fact-sheets/detail/food-safety accessed on 11-11-2019

Wilson, H. L., Kennedy, K. J., & Moffatt, C. R. M. (2018). Epidemiology of non-typhoid *Salmonella* infection in the Australian Capital Territory over a 10-year period. *Internal Medicine Journal*, *48*(3), 316–323. https://doi.org/10.1111/imj.13625

Winkler, H., Vinh, Q. T., Khanh, T. Q., Benker, A., Bois, C., Petry, R., & Zych, A. (2014). LED Components - Principles of Radiation Generation and Packaging. In *LED Lighting: Technology and Perception* (pp. 49–132). https://doi.org/10.1002/9783527670147.ch3

Witkin, E. M. (1976). Ultraviolet mutagenesis and inducible DNA repair in Eschericia Coli. *Bacteriological Reviews*, *40*(4), 869–907. https://doi.org/10.1007/BF00329935

Woodward, D. L., Khakhria, R., & Johnson, W. M. (1997). Human salmonellosis associated with exotic pets. *Journal of Clinical Microbiology*, *35*(11), 2786–2790.

Xie, L., Zheng, Z. A., Mujumdar, A. S., Fang, X. M., Wang, J., Zhang, Q., Ma, Q., Xiao, H. W., Liu, Y. H., & Gao, Z. J. (2018). Pulsed vacuum drying (PVD) of wolfberry: Drying kinetics and quality attributes. Drying Technology, 36(12), 1501–1514. https://doi.org/10.1080/07373937.2017.1414055

Yadav, B., Claudia, A., Govindan, B. N., Tsui, Y. Y., Mcmullen, L. M., & Roopesh, M. S. (2019). Cold plasma treatment of ready-to-eat ham : Influence of process conditions and storage on inactivation of Listeria innocua. *Food Research International*, *123*(December 2018), 276–285. https://doi.org/10.1016/j.foodres.2019.04.065

Zhao, J., Krishna, V., Hua, B., Moudgil, B., & Koopman, B. (2009). Effect of UVA irradiance on photocatalytic and UVA inactivation of Bacillus cereus spores. *Journal of*

Photochemistry and Photobiology B: Biology, *94*(2), 96–100. https://doi.org/10.1016/j.jphotobiol.2008.10.006

Zou, X.-Y., Lin, Y.-L., Xu, B., Cao, T.-C., Tang, Y.-L., Pan, Y., Gao, Z.-C., & Gao, N.-Y. (2019). Enhanced inactivation of E. coli by pulsed UV-LED irradiation during water disinfection. *Science of The Total Environment*, *650*(Pt 1), 210–215. https://doi.org/10.1016/j.scitotenv.2018.08.367