

The Pennsylvania State University  
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Department of Agricultural and Biological Engineering

**DECONTAMINATION OF BERRIES WITH OZONE AND PULSED UV-LIGHT**

A Thesis in  
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by

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## ABSTRACT

The consumption of fresh fruits and vegetables has increased by over the last twenty years. With an increase in consumption the number of foodborne illnesses associated with fruits and vegetables has also increased. Produce is now the second leading cause of foodborne illness in the United States. Small fruits, such as blueberries, raspberries, and strawberries are particularly vulnerable to contamination and currently undergo no processing prior to sale on the fresh market.

Ozone and pulsed UV-light are emerging technologies which have received increased attention. Both technologies have been approved for the treatment of raw commodities by the U.S. FDA and both have been shown to be effective biocides. This research investigated the use of gaseous ozone, aqueous ozone, and pulsed UV-light for the purpose of decontaminating *Escherichia coli* O157:H7 and *Salmonella* spp. on the surfaces of blueberries, raspberries, and strawberries.

Blueberries, strawberries, and raspberries were artificially contaminated with five strains of *Escherichia coli* O157:H7 and *Salmonella* spp. Fruits were treated with four ozone treatments; i) continuous ozone flow (5% wt/wt) for 2, 4, 8, 16, 32, and 64 min, ii) pressurized ozone (83 kPa) for 2, 4, 8, 16, 32, and 64 min, iii) continuous ozone (64 min) followed by pressurized ozone (64 min), and iv) vacuum followed by 64-min pressurized ozone. Combined continuous and pressurized treatment yielded high  $\log_{10}$  reductions of 3.6 and 3.8 CFU/g of

*Salmonella* and *E. coli* O157:H7, respectively, for raspberries, whereas 2.6 and 2.9 CFU/g of *Salmonella* and *E. coli* O157:H7, respectively, for strawberries. For blueberries, the highest  $\log_{10}$  reductions resulted after treatment with continuous ozone for *E. coli* O157:H7 and was 2.2 CFU/g and for *Salmonella* the highest reductions resulted after the 64-min pressurized treatment and were 3.0  $\log_{10}$  CFU/g.

The efficacy of ozone as a water additive for washing blueberries, raspberries, and strawberries was investigated. Pathogen inoculated fruit were treated with aqueous ozone at 20°C for 2 to 64 min at ozone concentrations of 1.7 to 8.9 mg/L, at 4°C for 64 min at a concentration of 21 mg/L, and with water as a control. Blueberries were treated with ozone at two different temperature had maximum  $\log_{10}$  reductions of 5.2 CFU/g of *E. coli* O157:H7 and 6.2 CFU/g of *Salmonella* at 4°C. Ozone was applied on raspberries at two different temperatures with a maximum  $\log_{10}$  reduction of 5.6 CFU/g of *E. coli* O157:H7 at 4°C and 4.5 CFU/g of *Salmonella* at 4°C. Washing with water (sparging with air as control) resulted in reductions of only 1  $\log_{10}$  CFU/g.

Pulsed UV-light was applied to blueberries, strawberries, and raspberries at varying UV doses and times. On raspberries, maximum reductions of *E. coli* O157:H7 and *Salmonella* were 3.9 and 3.4  $\log_{10}$  CFU/g at 72 and 59.2 J/cm<sup>2</sup>, respectively. On the surfaces of strawberries maximum reductions were 2.1 and 2.8  $\log_{10}$  CFU/g at 25.7 and 34.2 J/cm<sup>2</sup>, respectively. Maximum reductions of 4.3 and 2.9  $\log_{10}$  CFU/g were achieved on blueberries after a UV dose of 22.6 J/cm<sup>2</sup>

for *E. coli* O157:H7 and *Salmonella*, respectively. There was no observable damage to the fruits at these UV doses.

The inactivation data from the studies conducted on blueberries, raspberries, and strawberries inoculated with *Escherichia coli* O157:H7 and *Salmonella* after treatment with gaseous ozone, aqueous ozone, and pulsed UV-light were used to construct models to estimate the inactivation. Two models were constructed, a log-linear (based on first-order kinetics) and a Weibull model. Initial analysis indicating that the survival curves were non-linear, typically exhibiting a tailing effect, and that the log-linear model failed to accurately estimate the reductions. The Weibull model more accurately estimated the reductions and the concavity of the inactivation curve. Validation of the Weibull model produced correlation coefficient of 0.83 to 0.99 and slope of 0.76 to 1.26. The results indicated that first-order kinetics are not suitable for the estimation of microbial inactivation on berries treated with ozone or pulsed UV-light, but that the Weibull model can be successfully used to estimate the reductions of *E. coli* O157:H7 and *Salmonella* on blueberries, raspberries, and strawberries treated with ozone or pulsed UV-light.

The ability of pulsed UV-light to effectively inactivation microorganism in clear liquids has been well documented; however, the effect of opaque food materials on the penetration of pulsed UV-light has not been adequately studied. Inactivation data and energy penetration obtained from the treatment of agar and whey protein gels after treatment with pulsed UV-light was used to construct

several models to estimate the amount of energy penetrating the sample at a given depth and the inactivation of *E. coli* K12. The inactivation curves obtained indicated that the relationship between UV dose and inactivation was non-linear and the Weibull model was used to estimate these inactivations. The model further incorporated a modified exponential model to characterize the decay of UV energy through either agar or whey protein isolate. It was determined that energy measurements were not a good basis for the estimation of microbial inactivation and that each depth had to be treated as a unique scenario due to filtration of wavelengths by the material. The results indicated that pulsed UV-light can penetrate materials up to 10 mm in the depth, and that the Weibull model can be successfully used to model the inactivation of *E. coli* K12.

Gaseous ozone, aqueous ozone, and pulsed UV-light were all found to have a potential use for the decontamination of pathogenic microorganisms on the surfaces of blueberries, raspberries, and strawberries. Further studies need to be conducted on the sensory effects of these treatments as well as the effect on the shelf-lives of these fruits. Studies to characterize the surface of small fruits and how it impacts microbial inactivation are also needed.

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## Chapter 1

### Introduction

The consumption of fresh fruits and vegetables in the United States has been on the rise since the early 1980s. Between 1982 and 1997, the consumption of fresh fruit rose by 21% from 38.7 kg to 46.7 kg per person per year (FDA, 2001). As consumption has risen, so has the incidence of associated foodborne illnesses, which costs the U.S. over \$6.9 billion each year, of this \$3 billion results from *Salmonella* infections (ERS, 2004) and \$405 million from *E. coli* O157 infections (Frenzen et al., 2005). Fresh produce is currently the second most common vehicle for foodborne illness; from 1990 to 2003, there were 428 outbreaks composed of 23,857 cases of produce associated illness (Center for Science in the Public Interest, 2004).

Small fruits, such as blueberries, red raspberries, and strawberries are important and valuable agricultural commodities, and are worth over \$1 billion each year. Small fruits destined for the fresh market are not treated or processed prior to sale, due to negative quality affects and a decrease in shelf-life. Contamination of small fruits is possible at any point during production. There are many potential sources of contamination: soil, irrigation water, equipment, human harvesters, and food handlers (Yu et al., 2001). There have been several notable outbreaks of foodborne illness associated with berries; an outbreak of Hepatitis A was associated with the consumption of strawberries

(CDC, 1997a) and an outbreak of cyclosporiasis was associated with the consumption of raspberries originating from Guatemala (CDC, 1997b). In spite of the outbreaks traced back to berries, washing in water alone is not a viable solution for the prevention of foodborne illnesses. Therefore, new technologies need to be investigated in order to prevent the spread of foodborne illness resulting from the consumption of small fruits.

The most common decontamination methods make use of sanitizers such as chlorine and quaternary ammonium compounds. However, research has shown that these compounds and others to be ineffective at inactivating pathogens on the surfaces of small fruits (Yu et al., 2001). Hence, there is the need for novel processes that are capable of effectively inactivating pathogens while ensuring that the quality of the fruit remains unharmed. This study enlists the use of ozone and pulsed ultraviolet (UV) light as technologies that can potentially be used to decontaminate small fruits.

Ozone has been used as an antimicrobial agent since the late nineteenth century for the purification of drinking water (Applied Ozone Systems, 2004). Ozone has the ability to oxidize organic molecules and decays into harmless oxygen after a relatively short amount of time. In 2001, ozone was approved for the treatment of raw commodities (Federal Register, 2001). Ozone can be applied in either its gaseous form or used as an additive to water. The use of both gaseous ozone and aqueous ozone to extend the shelf-life of several fruits and vegetables has been demonstrated without having any adverse effects on

food quality (Nortan et al., 1965; Bazarova, 1992; Barth et al., 1995; Kim et al., 1999a; Kim et al., 1999b; Sharma et al., 2000a; Sharma et al., 2002b).

Disinfection with pulsed UV-light involves the use of light from the ultraviolet region of the electromagnetic spectrum, which is between wavelengths of 100 and 400 nm. UV-light inactivates a microorganism by damaging the DNA via the formation of thymine dimers, which prevents transcription thus inhibiting the replication of the cell, which leads to death (Miller et al., 1999). The use of pulsed UV-light is preferred to continuous UV-light, because it can provide a greater amount of instantaneous energy which can result in a shorter application time. Pulsed UV-light has been shown to successfully inactivate food pathogens on the surfaces of some foods (Hillegas and Demirci, 2003; Jun, 2003; Sharma and Demirci, 2003b).

Therefore, the goal of this research was to determine the efficacy of ozone and pulsed UV-light for the purpose of inactivating *Escherichia coli* O157:H7 and *Salmonella enterica* on the surfaces of blueberries, raspberries and strawberries.

## Chapter 2

### Literature Review

#### 2.1 Food Safety and Foodborne Illnesses

Each year there are an estimated 76 million foodborne illnesses which result in 5,200 deaths and 325,000 hospitalizations in the United States (CDC, 2003). These foodborne illnesses cost the U.S. economy billions of dollars in lost revenue and medical expenses. Five foodborne pathogens, *Campylobacter*, *Salmonella*, *Escherichia coli* O157, *E. coli* non-O157, and *Listeria monocytogenes* cost the U.S. \$6.9 billion in 2000, with *Salmonella* alone costing \$2.3 billion (ERS, 2004).

As food production becomes more centralized and detection methods more specific, new vehicles and pathogens of foodborne illness are emerging. Over the last 20 years 14 new pathogens have emerged in the U.S., and have been reported as having food as the predominate method of transmission (Table 2.1). Many of these new foodborne pathogens have very similar characteristics such as an animal reservoir which shows no sign of disease, the ability to spread rapidly and resistance to antimicrobials due to modern agricultural practices (Tauxe, 1997). Also, many of these new pathogens can result in deadly or chronic sequelae. *E. coli* O157:H7 is often associated with hemolytic uremic syndrome, *L. monocytogenes* can result in miscarriages and

meningitis, *Salmonella* can result in reactive arthritis, and *Campylobacter* can cause Guillain-Barrè syndrome, which is actually the most common cause of flaccid paralysis in the U.S. (Altekruse et al., 1997).

Table 2.1: Emerging foodborne pathogens in the United States in the last 20 years. (Tauxe, 1997)

<i>Campylobacter jejuni</i>
<i>Campylobacter fetus</i> ssp. <i>fetus</i>
<i>Cryptosporidium parvum</i>
<i>Cyclospora cayetanensis</i>
<i>Escherichia coli</i> 0157:H7
<i>Listeria monocytogenes</i>
Norwalk-like viruses
<i>Nitzschia pungens</i>
<i>Salmonella</i> Enteritidis
<i>Salmonella</i> Typhimurium DT 104
<i>Vibrio cholerae</i> 01
<i>Vibrio vulnificus</i>
<i>Vibrio parahaemolyticus</i>
<i>Yersinia enterocolitica</i>

In addition to emerging foodborne pathogens, the vehicles from which many contract these diseases are also new. Historically, foodborne illnesses were associated with meat and animal products such as unpasteurized milk, now more outbreaks are being associated with eggs and fresh produce. For example, the inside of an egg was once considered to be sterile and safe to consume, it is now known that infected hens are capable of laying eggs containing *Salmonella* (Tauxe, 1997). The emergence of new foodborne pathogens and transmission vehicles has been traced to changes in food production and human behavior.

The World Health Organization (2002) has identified the following as changes contributing to the emergence of foodborne pathogens:

- Globalization of the food supply
- Changes in food processing
- Introduction of pathogens into a new geographic area
- Travel and exposure to foreign foodborne hazards
- Genetic changes in microorganisms
- Changes in human population and lifestyle

The response of most food safety agencies to these emerging foodborne pathogens has focused on prevention at all steps of production. In 1998, the U.S. government instituted a national food safety program intent on ensuring the safety of the nations food supply from farm to table. As a part of this initiative, numerous actions to improve food safety were identified (FDA, 1997):

- Enhancing surveillance
- Improving responses to foodborne outbreaks
- Improving methods of risk assessment

One critical outcome of this report was to expand the implementation of the HACCP program (Hazard Analysis and Critical Control Points), which focuses on identifying possible points of contamination and preventing food contamination as opposed to responding to a contaminated product.



### 2.1.1 Fresh Produce

There has been a drastic increase in the consumption of fresh fruits and vegetables. Between 1982 and 1997 there was a 21% increase in fruit consumption and a 40% increase in the consumption of vegetables (FDA, 2001). As the consumption of produce has increased so has the number of associated foodborne illnesses, fresh produce is now the second leading vehicle for foodborne illnesses in the U.S., with an estimated 554 outbreaks from 1990 to 2003 (CSPI, 2004). The majority of these outbreaks are tied to fresh salads, which consist of numerous ingredients (Figure 2.1).

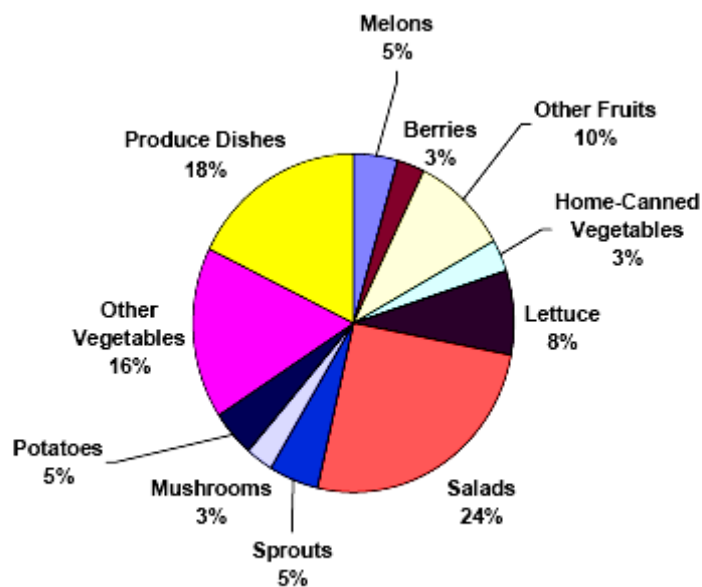


Figure 2.1. Vehicles of produce-related outbreaks from 1990 to 2003 (CSPI, 2004).

Most recently, an outbreak of *E. coli* O157:H7 was tied to contaminated washed and bagged spinach (FDA, 2006a). This outbreak sickened 199 people and resulted in 31 cases of hemolytic uremic syndrome, 102 hospitalizations, 3 deaths and affected 26 states. Also, a recent outbreak of *Salmonella* Typhimurium has been linked to the consumption of tomatoes in restaurants which sickened 183 people across 21 states (FDA, 2006b). The link between fresh produce consumption and foodborne illness is not recent. Illnesses tied to the consumption of fresh produce have been reported as far back as 1899 when an outbreak of typhoid fever was linked to the consumption of celery (Beuchat, 1998)

The contamination of fruits and vegetables has been associated with the use of wastewater and improperly composted manure as fertilizer as well as changes in packaging methods like modified and controlled atmospheres (Beuchat, 1998). The mechanisms by which produce can become contaminated are complex, with sources of contamination emanating from the farm to the consumer (Figure 2.2). Different pathogens have different natural reservoirs and can contaminate a product at different stages of production. Pathogens like *Salmonella*, *Campylobacter*, and *E. coli* typically reside in the intestinal tracts of animals and naturally contaminate a product like chicken or beef or contaminate fresh produce through cross contamination or the use of animal feces as fertilizer.

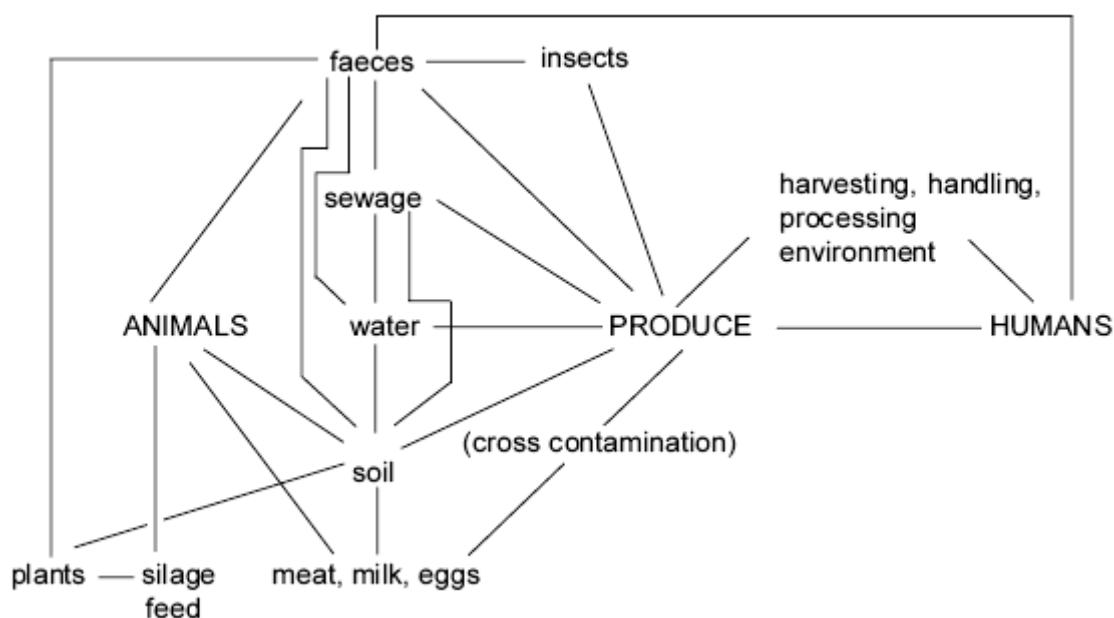


Figure 2.2. Mechanisms of contamination affecting fresh fruits and vegetables (Beuchat, 1996).

### 2.1.2 Food safety issues associated with berries

One particularly vulnerable category of fresh produce to contamination is berries. Typically, berries intended for the fresh market are harvested and packed in the field by hand (FDA, 2001). The berries are not washed or treated prior to market and can become contaminated from a variety of sources: improper sanitation, infected pickers, contaminated irrigation water, and manure fertilized fields are all potential culprits for contamination (Han et al., 2004)

In the U.S. there have been nine outbreaks of foodborne illness associated with the consumption of berries between 1973 and 1997; which

consisted of 1,815 illnesses and 24 hospitalizations (Table 2.2). Of the seven confirmed cases three were tied to strawberries contaminated with *Staphylococcus aureus* and hepatitis A, and six were associated with raspberries contaminated with *Cyclospora* (Sivapalasingam et al., 2004). There has been one reported outbreak of *L. monocytogenes* tied the consumption of blueberries and strawberries, however the source of this outbreak was never confirmed (Ryser and Marth, 1990).

An EPA Scientific Advisory Panel identified certain microorganisms as posing public health concerns due to contaminated produce; *Escherichia coli* O157:H7 and *Salmonella* were two of these organisms (EPA, 1997). While neither has been implicated in a berry associated outbreak they still pose a great risk. The U.S. Food and Drug Administration surveyed imported produce for the presence of pathogenic microorganisms and found that 1 out of 143 imported strawberry samples were positive for *Salmonella* (FDA, 1999). Furthermore, research has shown that pathogens like *E. coli* O157:H7, *Listeria monocytogenes*, and *Salmonella* are capable of surviving on the surfaces of strawberries for over 7 days (Flessa et al., 2005; Knusden et al., 2001).

Table 2.2. Berry-associated outbreaks. \*

Year	Pathogen	Number of cases	Berry type
1984	<i>Listeria monocytogenes</i> **	N/A	Blueberry and Strawberry
1985	<i>Staphylococcus aureus</i>	14	Strawberry
1990	Hepatitis A	51	Frozen strawberry
1995	<i>Cyclospora</i>	38	Raspberry
1995	<i>Cyclospora</i>	32	Raspberry
1996	<i>Cyclospora</i>	631	Raspberry
1997	<i>Cyclospora</i>	755	Raspberry
1997	Hepatitis A	258	Frozen strawberry

\*Sivapalasingam et al., 2004; Ryser and Marth, 1990

\*\*Unconfirmed

### 2.1.3 Pathogenic foodborne microorganisms

The latest Centers for Disease Control foodborne disease surveillance report (Lynch et al., 2006) indicated that the most notable outbreaks between 1998 and 2002 were caused by *E. coli* O157:H7, *Salmonella*, *Cyclospora cayetanensis*, and hepatitis A. Furthermore, *Listeria monocytogenes* caused more deaths (43%) and had a higher fatality rate than any other pathogen.

Bacterial pathogens cause 32% of all foodborne illness cases and resulted in 84% of all foodborne illness associated deaths. *Salmonella* accounted for 13% of all foodborne illnesses and 44% of bacterial illnesses (Figure 2.3). Furthermore, *Salmonella* resulted in 22% of all foodborne illness associated deaths.

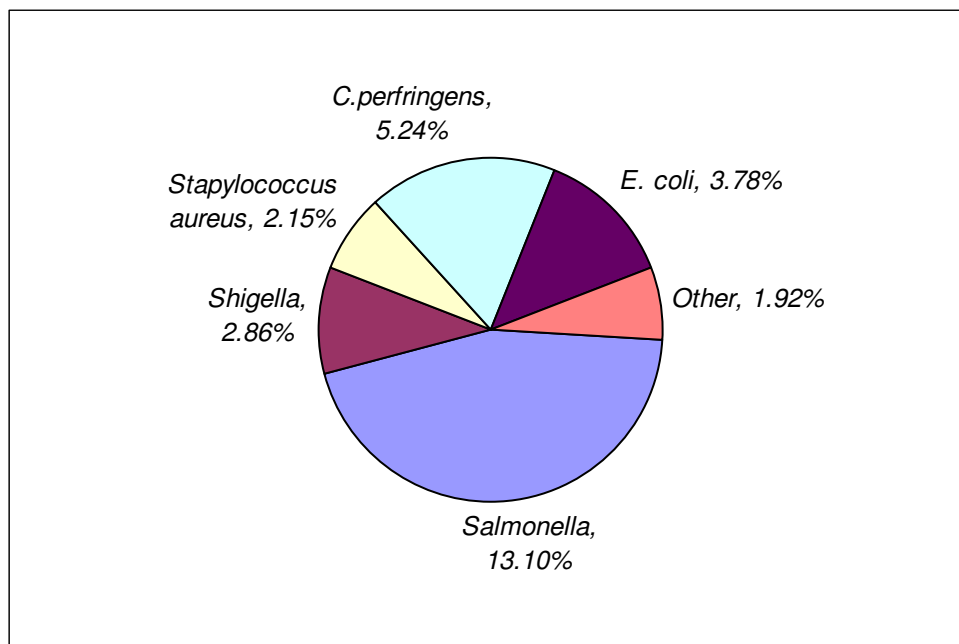


Figure 2.3. Causes of bacterial foodborne illnesses from 1998 to 2002 (Lynch et al., 2006).

#### 2.1.4 *Escherichia coli* O157:H7

*E. coli* O157:H7 was first identified as a pathogen in 1982 after an outbreak in Oregon causing 25 people to become ill and 16 requiring hospitalization linked to the consumption of hamburger from a fast food restaurant. Typing of the bacteria did not match any previously known type of etiological agent and a new serotype of *E. coli* was identified (Neill, 1989). The emergence of *E. coli* O157:H7 is thought to be due to an interaction of a pathogenic *E. coli* with *Shigella* due to its production of a particularly virulent toxin often referred to as verotoxin or shiga-like toxin. This toxin is closely related, both functionally and antigenically to the toxin produced by *Shigella*

*dysenteriae* (Todor, 2006). This toxin is closely related, both functionally and antigenically to the toxin produced by *Shigella dysenteriae*.

Each year *E. coli* O157:H7 causes 73,000 cases resulting in 61 deaths per year (CDC, 2006) and costs the U.S economy approximately \$405 million in lost productivity and medical expenses (Frenzen et al., 2005). *E. coli* O157:H7 is a Gram-negative, facultative anaerobic, rod shaped bacterium (Figure 2.4) and the only strain in the virotype enterohemorrhagic *E. coli* (EHEC). Unlike other foodborne pathogens, *E. coli* O157:H7 can survive in relatively low pH environments (< 4.4) and illness can occur with fewer than 100 cells (Bell and Kyriakides, 1998).

*E. coli* O157:H7 has a typical incubation period of 2 to 8 days and causes diarrhea and abdominal cramps which typically last 5 to 10 days. An *E. coli* O157:H7 infection is not typically fatal or life-threatening, but in a small segment of the population it can lead to the development of potentially fatal sequelae. Persons with compromised immune systems, children, and the elderly are more susceptible to the sequela known as hemolytic uremic syndrome which follows 2%-7% of the infections (CDC, 2006).

Between 1982 and 2002, *E. coli* O157:H7 has caused 350 outbreaks, with 183 associated with food. The most commonly associated vehicle for foodborne *E. coli* O157:H7 infection has been ground beef, accounting for 41% of the outbreaks (Figure 2.5). The outbreak which garnered the public's attention and brought forth the link between *E. coli* O157:H7 and ground beef was in 1993 when 230 people were sickened after consuming undercooked hamburger from

Jack in the Box (CDC, 1993). Dry-cured salami was implicated in an outbreak in 1994 which sickened 20 people in Seattle, Washington (CDC, 1995). Numerous outbreaks have been prevented due to recalls such as that in 1997 of frozen ground beef. Approximately 1.2 million pounds of frozen ground beef was recalled by Hudson foods due to suspected *E. coli* O157:H7 contamination (USDA, 1997).



Figure 2.4. *Escherichia coli* O157:H7 (Kunkel, 2006).



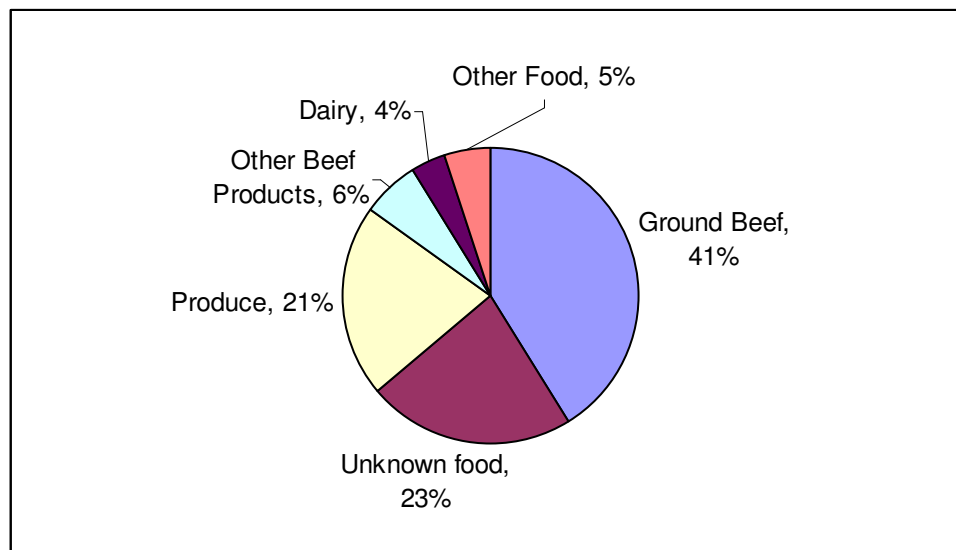


Figure 2.5. Outbreaks of foodborne *E. coli* O157:H7 by transmission vehicle from 1982 to 2002 (Rangel et al., 2005).

Produce was the second most implicated known vehicle of foodborne illness accounting for 21% of the outbreaks. In 1997, the consumption of alfalfa sprouts was linked to *E. coli* O157:H7 illnesses in Michigan and Virginia, which sickened 60 people (CDC, 1997c). The consumption of apple cider and juice has also been the cause of numerous outbreaks such as the 1996 outbreak, which caused 66 people to become ill (CDC, 1997d). Most recently, an outbreak of *E. coli* O157:H7 was linked to prewashed bagged spinach, which sickened 199 people and resulted in 31 cases of hemolytic uremic syndrome, 102 hospitalizations, 3 deaths and affected 26 states (FDA, 2006a).

### 2.1.5 *Salmonella*

*Salmonella* is a Gram-negative, facultative anaerobic, rod-shaped bacterium (Figure 2.6). It is typically found in the intestinal tracts of mammals and birds. There are only two species of *Salmonella*, which are known as *enterica* and *bongori*, this is due to the fact that 95-99% of the genetic material of *Salmonella* is identical. *Salmonella enterica* is further broken down in six subspecies: *enterica*, *salamae*, *arizonae*, *diarizonae*, *houtenae*, and *indica*. Furthermore, there are 2,541 serotypes of *Salmonella* and 60% of these serotypes are members of the *enterica* subspecies (CDC, 2005). The most commonly encountered serotypes of *Salmonella* are Enteritidis, Typhimurium, and Typhi.

Salmonellosis results from the ingestion of viable cells, typically  $10^8$  cells are needed to cause gastroenteritis, however it has been reported that as few as 100 cells can cause gastroenteritis as well (Lindquist, 2003). Salmonellosis is usually accompanied by the following symptoms: diarrhea, fever, abdominal cramps, and nausea which results from the production of enterotoxin in the intestines. Each year there is an estimated 40,000 cases of salmonellosis, which cause approximately 600 deaths (CDC, 2005). The most common single source of salmonellosis is eggs, followed by poultry and vegetables (Figure 2.7).

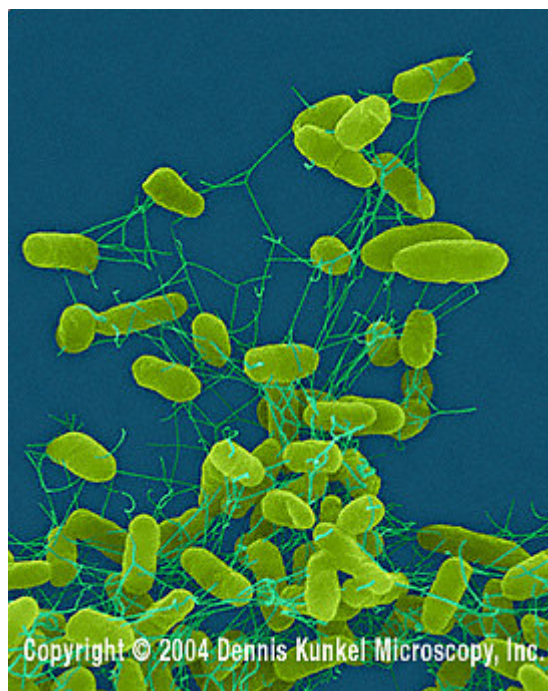


Figure 2.6. *Salmonella enterica* (Kunkel, 2006).

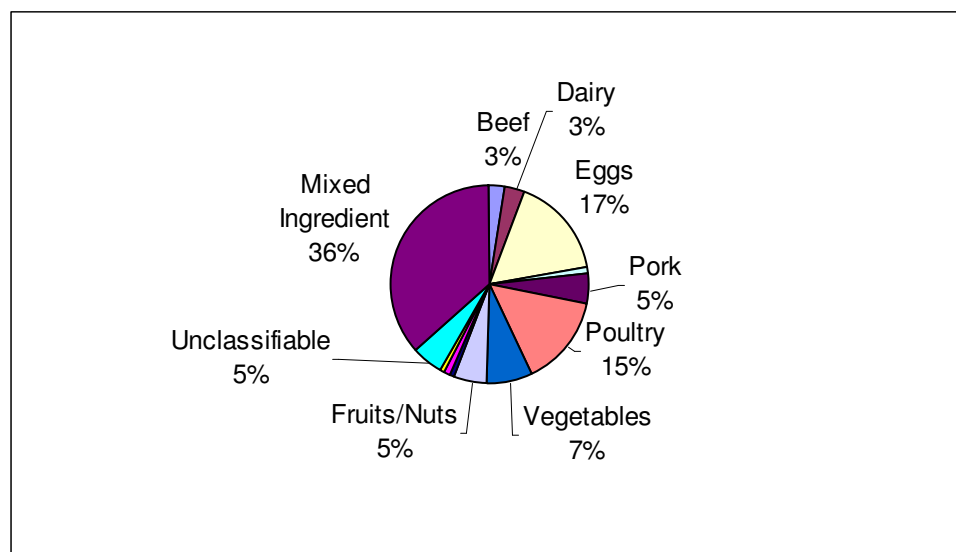


Figure 2.7. Known vehicles of salmonellosis, 1998-2002 (CDC, 2006).

An outbreak of salmonellosis associated with the consumption of raw eggs was reported in 1995 in Washington D.C., New York, and Indiana. A total of 205 people became ill and the serotype *S. Enteritidis* was implicated (CDC, 1996). Outbreaks of salmonellosis have also been reported due to the consumption of ice cream prepared using raw eggs. Two outbreaks were reported in 1994, one associated with commercially prepared ice cream and the other with homemade ice cream. The outbreak associated with commercially prepared ice cream resulted in 142 cases of illness tied to *S. Enteritidis* (CDC, 1994). Most recently, an outbreak of *S. Typhimurium* was reported and linked to the consumption of tomatoes in restaurants which sickened 183 people across 21 states (FDA, 2006b). To date there have been no outbreaks of salmonellosis linked to the consumption of small fruits, but a 1999 FDA survey found that samples of imported strawberries contained *Salmonella* in one out of 143 samples (FDA, 1999).

## **2.2 Production of fresh produce**

The production and value of fresh produce has increased drastically since the late 1980's. In 1987 the value of fresh produce was \$34.6 billion and in 1997 it was worth \$70.8 billion (FDA, 2001). The amount of fresh produce being imported into the United States has also increased within the same time period. In 1987 \$2.0 billion of produce was imported and \$4.1 billion was imported in 1997 (FDA, 2001). The production and sale of fruits has also increased. In

1999, 29.2 million tons of fruit was produced and non-citrus fruits had a value of 5.6 billion dollars. In 2003, 813 thousand tons of cultivated blueberries, raspberries, and fresh strawberries were produced which were worth approximately \$1.6 billion (NASS, 2003).

### **2.2.1 Berry production**

In the United States, strawberries are the eighth most produced commercial fruit with 711 thousand tons of fresh product produced each year with a worth of approximately \$1.4 billion (NASS, 2003). There are many different species of strawberries that are commercially grown which belong to the genus *Fragaria*. The harvest is done entirely by hand. The berries are picked, sorted, and packaged by hand without any washing or treatment (California Strawberry Commission, 2004).

Blueberries are one of the few fruits indigenous to North America, which produces approximately 90% of the world's blueberries totaling approximately 87 thousand tons with a worth of over \$221 million (NASS, 2003). The cultivated blueberry belongs to the species *Vaccinium corymbosum* or *V. ashei* and is more commonly known as the northern highbush blueberry. Approximately 50% of the blueberries produced are committed to the fresh market. The fruit is hand picked and immediately shipped to packing houses and then to stores. There is no treatment or washing of the berry prior to shipping, due to the belief that

washing will propagate mold growth and shorten the shelf life of the product (U.S. Highbush Blueberry Council, 2002).

Approximately, 14 thousand tons of raspberries are produced each year in the United States with a value of \$65 million. Almost 90% of the raspberries produced are destined for the fresh market (NASS, 2003). The most commonly grown variety is *Rubus idaeus*, which is the red variety. Raspberries are a very fragile fruit and are picked only by hand and those fruits going to the fresh market are not processed or treated (Oregon Raspberry and Blackberry Council, 2004).

### **2.3 Decontamination of fresh produce**

There are a wide variety of chemical and non-chemical methods available for decontaminating produce. Many have a long history of use within the industry and some are considered novel approaches. The efficacy of such treatments greatly depends on the type of produce being treated as well as the microorganisms of concern. Some of the most commonly used chemical methods of decontamination include: chlorine, chlorine dioxide, bromine, trisodium phosphate, quaternary ammonium compounds, organic acids, and hydrogen peroxide. Lately attention has been drawn to novel technologies, many non-thermal, for the purpose of decontaminating fresh produce, which include irradiation, ozone, electrolyzed oxidizing water, and ultra-violet light.

### 2.3.1 Chlorine

Chlorine based compounds have long been used as methods of decontaminating water and processing equipment; and much research has been conducted on the efficacy of chlorine based compounds for the decontamination of fresh fruits and vegetables. The amount of free chlorine usually applied is between 50 and 200 ppm for times of 1 to 2 minutes (Beuchat, 1998) and can be applied either as elemental chlorine or hypochlorites. Research has found that the efficacy of chlorine for the purpose of decontaminating fresh produce is mixed and depends greatly on product type as well as processing conditions such as pH and temperature. Zhang and Farber (1996) observed that reductions of *L. monocytogenes* on cabbage and lettuce washed with 200 ppm chlorine were slightly higher at 22°C versus 4°C, with reductions of 1.2 versus 0.9 log<sub>10</sub> CFU/g on cabbage and 1.7 versus 1.3 log<sub>10</sub> CFU/g on lettuce.

The type of produce also greatly effects the efficacy of chlorine. Tomatoes inocuated with *Salmonella* were treated with 200 ppm sodium hypochlorite at 35°C and found that after 120 s a 4 log<sub>10</sub> reduction was observed on the smooth surface of the fruit. However, on the stem scar and puncture wounds reductions were only 2.6 and 1.5 log<sub>10</sub>, respectively, after 120 s (Yuk et al., 2005). *Salmonella* inoculated cut canteloupe cubes treated with 2,000 ppm of chlorine only resulted in a 1 log<sub>10</sub> reduction, which was attributed to high levels of organic matter (Beuchat, 1997).

Other inadequacies in the use of chlorine as a disinfectant specifically for fruits and vegetables have been identified. The composition of the tissue of fruits and vegetables has been reported to neutralize chlorine and the cracks and crevices normally associated with fruit and vegetable products are believed to interfere with the treatment (Beuchat, 1998). Also, a linkage between chlorinated water and cancer was detected in 1970's which was tied to the presence of trihalomethanes (Simpson et al., 2006).

### **2.3.2 Chlorine dioxide**

Chlorine dioxide is another method of chlorine application, and its activity is reported to be less affected by pH and the presence of organic matter, and is reported to possess 2.5 times the oxidizing power of chlorine. Also, it is reported to produce less hazardous byproducts than chlorine. It has been found to be very effective when used to sanitize surfaces and water, with reductions of 5 log<sub>10</sub> on surfaces within one minute (Simpson et al., 2006).

Its effectiveness for the purpose of decontaminating produce inoculated with pathogens has had mixed results. Zhang and Farber (1996) evaluated chlorine dioxide for the purpose of decontaminating lettuce and cabbage inoculated with *L. monocytogenes*. After treatment for 10 min with 5 ppm chlorine dioxide, maximum reductions of 1.1 log<sub>10</sub> CFU/g were observed. Han et al. (2001) found that when green peppers were treated with 3 mg/L of chlorine dioxide a reduction of 0.74 log<sub>10</sub> CFU/g of *L. monocytogenes* was achieved.



Chlorine dioxide can also be applied in its gaseous form which has been more effective than when used as an aqueous solution. When used on green peppers at a concentration of 3 mg/L a reduction of 1.2 log<sub>10</sub> CFU/g of *L. monocytogenes* was observed (Han et al., 2001). Gaseous chlorine dioxide was also investigated for the inactivation of *Salmonella* on strawberries. Gas at a total of 100 mg resulted in a reduction of 4.76 log<sub>10</sub> CFU/strawberry (Yuk et al., 2006a). Sy et al. (2005) also investigated the use of gaseous chlorine dioxide for the inactivation of *Salmonella*, yeast, and mold on the surfaces of blueberries, raspberries, and strawberries. On blueberries, reductions as high as 3.7 log<sub>10</sub> CFU/g of *Salmonella*, 2.5 log<sub>10</sub> CFU/g of mold and 1.4 log<sub>10</sub> CFU/g of yeast were observed. A log<sub>10</sub> reduction of 4.4 CFU/g *Salmonella* was observed on strawberries after chlorine dioxide treatment and reductions of yeast and mold were 1.4 and 4.2 log<sub>10</sub> CFU/g, respectively. Reductions on raspberries were the lowest with a reduction of 1.5 log<sub>10</sub> CFU/g *Salmonella* and reductions of 2.6 and 3.0 log<sub>10</sub> CFU/g of yeast and mold, respectively.

In addition to mixed efficacy, chlorine dioxide is an unstable gas and currently is not permitted for use on cut produce or products without intact membranes and products must be followed with a potable water rinse or further processing (FDA, 2001).

### 2.3.3 Bromine

Bromine has been identified as a potential sanitizer for produce; however, little research has been conducted pertaining to its efficacy. Several studies have noted that there may be a possible synergy between bromine and chlorine (Beuchat, 1998). It has been found to be effective against *E. coli*, *Salmonella*, and *Staphylococcus aureus* when used at 200 ppm, but not effective against *Pseudomonas aeruginosa* and *Bacillus cereus* spores (FDA, 2001). Also, there is very little information regarding the formation of potentially hazardous by-products.

### 2.3.4 Trisodium phosphate

Trisodium phosphate (TSP) has been shown to be effective at decontaminating non-food surfaces, with reductions as high as 6 log<sub>10</sub> after 30 s at room temperature at a concentration of 1% (FDA, 2001). It has also been evaluated for the decontamination of selected produce items. Zhuang and Beuchat (1996) found that when tomatoes were treated with 15% TSP levels of *Salmonella* Montevideo were reduced to undetectable levels after 15 s of contact. Similar results were noted on alfalfa seeds and after treatment with 4% TSP for 30 s *E. coli* O157:H7 was undetectable (Taormina and Beuchat, 1999).

However, TSP has been found to be less effective on cut-produce surfaces as well as against *L. monocytogenes*. Populations of *Salmonella* Chester were reduced by 1 log<sub>10</sub> on apple slices treated with 2% TSP for 5 min

(Liao and Sapers, 2000). Shredded lettuce inoculated with *L. monocytogenes* treated with 2% TSP exhibited no decrease in levels of the bacteria (Zhang and Farber, 1996).

### **2.3.5 Quaternary ammonium compounds**

Quaternary ammonium compounds (quats) have been successfully used to sanitize food processing equipment as well as walls and floors of processing plants (Beuchat, 1998). Quats are colorless, odorless, stable at high temperatures, non-corrosive, and stable in the presence of organic material (FDA, 2001). Quats have been identified as being possible compounds for the decontamination of uncut produce where the skin, rind, or peel will be removed (Beuchat, 1998). However, little research has been conducted on the ability of quats to decontaminate pathogenic bacteria on fresh produce.

### **2.3.6 Organic acids**

Organic acids are naturally occurring in fruit and can be applied to the surfaces of fruits and vegetables to decontaminate them. Such acids include acetic, citric, succinic, malic, tartaric, lactic, benzoic, and sorbic acids. The application of lemon juice (citric acid) was found to reduce populations of *Salmonella* Typhi on papaya and jicama temporarily, but bacterial growth resumed within several hours (Escartin et al., 1989). Acetic acid has been used

to inactivate *Yersinia enterocolitica* on fresh parsley. Leaves were soaked in 2% acetic acid for 15 min, which resulted in a 7 log<sub>10</sub> reduction (Karapinar and Gonul, 1992). The antimicrobial efficacy of organic acids varies greatly depending upon the type of acid used. It has been reported that citric acid was much less effective at preventing the growth of pathogenic microorganisms on mixed salad vegetables than tartaric acid (Shapiro and Holder, 1960).

The combination of organic acids and chlorine has also been shown to be effective. Zhang and Farber (1996) combined lactic acid with 100 ppm chlorine and used it to treat shredded lettuce inoculated with *L. monocytogenes*; they found this combination to be much more effective than lactic acid or chlorine alone. Reductions were 1.1 log<sub>10</sub> CFU/g greater than the control water treatment after 10 min of treatment. Organic acids have great potential for home use or the production of acidified foods, but as surface sanitizers they show little long term efficacy.

### **2.3.7 Hydrogen peroxide**

The use of hydrogen peroxide for the purpose of decontamination has been documented and can be used as either a vapor or an aqueous solution. Hydrogen peroxide vapor was found to greatly decrease microbial populations on cantaloupe, grapes, prunes, raisins, and walnuts (Sapers and Simmons, 1998). The use of 2% hydrogen peroxide for the decontamination of *Salmonella* on alfalfa spouts was concluded to result in reductions comparable to 200 ppm

chlorine, with reductions of 2 log<sub>10</sub> CFU/g after 2 min of treatment (Beuchat, 1997). As with many chemical sanitizers the efficacy depends greatly on the type of product being treated. Cantaloupe cubes treated with hydrogen peroxide only exhibited a reduction of 1 log<sub>10</sub> CFU/g and appeared bleached after the treatment (Beuchat, 1998).

### **2.3.8 Irradiation**

The use of gamma rays from radioactive isotopes has been a means of extending the shelf-life of foods but has not been thoroughly evaluated for the purpose of inactivating pathogenic microorganisms (Beuchat, 1998). Bari et al. (2005) evaluated the efficacy of ionizing radiation for the purpose of decontaminating fresh produce inoculated with *L. monocytogenes*. A dose of 1 kGy resulted in reductions of 4.8 and 4.6 log<sub>10</sub> CFU/g on broccoli and mung bean sprouts and reductions of 5.3 and 4.1 log<sub>10</sub> CFU/g on cabbage and tomatoes. The authors did not detect any noticeable changes in the color, texture, taste, or overall acceptability of produce after several days of storage. It has been reported that many fresh produce items do not respond well to the irradiation doses needed to extend shelf-life. However, it was noted that the strawberry was the only domestic fruit, which had the potential to respond positively to irradiation as a means of extending shelf-life (Maxie et al., 1971). Despite the potential of irradiation as a method of decontamination there is still a great deal of public opposition to the treatment. A study done by the USDA-ERS found that the

likelihood of consumers to purchase irradiated food has decreased over the last several years, with 70% willing to purchase irradiated food in 1996 to 50% willing to purchase in 2000 (Frenzen et al., 2000).

### **2.3.9 Electrolyzed oxidizing water**

Electrolyzed oxidizing water (EO water) is a relatively new innovation that was developed in Japan. Research in Japan has revealed that EO water has strong bactericidal and virucidal properties. EO water has been used in various fields including medical sterilization and agriculture (Wullaert, 1997).

EO water is produced by passing an electrical current through a dilute salt solution where a permeable membrane separates the anode and the cathode. The anode attracts the chloride ions contained in the solution and produces hypochlorous acid. This acidic solution has a pH of 2.6 and 50-80 ppm of available chlorine. The cathode attracts the sodium ions and produces sodium hydroxide and hydrogen gas. This alkaline solution has a pH of 11.4 (Venkitanarayan et al., 1999a). The oxidation reduction potential (ORP) of EO water is 1150 mV for acidic water and -795 mV for alkaline EO water. The combination of high ORP, low pH and concentrations of chlorine species is believed to be the mechanism for the inactivation of microorganisms in acidic EO water (Len et al., 2000; Park et al., 2002). Len et al. (2000) found that there was a significant correlation ( $R^2=0.95$ ) between the microbial activity and concentration of HOCl in acidic EO water.

The efficacy of EO water has been shown in the treatment of bacterially contaminated foods and solutions. Acidic EO water was effective in inactivating *Salmonella* Enteritidis and other foodborne pathogens in solution (Venkitanarayan et al., 1999a). They found that a 7 log<sub>10</sub> CFU/ml reduction was achieved after 5 min. Acidic EO water was effective in washing poultry, and a 1.06 log<sub>10</sub> CFU/ml reduction was achieved after 7 days of storage (Fabrizio et al., 2002). EO water has also been shown to reduce microorganisms on fresh fruits and vegetables. Koseki et al. (2001) used alkaline and acidic EO water to decontaminate lettuce and found a 2 log<sub>10</sub> CFU/g reduction of aerobic bacteria. A 52.6% reduction of fruit rot by *Botryosphaeria berengeriana* on the surfaces of pears was found using acidic EO water (Al-Haq et al., 2002). The reduction of pathogens on the surfaces of tomatoes was also evaluated using acidic EO water. A 7.85 log<sub>10</sub> CFU/tomato reduction of *E. coli* O157:H7 and a 7.46 log<sub>10</sub> CFU/tomato reduction of *S. Enteritidis* was found, as well as a 7.54 log<sub>10</sub> CFU/tomato reduction of *L. monocytogenes* (Bari et al., 2003). Sharma and Demirci (2003a) found a 1.05–2.72 log<sub>10</sub> CFU/g reduction of *E. coli* O157:H7 when washing alfalfa sprouts with acidic EO water. EO water has been used in the cleaning and sanitation of solid surfaces. Acidic EO water was used to disinfect plastic cutting boards. A 5 log<sub>10</sub> CFU/100 cm<sup>2</sup> reduction was achieved for both *E. coli* O157:H7 and *L. monocytogenes* (Venkitanarayan et al., 1999b). Both acidic and alkaline EO water were used to clean and sanitize pipeline milking systems, consisting of rubber, PVC, stainless steel, and polysulfone

(Walker et al., 2003). Depending on the material, the  $\log_{10}$  reductions ranged between 6.4 and 7.3 CFU.

### **2.3.10 Ozone**

Ozone is a bluish gas which is soluble in water, and has been used to purify drinking water since the late nineteenth century (Graham, 1997). Ozone is naturally found in the earth's atmosphere as a result of either lightning or high-energy ultraviolet light which ruptures the oxygen molecules, creating singlet oxygen molecules which then bond with diatomic oxygen molecules to form triatomic oxygen ( $O_3$ ) or ozone (Figure 2.8).

Ozone has the ability to oxidize organic materials which makes it a suitable agent for disinfection. Ozone impacts a microorganism by oxidizing the organic molecules contained in its cell membrane, which causes the membrane to weaken and eventually rupture (Figure 2.9). Unlike other commonly used disinfectants, such as chlorine or organic acids, ozone directly lyses the cell; there is no need for the biocide to be transported across the membrane and into the cell (Pryor and Rice, 1999).

More specifically, it is believed that ozone oxidizes the unsaturated lipids contained in the cellular envelope. Additionally, for Gram-negative microorganisms, both the lipoprotein and the lipopolysaccharide layers are destroyed which results in increased cellular permeability (Das et al., 2006).



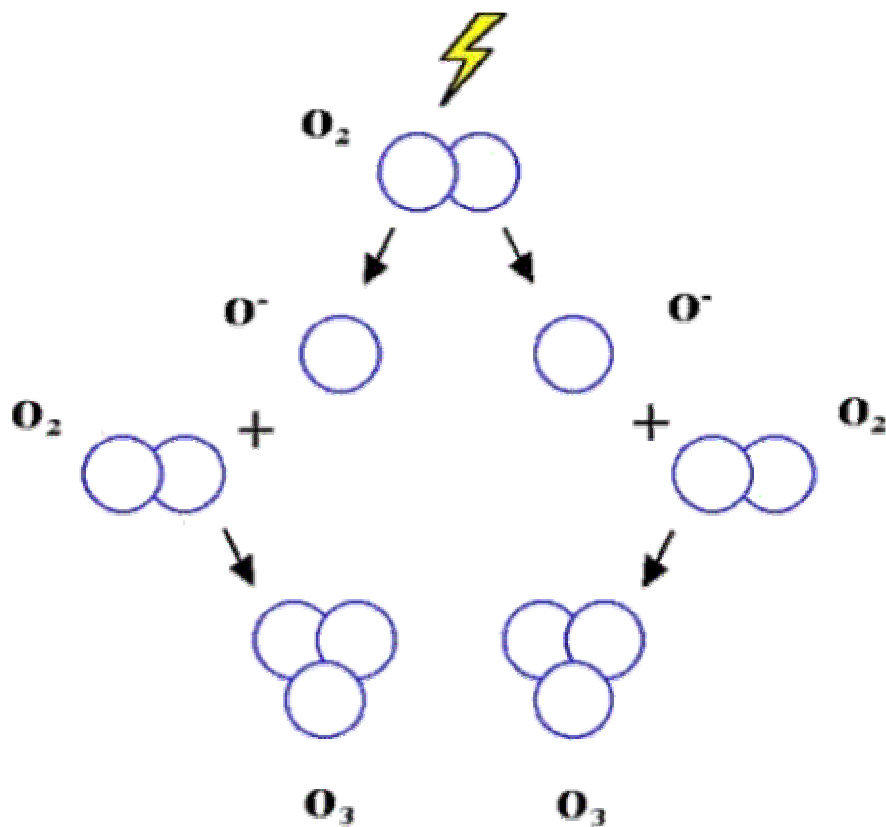


Figure 2.8. Ozone Formation (Ozone Solutions, 2004).

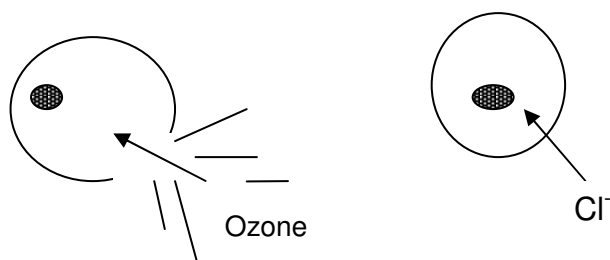


Figure 2.9 Comparison of mode of disinfection (Pryor and Rice, 1999).

Ozone is produced artificially by one of two methods; corona discharge or UV-light. Corona discharge mimics ozone's generation in the earth's lower atmosphere. For this process a corona is formed by applying a direct electric current (Figure 2.10) across two metallic electrodes which are separated by an air gap and a dielectric insulator. Oxygen rich dry air is then passed through this gap where the molecules dissociate and then recombine to form ozone. UV-light can also be used to generate ozone which mimics the compound's formation in the earth's upper atmosphere. This process utilizes an electromagnetic wavelength of 185 nm. Corona discharge (CD) is the preferred method of ozone generation because of a variety of factors. CD produces more ozone per hour and produces ozone at a higher concentration. CD produces approximately 1.9 lbs per hour at a concentration of up to 10% whereas UV-light produces only 2 g per hour at a maximum concentration of 0.2%. Also, CD is more cost effective than UV-light, the generator has a much greater life and requires less energy than UV-light (Ozone Solutions, 2004).

The use of ozone as a disinfectant for food products was approved in 2001 for the treatment of raw commodities (Federal Register, 2001). The effects of ozone on different food substances have been evaluated and can play a vital role in the success of ozone to inactivate microorganisms in or on the food. In recent years, ozone's effects have been investigated for use on meats, poultry, fruits, and vegetables. The effectiveness of ozone has been documented when used in its gaseous form or aqueous form.

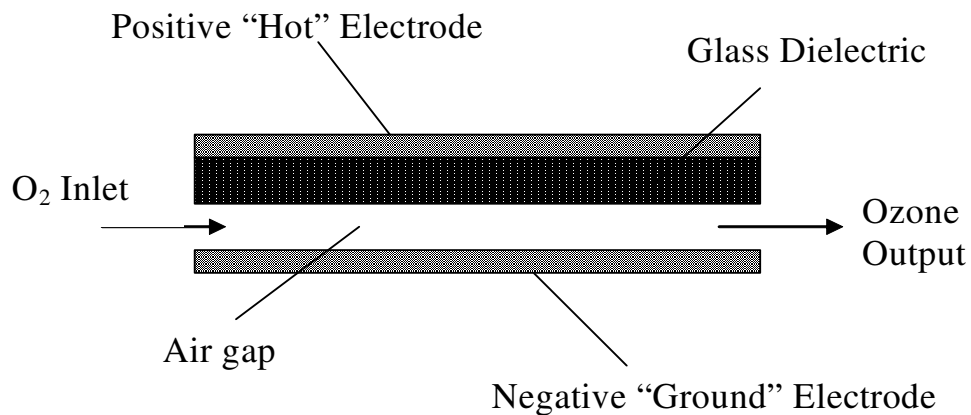


Figure 2.10. Corona Discharge (Pryor and Rice, 1999).

### 2.3.10.1 Gaseous ozone

The use of ozone in its natural state has been shown to be an effective bactericide against many foodborne pathogens. Kowalski et al. (1998) showed that a 4 log<sub>10</sub> reduction of *E. coli* and *S. aureus* could be achieved when Petri plates were exposed to gaseous ozone at a concentration of 1500 ppm for 480 s. Whistler and Sheldon (1989a) investigated the effect of ozone gas on *E. coli* and *S. Typhimurium* on Petri dishes. Dishes were exposed to ozone concentrations between 1.5 and 1.65% wt/wt and observed reductions between 4 and 7 log<sub>10</sub> CFU/ml, respectively. Gaseous ozone has also been found to effectively inactivate spores although greater times or concentrations must be utilized. *Bacillus subtilis* spores exposed to 3 ppm of ozone were reduced by 6 log<sub>10</sub>

after 1 hour of treatment (Ishizaki et al., 1986). Ishizaki also noted that the relative humidity in the treatment chamber increased the activity of ozone, with higher relative humidity's yielding higher activity. Gaseous ozone was also found to decrease the growth of *Botrytis cinerea* during storage. Cultures on agar were exposed to 1.5 ppm of ozone at 2°C and a significant decrease in the growth rate was observed (Nadas et al., 2003).

The use of gaseous ozone has been shown to be effective when applied to animal products. Whistler and Sheldon (1989b) investigated the use of ozone in conjunction with misted water to treat hatching eggs. They found that after 2 h of treatment there was a 2.5 log<sub>10</sub> CFU/g reduction in natural microflora, but that hatchability was reduced. Bailey et al. (1996) also used gaseous ozone to treat hatching chicken eggs. They observed a 75% to 99% reduction in *Salmonella*. Ozone's effectiveness for the decontamination on shell eggs was documented by Rodriguez-Romo and Yousef (2005). They found that when gaseous ozone was used at either 4°C or 8°C in combination with a pressure of 15 psi resulted in a 5.9 log<sub>10</sub> CFU/g reduction of *S. Enteritidis*. The moisture of the air was found to affect the bacteriocidal activity of ozone. Ozone in conjunction with distilled water, sprayed into the treatment chamber, significantly increased the efficacy of ozone for the purpose of decontaminating *S. Enteritidis* on shell eggs (Davies and Breslin, 2003)

When ozone was used to decontaminate chicken breasts inoculated with *S. Infantis* and *P. aeruginosa*. A 97% reduction was observed for *Salmonella* and a 95% reduction of *Pseudomonas* was observed after treatment with 2000

ppm ozone gas, although there was no reduction in the natural spoilage microflora (Al-Haddad et al., 2005). Kaess and Weidemann (1968) used ozone to treat beef carcasses and found that *Pseudomonas* spp. was significantly decreased on the carcasses treated with 0.02 ppm ozone. However, Fournard and Lauret (1972) found that there was little reduction in *Microbacterium thermosphactum*, *Lactobacillus* spp., *P. fluorescens*, and *Leuconostoc* when carcasses were treated with 100 ppm ozone gas for 30 min. They also noticed adverse quality effects after ozone treatment.

The efficacy of gaseous ozone to increase the shelf life of fruits and vegetables has also been investigated. Apples exposed to 0.06 ppm of ozone in a humidified chamber for 4 h daily exhibited a significant reduction in spoilage and weight loss (Bazarova, 1992). Blackberries exposed to 0.1 and 0.3 ppm of ozone for 12 days at 2°C showed an 80% reduction in *Botrytis cinerea* spoilage without any adverse effects on fruit quality (Barth et al., 1995). However, when cranberries were stored in ozonated conditions for 5 weeks a significant increase in rot and overall quality loss were observed (Nortan et al., 1965). Strawberries stored in an atmosphere containing 1.5 ppm ozone exhibited reduced decay, weight loss, and fruit softening; although, a loss in fruit aroma was noted (Nadas et al., 2003). Carrots stored in an atmosphere containing between 7.5 and 60 ppm ozone exhibited a decrease in fungal surface growth at 60 ppm, but as with many fruits and vegetables a loss of quality was observed. Ozone treated carrots weighed less and displayed a less intense color (Liew and Prange, 1994). Singh et al. (2002) also evaluated the effects of gaseous ozone for

decontaminating baby carrots and shredded lettuce inoculated with *E. coli* O157:H7. They found reductions of 1.79 and 2.64 log<sub>10</sub> CFU/g on lettuce and carrots, respectively, after treatment with 7.6 ppm ozone for 15 min. Tomatoes contaminated with *S. Enteritidis* were treated with ozone at concentrations between 5 and 20 ppm. Complete inactivation was observed after 15 min when a concentration of 20 ppm was utilized; however, there was a loss in fruit color (Das et al., 2006)

Gaseous ozone has also been used to decontaminate pistachios inoculated with *E. coli* and *B. cereus* (Akbas and Ozdemir, 2006). They observed 3.0 and 2.0 log<sub>10</sub> CFU/g reductions of *E. coli* and *B. cereus*, respectively after treatment with 1.0 ppm ozone for 360 min. Furthermore, they observed no physico-chemical changes in the nuts.

#### **2.3.10.2 Aqueous ozone**

The use of ozone as an additive to water commonly referred to as ozonated water or aqueous ozone has been used as an effective bactericidal agent. Ozone has been used treat drinking water since the late 19<sup>th</sup> century and is still used today to treat drinking water. Its ability to inactivate microorganisms in solution has been well documented. Kim and Yousef (2000) exposed both pathogenic and spoilage food-associated microorganisms to ozone in a batch-reactor system. They observed a 3.8 log<sub>10</sub> CFU/ml reduction of *E. coli* O157:H7 after exposure to 1.0 mg/L of ozone for 30 s. Reductions of 5 log<sub>10</sub> CFU/ml of *P.*

*fluorescens* were achieved after 30 s exposure to 1.2 mg/L ozone and a 7 log<sub>10</sub> CFU/ml reduction of *Leuconosotc mesenteroids* were achieved after 30 s at a concentration of 4 mg/L. Reductions of *L. monocytogenes* were achieved at lower concentration levels; reductions of 4.6 and 5.7 CFU/ml were observed after 30 s with 0.6 and 0.8 mg/L ozone, respectively. Finch et al. (1988) demonstrated that log<sub>10</sub> reductions of 6.5 CFU/ml could be achieved for *E. coli* in phosphate buffer at an ozone concentration of 0.8 mg/L after 120 s of exposure. Aqueous ozone's effect on *Salmonella* Enteritidis in distilled water was established by Dave et al. (1998). They found that when *S. Enteritidis* was exposed to 1.5 mg/L of ozone there was a log<sub>10</sub> reduction of 6 CFU/ml. Chen et al., 1992 determined the inhibition time and optimal ozone concentration necessary to inactivate several food pathogens and spoilage microorganisms which is summarized in Table 2.3.

The use of ozone to inactivate aflatoxin producing molds in phosphate buffer has also proven effective. *Aspergillus flavus* and *A. parasiticus* exposed to 1.74 mg/L ozone, exhibited D-values of 1.54 and 1.71 min, respectively at a pH of 7.0 (Beuchat et al., 1998)

Table 2.3. Inhibition time and ozone concentration necessary for completely inactivating pathogens in ozonated saline\*.

Microorganism	Time (min)	Ozone concentration (mg/L)
<i>Vibrio cholerae</i>	17	0.95
<i>V. parahaemolyticus</i>	12	0.81
<i>Falvobacterium aquatile</i>	27	1.00
<i>Pseudomonas aeruginosa</i>	9	0.34
<i>P. putida</i>	11	0.78
<i>P. fluorescens</i>	13	1.07
<i>Escherichia coli</i>	9	0.50
<i>Salmonella Typhimurium</i>	17	0.54
<i>Staphylococcus aureus</i>	5	0.30

\*Chen et al., 1992

Ozone is a very strong oxidizer of organic material, whether it is a microorganism or a food material. The presence of certain food components decreases the efficacy of an aqueous ozone treatment. Reductions of *E. coli* in the presence of locust bean gum, caseinate, and whipping cream were significantly lower when exposed to 0.4 mg/L ozone for 2 min; reductions were less than 1 log<sub>10</sub> CFU/ml. Similar results were noted for *S. aureus* and *B. stearothermophilus* (Guzel-Seydim et al., 2004). This research concluded that the presence of starch did not decrease the efficacy of the treatment but that food components high in fat did decrease the efficacy of aqueous ozone.

The use of aqueous ozone for the processing of muscle food has been investigated by several researchers. Sheldon and Brown (1986) explored the use of ozone as an additive to poultry chiller water. They found that at concentrations between 3.0 and 4.5 mg/L the microbial loads in the water were reduced by 2 log<sub>10</sub> CFU/ml after 45 min of exposure. Fabrizio et al. (2002)



investigated the use of ozonated water to immerse poultry carcasses. Immediately after immersion there was a 0.74 log<sub>10</sub> CFU/ml reduction, and after 7 d of storage population of *S. Typhimurium* were undetectable. Beef trimmings inoculated with *E. coli* and *S. Typhimurium* were treated with aqueous ozone (Strivarius et al., 2002). After treatment with 1% ozone for 7 min no significant reduction in either *E. coli* or *S. Typhimurium* was observed. After treatment for 15 min a reduction of 0.78 log<sub>10</sub> CFU/g of *S. Typhimurium* was observed; however there was still no reduction in *E. coli*. The reduction of *Clostridium perfringens* spores via aqueous ozone was studied by Norvak and Yuan (2004) on beef surfaces. A reduction of 1 log<sub>10</sub> spores/g was achieved after 5 min of 5 mg/L ozone and when ozone was combined with a heat treatment of 60°C for 30 min a reduction of 1.49 log<sub>10</sub> spores/g was attained.

The efficacy of ozone for the treatment of seafood has also been evaluated. Shrimp were treated with 5 mg/L of ozonated saline at 5°C and reductions of 2.19, 0.19, 1.73, and 0.73 log<sub>10</sub> CFU/g were achieved for *E. coli*, *Salmonella Typhimurium*, *Staphylococcus aureus*, and *Vibrio cholerae* (Chen et al., 1992). Mussels treated with aqueous ozone at 1 mg/L, for 60 and 90 min, and vacuum packaged were evaluated in terms of the reduction of several spoilage microorganisms. A 0.7 and 0.8 log<sub>10</sub> CFU/g reduction of mesophilic bacteria and *Pseudomonas*, respectively, was achieved after a 90 min treatment. Reductions of 0.9, 1.8, 0.8, and 0.8 log<sub>10</sub> CFU/g of mesophilic bacteria, hydrogen-sulfide producing bacteria, lactic acid bacteria, and *Enterobacteriaceae* were achieved, respectively, after 12 d of storage (Manoussaris et al., 2005).

Aqueous ozone's effect on decontaminating produce has also been investigated. Sharma et al. (2002a) investigated the use of ozone under pressure for decontaminating alfalfa seeds inoculated with *E. coli* O157:H7. Reductions of 1.56 to 1.62 log<sub>10</sub> CFU/g were observed at pressures of 8 and 12 psi, respectively. The use of aqueous ozone combined with heat for the purpose of decontaminating alfalfa seeds was also investigated. A reduction of 2.21 log<sub>10</sub> CFU/g was achieved after 64 min of continuous sparging and when coupled with a heat treatment at 60°C for 3 h the population of *E. coli* O157:H7 was reduced by 4.8 log<sub>10</sub> CFU/g (Sharma et al., 2002b).

The effects of aqueous ozone, alone and combined with other compounds, on lettuce has been extensively studied. Kim et al. (1999a) evaluated the effects of ozone on the inactivation of microflora on lettuce. Shredded lettuce was exposed to ozone for 3 min, and a 1.4 to 1.8 log<sub>10</sub> CFU/g reduction of mesophilic or psychrotrophic bacteria was observed, respectively. When exposed for 5 min, the reductions were increased to 3.9 and 4.6 log<sub>10</sub> CFU/g, respectively. Koseki and Isobe (2006) evaluated aqueous ozone on fresh-cut iceberg lettuce. After 5 min of treatment with 5 mg/L ozone a 1.4 log<sub>10</sub> CFU/g reduction of aerobic bacteria was observed. Furthermore, when the ozone treatment was compared to a heated water treatment followed by ozone and a 200 mg/L sodium hypochlorite treatment, no difference in the reduction of aerobic bacteria was detected. Shredded lettuce and baby carrots were treated with aqueous ozone at concentrations between 5.2 and 16.5 mg/L (Singh et al., 2002). Reductions of *E. coli* O157:H7 of 1.51 and 1.85 log<sub>10</sub> CFU/g on shredded

lettuce and baby carrots, respectively, were achieved after treatment with 16.5 mg/L ozone for 15 min. Lettuce was also treated with a combination of ozone and chlorine and evaluated for aerobic microorganisms and sensory attributes. Reductions of 2.5 log<sub>10</sub> were obtained after treatment with 7.5 mg/L ozone and 200 mg/L chlorine. The researchers noted that an ozone/chlorine treatment extended the shelf-life of the lettuce and had a positive consumer response (Garcia et al., 2003). The ability of ozone to decontaminate lettuce inoculated with *E. coli* O157:H7 and *L. monocytogenes* was evaluated by Yuk et al. (2006b). They found that reductions of 1.09 and 0.94 log<sub>10</sub> CFU/g of *E. coli* O157:H7 and *L. monocytogenes*, respectively, were possible after treatment with 5 mg/L ozone for 5 min. When ozone was combined with organic acids reductions significantly increased. A combination of 3 mg/L ozone and 1% citric acid resulted in reductions of 2.31 and 1.84 log<sub>10</sub> CFU/g of *E. coli* O157:H7 and *L. monocytogenes*, respectively, after 1 min of treatment. However, the researchers noted that a residual antimicrobial effect was not present after 10 d of storage.

The decontamination of black pepper using aqueous ozone has also been studied (Zhao and Cranston, 1995). Black pepper treated with ozone at a concentration of 6 mg/L exhibited a reduction of 4 log<sub>10</sub> CFU/g of total aerobic bacteria. They also noted that higher moisture contents led to higher orders of microbial inactivation.

The successful history of ozone, both gaseous and aqueous, at inactivating spoilage and pathogenic microorganisms has been well documented.

Since the efficacy of ozone greatly depends on the microorganisms of interest as well as the surface being treated, much research is still needed to evaluate ozone's full potential as a sanitizer.

### **2.3.11 Ultra-violet light**

The use of ultra-violet (UV) light for the purpose of disinfection involves the use of light from the ultra-violet region of the electromagnetic spectrum (Figure 2.11). This region is between 100 and 400 nm, and can be divided into 4 regions; vacuum (100-200 nm), UV-C (200-280 nm), UV-B (280-315), and UV-A (315-400 nm). The wavelengths between 200 and 280 nm are believed to have a germicidal effect, with the optimum inactivation achieved at 254 nm (FDA, 2000). The photons produced via UV-light are high in energy, between 11975 and 299 kJ/mol, and can cause the ionization of molecules. The absorption of this energy causes molecules to enter an excited state which can induce chemical changes (Demirci and Krishnamurthy, 2006). One such chemical change is the formation of thymine (Figure 2.12), which prevents transcription and replication of the DNA thereby leading to the cell's death (Miller et al., 1999). Treatment with UV light can be performed using either a continuous light source or with a pulsed light source.

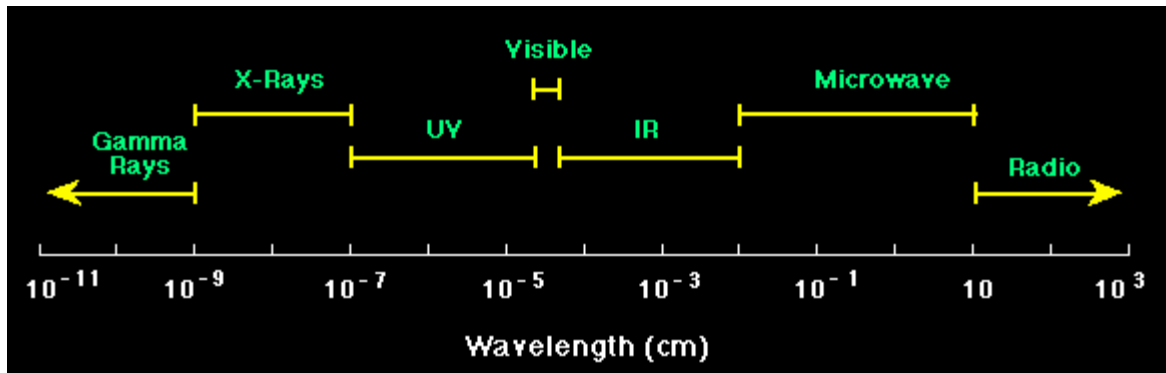


Figure 2.11. Electromagnetic Spectrum (University of Tennessee).

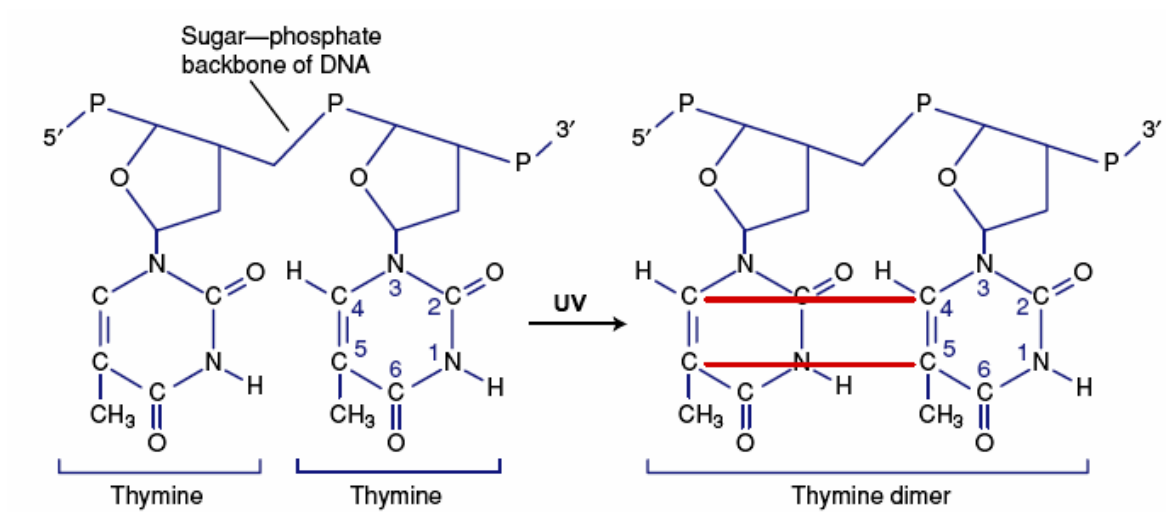


Figure 2.12. Formation of Thymine Dimers (Ananthaswamy, 1997).

### 2.3.11.1 Continuous UV-light

The effects of exposing microorganisms to a constant ultra-violet light source have been demonstrated for various species of organisms and foods. Hoyer (1998) evaluated the effect of 254 nm UV light on various microorganisms suspended in water and determined the dose required to achieve a 4 log<sub>10</sub> reduction, which is summarized in Table 2.4. This study illustrated the difference in the susceptibility of organisms to UV-light and further demonstrated the difference between strains of the same microorganism.

Table 2.4. Energy required to achieve a 4 log<sub>10</sub> reduction of various microorganisms in water.\*

Microorganism	Energy Required (J/cm <sup>2</sup> )
<i>Escherichia coli</i> ATCC 11229	0.01
<i>E. coli</i> ATCC 23958	0.005
<i>E. coli</i> NCTC 5934	0.009
<i>E. coli</i> NCIB 9481	0.01
<i>E. coli</i> wild type	0.011
<i>Enterobacter cloacae</i>	0.01
<i>Klebsiella pneumoniae</i>	0.011
<i>Citrobacter freundii</i>	0.008
<i>Yersinia enterocolitica</i>	0.01
<i>Salmonella</i> Typhi	0.014
<i>S. Typhimurium</i>	0.013
<i>Staphylococcus aureus</i> phage A994	0.038

\*Hoyer, 1998

The efficacy of UV-light for inactivating pathogens in liquid foods has been successful, depending upon the type of medium. Peptone water, apple juice, and egg white inoculated with *E. coli* O157:H7 were treated with UV-light (Ngadi et al., 2003). UV doses between 0 and 23.4 J/cm<sup>2</sup> were evaluated and reductions of 6.0, 4.2, and 4.2 log<sub>10</sub> CFU/ml were achieved in peptone water, apple juice, and egg white. The researchers noted that the pH of the medium did not influence the degree of inactivation. Mango nectar inoculated with *Saccharomyces cerevisiae* was treated with 7.5 to 45 J/cm<sup>2</sup> UV doses. Reductions between 1.2 and 2.7 log<sub>10</sub> CFU/ml were achieved, respectively (Guerrero-Beltran and Barbosa-Canovas, 2006).

The effect of continuous UV-light on the inhibition of pathogens on produce has been thoroughly investigated. The surfaces of tomatoes, green leaf lettuce, and Red Delicious apples were inoculated with cultures of *Salmonella* and *E. coli* O157:H7 (Yuan et al., 2004). Samples of fruit were exposed to doses of UV-light ranging from 5.4 to 86.4 J/cm<sup>2</sup>. A reduction of 2.19 log<sub>10</sub> CFU/g of *Salmonella* was achieved on the surfaces of tomatoes receiving 86.4 J/cm<sup>2</sup>. On the surfaces of leaf lettuce, reductions of 2.79 and 2.65 log<sub>10</sub> CFU/g of *E. coli* O157:H7 and *Salmonella*, respectively, were attained at 86.4 J/cm<sup>2</sup>. The highest reduction was observed on the surface of the Red Delicious apple, which was 3.3 log<sub>10</sub> CFU/g of *E. coli* O157:H7 after a dose of 86.4 J/cm<sup>2</sup>. Allende and Artes (2003) investigated the efficacy of UV-light on the storage life of “Lollo Rosso” lettuce. The investigators found that exposure to UV-light, at 0.407 and 0.814

J/cm<sup>2</sup>, decreased populations of psychrotrophic bacteria, coliforms, yeasts, and molds; however, treatment did increase the population of lactic acid bacteria.

The effects of UV-C light (200-280 nm) combined with heat treatment on storage rot on strawberries and sweet cherries were investigated by Marquenie et al. (2002). Fruit inoculated with *Botrytis cinerea* and *Monilinia fructigena* were exposed to temperature between 40 and 48°C and UV doses of 0.05 to 1.5 J/cm<sup>2</sup>. At doses of 0.05 J/cm<sup>2</sup> or higher a significant retardation of fungal growth was reported for both strawberries and sweet cherries. However, at doses of 1.0 and 1.5 J/cm<sup>2</sup> negative effects were seen on the calyx of the strawberry.

Boysenberries treated with 0.92 J/cm<sup>2</sup> exhibited less druplet damage and firmer texture which was equivalent to a 45°C heat treatment for 1 h. They also noted that treated berries had reduced softening during storage as well as lower respiration rates and decreased anthocyanin leakage (Vicente et al., 2004).

Baka et al. (1999) observed similar benefits of UV-C light treatment on strawberries. Strawberries treated with 0.1 J/cm<sup>2</sup> had their storage life increased by 5 days and also exhibited lower respiration rates, firmer texture, and increased anthocyanin content.

### **2.3.11.2 Pulsed UV-light**

When UV-light is applied in short pulses, as short as several nanoseconds, several times per second it is referred to as pulsed UV-light.

Typically each pulse lasts between 100 ns and 2 ms and is 20,000 times brighter



than sunlight at the earth's surface (Dunn et al., 1995). Pulsed UV-light produces greater instantaneous energy than continuous UV-light, but produces the same amount of energy overall, which can result in shortened treatment times.

Table 2.5 illustrates the major differences between pulsed UV-light and continuous UV-light. A study conducted by McDonald et al. (2000) evaluated the effectiveness of both continuous and pulsed UV-light for the purpose of decontaminating surfaces. This study showed that the amount of energy necessary to inactivate *B. subtilis* spores,  $3 \log_{10}$  CFU/ml, was much less for pulsed UV-light, which required  $0.008 \text{ J/cm}^2$ , compared to continuous UV-light which required  $0.016 \text{ J/cm}^2$ .

The efficacy of pulsed UV-light for the purpose of decontaminating surfaces and inactivating microorganisms in liquid has been well documented. Rowan et al. (1999) investigated the effects of pulsed UV-light on food-related microorganisms. Populations of *L. monocytogenes*, *E. coli*, *Salmonella* Enteritidis, *Pseudomonas aeruginosa*, *B. cereus*, and *Staphylococcus aureus* that were seeded on tryptone soya-yeast agar were exposed to pulsed UV-light emitting either high or low content UV-light. Reductions between 2 and  $6 \log_{10}$  CFU/ml were attained after 200 pulses with low UV content and high UV content, respectively. Anderson et al. (2000) reported  $8 \log_{10}$  CFU/ml reductions when populations of *B. cereus*, *E. coli*, and *Salmonella* Enteritidis were exposed to 1000 pulses of high intensity UV-light. They also reported a  $4.5 \log_{10}$  CFU/ml reduction in *Aspergillus niger* when exposed to the same light.

Table 2.5. Comparison of pulse and continuous UV-light\*.

Parameter	Pulsed UV-Light	Continuous UV-Light
Wavelength	100 to 1100 nm	254 nm
Instantaneous energy	Magnified several thousand fold	Less
Inactivation mechanism	Damage to cells by photochemical changes and by localized heating	Photochemical DNA damage by thymine dimer formation
Natural cooling of lamp	Enables lamp to cool between pulses	No
Mercury	Provides mercury free alternative	Commonly used as source
Inactivation energy	Up to 4 times increased inactivation efficiency as compared to continuous UV-light	Normal
Temperature increase during UV treatment	Significant temperature increase due to infrared	No significant temperature increase

\*Demirci and Krishnamurthy, 2006

Krishnamurthy et al. (2004a) investigated the use of pulsed UV-light to inactivate *Staphylococcus aureus* in buffer solution and on agar seeded plates. They found a 7 to 8 log<sub>10</sub> CFU/ml reduction of *S. aureus* on seeded agar plates and buffer solution at treatment times less than 5 s, without a significant increase in temperature. The inactivation of *S. aureus* in milk using pulsed UV-light has been successful (Krishnamurthy et al., 2004b). Reductions as high as 8.55 log<sub>10</sub> CFU/ml were achieved at treatment times of 180 s. The researchers observed

that the turbidity of a solution greatly impacts the ability of pulsed UV-light to penetrate a food material and inactivate microorganisms contained within it. The effects of pulsed UV-light on the inactivation of *Clostridium sporogenes* in honey was investigated by Hillegas and Demirci (2003). They found that when a 2 mm deep sample of honey was exposed to pulsed UV-light at a distance of 8 cm an 86.7% reduction was seen after 45 s.

The efficacy of pulsed UV-light to inactivate pathogens on raw salmon fillets has also been investigated (Ozer and Demirci, 2005). The distance from the UV strobe and treatment time were evaluated and a maximum reduction of 1.09 log<sub>10</sub> CFU/g of *E. coli* O157:H7 at 8 cm from the light for 60 s was observed. A maximum reduction of *L. monocytogenes* was 1.02 log<sub>10</sub> CFU/g was achieved after 60 s of treatment at 8 cm from the UV source.

Jun et al. (2003) investigated the use of pulsed UV-light to inactivate *Aspergillus niger* spores in cornmeal. At a treatment time of 100 s, a log reduction of 4.95 CFU/g was achieved as long as the distance between the sample and the strobe was 8 cm. Sharma and Demirci (2003b) exposed alfalfa seeds inoculated with *E. coli* O157:H7 to pulsed UV-light. They found that when a seed layer thickness of 1.02 mm was treated for 30 s a 4.80 log<sub>10</sub> CFU/g reduction was achieved. Celery, celeriac, green paprika, soybean spouts, radicchio, carrots, iceberg lettuce, and white cabbage have all been treated with pulsed UV-light and analyzed in terms of mesophilic bacteria reduction. Reductions of 0.90, 0.21, 0.56, 0.65, 0.79, 1.64, 1.97, 2.04, 0.84, 1.64 log<sub>10</sub> CFU/cm<sup>2</sup> were achieved of celery, celeriac, green paprika, soybean spouts,

radicchio, carrots, iceberg lettuce, and white cabbage, respectively (Gomez-Lopez et al., 2005). They also conducted a sensory analysis of processed iceberg lettuce and white cabbage using a semi-trained panel. The pulsed UV-light treated iceberg lettuce received higher scores by the panelists than the control sample. Pulsed UV-light has also been evaluated as a method of extending the shelf-lives of fresh fruits by inactivating molds on the surface. Apples, kiwi, oranges, lemons, nectarines, peaches, pears, raspberries, and table grapes were all inoculated with several types of mold and treated with pulsed UV-light at doses between 1 and 3 J/cm<sup>2</sup> (Lagunosa-Solar et al., 2006). Complete inactivation of molds was observed and thus significantly extended the shelf-lives of these fruits.

The success of pulsed UV light for the inactivation of both spoilage and pathogenic microorganisms has been well documented. The efficacy of UV-light, both pulsed and continuous, depends greatly on the surface being treated and research is still needed to evaluate the full potential of pulsed UV-light.

#### **2.4 Decontamination of berries**

Limited research has been conducted for the purpose of evaluating treatments for the reduction of illness causing microorganisms. The majority of research has concentrated on the extension of shelf life and the reduction of rot on strawberries. Wszelaki and Mitcham (2003) evaluated the use of hot water drips, biological control, and controlled atmosphere on the reduction of gray mold

on strawberries. The combined use of these treatments did not significantly decrease spoilage; and furthermore, heat treatments did damage the fruit. Couey and Follstad (1966) also investigated the use of heat to control postharvest decay on strawberries. Strawberries were exposed to moist air at 44°C for 40 or 60 min. It was noted that decay caused by *Botrytis cinerea* and *Rhizopus stolonifer* was controlled by both treatments; however, there was some injury of the fruit caused by treatment at 60 min, although there was no negative effect on flavor or texture. The effects of continuous UV-C light combined with heat treatment on storage rot on strawberries and sweet cherries were investigated by Marquenie et al. (2002). Fruit inoculated with *Botrytis cinerea* and *Monilinia fructigena* were exposed to temperatures between 40 and 48°C and UV doses of 0.05 to 1.5 J/cm<sup>2</sup>. At doses of 0.05 J/cm<sup>2</sup> or higher a significant retardation of fungal growth was reported for both strawberries and sweet cherries. However at doses of 1.0 and 1.5 J/cm<sup>2</sup> negative effects were seen on the calyx of the strawberry. Marquenie et al. (2003) evaluated the use of pulsed white light combined with UV-C and heat treatments on the reduction of storage rot on strawberries. Strawberries were treated with pulsed white light which consisted of 30 μs pulses at a frequency of 15 Hz for a time of 40 to 250 s, with a UV-C treatment of 0.05 or 1.0 J/cm<sup>2</sup>, and a heat treatment of 40 or 45°C for 3 or 15 min. It was noted that pulsed light treatments had no effect on the growth of *Botrytis cinerea*. They found that the combined treatment significantly reduced spoilage; however there was no significant decrease in spoilage when just pulsed light and UV-C light were used together. Baka et al. (1999) observed dissimilar

results regarding UV-C light treatment on strawberries. Strawberries treated with  $0.1 \text{ J/cm}^2$  had their storage life increased by 5 days and also exhibited lower respiration rates, firmer texture, and increased anthocyanin content.

Boysenberries treated with  $0.92 \text{ J/cm}^2$  of continuous UV-C light exhibited less druplet damage and firmer texture which was equivalent to a  $45^\circ\text{C}$  heat treatment for 1 h. They also noted that treated berries had reduced softening during storage as well as lower respiration rates and decreased anthocyanin leakage (Vicente et al., 2004).

Chemicals have been used to decontaminate berries inoculated with foodborne pathogens and other bacteria with limited success. Gulati et al. (2001) treated strawberries inoculated with feline calicivirus (FCV) (used as a surrogate for Norwalk virus) with sodium hypochlorite, quaternary ammonium compounds (quats), peroxyacetic acid, and hydrogen peroxide. A combination of 15% peroxyacetic acid and 11% hydrogen peroxide at 4 times the manufacturer's suggested strength resulted in a  $3 \log_{10}$  reduction of FCV titer. When quats were used at 4 times its recommended strength a  $\log_{10}$  reduction of 1.5 FCV titer was seen. At a free chlorine concentration of 800 ppm treatment with a sodium hypochlorite solution only resulted in a reduction of  $1.0 \log_{10}$  of FCV titer, which is 4 times greater than the FDA allowed concentration of sodium hypochlorite. Yu et al. (2001) investigated the effects of sodium hypochlorite, Tween 80, acetic acid, sodium phosphate, and hydrogen peroxide on the reduction of *E. coli* O157:H7 on strawberries. Strawberries treated with sodium hypochlorite (130 ppm free chlorine) resulted in a  $\log_{10}$  reduction of 1.3 CFU/g. When the free

chlorine concentration was increased to 1,300 ppm the  $\log_{10}$  reduction was only increased to 1.7 CFU/g. Treatment with Tween 80 resulted in  $\log_{10}$  reductions of 1.1 CFU/g and 1.2 CFU/g at concentrations of 100 and 200 ppm, respectively. Acetic acid at concentrations of 3 and 5% resulted in  $\log_{10}$  reductions of 1.47 CFU/g and 1.55 CFU/g, respectively. Treatment with 2 or 5% sodium phosphate resulted in  $\log_{10}$  reductions of 1.58 CFU/g and 1.85 CFU/g, respectively. The most effective treatment used was 3% hydrogen peroxide which resulted in a reduction of 2.18  $\log_{10}$  CFU/g. The efficacy of acidic electrolyzed oxidizing (EO) water was investigated for the purpose of decontaminating strawberries. EO water was compared to other decontamination methods like sodium hypochlorite, ozone, water, and alkaline plus acidic EO water. Treatment with acidic EO water, sodium hypochlorite, ozone, and a combined EO water treatment all resulted in undetectable coliform bacteria. However, the initial populations of coliform bacteria were only 2.4  $\log_{10}$  CFU/strawberry, so the true potential of these decontamination methods is not known. Although, when treated with tap water for 10 min only a 0.3  $\log_{10}$  CFU/strawberry reduction was observed (Koseki et al., 2004).

The use of gaseous methods of decontamination has also been investigated. Gaseous chlorine dioxide was investigated for the inactivation of *Salmonella* on strawberries. Gas at a total concentration of 100 mg resulted in a reduction of 4.76  $\log_{10}$  CFU/strawberry (Yuk et al., 2006a). Sy et al. (2005) also investigated the use of gaseous chlorine dioxide for the inactivation of *Salmonella*, yeast, and mold on the surfaces of blueberries, raspberries, and

strawberries. On blueberries, reductions as high as 3.7 log<sub>10</sub> CFU/g of *Salmonella*, 2.5 log<sub>10</sub> CFU/g of mold and 1.4 log<sub>10</sub> CFU/g of yeast. A log<sub>10</sub> reduction of 4.4 CFU/g *Salmonella* was observed on strawberries after chlorine dioxide treatment and reductions of yeast and mold were 1.4 and 4.2 log<sub>10</sub> CFU/g, respectively. Reductions on raspberries were the lowest with a reduction of 1.5 log<sub>10</sub> CFU/g *Salmonella* and reductions of 2.6 and 3.0 log<sub>10</sub> CFU/g of yeast and mold, respectively.

Gaseous ozone has also been used to extend the shelf-lives of berries. Blackberries exposed to 0.1 and 0.3 ppm of ozone for 12 days at 2°C showed an 80% reduction in *Botrytis cinerea* spoilage without any adverse effects on fruit quality (Barth et al., 1995). However, when cranberries were stored in ozonated conditions for 5 weeks a significant increase in rot and overall quality loss were observed (Nortan et al., 1965). Strawberries stored in an atmosphere containing 1.5 ppm ozone exhibited reduced decay, weight loss, and fruit softening; although, a loss in fruit aroma was noted (Nadas et al., 2003).

## **2.5 Predictive microbiology**

Microbial growth and inactivation models are helpful tools for the development of new foods and processing techniques. Microbial modeling describes microbial behavior using systems of mathematical expressions. Conventionally, the risk of microbial growth in a product was determined using inoculated pack studies or evaluating inactivation in microbial broth. However,



these two techniques do not allow for quantitative interpolation and the in-depth evaluation of multiple factors is not feasible (Whiting and Buchanan, 1994). Conversely, predictive microbial models allow for a more in-depth evaluation of the system and are classified into three levels. Primary models “describe how microbial numbers change with time in a specified environment” (Whiting and Buchanan, 1994). Primary models predict microbial growth with as few factors as possible to accurately model the distinct growth phases of the organisms, and are typically based on first-order kinetics (McKellar and Lu, 2004). This type of model is based on the assumption that all cells within a population have equal resistances to a given treatment. One commonly used set of first-order models are based on the Monod equation. Ludikhuyze et al. (1998) used a modified Monod equation to evaluate the inactivation kinetics of soybean lipoxygenase. Their model incorporated a modified rate constant which incorporated the effects of both temperature and pressure. Mussa et al. (1999) combined a first-order kinetic model with the Arrhenius model to examine the effects of high-pressure processing of *L. monocytogenes* on pork.

The assumption that microorganisms die in an exponential fashion has been debated by the observance of shoulders and tails in inactivation curves. Eight different shapes of survival curves (Figure 2.13) have been reported in the literature and were summarized by Geeraerd et al. (2005). Non-linear primary models are typically divided into two classes: shoulder and tailing (McKellar and Lu, 2004). These models account for either the lag phase encountered when trying to inactivate microorganisms or a levelling-off of the inactivation rate.

Shouldering or tailing is usually attributed to clumping of microorganisms or resistant subpopulation and has been observed more in the inactivation of vegetative microorganisms as opposed to spores (Xiong et al., 1999).

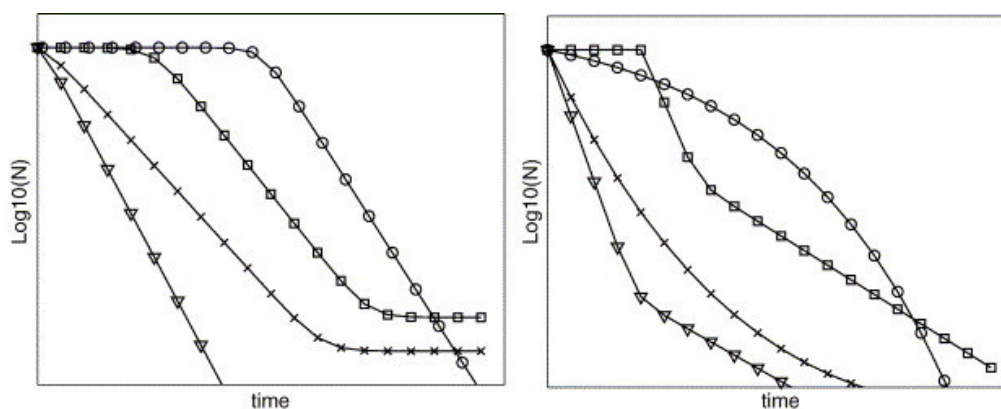


Figure 2.13. Commonly observed inactivation curves (Geeraerd et al., 2005).

Ross et al. (1998) used a non-linear tailing model to describe the inactivation of *Enterococcus faecium* in whole milk using HTST pasteurization. Huang and Juenja (2001) used a non-linear tailing model to demonstrate the thermal inactivation of *E. coli* O157:H7 in ground beef. Xiong et al. (1999) evaluated several non-linear inactivation models for the purpose modeling the thermal inactivation of *L. monocytogenes*. They evaluated the Baranyi and modified Gompertz models and found that the both models could be used to describe curves with a lag phase, a tail, and those that were sigmoidal in shape with  $R^2$  values of 0.99 for both the Baranyi and modified Gompertz.

Recently, the theory of microbial inactivation has strayed from the idea of inactivations as deterministic events (those with no uncertainty), but rather that they are probabilistic events (VanBoekel, 2004). The Weibull distribution is being

used to a greater extent to describe microbial inactivation, and uses the engineering principle of failure. Instead of a structural or mechanical failure, the failure is that of the microorganism (Peleg, 2006). This model assumes that the time required for a microorganism to die varies within the population and are statistically distributed. The Weibull distribution (Eqn 2.1) can be used to describe both concave upward and concave downward inactivation curves depending upon the value of the shape parameter ( $\beta$ ).

$$\log_{10}\left(\frac{N}{N_o}\right) = -\frac{1}{2.303} \left(\frac{t}{\alpha}\right)^\beta \quad \text{Eqn 2.1}$$

In addition to describing the shape of the inactivation curve the shape parameter can also be an indicator of the resistance of the microbial population. If the curve is concave upward ( $\beta < 1$ ) the remaining cells are resistant to the treatment, if the curve is concave downward ( $\beta > 1$ ) the remaining cells have a good probability of dying (van Boekel, 2002).

The Weibull model was applied to 55 sets of thermal inactivation data by van Boekel (2002). The author found that 53 out of 55 sets of data were non-linear and all could be modeled using the Weibull distribution function. Of these 53, 39 were found to be concave downward, 14 to be concave upward, and only 2 were truly linear with  $\beta=1$ . This model has also been used to describe the heat inactivation of *L. monocytogenes*, *Salmonella* Enteritidis, *S. Typhimurium*, *E. coli* O157:H7 and *Staphylococcus aureus* (Buzrul and Alpas, 2006). The authors compared the Weibull model to a first-order kinetic model and found that the Weibull model more accurately estimated the inactivation curve of all three

microorganisms. The correlation coefficients for the Weibull distribution were between 0.94 and 0.99 and for the first-order model the correlation coefficients were between 0.41 and 0.68. The Weibull was compared to the linear first-order kinetic model to describe the inactivation of several food pathogens in milk (Chen, 2007). The authors found that under all processing conditions the Weibull model more accurately estimated the microbial reductions with a MSE value of 6.1 for the first-order model and a value of 0.7 for the Weibull model.

Guan et al. (2006) also compared the Weibull distribution to other models to describe the inactivation of *E. coli* O157:H7 and *S. aureus* after high-pressure processing. The Weibull, log-logistic, and modified Gompertz model were all used. The authors found that the fit of the model depended on the microorganism and processing conditions and that the inactivation of these microorganisms followed a non-linear trend. The inactivation of *Listeria innocua* in buffer solution with pulsed electric fields was modeled using both the Weibull and first-order kinetic models (San Martin et al., 2006). The Weibull model was found to be far superior to the first-order model.

## **2.6 Summary of literature review**

Numerous outbreaks have been associated with the consumption of fresh produce. There are many methods of decontamination available such as chlorine, chlorine dioxide, and hydrogen peroxide. These methods have been shown to have varied efficacy and depend greatly on the microorganism of

concern and the produce item being decontaminated. Research has been conducted on the efficacy of alternative methods of decontamination. Ozone is one technology and has been shown to be effective at extending the shelf-life and decontaminating certain fresh fruits and vegetables. UV light has also shown promise for the decontamination of fresh produce.

Berries have been implicated in several multi-state outbreaks of illness and are currently not washed prior to fresh-market sale. Berries are difficult fruits to decontaminate due to the presence of seeds, crevices, and hairs where microorganisms can attach to or allow them to be shielded from decontamination. Research has found that washing with water is ineffective at removing pathogens and that conventional sanitizers have shown very limited efficacy, resulting in no more than 2 logs of bacterial reduction.

Both ozone and UV light have been used to extend the shelf-lives of berries and have been shown not to damage fruit. However, these treatments have not been used to inactivate pathogenic microorganisms on the surfaces of berries. Therefore, the objective of this research was to evaluate the efficacy of gaseous ozone, aqueous ozone, and pulsed UV-light for the decontamination of *Escherichia coli* O157:H7 and *Salmonella* on the surfaces of blueberries, raspberries, and strawberries. Furthermore, additional research was conducted to gain a better insight into the technologies through inactivation modeling and characterization studies.

## Chapter 3

### Utilization of gaseous ozone for the decontamination of *Escherichia coli* O157:H7 and *Salmonella* on berries

#### Abstract

Each year there are approximately 76 million foodborne illnesses and fresh produce is the second most common vehicle for such illnesses. Prior to market small fruits are not washed or treated in any manner so as to extend their shelf life. Washing alone is not a viable option and the use of novel technologies needs to be investigated. One such technology is ozone which has been used to treat drinking water since the late nineteenth century. The efficacy of gaseous ozone to decontaminate pathogens on blueberries, strawberries, and raspberries, was investigated in this study. Blueberries, strawberries and raspberries were artificially contaminated with five strains of *Escherichia coli* O157:H7 and *Salmonella* spp. Fruits were treated with four ozone treatments; i) continuous ozone flow (5%, wt/wt) for 2, 4, 8, 16, 32, and 64 min, ii) pressurized ozone (83 kPa) for 2, 4, 8, 16, 32, and 64 min, iii) continuous ozone (64 min) followed by pressurized ozone (64 min), and iv) vacuum followed by 64-min pressurized ozone. Combined continuous and pressurized treatment yielded high log<sub>10</sub> reductions of 3.6 and 3.8 CFU/g of *Salmonella* and *E. coli* O157:H7, respectively, for raspberries, whereas 2.6 and 2.9 CFU/g of *Salmonella* and *E.*

*coli* O157:H7, respectively, for strawberries. For blueberries, the highest log<sub>10</sub> reductions resulted after treatment with continuous ozone for *E. coli* O157:H7 and was 2.2 CFU/g and for *Salmonella* the highest reductions resulted after the 64-min pressurized treatment and was 3.0 log<sub>10</sub> CFU/g . These results demonstrate that gaseous ozone has the potential to be used for the decontamination of small fruits.

### 3.1 Introduction

In the United States the consumption of fresh fruits has risen from 38.7 to 46.7 kg per person per year over the last 15 years (FDA, 2001). The increased consumption has caused an increase in the number of foodborne illnesses associated with fresh produce. Fresh produce is now the second most common vehicle for foodborne illness with 428 outbreaks between 1990 and 2003 associated with produce (CSPI, 2004).

Small fruits, such as raspberries and strawberries, have been implicated in several outbreaks. Raspberries have been implicated in at least five outbreaks of *Cyclospora cayetanensis* (CDC, 1997b) and strawberries have been implicated in three outbreaks of Hepatitis A (CDC, 1997a). An outbreak of listeriosis has also been tied to the consumption of blueberries (Ryser and Marth, 1990). A U.S. Food and Drug Administration (FDA) survey found that 1 out of 143 imported strawberry samples tested positive for *Salmonella* (FDA, 1999). Also, research has shown that both *Salmonella* and *Escherichia coli* O157:H7

are capable of surviving on fresh strawberries for over 7 days (Knudsen et al., 2001).

Throughout the production of small fruits, the opportunity for contamination exists due to improper sanitation, infected pickers, contaminated irrigation water, and manure fertilized fields (Han et al., 2004). In spite of these risks, small fruits are not washed prior to delivery to market, due to the negative affect on fruit quality and shelf life. Washing alone has been shown to have limited efficacy at removing both spoilage and pathogenic bacteria from the surfaces of produce (FDA, 2001), and conventional sanitizers have also shown limited efficacy. Yu et al. (2001) compared five sanitizers for the purpose of reducing populations of *E. coli* O157:H7 on strawberries. Of these five sanitizers the most effective was found to be hydrogen peroxide which gave a reduction of 2.2 CFU/g.

Gaseous decontamination methods have been shown to be more successful for the decontamination of pathogens on the surfaces of small fruits. Han et al. (2004) used chlorine dioxide gas for the decontamination of *E. coli* O157:H7 and *Listeria monocytogenes* on the surfaces of strawberries. Reductions of 3.0 and 3.6 CFU/fruit were seen at a concentration of 0.6 mg/L ClO<sub>2</sub> for *E. coli* O157:H7 and *L. monocytogenes*, respectively. Sy et al. (2005) also investigated the use of gaseous chlorine dioxide for the purpose of decontaminating small fruits inoculated with *Salmonella*. They found log<sub>10</sub> reductions of 3.6 CFU/g of *Salmonella* on strawberries when a concentration of 8.0 mg/L and a time of 120 min. For raspberries, they achieved a maximum log<sub>10</sub>



reduction of 1.52 CFU/g of *Salmonella* at the same conditions. Research seems to indicate that chlorine dioxide may be an effective gaseous decontamination method for small fruits, however, after treatment residual chlorine remains on the fruits.

Since conventional sanitizers have shown little efficacy and gaseous sanitizers such as chlorine dioxide have shown promise, other dry technologies need to be investigated. Ozone is one such technology and has been used as an antimicrobial agent since the late nineteenth century to purify drinking water (Graham, 1997). Ozone has the ability to oxidize organic materials and decays in a short amount of time into harmless oxygen, which means no chemical residues are left behind. Therefore, the application of ozone is considered to be a process rather than a chemical additive. In 2001, ozone was approved for the treatment of raw commodities (Federal Register, 2001). Ozone gas has been shown to be an effective bactericide for several foodborne pathogens. Whistler and Sheldon (1989) found that reductions of up to 7 log<sub>10</sub> CFU/ml of both *E. coli* and *Salmonella* Typhimurium were possible when Petri dishes were exposed to 1.65 % (wt/wt) gaseous ozone. The use of ozone gas to extend the shelf life of fruits has been shown. Bazarova (1982) found that when apples were exposed to 0.06 ppm of ozone gas for 4 h each day that there was a reduction in both weight loss and spoilage. An 80% reduction in the spoilage of blackberries by *Botrytis cinerea* was seen when the fruits were exposed to 0.1 and 0.3 ppm ozone gas for 12 days (Barth et al., 1995). Based on this previous research and the potential food safety risk associated with small fruits, this research was

undertaken to investigate the efficacy of gaseous ozone for the purpose of decontaminating *E. coli* O157:H7 and *Salmonella* on the surfaces various berries.

## **3.2 Materials and Methods**

### **3.2.1 Preparation of inoculum**

Five strains of nalidixic acid resistant *E. coli* O157:H7 and *Salmonella* were obtained from the Center for Food Safety at the University of Georgia. The *E. coli* O157:H7 strains were: 932 (human isolate), 994 (salami isolate), E0018 (calf fecal isolate), H1730 (human isolate from outbreak associated with lettuce), and F4546 (human isolate from outbreak associated with alfalfa sprouts). The *Salmonella* serotypes used were: Agona (human isolate from outbreak associated with alfalfa sprouts), Baildon (human isolate from outbreak associated with diced tomatoes), Gaminara (orange juice isolate), Michigan (human isolate associated with cantaloupe outbreak), and Montevideo (human isolate associated with tomato outbreak). Cultures were grown in tryptic soy broth (Difco, Detroit, MI) supplemented with 50 µg/ml nalidixic acid (Fisher Scientific Co., Fair Lawn, NJ) at 37°C for 24 h. A mixture of *E. coli* O157:H7 or *Salmonella* strains were prepared by combining 10 ml of each culture and centrifuging for 15 min at 3,300 x *g* and 4°C. The supernatant was discarded and the cells were

resuspended in 10 ml of 0.1% peptone water (Difco) to yield an approximate population of  $10^8$  CFU/ml.

### **3.2.2 Inoculation of berries**

Blueberries, red raspberries and strawberries were purchased from a local grocery store and left at room temperature for 1 h prior to inoculation. To inoculate the blueberries and raspberries, 25  $\mu$ L of inoculum was deposited on the skin of each fruit. For strawberries, 50  $\mu$ L of inoculum was deposited on the skin of each strawberry, approximately midway between the calyx and cap (Yu et al., 2001). The fruits were dried in a laminar flow hood for 24 h before the treatment to allow for proper attachment of the microorganisms. The inoculated blueberries, raspberries and strawberries had approximately  $10^5$  CFU/g fruit of both *E. coli* O157:H7 and *Salmonella*.

### **3.2.3 Production and delivery of ozone**

Ozone gas was generated using a lab-scale ozone generator (Model No. H-50, Hess Machines International, Ephrata, PA) equipped with an oxygen concentrator. Gas was delivered at a flow rate of 0.34 m<sup>3</sup>/h and a concentration of 5.00% (wt/wt) which was measured using a Teledyne 450H bench top analyzer (Teledyne Technologies Inc., Los Angeles, CA). A 1 L beaker containing the fruit sample was placed in a 17-L pressure vessel (Model No.

1915X, Wisconsin Aluminum Foundry Co. Inc, Manitowoc, WI) which was connected to the ozone gas line (Figure 3.1). After the treatment, the ozone gas was passed through a 2% (wt/v) potassium iodide solution to prevent ozone from being released into the environment. Furthermore, the ozone treatment was performed in a fume hood for safety considerations.

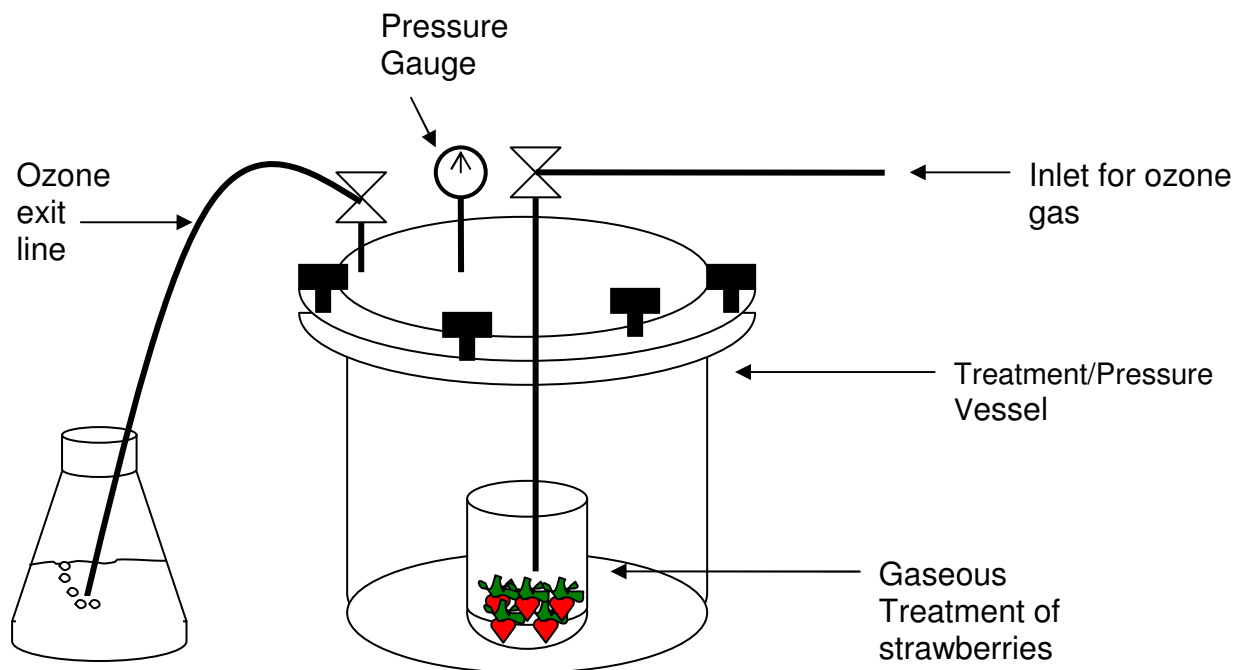


Figure 3.1. Schematic of ozone treatment setup.

### **3.2.4 Treatment with ozone**

A batch of 5 strawberries or 18 raspberries or blueberries was used for each ozone treatment based on size constraints. Fruits were subjected to 4 different ozone treatments:

- i. Fruits were exposed to continuous ozone flow for 2, 4, 8, 16, 32, and 64 min.
- ii. Fruits were placed in the treatment vessel with the exit line closed and ozone gas was introduced until a pressure of 83 kPa was achieved, which took approximately 3 min. Once pressurized, fruits were held for 2, 4, 8, 16, 32, and 64 min under this pressure.
- iii. Fruits were first exposed to a continuous flow of ozone gas for 64 min followed by the pressurization of the treatment vessel with ozone gas to 83 kPa and held for an additional 64 min.
- iv. Fruits were exposed to a vacuum of -85 kPa and then ozone gas was used to pressurize the treatment vessel to 83 kPa and held for 64 min.

### **3.2.5 Microbial analysis**

After treatment strawberries were placed in 100 ml of Dey-Engley Neutralizing (D/E) Broth (Difco) and blueberries and raspberries were placed in 50 ml D/E broth and pummeled for 1 min in a stomacher. The homogenate was then serially diluted in 0.1% peptone water (Difco) and spiral plated on tryptic soy

agar (Difco) supplemented with 50 µg/ml of nalidixic acid with an Autoplate 4000 (Spiral Biotech, Norwood, MA). Plates were incubated at 37°C for 24 h and then enumerated using Q-count (Version 2.1, Spiral Biotech, Norwood, MA).

Reductions of bacteria were calculated on a per gram of fruit basis. Random colonies of *E. coli* O157:H7 and *Salmonella* were confirmed serologically using RIM *E. coli* O157:H7 latex test (Remel Microbiology Products, Lenexa, KS) and *Salmonella* O Antiserum A-1 latex agglutination test (Remel).

### **3.2.6 Color analysis**

To determine whether treatment with ozone had any negative effects on quality, the fruit color analysis was performed on fruits from the treatment with the highest microbial reduction. A Minolta Chromo Meter CR200 colorimeter (Minolta, Ramsey, NJ) was used to measure the L\*a\*b\* color space. The color space uses the following parameters: L\* indicates the lightness, a\* and b\* are the chromaticity coordinates. Value -a\* indicates a green color, +a\* a red color, -b\* a blue color, and +b\* a yellow color. Prior to use, the chromameter was calibrated using a white tile. Two randomly selected spots were analyzed and averaged to get an overall measurement for each fruit and replicated three times.

### 3.2.7 Statistical analysis

All experiments were replicated three times and MINITAB statistical software (Version 13, MINITAB, State College, PA) was used to analyze the mean  $\log_{10}$  reductions. A one-way ANOVA with a 95% confidence level was used to compare the treatment times and scenarios. A Tukey's pairwise comparison was also performed to determine significant differences.

## 3.3 Results and Discussion

In this study, the efficacy of gaseous ozone for the inactivation of *E. coli* O157:H7 and *Salmonella* on blueberries, raspberries and strawberries was evaluated. Ozone was applied in four different treatment types to evaluate the effect of continuously flowing 5.00% (wt/wt) ozone gas, stagnant pressurized ozone gas, a combination of the continuous and pressurized and finally, vacuum followed by pressurized ozone gas.

### 3.3.1 Treatment of blueberries

The efficacy of gaseous ozone on *E. coli* O157:H7 and *Salmonella* inoculated on the surfaces of blueberries was evaluated at treatment times of 2, 4, 8, 16, 32 and 64 min. Reductions of *Salmonella* are presented in Table 3.1 and ranged from 0.3 to 1.0  $\log_{10}$  CFU/g for treatment times of 4 and 64 min, respectively. A treatment time of 64 min resulted in a significantly higher

reduction of *Salmonella* than the lower treatments except for 32 min. Reductions of *E. coli* O157:H7 are found in Table 3.2, and were between 0.4 and 2.2 log<sub>10</sub> CFU/g for times of 4 and 64 min, respectively. The 64-min treatment resulted in significantly higher log<sub>10</sub> reductions than the 2 through 16-min treatments, but not the 32-min treatment.

The second treatment scenario was to evaluate the effect of pressurized ozone; samples were held for 2, 4, 8, 16, 32, and 64 min and reductions can be found in Tables 3.1 and 3.2, for *Salmonella* and *E. coli* O157:H7, respectively. Reductions of *Salmonella* were between 0.3 and 3.0 log<sub>10</sub> CFU/g for times of 2 and 64 min, respectively. Treatment times of 32 and 64 min resulted in significantly higher reductions than the lower treatment times with reductions of 2.2 and 3.0 log<sub>10</sub> CFU/g, respectively. Reductions of *E. coli* O157:H7 ranged from 0.4 to 1.4 log<sub>10</sub> CFU/g for treatment times of 4 and 64 min, respectively. The 64-min treatment resulted in significantly higher reductions than other treatment times except for the 32-min treatment.

The combined ozone treatments, continuous followed by pressurized and vacuum followed by pressurized ozone, did not result in an increase in log<sub>10</sub> reductions for either *Salmonella* or *E. coli* O157:H7. Reductions of *Salmonella* were 1.9 and 0.3 log<sub>10</sub> CFU/g for continuous followed by pressurized and vacuum followed by pressurized, respectively. Reductions of *E. coli* O157:H7 were 1.1 and 0.1 log<sub>10</sub> CFU/g for continuous followed by pressurized and vacuum followed by pressurized, respectively.



Table 3.1. Population reductions of *Salmonella* on blueberries treated with gaseous ozone.

Treatment Type	Treatment Time (min)	Log <sub>10</sub> Reductions <sup>a,b,c</sup>
Continuous Ozone	2	0.0 ± 0.0A
	4	0.3 ± 0A
	8	0.4 ± 0.1A
	16	0.5 ± 0.1AB
	32	0.8 ± 0.2BC
	64	1.0 ± 0.1C
Pressurized Ozone	2	0.3 ± 0.3A
	4	0.3 ± 0.2A
	8	0.5 ± 0.4A
	16	0.7 ± 0.1A
	32	2.2 ± 0.7B
	64	3.0 ± 0.3B
Continuous Ozone followed by Pressurized Ozone	64 + 64	1.9 ± 0.7
Vacuum followed by Pressurized Ozone	64	0.3 ± 0.2

<sup>a</sup> Within the same column and treatment, values not followed by the same letter are significantly different.

<sup>b</sup> Average weight of blueberry sample is 30.0 ± 2.5 g.

<sup>c</sup> Values are mean reductions ± standard deviation.

Table 3.2. Population reductions of *E. coli* O157:H7 on blueberries treated with gaseous ozone.

Treatment Type	Treatment Time (min)	Log <sub>10</sub> Reductions <sup>a,b,c</sup>
Continuous Ozone	2	0.5 ± 0.0A
	4	0.4 ± 0.2A
	8	1.0 ± 0.1AB
	16	1.3 ± 0.4B
	32	1.3 ± 0.4B
	64	2.2 ± 0.2C
Pressurized Ozone	2	0.5 ± 0.3A
	4	0.4 ± 0.1A
	8	0.7 ± 0.2A
	16	0.7 ± 0.2A
	32	1.0 ± 0.3AB
	64	1.4 ± 0.3B
Continuous Ozone followed by Pressurized Ozone	64 + 64	1.1 ± 0.6
Vacuum followed by Pressurized Ozone	64	0.1 ± 0.0

<sup>a</sup> Within the same column and treatment, values not followed by the same letter are significantly different.

<sup>b</sup> Average weight of blueberry sample is 30.0 ± 2.5 g.

<sup>c</sup> Values are mean reductions ± standard deviation.

When comparing these results to those of other gaseous treatments, such as chlorine dioxide, the reductions were comparable. After a gaseous ozone at 83 kPa for 32 min a 2.2 log<sub>10</sub> reduction of *Salmonella* was achieved, on blueberries treated with gaseous chlorine dioxide as reduction of 2.95 log<sub>10</sub> CFU/g was observed after 30 min and a reduction 3.56 log<sub>10</sub> CFU/g of *Salmonella* after 60 min (Sy et al., 2005). Even though the results obtained with chlorine dioxide treatment may be slightly higher there are drawbacks to chlorine dioxide such as the presence of a chlorine residue which is not true for ozone.

### **3.3.2 Treatment of raspberries**

To investigate the lethality of ozone on pathogenic microorganisms, inoculated raspberries were exposed continuously to ozone gas for 2, 4, 8, 16, 32, and 64 min. Reductions for *Salmonella* can be seen in Table 3.3, where log<sub>10</sub> reductions ranged from 0.1 to 1.6 CFU/g. Treatment times of 16, 32, and 64 min were determined to produce log<sub>10</sub> reductions that were significantly higher than lower treatment times. However, there was no significant difference in the reductions resulting from these treatments, which had log<sub>10</sub> reductions of 1.5, 1.3, and 1.6 CFU/g for 16, 32, and 64 min, respectively. Reductions of *E. coli* O157:H7 are shown in Table 3.4 and ranged from 0.3 to 2.6 log<sub>10</sub> CFU/g. Treatment times of 32 and 64 min produced log<sub>10</sub> reductions which were significantly higher than other treatment times.

Table 3.3. Population reductions of *Salmonella* on raspberries treated with gaseous ozone.

Treatment Type	Treatment Time (min)	Log <sub>10</sub> Reduction (CFU/g) <sup>a,b,c</sup>
Continuous Ozone	2	0.1 ± 0.1 A
	4	0.2 ± 0.2 A
	8	0.5 ± 0.5 A
	16	1.5 ± 0.3 B
	32	1.3 ± 0.3 B
	64	1.6 ± 0.3 B
Pressurized Ozone	2	0.1 ± 0.1 A
	4	0.3 ± 0.2 AB
	8	0.8 ± 0.2 BC
	16	1.1 ± 0.4 C
	32	1.3 ± 0.5 CD
	64	2.0 ± 0.3 D
Continuous Ozone followed by Pressurized Ozone	64 + 64	3.6 ± 0.4
Vacuum followed by Pressurized Ozone	64	2.9 ± 0.5

<sup>a</sup> Within the same column and treatment, values not followed by the same letter are significantly different.

<sup>b</sup> Average weight of raspberry sample is 17.5±2.5 g.

<sup>c</sup> Values are mean reductions ± standard deviation.

Table 3.4. Population reductions of *E. coli* O157:H7 on raspberries treated with gaseous ozone.

Treatment Type	Treatment Time (min)	Log <sub>10</sub> Reduction (CFU/g) <sup>a,b,c</sup>
Continuous Ozone	2	0.0 ± 0.0 A
	4	0.3 ± 0.2 A
	8	0.9 ± 0.3 B
	16	1.0 ± 0.2 B
	32	1.9 ± 0.2 C
	64	2.6 ± 0.4 D
Pressurized Ozone	2	0.3 ± 0.0 A
	4	0.7 ± 0.0 AB
	8	0.7 ± 0.5 ABC
	16	0.9 ± 0.4 ABC
	32	1.7 ± 0.7 BC
	64	2.8 ± 0.7 D
Continuous Ozone followed by Pressurized Ozone	64 + 64	3.8 ± 0.2
Vacuum followed by Pressurized Ozone	64	3.3 ± 0.5

<sup>a</sup> Within the same column and treatment, values not followed by the same letter are significantly different.

<sup>b</sup> Average weight of raspberry sample is 17.5±2.5 g.

<sup>c</sup> Values are mean reductions ± standard deviation.

Log<sub>10</sub> reductions were 1.9 and 2.6, for 32 and 64 min, respectively. When comparing the lethality of ozone gas to other novel gaseous treatments, such as chlorine dioxide, the use of gaseous ozone appears to produce higher log<sub>10</sub> reductions. Sy et al. (2005) treated raspberries inoculated with *Salmonella* with chlorine dioxide for 60 min and obtained a maximum reduction of 1.1 CFU/g. The comparable treatment time for continuously applied ozone gas in this study of 64 min produced reductions of 1.6 CFU/g for *Salmonella* and 2.6 CFU/g for *E. coli* O157:H7, although no comparable data is available for the decontamination of *E. coli* O157:H7 on raspberries.

The third treatment which was used to decontaminate raspberries was a combined continuous and pressurized ozone treatment; where raspberries were exposed to 64 min of continuous ozone and then exposed to 64 min of pressurized ozone treatment. Reductions of *Salmonella* and *E. coli* O157:H7 are shown in Tables 3.5 and 3.6, respectively. A log<sub>10</sub> reduction of 3.6 CFU/g was achieved for *Salmonella* and a reduction of 3.7 log<sub>10</sub> CFU/g was attained for *E. coli* O157:H7. The final treatment involved replacing the air in the pores of the fruits with ozone via a vacuum. Log<sub>10</sub> reductions were 2.9 and 3.3 CFU/g for *Salmonella* and *E. coli* O157:H7, respectively.

Based on the data, it appears that for both *Salmonella* and *E. coli* O157:H7 the most effective treatment is the 64 min continuous ozone exposure followed by the 64 min pressurized ozone. This treatment resulted in log<sub>10</sub> reductions of 3.56 and 3.8 for *Salmonella* and *E. coli* O157:H7, respectively. When the four treatment scenarios producing maximum log<sub>10</sub> reductions were

analyzed using a Tukey's comparison, it was concluded that for *E. coli* O157:H7 that the combined continuous and pressurized ozone treatment produced significantly ( $P < 0.05$ ) higher  $\log_{10}$  reductions than the 64-min continuous ozone treatment or the 64-min 83 kPa ozone treatment with a  $\log_{10}$  reduction of 3.8 CFU/g compared to 2.6 and 2.8 CFU/g, respectively.

### 3.3.3 Treatment of strawberries

Strawberries inoculated with *E. coli* O157:H7 and *Salmonella* were treated with continuously applied 2% ozone gas for 2, 4, 8, 16, 32 and 64 min.

Reductions of *Salmonella* are found in Table 3.5, and ranged from 0.1 to 0.9  $\log_{10}$  CFU/g. Treatment times of 32- and 64-min resulted in significantly ( $P < 0.05$ ) higher  $\log_{10}$  reductions than other treatment times, with reductions of 0.4 and 0.9  $\log_{10}$  CFU/g, respectively. However, there was no significant ( $P > 0.05$ ) difference between the two treatments. Somewhat higher reductions were seen for *E. coli* O157:H7 with reductions ranging from 0.3 to 1.8  $\log_{10}$  CFU/g (Table 3.6). As with *Salmonella*, the 32- and 64-min treatments were determined to result in significantly ( $P < 0.05$ ) higher reductions of *E. coli* O157:H7 than other treatment times. Reduction of 1.5 and 1.8  $\log_{10}$  CFU/g were attained for 32- and 64-min treatments, respectively. Still, there was no significant ( $P > 0.05$ ) between the two treatment times. When the lethality of gaseous ozone was compared to other gaseous decontamination methods, chlorine dioxide, ozone resulted in slightly lower reductions.

Table 3.5. Population reductions of *Salmonella* on strawberries treated with gaseous ozone.

Treatment Type	Treatment Time (min)	Log <sub>10</sub> Reduction (CFU/g) <sup>a,b,c</sup>
Continuous Ozone	2	0.0 ± 0.0 A
	4	0.1 ± 0.1 AB
	8	0.1 ± 0.1 AB
	16	0.1 ± 0.1 AB
	32	0.4 ± 0.4 B
	64	0.9 ± 0.1 C
Pressurized Ozone	2	0.3 ± 0.1 A
	4	0.6 ± 0.1 AB
	8	0.8 ± 0.4 AB
	16	0.9 ± 0.5 AB
	32	1.4 ± 0.5 BC
	64	2.2 ± 0.3 C
Continuous Ozone followed by Pressurized Ozone	64 + 64	2.6 ± 0.9
Vacuum followed by Pressurized Ozone	64	1.7 ± 0.2

<sup>a</sup> Within the same column and treatment, values not followed by the same letter are significantly different.

<sup>b</sup> Average weight of strawberry sample is 110±5 g.

<sup>c</sup> Values are mean reductions ± standard deviation.



Sy et al. (2005) achieved  $\log_{10}$  reductions of 3.3 CFU/g of *Salmonella* on the surfaces of strawberries with chlorine dioxide at time of 60 min, while reductions of *Salmonella* in this study, for continuously applied ozone, were 0.9  $\log_{10}$  CFU/g. Han et al. (2004) achieved  $\log_{10}$  reductions of 4.5 CFU/g *E. coli* O157:H7 with chlorine dioxide gas at a time of 30 min, while in this study exposure for 32 min yielded a maximum reduction of 1.4  $\log_{10}$  CFU/g and at 64 min yielded a  $\log_{10}$  reduction of 1.8 CFU/g for *E. coli* O157:H7.

When strawberries were treated with 83 kPa pressurized ozone,  $\log_{10}$  reductions increased for both *Salmonella* and *E. coli* O157:H7 (Tables 3.5 and 3.6) with reductions ranging from 0.3 to 2.2 CFU/g and 0.2 to 2.3 CFU/g, respectively. For strawberries inoculated with *Salmonella*, the treatments times of 32- and 64-min were determined to produce significantly ( $P < 0.05$ ) higher reductions than other treatment times, with reductions of 1.4 and 2.2  $\log_{10}$  CFU/g, respectively. However, there was no significant ( $P > 0.05$ ) difference between the treatments. For fruits inoculated with *E. coli* O157:H7, the only treatment time that produced a significantly ( $P < 0.05$ ) higher reduction was the 64-min treatment with a reduction of 2.3  $\log_{10}$  CFU/g. When comparing this treatment scenario to just continuous ozone flow, the disparity in the reductions between *Salmonella* and *E. coli* O157:H7 is no longer significant. The reductions of *Salmonella* achieved using the pressurized ozone are significantly ( $P < 0.05$ ) higher than reductions observed with just ozone exposure.

Table 3.6. Population reductions of *E. coli* O157:H7 on strawberries treated with gaseous ozone.

Treatment Type	Treatment Time (min)	Log <sub>10</sub> Reduction (CFU/g) <sup>a,b,c</sup>
Continuous Ozone	2	0.3 ± 0.2 A
	4	0.5 ± 0.3 A
	8	0.7 ± 0.3 AB
	16	0.8 ± 0.4 AB
	32	1.5 ± 0.6 BC
	64	1.8 ± 0.2 C
Pressurized Ozone	2	0.4 ± 0.3 A
	4	0.2 ± 0.3 AB
	8	0.6 ± 0.4 ABC
	16	1.1 ± 0.2 ACD
	32	1.5 ± 0.3 D
	64	2.3 ± 0.1 E
Continuous Ozone followed by Pressurized Ozone	64 + 64	2.9 ± 0.4
Vacuum followed by Pressurized Ozone	64	0.9 ± 0.0

<sup>a</sup> Within the same column and treatment, values not followed by the same letter are significantly different.

<sup>b</sup> Average weight of strawberry sample is 110±5 g.

<sup>c</sup> Values are mean reductions ± standard deviation.

Indicating that for *Salmonella* pressure plays an integral role the inactivation of the organism. While for *E. coli* O157:H7, this does not appear to be the case. There is no significant ( $P>0.05$ ) difference in  $\log_{10}$  reductions when comparing the two treatments.

To further investigate the possible synergistic effect between ozone and pressure, strawberries were treated with continuously applied ozone gas for 64-min, which was followed by a 64-min pressurized ozone treatment.  $\log_{10}$  reductions of *Salmonella* averaged 2.6 CFU/g (Table 3.5) and for *E. coli* O157:H7 averaged 2.9 CFU/g (Table 3.6). When this treatment was compared to the 64-min continuous treatment, it was determined that for *Salmonella* the combined ozone treatment resulted in significantly ( $P<0.05$ ) higher  $\log_{10}$  reductions, 0.9 versus 2.6 CFU/g. However, there was no significant ( $P>0.05$ ) difference in the 64-min pressurized ozone treatment and the combined ozone treatment. Although the combined ozone treatment resulted in significantly ( $P<0.05$ ) higher  $\log_{10}$  reductions for *E. coli* O157:H7 than both the 64-min continuous ozone treatment and the 64-min pressurized ozone treatment; 2.9 CFU/g versus 1.8 and 2.3 CFU/g, respectively. The final treatment, which made the supposition that by replacing the air in the crevices of the fruit with ozone would increase the lethality of the treatment, did not result in significantly ( $P>0.05$ ) higher  $\log_{10}$  reductions than the other treatment scenarios.  $\log_{10}$  reductions were 1.7 and 0.8 CFU/g for *Salmonella* and *E. coli* O157:H7, respectively. For *Salmonella*, there was no significant ( $P>0.05$ ) difference between this treatment and the other treatments tested. For *E. coli* O157:H7,

this treatment resulted in significantly ( $P < 0.05$ ) lower  $\log_{10}$  reductions than the other treatments.

### 3.3.4 Color analysis

Fruits from the most effective treatment times were analyzed for color to determine whether ozone treatment had any negative effects. A 64-min continuous ozone treatment followed by a 64-min pressurized treatment was selected. Treated raspberries were similar in color compared to untreated raspberries with mean  $L^*$ ,  $a^*$ ,  $b^*$  values of 34.24, +26.67, and +14.55 compared to the untreated raspberries which had values of 31.13, +26.31, and +12.35, respectively (Table 3.7). For strawberries the mean  $L^*$ ,  $a^*$ ,  $b^*$  values were 37.81, +32.44, and +21.85, which were not significantly different from the untreated strawberries which had values of 36.15, +34.25, and +22.27, respectively. However, there were significant differences observed in  $L^*$ ,  $a^*$ ,  $b^*$  values of treated versus untreated blueberries. The mean  $L^*$ ,  $a^*$ ,  $b^*$  values of treated blueberries were 30.55, +2.38, and -2.85, respectively, and untreated blueberries had values of 34.06, +0.91, and -5.94. These  $L^*$   $a^*$   $b^*$  values indicate that the treated blueberries had less of a blue color based on a smaller  $b^*$  values, were more red based on a higher  $a^*$  value, and were not as light as the untreated blueberries based on the  $L^*$  value. This decrease in the lightness or white may be due to the removal of the bloom on the surface of the berry, which is a white antimicrobial coating. Color measurements were taken immediately after

treatment so it is not known whether there is a long term impact on the color of the fruit during storage.

Table 3.7. L\*a\*b\* color readings for blueberries, raspberries, and strawberries after ozone treatment.

		L * value <sup>c,d,e</sup>	a * value	b * value
Blueberry <sup>a</sup>	Untreated	34.06 ± 2.53A	0.91 ± 0.83A	-5.94 ± 1.43A
	Treated	30.55 ± 2.70B	2.38 ± 1.71B	-2.85 ± 1.60B
Raspberry <sup>a</sup>	Untreated	31.13 ± 1.41A	26.31 ± 0.92A	12.35 ± 0.83A
	Treated	34.24 ± 1.02A	26.67 ± 1.21A	14.55 ± 2.51A
Strawberry <sup>b</sup>	Untreated	36.15 ± 1.26A	34.25 ± 1.20A	22.27 ± 1.67A
	Treated	37.81 ± 1.13A	32.44 ± 0.89A	21.85 ± 1.32A

<sup>a</sup>Each replication consisted of 9 samples with 2 readings per sample.

<sup>b</sup>Each replication consisted of 3 samples with 2 readings per sample.

<sup>c</sup>Treatment was 64-min continuous ozone followed by 64-min at 12-psi.

<sup>d</sup>Values within the same row followed by the same letter are not significantly different (P>0.05).

<sup>e</sup>Values are mean ± standard deviation

### 3.4 Conclusions

The results presented here indicate the use of ozone gas as decontamination method for small fruits shows promise for the inactivation of both *Salmonella* and *E. coli* O157:H7. Continuously applied ozone gas resulted in maximum log<sub>10</sub> reductions of 1.0, 1.5 and 0.9 CFU/g of *Salmonella* on blueberries, raspberries, and strawberries, respectively. For blueberries, raspberries, and strawberries inoculated with *E. coli* O157:H7, maximum log<sub>10</sub>

reductions of 2.2, 2.6, and 1.8 CFU/g were achieved. Ozone treatment combined with pressure yielded higher reductions in both *E. coli* O157:H7 and *Salmonella*. Reduction on blueberries of 3.0 and 1.4 log<sub>10</sub> CFU/g were achieved for *Salmonella* and *E. coli* O157:H7, respectively. Reductions on raspberries of 1.9 and 2.8 log<sub>10</sub> CFU/g were achieved for *Salmonella* and *E. coli* O157:H7, respectively. Reductions on strawberries were 2.2 and 2.3 for *Salmonella* and *E. coli* O157:H7, respectively. Combined continuous and pressurized treatment yielded even higher log<sub>10</sub> reductions of 3.6 and 3.8 CFU/g of *Salmonella* and *E. coli* O157:H7, respectively, for raspberries, whereas 2.6 and 2.9 CFU/g of *Salmonella* and *E. coli* O157:H7, respectively, for strawberries. However, this was not the case for blueberries where reductions were 1.9 and 1.1 log<sub>10</sub> CFU/g for *Salmonella* and *E. coli* O157:H7, respectively. The final treatment consisted of a vacuum followed by a pressurized treatment which did not produce a significantly higher log<sub>10</sub> reduction for either fruit. There was no observable difference in color between untreated and treated raspberries and strawberries, however there was an observable difference in treated versus untreated blueberries. A difference in the way in which each fruit responded to the treatments varied greatly and was most likely due to the shape of the fruit, location or presence of seeds and drupelets, as well as the distribution of cells on the fruit or within the crevices.

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## Chapter 4

### **The efficacy of aqueous ozone for the decontamination of *Escherichia coli* O157:H7 and *Salmonella* on berries**

#### **Abstract**

The efficacy of ozone as a water additive for washing blueberries, raspberries, and strawberries was investigated in this research. Pathogen inoculated fruit were treated with aqueous ozone at 20°C for 2 to 64 min at ozone concentrations of 1.7 to 8.9 mg/L, at 4°C for 64 min at a concentration of 21 mg/L, and with water as a control. Blueberries were treated with ozone at two different temperature had maximum log<sub>10</sub> reductions of 5.2 CFU/g of *E. coli* O157:H7 and 6.2 CFU/g of *Salmonella* at 4°C. Ozone was applied on raspberries at two different temperatures with a maximum log<sub>10</sub> reduction of 5.6 CFU/g of *E. coli* O157:H7 at 4°C and 4.5 CFU/g of *Salmonella* at 4°C. Washing with water (sparging with air) as a control resulted in reductions of only 1 log<sub>10</sub> CFU/g. The results presented here indicate that aqueous ozone has a potential to be used as decontamination method for small fruits.

## 4.1 Introduction

Each year *Escherichia coli* O157 infections cost the United States approximately \$405 million (Frenzen et al., 2005) and *Salmonella* infections result in \$3 billion in medical expenses and lost productivity (ERS, 2005). Fresh produce has been increasingly implicated in many of these infections; it is now the second leading cause of foodborne illness having been responsible for 428 outbreaks between 1990 and 2003 (CSPI, 2004).

Concern about the microbial safety of small fruits has arisen in recent years due to their implication in several notable outbreaks. Strawberries have been responsible for three separate outbreaks of Hepatitis A (CDC, 1997a) and raspberries have been to blame for five outbreaks of cyclosporiasis (CDC, 1997b). The production practices of these small fruits make them especially vulnerable to contamination. The fruits are not washed or treated prior to market and can become contaminated from a variety of sources: improper sanitation, infected pickers, contaminated irrigation water, and manure fertilized fields are all potential culprits for contamination (Han et al., 2004). Research has shown that pathogens like *E. coli* O157:H7, *Listeria monocytogenes* and *Salmonella* are capable of surviving on the surfaces of strawberries for over 7 days (Flessa et al., 2005; Knudsen et al., 2001) and a FDA survey found that 1 out of 143 samples of imported strawberries were positive for *Salmonella* contamination (FDA, 1999).

The use of water or commercial sanitizers has shown very limited efficacy. Yu et al. (2001) evaluated the efficacy of five different sanitizers for the purpose

of decontaminating *E. coli* O157:H7 on strawberries. They investigated sodium hypochlorite, Tween 80, acetic acid, sodium phosphate, and hydrogen peroxide at varying levels. The maximum reduction was reported as 2.2 log<sub>10</sub> CFU/g using hydrogen peroxide at a concentration of 3%. They also found that when strawberries were treated with water alone a reduction of 0.8 log<sub>10</sub> CFU/g of *E. coli* O157:H7 was achieved.

The use of ozone as an aqueous sanitizer has shown promise based on past research and commercial uses. Ozone has been used to purify drinking water since the late nineteenth century (Graham, 1997), and is one of the most powerful oxidizers available. Ozone decays into oxygen in a relatively short amount of time so there is not harmful residue left behind, as is the case with some sanitizers, such as chlorine. In 2001, ozone was granted approval for the treatment of raw commodities (Federal Register, 2001). Gaseous ozone is generated on site with an ozone generator and dissolved into water to make aqueous ozone.

Aqueous ozone has shown to be an effective sanitizing agent for food-related microorganisms. Kim and Yousef (2000) found that a 3.8 log<sub>10</sub> CFU/g reduction in *E. coli* O157:H7 could be achieved with 1.0 mg/L aqueous ozone after only 10 s and Dave et al. (1998) attained a 6 log<sub>10</sub> CFU/ml of *Salmonella* Enteritidis using 1.5 mg/L aqueous ozone. Therefore, aqueous ozone is very efficient in killing suspended-cells in water even at very low ozone concentrations. However, ozone may not be as effective if microorganisms are on foods or in the crevices of the food surface. Therefore, the concentrations of

ozone and treatment times need to be increased. Research has shown that aqueous ozone is effective at inactivating *E. coli* O157:H7 on alfalfa sprouts and seeds at a concentration of 21 mg/L (Sharma et al., 2002).

Previous studies concerning the efficacy of aqueous ozone have found that its ability to inactivate microorganisms is very dependent on the food product involved and results have been somewhat inconsistent. Reports on the efficacy of aqueous ozone for the purpose of treating lettuce have found reductions ranging from 4.6 log<sub>10</sub> CFU/g to just 1.5 log<sub>10</sub> CFU/g (Kim et al., 1999; Koseki et al., 2001). Kim et al. (1999) reported the reduction of 4.6 log<sub>10</sub> CFU/g of aerobic microorganisms at lower concentration, 1.3 mg/L, and 5-min treatment time, while Koseki et al. (2001) reported a reduction of 1.5 log<sub>10</sub> CFU/g after treatment with 5 mg/L aqueous ozone for 10-min treatment time. The roughness of the surface and presence of achenes make strawberries, and other small fruits, difficult to decontaminate (Yu et al., 2001). Also, the space between the drupelets of the raspberries makes them difficult to decontaminate as well.

Based on previous research and the ineptitude of conventional sanitizers this research was undertaken to determine if the use of aqueous ozone would be a suitable treatment method for various berries.

## 4.2 Materials and Methods

### 4.2.1 Preparation of inoculum

Five strains of nalidixic acid resistant *E. coli* O157:H7 and *Salmonella* were obtained from the Center for Food Safety at the University of Georgia. The *E. coli* O157:H7 strains were: 932 (human isolate), 994 (salami isolate), E0018 (calf fecal isolate), H1730 (human isolate from outbreak associated with lettuce), and F4546 (human isolate from outbreak associated with alfalfa sprouts). The *Salmonella* serotypes used were: Agona (human isolate from outbreak associated with alfalfa sprouts), Baildon (human isolate from outbreak associated with diced tomatoes), Gaminara (orange juice isolate), Michigan (human isolate associated with cantaloupe outbreak), and Montevideo (human isolate associated with tomato outbreak). Cultures were grown in tryptic soy broth (Difco, Detroit, MI) supplemented with 50 µg/ml nalidixic acid (Fisher Scientific Co., Fair Lawn, NJ) at 37°C for 24 h. A mixture of *E. coli* O157:H7 or *Salmonella* strains were prepared by combining 10 ml of each culture and centrifuging for 15 min at 3,300 x *g* and 4°C. The supernatant was discarded and the cells were resuspended in 10 ml of 0.1% peptone water (Difco) to yield an approximate population of 10<sup>8</sup> CFU/ml.

#### 4.2.2 Inoculation of small fruits

Fresh blueberries, red raspberries, and strawberries were purchased from a local grocery store and left at room temperature for 1 h prior to inoculation. To inoculate the blueberries and raspberries, 25  $\mu\text{L}$  of inoculum was deposited on the skin of each fruit. For strawberries, 50  $\mu\text{L}$  of inoculum was deposited on the skin of each strawberry, approximately midway between the calyx and cap (Yu et al., 2001). The fruits were dried in a laminar flow hood for 24 h before the treatment to allow for attachment of the microorganisms. Both inoculated raspberries and strawberries had approximately  $10^5$  CFU/g of both *E. coli* O157:H7 and *Salmonella*.

#### 4.2.3 Production and delivery of ozone

Ozone gas was generated using a lab-scale ozone generator (Model No. H-50, Hess Machines International, Ephrata, PA) equipped with an oxygen concentrator. Gas was delivered at a flow rate of 0.34  $\text{m}^3/\text{h}$  and an ozone concentration of 5% (wt/wt) which was measured using a bench-top ozone analyzer (Model 450H, Teledyne Technologies Inc., Los Angeles, CA). A 500 ml flask containing sterile deionized water was sparged with ozone (Figure 4.1) for various times and temperatures, and excess ozone was passed through a 2% (wt/v) potassium iodide solution to prevent ozone from being released into the environment. Furthermore, the ozone treatment was performed in a fume hood for safety considerations.

#### 4.2.4 Treatment with ozone

A sample of five inoculated strawberries or eighteen inoculated blueberries or raspberries was placed in a flask containing 500 ml of sterile deionized water and a stainless steel sparger with a pore size of 10  $\mu\text{m}$  (Figure 4.1). Fruits were subjected to ozone treatments, at 20°C and at 4°C. The effect of ozone solubility was investigated in this study by lowering the treatment water temperature; because it is a known fact that solubility of gases increases in solutions as temperature decreases. Ozone was sparged into the water for times of 2, 4, 8, 16, 32 and 64 min at 20°C and sparged for 64 min at 4°C. Water sparged with air for 64 min was used as a control for both treatment scenarios. Temperature was maintained by placing flasks in a water bath at the appropriate settings.

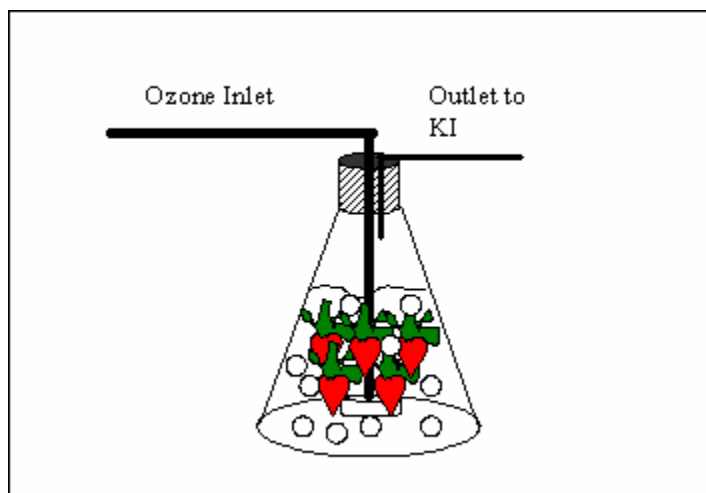


Figure 4.1 Schematic of ozone treatment.

#### 4.2.5 Aqueous ozone analysis

The final ozone concentration in the treatment flask, but with no fruits, was measured for each sparging time. Actual ozone concentrations during treatment were expected to be less due to the degradation of ozone during the treatment by microorganisms and other organic matters. Ozone concentration was measured using direct UV absorption at 258 nm. The concentration was calculated using the following formula (Sharma et al., 2001).

$$c = \frac{48,000 * A}{2,900} \quad \text{Eqn. 4.1}$$

where,  $c$  = concentration of ozone in water (mg/L)

$A$  = absorbance value at UV 258 nm

Furthermore, the volumetric mass transfer coefficient ( $k_L a$ ) was determined using Eqn. 4.2 (Demirci, 2002). This value is a measure of the rate of ozone use in the system and can be helpful when scaling up a process.  $k_L a$  was determined using the following formula:

$$\frac{dC}{dt} = k_L a (C^* - C) \quad \text{Eqn. 4.2}$$

where  $k_L a$  :the volumetric mass transfer coefficient ( $\text{min}^{-1}$ )

$C^*$  :the liquid-phase ozone concentration at equilibrium (mg/L)

$C$  :the ozone concentration at a given time (mg/L)



#### 4.2.6 Microbial analysis

After treatment, strawberries were placed in 50 ml of Dey-Engley Neutralizing (D/E) Broth (Difco) and blueberries and raspberries were placed in 25 ml D/E broth and pummeled for 1 min in a stomacher. The homogenate was then serially diluted in 0.1% peptone water (Difco) and spiral plated on tryptic soy agar (Difco) supplemented with 50 µg/ml of nalidixic acid (TSBN) with an Autoplate 4000 (Spiral Biotech, Norwood, MA). Plates were incubated at 37°C for 24 h and then enumerated using Q-count (Version 2.1, Spiral Biotech, Norwood, MA). Reductions of bacteria were calculated on a per gram of fruit basis. Random colonies of *E. coli* O157:H7 and *Salmonella* were confirmed serologically using RIM *E. coli* O157:H7 latex test (Remel Microbiology Products, Lenexa, KS) and *Salmonella* O Antiserum A-1 latex agglutination test (Remel). Enrichments were performed for samples demonstrating zero plate counts. For both *E. coli* O157:H7 and *Salmonella* 1 ml of 0.1% peptone water was transferred to 9 ml of TSBN and incubated at 37°C for 24 h. After incubation 1 ml of the TSBN was transferred to 9 ml either TT Broth Base Hajna (Difco) or MacConkey broth (Difco), for *Salmonella* and *E. coli* O157:H7, respectively. TT Broth Base Hajna was incubated at 45°C for 48 h. A loopful of enrichment solution was then streaked onto Xylose Lysine Desoxycholate (XLD) agar (Difco) and incubated for 24 h at 37°C. Colonies were again confirmed using *Salmonella* O Antiserum A-1 latex agglutination test. MacConkey broth tubes were incubated for 24 h at 37°C and then a loopful of solution was streaked onto

MacConkey agar plates which were incubated for 24 h at 37°C. Colonies were then confirmed at *E. coli* O157:H7 using a RIM *E. coli* O157:H7 latex test.

#### **4.2.7 Color analysis**

To determine whether treatment with ozone had any negative effects on quality, the fruit color analysis was performed on fruits from the treatment with the highest microbial reduction. A Minolta Chromo Meter CR200 colorimeter (Minolta, Ramsey, NJ) was used to measure the L\*a\*b\* color space. The color space uses the following parameters: L\* indicates the lightness, a\* and b\* are the chromaticity coordinates. Value -a\* indicates a green color, +a\* a red color, -b\* a blue color, and +b\* a yellow color. Prior to use, the chromameter was calibrated using a white tile. Two randomly selected spots were analyzed and averaged to get an overall measurement for each fruit and replicated three times.

#### **4.2.8 Statistical analysis**

All experiments were replicated three times and MINITAB (Version 13, MINITAB, State College, PA) statistical software was used to analyze the mean log<sub>10</sub> reductions. A one-way ANOVA with a 95% confidence level was used to compare the treatment times and scenarios. A Tukey's comparison was also performed to determine significant differences using a P-value less than or equal to 0.05.

### 4.3 Results and Discussion

In this study, the efficacy of ozone for the decontamination of *E. coli* O157:H7 and *Salmonella* on blueberries, raspberries, and strawberries was evaluated by sparging ozone gas (5%, wt/wt) through water at 4 or 20°C. The effects of ozone when used as an additive to water were investigated at two different temperatures. Control fruits were treated similarly, except with water being sparged with air.

Final ozone concentrations were measured in the treatment flask with no fruits and were 1.7, 1.8, 3.7, 7.6, 7.9, and 8.9 mg/L for 2-, 4-, 8-, 16-, 32-, and 64-min, respectively at 20°C, whereas, the final ozone concentration at 4°C was 21 mg/L for a 64-min treatment time. The volumetric mass transfer coefficient ( $k_{La}$ ) was determined at 20°C to further characterize the system, and was 0.126 min<sup>-1</sup>.

#### 4.3.1 Treatment of blueberries

Blueberries inoculated with *E. coli* O157:H7 and *Salmonella* were treated with ozonated water at 20 or 4°C. At 20°C, reductions of *E. coli* O157:H7 ranged from 1.3 to 4.9 log<sub>10</sub> CFU/g for 2- and 64-min treatments, respectively (Table 4.1). The reduction achieved after 64-min of treatment was significantly higher than the reductions obtained at lower treatment times.

Table 4.1. Population reduction of *E. coli* O157:H7 and *Salmonella* on blueberries treated with aqueous ozone at 20°C.

Organism	Treatment Time (min)	Ozone Concentration mg/L	Log <sub>10</sub> Reduction (CFU/g) <sup>a,b,d</sup>
<i>E. coli</i> O157:H7	2	1.7	1.3 ± 0.2A
	4	1.8	1.3 ± 0.5A
	8	3.7	1.5 ± 0.1A
	16	7.6	2.5 ± 1.2A
	32	7.9	2.5 ± 1.1A
	64	8.9	4.9 ± 1.0B <sup>c</sup>
<i>Salmonella</i>	2	1.7	0.7 ± 0.1A
	4	1.8	1.7 ± 0.2AB
	8	3.7	2.4 ± 0.7AB
	16	7.6	3.5 ± 0.9BC
	32	7.9	4.9 ± 0.9C
	64	8.9	4.7 ± 1.3C <sup>c</sup>

<sup>a</sup>Within the same column and microorganism, values not followed by the same upper case letter are significantly different.

<sup>b</sup>Average weight of blueberry sample is 30.0 ± 2.5 g

<sup>c</sup>Detection limit was ~1.5 × 10<sup>1</sup> CFU/ml. Enrichments after treatment were positive for *E. coli* O157 and *Salmonella*, respectively.

<sup>d</sup>Values are mean reductions ± standard deviation.

Table 4.2. Population reduction of *E. coli* O157:H7 and *Salmonella* on blueberries after treatment with aqueous ozone or air at 20°C and 4°C for 64 min.

Organism	Treatment		Ozone Concentration (mg/L)	Log <sub>10</sub> Reduction <sup>a,b</sup>
<i>E. coli</i> O157:H7	20°C	Ozone	8.9	4.9 ± 1.0A
		Air	N/A	2.1 ± 0.5B
	4°C	Ozone	21	5.2 ± 0.0A
		Air	N/A	2.3 ± 0.0B
<i>Salmonella</i>	20°C	Ozone	8.9	4.7 ± 1.3A
		Air	N/A	1.9 ± 0.3B
	4°C	Ozone	21	6.2 ± 0.0A
		Air	N/A	1.1 ± 0.1B

<sup>a</sup>Within the same column and microorganism, values not followed by the same letter are significantly different.

<sup>b</sup>Values are mean reductions ± standard deviation.

When the temperature was decreased to 4°C the log<sub>10</sub> reductions increased to 5.2 log<sub>10</sub> CFU/g from 4.9 log<sub>10</sub> CFU/g at 20°C; furthermore, this treatment resulted in zero plate counts of *E. coli* O157:H7 with 2 out of 3 enrichments negative for *E. coli* O157:H7 (Table 4.2). At both 20 and 4°C, the log<sub>10</sub> reductions achieved after treatment with ozone were significantly higher than the treatment with air; 4.9 versus 2.1 log<sub>10</sub> CFU/g at 20°C, and 5.2 versus 2.3 log<sub>10</sub> CFU/g at 4°C.

Reductions of *Salmonella* were between 0.7 and 4.9 log<sub>10</sub> CFU/g for times of 2- and 32-min, respectively (Table 4.1) at 20°C. The treatment times of 32- and 64-min had significantly higher log<sub>10</sub> reductions, 4.9 and 4.7 CFU/g, respectively, than treatments less than 8 min. There was no significant difference between the 16-, 32-, and 64-min treatments. When the temperature was decreased to 4°C, the log<sub>10</sub> reduction increased to 6.2 CFU/g (Table 4.2). This treatment resulted in zero plate counts, and 2 out of 3 enrichments were negative for *Salmonella*. Both the 20 and 4°C ozone treatments resulted in significantly higher reductions than the air treatments, for which log<sub>10</sub> reductions were 1.9 and 1.1 CFU/g at 20 and 4°C, respectively.

#### **4.3.2 Treatment of raspberries**

Raspberries inoculated with either *E. coli* O157:H7 or *Salmonella* were treated with ozone at 20°C and 4°C. At 20°C, reductions of *E. coli* O157:H7 ranged from 2.6 to 4.8 log<sub>10</sub> CFU/g for 2- and 32-min treatment times, respectively (Table 4.3). Only the treatments at 32-min and 64-min, which demonstrated reductions of 4.8 log<sub>10</sub> CFU/g, resulted in significantly higher reductions than the 2-min treatment time. It should be noted, that the reductions after 16-min of treatment decreased when compared to those at 8-min or 32-min of treatment. The reason for the decrease is not known, but the low standard deviation indicates that it is most likely not experimental error.

Table 4.3. Population reductions of *E. coli* O157:H7 and *Salmonella* on raspberries treated with aqueous ozone at 20°C.

Organism	Treatment Time (min)	Ozone Concentration (mg/L)	Log <sub>10</sub> Reduction (CFU/g) <sup>a,b,d</sup>
<i>E. coli</i> O157:H7	2	1.7	2.6±0.0A
	4	1.8	3.6±1.4AB
	8	3.7	4.3±1.4AB
	16	7.6	2.7±0.6A
	32	7.9	4.8±0.3B <sup>c</sup>
	64	8.9	4.8±0.3B <sup>c</sup>
<i>Salmonella</i>	2	1.7	1.3±0.1A
	4	1.8	1.4±0.1A
	8	3.7	2.2±0.7AB
	16	7.6	2.7±0.9ABC
	32	7.9	3.5±1.1BC
	64	8.9	4.4±0.9C <sup>c</sup>

<sup>a</sup>Average weight of raspberry sample is 17.5±2.5 g.

<sup>b</sup>Within the same column and microorganism, values not followed by the same upper case letter are significantly different (P<0.05).

<sup>c</sup>Detection limit was ~1.5x10<sup>1</sup> CFU/ml. Enrichments after treatment were positive for *E. coli* O157:H7 and *Salmonella*, respectively.

<sup>d</sup>Values are mean reductions ± standard deviation.

Table 4.4. Population reduction of *E. coli* O157:H7 and *Salmonella* on raspberries after treatment with aqueous ozone or air at 20°C and 4°C for 64 min.

Organism	Treatment		Ozone Concentration (mg/L)	Log <sub>10</sub> Reduction <sup>a,b</sup>
<i>E. coli</i> O157:H7	20°C	Ozone	8.9	4.8±0.3A
		Air	N/A	1.2±1.0B
	4°C	Ozone	21	5.6±0.2A
		Air	N/A	1.3±0.9B
<i>Salmonella</i>	20°C	Ozone	8.9	4.4±0.9A
		Air	N/A	0.9±0.6B
	4°C	Ozone	21	4.5±0.0A
		Air	N/A	1.1±0.4B

<sup>a</sup> Within the same column and microorganism, values not followed by the same letter are significantly different (P<0.05).

<sup>b</sup> Values are mean reductions ± standard deviation.

By decreasing the treatment temperature to 4°C, the concentration of ozone increased from 8.9 mg/L to 21 mg/L for the 64-min treatment. This increased log<sub>10</sub> reductions to 5.6 CFU/g after treatment for 64-min compared to 4.8 CFU/g at 20°C for 64-min (Table 4.4). However, the log<sub>10</sub> reductions were not significantly different. At both conditions the log<sub>10</sub> reduction was significantly



higher with ozone than for air at 4°C, 5.6 log<sub>10</sub> CFU/g versus 1.3 log<sub>10</sub> CFU/g, and at 20°C, 4.8 log<sub>10</sub> CFU/g versus 1.2 log<sub>10</sub> CFU/g.

Reductions of *Salmonella* at 20°C ranged from 1.3 to 4.4 log<sub>10</sub> CFU/g for 2-min and 64-min, respectively (Table 4.3). The 64-min treatment time resulted in a significantly higher population reduction than the 2-, 4-, and 8-min treatments (1.3, 1.4, and 2.2 log<sub>10</sub> CFU/g compared to 4.4 log<sub>10</sub> CFU/g). When the temperature was lowered to 4°C, which increased the ozone concentration to 21 mg/L, the population reduction only increased to 4.5 log<sub>10</sub> CFU/g for *Salmonella* which was not significantly different than the 64-min 20°C treatment (4.4 log<sub>10</sub> CFU/g (Table 4.4)). As a control, raspberries were treated with air instead of ozone at both 20°C and 4°C to determine whether bacterial reduction was due to ozone presence or to the agitation supplied by the gas. The resulting reductions were 0.9 and 1.1 log<sub>10</sub> CFU/g for 20°C and 4°C, respectively. Statistical analysis indicated that these values were significantly lower than reductions resulting from ozone treatment.

#### **4.3.3 Treatment of strawberries**

Strawberries inoculated with *E. coli* O157:H7 and *Salmonella* were treated with aqueous ozone at both 20°C and 4°C. Reductions of *E. coli* O157:H7 were 1.0 to 2.9 log<sub>10</sub> CFU/g from times of 2-min to 64-min at 20°C (Table 4.5). The 64-min treatment resulted in significantly higher log<sub>10</sub> reductions than lower treatment times. At 4°C the log<sub>10</sub> reduction at 64-min was 2.6 CFU/g. Consistent

with what was observed for raspberries, the lethal portion of the treatment was determined to be ozone exposure as opposed to agitation, with ozone treatments demonstrating significantly higher  $\log_{10}$  reductions than treatment with air (Table 4.6). A  $\log_{10}$  reduction of 1.6 CFU/g resulted after a 64-min air treatment compared to the comparable ozone treatment with a reduction of 2.9 CFU/g. Reductions of *Salmonella* were between 0.5 and 3.3  $\log_{10}$  CFU/g from 2-min to 64-min at 20°C (Table 4.5). The 64-min treatment time resulted in significantly higher  $\log_{10}$  reductions than lower treatment times. As with *E. coli* O157:H7, reductions obtained at 4°C for 64-min did not result in significantly higher reductions than at 20°C for 64-min. An average reduction at 4°C of 2.4  $\log_{10}$  CFU/g was obtained compared to 3.3  $\log_{10}$  CFU/g at 20°C. The data presented here indicates the significant effect of ozone versus the agitation supplied by the gas. When fruits were treated with air sparging, a significantly lower  $\log_{10}$  reduction of *Salmonella* was observed. Air alone resulted in a 0.9  $\log_{10}$  CFU/g and a 1.4  $\log_{10}$  CFU/g reduction in *Salmonella* at 20°C and 4°C, respectively.

Table 4.5. Population reductions of *E. coli* O157:H7 and *Salmonella* on strawberries treated with aqueous ozone at 20°C.

Organism	Treatment Time (min)	Ozone Concentration (mg/L)	Log <sub>10</sub> Reduction (CFU/g) <sup>a,b,c</sup>
<i>E. coli</i> O157:H7	2	1.7	1.0±0.2A
	4	1.8	1.3±0.2AB
	8	3.7	1.5±0.2B
	16	7.6	1.8±0.3B
	32	7.9	1.7±0.1B
	64	8.9	2.9±0.7C
<i>Salmonella</i>	2	1.7	0.5±0.2A
	4	1.8	1.4±0.4AB
	8	3.7	1.5±1.1AB
	16	7.6	1.8±0.2B
	32	7.9	2.1±0.4B
	64	8.9	3.3±0.6C

<sup>a</sup>Average weight of strawberry sample is 110±5 g.

<sup>b</sup> Within the same column and microorganism, values not followed by the same letter are significantly different (P<0.05).

<sup>c</sup>Values are mean reductions ± standard deviation.

Table 4.6. Population reduction of *E. coli* O157:H7 and *Salmonella* on strawberries after treatment with aqueous ozone or air at 20°C and 4°C for 64 min.

Organism	Treatment		Ozone Concentration (mg/L)	Log <sub>10</sub> Reduction <sup>a,b</sup>
<i>E. coli</i> O157:H7	20°C	Ozone	8.9	2.9±0.7A
		Air	N/A	1.6±0.6B
	4°C	Ozone	21	2.6±0.1A
		Air	N/A	1.3±0.5B
<i>Salmonella</i>	20°C	Ozone	8.9	3.3±0.6A
		Air	N/A	0.9±0.1B
	4°C	Ozone	21	2.4±0.5C
		Air	N/A	1.4±0.2B

<sup>a</sup> Within the same column and microorganism, values not followed by the same letter are significantly different (P<0.05).

<sup>b</sup> Values are mean reductions ± standard deviation.

#### 4.3.4 Color analysis

Fruits from the most effective treatment times were analyzed for color to determine whether ozone treatment had any negative effects. The treatment selected for blueberries, raspberries, and strawberries, was the 64-min treatment at 20°C. Blueberries treated with ozone had mean L\*, a\*, b\* values of 35.25, -

1.71, and -2.41 compared to the untreated values of 35.95, -1.32, and -2.56 which were not significantly different (Table 4.7). Treated raspberries had a mean L\*, a\*, b\* values of 29.64, +21.94, and +11.77 compared to the untreated raspberries which had values of 29.20, +20.25, and +11.66, respectively. None of the differences were significant. For strawberries the mean L\*, a\*, b\* values were 34.10, +30.08, and +19.36, which were not significantly different from the untreated strawberries which had values of 33.17, +25.94, and +17.30, respectively. It is not know what the long term effect aqueous ozone may have on the color of the berries since measurements were taken immediately after treatment.

Table 4.7. L\*a\*b\* color readings for blueberries, raspberries, and strawberries after ozone treatment.

		L * value <sup>c,d,e</sup>	a * value	b * value
Blueberry <sup>a</sup>	Untreated	35.95 ± 2.71A	-1.32 ± 1.14A	-2.56 ± 1.36A
	Treated	35.25 ± 2.08A	-1.71 ± 1.07A	-2.41 ± 0.99A
Raspberry <sup>a</sup>	Untreated	29.20 ± 1.85A	20.25 ± 2.22A	11.66 ± 1.68A
	Treated	29.64 ± 2.93A	21.94 ± 3.07A	11.77 ± 2.18A
Strawberry <sup>b</sup>	Untreated	33.17 ± 1.94A	25.94 ± 4.15A	17.30 ± 3.19A
	Treated	34.10 ± 1.96A	30.08 ± 1.77A	19.36 ± 2.71A

<sup>a</sup>Each replication consisted of 9 samples with 2 readings per sample.

<sup>b</sup>Each replication consisted of 3 samples with 2 readings per sample.

<sup>c</sup>Treatment was 64-min at 20°C.

<sup>d</sup>Values within the same row followed by the same letter are not significantly different (P>0.05).

<sup>e</sup>Values are means ± standard deviation.

#### 4.4 Conclusions

The research presented here indicates that aqueous ozone is a promising sanitizer for berries. Blueberries were treated with ozone at two different temperatures and had maximum  $\log_{10}$  reductions of 5.2 CFU/g of *E. coli* O157:H7 and 6.2 CFU/g of *Salmonella* at 4°C. Ozone was applied on raspberries at two different temperatures with a maximum  $\log_{10}$  reduction of 5.6 CFU/g of *E. coli* O157:H7 at 4°C and 4.5 CFU/g of *Salmonella* at 4°C. The results obtained using aqueous ozone were significantly higher than washing with water alone which produced a maximum reduction of 1.3 and 1.1  $\log_{10}$  CFU/g for *E. coli* O157:H7 and *Salmonella*, respectively.  $\log_{10}$  reductions on strawberries were slightly lower with maximum reductions of 2.9 and 3.3 CFU/g of *E. coli* O157:H7 and *Salmonella*, respectively, at 20°C and were significantly higher than washing with water alone. The treatment temperature made no significant difference on the efficacy of the sanitizer for raspberries and strawberries, so treatment could be performed at room temperature, 20°C, resulting in less energy use. There was a noticeable difference in the 4°C treatment versus the 20°C treatment in the number of positive enrichments. No difference was observed in the color of the fruits after treatment visually or with the colorimeter; however a textural difference was observable for raspberries but this was not quantified. This study demonstrated that aqueous ozone could be an effective sanitizer for small fruits.

#### 4.5 References

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## Chapter 5

### **The efficacy of pulsed UV-light for the decontamination of *Escherichia coli* O157:H7 and *Salmonella* on berries.**

#### **Abstract**

Small fruits are increasingly being implicated in outbreaks of foodborne illness, and fresh produce is now the second leading cause of foodborne illness in the U.S. Conventional methods of decontamination are not effective, and there is a need to evaluate novel technologies. Pulsed ultra-violet (UV)-light is one such technology. In this study, pulsed UV-light was applied to blueberries, strawberries, and raspberries at varying UV doses and times. On raspberries, maximum reductions of *Escherichia coli* O157:H7 and *Salmonella* were 3.9 and 3.4 log<sub>10</sub> CFU/g at 72 and 59.2 J/cm<sup>2</sup>, respectively. On the surfaces of strawberries maximum reductions were 2.1 and 2.8 log<sub>10</sub> CFU/g at 25.7 and 34.2 J/cm<sup>2</sup>, respectively. Maximum reductions of 4.3 and 2.9 log<sub>10</sub> CFU/g were achieved on blueberries after a UV dose of 22.6 J/cm<sup>2</sup> for *E. coli* O157:H7 and *Salmonella*, respectively. There was no observable damage to the fruits at these UV doses. The results obtained in this study indicate that pulsed UV-light has the potential to be used as a decontamination method for blueberries, raspberries, and strawberries.

## 5.1 Introduction

Each year foodborne illnesses cost the U.S. economy \$6.9 billion in loss productivity and medical expenses (ERS, 2005). Fresh produce has been increasing as the vehicle of transmission over the last decade with 23,857 cases reported between 1990 and 2003 with 16% of these cases tied to fruits (CSPI, 2004). An estimated 73,000 cases of *Escherichia coli* O157 infections (**Error! Reference source not found.**) and 2,000,000 salmonellosis infections are reported each year in the U.S.

With increasing numbers of outbreaks tied to fresh foods, there is a need to evaluate novel processing technologies that do not destroy the integrity of the product: ultra-violet (UV) light is one such technology. UV-light inactivates a microorganism by damaging its DNA, so that pyrimidine dimers form which prevents the cell from replicating (Rowan et al., 1999). UV-light can be applied in two ways, continuously or as a pulse. The benefit of applying pulsed UV-light is that processing times can be reduced due to an increase in instantaneous energy. Also, research has indicated that pulsed UV-light is a more efficient and effective method of application (Miller et al., 1999).

The efficacy of pulsed UV-light has been documented both for inactivating foodborne microorganisms in suspension as well as in food. Rowan et al. (1999) investigated the effects of pulsed UV-light on food-related microorganisms. Populations of *Listeria monocytogenes*, *Escherichia coli*, *Salmonella* Enteritidis, *Pseudomonas aeruginosa*, *Bacillus cereus*, and *Staphylococcus aureus* that

were seeded on tryptone soya-yeast agar media were exposed to pulsed light having either high or low content UV light. Reductions between 2 and 6 log<sub>10</sub> CFU/ml were attained using 200 pulses with low UV content and high content, respectively. Krishnamurthy et al. (2004) investigated the use of pulsed UV light to inactivate *S. aureus* in buffer solution and on agar seeded plates. They found a 7 to 8 log<sub>10</sub> CFU/ml reduction of *S. aureus* on seeded agar plates and buffer solution at treatment times less than 5 s without significant temperature increase. Sharma and Demirci (2003) exposed alfalfa seeds inoculated with *E. coli* O157:H7 to pulsed UV-light. They found that when a seed layer thickness of 1.02 mm was treated for 30 s a 4.80 log<sub>10</sub> CFU/g reduction was achieved.

Small fruits, such as raspberries and strawberries, have been implicated in several notable outbreaks. Raspberries have been implicated in at least five outbreaks of *Cyclospora cayetanensis* (CDC, 1997b) and strawberries have been implicated in three outbreaks of Hepatitis A (CDC, 1997a). While there have been no recorded bacterial outbreaks associated with small fruits, the possibility exists, since the contamination routes responsible for previous outbreaks are the same for bacterial pathogens. A United States Food and Drug Administration (FDA) survey found that 1 out of 143 imported strawberry samples tested positive for *Salmonella* (FDA, 1999). Also, research has shown that both *Salmonella* and *E. coli* O157:H7 are capable of surviving on fresh strawberries for over 7 days (Knudsen et al., 2001).

Throughout the production of small fruits, the opportunity for contamination exists due to improper sanitation, infected pickers, contaminated

irrigation water, and manure fertilized fields (Han et al., 2004). In spite of these risks, small fruits are not washed prior to delivery to market, due to the negative effect on fruit quality and shelf life. However, washing alone has been shown to have limited efficacy at removing both spoilage and pathogenic bacteria from the surfaces of produce and conventional sanitizers have also shown limited efficacy (Yuk et al., 2006; Han et al., 2004; Yu et al., 2001). Yu et al. (2001) compared five sanitizers for the purpose of reducing populations of *E. coli* O157:H7 on strawberries. Of these five sanitizers the most effective was found to be hydrogen peroxide which gave a reduction of 2.2 log<sub>10</sub> CFU/g.

The use of UV-C light for the purpose of extending the shelf-life of berries has been well documented. Boysenberries treated with 0.92 J/cm<sup>2</sup> exhibited druplet damage and texture which was equivalent to a 45°C heat treatment for 1 h. They also noted compared to untreated, that treated berries had reduced softening during storage, lower respiration rates and decreased anthocyanin leakage (Vicente et al., 2004). Baka et al. (1999) observed similar benefits of UV-C light treatment on strawberries. Strawberries treated with 0.1 J/cm<sup>2</sup> had a 5 day longer storage life, lower respiration rates, firmer texture, and increased anthocyanin content compared to untreated. It has also been shown that there is a hormetic effect from exposure to UV-C light which increases the resistance of a fruit to pathogens, which is believed to induce the formation of certain compound which can aid in the formation of phenolic compounds, like anthocyanins (Guerrero-Beltran and Barbosa-Canovas, 2004). Based on the history of small fruit associated foodborne outbreaks, the low efficacy of chemical sanitizers, and

the potential bactericidal as well as quality benefits the evaluation of pulsed UV-light for the purpose of decontaminating blueberries, raspberries, and strawberries was undertaken in this study.

## **5.2 Materials and Methods**

### **5.2.1 Preparation of inoculum**

Five strains of nalidixic acid resistant *E. coli* O157:H7 and *Salmonella* were obtained from the Center for Food Safety at the University of Georgia. The *E. coli* O157:H7 strains were: 932 (human isolate), 994 (salami isolate), E0018 (calf fecal isolate), H1730 (human isolate from outbreak associated with lettuce), and F4546 (human isolate from outbreak associated with alfalfa sprouts). The *Salmonella* serotypes used were: Agona (human isolate from outbreak associated with alfalfa sprouts), Baildon (human isolate from outbreak associated with diced tomatoes), Gaminara (orange juice isolate), Michigan (human isolate associated with cantaloupe outbreak), and Montevideo (human isolate associated with tomato outbreak). Cultures were grown in tryptic soy broth (Difco, Detroit, MI) supplemented with 50 µg/ml nalidixic acid (Fisher Scientific Co., Fair Lawn, NJ) at 37°C for 24 h. A mixture of *E. coli* O157:H7 or *Salmonella* strains were prepared by combining 10 ml of each culture and centrifuging for 15 min at 3,300 x *g* and 4°C. The supernatant was discarded and the cells were

resuspended in 10 ml of 0.1% peptone water (Difco) to yield an approximate population of  $10^8$  CFU/ml.

### **5.2.2 Inoculation of berries**

Fresh blueberries, red raspberries, and strawberries were purchased from a local grocery store and left at room temperature for 1 h prior to inoculation. To inoculate the blueberries and raspberries, 25  $\mu$ L of inoculum was deposited on the skin of each fruit. For strawberries, 50  $\mu$ L of inoculum was deposited on the skin of each strawberry, approximately midway between the calyx and cap (Yu et al., 2001). The fruits remained in a laminar flow hood for 24 h after inoculation to allow for attachment of the microorganisms. Both inoculated raspberries and strawberries had approximately  $10^5$  CFU/g of both *E. coli* O157:H7 and *Salmonella*.

### **5.2.3 Treatment with pulsed UV-light**

Pulsed light was produced using a laboratory scale pulsed-light system (Figure 5.1, Steripulse-XL 3000, Xenon Corp., Wilmington, MA). As per the manufacturer's specifications the system generated  $1.27 \text{ J/cm}^2$  per pulse of broad band energy (100 to 1100 nm) at 1.8 cm from the quartz window for an input of 3,800 V and with 3 pulses per second setting. It should be noted that the distance between the UV-strobe and the quartz window is 5.8 cm. Fruits were

treated at three different distances from the quartz window; 3, 8, and 13 cm for blueberries and raspberries and 5, 8, and 13 cm for strawberries, as measured from the bottom of the fruit. For strawberries a distance of 5 cm was used instead of 3 cm because at that height the system could not accommodate the fruits due to their larger size.

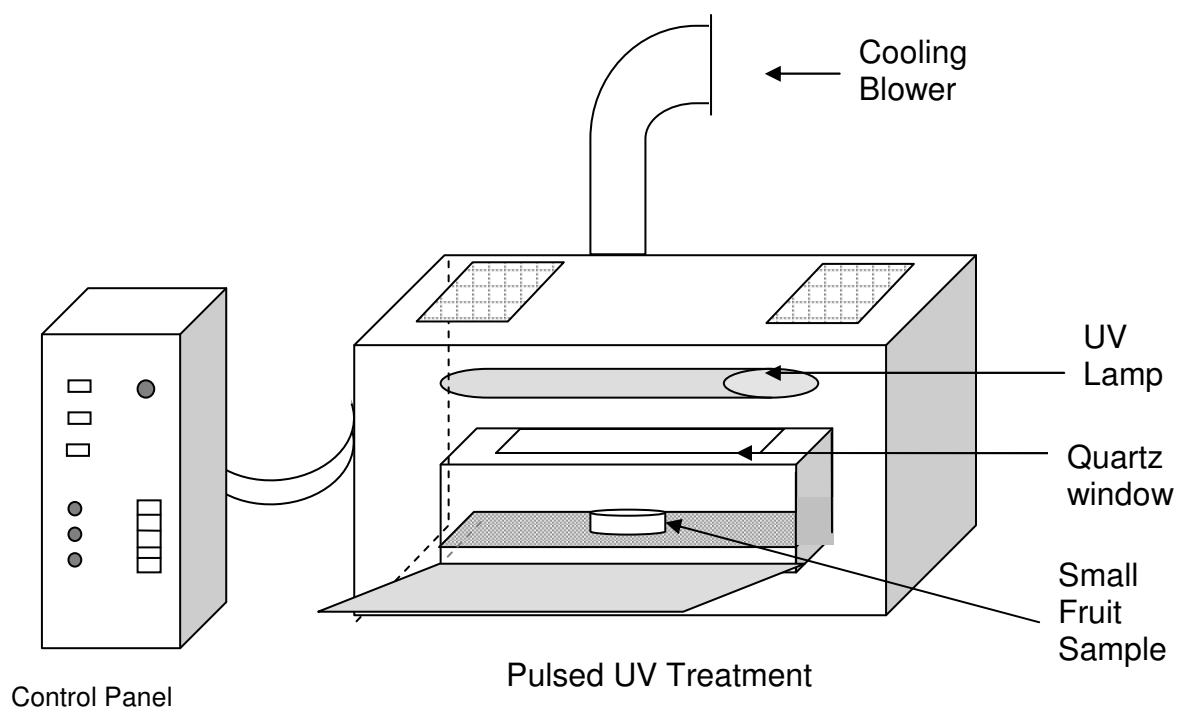


Figure 5.1. Schematic of pulsed UV system.



However, for blueberries a system voltage of 3,800 resulted in significant damage and the system was lowered to 2,400 V to minimize the damage. At each distance from the quartz window, 5, 10, 30, 45, and 60 s treatment times were evaluated. Furthermore, the temperature at the surface of the fruit was monitored using a K-type thermocouple (Omegaette HH306, Omega Engineering, Inc., Stamford, CT) by placing the thermocouple 1-2 mm under the surface of the fruit. The energy at each level was also measured at each treatment level using a Nova Laser Power energy monitor (Ophir Optronics Ltd., Wilmington, MA), which averaged the broad band energy across 30 pulses with the face of the sensor located at the same height as the bottom of the fruit.

#### **5.2.4 Microbial analysis**

After treatment, strawberries were placed in 50 ml of Dey-Engley Neutralizing (D/E) Broth (Difco) and blueberries and raspberries were placed in 25 ml D/E broth and pummeled for 1 min in a stomacher. The homogenate was then serially diluted in 0.1% peptone water (Difco) and spiral plated on tryptic soy agar (Difco) supplemented with 50 µg/ml of nalidixic acid with an Autoplate 4000 (Spiral Biotech, Norwood, MA). Plates were incubated at 37°C for 24 h and then enumerated using Q-count (Version 2.1, Spiral Biotech, Norwood, MA). Reductions of bacteria were calculated on a per gram of fruit basis. Random colonies of *E. coli* O157:H7 and *Salmonella* were confirmed serologically using

RIM *E. coli* O157:H7 latex test (Remel Microbiology Products, Lenexa, KS) and *Salmonella* O Antiserum A-1 latex agglutination test (Remel).

### 5.2.5 Color analysis

As a preliminary measurement of fruit quality immediately after pulsed UV-light treatment a color analysis was performed on fruits from the treatment with the highest microbial reduction. A Minolta Chromo Meter CR200 colorimeter (Minolta, Ramsey, NJ) was used to measure the L\*a\*b\* color space. The color space uses the following parameters: L\* indicates the lightness, a\* and b\* are the chromaticity coordinates. Value -a\* indicates a green color, +a\* a red color, -b\* a blue color, and +b\* a yellow color. Prior to use, the chromameter was calibrated using a white tile. Three randomly selected spots were analyzed and averaged to get an overall measurement for each fruit and replicated three times.

### 5.2.6 Statistical analysis

All experiments were replicated three times and MINITAB statistical software (Version 13, MINITAB, State College, PA) was used to analyze the mean log<sub>10</sub> reductions. A one-way ANOVA with a 95% confidence level was used to compare the treatment times and distance from UV-light. A Tukey's comparison was also performed to determine significant differences using a P-value less than or equal to 0.05. Furthermore, a general linear model was used

to determine the significant factor involved in inactivation and determine if there was any interaction between factors.

## 5.3 Results and Discussion

### 5.3.1 Treatment of raspberries

Raspberries inoculated with *E. coli* O157:H7 and *Salmonella* were treated with pulsed UV-light at fluencies of 0.19, 0.33, and 0.40 J/cm<sup>2</sup>/pulse which corresponded to 13, 8, and 5 cm from the quartz window, respectively. Raspberries were treated at times of 5, 10, 30, 45, and 60 s, which resulted in maximum total UV doses of 34.2, 59.4, and 72 J/cm<sup>2</sup> for 13, 8, and 5 cm, respectively.

On raspberries inoculated with *E. coli* O157:H7 reductions after pulsed UV treatment were between 0.4 and 3.9 log<sub>10</sub> CFU/g at fluencies of 2.9 to 72 J/cm<sup>2</sup>, respectively (Table 5.1). At a distance of 3 cm from the quartz window reductions ranged from 0.9 to 3.9 log<sub>10</sub> CFU/g at times of 5 and 60 s, respectively. ANOVA analysis indicated that a treatment of 60 s resulted in significantly higher log<sub>10</sub> reductions than the lower treatment times. Slightly lower log<sub>10</sub> reductions were observed at treatments conducted at 8 cm from the quartz window; reductions were between 0.7 and 3.0 log<sub>10</sub> CFU/g at times of 5 and 60 s, respectively. The treatment at 8 cm for 60 s resulted in a significantly higher log<sub>10</sub> reduction (3.0 log<sub>10</sub> CFU/g) than the other treatment times.

Table 5.1. Log reductions of *E. coli* O157:H7 and *Salmonella* on raspberries after pulsed UV-light treatment.

Distance from Quartz window <sup>a</sup>	Treatment Time (s)	UV-dose (J/cm <sup>2</sup> )	Log <sub>10</sub> Reduction <sup>b,c,d,e</sup>	
			<i>E. coli</i> O157:H7	<i>Salmonella</i>
3 cm	5	6.0	0.9 ± 0.4A	1.0 ± 0.0A
	10	12.0	1.2 ± 0.1 A	1.2 ± 0.2A
	30	36.0	2.0 ± 0.5A	1.8 ± 0.2A
	45	54.0	2.1 ± 0.5A	2.4 ± 0.6AB
	60	72.0	3.9 ± 0.9B	3.4 ± 1.3B
8 cm	5	4.9	0.7 ± 0.2A	1.2 ± 0.3 A
	10	9.9	0.7 ± 0.4A	1.2 ± 0.2A
	30	29.7	1.5 ± 0.3A	1.7 ± 0.3A
	45	44.5	1.5 ± 0.2A	1.9 ± 0.2A
	60	59.4	3.0 ± 0.6B	3.4 ± 0.9B
13 cm	5	2.9	0.4 ± 0.3A	0.3 ± 0.1 A
	10	5.7	0.9 ± 0.4B	0.6 ± 0.1A
	30	17.1	1.6 ± 0.3B	1.1 ± 0.5A
	45	25.7	1.5 ± 0.3B	2.2 ± 0.1B
	60	34.2	2.6 ± 0.2C	2.9 ± 0.5B

<sup>a</sup>Distance from quartz window to UV strobe is 5.8 cm.

<sup>b</sup>Average weight of raspberry sample is 17.5±2.5 g.

<sup>c</sup>Within the same column and microorganism, values not followed by the same letter are significantly different (P<0.05).

<sup>d</sup>Original populations of 10<sup>6</sup> CFU/g.

<sup>e</sup>Values are mean reductions ± standard deviation.

The final treatment distance was 13 cm from the quartz window, which resulted in reductions of 0.4 to 2.6 log<sub>10</sub> CFU/g at times of 5 and 60 s, respectively. Again the 60-s treatment produced significantly higher log<sub>10</sub> reductions than the lower treatment times.

An analysis of variance using a general linear model indicated that both distance from the quartz window and treatment time were significant factors in the inactivation of *E. coli* O157:H7 on raspberries, but there was no significant interaction. The analysis was also used to compare reductions at the three treatment distances, and it was concluded that a distance of 3 cm resulted in significantly higher log<sub>10</sub> reductions than the 8 and 13-cm distances. However, there was no significant difference between reductions at 8 and 13 cm from the quartz window.

Reductions of *Salmonella* on raspberries ranged from 0.3 to 3.4 log<sub>10</sub> CFU/g at fluencies of 2.9 to 72 J/cm<sup>2</sup>, respectively (Table 5.1). At a distance of 3 cm from the quartz window reductions were between 1.0 and 3.4 log<sub>10</sub> CFU/g at 5 and 60 s, respectively. The ANOVA analysis indicated that there were no treatments that resulted in significantly higher reductions than other treatment times at this level. Reductions at 8 cm from the quartz window were between 1.2 and 3.4 log<sub>10</sub> CFU/g for 5 and 60-s treatments, respectively. At this treatment distance, a time of 60 s produced significantly higher log<sub>10</sub> reductions than other treatment times. The furthest treatment distance, 13 cm, resulted in reductions between 0.3 and 2.9 log<sub>10</sub> CFU/g for 5 and 60-s treatments, respectively. A general linear model was used to determine if distance, time, and an interaction

between distance and time were significant factors in the reduction of *Salmonella* on raspberries. From this analysis, it was concluded that both distance and time were significant factors, but there was no significant interaction. It was also determined that there was no significant difference between the reductions obtained at 3 and 8 cm from the quartz window, and that there was a significant difference between reductions at 8 and 13 cm. From this analysis, it was concluded that the “best” treatment was at 8 cm from the quartz window with a treatment time of 60 s which resulted in a reduction of 3.4 log<sub>10</sub> CFU/g.

Finally, it should be noted that there was a significant increase in the temperature of the raspberry after pulsed UV treatment at all distances from the light. Maximum increases of 60, 55, and 30°C (fruit temperature of 80, 75, and 48°C) were observed at 3, 8, and 13 cm, respectively, after the 60-s treatment. Thus, it is especially important to note that there is a thermal component to the inactivation of pathogens using pulsed UV-light in addition to the UV radiation.

### **5.3.2 Treatment of strawberries**

Reductions of *E. coli* O157:H7 and *Salmonella* on strawberries treated with various fluencies of pulsed UV-light can be seen in Table 5.2. Maximum UV doses were 34.2, 59.4, and 64.8 J/cm<sup>2</sup> at 13, 8, and 5 cm, respectively, after treatment for 60 s.

Table 5.2. Log reductions of *E. coli* O157:H7 and *Salmonella* on strawberries after pulsed UV-light treatment.

Distance from Quartz window <sup>a</sup>	Treatment Time (s)	UV-dose (J/cm <sup>2</sup> )	Log <sub>10</sub> Reduction <sup>b,c,e,f</sup>	
			<i>E. coli</i> O157:H7	<i>Salmonella</i>
5 cm	5	5.4	0.9 ± 0.6A <sup>d</sup>	1.1 ± 0.6A <sup>d</sup>
	10	10.8	1.2 ± 0.1A <sup>d</sup>	1.6 ± 0.6A <sup>d</sup>
	30	32.4	2.3 ± 0.7AB <sup>d</sup>	1.9 ± 0.4A <sup>d</sup>
	45	48.6	2.6 ± 0.3B <sup>d</sup>	2.9 ± 1.0AB <sup>d</sup>
	60	64.8	3.3 ± 0.7B <sup>d</sup>	4.3 ± 1.2B <sup>d</sup>
8 cm	5	4.9	1.3 ± 0.0A	1.1 ± 0.3A
	10	9.9	1.3 ± 0.4A	2.1 ± 0.0AB
	30	29.7	1.7 ± 0.4A	2.1 ± 0.2AB
	45	44.5	1.7 ± 0.3A <sup>d</sup>	2.6 ± 0.1BC <sup>d</sup>
	60	59.4	2.3 ± 1.1A <sup>d</sup>	3.9 ± 1.2C <sup>d</sup>
13 cm	5	2.9	0.8 ± 0.2A	1.1 ± 0.1A
	10	5.7	1.2 ± 0.2AB	1.1 ± 0.5A
	30	17.1	1.5 ± 0.3BC	1.5 ± 0.4A
	45	25.7	2.1 ± 0.2D	2.1 ± 0.3AB
	60	34.2	2.0 ± 0.1CD	2.8 ± 0.6B

<sup>a</sup>Distance from quartz window to UV strobe is 5.8 cm.

<sup>b</sup>Average weight of strawberry sample is 110±5 g.

<sup>c</sup>Within the same column and microorganism, values not followed by the same letter are significantly different (P<0.05).

<sup>d</sup>Treatments resulted in significant damage to the fruit.

<sup>e</sup>Original populations of 10<sup>6</sup> CFU/g.

<sup>f</sup>Values are mean reductions ± standard deviation.

At a treatment level of 5 cm, the highest reductions were observed, but there was significant damage to the calyx of the fruit. Similar damage was observed at 8 cm after 45 and 60-s treatments. Therefore reductions related to these treatment scenarios will not be discussed. Reductions of *E. coli* O157:H7 were from 0.8 to 2.1 log<sub>10</sub> CFU/g, for 5 s and 45 s at 13 cm, respectively. At a distance of 8 cm from the quartz window, reductions were between 1.3 and 1.7 for 5 and 30-s treatments, respectively. There was no significant difference between the reductions of obtained at 8 cm. At 13 cm reductions were between 0.8 and 2.1 for 5 and 45-s treatments, respectively. Analysis indicated that the reduction obtained after 45 s of treatment yielded significantly higher reductions than the lower treatment times. A general linear model was used to determine the significant factors involved in the inactivation of *E. coli* O157:H7 on strawberries. When the reductions resulting from damage to the fruit were removed from the model, the only significant factor in reduction was treatment time; and furthermore, there was no significant difference in the reductions obtained at 8 cm versus reductions obtained at 13 cm. Reductions of *Salmonella* at a distance of 8 cm from the quartz window were between 1.1 and 2.1 log<sub>10</sub> CFU/g at 5 and 30 s, respectively (Table 5.2). There were no treatment times at this distance that resulted in significantly higher reductions, where no damage was observed. At a distance of 13 cm, reductions were between 1.1 and 2.8 log<sub>10</sub> CFU/g for 5 and 60 s, respectively. The reduction obtained at 60 s was significantly higher than the treatments times between 5 and 30 s, there was no significant difference between reductions at 60 s and 45 s, which were 2.8 and 2.1 log<sub>10</sub> CFU/g,



respectively. Again a general linear model was used to determine the significant factors involved in the inactivation of *Salmonella* on strawberries. With the reductions resulting from damage to the fruit were removed from the model, both the distance from the light and the treatment time were significant factors involved in the inactivation of the microorganism. Furthermore, the interaction term was also significant in the model and there was a significant difference between reductions obtained at 8 cm versus 13 cm.

As with raspberries, there was a significant increase in the temperature of the fruit after treatment with pulsed UV-light. At the treatment times which produced the maximum  $\log_{10}$  reductions resulting in no observable damage to the fruit temperature increases were 18.6 and 24.4°C (fruit temperatures of 45 and 40°C) at a distance of 8 cm for 30 s and 13 cm for 60 s, respectively.

### **5.3.3 Treatment of blueberries**

When blueberries were treated with pulsed UV-light generated at 3,800 V significant damage was observed. Therefore, the voltage for the pulsed UV-light treatment was lowered to 2,400 V. This voltage produced fluencies of 0.07, 0.11, and 0.18 J/cm<sup>2</sup>/pulse which corresponded to 13, 8, and 3 cm from the quartz window (Figure 5.2). Maximum UV doses were 32.4, 22.6, and 12.8 J/cm<sup>2</sup> at 3, 8, and 13 cm, respectively. At a treatment distance of 3 cm, the highest  $\log_{10}$  reductions were observed, but there was observable damage to the fruit.

Reductions of *E. coli* O157:H7 at 8 cm from the quartz window were between 1.1 and 4.3 log<sub>10</sub> CFU/g (Table 5.3). At this distance the only a significant difference existed between reductions obtained after 5 s and those after 60 s. At a distance of 13 cm reductions were between 1.3 and 3.0 log<sub>10</sub> CFU/g, with reductions at 30 and 60 s identical at 3.0 log<sub>10</sub>. There was no significant difference between any of the treatments at this distance from the quartz window. When a general linear model was used to compare the treatment distances it was concluded that there was no significant difference between reductions obtained at 8 cm and those obtained at 13 cm.

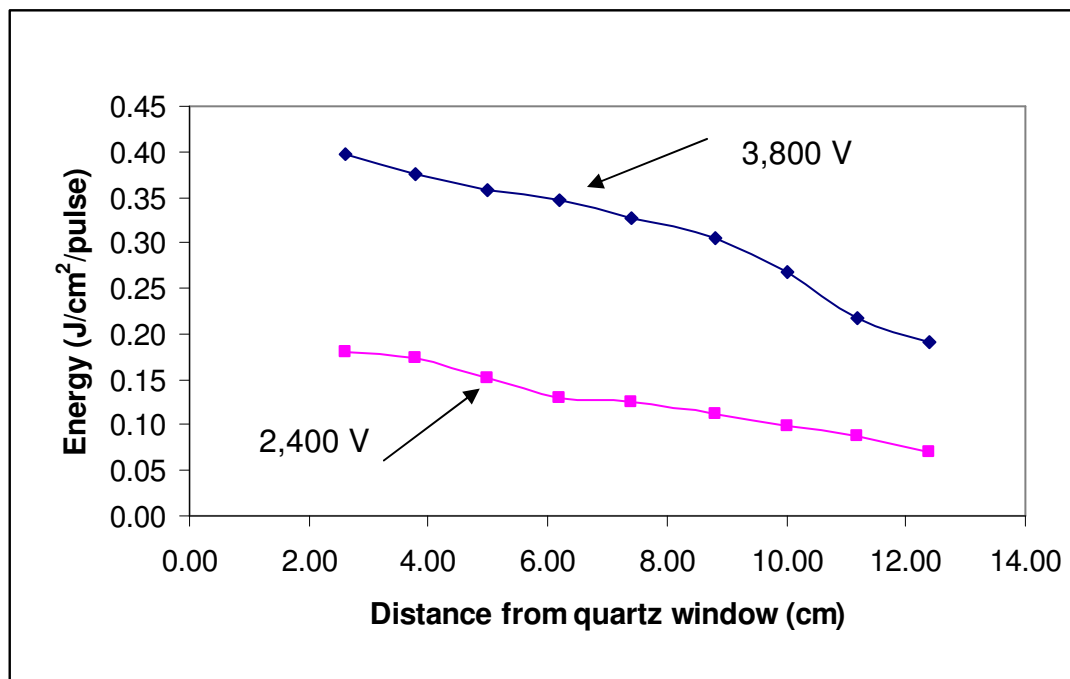


Figure 5.2. Broad band energy as a function of distance.

Table 5.3. Log reductions of *E. coli* O157:H7 and *Salmonella* on blueberries after pulsed UV-light treatment.

Distance from Quartz window	Treatment Time (s)	UV-dose (J/cm <sup>2</sup> )	Log <sub>10</sub> Reduction <sup>a,b,c,e,f</sup>	
			<i>E. coli</i> O157:H7	<i>Salmonella</i>
3 cm	5	2.7	1.3 ± 0.6A <sup>d</sup>	1.3 ± 0.3A <sup>d</sup>
	10	5.4	2.4 ± 1.3A <sup>d</sup>	1.4 ± 0.2A <sup>d</sup>
	30	16.2	3.1 ± 1.2A <sup>d</sup>	2.6 ± 0.3A <sup>d</sup>
	45	24.3	3.2 ± 0.6A <sup>d</sup>	2.6 ± 0.5A <sup>d</sup>
	60	32.4	4.9 ± 2.4B <sup>d</sup>	3.8 ± 2.3A <sup>d</sup>
8 cm	5	1.9	1.1 ± 0.2A	1.1 ± 0.8A
	10	3.8	2.4 ± 0.8AB	1.8 ± 0.8A
	30	11.3	2.7 ± 1.0AB	2.8 ± 0.4A
	45	16.9	3.3 ± 0.7AB	2.8 ± 0.9A
	60	22.6	4.3 ± 1.2B	2.9 ± 0.5A
13 cm	5	1.1	1.3 ± 0.8A	1.0 ± 0.1A
	10	2.1	2.1 ± 1.1A	1.0 ± 0.1A
	30	6.4	2.6 ± 0.9A	2.1 ± 0.0B
	45	9.6	3.0 ± 0.2A	2.4 ± 0.4B
	60	12.8	3.0 ± 0.6A	2.6 ± 0.4B

<sup>a</sup>Distance from quartz window to UV strobe is 5.8 cm.

<sup>b</sup>Average weight of blueberry sample is 30±2.5 g.

<sup>c</sup>Within the same column and microorganism, values not followed by the same upper case letter are significantly different (P<0.05).

<sup>d</sup>Treatments resulted in significant damage to the fruit.

<sup>e</sup>Original populations of 10<sup>6</sup> CFU/g.

<sup>f</sup>Values are mean reductions ± standard deviation.

Reductions of *Salmonella* at 8 cm from the quartz window were slightly lower than those of *E. coli* O157:H7 (Table 5.3) at 1.1 and 2.9 log<sub>10</sub> CFU/g. There was no significant difference between any of the treatment times at this distance from the quartz window. At a distance of 13-cm, reductions were between 1.0 and 2.6 log<sub>10</sub> CFU/g. Reductions obtained after 5 and 10 s were significantly lower than the other treatments.

When results were compared between levels using a general linear model it was concluded that no significant difference existed between reductions obtained at 8 cm and those at 13 cm.

#### **5.3.4 Color analysis**

Fruits from the most effective treatments were analyzed immediately after treatment to determine if pulsed UV-light had any negative effects on the color of the fruit as the quality indicator. For raspberries and blueberries the treatment which was chosen was the 60 s treatment at 8 cm from the quartz window. The treated raspberries had L\*, a\*, and b\* values of 31.29, +23.16, and +12.99 compared to the untreated raspberries which had values of 29.20, +20.25, and +11.66, respectively (Table 5.4). None of the differences were significant. The treated blueberries had L\*, a\*, and b\* values of 34.14, -1.64, and -2.51 compared to the untreated blueberries which had values of 35.95, -1.32, and -2.56, respectively. None of the differences were significant. The treatment used to strawberries was at 8 cm from the quartz window for 30 s. The treated

strawberries had L\*, a\*, and b\* values of 30.35, +25.59, and +17.65 compared to the untreated strawberries which had values 33.17, +25.94, and +17.30, respectively. None of the values were significantly different between the treated and untreated strawberries.

Table 5.4. L\*a\*b\* color readings for blueberries, raspberries, and strawberries after pulsed UV-light treatment.

		L * value <sup>e,f</sup>	a * value	b * value
Blueberry <sup>a,c</sup>	Untreated	35.95 ± 2.71A	-1.32 ± 1.14A	-2.56 ± 1.36A
	Treated	34.15 ± 1.96A	-1.64 ± 1.13A	-2.51 ± 1.02A
Raspberry <sup>a,c</sup>	Untreated	29.20 ± 3.85A	20.25 ± 2.22A	11.66 ± 1.68A
	Treated	31.29 ± 3.85A	23.16 ± 3.93A	12.99 ± 2.83A
Strawberry <sup>b,d</sup>	Untreated	33.17 ± 1.94A	25.94 ± 4.15A	17.30 ± 3.19A
	Treated	30.35 ± 2.39A	25.59 ± 2.51A	17.65 ± 2.61A

<sup>a</sup>Each replication consisted of 9 samples with 2 readings per sample.

<sup>b</sup>Each replication consisted of 3 samples with 2 readings per sample.

<sup>c</sup>Treatment 8 cm for 60 s.

<sup>d</sup>Treatment at 8 cm for 30 s.

<sup>e</sup>Values within the same row followed by the same letter are not significantly different (P>0.05).

<sup>f</sup>Values are mean reductions ± standard deviation.

There have been few studies evaluating the effects of ultra-violet radiation, in either its continuous or pulsed forms, on the decontamination of fresh produce. Yuan et al. (2003) evaluated continuous UV-C light on the decontamination of *E. coli* O157:H7 and *Salmonella* on the surfaces of apples and tomatoes.

Reductions of 3.3 log<sub>10</sub> CFU/apple were achieved after treatment with 86.4 J/cm<sup>2</sup> and reductions of 2.19 log<sub>10</sub> CFU/tomato of *Salmonella*. The results presented in this study are somewhat comparable. Maximum reductions *E. coli* O157:H7 and *Salmonella* on raspberries were achieved at a UV dose of 72 and 59 J/cm<sup>2</sup>, respectively, which resulted in reductions of 3.9 and 3.4 log<sub>10</sub> CFU/g, respectively.

There have been several studies looking at the efficacy of sanitizers for the decontamination of pathogens on strawberries. Yuk et al. (2001) evaluated chlorine dioxide gas and found a 4.6 log<sub>10</sub> CFU/berry reduction after 1-h treatment. A variety of “wet” sanitizers have been evaluated. Acidic electrolyzed oxidizing water produced a 2.4 log<sub>10</sub> CFU/fruit of coliform bacteria (Koseki et al., 2001). Yu et al. (2001) evaluated sodium hypochlorite (200 ppm), Tween 80 (200 ppm), 5% acetic acid, 5% sodium phosphate, and 3% hydrogen peroxide on the ability to decontaminate *E. coli* O157:H7 on strawberries. These sanitizers produced reductions of 1.34, 1.16, 1.57, 1.58, and 2.15 log<sub>10</sub> CFU/g, respectively.

#### **5.4 Conclusion**

The results presented in this study indicate that pulsed UV-light may be an effective mode of decontamination for small fruits such as blueberries, raspberries, and strawberries. These reductions are comparable if not greater than reductions obtained via other methods of decontamination. Pulsed UV-light has the added benefit of a relatively short treatment time compared to chemical

treatments. Maximum reductions of *E. coli* O157:H7 and *Salmonella* were achieved after 60 s of pulsed UV-light treatment. Reductions of 3.9 and 3.4 log<sub>10</sub> CFU/g were achieved on raspberries after a UV dose of 72 and 59.4 J/cm<sup>2</sup>, respectively. On strawberries reductions of 2.1 and 2.8 log<sub>10</sub> CFU/g were achieved after 25.7 and 34.2 J/cm<sup>2</sup> of UV exposure. Maximum reductions of 4.3 and 2.9 log<sub>10</sub> CFU/g were achieved on blueberries after a UV dose of 22.6 J/cm<sup>2</sup> for *E. coli* O157:H7 and *Salmonella*, respectively. This variation in the reductions of microorganisms on the surfaces of small fruits is most likely due to the irregular surface structure of the fruits, which could include the achenes of the strawberry and the space between drupelets in raspberries which can shield microorganisms. Less variation is exhibited in the reductions on blueberries due to its more uniform structure. Also it has been reported that only partial disinfection can be obtained due to shielding or shadowing effects (Lagunas-Solar et al., 2006). This research indicates that pulsed UV-light could be an effective decontamination agent for blueberries, raspberries and strawberries; however, more research needs to be conducted on the quality and sensory characteristics of the fruits after treatment with pulsed UV-light.

## 5.5 References

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## Chapter 6

### Modeling of the Inactivation of *Escherichia coli* O157:H7 and *Salmonella* on Berries Resulting From Exposure to Ozone or Pulsed UV-Light

#### Abstract

Inactivation data from studies conducted on blueberries, raspberries, and strawberries inoculated with *Escherichia coli* O157:H7 and *Salmonella* after treatment with gaseous ozone, aqueous ozone, and pulsed UV-light were used to construct models to estimate the inactivation. Two models were constructed, a log-linear (based on first-order kinetics) and a Weibull model. Initial analysis indicated that survival curves were non-linear and that the log-linear model failed to accurately estimate the inactivations in most instances. The Weibull model more accurately estimated the reductions and the concavity of the inactivation curve. Validation of the Weibull model produced correlation coefficient of 0.83 to 0.99 and slope of 0.76 to 1.26. The results presented in this study indicated that first-order kinetics are not suitable for the estimation of microbial inactivation on berries treated with ozone or pulsed UV-light, but that the Weibull model can be successfully used to estimate the reductions of *E. coli* O157:H7 and *Salmonella* on blueberries, raspberries, and strawberries treated with ozone or pulsed UV-light.

## 6.1 Introduction

Concern about the microbial safety of small fruits has increased in recent years after several notable outbreaks. Raspberries have been implicated in numerous outbreaks of cyclosporiasis, with the most recent outbreak in 2000, which sickened 54 people (Ho et al., 2002). Strawberries have also been implicated in several outbreaks of Hepatitis A (CDC, 1997) and in a study of imported produce, strawberries were found to contain *Salmonella* in 1 out of 143 samples (FDA, 1999). Furthermore, research has shown that pathogens like *E. coli* O157:H7, *Listeria monocytogenes*, and *Salmonella* are capable of surviving on the surfaces of strawberries for over 7 days (Flessa et al., 2005; Knudsen et al., 2001). Currently, berries undergo no processing prior to market and research has shown that water and chemical sanitizers have limited efficacy. Therefore, there is a need to identify alternative methods of decontamination. Ozone and pulsed UV-light are potential technologies and have been shown to have a potential for the decontamination of berries.

Ozone when used in its gaseous form as an additive to water (aqueous ozone) has been successfully used to inactivate both pathogenic and spoilage microorganisms in solution and on various food products. The inactivation of *E. coli* in solution has been shown to follow first-order kinetics with respect to ozone concentration (Hunt and Marinas, 1997). However, ozone readily reacts with organic materials and reductions of microorganisms in the presence of locust

bean gum, caseinate, and whipping cream were all found to significantly decrease (Guzel-Seydim et al., 2004).

Pulsed UV-light has been shown to effectively inactivate microorganism in solution and on surfaces. However, some food materials present a much more difficult disinfection situation due to the presence of crevices and irregular shapes. The inactivation of microorganisms via ultra-violet light has been shown to follow first-order kinetics in solution (EPA, 2003), but have also been reported to display a sigmoidal shape with a shoulder and/or a tail (CFSAN-FDA, 2000).

Traditionally, microbial inactivation has been described by first-order kinetics which assumes that under a constantly applied method of inactivation the number of viable cells will decrease exponentially and that all cells have the same probability of death. This assumes that a straight line will be derived from a semilogarithmic plot. The frequent observance of shoulders, tails, and concavity in inactivation curves has led many to believe that the assumption of microbial inactivation as a first-order kinetic to be an exception rather than the norm (Peleg, 2006; Van Boekel, 2002).

The Weibull distribution is being used to a greater extent to describe microbial inactivation, and uses the engineering principle of failure. Instead of a structural or mechanical failure, the failure is that of the microorganism (Peleg, 2006). This model has been used to describe the heat inactivation of *L. monocytogenes*, *S. Enteritidis*, *S. Typhimurium*, *E. coli* O157:H7 and *Staphylococcus aureus*, which exhibited a tailing concave survival curves (Buzrul and Alpas, 2006). This model has been successfully used to model other novel

processing technologies. The inactivation of *Staphylococcus aureus* during high pressure processing was successfully modeled using a Weibull distribution (Guan et al., 2006). The inactivation of *Listeria innocua* with pulsed electric fields was successfully estimated using the Weibull distribution (San Martin et al., 2006). The authors also pointed out that the estimates obtained using the Weibull distribution were better than those estimates obtained using models based on first-order kinetics.

The goal of this study was to develop a model to estimate the reductions of *E. coli* O157:H7 and *Salmonella* on blueberries, raspberries, and strawberries after ozone or pulsed UV-light treatment.

## **6.2 Materials and Methods**

### **6.2.1 Preparation of inoculum**

Five strains of nalidixic acid resistant *E. coli* O157:H7 and *Salmonella* were obtained from the Center for Food Safety at the University of Georgia. The *E. coli* O157:H7 strains were: 932 (human isolate), 994 (salami isolate), E0018 (calf fecal isolate), H1730 (human isolate from outbreak associated with lettuce), and F4546 (human isolate from outbreak associated with alfalfa sprouts). The *Salmonella* serotypes used were: Agona (human isolate from outbreak associated with alfalfa sprouts), Baildon (human isolate from outbreak associated with diced tomatoes), Gaminara (orange juice isolate), Michigan (human isolate

associated with cantaloupe outbreak), and Montevideo (human isolate associated with tomato outbreak). Cultures were grown in tryptic soy broth (Difco, Detroit, MI) supplemented with 50 µg/ml nalidixic acid (Fisher Scientific Co., Fair Lawn, NJ) at 37°C for 24 h. A mixture of *E. coli* O157:H7 or *Salmonella* strains were prepared by combining 10 ml of each culture and centrifuging for 15 min at 3,300 x *g* and 4°C. The supernatant was discarded and the cells were resuspended in 10 ml of 0.1% peptone water (Difco) to yield an approximate population of 10<sup>8</sup> CFU/ml.

### **6.2.2 Inoculation of berries**

Blueberries, red raspberries, and strawberries were purchased from a local grocery store and left at room temperature for 1 h prior to inoculation. To inoculate the blueberries and raspberries, a 25 µL droplet of inoculum was deposited on the skin of each fruit. For strawberries, a 50 µL droplet of inoculum was deposited on the skin approximately midway between the calyx and cap (Yu et al., 2001). The fruits were dried in a laminar flow hood for 24 h before the treatment to allow for proper attachment of the microorganisms. The inoculated blueberries, raspberries, and strawberries had approximately 10<sup>5</sup> CFU/g fruit of both *E. coli* O157:H7 and *Salmonella*.

### 6.2.3 Production of ozone

Ozone gas was generated using a lab-scale ozone generator equipped with an oxygen concentrator (Model No. H-50, Hess Machines International, Ephrata, PA). Gas was delivered at a flow rate of 0.34 m<sup>3</sup>/h and a concentration of 5.00% (wt/wt) which was measured using a Teledyne 450H bench top analyzer (Teledyne Technologies Inc., Los Angeles, CA). A 1 L beaker containing the sample was placed in a 17-L pressure vessel (Model No. 1915X, Wisconsin Aluminum Foundry Co. Inc, Manitowoc, Wisc.) which was connected to the ozone gas line or a 500 ml flask containing 20°C sterile deionized water was sparged with ozone. After the treatment, the ozone gas was passed through a 2% (wt/v) potassium iodide solution to prevent ozone from being released into the environment. Furthermore, the ozone treatment was performed in a fume hood for safety considerations.

### 6.2.4 Treatment with ozone

A batch of 5 strawberries or 18 raspberries and blueberries was used for each ozone treatment based on size constraints. Fruits were subjected to a continuous supply of 5% (wt/wt) ozone gas for time of 2, 4, 8, 16, 32, and 64 min.

Batches of berries were placed 500 ml of sterile deionized water and a stainless steel sparger with a pore size of 10 µm. Ozone was sparged into the water for times of 2, 4, 8, 16, 32 and 64 min at 20°C. Temperature was maintained by placing flasks in a water bath at the appropriate settings.



### **6.2.5 Treatment with pulsed UV-light**

Pulsed light was produced using a laboratory scale, batch-fed pulsed-light system (Steripulse-XL 3000, Xenon Corp., Wilmington, MA). The system generated  $1.27 \text{ J/cm}^2$  per pulse for an input of 3,800 V and with 3 pulses per second setting at 1.8 cm from the quartz window per the manufacturer's specifications. The distance between the UV-strobe and the quartz window is 5.8 cm. Fruits were treated at three different distances from the quartz window measured from the bottom of the fruit; 3 cm, 8 cm, and 13 cm for raspberries and blueberries, and 5 cm, 8 cm, and 13 cm for strawberries. For strawberries a distance of 5 cm was used instead of 3 cm due to their larger size. Furthermore, the surface of the fruit which was inoculated was oriented to receive full light exposure. At each distance from the quartz window, 5, 10, 30, 45, and 60 s treatment times were evaluated.

### **6.2.6 Microbial analysis**

After treatment strawberries were placed in 100 ml of Dey-Engley Neutralizing (D/E) Broth (Difco) and blueberries and raspberries were placed in 50 ml D/E broth and pummeled for 1 min in a stomacher. The homogenate was then serially diluted in 0.1% peptone water (Difco) and spiral plated on tryptic soy agar (Difco) supplemented with 50  $\mu\text{g/ml}$  of nalidixic acid with an Autoplate 4000 (Spiral Biotech, Norwood, MA). Plates were incubated at  $37^\circ\text{C}$  for 24 h and then

enumerated using Q-count (Version 2.1, Spiral Biotech, Norwood, MA).

Reductions of bacteria were calculated on a per gram of fruit basis.

### 6.2.7 Models

Traditionally, microbial inactivation has been described by first-order inactivation kinetics (Eqn. 6.1) which will be termed the log-linear equation.

$$\log_{10}\left(\frac{N}{N_0}\right) = -kt$$

Where N = number of microorganisms at time t  
 $N_0$  = initial number of microorganisms,  
 k = first-order rate constant (time<sup>-1</sup>)

Eqn. 6.1

From this equation the classic “D-value” or the time necessary for a 1 log<sub>10</sub> reduction can be determined and is calculated as the reciprocal of the first-order rate constant. The log-linear equation is only appropriate for linear inactivation curves (where time is on the x-axis and log<sub>10</sub> (N/N<sub>0</sub>) is on the y-axis) and research has found that many inactivation curves are non-linear.

The second model used in the study is the Weibull model (Eqn. 6.2), which has been historically used in reliability engineering (van Boekel, 2002).

$$\log_{10}\left(\frac{N}{N_0}\right) = -\frac{1}{2.303}\left(\frac{t}{\alpha}\right)^\beta$$

where N = number of microorganisms  
 $N_0$  = initial number of microorganism  
 $\alpha$  = characteristic time  
 $\beta$  = shape parameter

Eqn. 6.2

A majority of survival curves exhibit concavity, either downwards or upwards, and the  $\beta$  parameter is used to describe this concavity. If  $\beta < 1$  the curve displays upward concavity and if  $\beta > 1$  the curve displays downward concavity. Also, the parameters  $\alpha$  and  $\beta$  can be used to calculate the reliable life,  $t_R$ , or the 90% percentile of the failure time distribution (Van Boekel, 2002), which is analogous to the D-value (Eq. 6.3).

$$t_R = \alpha * (2.303)^{\frac{1}{\beta}}$$

where  $\alpha$  = characteristic time  
 $\beta$  = shape parameter

Eqn. 6.3

The models were constructed using averages from two experimental data sets, and a third data set was used for validation. To achieve a best-fit, parameter estimation was performed by minimizing the sum of square error between the experimental and estimated  $\log_{10}$  reductions, using non-linear least squares regression method in Microsoft Excel 2000. Validation of the model was conducted by back-predicting the third experimental data set and performing a linear regression with the estimated versus the experimental data.

### 6.3 Results and Discussion

The reductions of *E. coli* O157:H7 and *Salmonella* on blueberries, raspberries, and strawberries after treatment with gaseous and aqueous ozone or pulsed UV-light was evaluated. Models estimating the reduction of *E. coli* O157:H7 and *Salmonella* in terms of ozone exposure time were developed, or

UV dose, which involved the application of two models; the log-linear model and the Weibull model.

### 6.3.1 Aqueous ozone models

The fraction of survivors ( $S = \frac{N}{N_o}$ ) after treatment with aqueous ozone is presented in Figure 6.1, where all treatments exhibit a similar trend; which is the tailing off of survivors. The first approach taken to model the data was a first-order kinetic or the log-linear model. Two sets of data were fit using linear regression and the root mean square error (RMSE) was used as a measure of goodness-of-fit (Table 6.1). To validate the model,  $\log_{10}(S)$  values were back predicted with the third data set and a linear regression was performed to determine the correlation coefficient ( $R^2$ ) (Figure 6.2). For *E. coli* O157:H7 the RMSE values were 0.23, 0.31, and 0.21 for blueberries, raspberries, and strawberries, respectively, and for *Salmonella* the RMSE values were 0.21, 0.18, and 0.23 for blueberries, raspberries, and strawberries, respectively. The failure of the model to accurately estimate  $\log_{10}(S)$  values can be seen in the correlation coefficients obtained using this model which was 0.80, 0.56, and 0.62 for blueberries, raspberries, and strawberries, respectively, when modeling the inactivation of *E. coli* O157:H7.

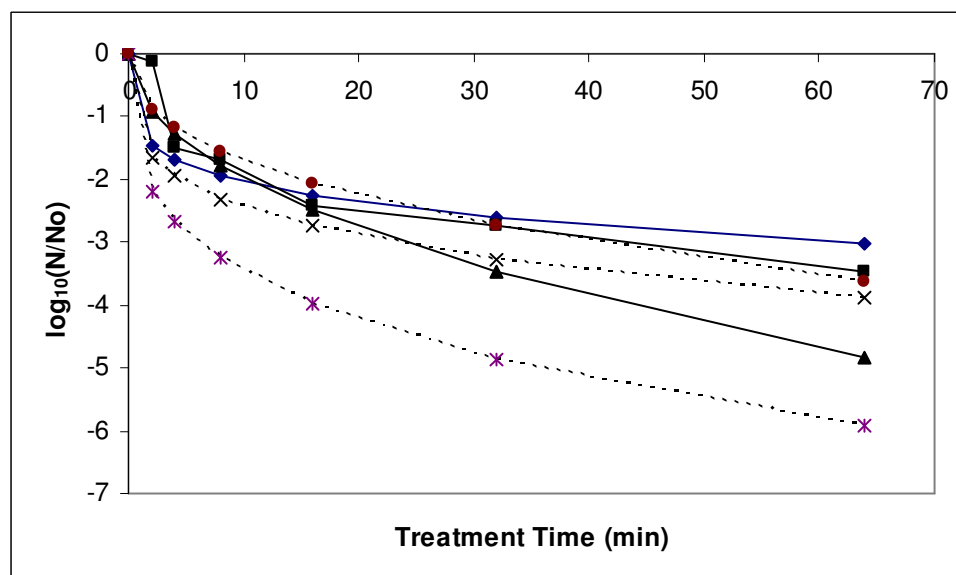


Figure 6.1. Influence of treatment time on microbial reduction after treatment with aqueous ozone on blueberries, raspberries, and strawberries. *E. coli* O157:H7 (---); (\*) Raspberry ;(x) Blueberry;(●) Strawberry. *Salmonella* (—);(▲) Raspberry;(◆) Blueberry;(■) Strawberry

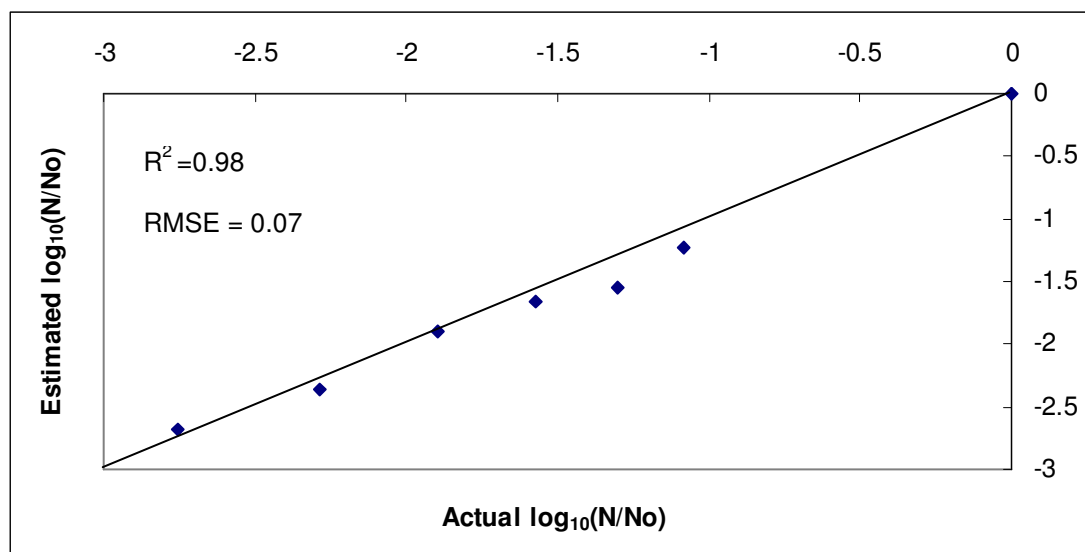


Figure 6.2. Example of Weibull model validation for the reduction of *E. coli* O157:H7 on strawberries after exposure to aqueous ozone.

For *Salmonella* correlation coefficients were 0.91, 0.66, and 0.86 for blueberries, raspberries, and strawberries, respectively. It was concluded that the RMSEs and correlation coefficients were not acceptable.

The ability of the Weibull model to more accurately estimate values of  $\log_{10}(S)$  can be seen in Table 6.1 were RMSE values for *E. coli* O157:H7 are less than those for the log-linear model. The RMSE values were 0.12, 0.08, and 0.07 for blueberries, raspberries, and strawberries, respectively. Also, the correlation coefficients were much higher at 0.94, 0.98, and 0.99 for blueberries, raspberries, and strawberries, respectively. The goodness-of-fit parameters obtained for the inactivation of *Salmonella* on blueberries, raspberries, and strawberries were 0.07, 0.17, and 0.12 for RMSE values and 0.93, 0.88, and 0.98 for the correlation coefficient. The fit of the Weibull model to the experimental data can be seen in Figures 6.3 and 6.4, for *E. coli* O157:H7 and *Salmonella*, respectively.

Table 6.1: Goodness-of-fit parameters of two models estimating reductions of *E. coli* O157:H7 and *Salmonella* on blueberries, raspberries, and strawberries after treatment with aqueous ozone.

Fruit	Microorganism	Models	RMSE	R <sup>2</sup>
Blueberry	<i>E. coli</i> O157:H7	Log-Linear	0.21	0.80
		Weibull	0.13	0.94
	<i>Salmonella</i>	Log-Linear	0.14	0.91
		Weibull	0.08	0.93
Raspberry	<i>E. coli</i> O157:H7	Log-Linear	0.26	0.56
		Weibull	0.09	0.95
	<i>Salmonella</i>	Log-Linear	0.09	0.66
		Weibull	0.17	0.88
Strawberry	<i>E. coli</i> O157:H7	Log-Linear	0.18	0.62
		Weibull	0.07	0.99
	<i>Salmonella</i>	Log-Linear	0.19	0.86
		Weibull	0.12	0.98

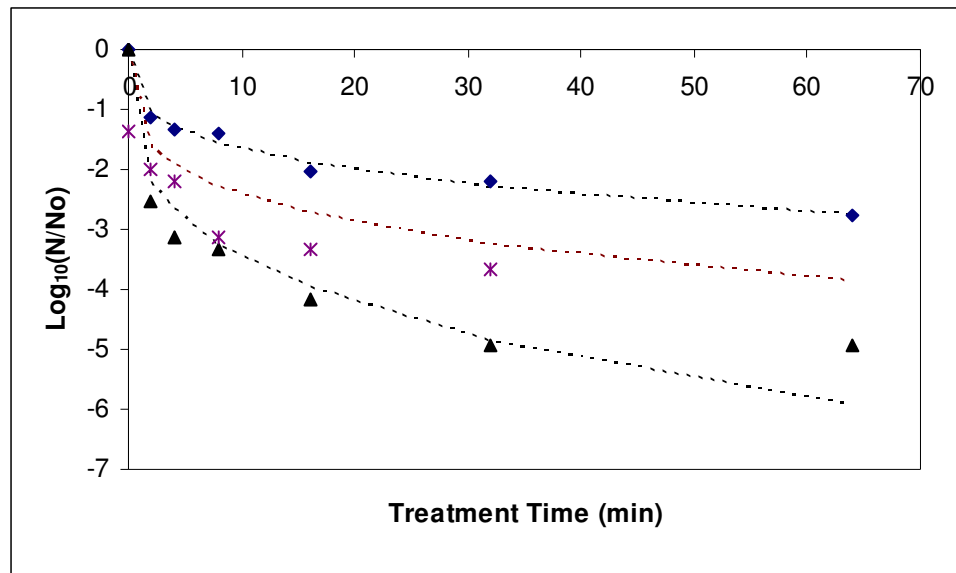


Figure 6.3. Inactivation of *E. coli* O157:H7 with aqueous ozone fitted with the Weibull model. (♦) Strawberry; (\*) Blueberry; (▲) Raspberry

The Weibull model consists of two parameters which are presented in Table 6.2, the alpha value corresponds to the mean of the distribution which describes death times, and can be used to determine a failure time or the time in which a 1 log<sub>10</sub> reduction will occur. Overall, reductions of *E. coli* O157:H7 occur faster than reductions of *Salmonella*, with  $t_R$  values of 0.27, 0.13, and 1.49 min for blueberries, raspberries, and strawberries, respectively; whereas  $t_R$  values for *Salmonella* were 0.32, 2.39, and 2.60 for blueberries, raspberries, and strawberries. The beta parameters, for both *E. coli* O157:H7 and *Salmonella* are all less than 1 and indicates that the remaining cells have less of a probability dying.

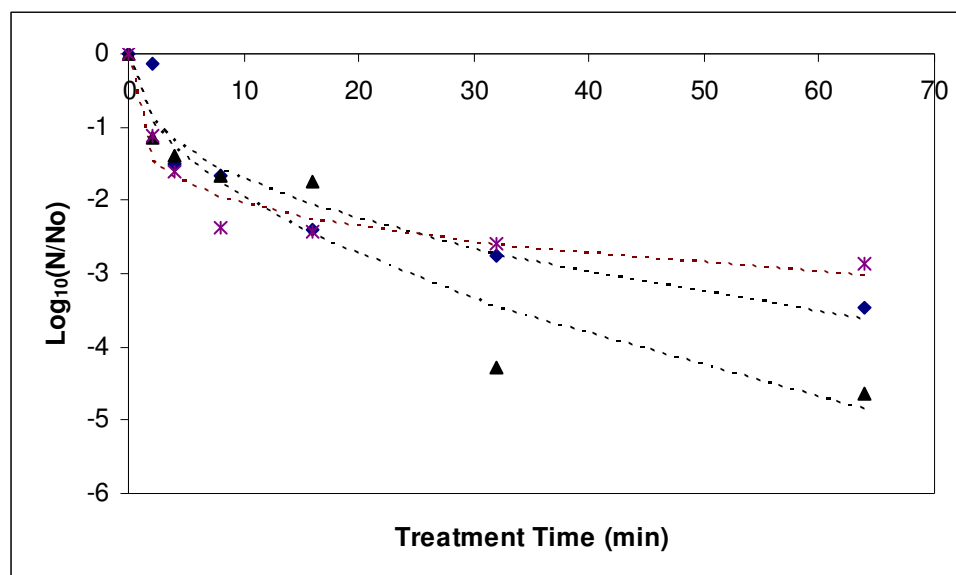


Figure 6.4. Inactivation of *Salmonella* with aqueous ozone fitted with the Weibull model. (♦) Raspberry; (\*) Blueberry; (▲) Strawberry

Table 6.2. Weibull model parameters for reductions of *E. coli* O157:H7 and *Salmonella* using aqueous ozone.

Fruit	Microorganism	$\alpha$	$\beta$	$t_R$ (min)
Blueberry	<i>E. coli</i> O157:H7	0.009	0.24	0.27
	<i>Salmonella</i>	0.006	0.21	0.32
Raspberry	<i>E. coli</i> O157:H7	0.007	0.29	0.13
	<i>Salmonella</i>	0.42	0.48	2.39
Strawberry	<i>E. coli</i> O157:H7	0.06	0.27	1.49
	<i>Salmonella</i>	0.32	0.40	2.60

### 6.3.2 Gaseous ozone models

The inactivation curves of *E. coli* O157:H7 and *Salmonella* after treatment with gaseous ozone can be seen in Figure 6.5. As with the inactivation curves



observed for aqueous ozone there is an observable tailing effect as the treatment time approaches 30 min for *Salmonella*; however, this is not observed for reductions of *E. coli* O157:H7. The Weibull model provided better estimates of microbial inactivation after treatment based on RMSE, slope and  $R^2$  values (Table 6.3). For example, the  $R^2$  values for the reduction of *E. coli* O157:H7 on raspberries is 0.98 for the log-linear model and 0.99 for the Weibull model. However, the Weibull model is a better fit than the log-linear model for *E. coli* O157:H7 (Figure 6.6).

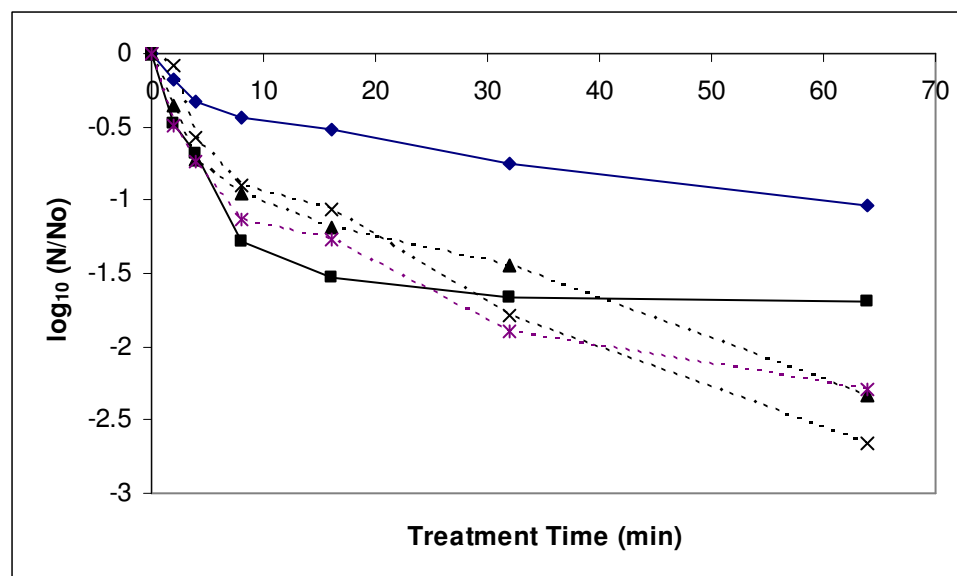


Figure 6.5. Influence of treatment time on microbial reduction after treatment with gaseous ozone on blueberries, raspberries, and strawberries. *E. coli* O157:H7 (---); (\*) Strawberry;(x) Raspberry; (▲) Blueberry; *Salmonella* (—); (■) Raspberry;(◆) Blueberry

Whereas, for *Salmonella* the  $R^2$  values for raspberries are 0.70 for the log-linear model and 0.98 for the Weibull model. The fit of the Weibull model to the data obtained for the inactivation of *Salmonella* can be seen in Figure 6.7.

As with the reductions obtained after aqueous ozone treatment the beta values is less than 1, which indicates that the majority of the remaining cells are resistant to the treatment (Table 6.4). The alpha values modeling this treatment exhibit no trend based on the microorganism, like was seen for the aqueous ozone treatments.

Table 6.3. Goodness-of-fit parameters of two models estimating reductions of *E. coli* O157:H7 and *Salmonella* on blueberries, raspberries, and strawberries after treatment with gaseous ozone.

Fruit	Microorganism	Models	RMSE	$R^2$
Blueberry	<i>E. coli</i> O157:H7	Log-Linear	0.12	0.85
		Weibull	0.02	0.98
	<i>Salmonella</i>	Log-Linear	0.006	0.87
		Weibull	0.001	0.97
Raspberry	<i>E. coli</i> O157:H7	Log-Linear	0.09	0.98
		Weibull	0.06	0.99
	<i>Salmonella</i>	Log-Linear	0.16	0.70
		Weibull	0.09	0.98
Strawberry	<i>E. coli</i> O157:H7	Log-Linear	0.12	0.90
		Weibull	0.06	0.99
	<i>Salmonella</i>	Log-Linear	NA*	NA
		Weibull	NA	NA

\*Not applicable

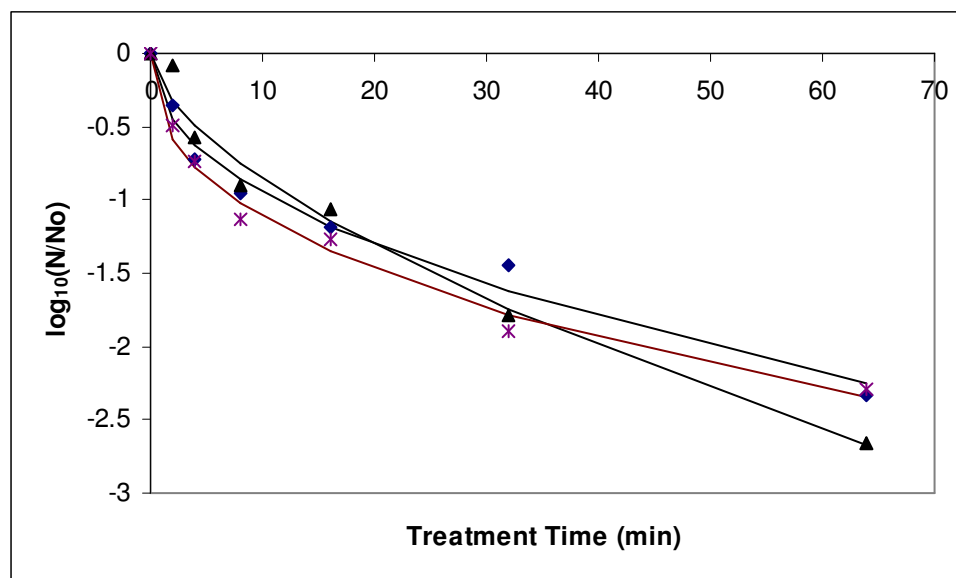


Figure 6.6. Inactivation of *E. coli* O157:H7 with gaseous ozone fitted with the Weibull model (◆)Blueberry; (\*) Strawberry; (▲) Raspberry.

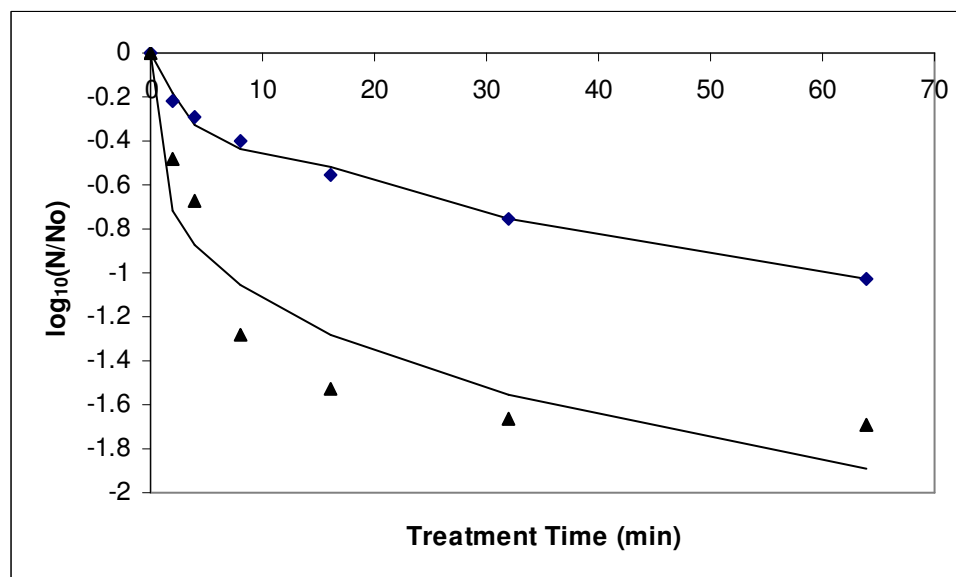


Figure 6.7. Inactivation of *Salmonella* with gaseous ozone fitted with the Weibull model (◆)Blueberry; (▲) Raspberry.

Also, the time necessary to achieve a  $\log_{10}$  reduction is much greater for the gaseous ozone treatments than the aqueous ozone treatments, with a maximum  $t_R$  of 60.33 min for *Salmonella* on blueberries. Overall, the Weibull model fits the data obtained after treatment with gaseous ozone better with RMSE values less than those for the log-linear model, and large correlation coefficients after model validation.

Table 6.4. Weibull model parameters for reductions of *E. coli* O157:H7 and *Salmonella* using gaseous ozone.

Fruit	Microorganism	$\alpha$	$\beta$	$t_R$
Blueberry	<i>E. coli</i> O157:H7	1.85	0.46	11.17
	<i>Salmonella</i>	9.44	0.45	60.33
Raspberry	<i>E. coli</i> O157:H7	3.27	0.61	12.81
	<i>Salmonella</i>	0.33	0.28	6.60
Strawberry	<i>E. coli</i> O157:H7	0.95	0.40	7.59
	<i>Salmonella</i>	NA*	NA	NA

\*Not applicable

### 6.3.3 Pulsed UV-light Models

Models describing the inactivation of *E. coli* O157:H7 and *Salmonella* on blueberries, raspberries, and strawberries were developed based on the treatment level that produced the highest  $\log_{10}$  reductions and resulted in minimal damage to the fruit. For raspberries this treatment level was selected as 3 cm from the quartz window which had energy doses between 6 and 72 J/cm<sup>2</sup> (Figure 6.8), blueberries the level was selected as 8 cm with doses between 1.8 and 22.5 J/cm<sup>2</sup>, and for strawberries the level was 13 cm with doses between 5.4

and  $64.4 \text{ J/cm}^2$ . Two models were compared the log-linear and Weibull models and the goodness-of-fit parameters can be seen in Table 6.5. Overall, the RMSE and  $R^2$  values obtained for the Weibull model are less than or greater than, respectively, those obtained for the log-linear model. From the inactivation curves it can easily be observed that none of the berry-microorganism combinations exhibit a linear trend.

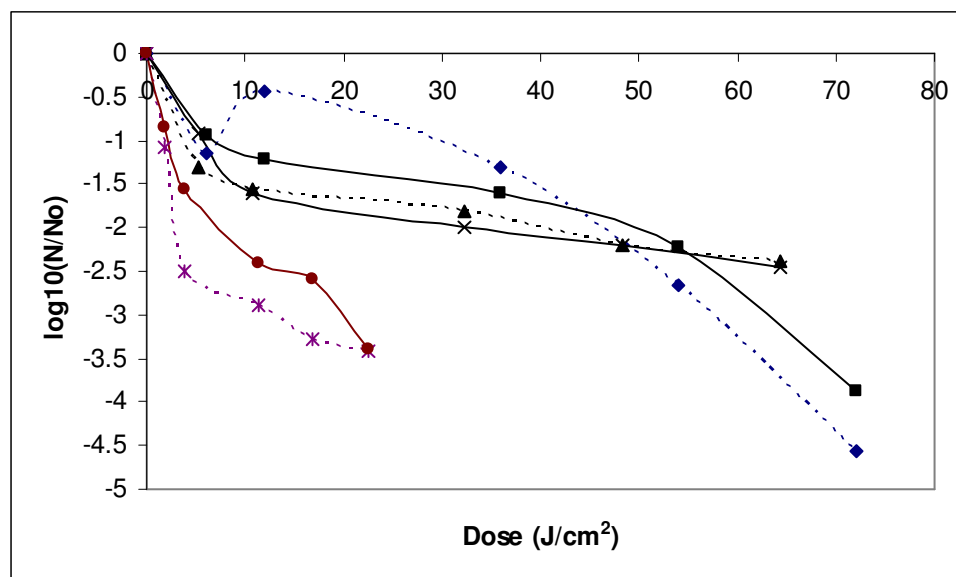


Figure 6.8. Influence of energy dose on microbial reduction after treatment with pulsed UV-light on blueberries, raspberries, and strawberries. *E. coli* O157:H7 (---); (\*) Blueberry; (♦) Raspberry; (▲) Strawberry. *Salmonella* (—); (■) Raspberry; (●) Blueberry; (X) Strawberry.

For *E. coli* O157:H7 the RMSE values obtained using the Weibull model were 0.07, 0.23, and 0.06 for blueberries, raspberries, and strawberries, respectively, with correlation coefficients of 0.94, 0.91, and 0.98, respectively.

This ability of this model to accurately estimate reductions of *E. coli* O157:H7 can be seen in Figure 6.9. As can be seen from Figure 6.8 the shape of the survival curves is different for each berry, with strawberry exhibiting a tailing effect. The Weibull model parameters (Table 6.5) can provide more insight into the shapes of these inactivation curves. For both blueberries and strawberries, the  $\beta$  parameter is less than 1, which accounts for its upward concavity, but also indicated that the remaining cells are more resistant to the treatment or are being shielded from the treatment due to the physical characteristics of the berry. The opposite can be observed for the inactivation of *E. coli* O157:H7 on raspberries; which display a downward concavity, with a  $\beta$  parameter greater than 1, which may be due to a different distribution of cells on the fruit.

Table 6.5. Goodness-of-fit parameters of two models estimating reductions of *E. coli* O157:H7 and *Salmonella* on blueberries, raspberries, and strawberries after treatment with pulsed UV-light.

Fruit	Microorganism	Models	RMSE	R <sup>2</sup>
Blueberry	<i>E. coli</i> O157:H7	Log-Linear	0.11	0.86
		Weibull	0.07	0.94
	<i>Salmonella</i>	Log-Linear	0.30	0.81
		Weibull	0.28	0.83
Raspberry	<i>E. coli</i> O157:H7	Log-Linear	0.57	0.80
		Weibull	0.23	0.91
	<i>Salmonella</i>	Log-Linear	0.31	0.50
		Weibull	0.06	0.92
Strawberry	<i>E. coli</i> O157:H7	Log-Linear	0.06	0.69
		Weibull	0.06	0.98
	<i>Salmonella</i>	Log-Linear	0.21	0.86
		Weibull	0.02	0.96

This trend can also be seen in the dose required for 1  $\log_{10}$  reduction ( $d_R$ ), with  $d_R$  values much less for blueberries and strawberries indicating that the most susceptible cells are inactivated quickly, but much larger  $d_R$  values are observed on raspberries illustrating the cumulative effect of the treatment.

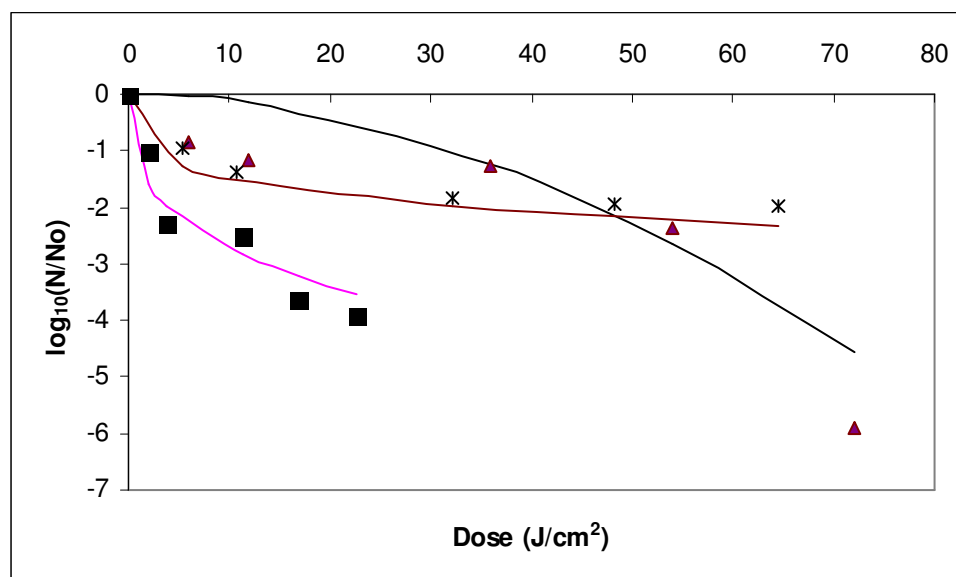


Figure 6.9. Inactivation of *E. coli* O157:H7 with pulsed UV-light fitted with the Weibull model (■) Blueberry; (\*) Strawberry; (▲) Raspberry.

Table 6.6. Weibull model parameters for reductions of *E. coli* O157:H7 and *Salmonella* using pulsed UV-light.

Fruit	Microorganism	$\alpha$	$\beta$	$d_R$
Blueberry	<i>E. coli</i> O157:H7	0.03	0.32	0.45
	<i>Salmonella</i>	2.52	0.93	6.13
Raspberry	<i>E. coli</i> O157:H7	20.5	1.87	32.10
	<i>Salmonella</i>	4.16	0.71	13.33
Strawberry	<i>E. coli</i> O157:H7	0.009	0.23	0.32
	<i>Salmonella</i>	0.05	0.32	0.69

Reductions of *Salmonella* exhibit a similar trend for the fit of the Weibull model, except that none of the curves exhibit downward concavity (Figure 6.10). All berries had  $\beta$  values less than 1 which indicated that cells were being shielded from the treatment.

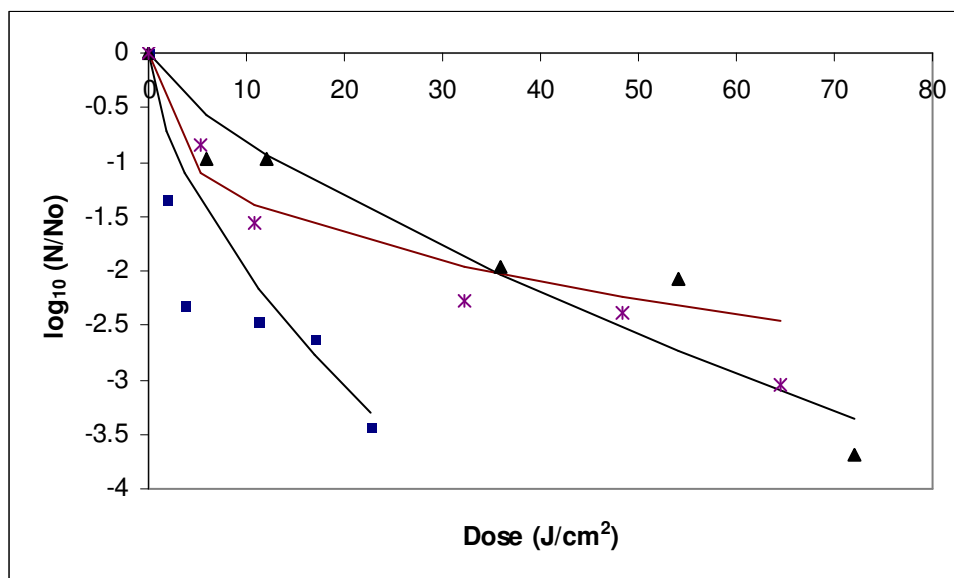


Figure 6.10. Inactivation of *Salmonella* with pulsed UV-light fitted with the Weibull model (■) Blueberry; (\*) Strawberry; (▲) Raspberry.

#### 6.4 Conclusion

The results presented here further illustrate the misconception that reductions of microorganisms within or on a food exhibit a log-linear trend. This was expected for both the aqueous ozone treatment and the pulsed UV treatment since the concentration or temperature, respectively, was increasing



with time; however, with the gaseous ozone treatment the concentration was constant which is the general assumption used in a log-linear model, and as the data shows the Weibull model produces better estimations of microbial reduction than the log-linear model. As noted by Van Boekel (2002) the majority of inactivation curves exhibit concavity. Only 1 out of 11 models approached a first-order kinetic with  $b$  values approaching 1. Van Boekel associates the concavity in the curves to an adaptation of the microorganisms to the treatment; within this research much of the “adaptation” may be attributable to the location of the microorganisms on the fruit. Microorganisms may have been shielded from the treatment by being located with the drupelets of the raspberries or under the achenes of the strawberries. In order to estimate the reductions of *E. coli* O157:H7 and *Salmonella* on raspberries and strawberries after treatment with ozone or pulsed UV-light, the Weibull model was successfully used, since it consistently and accurately estimated reductions of all fruits, treatment, and microorganisms. Further research into the affect of the physical structure of the food on microbial inactivation resulting from ozone and pulsed UV-light is needed.

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## Chapter 7

### **Pulsed UV-light penetration characterization and the inactivation of *Escherichia coli* K12 in solid model systems**

#### **Abstract**

With an ever increasing number of foodborne outbreaks in the U.S. tied to produce, novel processing technologies are needed. Pulsed ultraviolet (UV) light is one such technology, which provides broadband spectrum between 100 and 1100 nm, but more than 50% falls into the UV region. However, the efficacy of such a technology is not fully understood and there is some concern as to the penetrability of pulsed UV-light through materials, which are opaque. Inactivation and energy penetration data obtained from the treatment of agar (clear solid medium) and whey protein (opaque solid medium) gels after treatment with pulsed UV-light were used to construct several models to estimate the amount of energy penetrating the sample at a given depth and the inactivation of *E. coli* K12. The amount of broadband energy being transmitted through the materials decreased by as much as 60% at a depth of 10 mm. The inactivation curves obtained indicated that the relationship between energy dose and inactivation was non-linear and the Weibull model was used to estimate microbial inactivations. Three approaches were undertaken to determine the needed model parameters. The first model (Weibull Model 1) used a single set of values for the two Weibull distribution parameters, this model was found to accurately

estimate the reductions of *E. coli* K12 based on  $R^2$  and RMSE values. The second approach (Weibull Model 2) simultaneously solved for the extinction coefficient value in the exponential dose model in addition to the two Weibull distribution parameters. This model was found to increase the  $R^2$  values and decrease the RMSE values. However, this model was found to underestimate the dose. The third approach (Weibull Model 3) was to determine the two Weibull distribution parameters at each depth. This approach more accurately estimated reductions and dose, but was determined to be over-fitted. Each model had its advantages and disadvantages, but in all three it was observed that broadband energy was not a suitable predictor of microbial inactivation. The results presented in this study indicated that pulsed light can penetrate opaque materials up to 10 mm deep with decreasing energy levels, and that the Weibull model can be used to model the inactivation of *E. coli* K12 with correlation coefficients of 0.75 and 0.79.

## 7.1 Introduction

Each year illnesses resulting from the consumption of contaminated food costs the U.S. economy over \$6.9 billion in medical bills and lost productivity (ERS, 2005). There are numerous microorganisms of concern, which include *E. coli* O157:H7, *Salmonella* spp., *Staphylococcus aureus*, *Campylobacter jejuni*, and *Listeria monocytogenes*. With increasing concern over the safety of the nation's food supply there is a need to evaluate alternative technologies that can

maintain the integrity of a given food and not leave behind any toxic by-products. One such technology is pulsed UV-light. This technology involves pulsing broad spectrum light (100 to 1100 nm) several times per second lasting between 100 ns and 2 ms.

Pulsed UV-light has been reported to be more effective than conventional UV-light due to greater instantaneous energy. Pulsed UV-light has been shown to be highly effective at inactivating pathogens in liquids and on surfaces. Rowan et al. (1999) investigated the effects of pulsed UV-light on food-related microorganisms. Populations of *Listeria monocytogenes*, *Escherichia coli*, *Salmonella* Enteritidis, *Pseudomonas aeruginosa*, *Bacillus cereus*, and *Staphylococcus aureus* that were seeded on tryptone soya-yeast agar media were exposed to pulsed light having either high or low content UV light. Reductions between 2 and 6 log<sub>10</sub> CFU/ml were attained using 200 pulses with low UV content and high content, respectively. Krishnamurthy et al. (2004a) investigated the use of pulsed UV light to inactivate *S. aureus* in buffer solution and on agar seeded plates. They found a 7 to 8 log<sub>10</sub> CFU/ml reduction of *S. aureus* on seeded agar plates and buffer solution at treatment times less than 5 s without significant temperature increase.

Pulsed UV-light is very effective for decontaminating solid surfaces and clear liquids. However, UV-light decays exponentially as it passes through solids or opaque solutions. Krishnamurthy et al. (2004b) studied the inactivation of *S. aureus* milk with pulsed UV-light. They observed an increase in log<sub>10</sub> reductions with an increase in treatment time and a decrease in log<sub>10</sub> reductions as the

depth of the sample increased. Hillegas and Demirci (2003) found that when honey inoculated with *Clostridium sporogenes* at a depth of 2 mm was exposed to pulsed UV-light for 45 s, a reduction of 86.7% was observed, whereas honey at a depth of 8 mm pulsed for 45 s resulted in an insignificant reduction of *C. sporogenes*. Sharma and Demirci (2003) exposed alfalfa seeds inoculated with *E. coli* O157:H7 to pulsed UV-light. They found that when a seed layer thickness of 1.02 mm was treated for 30 s a 4.80 log<sub>10</sub> CFU/g reduction was achieved. However, as the thickness of the seed layer increased to 1.92, 3.61, and 6.25 mm reductions of only 1.05, 0.68, and 0.53 log<sub>10</sub> CFU/g were observed, respectively, after 30 s of pulsed UV-light treatment.

The inactivation of microorganisms via UV light has been shown to follow first-order kinetics in solution (EPA, 2003), but have also been reported to display a sigmoidal shape with a shoulder and/or a tail. The shoulder is where injury of the cells takes place followed by a rapid death phase and the cells eventually become resistant resulting in a tail (CFSAN-FDA, 2000). The sigmoidal inactivation curve has been observed predominately in cells suspended in clear liquids and has not been evaluated in solid and opaque media.

Even though previous studies have clearly demonstrated the effect of thickness during pulse UV-light treatment, they did not quantify the energy levels or inactivation at these varying depths. Therefore, the objectives of this study were to: 1) evaluate the penetrability of pulsed UV-light through solid materials of different opaqueness, and 2) model the inactivation of microorganisms at various depths.

## 7.2 Material and Methods

### 7.2.1 Preparation of inoculum

*Escherichia coli* K12 was obtained from the Gastroenteric Disease Center at the Pennsylvania State University and maintained on tryptic soy agar (Difco, Detroit, MI). The culture was maintained for long-term storage at -80°C in 20% glycerol solution. To prepare the inoculum the culture was grown in 500 ml of tryptic soy broth (Difco) for 24 h at 37°C. The culture was then centrifuged for 30 min at 3,300 x *g* and 4°C. The supernatant was discarded and the pellet was mixed with molten agar or whey protein isolate solution.

### 7.2.2 Preparation of model system

Two model systems were used in the study; agar and whey protein isolate (WPI) gels. Agar (4%, wt/v) was prepared using Bacto agar (Difco) and deionized water, the solution was boiled until fully dissolved and autoclaved for 20 min at 121°C. The agar solution was then cooled to approximately 40°C and the overnight grown culture was added to achieve a concentration of 10<sup>6</sup> CFU/g of gel. WPI (10%, wt/v) was prepared as follows; WPI (Davisco Foods International, Inc., Eden Prairie, MN) was added to deionized water and mixed for 3 h at room temperature to allow the protein to become fully hydrated. Since a typical WPI gel sets at a temperature of 80°C, it is not suitable for a microbiological study; however, denatured WPI (DWPI) was reported to gel at



much lower temperatures (Bryant and McClements, 2000). Therefore, WPI was denatured in a water bath at 90°C for 1 h, autoclaved for 20 min at 121°C, and then stored overnight at 4°C. NaCl solution (4M) was added to DWPI at a ration of 5% (v/v) to increase the firmness of the gel. Then the overnight grown culture was added to the gel to achieve a concentration of 10<sup>6</sup> CFU/g of gel. Finally, inoculated solutions were poured into sterile 70-mm aluminum dishes to form gels with depths of 10 mm.

### **7.2.3 Treatment with pulsed UV-light**

Pulsed UV-light treatment was performed using a laboratory scale, pulsed-light system (Figure 7.1, Steripulse-XL 3000, Xenon Corp., Wilmington, MA). Per the manufacturer's specifications, the system generated 1.27 J/cm<sup>2</sup> per pulse for an input of 3,800 V and with 3 pulses per second setting at 1.8 cm from the quartz window. This system utilizes a broad range of wavelengths which are between 100 and 1100 nm. The majority of this energy is in the UV region, making up 54% of the total wavelengths. The distance between the UV-strobe and the quartz window was 5.8 cm. Samples were treated with pulsed UV-light at 8-cm from the quartz window for times of 30, 60, 90, and 120 s.

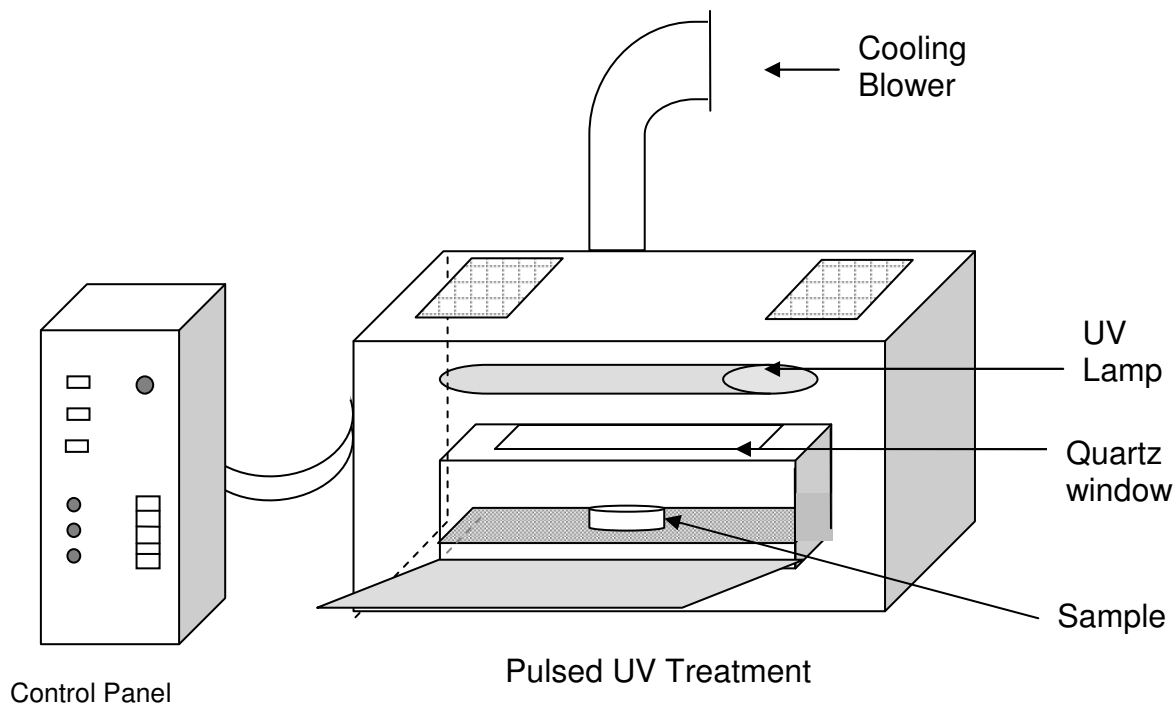


Figure 7.1. Schematic of pulsed UV system.

#### 7.2.4 Microbial analysis

The affects of energy penetrability on microbial inactivation through the materials was evaluated by removing three 16-mm diameter cylinders 10-mm in length from the gel and dividing them into 2 mm segments using sterile razor blades and forceps, which would correspond to depths of 0-2, 2-4, 4-6, 6-8, and 8-10 mm. The three segments from each depth were combined with 5 ml of 0.1% peptone water (Difco) and stomached in a filter bag for 2 min at 230 rpm. The homogenate was then serially diluted in 0.1% peptone water and spiral

plated on tryptic soy agar (Difco) with an Autoplate 4000 (Spiral Biotech, Norwood, MA). Plates were incubated at 37°C for 24 h and then enumerated using Q-count (Version 2.1, Spiral Biotech, Norwood, MA). Reductions of bacteria were calculated on a per gram of gel basis.

### **7.2.5 Measurement of energy**

The amount of broad band energy received at various depths was measured at 8-cm from the quartz window with a Nova Laser Power energy monitor (Ophir Optronics Ltd., Wilmington, MA), which averaged the energy level across 30 pulses. In order to do this, a set of gel samples were prepared in 70-mm diameter aluminum dishes with thicknesses of 2, 4, 6, 8, and 10 mm. The gels were removed from the dishes and placed over the sensor to measure the amount of energy passing through.

### **7.2.6 Temperature measurement**

The temperature increase during pulsed UV-light treatment was measured at various depths. A K-type thermocouple (Omegaette HH306, Omega Engineering, Inc., Stamford, CT) was used and was placed at depths of 1, 3, 5, 7, or 9 mm at the center of the sample.

### 7.2.7 Model development

To estimate microbial inactivation as a function of treatment time, material depth, and dose, the Weibull model (Eqn. 7.1) was selected, which has been successfully used in estimating microbial inactivations (Van Boekel, 2002).

$$\log_{10}(S) = \log_{10}\left(\frac{N}{N_0}\right) = -\frac{1}{2.303}\left(\frac{D}{\alpha}\right)^\beta$$

where

$$\begin{aligned} \log_{10}(S) &= \text{the fraction of survivors} && \text{Eqn. 7.1} \\ N &= \text{number of microorganisms after dose } D \text{ (CFU/g)} \\ N_0 &= \text{initial number of microorganisms (CFU/g)} \\ D &= \text{energy dose (J/cm}^2\text{)} \\ \alpha &= \text{characteristic dose (J/cm}^2\text{)} \\ \beta &= \text{shape parameter (unitless)} \end{aligned}$$

A majority of survival curves exhibit concavity, either downwards or upwards, and the  $\beta$  parameter is used to describe this concavity. If  $\beta < 1$  the curve displays upward concavity and if  $\beta > 1$  the curve displays downward concavity. Several approaches were undertaken to estimate the Weibull model parameters.

An additional model was developed to relate energy to treatment time and sample depth. As the depth of the material increases a decrease in the energy was observed as an exponential decay as in Eqn. 7.2. By incorporating time into the equation the dose that the gel received was calculated.

$$D = I_o e^{-kx} * t$$

where  $I_o$ =initial intensity at the top of the sample

(J/cm<sup>2</sup>/s)

k=extinction coefficient (mm<sup>-1</sup>)

x=material depth (mm)

t=time (s)

Eqn. 7.2

To determine the fraction of survivors ( $\log_{10}(S) = \log_{10} \frac{N}{N_o}$ ) the dose

model (Eqn. 7.2) was incorporated into Eqn 7.1. The models were constructed using an average of two experimental data sets, and the third data set was used for validation. Model fitting was performed by minimizing the sum-of-square error between the experimental and estimated survivor fractions, using non-linear least squares regression method in Microsoft Excel 2000. The model was validated by comparing it with the third experimental data set and by performing a linear regression with the calculated values versus the experimental data.

### 7.3 Results and Discussion

In this study the effect of material depth on the penetration of pulsed UV-light was evaluated. Agar and DWPI were evaluated in terms of energy dose and temperature increase. Furthermore, the effects of energy dose through the material on the inactivation of microorganisms were evaluated.

### 7.3.1 Effect of depth on dose and temperature

As the depth of the material increased from 2 to 10 mm, the amount of energy decreased from 0.61 J/cm<sup>2</sup>/s to 0.43 J/cm<sup>2</sup>/s in agar (Figure 7.2), which was a decline of 30%. In DWPI, the amount of energy decreased by 45% to 0.34 J/cm<sup>2</sup>/s at a depth of 10 mm. At 1 mm from the surface of the agar, the temperature increased by 43°C after 120 s of treatment with a total energy dose of 74 J/cm<sup>2</sup>; at the depth of 9 mm the temperature increase observed after 120 s was 32°C (Figure 7.3). Similar results were observed for DWPI with an increase of 43°C at 1 mm from the surface, and an increase of 25°C at 9 mm from the surface after 120 s of treatment.

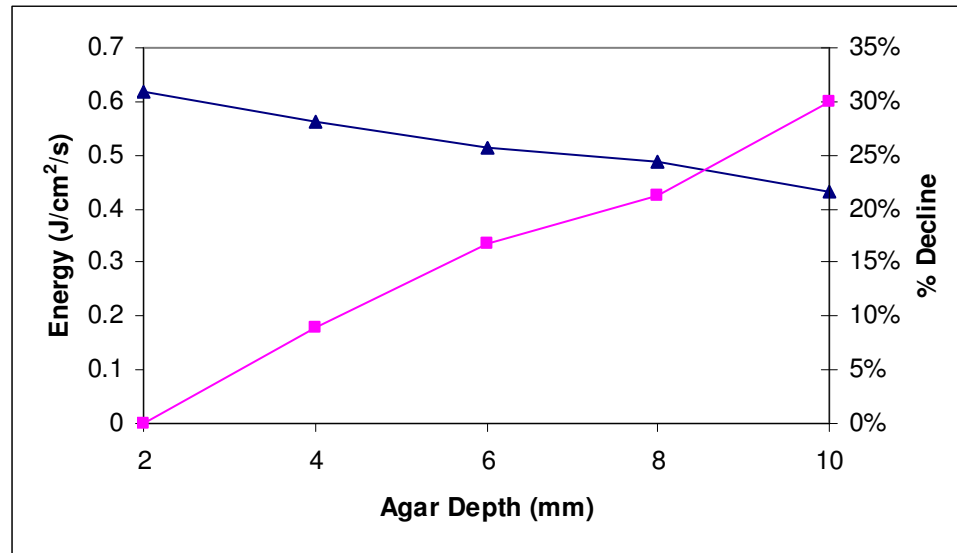
### 7.3.2 Inactivation of *E. coli* K12

The fraction of survivors ( $\log_{10} S$ ) after treatment with pulsed UV-light are presented in Figure 7.4. As the depth of the sample increased from 2 mm to 10 mm a change in the shape of the survival curves was observed for both 4% agar and 10% DWPI. At 2 mm and 4 mm the inactivation curve displays the classic sigmoid shape, which is frequently reported for UV inactivation (CFSSAN-FDA, 2000) and is more pronounced for agar than DWPI; as the depth increases to 10 mm the shape of the inactivation curve becomes more concave. At depths of 8 and 10 mm, almost 40% less energy is being transmitted to those cells, and the fraction of surviving microorganisms is much greater than depths of 2 or 4 mm.

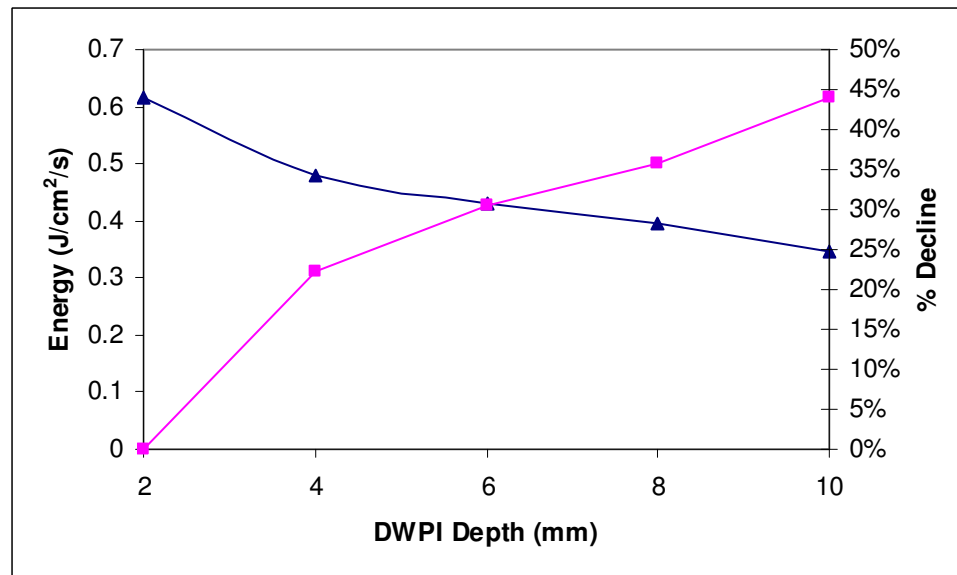
Also, it can be observed that the fraction of remaining cells is much greater for DWPI versus agar with maximum S values of -0.91 and -3.67  $\log_{10}$ , respectively.

### 7.3.3 Energy dose model

Equation 7.2 was used to model the energy dose received at depths of 2, 4, 6, 8, and 10 mm in both agar and DWPI gels. The energy measured at the 2 mm segment was assumed to be a 0 mm depth so that the role of reflection at the surface would not have to be taken into consideration. The model was found to be successful with RMSE and  $R^2$  values of 0.51 and 0.99, for agar, respectively; and 0.71 and 0.99 for DWPI, respectively (Figure 7.5). The k values describing the decline in energy with depth were found to be 0.03 and 0.05  $\text{mm}^{-1}$  for agar and DWPI, respectively. As expected, the k value is larger for DWPI than for agar indicating quicker decay of energy through the material. Figure 7.6 depicts the fit of Eqn 7.2 to the broadband energy measured in the system.



(a)



(b)

Figure 7.2. Radiant energy (■) and energy decline (▲) through agar and DWPI at various depths (a) 4% agar, (b) 10% DWPI.



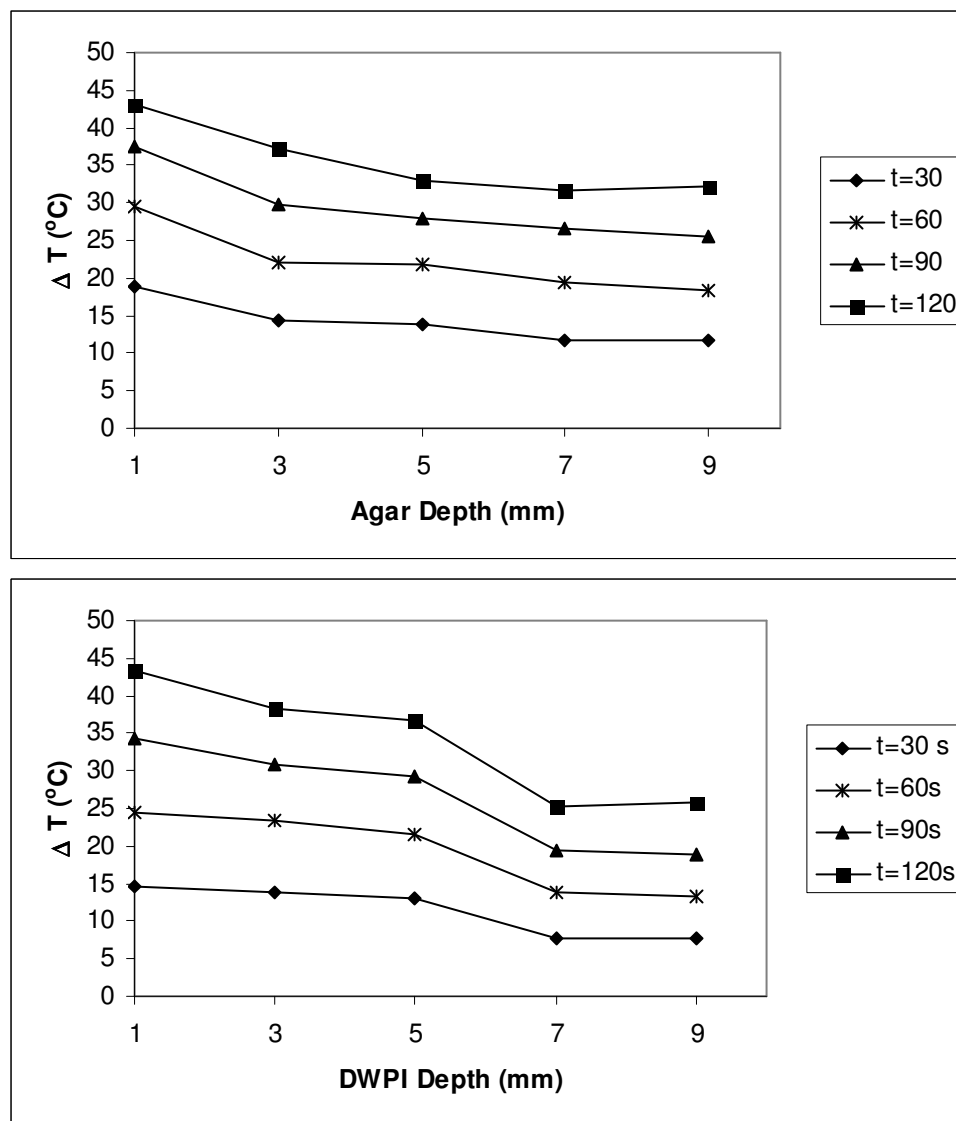
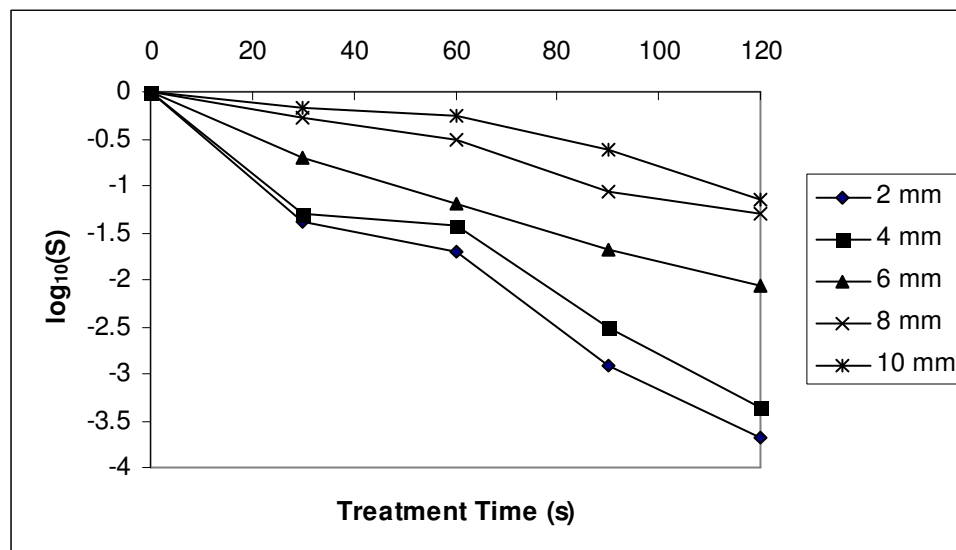
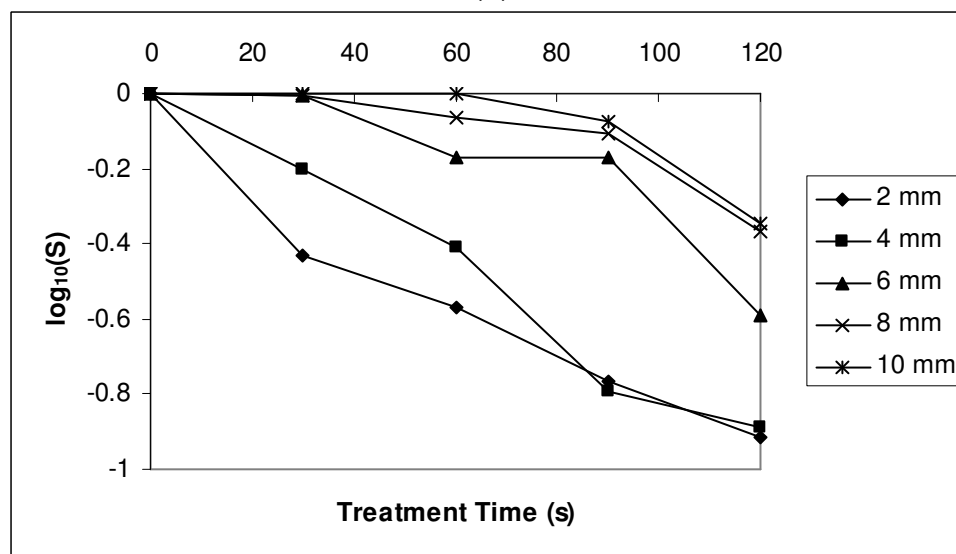


Figure 7.3. Temperature increase of material after treatment with pulsed UV-light  
(a) 4% agar, (b) 10% DWPI.

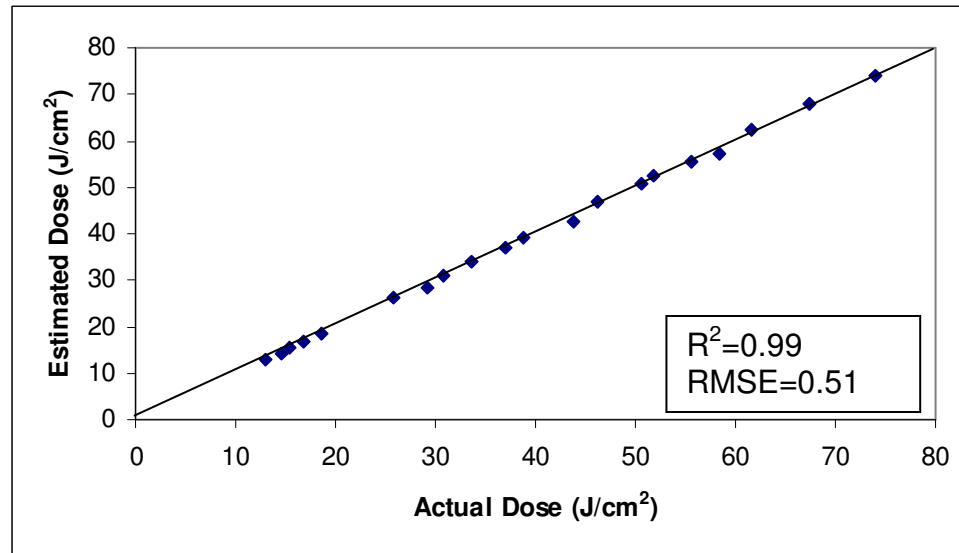


(a)

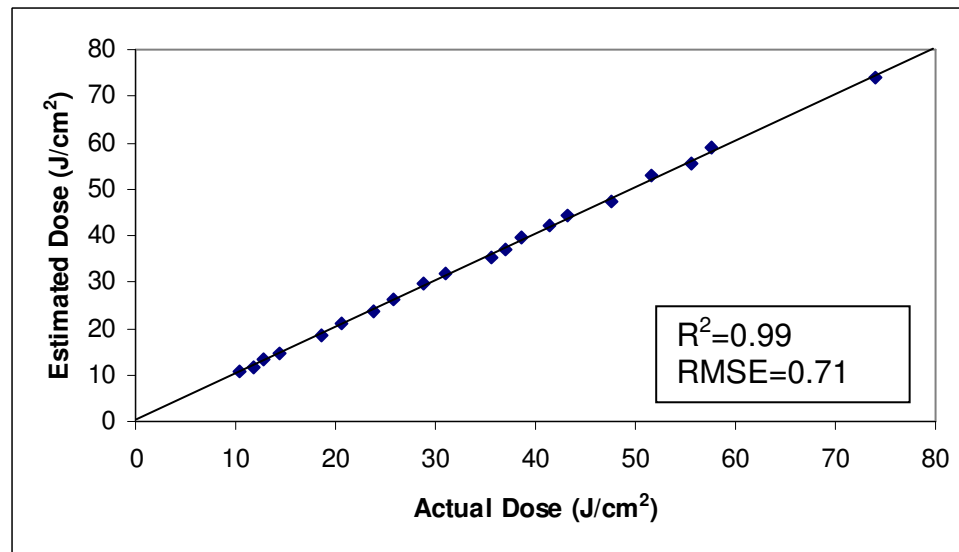


(b)

Figure 7.4. Inactivation of *E. coli* K12 after treatment with pulsed UV-light. (a) 4% agar, (b) 10% DWPI.

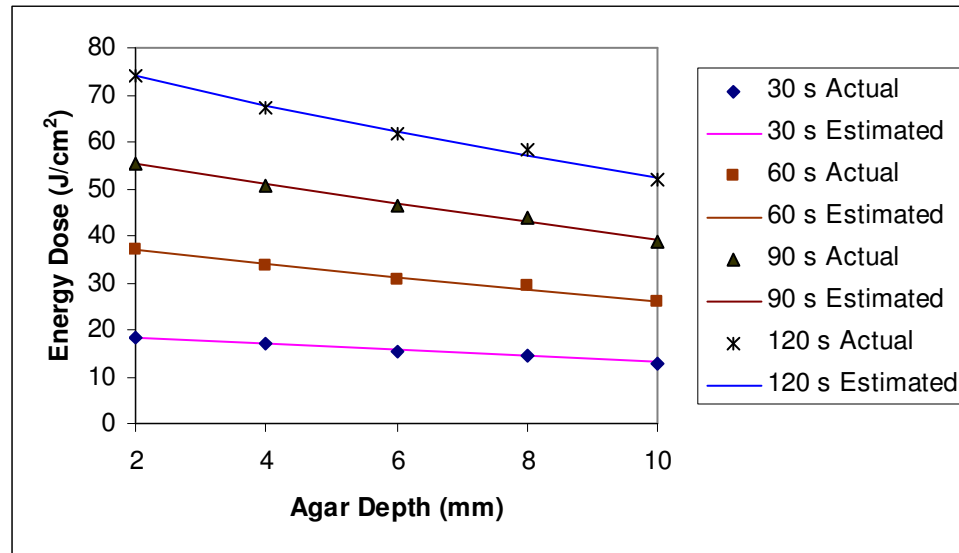


(a)

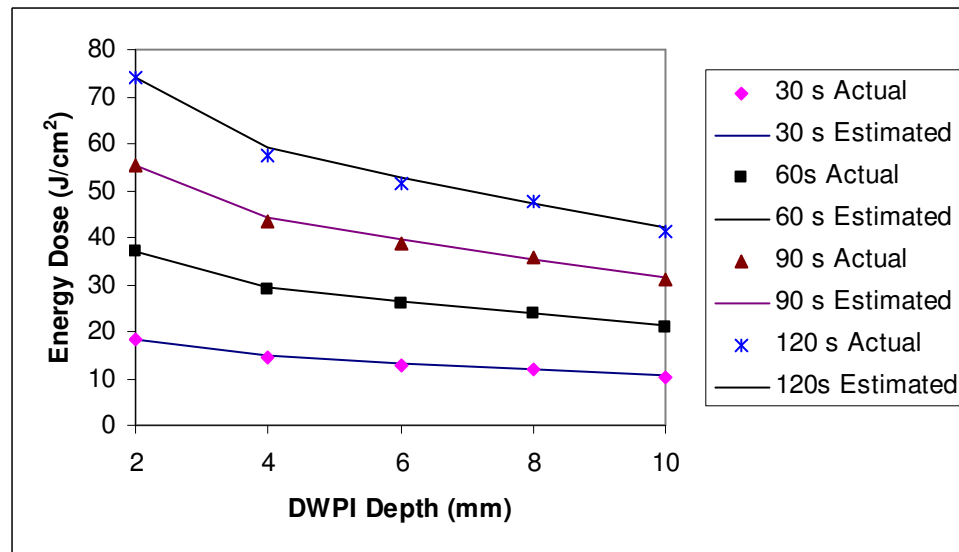


(b)

Figure 7.5. Validation of dose obtained using Eqn. 7.2 (a) 4% agar and (b) 10% DWPI.



(a)

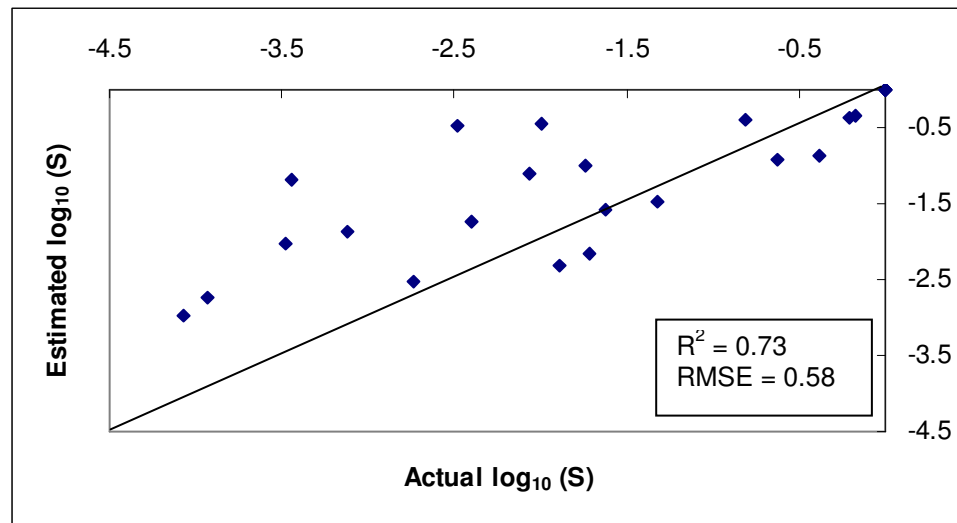


(b)

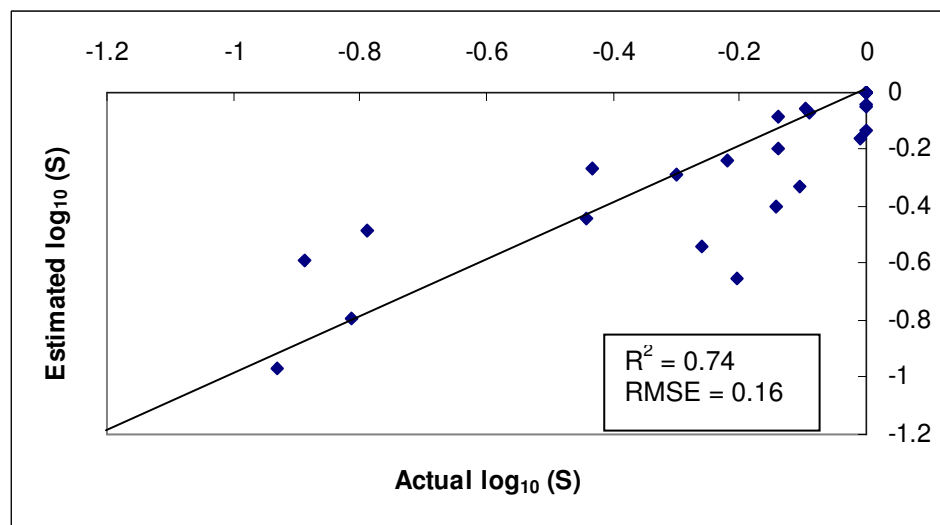
Figure 7.6. Fit of eqn. 7.2 to energy dose data for (a) 4% agar and (b) 10% DWPI.

### 7.3.4 Microbial inactivation model

As discussed earlier, there was an observed increase in the temperature of the gels during treatment; while this may affect the properties of the gels it most likely will not have a significant impact on microbial inactivation. Since the scope of this work was to model the affects of energy, temperature was not taken into account in the subsequent models. In order to estimate the microbial inactivation, the Weibull model (Eqn. 7.1) was used, which consists of two parameters,  $\alpha$  and  $\beta$ , which are characteristic dose and shape parameters, respectively. Several different approaches were taken to develop the best-fit model. The first approach was to estimate the inactivation using doses estimated using Eqn. 7.2 and subsequently using Eqn 7.1 to estimate the reductions, by finding a single value for  $\alpha$  and  $\beta$ , and will be referred to as Weibull Model 1. The root mean square error (RMSE) and coefficient of correlation ( $R^2$ ) for the model were 0.58 and 0.73 for 4% agar and 0.16 and 0.74 for 10% DWPI, respectively (Figure 7.7). The  $\alpha$  and  $\beta$  values for agar and DWPI were 16.2 J/cm<sup>2</sup> and 1.32, and 41.7 J/cm<sup>2</sup> and 1.73, respectively. The validation of Weibull Model 1 with the third inactivation data set for each individual depth indicates that the reductions estimated using this model do not correlate well the trend of inactivation (Figures 7.8 and 7.9). Therefore, this approach failed to estimate the shoulder and tailing effects observed in the experimental data.



(a)



(b)

Figure 7.7. Validation of  $\log_{10}(S)$  values for *E. coli* K12 using a single set of  $\alpha$  and  $\beta$  parameter values (a) 4% agar, (b) 10% DWPI.

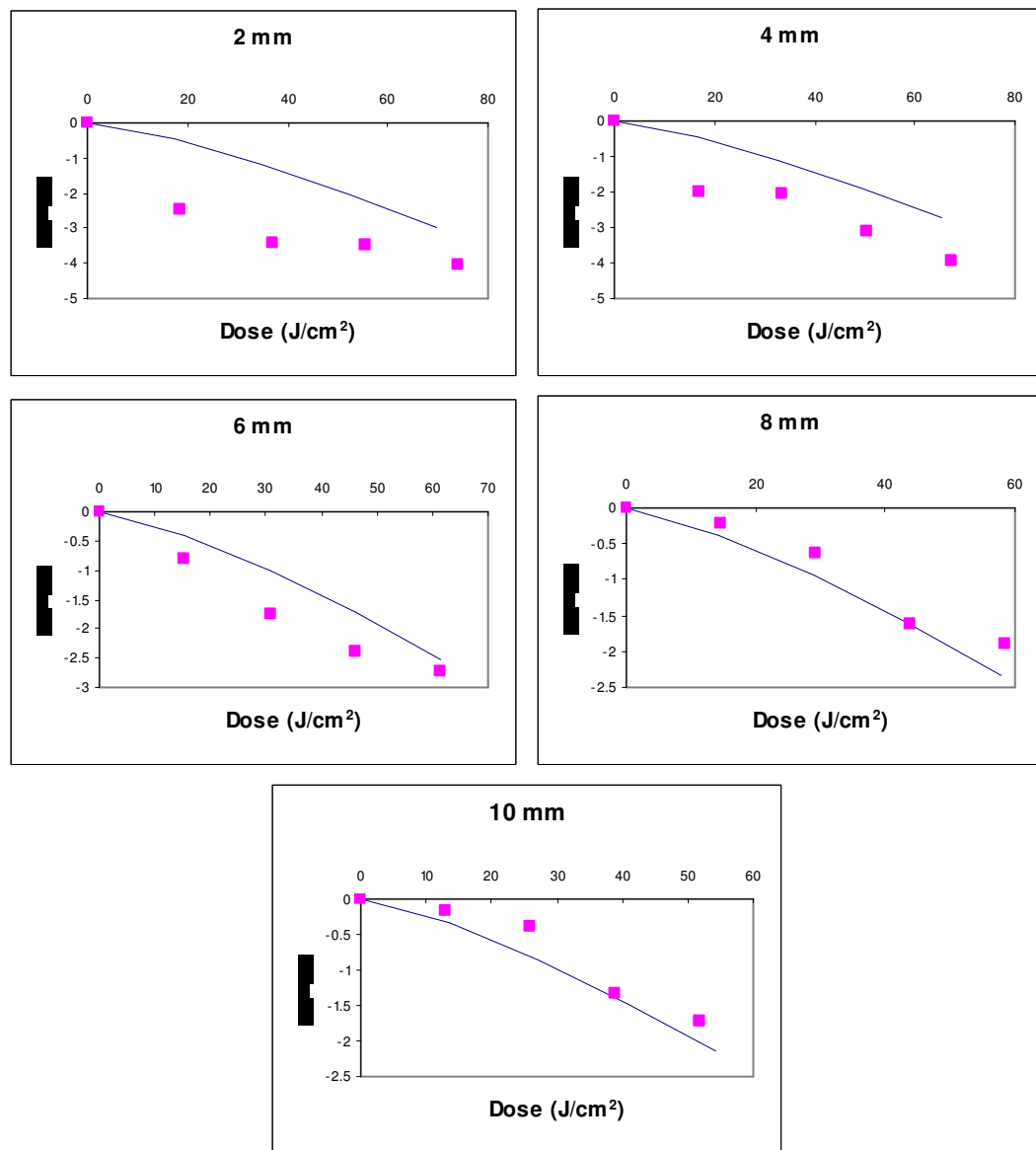


Figure 7.8: Fit of Weibull Model 1 with a single set of  $\alpha$  and  $\beta$  parameters to simulate inactivation of *E. coli* K12 in 4% agar at different depths: 2 mm, 4 mm, 6 mm, 8 mm, and 10 mm.

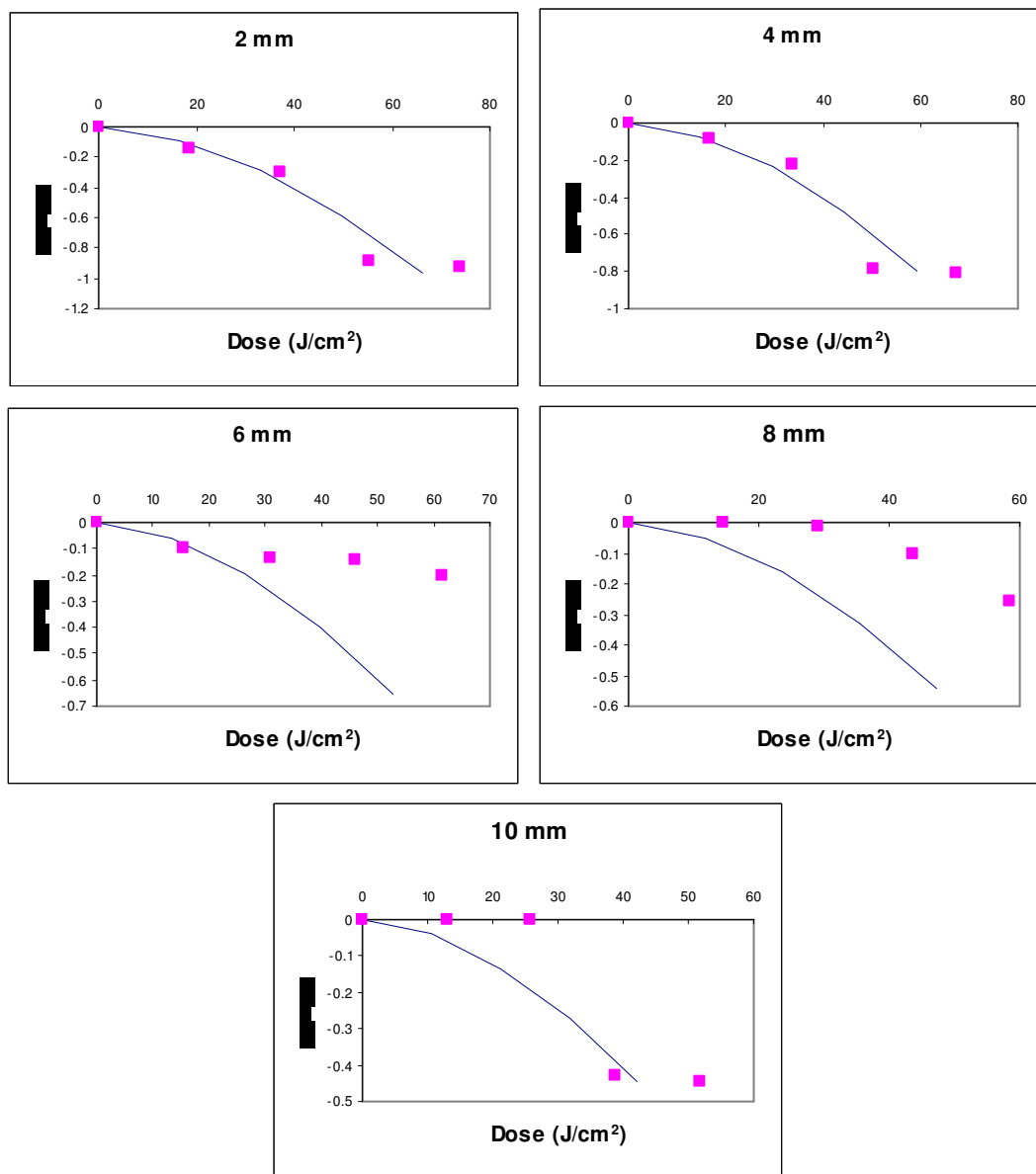


Figure 7.9: Fit of Weibull Model 1 with a single set of  $\alpha$  and  $\beta$  parameters to simulate inactivation of *E. coli* K12 in 10% DWPI at different depths: 2 mm, 4 mm, 6 mm, 8 mm, and 10 mm.

Furthermore, the inactivation data estimated using the Weibull Model 1 was compared to the actual inactivation data on the plots of  $\log_{10}(S)$  versus

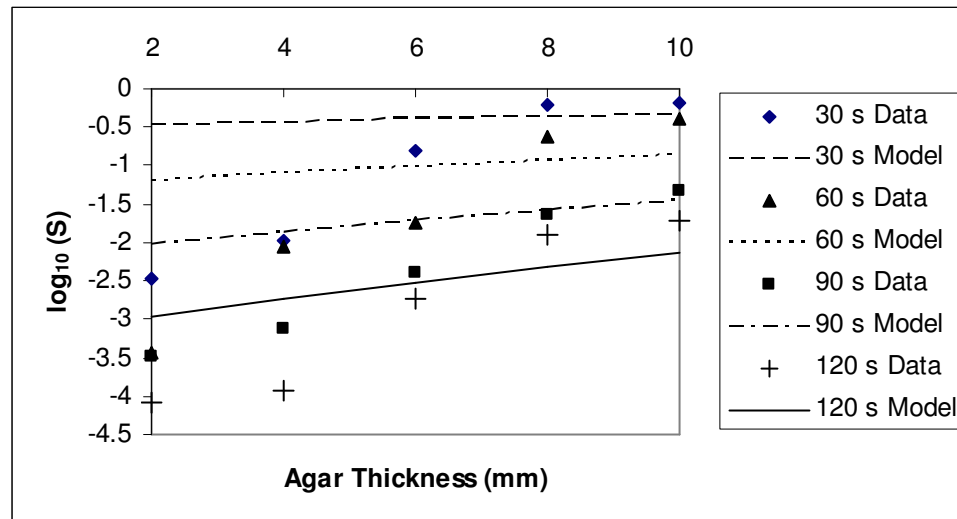


material thickness (Figure 7.10). This representation also indicates that Weibull Model 1 fails to estimate shoulders and tails seen in the data; the model appears to be linear which is not observed in the experimental data.

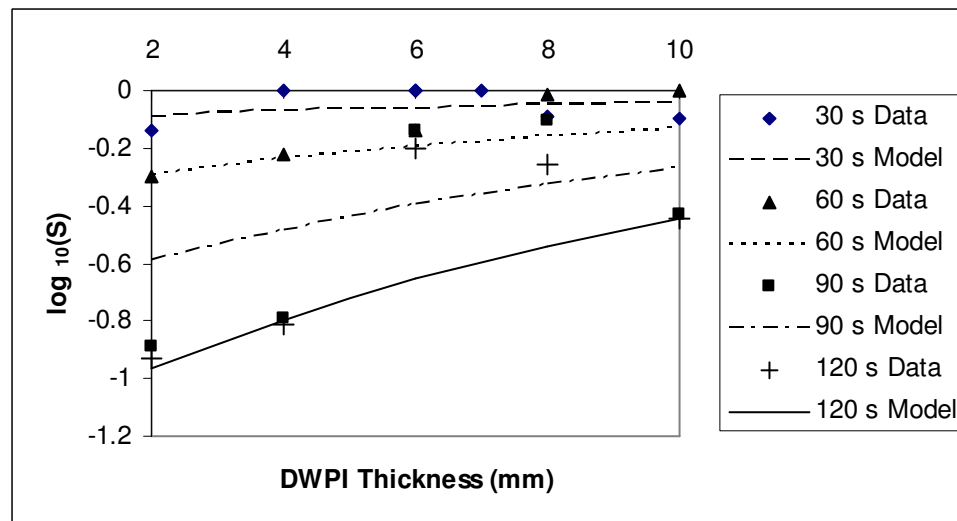
The second approach (Weibull Model 2) was undertaken to simultaneously estimate dose and the fraction of survivors, by solving for  $k$  (extinction coefficient),  $\alpha$  (characteristic dose), and  $\beta$  (shape parameter); by including an additional factor, the  $R^2$  of the model is expected to increase (Eqn. 7.3)

$$\log_{10}(S) = -\left(\frac{I_o e^{-kx} * time}{\alpha}\right)^{\beta} * \frac{1}{2.303} \quad \text{Eqn 7.3}$$

The  $k$ ,  $\alpha$  and  $\beta$  parameters for agar were found to be  $0.23 \text{ mm}^{-1}$ ,  $3.36 \text{ J/cm}^2$ , and  $0.73$ , respectively and for DWPI the values were  $0.20 \text{ mm}^{-1}$ ,  $28.1 \text{ J/cm}^2$  and  $0.9$ , respectively. This model was presented in the same formats as discussed earlier. As expected, the  $R^2$  increased and the RMSE values decreased for both agar and DWPI.  $R^2$  and RMSE values were  $0.84$  and  $0.48$  for agar and  $0.81$  and  $0.13$  for DWPI, respectively (Figure 7.11).



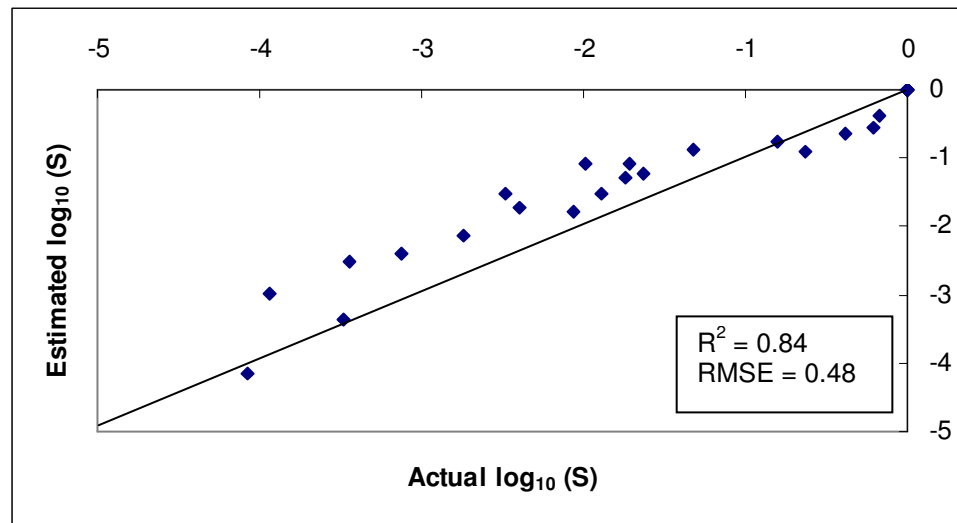
(a)



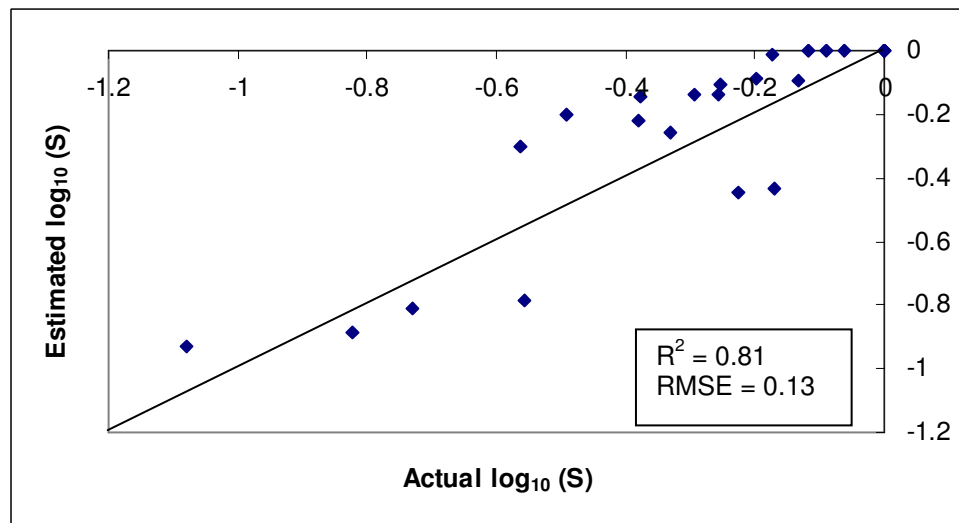
(b)

Figure 7.10. Fit of Weibull Model 1 with a single set of  $\alpha$  and  $\beta$  parameter values to simulate the inactivation of *E. coli* K12 (a) 4% agar, (b) 10% DWPI.

When comparing the calculated values with viewing the experimental data separated out at the individual depths (Figures 7.12 and 7.13), it is evident that in terms of dose, Weibull Model 2 does not fit well, either. It can be seen that the estimated doses are greatly underestimated as the depth of the sample increases, and indicates that perhaps using broadband energy as a predictor of microbial inactivation may not be suitable. There are many wavelengths involved in pulsed light (100 to 1100 nm) and each wavelength might not be filtered by the material equally. The representation of the calculated versus the experimental data for  $\log_{10}(S)$  with respect to depth also shows that Weibull Model 2 fits the inactivation data better than Weibull Model 1 (Figure 7.14). The model does contain a slight curve which appears to correspond with the data better.



(a)



(b)

Figure 7.11. Weibull Model 2 (i.e., with  $k$ ,  $\alpha$  and  $\beta$  parameters) validation for *E. coli* K12 (a) 4% agar, (b) 10% DWPI.

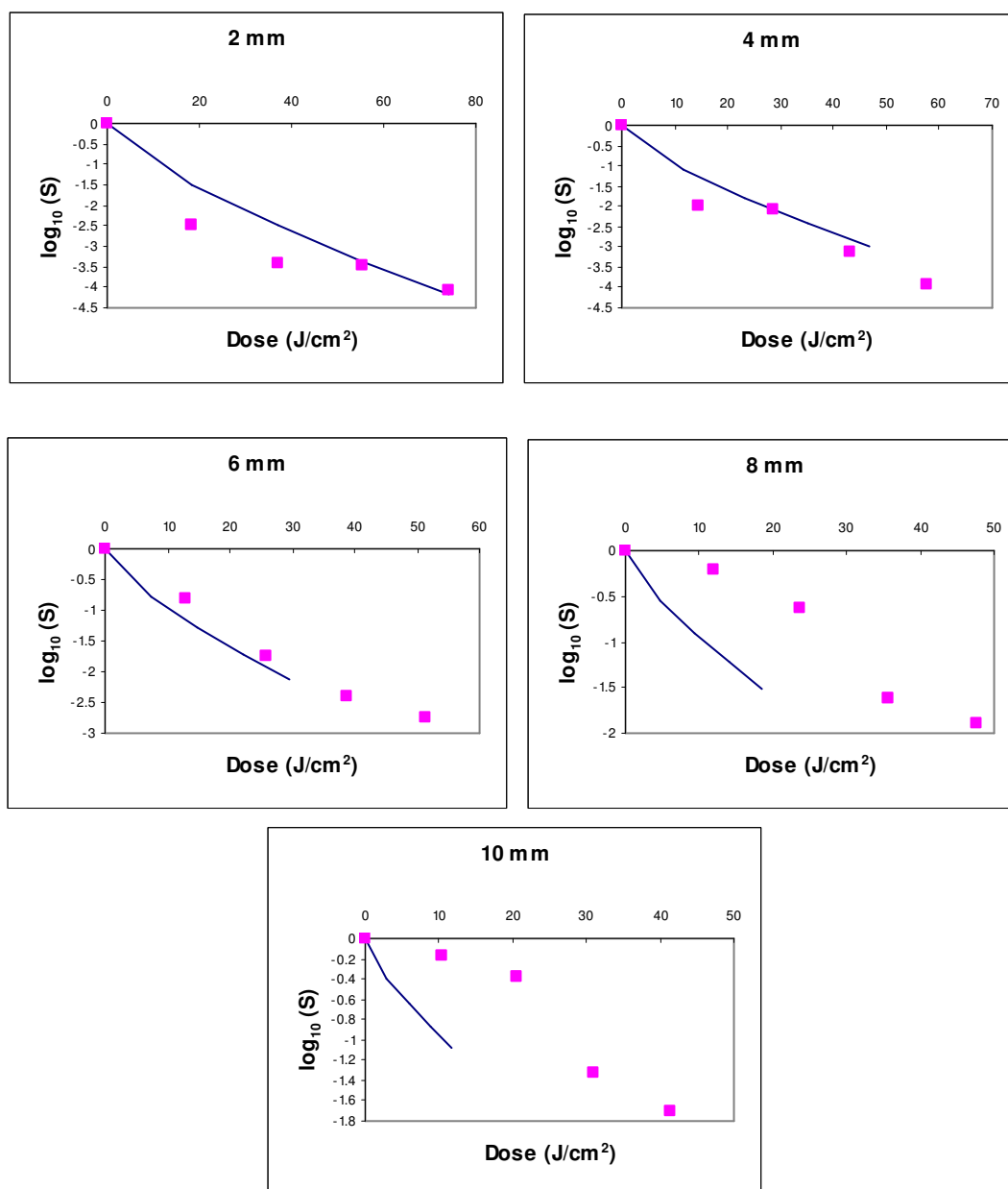


Figure 7.12: Fit of Weibull Model 2 (Eqn. 7.3) with  $k$ ,  $\alpha$  and  $\beta$  parameters to simulate the inactivation of *E. coli* K12 in 4% agar at different depths: 2 mm, 4 mm, 6 mm, 8 mm, and 10 mm.

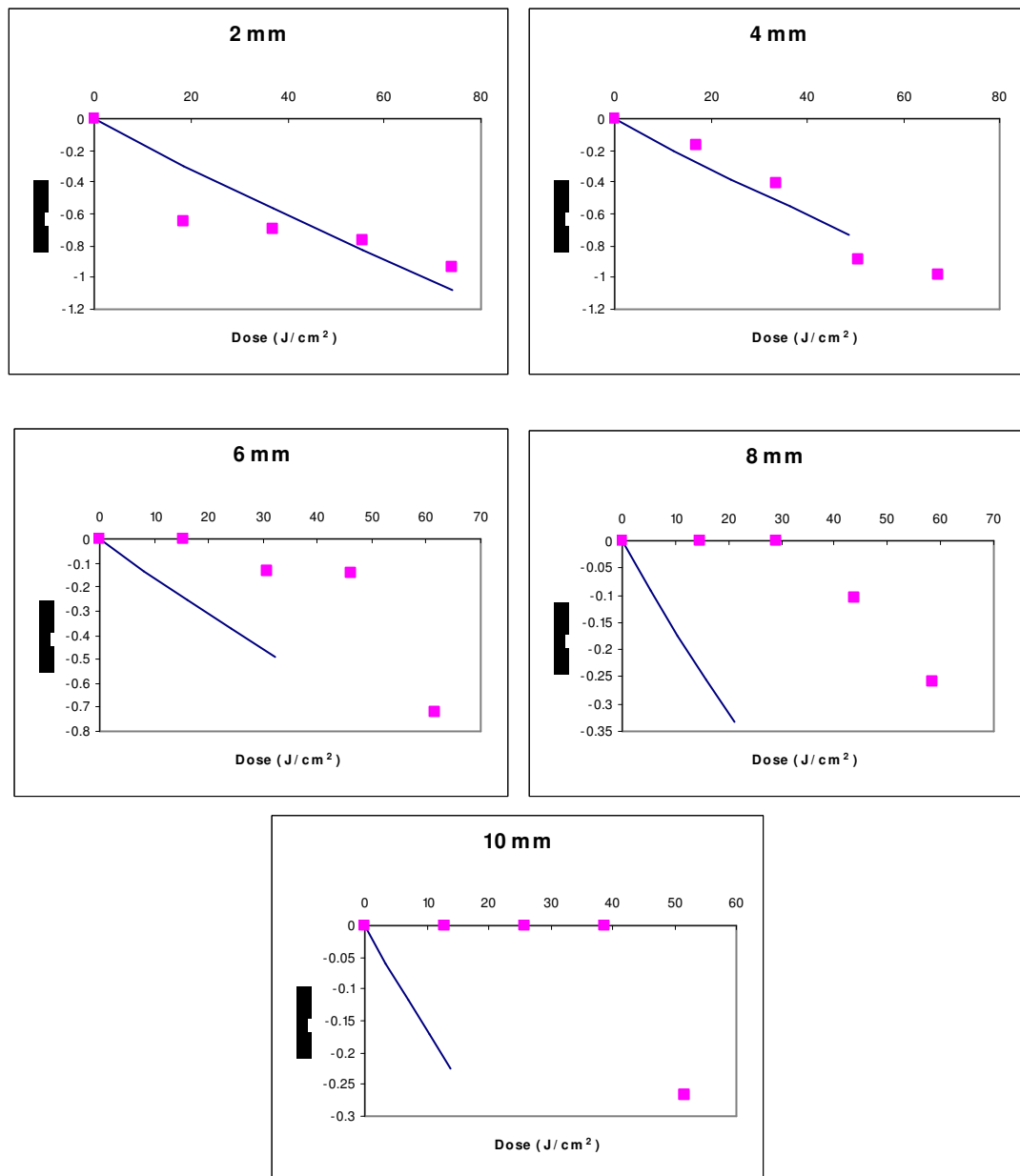


Figure 7.13: Fit of Weibull Model 2 (Eqn. 7.3) with  $k$ ,  $\alpha$  and  $\beta$  parameters to simulate the inactivation of *E. coli* K12 in 10% DWPI at different depths: 2 mm, 4 mm, 6 mm, 8 mm, and 10 mm.

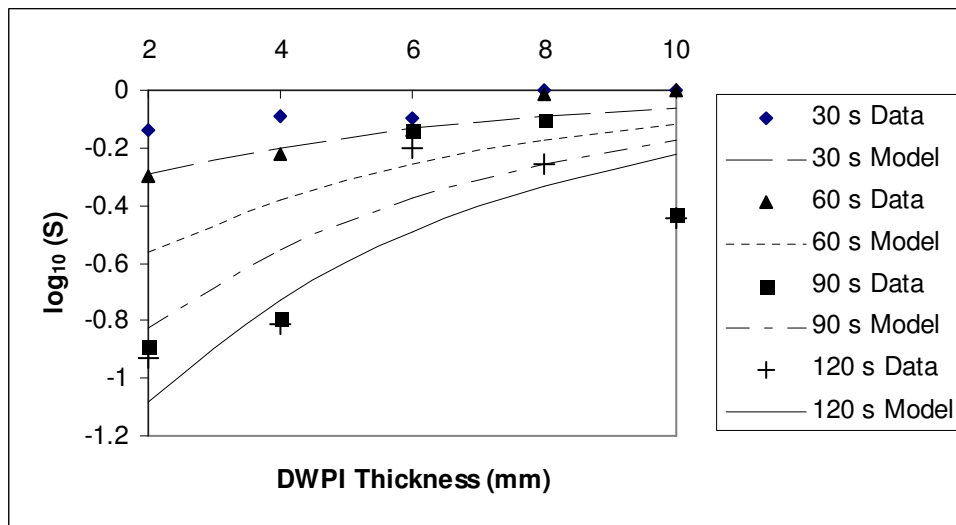
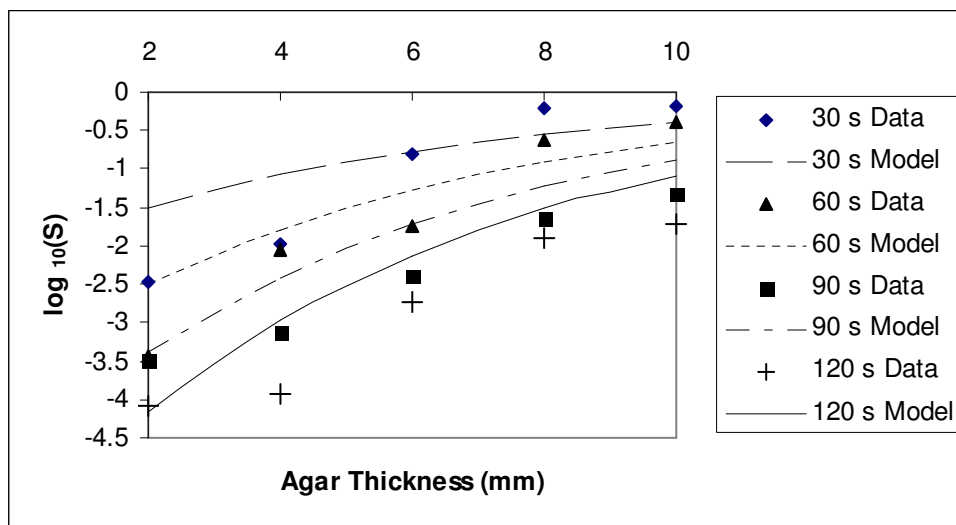


Figure 7.14. Fit of Weibull Model 2 with parameters  $k$ ,  $\alpha$  and  $\beta$  to simulate the inactivation of *E. coli* K12 (a) 4% agar, (b) 10% DWPI.

Based on the apparent problems using broadband energy dose as an indicator of microbial reduction, a third approach was formulated to estimate the model parameters. As pointed out earlier, wavelengths may be filtered at each depth, and perhaps not filtered equally. Therefore, third approach (Weibull Model 3) was undertaken to fit the individual depth data using non-linear least squares regression to find the  $\alpha$  and  $\beta$  parameters at each depth, which would minimize the sum of square error. This approach would allow for the different composition of wavelengths at each depth to be considered and essentially treat each depth as a unique scenario.

The  $\alpha$  and  $\beta$  parameters determined for each depth displayed sigmoidal curves and were modeled using a least squares polynomial fit which is represented in Eqns. 7.4-7.7, where  $x$  is the depth of the material.

$$\alpha(\text{agar}) = -0.0761x^4 + 1.7685x^3 - 13.497x^2 + 40.94x - 34.738$$

$$R^2=0.97 \quad \text{Eqn. 7.4}$$

$$\beta(\text{agar}) = -0.0019x^4 + 0.0511x^3 - 0.4384x^2 + 1.4413x - 0.6568$$

$$R^2=0.99 \quad \text{Eqn. 7.5}$$

$$\alpha(\text{DWPI}) = 0.0943x^4 - 2.5017x^3 + 22.213x^2 - 71.268x + 93.077$$

$$R^2=1 \quad \text{Eqn. 7.6}$$

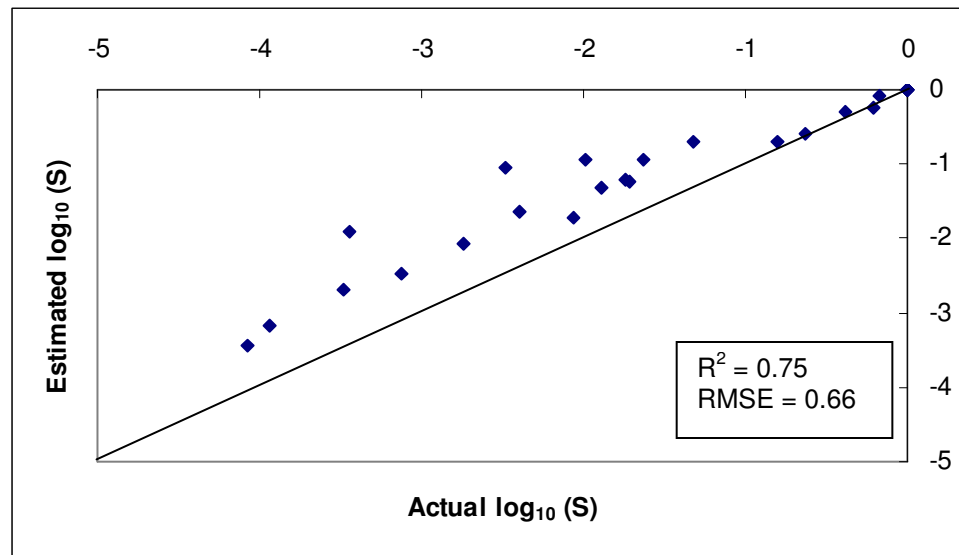
$$\beta(\text{DWPI}) = 0.0145x^4 - 0.352x^3 + 2.9765x^2 - 9.5064x + 10.27$$

$$R^2=1 \quad \text{Eqn. 7.7}$$

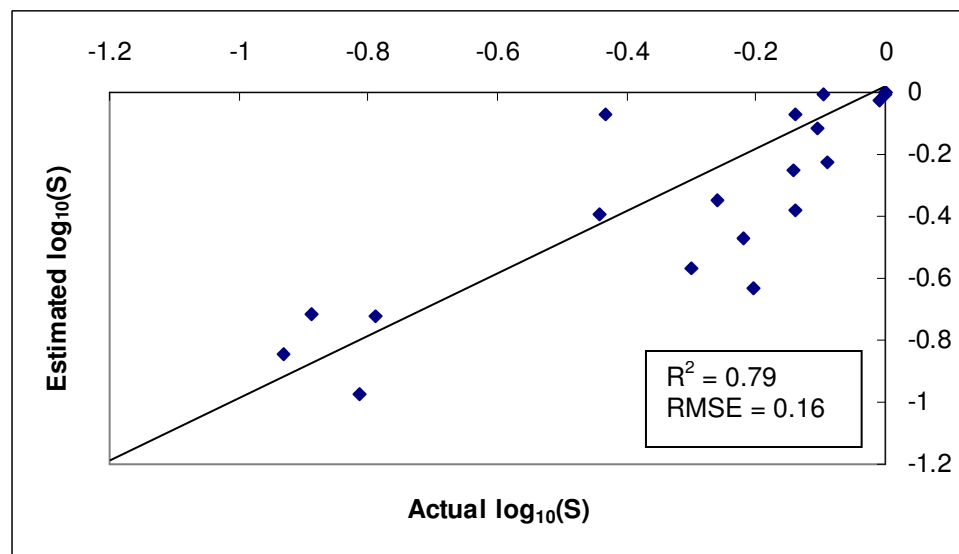


The correlations for  $\alpha$  and  $\beta$  were then incorporated into the Weibull model (Eqn. 7.1) along with the model developed to estimate the energy dose (Eqn. 7.2) received by the sample.

The validation of the model can be seen in Figure 7.15, where an  $R^2$  value of 0.75 was obtained and an RMSE value of 0.66 for agar, the goodness-of-fit parameters for DWPI were slightly better than those for agar with an  $R^2$  of 0.79 and a RMSE of 0.16. The calculated values were compared to the actual data in the same way as the previous two models. Figures 7.16 and 7.17 illustrate how the model fits the data points at each individual depth. When comparing these figures to those for the previous two models, the most noticeable aspect of this approach is that Weibull Model 3 more accurately estimates the dose. However, from Figure 7.18, it is observed that Weibull Model 3 is over-fitted (Figure 7.18). This is probably due to the inclusion of more parameters than needed to model the  $\alpha$  and  $\beta$  parameters. This is especially obvious when considering the DWPI model at treatment times of 90 and 120 s, where it appears that increasing the depth to 4 mm actually causes an increase in the reduction of the microorganism, which is not reflected in the data. Alternately, the polynomial correlations for  $\alpha$  and  $\beta$  (Eqns. 7.4-7.7), although simple, may not be entirely appropriate.



(a)



(b)

Figure 7.15. Weibull Model 3, i.e., with parameters determined at each depth, validation for *E. coli* K12 (a) 4% agar, (b) 10% DWPI.

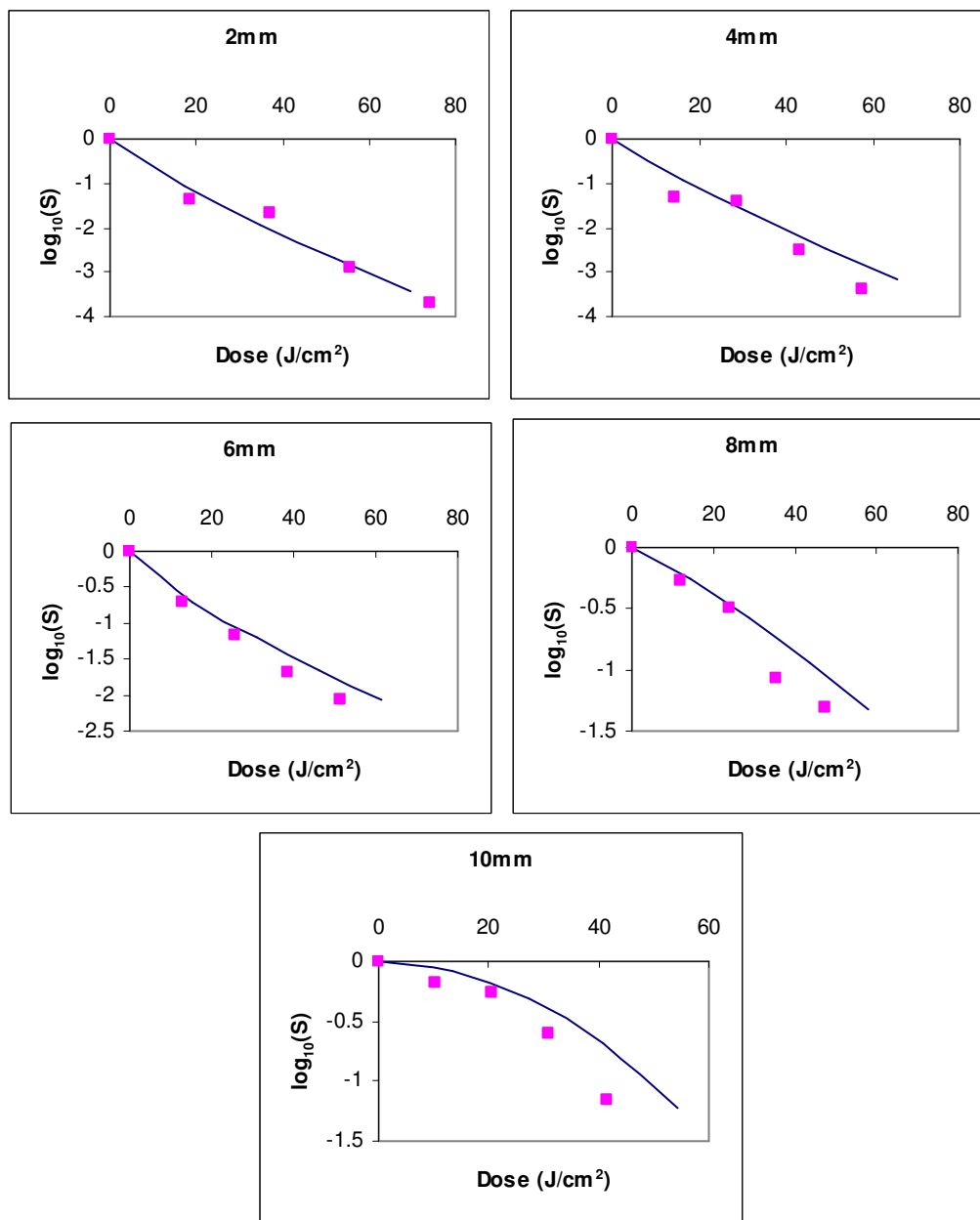


Figure 7.16: Fit of Weibull Model 3 with parameters determined at each depth to simulate the inactivation of *E. coli* K12 in 4% agar at different depths: 2 mm, 4 mm, 6 mm, 8 mm, and 10 mm.

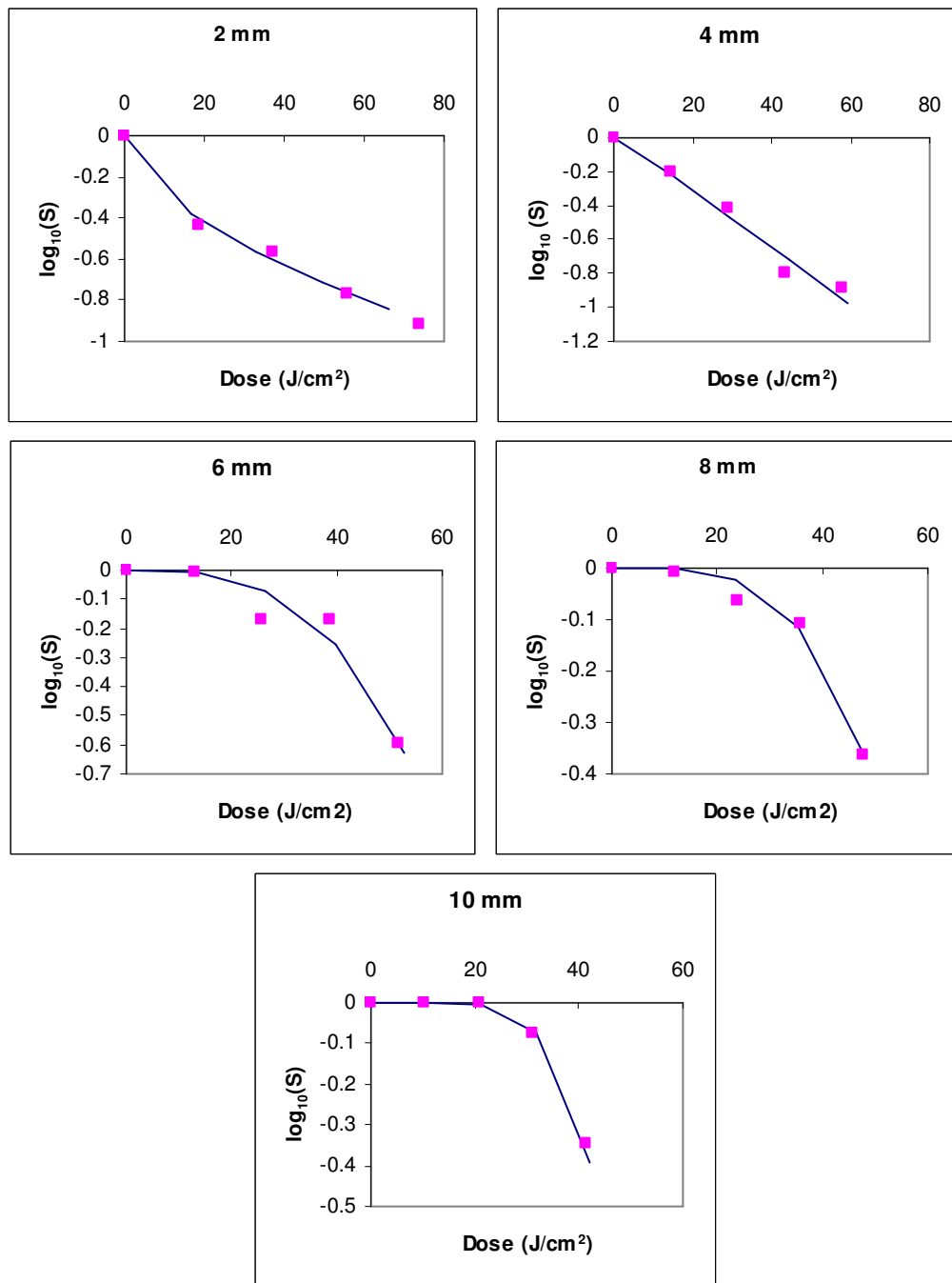
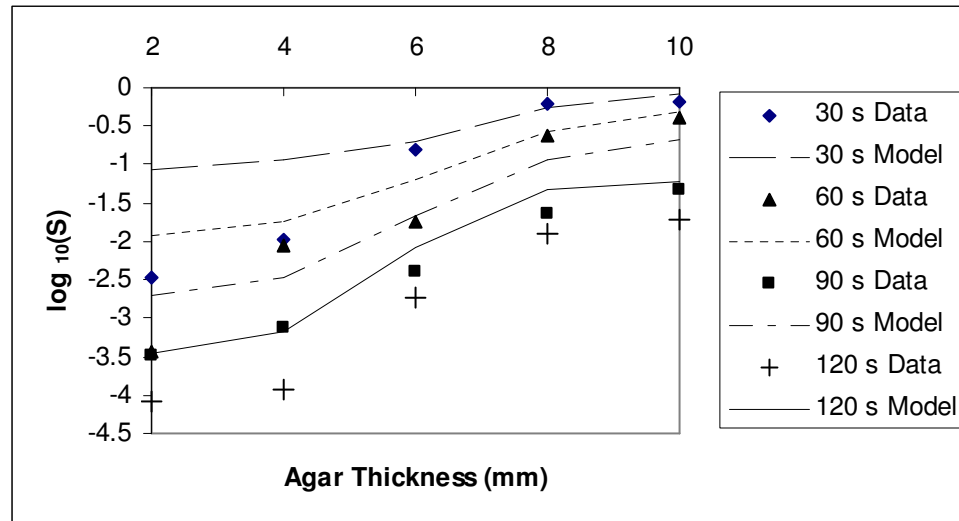
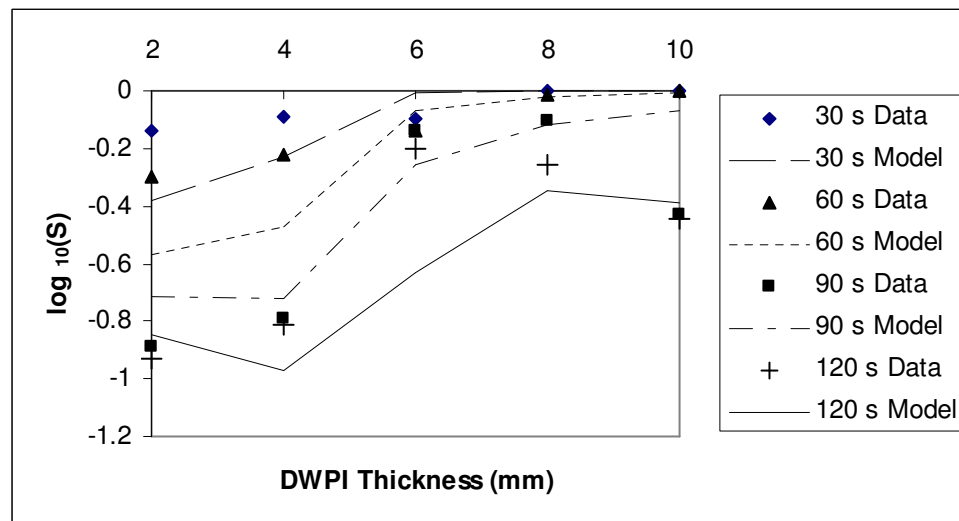


Figure 7.17: Fit of Weibull Model 3 with parameters determined at each depth to simulate the inactivation of *E. coli* K12 in 10% DWPI at different depths: 2 mm, 4 mm, 6 mm, 8 mm, and 10 mm.



(a)



(b)

Figure 7.18. Fit of Weibull Model 3 with parameters determined at each depth to simulate the inactivation of *E. coli* K12 (a) 4% agar, (b) 10% DWPI

The benefit of Weibull Model 3 is that it is capable of modeling the change in concavity of the survival curves change as the depth of the sample increases, as was noted earlier. This is further reflected in the model parameters obtained for the agar and DWPI gels (Table 7.1), where the  $\beta$  values are between 0.57 and 5.85. One issue observed in Weibull Model 3 was that the same dose did not result in the same reduction a every depth.

Table 7.1. Weibull Model 3 parameters for 4% agar and 10% denatured whey protein isolate (DWPI) gels.

	Depth	$\alpha$	$\beta$
Agar	2 mm	6.08	0.85
	4 mm	6.77	0.87
	6 mm	8.38	0.78
	8 mm	22.74	1.19
	10 mm	32.46	2.02
DWPI	2 mm	20.88	0.57
	4 mm	27.45	1.05
	6 mm	46.98	3.14
	8 mm	49.94	3.88
	10 mm	42.99	5.85

The preceding analyses based on Weibull Models 1, 2, and 3 reinforces the observation that using broadband energy as a predictor of inactivation is not suitable. Since at every depth, although the total energy is the same, the composition of the wavelengths is most likely not.

## 7.4 Conclusion

The results from this study illustrated the ability of pulsed UV-light to penetrate several millimeters into an opaque solid medium (10% DWPI). The shape of the inactivation curves of *E. coli* K12 exhibited different trends as the material depth increased, from sigmoid at lower depths to concave upward as the depth increased. Several approaches were taken to estimate the parameters used in the Weibull model. When comparing the three approaches, Weibull Model 1 was not a good fit since it did not accurately estimate the microbial reductions. This model appeared to be too linear, and this trend was not observed in the experimental data. Weibull Model 2 had the highest  $R^2$  values (Table 7.2) of all the models and accurately estimated the microbial reductions; however, it underestimated the broadband dose received by the sample. Weibull Model 3 was able to estimate both the microbial reductions and the dose received; however, this model was over-fit and that resulted in some inconsistencies.

One issue which was identified from the construction and evaluation of these models was that total broadband energy dose is not an appropriate predictor of microbial inactivation. Since UV-C light (200-280 nm) is believed to be the germicidal component of the system, a way to accurately measure this is needed. In addition, information and knowledge of how this light is filtered and absorbed through the material, and the effect on microbial inactivation is essential. In this study, it was also observed that there was an increase in temperature which is most likely due to infrared radiation and was not

incorporated into the model. By identifying a way to accurately measure dose, the fit of the Weibull model should improve.

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## Chapter 8

### Conclusions and scope for future research

In this study, the decontamination of blueberries, raspberries, and strawberries inoculated with *Escherichia coli* O157:H7 and *Salmonella* with ozone and pulsed UV-light was evaluated. The first study evaluated the efficacy of gaseous ozone applied in four different treatment scenarios. Ozone at a concentration of 5% (wt/wt) was applied continuously or under pressure (83 kPa) for 2 to 64 min. *Salmonella* appeared to be more resistant than *E. coli* O157:H7 to ozone exposure with reductions of 1.0, 1.6, and 0.9 log<sub>10</sub> CFU/g on blueberries, raspberries, and strawberries, respectively. Reductions of *E. coli* O157:H7 were 1.4, 2.6, and 1.8 log<sub>10</sub> CFU/g on blueberries, raspberries, and strawberries, respectively. When ozone was pressurized, reductions of *Salmonella* increased to 3.0, 2.0 and 2.2 log<sub>10</sub> CFU/g on blueberries, raspberries, and strawberries, respectively. Whereas, reductions of *E. coli* O157:H7 did not really increase and were 1.4, 2.8, and 2.3 log<sub>10</sub> CFU/g on blueberries, raspberries, and strawberries, respectively. The third treatment consisted of a combination of continuous and pressurized ozone which increased log reductions on raspberries and strawberries but had little effect on reductions of *E. coli* O157:H7 or *Salmonella*. On raspberries, reductions were 3.6 and 3.8 log<sub>10</sub> CFU/g for *Salmonella* and *E. coli* O157:H7, respectively. On strawberries, reductions were 2.6 and 2.9 log<sub>10</sub> CFU/g for *Salmonella* and *E. coli* O157:H7, respectively. The final gaseous ozone treatment was to place fruits under a

vacuum followed by a pressurized ozone treatment. This treatment had little effect and did not significantly increase reductions of *E. coli* O157:H7 or *Salmonella* on blueberries, raspberries, and strawberries.

The second study evaluated the effect of using ozone as an additive to water at 20 and 4°C. By decreasing the temperature of the water the concentrations of ozone increased to 21 ppm from 9 ppm at 20°C. On blueberries and raspberries, zero plate counts were observed after treatment for 64 min at 4°C with only 1 out of 3 enrichments positive for pathogenic microorganisms. Aqueous ozone did not have as great of an effect on strawberries with none of the treatments resulting in zero plate counts. Also in this study the effect of water sparged with air was evaluated as a control. It was observed that treating fruits with water sparged with air had very little effect on the inactivation of *E. coli* O157:H7 or *Salmonella* with reductions of around 1 log<sub>10</sub> CFU/g.

The other technology evaluated in this study for the purpose of decontaminating berries was pulsed UV-light. Pulsed UV-light was evaluated at varying distances and treatment times. This treatment was found to produce reductions as high as 3.4 and 3.9 log<sub>10</sub> CFU/g of *Salmonella* and *E. coli* O157:H7, respectively, on raspberries without any damage to the fruit. Strawberries and blueberries, treated with pulsed UV-light did exhibit damage at closer distances from the light source. The treatments that did not damage the fruit resulted in reductions of 2.1 and 2.8 log<sub>10</sub> CFU/g of *E. coli* O157:H7 and

*Salmonella*, respectively, on strawberries. On blueberries, maximum reductions were 4.3 and 2.9 log<sub>10</sub> CFU/g of *E. coli* O157:H7 and *Salmonella*, respectively.

The results obtained from the ozone and pulsed UV-light treatments were used to create models to estimate the microbial reductions. Two models were evaluated, a first-order linear model and the non-linear Weibull model. The first-order model was found to inadequately estimate reductions of *E. coli* O157:H7 and *Salmonella*. The Weibull model was found to be an acceptable model for estimating reductions of *E. coli* O157:H7 and *Salmonella* on blueberries, raspberries, and strawberries treated with gaseous ozone, aqueous ozone, and pulsed UV-light; with correlation coefficients between 0.83 and 0.99 and slope values of 0.76 to 1.26.

The final element of this study was to evaluate the effects of material thickness on UV energy penetration and microbial inactivation. The inactivation curves obtained indicated that the relationship between UV dose and inactivation was non-linear and the Weibull model was used to estimate these inactivations. The model further incorporated a modified exponential model to characterize the decay of UV energy through either agar or whey protein isolate. Furthermore, it was concluded that energy could not be directly correlated to microbial inactivation in the case of pulsed UV-light, due to the filtering effects of the materials used. Each depth studied had to be considered a unique environment and had to be modeled as such.

In this study, several novel technologies were evaluated for the purpose of decontaminating pathogens on berries. Both ozone and pulsed UV-light were

found to successfully inactivate *E. coli* O157:H7 and *Salmonella* without noticeable damage to the fruit.

Further research needs to be conducted with gaseous ozone at higher pressures in order to force the ozone further into the seeds and crevices of berries. The effects of both gaseous and aqueous ozone on the shelf-lives of blueberries, raspberries, and strawberries needs to be evaluated, as well as effects on the sensory characteristics of the fruits.

The use of pulsed UV-light was effective at reducing pathogens on only one exposed surface of the berry. Additional research needs to be conducted to determine a way of turning the fruit so that all sides receive an adequate amount of energy to decontaminate them. Also as with ozone, the effects of pulsed UV-light on the shelf-life and sensory attributes of the fruits need to be evaluated.

For all technologies, the role of the fruits structure needs to be considered and studied further. The surfaces need to be characterized to understand how the cells might be arranged so that strategies could be developed to reach those cells. Also, the achnes, drupelets, and crevices of the fruits needs to be characterized to understand how these characteristics might impact the ability of the sanitizer to infiltrate them and to further understand the shadowing affect that is encountered during pulsed UV-light treatment.

Furthermore, the range of pathogenic microorganisms needs to be expanded. In this study only Gram-negative microorganisms were studied and there was an observed difference in the sensitivity of these microorganisms. Gram-positive microorganisms need to be studied as well as viral and parasitic

pathogens to ensure that these technologies can be used to produce a safe product. Finally, for all technologies scale-up studies need to be conducted. The efficacy of ozone and pulsed UV-light needs to be determined on a pilot-scale and optimized to achieve maximum reductions within a minimum treatment time.

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## Appendix A

### Sensory evaluation of blueberries treated with ozone and pulsed UV-light

Gaseous ozone, aqueous ozone, and pulsed UV-light were evaluated as possible technologies for the decontamination of blueberries. From previous studies, best treatment scenarios were selected based on  $\log_{10}$  reductions of *E. coli* O157:H7 and *Salmonella*. The treatments which were selected were as follows:

- Gaseous ozone: 64-min continuous 5% (wt/wt) ozone
- Aqueous ozone: 64-min at 20°C (8.9 ppm)
- Pulsed UV-light: 11.3 J/cm<sup>2</sup> UV dose which corresponded to 8 cm from the quartz window for 30 s.

Treated and untreated blueberries were evaluated, 3 days after treatment so that no ozone residue could be detected, to determine if an overall significant difference was detectable by an untrained sensory panel. A duo-trio test was selected due to its ease of use. Furthermore, this test utilizes a constant reference sample which aids in avoiding panelist confusion.

Forty panelists were selected and asked to determine if a sample was the same or different from the presented reference. There is a 50% chance of a panelist “guessing” the correct answer, so while the results may appear that there is a difference, statistically a difference was not detectable. The results obtained from this test are presented in Table A.1.

Table A.1. Responses of untrained panelists from the duo-trio test.

	Aqueous Ozone	Gaseous Ozone	Pulsed UV-Light
# Same Responses	14	18	17
# Different Responses	26	22	23

The results indicate that a difference in the treated versus the untreated blueberries was not detectable. In order for a difference to be detected the number of same responses would have to be greater than or equal to 26 at the 5% level of significance.

Based on these results it was concluded that treatment with gaseous ozone, aqueous ozone, or pulsed UV-light had no effect on the overall sensory characteristics of blueberries.

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## Publications

Bialka, K.L. and A. Demirci. 2006. The efficacy of aqueous ozone for the decontamination of *Escherichia coli* O157:H7 and *Salmonella* spp. on raspberries and strawberries. *Journal of Food Protection* (Accepted)

Bialka K.L. and A. Demirci. 2006. The utilization of gaseous ozone for the decontamination of raspberries and strawberries. *Journal of Food Protection* (Accepted)

Bialka, K.L. and A. Demirci. 2006. Pulsed ultraviolet-light for the disinfection of small fruits. *Journal of Food Protection*.