

**Application of Far Infrared Radiation and Ethanol Vapor as
Alternative Treatment Methods for Reduction of *Salmonella*
enterica Tennessee in Dried, Ground Spices**

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Thesis submitted to the faculty of Virginia Polytechnic Institute and State University in partial fulfillment of the requirements for the degree of

Masters of Science

in

Biological Systems Engineering

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© May 6, 2013

Blacksburg, Virginia

Keywords: Spices, *Salmonella*, Ethanol, Far Infrared Radiation

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ABSTRACT

The consumption of spiced food is steadily increasing, subsequently leading to increased incidence of spice-related food illnesses. Many outbreaks can be traced to human pathogens that can survive in low moisture content of spices, prompting development of additional inactivation treatments that reduce bacterial pathogens while maintaining spice quality. Spices are currently treated by fumigation with ethylene oxide, pasteurization with ionizing radiation, or steam treatment. However, these treatments exhibit flaws pertaining to consumer preference, regulatory issues, and quality degradation. In this study, two novel treatments were evaluated for reduction of *Salmonella enterica* Tennessee: far infrared radiation (FIR), a short time – high temperature treatment, and pasteurization with ethanol vapor (EV). Both treatments were effective in reducing levels of *Salmonella* Tennessee between 3-5 logs. FIR treatment showed increased efficacy at longer treatment times with a maximum reduction of 5 log CFU/g in paprika at 24s. EV reduced *Salmonella* Tennessee by 3 log CFU/g within 120s when applied to inoculated paprika and black pepper without detrimentally affecting spice quality. However, the samples receiving FIR treatments suffered reductions in volatile content and color changes to the spices. High levels (up to 1% w/w) of residual ethanol were also detected on samples treated for 300s. Concluding, both treatment show similar results when comparing efficacy; however, based on the magnitude of change in volatile content associated with FIR being significantly greater than those samples receiving EV, FIR treatment requires additional research before recommending for use with dried, ground paprika, black pepper, or sage.

Acknowledgements

In 2010, I contacted Dr. Kumar Mallikarjunan expressing my interest in returning to Virginia Tech on the chance he was willing to help me pursue a graduate degree in food engineering. I enrolled as his student shortly thereafter. His enthusiasm and expertise on the subject of food engineering had intrigued me since I first had the opportunity to meet him as an undergraduate in 2004. I want to thank him for taking the time to be my advisor and sharing his knowledge of the food engineering profession with me.

I would also like to thank Dr. Monica Ponder for allowing me to use her lab in Fralin for the bulk of the work in Chapter 3 of this thesis. Her help and time was invaluable; I could not have done this without her. A special thanks goes out to all of Dr. Ponder's graduate students as well. The help they provided will be remembered kindly. Thanks to Dr. Greg Welbaum for his assistance in my research. I enjoyed getting to know him and a little bit more about horticulture. The funding and feedback he provided was instrumental in the completion of my studies.

I need to also thank many members of the BSE and FST departments. Thanks to all of Dr. Kumar's graduate students, past and present, for their various bits of help and encouragement throughout the last few years. Thanks to Amy Egan and Laura Lehmann for assistance with all the various pieces of lab equipment and supplies that kept me going. Thanks to Dr. Ryan Senger and Dr. Rob Williams for serving on my committee and being so friendly and willing to talk when I needed them. Thanks to Harriet Williams and Brad Smith for their time spent teaching me.

Finally, thanks to my wife, Erin, for being kind and understanding on those late nights and weekends when I was in the lab. The push she provided helped me during the times when I thought this would never end.

I apologize to anyone not mentioned by name, or I may have forgotten, as I am already out of space. Your contributions are greatly appreciated and not forgotten.

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CHAPTER 1: INTRODUCTION

1.1 Background

The world spice market has been increasing for several decades, with a 60% increase seen in spice consumption in the United States from 1980-2000 (Vij, Ailes et al. 2006). Unfortunately, this increase in consumption is associated with increased numbers of spice related illnesses (McKee 1995, Banerjee and Sarkar 2003, Sagoo, Little et al. 2009, Zweifel and Stephan 2012). Contamination of two of the most popular spices in the world, black pepper and paprika, have resulted in many of these illnesses (McKee 1995, Tainter and Grenis 2001, Keller, VanDoren et al. 2013). In 1993, over 1000 illnesses were reported in Germany as a result of consumption of potato chips coated with contaminated paprika (Lehmacher, Bockemuhl et al. 1995). Numerous other outbreaks have been associated with spices; the FDA monitored 21 recalls of spices between 1970 and 2003 (Vij, Ailes et al. 2006). The most recent outbreak saw over 600,000 pounds of salami seasoned with *Salmonella* Montevideo infested black and red pepper recalled (CDC 2010).

Decontamination methods for spices currently include: fumigation with ethylene oxide, irradiation from ionizing radiation, and steam treatment. Ethylene oxide (EO) is the most common post-harvest treatment, with an estimated 85% of all spices in the United States receiving ethylene oxide treatment (ASTA 2011). The use of EO is strictly regulated and it must be listed as a food additive (ASTA 2011). Irradiation is effective, but spices treated in bulk must be marked with the radiation treatment symbol. However, products where spices compose a minor component do not require the irradiation symbol. Dried foods such as spices, show much lower levels of susceptibility to irradiation than foods with higher levels of water (Sádecká 2007). As a result, spices can withstand high levels of irradiation before changes begin to impact quality; therefore, spices can receive one of the highest allowable doses of radiation, 30 kGy (Alam Kham and Abraham 2010). Several political groups also protest the use of irradiation and it remains an unpopular method among consumers (Alam Kham and Abraham 2010). Steam is a

new method, but it requires heating of the spice, which can result in loss of quality of the product. Several studies have shown steam produces some color changes and volatile losses (Kispéter, Bajúsz-Kabók et al. 2003, Waje, Kim et al. 2008, Rico, Kim et al. 2010).

The use of FIR and ethanol vapor may solve some of the limitations of the current treatment methods. FIR has been used extensively for several decades in the food industry to control insect larvae in grains. It has also been tested for the use in drying of fruits and vegetables, baking, and roasting (Sakai and Hanzawa 1994, Nowak and Lewicki 2004, Sharma, Verma et al. 2005, Wang and Sheng 2006). Most recently, FIR, along with near infrared radiation (NIR) has been used to inactivate pathogens in liquid media (Krishnamurthy, Khurana et al. 2008, Krishnamurthy, Tewari et al. 2010) and solid food products (Staack, Ahrné et al. 2008, Erdogdu and Ekiz 2011). Ethanol has been approved as a food additive and is, in small amounts, safe for consumption. It is commonly used in its liquid form as an antimicrobial agent. However, It can be used in vapor form to delay ripening of fruits and to elongate the shelf life of baked goods (Dao and Dantigny 2011).

1.2 Rationale and Significance

An increase in spice consumption has led to an increase in spice related illnesses. To prevent such illness, spices should undergo treatment. Consumer preference for these treatments is low which leaves room for new treatments to take their place. Ethanol vapor and far infrared radiation (FIR) were selected as potential treatment methods. FIR has been commonly used in the food industry since the 1960s. Ethanol has been approved for human consumption, making it safer than current chemical fumigation techniques. These new treatment methods were vetted by investigating the influence they have on three factors influencing quality: water activity, color, and volatile profile.

1.3 Hypotheses

It is hypothesized that high temperature-short time FIR treatments will inactivate living pathogen cells without compromising quality. By varying the treatment time as well as sample placement

in the treatment chamber it is expected to find a suitable combination that will balance a high inactivation rate while maintaining spice quality. Furthermore, it is believed that a low temperature treatment with ethanol vapor will maintain all quality parameters while inactivating pathogens. Due to the highly volatile nature of ethanol, high concentrations of any residual chemical vapor should evaporate.

1.4 Objectives

This project set out to improve the safety of spices by studying the effectiveness of alternative treatment methods on reducing levels of bacteriological contamination in spices. Specific objectives are as follows:

1. Construct a treatment chamber and develop associated methodology for treating spices with far infrared radiation and ethanol vapor.
2. Apply novel treatments to inoculated spices and evaluate the ability of the treatments to inactivate *Salmonella enterica* Tennessee.
3. Quantify any quality losses based on changes in water activity, color values, and head space volatile analysis.

1.5 Thesis Outline

Testing of these new spice treatment methods included testing with live strains of pathogenic *Salmonella* Tennessee. After determining the treatment parameters required to reduce levels of viable *Salmonella* Tennessee, the effect on quality was investigated. Color was measured with a Minolta CR-300 chroma meter. Volatile profile was captured with the use of solid phase micro-extraction headspace sampling and gas chromatography (HS-SPME-GC). Water activity was also measured and checked for changes. Mathematical modeling was used to fit the inactivation curves to Bigelow and Weibull models.

This thesis consists of five chapters. Chapter one allows the reader to familiarize themselves with the topic and gain background knowledge on the issues being researched. Chapter two is a

literature review and details previous and current work occurring in areas relevant to the topic. Current spice treatment methods, including the proposed methods and information about food borne illness and contaminated spices are presented. Chapter three presents the completed work on *Salmonella* Tennessee reduction by exposure to far infrared radiation and ethanol vapor. Chapter four focuses on the impact on selected quality parameters post-treatment. Chapter five is for conclusions and a wrap up of all completed work including recommendations for future work.

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CHAPTER 2

Literature Review

2.1 Introduction

Recent incidences of food-borne illness have brought the issue of food safety and food security to the forefront of the public arena. Legislation, including the new Food Safety Modernization Act, holds potential to shape future trends in food safety and food manufacturing (H.R.2751 2011). Therefore, it is important to develop technologies that can meet the new regulations and improve customer health. One food item often being affected are spices.

2.2 Spice Use and Consumption Trends

The FDA (CPG § 525.750) defines a spice as:

Aromatic vegetable substances, in the whole, broken, or ground form, whose significant function in food is seasoning rather than nutrition.

This can include the seeds, leaves, bark, flower buds, roots, stalks, or any other part of the plant. The term *spice* has also come to encompass herbs as well. Condiments can also be included in this definition, as they are typically blends of spices. Dried vegetable seasonings are also spices (e.g. onion powder, garlic powder).

These seasonings have been used for the length of human history by all cultures and in all aspects of the food industry and at some points have been valued for their weight in gold (McKee 1995). Therefore, spices hold a very important role in food consumption – not only from

the taste or sensory aspect of food, but also from the psychological, emotional, and historical side as well. Currently, there are many types of spices available to consumers. The International Trade Centre declares over 1.547 million tons of spices were traded in 2004 (Zijlstra-Adriano 2006). The same report states the worldwide spice market is valued at almost US\$3 billion with that number growing over the study period, 2000 to 2004, by 1.9% annually.

Only a few markets dominate the world spice market. The United States, the European Union, Japan, and Singapore comprise 64% of the total market of all imported spices in 2004. The United States accounts for 21% of this total (Zijlstra-Adriano 2006). Furthermore, spice consumption in the United States increased 60% over the 20 year span of 1980 to 2000 (1.0 to 1.6 kg/person/year)(Vij, Ailes et al. 2006). The most consumed spices over the last decade have been capsicum (chilies), peppers, ginger, spice seeds, and spice mixtures (Zijlstra-Adriano 2006).

2.3 Spice Information and Harvesting Production Methods

2.3.1 Black Pepper – *Piper nigrum* is a woody perennial vine that is commonly grown in warm tropical climates, being native to Southern India. The pepper vine is cultivated for its fruit, or peppercorn. Peppercorns are a small drupe approximately 1 cm in diameter, being dark red when fully mature, and contain a single seed. Peppercorns are commonly ground for use and come in three different varieties: black pepper (unripe, dried pepper), white pepper (mature, shelled pepper), and green pepper (immature pepper). Pepper is often hand harvested and sun-dried, with conditions varying depending on the type of pepper. Pepper is a widely popular spice in the United States with 66,000 tons and US\$114 million being sold in 2004. This accounts for 24% of the entire world supply of pepper imports (Zijlstra-Adriano 2006). Pepper has established itself as a universal spice in nearly all food applications.

2.3.2 Paprika – *Capsicum annuum* is the ground form of dried capsicum, or chili peppers. The pepper plants are native to Southwestern United States or Mexico. There are many types of paprika – ranging from sweet to mild to hot, with others being used more for the red color contribution they add to the food than the flavor. The pigment responsible for the color is the

carotenoid *capsanthin*. Capsicum is the most widely traded spice in the world. Over 370,000 tons were traded in in 2004, with the United States consuming 24% of this total (Zijlstra-Adriano 2006). Traditionally, the highest quality paprika was found in Spain and Hungary; but now domestic paprika has surpassed imported paprika for consumer preference, accounting for 70.8% of the total (Tainter and Grenis 2001). The majority of domestic paprika is grown in New Mexico. Technological advances have reduced the number of producers using sun-drying as the method of pepper dehydration. This has improved quality and cleanliness, but costs more.

2.3.3 Sage – *Salvia officinalis* is a perennial bush growing about a meter in height and native to the Mediterranean region. The leaves range from silvery-gray to blue-green in color with a hairy texture and are about 5 cm in length. The leaves are dried and processed to a final product that can be rubbed or ground for use. Sage has been used for a long time for medical treatments as well as an herb for cooking. Sage is much different from the previously listed spices as it is much more herbaceous and a leaf and not a fruit. US\$4.4 million of sage was imported in 1999 from mostly Eastern European countries (Tainter and Grenis 2001). It is commonly used as a seasoning in the meat packing industry.

2.4 The Role of Microbiology with Food and Spices

The globalization of the world's food supply has led to the sourcing of many ingredients from foreign countries – most with varying regulations on food safety. Spices, as well as most agricultural products, can be exposed to dirt, insects, and animal waste before harvesting. Furthermore, many spices are produced in tropical climates where the conditions for microbial growth are readily available. The role of spices in the microbiology world is two-fold; spices can contain antimicrobial agents (Zaika 1988, Ceylan and Fung 2004, Sofia, Prasad et al. 2007, Joe, Jayachitra et al. 2009) and be used for preservation of foodstuffs as well as harbor pathogens in the form of bacteria, mold, and fungus (McKee 1995, Banerjee and Sarkar 2003, Vij, Ailes et al. 2006, Sagoo, Little et al. 2009).

2.4.1 Costs Associated with Food Borne Illness

The CDC estimates there are 48 million cases of food borne related disease every year in the United States accounting for 128,000 hospitalizations, 3000 deaths, and billions of dollars in lost wages, lawsuits, and elevated health care costs (Scallan, Hoekstra et al. 2011). Due to the organic nature of spices, it is almost impossible to eliminate all of the disease causing pathogens. The volatile aspect of spices also prevents several of the most common pathogen mitigation techniques from being used (i.e. pasteurization, blanching). Spices are generally dried to reduce water activity and to lengthen shelf life.

2.4.2 Food Borne Outbreaks Related to Spices

There have been several recent outbreaks associated with consumption of tainted spices. From 1970 to 2003, there were 21 FDA recalls of spices, with all but one being the result of *Salmonella* contamination (Vij, Ailes et al. 2006). They go on to state that 16 of these 21 recalls occurred during the 2000 to 2003 calendar years. It is estimated that 6.6% of spices imported into the United States, between 2007 and 2009, contained detectable levels of *Salmonella*; this is almost twice as great as all other imported foods tracked by the FDA (Van Doren, Kleinmeier et al. 2013). This increase in contamination rate could be tied to an increase in spices, but could also be a result of a more robust and effective disease surveillance system. Black pepper is typically the 'dirtiest' spice, with total aerobic plate counts exceeding 10^6 CFU/g (Tainter and Grenis 2001). Paprika is also known to be highly contaminated with aerobic counts occasionally reaching 10^6 CFU/g (McKee 1995).

Most recently, deli meat seasoned with black and red pepper was found to contain *Salmonella* Montevideo. This strain was traced back to 272 cases and 44 states between the summer of 2009 and spring of 2010 (CDC 2010). Brazilian black pepper was banned from import in the 1980s after issues with *Salmonella* contamination (Tainter and Grenis 2001). Germany had over 1000 cases of salmonellosis in 1993 from paprika flavored potato chips (Lehmacher, Bockemuhl et al. 1995). Studies in India, UK, Japan, and Mexico all show high levels of contamination in spices on grocery store shelves (Garcia, Iracheta et al. 2001, Banerjee and Sarkar 2003, Hara-Kudo, Ohtsuka et al. 2006, Sagoo, Little et al. 2009).

2.4.3 *Salmonella*

Salmonella is a Gram negative, rod shaped, motile anaerobic bacteria of the family *Enterobacteriaceae*. *Salmonella* can be classified as either typhoidal or nontyphoidal. Typhoidal cases are much more serious and may result in bloody stools (Lund, Baird-Parker et al. 2000). Nontyphoidal salmonellosis, the more common disease variant, may lead to gastroenteritis within as few as 8 hours and last only 48 hour after exposure to the bacteria (Lund, Baird-Parker et al. 2000). *Salmonella* carries a high infectious dose, estimated between 4-8log (Kothary and Babu 2001).

It is estimated that nontyphoidal *Salmonella* causes over one million cases of food borne illness in the United State every year (Scallan, Hoekstra et al. 2011). It is also estimated to be the leading cause of hospitalizations (19,336) and deaths (378) associated with food borne illness each year (Scallan, Hoekstra et al. 2011). The prevalence of *Salmonella* in the environment is a direct cause of the continual occurrence of food borne illness. *Salmonella* has been traced back to many products including, but not limited to: meat (beef, pork, chicken, turkey), eggs, shellfish, milk, cheese, vegetables (sprouts, tomatoes, onion, radish, carrots), fruits (cantaloupe, watermelon), and chocolate (Lund, Baird-Parker et al. 2000).

Until recently, it was believed that *Salmonella* along with other vegetative bacteria required high levels of water for viability. However, recent outbreaks associated with peanut butter have shown, this is not the case. Low moisture foods include chocolate, dried milk, dried eggs, peanut butter, infant formula, pasta, spices, cereal, flour, and pet food. Studies have shown that these low moisture foods have the potential to harbor and maintain a population of pathogenic *Salmonella* cells if the product should become introduced to contamination (Podolak, Enache et al. 2010). Furthermore, *Salmonella* cells have shown resistance to thermal treatment methods when found in low moisture food (Podolak, Enache et al. 2010).

2.5 Current Treatment Methods

Instituting critical control points in the spice processing chain can reduce the overall levels of filth in spices and minimize opportunities of contaminants being introduced. However, due to the possibility of high levels of contamination with bacteria, it is often necessary to apply further processing steps to spices. Several treatment methods are available for reduction of pathogens in spices; however, the FDA has not approved all of them. These methods, or post-processing techniques, while successful may possess certain limitations and restrictions. Bacterial spores and mycotoxins are more difficult to treat (ASTA 2011). The common methods include chemical fumigation, steam sterilization, and treatment using gamma radiation. This section describes each of these methods.

2.5.1 Fumigation

Application of various gases to spices was the most widely used method for microbial inactivation (Lund, Baird-Parker et al. 2000). During treatment, sealed chambers are evacuated and the desired fumigant, in pure or blended form, is released and allowed to contact the spice for a desired amount of time. Often, an aeration step is required to reduce any trace levels of residual fumigant to desired levels. There are several chemicals that can be used to fumigate spices. The most common of these are ethylene oxide and propylene oxide. Both are detailed below.

2.5.1.1 Ethylene Oxide

Ethylene Oxide (C₂H₄O) is a colorless, flammable gas with a sweet smell. It is widely used as an industrial intermediate in the production of ethylene glycol (antifreeze). In the gaseous state, ethylene oxide can be used as a fumigant for sterilizing and disinfecting medical devices and other organic materials (i.e. food items). Ethylene oxide also has uses as an insecticide. Ethylene oxide (EO) has proven to be effective at concentration ranges of 400 – 1000 mg/L (Lund, Baird-Parker et al. 2000). Depending on treatment conditions, a 1 to 4 log reduction in aerobic bacteria was observed (Lund, Baird-Parker et al. 2000). Many factors contribute to the efficacy of the

fumigation treatment: concentration of EO, temperature, relative humidity of environment, moisture content of spice, penetration ability of EO into the sample, etc. The spice industry uses an estimated 800,000 pounds of EO annually for spice disinfecting (ASTA 2011). It is estimated that up to 85% of all spices in the United States are treated with EO (ASTA 2011).

There exist many problems with the use of ethylene oxide. In 1985, it was labeled as a *possible carcinogen*. Much evidence exists that demonstrates ethylene oxide's potential to cause genetic damage. This resulted in ethylene oxide being labeled a *known human carcinogen* in 2000 (Program 2011). Ethylene oxide use is now strictly monitored by multiple government organizations. The FDA has issued regulations on EO as a food additive which are listed under 21 CFR 172, 173, 175, 176, and 178. Specific labeling is required for products receiving EO as a treatment. The FDA has placed a 7 ppm limit on residues of EO when used as a fumigant on spices (ASTA 2011).

2.5.1.2 Propylene Oxide

Propylene Oxide (C₃H₆O) is a relative to EO. Propylene oxide (PPO) has been approved for treatment of spices with up to 300 ppm residue (ASTA 2011). PPO is a much less common fumigant for spices due to it being less effective, however it is growing in popularity due to the stricter regulations placed on EO (Tainter and Grenis 2001). Yet, PPO treatment should not be viewed as best method for pathogen reduction as it will most likely meet the same fate as EO as regulations are starting to take hold and limiting the use of PPO.

2.5.2 Irradiation

Irradiation has found a myriad of uses in the food industry. It has also been widely studied for efficacy and safety. Irradiation is the process of applying ionizing radiation to food stuffs. This radiation destroys the DNA integrity of contaminants without making the irradiated sample radioactive (Sádecká 2007). It is currently used for destroying pathogens and insects, as well as delaying ripening and preventing sprouting in fruits and vegetables. The ability to treat foods in their final packaging state prevents any recontamination and is a benefit seldom seen in other

methods. The dose and time of irradiation a food item will receive is based upon regulations set by the FDA and other respective food governing agencies for upper limits of application. Spices typically are shelf-stable and therefore can receive a larger dose of irradiation, up to 30 kGy (21 CFR 179.26 Irradiation in the Production, Processing and Handling of Food). Irradiation has been classified as a food additive and therefore food items receiving this treatment must be labeled as such.

Despite the success in the use of irradiation as a treatment method for spices, there remains a negative public perception with regards to use of this technology in food. While there are no proven risks associated with the use of irradiation at the current allowable limits as set by the FAO/WHO Codex Alimentarius, health concerns remain tied to the issue of using radiation on the food supply. The lack of consumer knowledge on the topic of food irradiation limits the use of this technology.

2.5.3 Steam

Treatment of spices with steam (super-heated, saturated, or dry) has shown success in achieving 1-5 log₁₀ reductions in spices. Steam has the benefit of not having to deal with regulations or strict labeling protocols, but has operating restrictions. Steam treatment is popular for European Union countries where fumigation with EO and PPO is not approved. Due to the high temperature of the steam, care must be taken not to change the color and volatile oil content of the spices. Care also must be given to prevent increasing the moisture content of the spice. This is to prevent bacteria or mold growth at higher levels of moisture and to prevent having to add a subsequent step of drying. Clumping may also be noticed with application of steam.

Two separate studies (Waje, Kim et al. 2008, Rico, Kim et al. 2010) showed irradiated spices had lower levels of bacterial contamination than those treated with steam. Waje et al was able to note a decrease in the piperine content of black pepper while Rico et al found lower sensory acceptability as well as greater color change in the steamed samples. A third study (Kispéter, Bajúsz-Kabók et al. 2003) indicated saturated steam produced a color change in paprika.

2.5.4 Microwave heating

The use of microwaves for pathogen inactivation in spices has been studied previously with conflicting results. An early paper stated that microwave treatment of six different spices, including black pepper and paprika, produced no statistically significant difference in the pathogen populations (Vajdi and Pereira 1973). They found many pockets of localized heat due to the low moisture levels associated with dried spices. A more recent study showed promise with radio frequency (RF) heating. Heating red and black pepper samples for 40 seconds was able to achieve a 2.8 to 5 log₁₀ reduction in *Salmonella* Typhimurium and *E. coli* O157:H7 without effecting spice color (Kim, Sagong et al. 2012). No volatile analysis was performed in this study.

2.6 Proposed Methods (Ethanol Vapor and Far Infrared Radiation)

Two separate methods are proposed to reduce pathogen levels in spices. A high temperature – low time treatment utilizing far infrared radiation (FIR) will be investigated. Fumigation with ethanol vapor will also be investigated. It is hoped that these methods will be able to successfully reduce pathogens without impairing final product quality.

2.6.1 Infrared Heating and its Uses in Food Industry

Radiation heat transfer is the transfer of heat through electromagnetic radiation. This method does not typically heat the medium in which the radiation is transferred through. Infrared radiation (IR) is a type of radiation heat transfer. Energy is transferred in waveform (0.78μm to 1000μm) between visible light and microwave wavelengths. This energy is responsible for the heating observed as the result of the application of IR. There are three specific sub-regions of IR. They can be defined as near-infrared (NIR), mid-infrared (MIR), and far-infrared (FIR). The wavelengths associated with each sub-region each region are as follows: NIR: 0.78-1.4μm; MIR: 1.4-3μm; FIR: 3-1000μm (Sakai and Hanzawa 1994).

Any material may emit infrared radiation. The energy produced is dependent on the temperature and emissivity of the radiation source and associated wavelengths or radiation. A perfect material that emits and absorbs the maximum amount of radiation is called a *black body*. Plank's law describes the emitted radiative power ($E_{B\lambda}$) of a black body at temperature T at wavelength λ :

$$E_{B\lambda} = \frac{3.7418 \times 10^{-16}}{\lambda^5 [e^{1.4388 \times 10^{-2} / \lambda T} - 1]} \quad (1)$$

This emitted radiation is a function of temperature and wavelength. The emitted radiation increases with an increase in temperature. Wein's Displacement Law describes the wavelength at which the emission of radiation from an ideal black body (λ_{\max}) at a constant temperature (T) is at a maximum:

$$\lambda_{\max} T = 2.898 \times 10^{-3} \text{ m K} \quad (2)$$

Adjusting the emissive power (E_B) for a real surface with emissivity, ϵ , and integrating yields the Stefan-Boltzmann Law:

$$E_B = \epsilon \sigma T^4 \quad (3)$$

where: σ is the Stefan-Boltzmann Constant ($5.676 \times 10^{-8} \text{ W/m}^2 \text{ K}^4$)

2.6.2 Far Infrared Radiation (FIR)

FIR has been successfully used as an alternative drying method for production of dehydrated fruits and vegetables (Nowak and Lewicki 2004, Sharma, Verma et al. 2005, Wang and Sheng 2006). It is possible to lower costs, reduce drying times, improve energy efficiency, and increase rehydration rates when substituting FIR for conventional drying or freeze drying (Sakai and Hanzawa 1994, Krishnamurthy, Khurana et al. 2008). The use of FIR in baking and roasting, among other unit operations, was evaluated by several groups with success and is summarized by Sakai and Hanzawa (1994). In bread baking, FIR heaters produced superior crust formation accompanied by a more preferential crust color when compared to other types of heaters, including NIR. Furthermore, FIR heaters were shown to reduce baking time, energy costs, and the heater footprint over other methods. Roasting of coffee and tea was successfully done with

FIR. Sweet potatoes were roasted with FIR to give the most preferential taste. FIR thawing of oysters limited drip loss. Eggs were ‘hard-boiled’ using FIR to eliminate the need for large water baths while minimizing contamination and breakage.

Due to the largely heterogeneous nature of spices, FIR is a more effective treatment choice due to the reflective nature of NIR. Only 10% of FIR radiation is reflected by foods with a rough surface as compared to up to 50% of NIR radiation (Krishnamurthy, Khurana et al. 2008). This allows for a more efficient heating treatment with a shorter exposure time as the total incidence of radiation absorbed (α) and reflected (ρ) for a perfect black body must sum to 1.0 (Geankoplis 2005):

$$\alpha + \rho = 1.0 \quad (4)$$

It is important to match the material to be treated with IR to the appropriate temperature and wavelengths. Water, along with proteins, sugars, and lipids all have IR adsorption bands between 3 to 10 μ m (Sandu 1986). Therefore, in general, the FIR region of IR contains wavelengths acceptable for absorption of energy by the main constituents on food products. This makes a FIR oven more efficient and provides more uniform heating than conventional NIR ovens (Sakai and Hanzawa 1994). The surrounding atmosphere is also largely unaffected by the heating and remains at ambient conditions.

2.6.3 FIR to reduce pathogens

Many parameters must be considered when attempting to reduce or inactivate pathogens with IR heating. Infrared power level, temperature of sample, peak wavelength of IR source, sample depth, moisture content of sample, type of pathogen, and type of food sample are among the possible factors that must be controlled (Krishnamurthy, Khurana et al. 2008). FIR has been used with some effect on many different substrates to reduce or eliminate bacteria, fungus, insects, and yeasts. IR treatments have been common for many years in treating insect infestations in stored grains (Kirkpatrick and Cagle 1978, Mourier and Poulsen 2000). FIR has also proven effective on *Bacillus Subtilis*, *Staphylococcus aureus*, *Escherichia coli* in both solid and liquid nutrient media (Krishnamurthy, Khurana et al. 2008, Krishnamurthy, Tewari et al. 2010).

Listeria monocytogenes levels were reduced in meat and poultry with IR treatment (Huang and Sites 2008, Ha, Ryu et al. 2012). FIR was used to reduce aerobic bacteria, mold, and yeast on cumin and black pepper seeds while not impairing product quality (Erdogdu and Ekiz 2011, Erdođdu and Ekiz 2013). The effects of IR treatment at varying pH, temperature, and water activity conditions was studied an attempt to reduce levels of *Bacillus cereus* spores in paprika (Staack, Ahrné et al. 2008).

2.6.4 Ethanol Vapor

Ethanol (CH₃CH₂OH), also known as ethyl alcohol, is a flammable, colorless liquid. Ethanol boils at 78°C. Ethanol holds uses as a fuel, solvent, and sterilization agent. It may also be found in its liquid form in many household items. Ethanol, in limited doses, is considered safe for human consumption and therefore placed on the Generally Recognized as Safe (GRAS) list (21 CFR Part 184 §184.1293). Ethanol is the component in beer, wine, and liquors that produces intoxication when imbibed. Ethanol, along with other alcohols such as: isopropyl and n-propanol are widely used as anti-microbial agents. Vegetative cells are destroyed by disruption of proteins in the cellular membrane (McDonnell and Russell 1999). This results in interruption of cellular metabolism and results in cell lysis (McDonnell and Russell 1999). Optimum concentration is between 60-90% ethanol by volume.

Liquid sterilization is common; however, ethanol vapor has been used in some instances. Limited research is available on the use of ethanol vapor in the food industry; however, ethanol vapor has been applied to grapes, tomatoes, cherries, and other fruit to prevent mold growth and product decay (Ritenour, Mangrich et al. 1997, Chervin, Westercamp et al. 2005, Tzortzakis and Economakis 2007, Bai, Plotto et al. 2011). The use of ethanol vapor as a sterilization agent has been shown to be effective in spices (Wistreich, Thundiyil et al. 1975). Wisteich et al filed a patent that used ethanol at a concentration of 80% and held it in contact with the spice sample for 5 to 10 hours at a temperature of 78-150°C. Further study is required to evaluate if these treatment conditions can be optimized and the time reduced. Quality parameters were not mentioned in the patent filing.

2.7 Modeling of Microbial Inactivation

Mathematical modeling creates a description of a system that can be used to predict or confirm the behavior of complex systems. Collected data is often displayed graphically and mathematical equations are tested for fit. The developed equations represent the relationship between specific variables. There are several mathematical models that can be used to describe the inactivation kinetics of microbial cells. These models can be used to calculate times and temperatures that must be achieved to ensure product safety.

2.7.1 Bigelow Model

The Bigelow model is the simplest of all inactivation models as it follows first order reaction kinetics. The decimal reduction time, or D-value, is calculated through the log-linear model of pathogen inactivation versus time (Bigelow 1921) and follows the model as described by Eq. 5:

$$\log\left(\frac{N_t}{N_0}\right) = -\frac{t}{D} \quad (5)$$

Where: t is the treatment time

N_t is the number of surviving bacteria at time t

N_0 is the initial number of bacteria at time zero

D is the D-value, or thermal death time, in seconds

2.7.2 Weibull Model

Recent studies have shown that many times, microbial inactivation curves do not follow a linear model; there are often a tailing or shouldering effects (Iii, Wiener et al. 1978, Peleg and Cole 1998, van Boekel 2002). Using a linear model on non-linear data can produce inaccurate D-values resulting in over or under-processing food products. The Weibull model allows data to be fit for many shapes, including sigmoidal and curvilinear (Eq. 6) (Peleg and Cole 1998). Scale and shape parameters can be used to calculate a thermal reduction time similar to a D-value (Eq 7) (Peleg 1999).

$$\log\left(\frac{N_t}{N_0}\right) = -\alpha t^\beta \quad (6)$$

Where: t is the treatment time

N_t is the number of surviving bacteria at time t

N_0 is the initial number of bacteria at time zero

α is the scale factor

β is the shape factor

$$t_{\text{lethal},d} = \left(\frac{d}{\alpha}\right)^{\frac{1}{\beta}} \quad (7)$$

Where: $t_{\text{lethal},d}$ is the time to reach the desired reduction in d

d is the decade reduction number

α is the scale factor

β is the shape factor

2.7.3 Accuracy Factors

Confirming the accuracy of the model is important to demonstrate valid methods. By comparing the sums of squares of observed and predicted values, one may check the fit of each model and select the one with ‘best fit’ (Eq. 8).

$$\text{Log } A_f = \frac{\sum \left| \log\left(\frac{\text{predicted}}{\text{observed}}\right) \right|}{n} \quad (8)$$

Where: A_f is the accuracy factor

Predicted is the value calculated from the regression equation

Observed is the value observed through experimentation

n is the number of trials

2.8 Conclusion

A great deal of data points to spices and spiced foods becoming increasingly important in the effort to ensure safety of food. However, environmental conditions and traditional growing techniques often lead to spices which may be harmful when consumed. Many studies have been conducted on the potential to reduce pathogens with far infrared radiation, but none on ground paprika, sage, or black pepper. The use of ethanol vapor as a fumigant for pathogen control also

has not yet been extensively studied. This report will attempt to fill in the knowledge gaps for both ethanol vapor and FIR.

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CHAPTER 3

Effect of Far Infrared Radiation and Ethanol Vapor Fumigation Treatment on Survival of *Salmonella enterica* Tennessee in Dried, Ground Spices

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Abstract

Microbial contamination of spices can lead to outbreaks of foodborne illness. The use of far infrared radiation (FIR) and ethanol vapor (EV) were evaluated for the reduction of the human pathogen *Salmonella enterica* Tennessee applied to dried, ground black pepper (*Piper nigrum*), paprika (*Capsicum annuum*), and sage (*Salvia officinalis*). Spices were heated with FIR to ~350°C for short periods of time (8-24 seconds) at various positions within the treatment chamber. Alternatively, hot ethanol vapor was circulated in the chamber for intervals ranging from 30-300 seconds. Both FIR and EV treatments proved effective against *Salmonella* Tennessee. FIR achieved a greater than 3-log CFU/g reduction in less than 40 seconds for all treatment distances. A tailing effect was observed showing decreased treatment efficacy at extended treatment times. EV treatments between 1-5 minutes provided equivalent reductions to FIR. A greater degree of non-linearity and tailing was noticed with the EV treatment. Both treatments were more effective on paprika, achieving a 5-log CFU/g reduction. Bigelow and Weibull models were used to calculate decimal reduction times. The Weibull model proved to be acceptable for both treatments with accuracy factors not exceeding 3.0.

3.1 Introduction

Spiced foods have increased in popularity over the last few decades with a 60% increase in spice consumption in the United States between 1980 to 2000 (Vij, Ailes et al. 2006). This is a result of changing demographics and a higher demand for ethnic, ready-to-eat, low-fat, and low-sodium foods (McKee 1995). This trend also registered in other countries, which has led to an increased prevalence in spice-related foodborne illness and a corresponding rise in public awareness regarding outbreaks associated with spices (McKee 1995, Banerjee and Sarkar 2003, Sagoo, Little et al. 2009, Zweifel and Stephan 2012). Twenty one recalls and outbreaks associated with contaminated spices in the United States occurred between 1970 and 2003 (Vij, Ailes et al. 2006). Zweifel and Stephan (2012) summarized two recent outbreaks that were traced to contaminated red, black, and white pepper and led to over 350 combined cases of illness in 44 states. Microbial counts from black pepper and paprika have both been found to occasionally contain bacterial levels as high as 6 log CFU/g (McKee 1995, Tainter and Grenis 2001). Since spices are often added after meal preparation is complete, there is often no step in place to kill any human pathogens. As a result, spices often require a decontamination step in the processing chain to ensure product quality and maintain safety.

Current treatment methods are inadequate and can be improved. The use of irradiation as a sterilization technique, while effective, is generally not a popular method among consumers (Alam Kham and Abraham 2010). Fumigation with ethylene oxide is another common method that is under scrutiny due to possible associated health risks (Program 2011). The use of steam is a new method being employed, especially in the organic spice sector. However, several studies (Kispéter, Bajúsz-Kabók et al. 2003, Waje, Kim et al. 2008, Rico, Kim et al. 2010) found color changes and reduced volatile levels after treatment. An effective treatment must reduce pathogen levels while maintaining volatile profile, color, and physical characteristics of the spice.

Far infrared radiation has been used for drying fruits and vegetables, baking bread, and roasting coffee (Sakai and Hanzawa 1994, Nowak and Lewicki 2004, Sharma, Verma et al. 2005, Wang and Sheng 2006). While FIR has long been used to treat grain for inactivating insect larvae, new research has demonstrated the potential of FIR to reduce numbers of bacterial pathogens. FIR

effectively reduced numbers of *Staphylococcus aureus*, *Escherichia coli*, *Listeria monocytogenes*, yeasts, and molds in liquid buffer samples, as well as solid foods including strawberry, onion, eggs, meat, and wheat (Krishnamurthy, Khurana et al. 2008, Krishnamurthy, Tewari et al. 2010). Pathogen inactivation tests carried out on cumin and paprika showed minimal quality loss (Staack, Ahrné et al. 2008, Erdogdu and Ekiz 2011).

Ethanol has been studied extensively as a preservative because it disrupts proteins in cell membranes, resulting in leakage of cytoplasm and eventually cell lysis and death (Ingram 1990, McDonnell and Russell 1999). Ethanol has many applications in the bread and baking industry to prevent fungi and mold growth when incorporated directly in the product or with the use of ethanol vapor packs (Dao and Dantigny 2011). The FDA has approved ethanol spray to pizza crusts (21 CFR § 184.1293) as an antimicrobial treatment. Ethanol vapor and immersion are used as a post-harvest treatment to prevent decay of apples, berries, peaches, grapes, and some citrus products (Dao and Dantigny 2011).

The objective of this study was to determine if treatment with far infrared radiation and ethanol vapor can significantly reduce the population of *Salmonella enterica* Tennessee on dried, ground spices. Black pepper (*Piper nigrum*), paprika (*Capsicum annuum*), and sage (*Salvia officinalis*) were inoculated with *Salmonella* Tennessee and subjected to various treatment times and distances in an effort to identify the most effective inactivation treatment. In this paper, *inactivation* is defined as the ability of each treatment to reduce the quantity of *Salmonella* Tennessee cells recovered post-treatment.

3.2 MATERIALS AND METHODS

3.2.1 Materials:

Bulk quantities of untreated paprika, black pepper, and sage were obtained from McCormick & Company (Hunt Valley, MD), and stored in airtight plastic bags at a constant room temperature (22°C) until needed. Anhydrous ethanol (Fisher Scientific, Waltham, MA) was used at a concentration of 95%. Peptone buffer was purchased from Sigma (St. Louis, MO). Nalidixic acid

was obtained from FisherBiotech (Fair Lawn, NJ). Tryptic soy agar and tryptic soy broth were from BD Diagnostic Systems (Sparks, MD).

3.2.2 Experimental Chamber Setup:

A Thermo Fisher Scientific Isotemp Oven Model 630G (Waltham, MA) with dimensions 15"x19"x20" was utilized as a treatment chamber (Figure 3.1). For FIR treatment, a liquid propane (LP) powered, 12"x12" catalytic heater from Bruest Catalytic Heaters (Independence, KS) was installed in the horizontal position at the top of the treatment chamber. A 1/8 HP Gast 22D diaphragm pump (Benton Harbor, MI) was used to create positive pressure and force airflow through 3/8" food-grade vinyl tubing. Electric preheating of the heater lasted 15 minutes, after which the LP was started and the heater was allowed to reach treatment temperature of 350°C. The temperature was monitored at the heater surface with Type K thermocouples (Omega Engineering, Inc., Stamford, CT) and measured using an Omega HH21 handheld thermometer (Stamford, CT) and experiments were conducted at a 350±10°C. During ethanol vapor treatment, the heater component was removed from the oven for safety reasons. Ethanol was heated to boiling under agitation in a one-liter filter flask located outside the treatment chamber within a chemical fume hood. A 1/8 HP Gast 22D diaphragm pump (Benton Harbor, MI) was used to aspirate the heated ethanol and force airflow through 3/8" food-grade vinyl tubing from the flask and into the treatment chamber at a flow rate of 7.5 mL/min. During treatment, the door to the chamber remained closed and locked, unless loading or unloading samples, to prevent EV from escaping the chamber. A second identical pump was used to withdraw vapor and condense the used ethanol by means of passing the tubing through an ice bath (Figure 3.2). This was done within a chemical fume hood. The treatment chamber maintained an internal temperature of 50±5°C during the experiments by continuous pumping of heated vapor. Thermocouples (Omega Type K) were attached to the sample holding rack.

3.2.3 Treatment Procedure and Experimental Design:

Each spice sample was weighed to 3.0±0.1 g and uniformly spread in a thin layer, less than 2 mm thick, on a sheet of 11 cm diameter filter paper (Whatman International Ltd., Maidstone, England). After treatment, each sample was sealed in a sterile, 8 mL screw cap glass vial and stored at 4°C until analysis.

Far Infrared Radiation

A full factorial design of five treatment times (8, 12, 16, 20, and 24s) and three treatment distances (4, 6, and 8 in) was used to treat black pepper, paprika, and sage. Each sample was placed onto a sample tray (Figure 3.1) and treated for the predetermined time. After each set of times was completed, the sample tray was adjusted to the next treatment distance.

Ethanol Vapor

Paprika and black pepper samples were treated with ethanol vapor for five treatment times (30, 60, 90, 120, 150, and 300s). Samples were placed into the chamber and heated ethanol was introduced (Figures 3.2 & 3.3). Prior to each sample, the chamber was allowed to equilibrate for 5 min to ensure a constant level of vapor in the chamber.

3.2.4 Preparation and Inoculation of Spice with *Salmonella* Tennessee

Salmonella enterica Tennessee previously isolated from peanut butter during the peanut butter outbreak of 2006 (Sheth et al. 2011) was inoculated from a -80°C stock into tryptic soy broth, and incubated shaking (125 RPM) at 37°C for 24 hours. The strain was rendered resistant to nalidixic acid (Nal) by passage through increasing concentrations of Nal. This was done until a final concentration of 50 ppm was achieved in order to discriminate between native spice microbiota and the inoculated strain. Freezer stocks were made with these cells, and maintained at -80°C for all future studies. Bacterial cells were grown in 125 mL Erlenmeyer flasks containing tryptic soy broth (TSB) and 50ppm nalidixic acid (TSB+Nal). Shaking was done at 125 RPM for 48 hours, at 37°C. Cells were then centrifuged at 4000 x g (Eppendorf Centrifuge 5810, Germany) for 12 minutes, the supernatant was decanted, and the cell pellets were resuspended into 10 mL of TSB+Nal.

Each sample was inoculated with 1 mL inoculum per 10 g of spice using a 16 gauge needled syringe to apply the inoculum to the spice while simultaneously mixing to distribute the inoculum throughout the product. Spices were allowed to dry in an ABSL2 cabinet for one hour to reach a uniform moisture distribution before performing trials. One hour after inoculation the water activity of paprika, black pepper, and sage was 0.448, 0.420, and 0.428, respectively.

3.2.5 Enumeration of *S. Tennessee*

S. Tennessee were enumerated from each spice and treatment combination by placing 2 g of spice with 18 mL of 0.1 M peptone buffer, into a lab blender bag (Fisher Scientific, Waltham, MA) and mixing for 90s at a speed of 7 in a BagMixer lab blender (Interscience, Saint Nom, France). Following mixing a standard 10-fold serial dilution of the spice mixture was performed in 0.1M peptone buffer and aliquots (100 µl) were plated in triplicate on tryptic soy agar with 50 ppm nalidixic acid. Plates were incubated at 37°C for 48 hours. Detection limit of plate count procedure was 2 log CFU/g. Enrichments were also made for each treatment by adding 0.25 g of treated spice to 10 ml of TSB+nal and incubated for 48 hours. The enrichment was then streaked onto TSA-Nal to check for growth of the inoculated strain and to confirm that a complete kill was achieved when plate counts were below the limit of detection.

3.2.6 Modeling Inactivation of *Salmonella Tennessee*

Two models, Bigelow and Weibull, were used to describe the inactivation kinetics of *Salmonella Tennessee*. Thermal death times and decade reduction times were calculated and checked for accuracy.

Bigelow Model and Calculation of D-Value

A first order reaction was used to create a log-linear model of pathogen inactivation versus time. The D-Value was calculated as the reciprocal of the slope of the regression line (Bigelow 1921):

$$\log\left(\frac{N_t}{N_0}\right) = -\frac{t}{D} \quad (1)$$

Where: t is the treatment time

N_t is the number of surviving bacteria at time t

N_0 is the initial number of bacteria at time zero

D is the D-value, or thermal death time, in seconds

Weibull Modeling and Calculation of Shape and Scale Parameters

It has been shown that inactivation curves commonly do not follow the log-linear model.

Weibull analysis was used to account for any shouldering or tailing effects seen in the reduction counts of *Salmonella Tennessee*. These counts were transformed to Log CFU/g and plotted

against time as a power curve based on the survival function (Peleg and Cole 1998) and then the parameters were used to calculate decade reduction times (Peleg 1999). Eq. 3 requires the assumption of constant temperature of the sample. The resulting times were then divided by the corresponding number of log reductions to produce an equivalent D-value (D_{eq}) for the Weibull model that could be compared to the D-value for Bigelow.

$$\log\left(\frac{N_t}{N_0}\right) = -\alpha t^\beta \quad (2)$$

Where: t is the treatment time

N_t is the number of surviving bacteria at time t

N_0 is the initial number of bacteria at time zero

α is the scale factor

β is the shape factor

$$t_{lethal,d} = \left(\frac{d}{\alpha}\right)^{\frac{1}{\beta}} \quad (3)$$

Where: $t_{lethal,d}$ is the time to reach the desired reduction in d

d is the decade reduction number

α is the scale factor

β is the shape factor

These models were checked with the previously designed accuracy factor (Ross 1996):

$$\log A_f = \frac{\sum |\log\left(\frac{\text{predicted}}{\text{observed}}\right)|}{n} \quad (4)$$

Where: A_f is the accuracy factor

Predicted is the value calculated from the regression equation

Observed is the value observed through experimentation

n is the number of trials

3.2.7 Statistical Analysis

Each treatment was replicated four times. Bacterial counts were transformed to log CFU/g to account for non-normal distribution. Data were analyzed using JMP 10 (SAS Institute, Cary, NC) through ANOVA to test the effect of FIR and EV treatment times on survival rates of

Salmonella Tennessee. Tukeys HSD mean testing was used to compare log CFU/g treatment means of surviving populations of *Salmonella* Tennessee. Weibull curve fitting was done with Excel 2010 (Microsoft, Seattle, WA). P-values less than 0.05 were considered significant.

3.3 RESULTS AND DISCUSSION

Efficacy of treatments was determined by quantifying the number of *Salmonella* Tennessee cells recovered on TSA+Nal after each treatment compared to the untreated control. The average recovery of *Salmonella* Tennessee from untreated samples of paprika was 7 log CFU/g, while 6 log CFU/g were recovered from black pepper and sage controls despite the same initial inoculum. Oils found in black pepper and sage have shown antimicrobial activity and may inhibit the growth of bacteria and be the result of lower control counts (Tainter and Grenis 2001, Ceylan and Fung 2004). A minimum target of 5 log CFU/g reduction is desired to create a safe food product and defines a successful decontamination treatment.

3.3.1 Far Infrared Radiation

Treatment with FIR significantly reduced amounts of *Salmonella* Tennessee recovered from all three tested spices, with extended periods of exposure associated with greater reductions (paprika: $p = 0.000$, $R^2 = 0.88$; black pepper: $p = 0.000$, $R^2 = 0.86$; sage: $p = 0.000$, $R^2 = 0.83$). As expected, longer treatment times and distances closest to the heat source produced spices with lower numbers of *Salmonella* Tennessee. A maximum reduction of 5 log CFU/g *Salmonella* Tennessee was achieved in some paprika samples (Fig. 3.4), while maximum reductions in black pepper (Fig. 3.5) and sage (Fig. 3.6) were lower with only a few samples exceeding a 3 log CFU/g reduction. The FIR sample treatment times were capped at 24 seconds because smoke and burned samples were common with longer duration treatments.

Complete pathogen inactivation was never achieved in any of the FIR time and treatments due to the burning in order to preserve spice quality.

The resulting curves exhibited no lag time or shouldering, and displayed a rapid destruction of bacterial cells following a curvilinear microbial destruction curve (Fig. 3.4-3.8). Furthermore, the concave-up shape, as defined by Peleg (1999), observed in black pepper and sage is

characteristic of cells adapting to treatment conditions. Peleg (1998) states a concave-up curve should yield a shape factor, β , of less than 1. Black pepper β -values ranged from 0.55 to 0.83, while sage ranged from 0.63 to 0.84 (Table 3.1). Interestingly, paprika, the spice exhibiting the greatest amount of reduction, more closely fit the linear Bigelow model and produced Weibull shape factors consistent with linear distributions ($\beta=1$) with β -values ranging from 0.94 to 1.12 (Table 3.1). Accuracy factors for paprika are similar for both models, as are the comparisons of D and D_{eq} (Tables 3.1 & 3.3); meaning, it would be suitable to use either the Bigelow or Weibull model for paprika when treated with FIR. Accuracy factors for black pepper and sage showed best fit for the Weibull model for all FIR samples. There was a significant difference between the two models as the Weibull model showed log reduction times (D_{eq}) increased with each successive log reduction. (Table 3.1 & 3.3). Correlation coefficients, R^2 , ranged from 0.62 to 0.82.

A thorough list was compiled for use in describing thermal inactivation of vegetative cells using the Weibull model (van Boekel 2002). However, most of the included references dealt with liquid foods. When solid foods were tested, the shape factor, β , was greater than 1.0, indicative of a delay in bacterial inactivation. No such delay, or shouldering, was observed in datasets for black pepper, paprika or sage (Table 3.1). There is limited data on the use of Weibull modeling on solid and powdered foods such as spices. Thermal inactivation of *Cronobacter* and *Salmonella* Enteritidis in powdered infant formula (PIF) showed tailing effects similar to those seen in this study, with β -values for *Salmonella* Tennessee in PIF ranging from 0.26 to 0.70 (Kandhai, Reij et al. 2010). The β -values calculated for spices in this study ranged from 0.49 to 0.85, excluding FIR paprika values of 1.0. Inactivation of *Cronobacter sakazaki* and *Cronobacter* MC10 had β -values at or slightly over 1.0 for several temperatures in PIF (Kandhai, Reij et al. 2010).

3.3.2 Ethanol Vapor

Treatment with ethanol vapor was associated with significant reductions of *Salmonella* Tennessee in spices, with longer treatment times yielding greater reductions (paprika: $p = 0.000$, $R^2 = 0.84$; black pepper: $p=0.000$, $R^2 = 0.88$) compared to the untreated control. Sage was not treated with EV due to extensive testing of the more popular spices black pepper and paprika.

Salmonella Tennessee reduction on paprika approached 5 log CFU/g after 120 seconds (Figure 3.7), and black pepper yielded a 4 log CFU/g reduction in *Salmonella* Tennessee at 120 seconds (Figure 3.8). Tailing was more evident after EV treatment; therefore, the Bigelow model becomes inaccurate with large accuracy factor values of 204 and 91 for paprika and black pepper, respectively. All Weibull model A_f values were under 3.0. D-values were ten times larger than associated D_{eq} times (Tables 3.4 & 3.5). Inactivation curves (Figs 3.7 & 3.8) exhibited the same concave-up shape observed for FIR treatment. β -values were again less than 1.0, showing the ability of *Salmonella* Tennessee to adapt at longer more intense treatments. The predicted Weibull model inactivation times proved to be accurate for the 3 log reduction times. The predicted time to achieve a single decade reduction was calculated to be 8.04s for paprika and 11.33s for black pepper. The shortest EV treatment was 30 seconds; therefore, single log reduction times cannot be confirmed. A reduction of 3 log CFU/g could be achieved with treatment under 100s, with paprika at 75s and black pepper at 97s.

Total inactivation of *Salmonella* Tennessee occurred between 10-15 minutes of ethanol vapor exposure (>600 seconds), as no growth was detected in enrichments for these treatments. However, the EV condensed on the sample creating a “wet spice” that was deemed unsatisfactory and testing was capped at 300s. Limiting the condensation may be possible by increasing the chamber temperature. A redesign of the treatment chamber and ethanol delivery system may aid in prevention of spice wetting and save resources while promoting increased safety. Currently, the chamber is fed with ethanol regardless if a sample is in place or not. Introducing cutoff valves in the ethanol supply line would allow the chamber to be purged between samples. This would also allow for individualistic recovery of ethanol for each sample. Institution of an individual batch system as opposed to the continuously fed chamber would result in a drastic increase in processing time.

Similar research on pathogen inactivation in spices showed processing times often took several minutes as opposed to treatment times of only a few seconds as found in this study. Paprika showed a 4.5 log CFU/g reduction of *Bacillus cereus* spores in 4.5 minutes (Staack, Ahrné et al. 2008). Bacterial cocktails required 4.7 min and 3.5 min to achieve a 3 log CFU/g reduction of total mesophilic aerobic bacteria (TMAB) on the surface of whole peppercorns at 300°C and

350°C (Erdoğan and Ekiz 2013). The same bacterial cocktail needed 1.57 min to achieve a 1.5 log CFU/g reduction of TMAB on whole cumin seeds (Erdoğan and Ekiz 2011).

3.3.3 Limitations of Modeling

The lower limit of bacterial detection, 10^2 , played a large role in shaping the results. The ability of paprika to be recovered at higher initial counts allowed it to be tracked longer and have a better defined inactivation curve. These inactivation curves became skewed heavily to the left, producing a large tailing effect (Fig. 3.5-3.8), indicative of surviving members of the microbial population becoming tolerant to treatment. This impaired the linear modeling technique (Eq. 1) and yielded poor accuracy, except in the case of FIR paprika. Weibull analysis adjusts for this skewness and fit the data well using a power function (Eq. 2).

The $t_{lethal,d}$ value operates under the assumption that the sample temperature remains constant. It is not possible to keep the spice at a constant temperature during FIR treatment, but different methods can minimize the impact that temperature change has on the results. The brevity of treatments is one way to limit the temperature fluctuation in a sample. Maximum treatment temperatures at the spice surface at the end of treatment were 60°C for paprika, 56°C for black pepper, and 52°C for sage. Despite the short treatment times, there appears to be a correlation of α and β values and treatment distance to the heater. For all FIR treatments, the scale factor, α , decreased by decreasing the intensity of heat (i.e. moving the sample further away from the heat source and therefore reducing temperature influences). EV treatment produced an α -value that is: (1) significantly larger than FIR paprika, and (2) between the 6" and 8" samples for black pepper (Tables 3.1 & 3.2). The shape factor, β , appears to be independent for FIR paprika, but increases with decreased heat intensity for other FIR treated spices. Increasing the β -value closer to 1.0 shows increased linearity of inactivation times at further heater distances. The EV β -values for paprika and black pepper are 0.49 and 0.51, respectively. This is indicative of a higher degree of tailing and less linearity with increased D-values. Inactivation times for FIR were considerably lower than equivalent reduction times for EV.

3.4 CONCLUSIONS

This research studied the feasibility of using far infrared radiation (FIR) and ethanol vapor (EV) as a decontamination method for black pepper, paprika, and sage. Both treatments were successful in achieving the desired minimum 3-log CFU/g reduction. A 3-log or greater reduction in black pepper, paprika, and sage should occur within 100 seconds of treatment time. Both treatments had a greater effect on paprika and resulted in a higher degree of lethality than black pepper or sage. In fact, FIR paprika was best fit was with the Bigelow model and produced a nearly linear fit; however, using the Weibull model with a β -value of 1.0 could effectively replace the use of the Bigelow model in this case. Therefore, Weibull model appears to be acceptable to use as a modeling method to predict inactivation of *Salmonella* Tennessee in dried, ground spices.

The inability of reaching a total kill is likely to have skewed the results of this study. A large part of the tailing and associated impact on the model fitting can be attributed to reaching the limit of detection. Further experimentation should be performed to improve the model fit. Increasing the level of inoculum or improving the recovery to >6 log CFU/g *Salmonella* Tennessee at current inoculum levels on black pepper and sage would improve the limited knowledge base in this area. This paper demonstrates the potential of FIR and EV for use in pathogen inactivation on black pepper, paprika, and sage.

3.5 TABLES & FIGURES



Figure 3.1. Far Infrared treatment chamber showing a black pepper sample in the four inch position on the sample holding rack. The sample tray was lowered from this position to the 6” and 8” treatment positions. The sample and filter paper rests on a thermocouple. The heater surface is also temperature monitored.



Figure 3.2. Ethanol treatment chamber showing a paprika sample on the sample holding rack. Note the 3/8" gas inlet on the back wall for application of vapor to chamber. The sample and filter paper rests on the thermocouple.



Figure 3.3 Tubing display for ethanol vapor delivery and condensation beaker for vapor withdrawal.

Table 3.1 Comparison of Bigelow and Weibull parameters for inactivation of *Salmonella* Tennessee in spices by far infrared radiation.

Paprika

Distance (in)	Bigelow Model			Weibull Model			
	D	R ²	A _f	α	β	R ²	A _f
4	4.02	0.72	2.26	0.19	1.06	0.82	2.33
6	6.65	0.74	1.62	0.10	1.12	0.80	1.72
8	12.72	0.71	1.34	0.098	0.94	0.70	1.22

Black Pepper

Distance (in)	Bigelow Model				Weibull Model		
	D	R ²	A _f	α	B	R ²	A _f
4	10.40	0.66	31.09	0.67	0.55	0.72	1.71
6	10.45	0.74	6.75	0.41	0.64	0.74	1.35
8	13.83	0.69	2.92	0.16	0.83	0.73	1.46

Sage

Distance (in)	Bigelow Model				Weibull Model		
	D	R ²	A _f	α	B	R ²	A _f
4	8.18	0.68	1.52	0.48	0.63	0.62	1.52
6	12.51	0.71	1.12	0.36	0.63	0.76	1.12
8	13.11	0.81	1.21	0.14	0.84	0.75	1.21

D: D-value in seconds

A_f: Accuracy factor

α and β: Scale and shape factors of the Weibull distribution

Table 3.2 Lethality times (s) for specific levels of decade reduction of *Salmonella* Tennessee based on Weibull model after treatment with far infrared radiation.

Decade Reduction (d)	Paprika			Black Pepper			Sage		
	Distance (in)			Distance (in)			Distance (in)		
	4	6	8	4	6	8	4	6	8
5	21.9	32.9	65.6	38.5	51.3	62.2	41.2	65.1	70.6
4	17.7	26.9	51.7	25.6	36.1	47.6	29.0	45.0	54.1
3	13.5	20.8	38.1	15.2	23.0	33.6	18.3	28.7	38.4
2	9.21	14.5	24.7	7.30	11.9	21.0	9.63	15.2	23.7
1	4.79	7.81	11.8	2.07	4.09	8.96	3.21	5.00	10.4

Table 3.3 Equivalent decade reduction times (s) for specific levels of log CFU/g reduction of *Salmonella* Tennessee based on Weibull model after treatment with far infrared radiation.

Decade Reduction (d)	Paprika			Black Pepper			Sage		
	Distance (in)			Distance (in)			Distance (in)		
	4	6	8	4	6	8	4	6	8
5	4.30	6.58	13.1	7.70	10.3	12.4	8.24	13.0	14.1
4	4.43	6.73	12.9	6.40	9.03	11.9	7.25	11.3	13.5
3	4.50	6.93	12.7	5.07	7.66	11.2	6.10	9.57	12.8
2	4.61	7.25	12.4	3.65	5.95	10.5	4.81	7.60	11.9
1	4.79	7.81	11.8	2.07	4.09	8.96	3.21	5.00	10.4

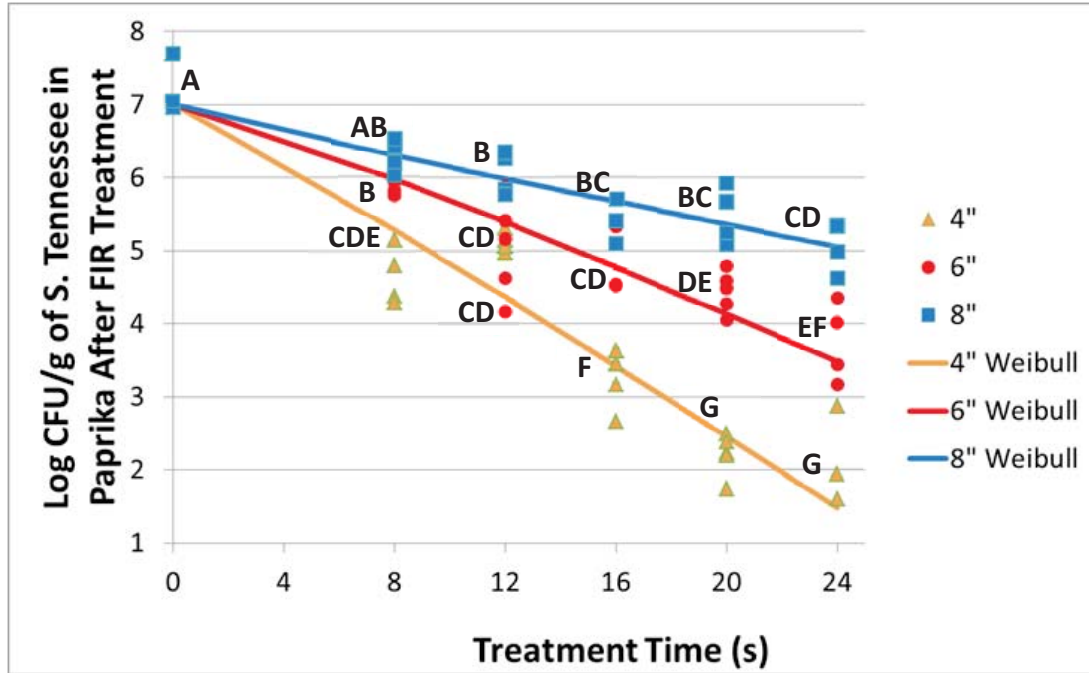


Figure 3.4. Effect of far infrared radiation on inactivation of *Salmonella* Tennessee in paprika. Varying letters indicate statistically different treatment means. Cells recovered on TSA+nal. N=4.

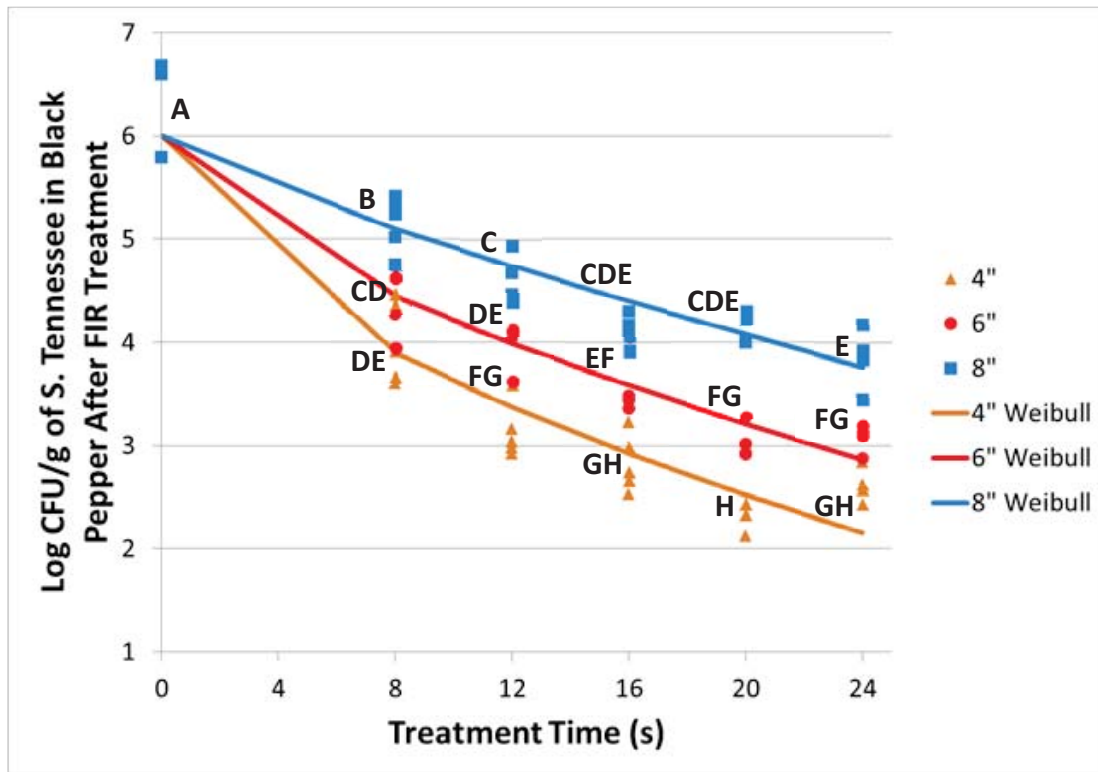


Figure 3.5. Effect of far infrared radiation on inactivation of *Salmonella* Tennessee in black pepper. Varying letters indicate statistically different treatment means. Cell recovered on TSA+nal. N=4.

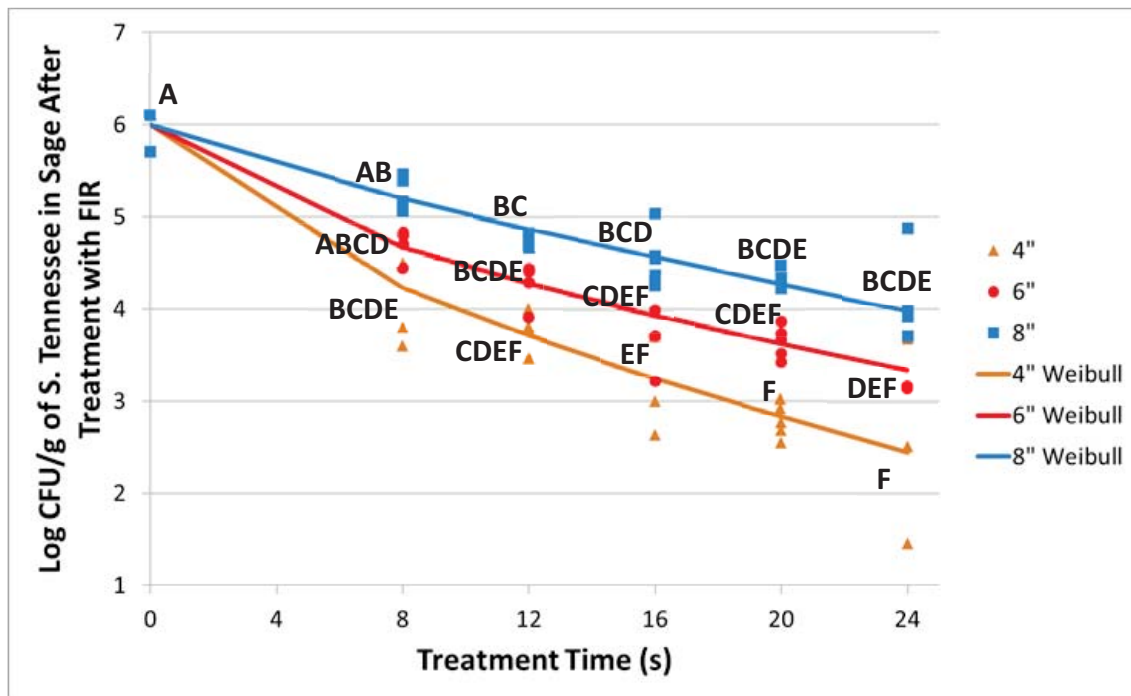


Figure 3.6. Effect of far infrared radiation on inactivation of *Salmonella* Tennessee in sage. Varying letters indicate statistically different treatment means. Cell recovered on TSA+nal. N=4.

Table 3.4 Comparison of Bigelow and Weibull parameters for inactivation of *Salmonella* Tennessee in spices by ethanol vapor.

Spice	Bigelow Model			Weibull Model			
	D	R ²	A _f	A	β	R ²	A _f
Paprika	88.50	0.48	204.5	0.36	0.49	0.64	2.53
Black Pepper	101	0.53	91.47	0.29	0.51	0.76	2.95

D: D-value in seconds

A_f: Accuracy factor

α and β: Scale and shape factors of the Weibull distribution

Table 3.5 Lethality times (s) for specific levels of decade reduction of *Salmonella* Tennessee based on Weibull model after treatment with ethanol vapor.

Decade Reduction (d)	Paprika		Black Pepper	
	Lethality Time (s)	D _{eq} (s)	Lethality Time (s)	D _{eq} (s)
5	214.8	43.0	265.9	53.2
4	136.2	34.1	171.6	42.9
3	75.7	25.2	97.7	32.6
2	33.1	16.6	44.1	22.1
1	8.04	8.04	11.3	11.3

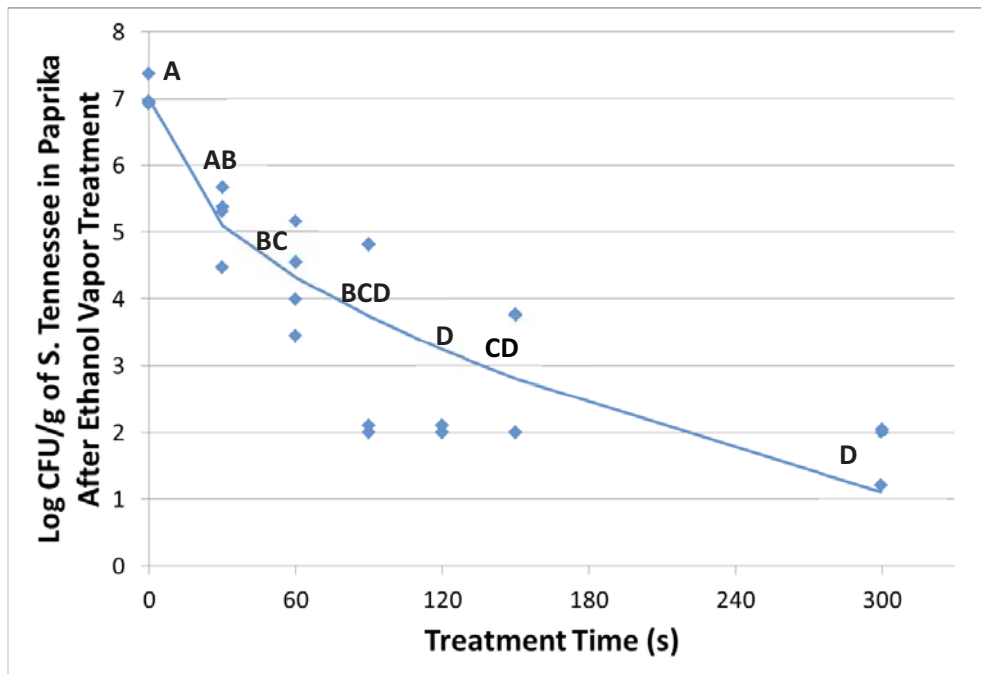


Figure 3.7. Effect of ethanol vapor on survivability of *Salmonella Tennessee* on paprika. Varying letters indicate statistical significant differences in treatment means. Cells recovered on TSA+nal. N=4.

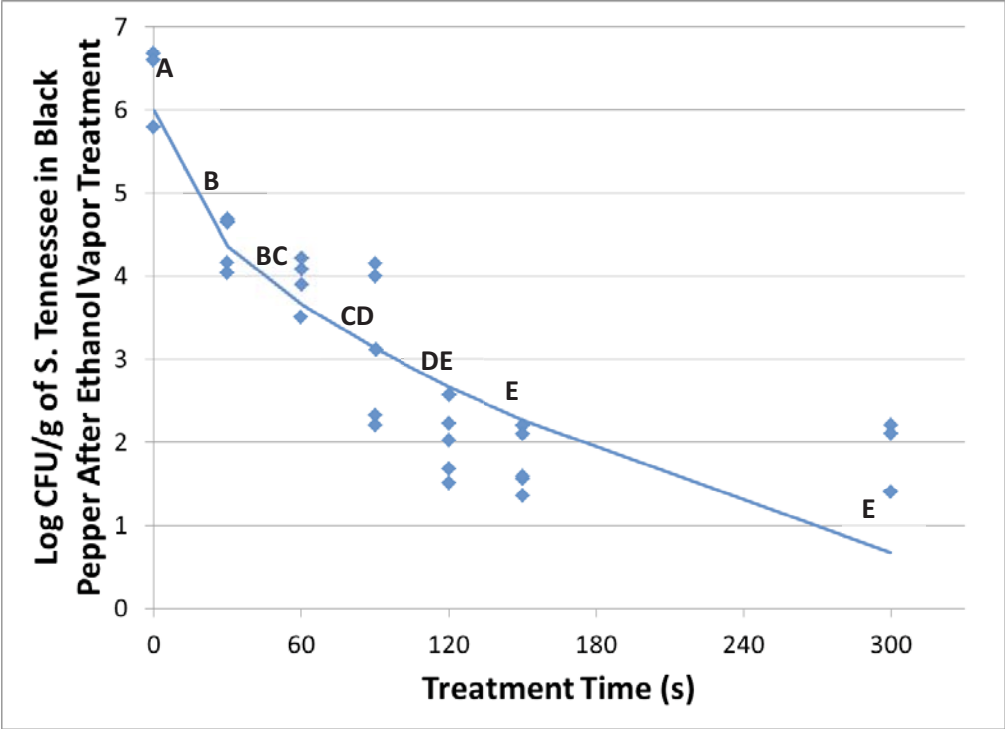


Figure 3.8 Effect of ethanol vapor on survivability of *Salmonella Tennessee* on black pepper. Varying letters indicate statistical significant differences in treatment means. Cells recovered on TSA+nal. N=4.

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CHAPTER 4

Effect of Far Infrared Radiation and Ethanol Vapor Treatment on Quality of Dried, Ground Spices

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Abstract

Far infrared radiation (FIR) and ethanol vapor (EV) have been found to be effective in reducing occurrence of *Salmonella enterica* Tennessee in black pepper (*Piper nigrum*), paprika (*Capsicum annuum*), and sage (*Salvia officinalis*). Many subtle traits, which are important in spices, can be deteriorated after harsh treatments and a successful adoption of these methods requires a confirmation that quality is not reduced in the final product. This study was conducted to evaluate post-treatment quality changes in water activity, color, and volatile profile in these spices. No significant differences were detected in water activity after EV treatment; however, a sharp decline in a_w after FIR treatment was observed to correspond to longer treatment times. Each spice exhibited small degrees of color change after treatment; however, the magnitudes of these changes were insignificant and not visible to the naked eye. FIR treatment severely altered volatile profiles, including samples with >40% loss in total volatiles for paprika and black pepper. EV treated spice samples exhibited high levels of residual ethanol post-treatment, but displayed no other changes to volatile composition up to 120s exposure.

4. 1 Introduction

There has been a significant increase in the number of food borne outbreaks and recalls associated with contaminated spices in recent years (Vij, Ailes et al. 2006). Between 1970 and 2003, there were twenty one recalls associated with spices, twenty of which were associated with *Salmonella* (Vij, Ailes et al. 2006). Three of the most recent recalls associated with spices occurred between 2007 and 2010 and were the result of *Salmonella* contaminated pepper (Van Doren, Kleinmeier et al. 2013). The spice processing chain contains many control points where contamination may be introduced into the food chain. To reduce the chance of illness, spices must undergo a treatment to reduce levels of harmful microorganisms.

Current treatments include the use of gamma irradiation, fumigation with ethylene oxide, or steam. Each of these treatments includes some level of inadequacies in their use. Irradiation suffers from negative public perception and food safety concerns (Alam Kham and Abraham 2010). Ethylene oxide can produce carcinogenic by-products after treatment and is banned in several European countries (Program 2011). Both of these treatments, as well as steam, also show certain levels of quality degradation in color and volatile oil (Vajdi and Pereira 1973, Farkas and Andrassy 1988, Kispéter, Bajúsz-Kabók et al. 2003, Waje, Kim et al. 2008, Rico, Kim et al. 2010). Preliminary work has shown that heat treatment with far infrared radiation (FIR) and fumigation with ethanol vapor (EV) can effectively reduce levels of *Salmonella* Tennessee in paprika, black pepper, and sage by 3-5 log CFU/g (Nimitz, Mallikarjunan et al. 2013).

FIR has previously been used as a tool for inactivation of insect larvae in stored grains, but new research has shown the possibility of utilizing FIR for drying of fruits and vegetables, as well as baking and roasting foods (Cogburn, Brower et al. 1971, Kirkpatrick and Cagle 1978, Sakai and Hanzawa 1994, Nowak and Lewicki 2004, Sharma, Verma et al. 2005, Wang and Sheng 2006). Bacterial control with FIR has recently been investigated and has shown the ability to reduce pathogenic strains. FIR has successfully inactivated mold, yeast, and bacteria in liquid buffer, as well as several solid foods (Krishnamurthy, Khurana et al. 2008, Krishnamurthy, Tewari et al. 2010). Application of FIR to cumin, paprika, and black pepper showed minimal quality losses coupled with reductions in bacteria equivalent or greater than 2.5 log CFU/g (Staack, Ahrné et al.

2008, Erdogdu and Ekiz 2011, Erdoğan and Ekiz 2013). Ethanol is nontoxic to humans and acts as an antimicrobial by disrupting the cellular structure of cells causing cell lysis and death (Ingram 1990, McDonnell and Russell 1999). Ethanol has been used to prevent fungi and mold in baked goods, either through product incorporation or use of EV packets (Dao and Dantigny 2011). EV has also been used to delay the ripening of fruit (Dao and Dantigny 2011). The use of EV on spices was shown to be effective in reducing bacteria levels, but no follow up research was performed (Wistreich, Thundiyil et al. 1975).

The goal of this work was to determine the impact of treatment with far infrared radiation and fumigation with ethanol vapor on physical and quality characteristics in paprika, sage, and black pepper. Color, water activity, and volatile peak analysis were all investigated post-treatment and compared to untreated control samples.

4.2 MATERIALS AND METHODS

4.2.1 Materials:

Untreated bulk quantities of paprika, black pepper, and sage were obtained from McCormick & Company (Hunt Valley, MD). Spices were stored in airtight plastic bags at a constant room temperature (22°C) until needed. Anhydrous ethanol (Fisher Scientific, Waltham, MA) was used at a concentration of 95%.

4.2.2 Experimental Chamber Setup:

A Thermo Fisher Scientific Isotemp Oven Model 630G (Waltham, MA) was utilized as a treatment chamber. The treatment chamber was fitted with a catalytic heater and configured for FIR trials as described by Nimitz (2013). During ethanol vapor treatment, the heater was removed from the oven and ethanol was supplied to the treatment chamber as described by Nimitz et al. (2013).

4.2.3 Treatment Procedure and Experimental Design:

For treatment, each sample was weighed to within 3.0 ± 0.1 g. Samples were spread on a piece of 11-cm diameter filter paper (Whatman International Ltd., Maidstone, England) to a thickness of

approximately 2-mm. Post-treatment, samples were stored in clean, glass 8mL screw cap vials at 4°C until analysis.

Far Infrared Radiation

Paprika, black pepper, and sage were tested. A full factorial design was used with three different ranges for sample distance to heat source (4, 6, 8 inches) and five different times of treatment (8, 12, 16, 20, 24 seconds). Samples were individually placed on the sample holding rack and treated for specific durations. When each set of times were complete, the sample rack was adjusted to the next desired distance. A ring stand and clamp was used to adjust the distance of the sample holder to the heater in the vertical direction (Nimitz, Mallikarjunan et al. 2013).

Ethanol Vapor

Paprika and black pepper samples were treated with vapor. Sage was omitted from EV treatment so emphasis could be placed on the more popular and more often contaminated spices of paprika and black pepper. Ethanol was heated to boiling and aspirated into the chamber. The treatment chamber was allowed to stabilize for five minutes between samples, in an attempt to equilibrate the concentration of atmospheric ethanol. Samples were placed on the sample holding rack and the door was closed and treatment was administered for a predetermined time (30, 60, 90, 120, 150, or 300 seconds) (Nimitz, Mallikarjunan et al. 2013).

4.2.4 Evaluation of Spice Quality

Effects on quality of each treatment time were judged by analyzing volatile losses, color changes, and water activity using the methods described below.

Water Activity

Water activity readings were taken from 1g samples by a Decagon CX-2 Water Activity Meter (Decagon Devices, Inc., Pullman, WA) in triplicate.

Color Analysis

Color was measured with a Minolta CR-300 chromameter (Minolta Camera Ltd., Osaka, Japan) on the CIE L*a*b* color scale in triplicate. Color readings were taken through a clear, 1/16”

thick piece of Plexiglas to ensure a flat surface for color readings and prevent spice dust from contaminating the lens of the chromameter. A white tile with color coordinates: $L^* = 97.23$; $a^* = 0.14$; $b^* = 1.82$ was used for calibration before each run.

Color change (ΔE) was calculated using Equation 1. Change in chroma (ΔC) was calculated using Equation 2. Change in hue angle (ΔH) was calculated with Equation 3.

$$\Delta E = \sqrt{\Delta L^{*2} + \Delta a^{*2} + \Delta b^{*2}} \quad (\text{Eq. 1})$$

$$\Delta C = \sqrt{\Delta a^{*2} + \Delta b^{*2}} \quad (\text{Eq. 2})$$

$$\Delta H = \arctan\left(\frac{b^*}{a^*}\right) - \arctan\left(\frac{b^0}{a^0}\right) \quad (\text{Eq. 3})$$

Volatile Analysis

Headspace solid phase microextraction gas chromatography (HS-SPME-GC) was used to determine if the volatile profile of samples had changed. A Shimadzu GC-2010 gas chromatograph (Kyoto, Japan) with an FID detector, running GC Solution software (Shimadzu Scientific Instruments, Kyoto, Japan) was used. A Restek Stabilwax (Bellafonte, PA) 30m x 0.25mm i.d. column was used for compound separation. The Kovats index was used for peak identification.

One gram of spice sample was placed in 4 mL clear vials sealed with a screw cap (PN: 27136 Supelco Bellafonte, PA) and SPME Supelco Thermoseal septum (Supelco Bellafonte, PA PN: 2-7192) and heated at 60°C for 30 minutes during absorption to the SPME fiber using a manual fiber holder (PN: 57330-U Supelco Bellafonte, PA). Splitless desorption was performed for 5 minutes. Helium was used as a carrier gas with a flow rate of 1 mL/min. Table 4.1 outlines the parameters for each spices used during HS-SPME-GC. Paprika used a Polydimethylsiloxane (PDMS100) fiber (Supelco, Bellafonte, PA) with a 100 μm thick coating and black pepper had a composite Divinylbenzene/Carboxen/Polydimethylsiloxane (DVB/CAR/PDMS) Stableflex fiber (Supelco Bellafonte, PA).

Six volatiles and seven peaks were tracked for paprika (2,3 butanediol appeared twice) (Table 4.2) and eight volatiles were tracked for black pepper (Table 4.3). Each treatment was run in triplicate. Specific identified peaks were integrated for peak area and averaged. Samples were

standardized based on total peak area to give a percentage of each component remaining in the spice after treatment.

4.2.5 Statistical Analysis

Each treatment was replicated three times. Data were analyzed using JMP 10 (SAS Institute, Cary, NC) through ANOVA to test the effect of FIR and EV treatments on water activity, color, and volatile profile. Tukeys HSD mean testing was used to compare treatment means. P-values less than 0.05 were considered significant.

4.3 RESULTS AND DISCUSSION

4.3.1 Water Activity, (a_w)

Water activity (a_w) is known to closely tie in with product quality. Rockland and Nishi (1980), state that small changes in a_w can have influence on physical properties of food products. Color, flavor, aroma, texture, and shelf stability are all quality parameters which can be affected due to a change in a_w . It is important to maintain a constant a_w during and after treatment to ensure retention of product quality.

Control samples in paprika, black pepper, and sage were found to have an average a_w of 0.49 ± 0.02 , 0.36 ± 0.05 , and 0.43 ± 0.08 , respectively (Fig. 4.1-4.5). No statistical differences were found in a_w after treatment with ethanol vapor for paprika ($p=0.11$) or black pepper ($p=0.97$). However, large statistical differences were found in a_w after treatment with FIR at all different treatments for paprika ($p=0.000$), black pepper ($p=0.000$), and sage ($p=0.000$). Longer treatment times resulted in lower average a_w for all spices.

Water activity values calculated in this study, treated and untreated, are considerably lower than reported values in reference texts. Reported water activity values for pure ground black pepper are 0.715 (Barbosa-Cánovas, Fontana et al.). Decagon, the manufacturer of the water activity meter used for this study, produced two separate reference values for black pepper, 0.516 and 0.409 (Decagon Devices 2010, Carter 2013). The observed value for black pepper in this study was 0.360. Domestic paprika has two grinds, 100 and 150; their standard reported a_w values are

0.611 and 0.523, respectively (Carter 2013). Paprika was observed to be 0.488 in this study. A reference for sage could not be found.

These levels are well below the stated a_w range of 0.91-0.95 that is an accepted minimum value for *Salmonella* growth (Barbosa-Cánovas, Fontana et al.). In fact, no microbes should be able to grow at a_w values less than 0.60 (Barbosa-Cánovas, Fontana et al.). However, viable bacteria survived and grew on spices in this study. *Salmonella* has been shown to be resilient enough to survive for up to 8 months in paprika and one year in black pepper at doses high enough to cause serious illness (Lehmacher, Bockemuhl et al. 1995, Keller, VanDoren et al. 2013).

During handling of the spice, it was noticed that clumping and caking would form after treatment at the more intense FIR treatments. These more intense treatments resulted in a hygroscopic sample and is responsible for moisture absorption from the environment into the sample. This reabsorption of moisture results in caking. FIR treatment changes the water activity of the spice and therefore could change the physical properties of the spice as well. Furthermore, it has been shown that *Salmonella* exhibits a higher degree of heat tolerance at lower levels of water activity (Mattick, Jorgensen et al. 2001, Podolak, Enache et al. 2010). The most effective treatments, as shown by Nimitz (2013), resulted in final products with low levels of moisture. These treatments showed the inability to achieve a full kill. The observed tailing of the microbial inactivation curve may be related to the low a_w levels in treated spice (Nimitz, Mallikarjunan et al. 2013). Stack et al (2008 quality) showed radiation heat transfer is the dominant method of heat transfer in powders up to product depths of 1 mm. The penetrative depth increased as a_w was decreased. The same group also found that under a transparent, quartz glass shield, near infrared radiation (NIR) could be used to control a_w levels in paprika (Stack, Ahrné et al. 2008). This shield maintained a constant a_w in the bulk of the sample, but resulted in a decrease on the sample surface.

4.3.2 Effect on Spice Color

The full range of the CIE $L^*a^*b^*$ color scale was used. Each spice were assigned values for L^* (lightness), a^* (redness-greenness), and b^* (yellowness-blueness). Hue angle (ΔH) and chroma (ΔC^*) were used for analyzing the redness and yellowness, while ΔL^* and total color change

(ΔE) accounted for any sample darkening after treatment. Paprika exposed to FIR did not show significant darkening (ΔL^*) ($p=0.412$) or show a significant change in chroma (ΔC^*) ($p=0.06$) (Fig. 4.11). Statistically significant differences in color change (ΔE) and hue angle change (ΔH) were measured for paprika samples ($p=0.00$ and $p=0.04$, respectively). However, there does not appear to be any trend associated with the treated samples. ΔE values were highest at the 6'' treatment position and the 8s and 24s times. Change in hue angle was at its maximum at the 8''-24s sample. Paprika fumigated with ethanol showed no significant changes to the L^* component color or chroma values (ΔC^*) ($p=0.20$ and $p=0.10$, respectively) (Fig. 4.14). Small but statistically significant differences ($p=0.05$) in color change, ΔE , were measured for paprika samples where treatment times exceeded 120 seconds. This can likely be associated with color leeching from samples, as shown in Figure 4.6. The filter paper was noticeably stained red, with increasing saturation with increased treatment time. This staining was not observed during FIR treatment. Despite being statistically significant ($p=0.02$), hue angle differences were less than 0.1 and therefore negligible. Paprika is a spice commonly used to color foods, as well as add spice. These results are positive as the deep red color is mostly unaffected by both treatments.

FIR treatment of black pepper resulted in a large degree of variation in the samples. Treatments resulted in a spice that was darker in color (L^*) ($p=0.000$) (Fig. 4.12). The darkest samples occurred at the closest distance (4 in) and the longest times (20s and 24s). The largest decrease in L^* was a decrease from a control value of 55.95 to 51.10 for the 4''-20s treatment. There was a significant color change (ΔE) ($p=0.00$), significant chroma change (ΔC) ($p=0.00$), and significant hue angle change (ΔH) ($p=0.01$) in black pepper after FIR treatment (Fig. 4.12). Hue angle change is unimportant for FIR black pepper and a nonfactor as the changes could be rounded to zero in most cases (Fig. 4.12d). ΔE showed no notable trend for describing the changes as seen in color readings. Ethanol treatment of black pepper resulted in a spice that was slightly darker when treated for the maximum time of 300s, $\Delta L^* = -2.11$ ($p=0.04$). No significant difference was found with ΔC^* or ΔH ($p=0.34$ and $p=0.30$, respectively) (Fig. 4.15). A small but significant color change, $\Delta E = 1.28$, was measured after 300 seconds ($p=0.03$). No other color changes were found in this sample set. Color change in black pepper can most likely be credited to the change in darkness as well as the color leeching as seen in Figure 4.7. Longer treatment times resulted in larger a degree of filter paper staining. No staining was evident after FIR treatment.

Sage was only treated with FIR. Sage showed no darkening ($p=0.55$), hue angle change ($p=0.61$), or overall color change ($p=0.65$) (Fig. 4.13). Chroma showed a significant change in color ($p=0.00$); however, the smallest and largest values occurred at the same treatment distance and at 16 and 20s. It is highly unlikely that this is a real phenomenon. It is more likely the result of sampling error.

Each treated spice had some statistically significant color change. However, many of these changes occurred on such a small order of magnitude that no real, observable changes can be noticed. A direct comparison showed no major change in color for any sample and most likely no change that would register at a consumer scale as unacceptable (Fig. 4.8-4.10). A small degree of color change associated with browning can be expected with the addition of heat to any sample. The intensity of color change compares favorably with changes seen in paprika and black pepper after treatment with steam or irradiation. Paprika showed ΔE values after treatment with high pressure steam and 5 kGy dose of irradiation to be 1.25 and 2.5, respectively (Kispéter, Bajúsz-Kabók et al. 2003). These values increased to 3.7 and 2.9 after 12 weeks of storage, while the control sample registered an increase in ΔE of 3.0 after the same storage time (Kispéter, Bajúsz-Kabók et al. 2003). The maximum ΔE values found in this study for paprika, black pepper, and sage was 2.45, 4.92, and 1.52, respectively. Rice et al. (2010) showed the effects of steam and irradiation treatment on color changes in paprika to have a lower degree of change after steam and irradiation, but a similar increase in color change for the control sample was noticed after six months of storage. Black pepper treated with steam or 10 kGy dose of irradiation also appeared to have similar color change values of 2.08 for steam and 0.60 for irradiation (Waje, Kim et al. 2008). Ethylene oxide also displayed a small post-treatment color change, of 3.67, in paprika (Vajdi and Pereira 1973). For high intensity FIR treatments, color can be expected to change rapidly as radiated power is proportional to the fourth power of temperature. Other sources of color change may come from the significant reduction in water activity or change in particle size due to clumping or caking of the spice powder (Staack, Ahrné et al. 2008).

4.3.3 Effect on Volatiles

Paprika and black pepper samples were compared using headspace sampling to determine if FIR and EV altered the aroma profiles. Six volatile components of interest were evaluated for paprika (Table 4.2). After FIR treatment, 3-methyl butanal, a compound responsible for a chocolate and malty aroma, was undetectable in most treated samples, indicative of a major loss of compound (Fig. 4.16a). 3-Methyl butanal is derived from the Strecker degradation reactions of leucine and isoleucine and are responsible for many of the highly aromatic compounds found in thermally processed foods, including paprika (Vidal AragÓN, Lozano et al. 2005). The combination of low boiling temperature and possibility of continued thermal degradation of these products may account for overall low recovery. For compounds that could be properly evaluated, significant differences were found after FIR treatment except 3 Dihydro-3,5 Dihydroxy-6 Methyl-4H-Pyran-4-one (Fig. 4.16e). Longer treatments showed the greatest degree of volatile loss ($p=0.000$). However, there appears to be no relationship of volatile loss based on independently changing distances from the heater ($p=0.818$).

Paprika samples treated with EV retained their respective volatile levels to a much higher degree when compared to FIR. 3-methyl butanal and ethanol were the only volatiles in paprika to register a significant change when compared to untreated controls ($p = 0.002$ and $p= 0.01$, respectively). In fact, ethanol concentrations were almost undetectable in control paprika which resulted in almost a 1000 fold increase in ethanol concentration in the 300s sample based on peak area. All treatments increased the percentage of ethanol recovered from the sample ($p = 0.000$) to over 30% while not being uncommon for samples analyzed to be 50% ethanol vapor (Fig. 4.19). Mean comparisons found no differences in any other volatiles tracked for paprika after EV treatment (Fig. 4.18) (all p -values > 0.22).

Seven compounds were tracked for FIR and EV treatment of black pepper (Table 4.3). Analysis of black pepper components shows β -caryophyllene accounts for a large portion of total volatiles ($>30\%$) and is responsible for a spicy characteristic of pepper. All other components contributed lesser percentage and were lower molecular weight compounds that eluted at a shorter retention time. Black pepper samples showed up to 40% loss of total volatile levels after FIR exposure

(Fig. 4.17h) ($p=0.000$) Each individual component decreased in quantity after FIR treatment (Fig. 4.17). As seen with paprika, increased treatment time yield spices with lower volatile recovery ($p=0.002$). Sabinene and β -Pinene appeared to be the most sensitive to heat as an 80% loss occurred at times as low as 12s.

Ethanol vapor treatment showed no statistically significant data indicating a change in volatile characteristics in black pepper up to 120 seconds (Fig. 4.20). There is a statistically significant increase in residual ethanol concentration after 60 seconds and an increase in l-limonene, α -thujene, α -pinene, and β -pinene in samples at 300s treatment times (Figures 4.20 & 4.21). It is possible this increase could be the result of a reaction with the ethanol vapor, but further study is needed in that area. Certain terpenes in black pepper have been shown to increase in quantity after heat treatment (Sádecká 2010). These compounds did not register an increase after FIR treatment, but are believed to be a result of thermal isomerization (Sádecká 2010). The longer EV treatments resulted in black pepper samples with high amounts of residual ethanol. Ethanol levels are over 25 \times higher in 300 sec samples than control samples and over 15 \times higher in samples treated for 150 seconds. These levels remain much lower than paprika. Black pepper contained approximately 30% ethanol by overall volatile levels, while paprika commonly exceeded 50% ethanol.

Further study is needed to calculate the effect that high levels of residual ethanol may play in affecting the flavor or aroma profiles of the spices. While ethanol is on the GRAS list for human consumption, these levels may be too high to be acceptable by either consumers or regulators. To test for the concentration of residual ethanol in each sample, spiked spice samples with varying concentrations of ethanol (0.2%, 0.5%, 1%, 2% w/w) were analyzed (data not shown). This produced a gradient where the concentration of the high levels of residual ethanol could be compared to known concentrations. Certain bakery products are allowed up to 2% ethanol by weight incorporated into them, as a preservative, before sensory attributes are altered (Dao and Dantigny 2011). It was assumed the same would be true for spices. It was desired to have ethanol concentrations less than 2% w/w.

Using the gradient determined above, it was determined a paprika sample with an ethanol concentration of 30% total volatiles would be at a concentration of 0.2% w/w, while 60% ethanol would account for an overall concentration of 0.5% w/w of ethanol. Figure 4.19 shows that most treatments for paprika fell between 40-60% ethanol by total volume of volatiles, meaning an overall dry weight percentage would be less than 0.5%. Ethanol concentration in black pepper samples equating to 10% of total volatile area (approximately 60s treatment) converts to only 0.2% ethanol on a w/w basis. A 20% ethanol by total volatiles and 30% ethanol by total volatiles equates to 0.5% and 1.0% ethanol on a per weight basis, respectively. No black pepper samples exceeded 30% ethanol by total volatiles, and the recommended treatment time of 120s was under 20%, resulting in weight concentrations of less than 1% ethanol. This meets the goal of under 2% w/w. Residues of ethylene oxide (EO) in food are set at a strict limit of 7ppm by the FDA (ASTA 2011). Prior to regulation, EO levels were commonly found to exceed this limit and top 100ppm with values as high as 580ppm (Jensen 1988). To prevent high levels of EO residues and toxic byproducts, spices and other foods must undergo an aeration step. However, due to the length, this aeration step leads to volatile losses in the spice (Farkas and Andrassy 1988). This extra step may be applicable in this case as it may be able to reduce levels of residual ethanol.

Limited work has been done on analyzing how the volatile compounds in spices react to specific treatment conditions. It is possible that increasing the temperature of the treatment chamber during EV application could reduce the level of residual vapor condensation within each spice sample. Reduction of condensation will reduce raw material costs associated with treatment due to vapor losses. However, EV treatment benefits from being a low temperature treatment method. It remains to be seen how increased temperature would further affect spice quality. Other groups have found that the intensity of individual aromatic compound extracts in black pepper decreased by 90% after 3 min of steam treatment (Sádecká 2010). Volatile oil content in black pepper was reduced by over 30% after treatment with ethylene oxide, while nonvolatile oils in paprika were reduced 20% post-treatment (Vajdi and Pereira 1973, Farkas and Andrassy 1988) This study found a 40% decrease in total volatile component quantity in some of the paprika and black pepper samples when treated with FIR (Fig. 4.16 and 4.17). Using whole seeds or unground spice may reduce the degradation of volatile profile. Volatile oil content was preserved in whole cumin and black pepper seeds after FIR treatment (Erdogdu and Ekiz 2011,

Erdođdu and Ekiz 2013). The selected compounds in this study were selected based on previous literature and not a sensory study. Without a sensory study or GC-O analysis it remains difficult to determine if these spices are destroyed or worthless. It remains to be seen how much each individual component plays into the overall volatile profile.

CONCLUSIONS

Far infrared radiation and ethanol vapor have been shown to be adequate decontamination methods for reducing *Salmonella* Tennessee in paprika, black pepper, and sage (Nimitz, Mallikarjunan et al. 2013). It was found that ethanol vapor treatment successfully retained most associated quality parameters (color, a_w , and volatile profile) up to two minutes of treatment. It was shown by Nimitz et al. (2013) that ethanol vapor application could achieve over a 3 log reduction of *Salmonella* Tennessee in less than 100s in paprika and black pepper. Pathogen inactivation times were much lower for FIR treatment, but quality parameters were greatly affected in terms of volatile retention and water activity. Water activity was greatly reduced for all treatments. Small changes in water activity can have an impact on spice color as well. FIR did not affect the redness of paprika, but did lead to slightly darker black pepper.

Volatile analysis showed 3-methyl butanal as a very sensitive compound for paprika. FIR resulted in a total loss for even short time treatments, while EV samples suffered a 75% loss. It is unsure how important this compound is to the sensory characteristics of the paprika. Black pepper and paprika both suffered over a 40% loss in total volatiles with most individual compounds being reduced at a similar rate.

At this moment, research findings show EV as the preferential treatment to FIR. Further study is needed to minimize loss of water and volatiles. There exists the possibility that rectifying the underlying issues of significant degradation of volatiles and developing a procedure to maintain constant water activity levels may auto-correct the minor issues found with color. Additionally, further studies are required to investigate the effect of high levels of residual ethanol on the food product and consumer acceptability. Increasing the temperature of the treatment chamber could

prevent condensation of the vapor and therefore reduce the concentration of residual ethanol. However, ethanol treatment is beneficial because it occurs at low temperature.

A sensory study would be desirable to determine if any off-flavors could be detected due to either the residual levels of ethanol or change water activity. Shelf-life studies should also be carried out to investigate how any residual ethanol may affect color and volatiles long term. It is estimated that after treatment, ethanol represents 0.5% of the final spice product by weight. Allowing the spices to breathe after treatment may release some of the residual ethanol trapped in the sample to be released.

4.5 TABLES & FIGURES

Table 4.1. Head Space-Solid Phase Microextraction-Gas Chromatography parameters for analysis of volatiles in spices on a Shimadzu GC-2010.

Parameter	Paprika	Black Pepper
Fiber	PDMS100	DVB/CAR/PDMS
Inlet Temperature (°C)	230	250
Column Profile	50°C for 5 min; 4°C/min to 180°C	40°C for 5 min; 6°C/min to 250°C
FID Detector Temperature (°C)	280	320

Table 4.2 Aromatic compounds and retention times of paprika as produced by a Shimadzu GC-2010 gas chromatograph. Selected compounds based on previous work (Keller, Flath Robert et al. 1981, van Ruth and Roozen 1994, Cremer and Eichner 2000, Zimmermann and Schieberle 2000).

Compound	Retention Time (min)	Aroma Characteristics
3-methyl butanal	3.50	Chocolate/ Malty
Ethanol	3.95	Alcoholic
Trimethyl pyrazine	21.37	Roasted/ Baked/ Nutty
2,3 butanediol	24.3	Sweet
Propanoic acid	24.56	Sweaty/ Sour
2,3 butanediol	24.70	Sweet
3 dihydro-3,5 dihydroxy - 6 methyl-4H-Pyran-4-one	42.06	Caramel-like

Table 4.3 Aromatic compounds and retention times of black pepper as produced by a Shimadzu GC-2010 gas chromatograph. Selected compounds based on previous work (Jirovetz, Buchbauer et al. 2002, Mamatha, Prakash et al. 2008).

Compound	Retention Time (min)	Aroma Characteristics
Ethanol	4.89	Alcohol
α -pinene	6.93	Piney, sharp, woody
α -thujene	7.13	Herbal, green
β -pinene	9.20	Terpene-like, dry, spicy
Sabinene	9.67	Peppery, oily
l-limonene	12.07	Piney, Turpentine
γ -terpinene	13.44	Citrusy, fresh
β -caryophyllene	22.32	Spicy, woody

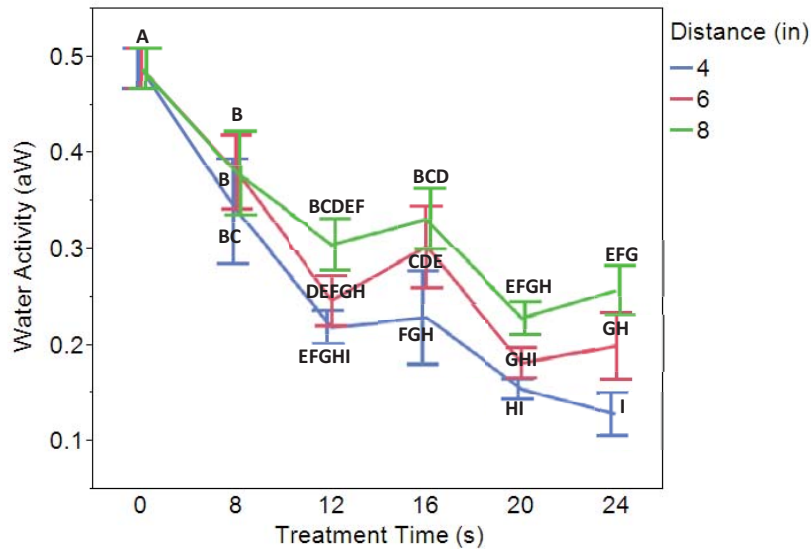


Figure 4.1. Water activity of paprika samples receiving FIR treatment, shown with standard deviations. A decrease in a_w was recorded for all treatments ($p = 0.000$). Treatments without similar capital letters are statistically different. $N=3$.

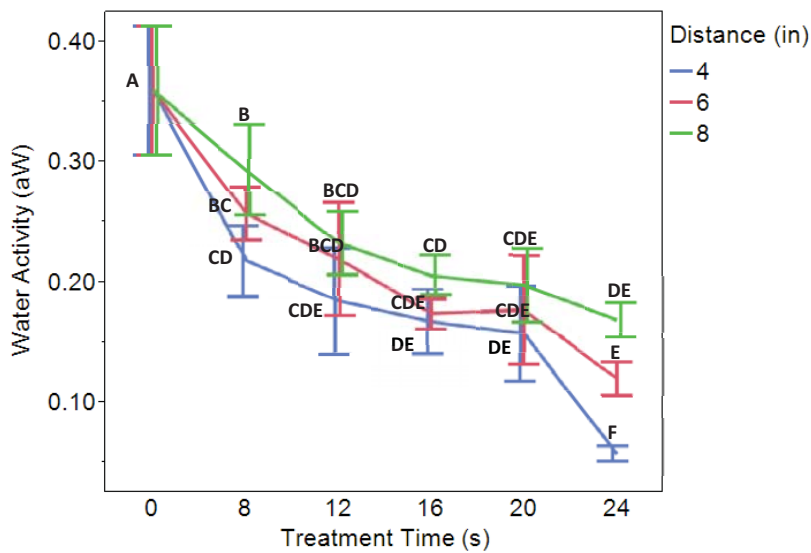


Figure 4.2. Water activity in black pepper samples receiving FIR treatment, shown with standard deviations. A decrease in a_w was recorded for all treatments ($p = 0.000$). Treatments without similar capital letters are statistically different. $N=3$.

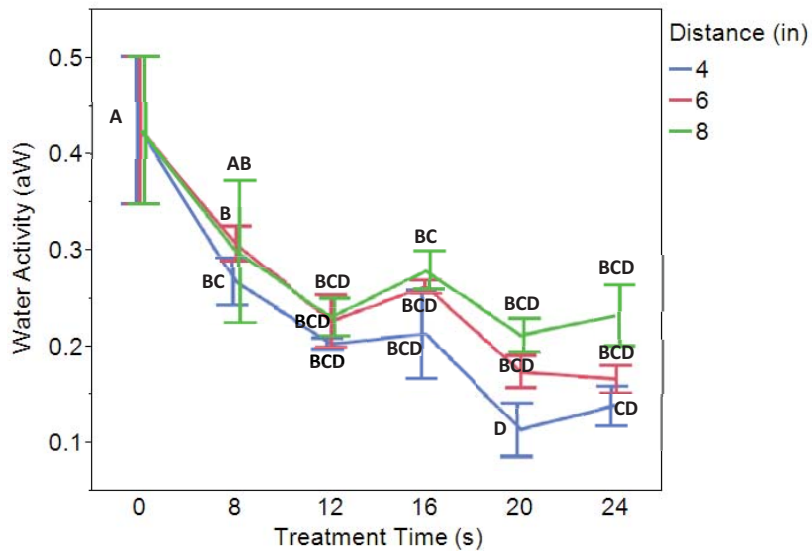


Figure 4.3. Water activity in sage samples receiving FIR treatment, shown with standard deviations. A decrease in a_w was recorded ($p = 0.000$). Treatments without similar capital letters are statistically different. $N=3$.

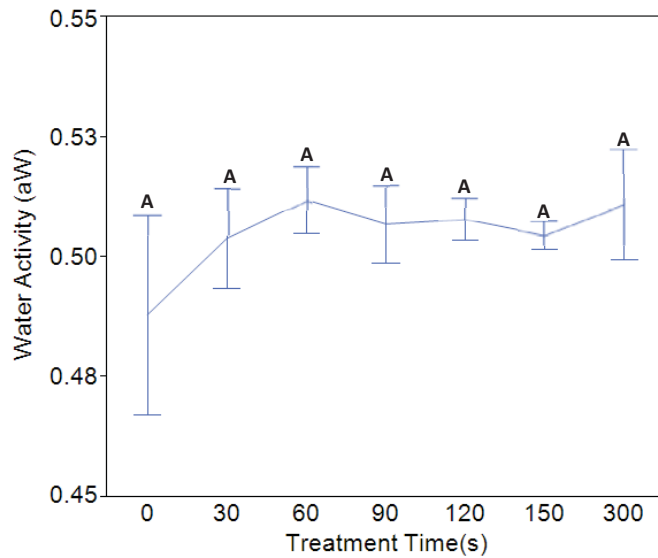


Figure 4.4 Water activity in paprika samples receiving EV treatment, shown with standard deviations ($p = 0.114$). Treatments without similar capital letters are statistically different. $N=3$.

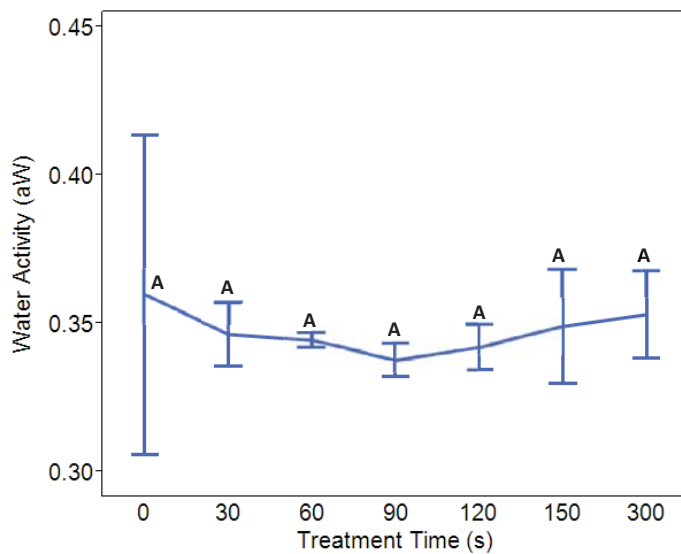


Figure 4.5 Water activity in black pepper samples receiving EV treatment, shown with standard deviations ($p = 0.966$). Treatments without similar capital letters are statistically different. $N=3$.



Figure 4.6. Filter paper leeching for paprika when treated with ethanol. Top row, left to right: control, 30s, 60s, 90s. Bottom row, left to right: 120s, 150s, 300s.

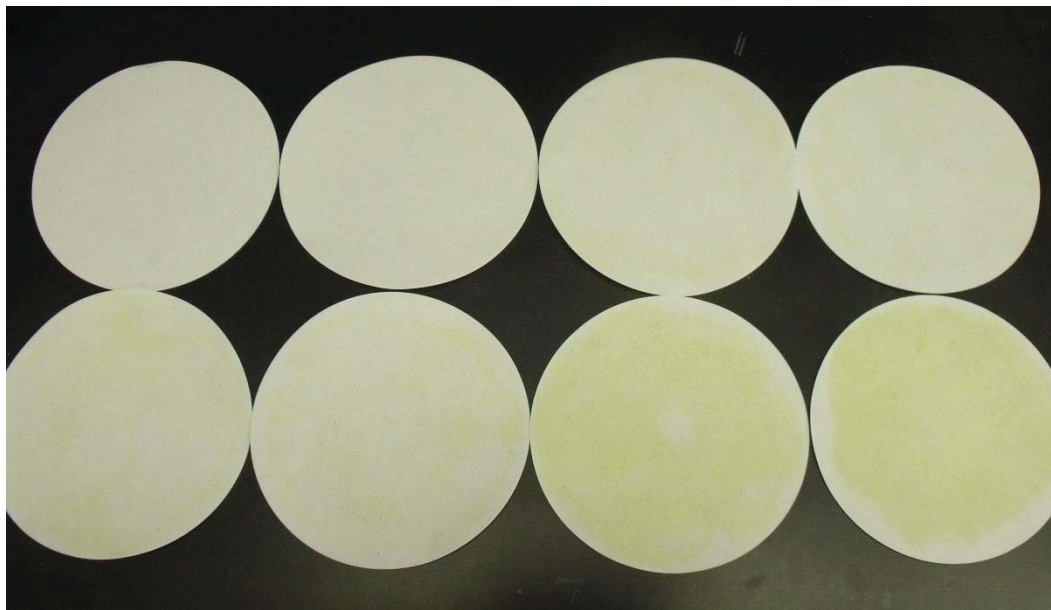


Figure 4.7. Color leeching of black pepper as a result of ethanol vapor treatment. Top row, from left to right: 0 sec, 30 sec, 60 sec, 90 sec. Bottom row, from left to right: 120 sec, 150 sec, 300 sec, 480 sec.

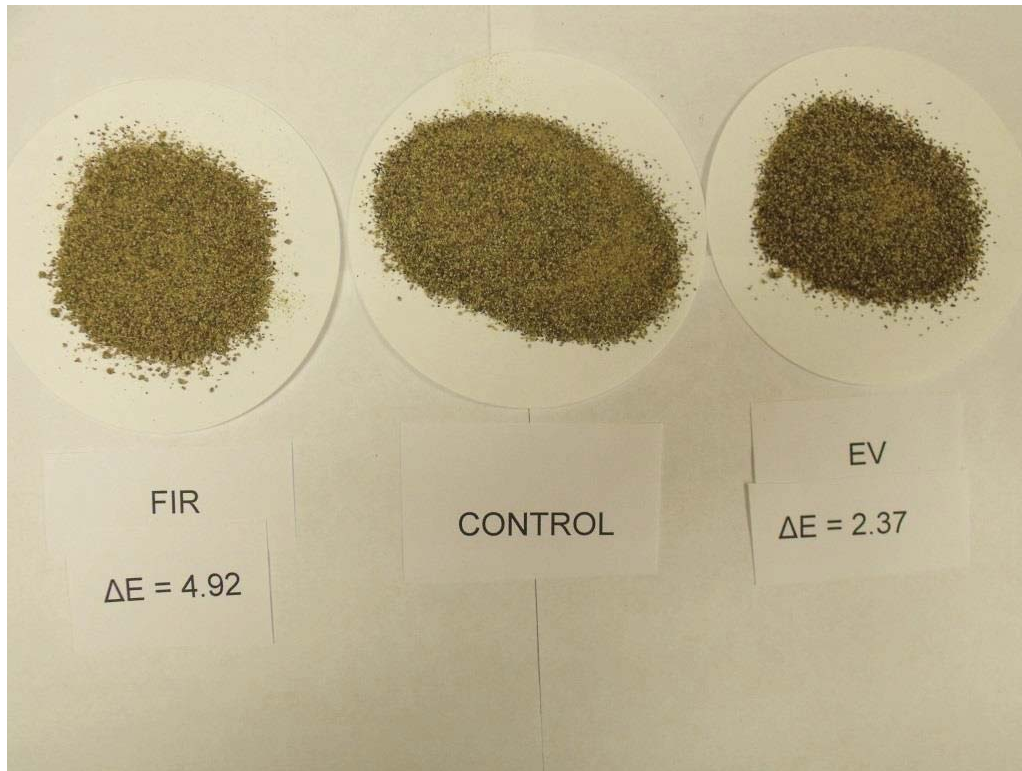


Figure 4.8. Black pepper samples showing maximum color change for each treatment. FIR is 4''-20s sample with $\Delta E = 4.92$. EV is 300s sample with $\Delta E = 2.37$.

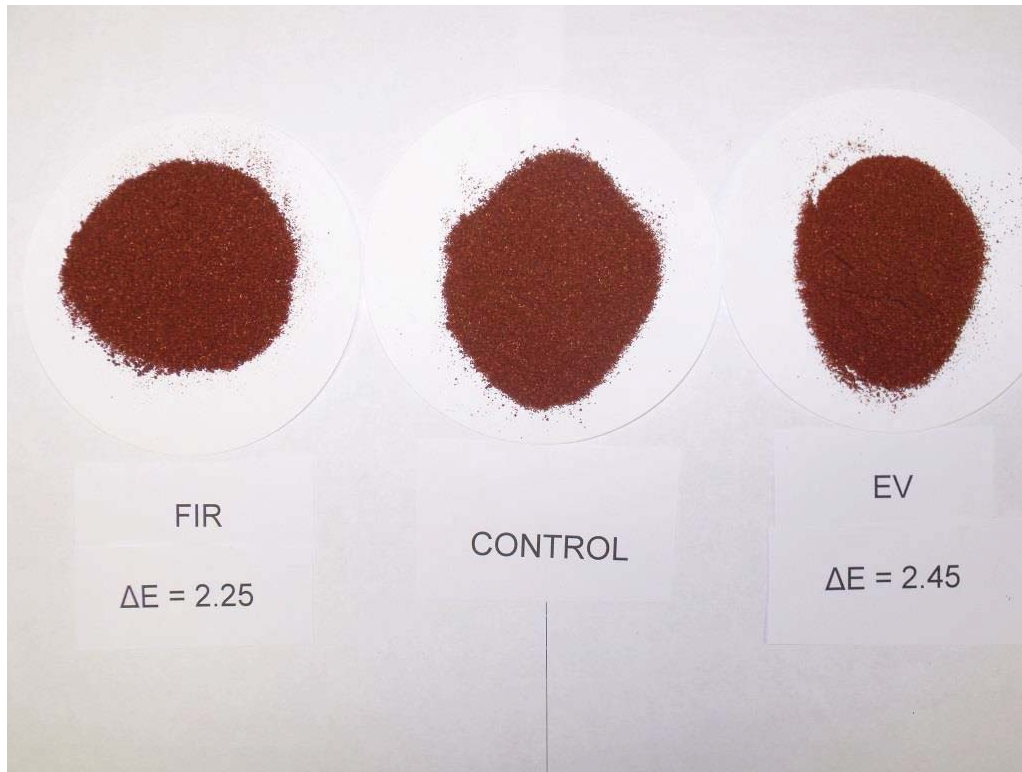


Figure 4.9. Paprika samples showing maximum color change for each treatment. FIR is for 6"-24s sample with $\Delta E = 2.25$. EV is 300s sample with $\Delta E = 2.45$.

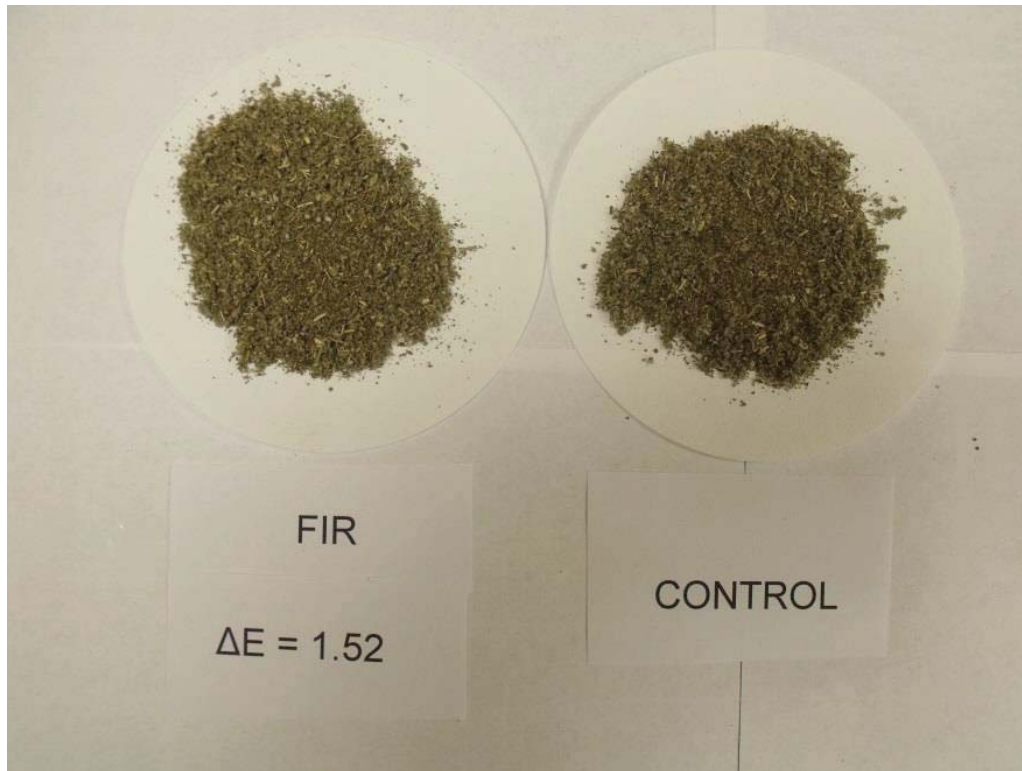


Figure 4.10. Sage samples showing maximum color change after FIR treatment. Sample is 4”16s with $\Delta E = 1.52$.

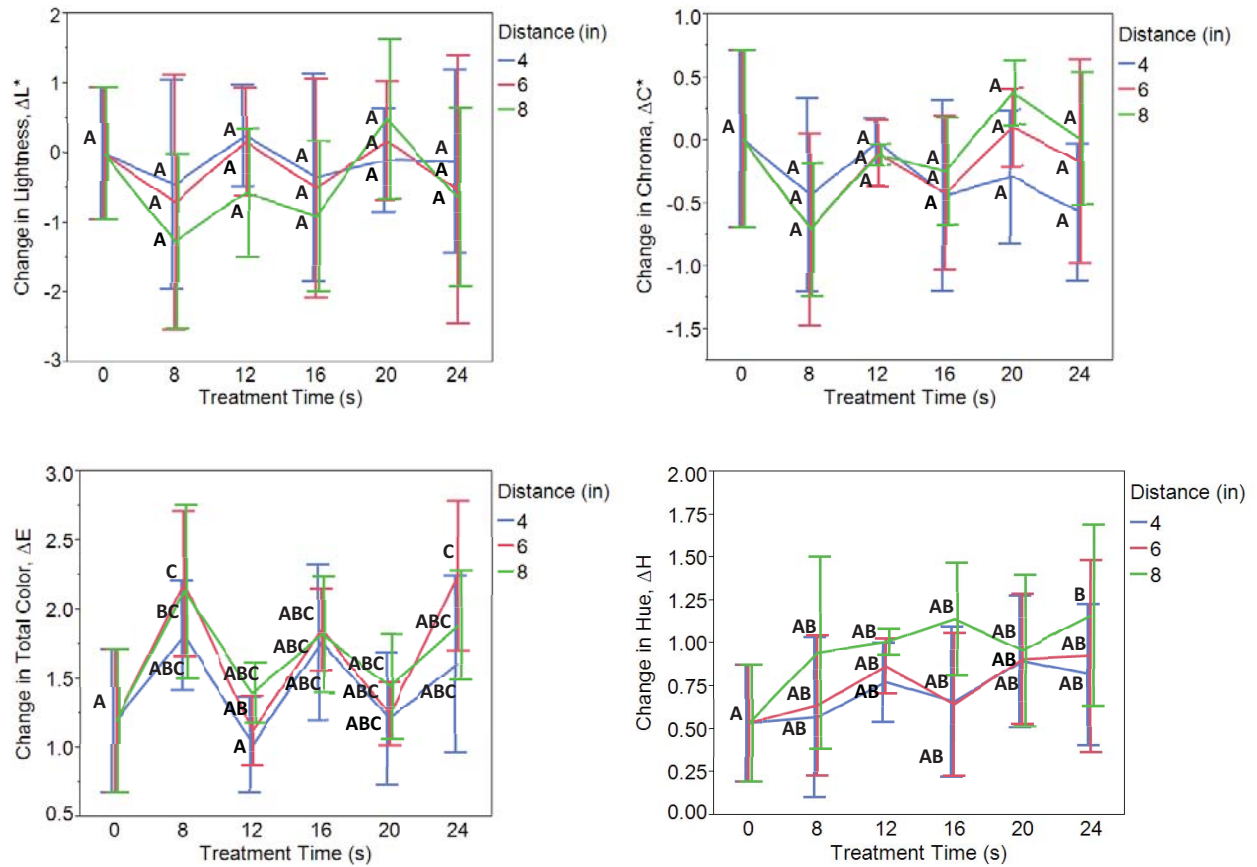


Figure 4.11. Mean color change values in paprika samples after receiving FIR treatment, shown with standard deviations. Treatments with different capital letters signify statistical significance. N=3.

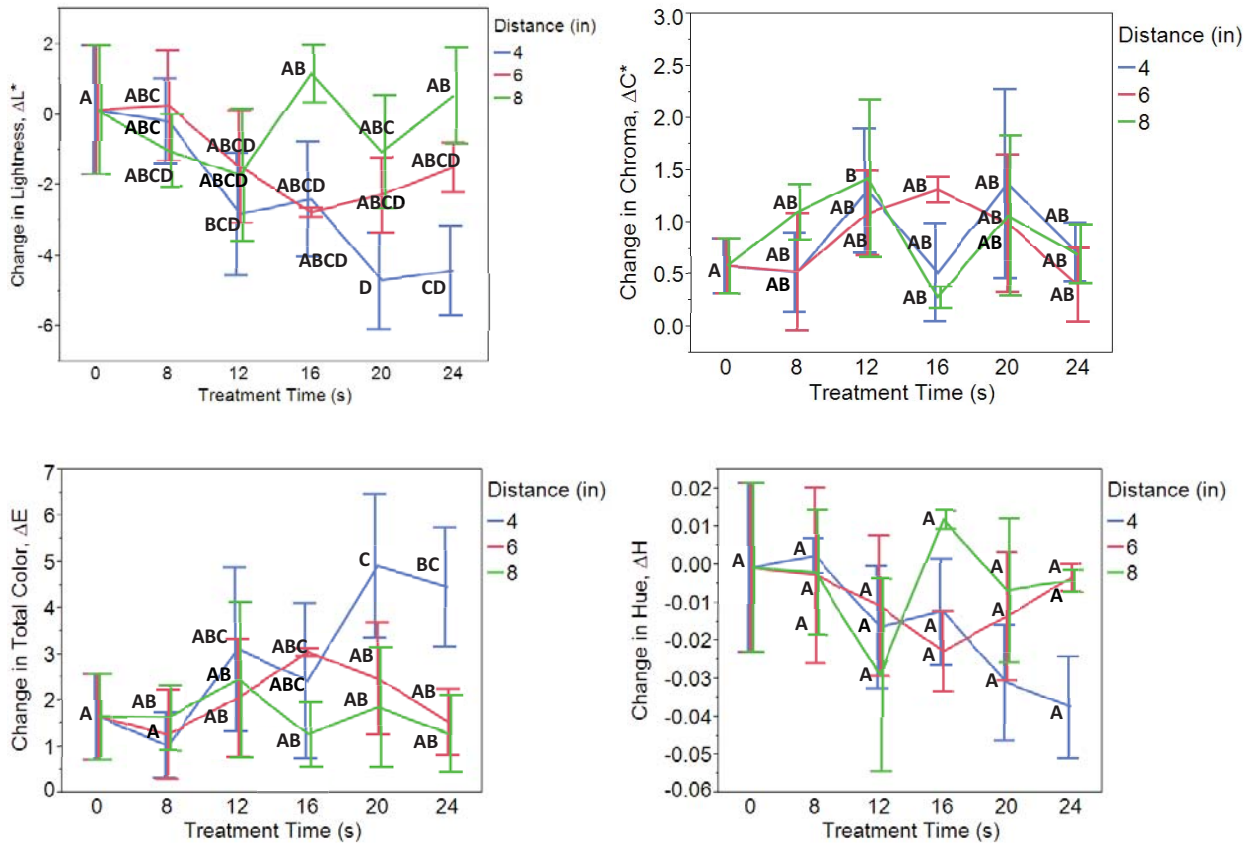


Figure 4.12. Mean color change values in black pepper after receiving FIR treatment, shown with standard deviations. Treatments with different capital letters signify statistical significance. N=3.

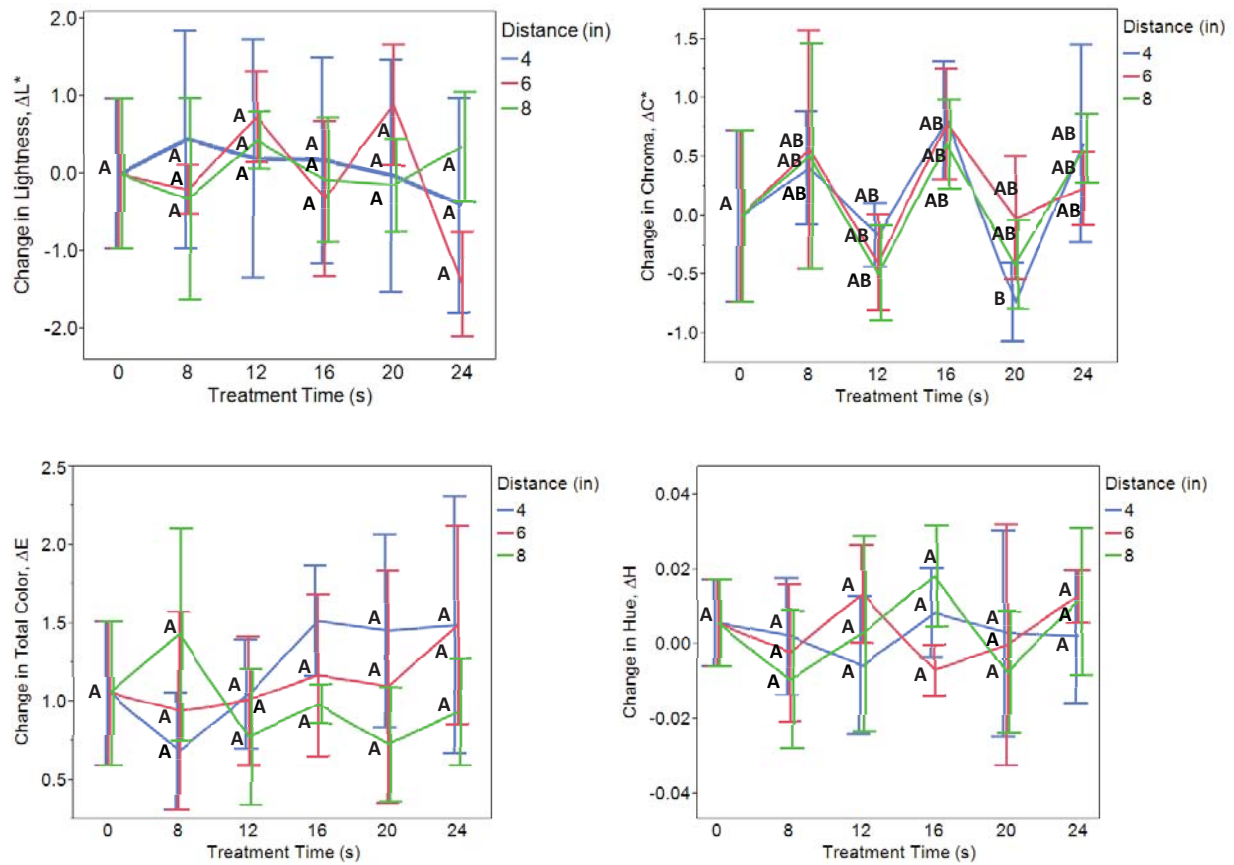


Figure 4.13. Mean color change values in sage after receiving FIR treatment, shown with standard deviations. Treatments with different capital letters signify statistical significance. N=3.

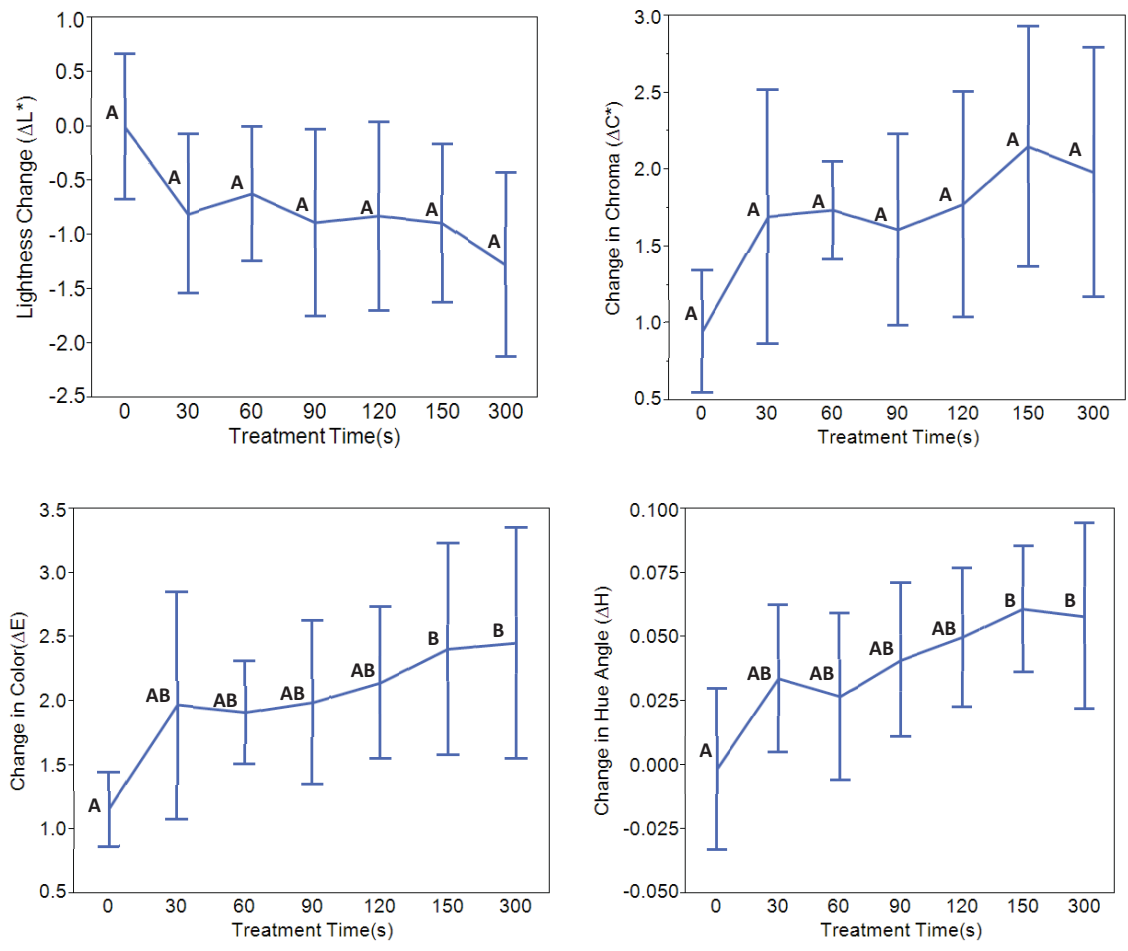


Figure 4.14. Mean color change values in paprika after receiving EV treatment, shown with standard deviations. Treatments with different capital letters signify statistical significance. N=3.

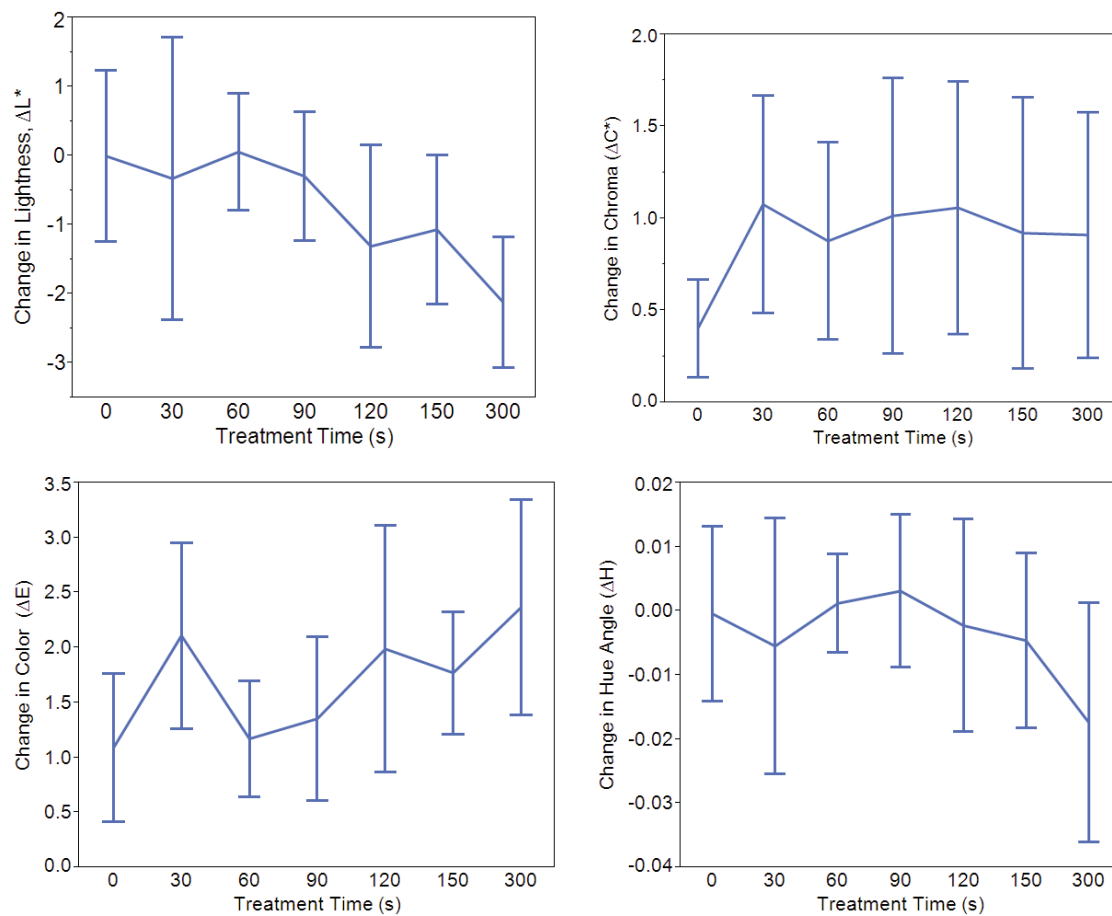


Figure 4.15. Mean color change values in black pepper after receiving EV treatment, shown with standard deviations. Treatments with different capital letters signify statistical significance. N=3.

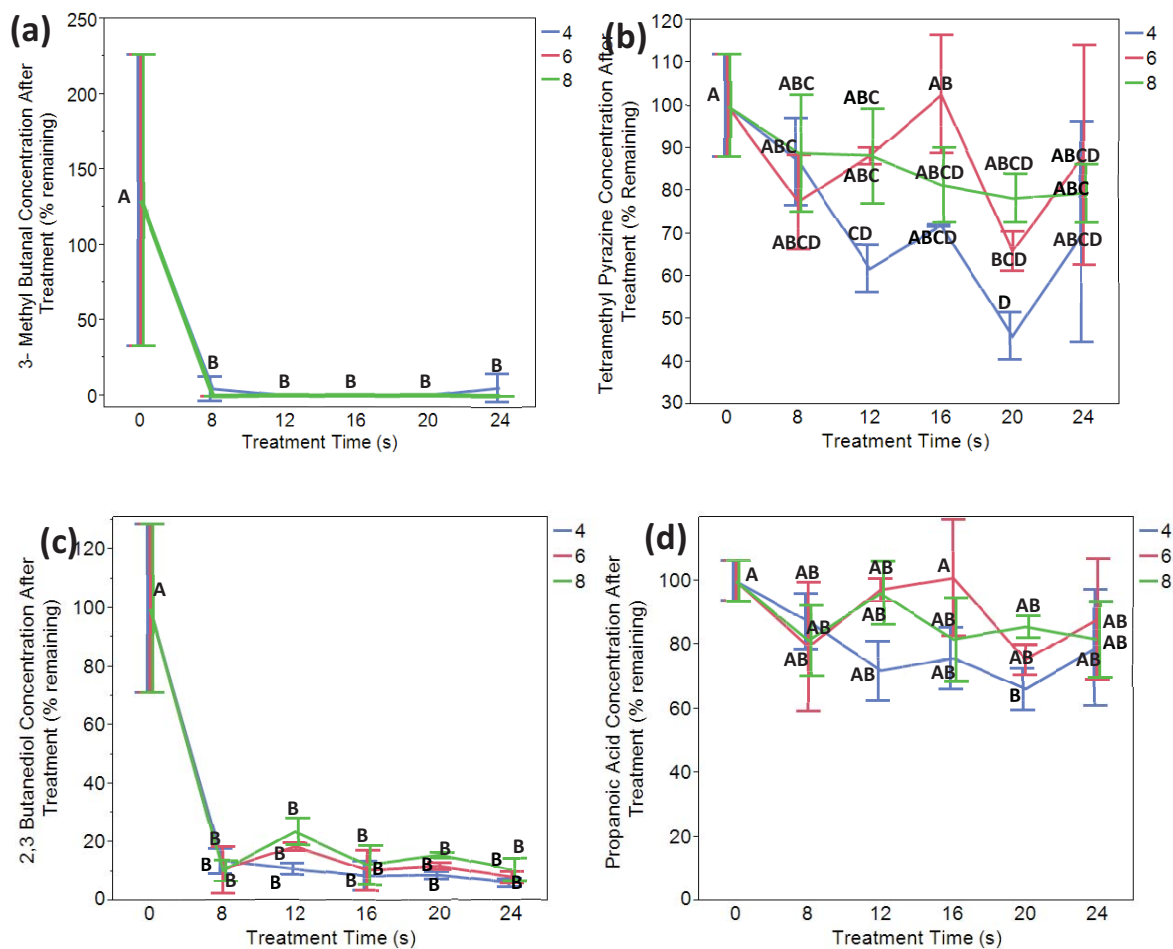


Figure 4.16 (a-d). Integrated peak area for volatiles in paprika when treated with FIR under various conditions, shown with standard deviations. Varying letters indicate statistically different treatment means. No significant differences were found in the aroma profiles of selected volatiles at the 0.05 level. (a) 3-Methyl Butanal – retention time: 3.50 min ($p=0.000$) (b) Tetramethyl Pyrazine – retention time: 21.37 min ($p = 0.000$); (c) 2,3 Butanediol – retention time: 24.3 min ($p = 0.000$); (d) Propanoic Acid – retention time: 24.56 min ($p = 0.001$). $N=3$.

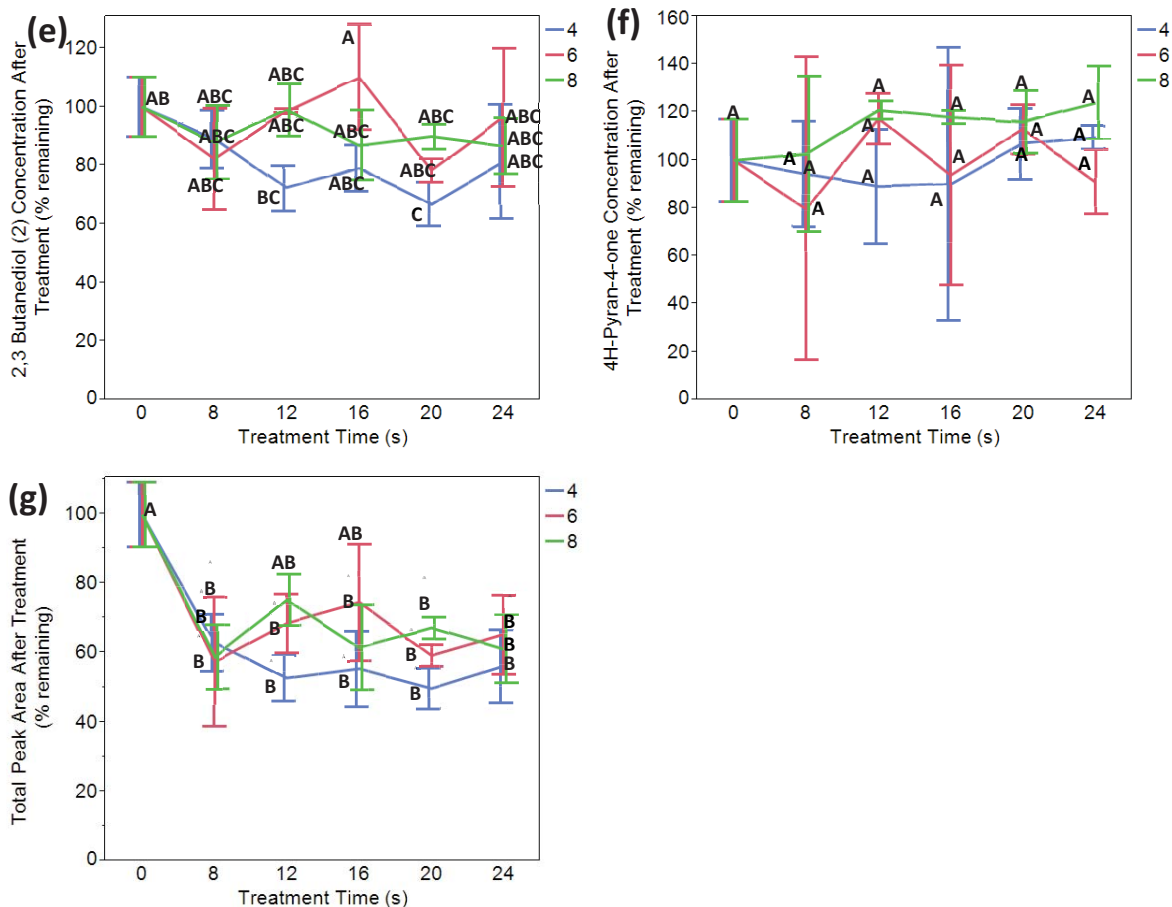


Figure 4.16 cont. (e-g). Integrated peak area for volatiles in paprika when treated with FIR under various conditions, shown with standard deviations. Varying letters indicate statistically different treatment means. No significant differences were found in the aroma profiles of selected volatiles at the 0.05 level. (e) 2,3 Butanediol (2) – retention time: 24.7 min ($p = 0.003$); (f) 3 Dihydro-3,5 Dihydroxy-6 Methyl-4H-Pyran-4-one – retention time: 42.06 min ($p = 0.622$); (g) Total peak area ($p = 0.000$). $N=3$.

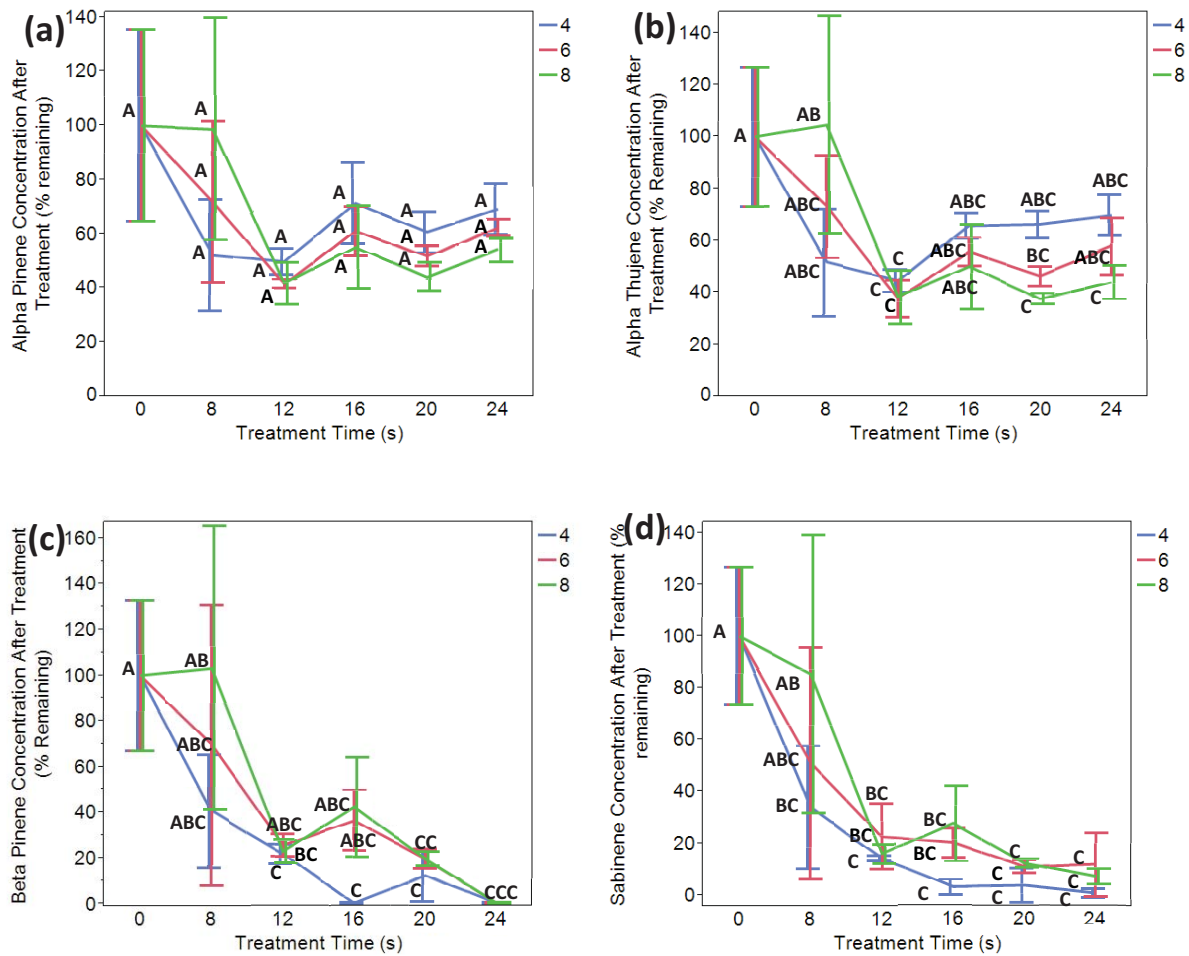


Figure 4.17(a-d). Peak area for volatile measurements for black pepper when treated for various lengths of time with FIR, showing standard deviations. Varying letters indicate statistically different treatment means. (a) α -Pinene – retention time: 6.93 min ($p = 0.002$); (b) α -Thujene – retention time: 7.13 min ($p = 0.000$); (c) β -Pinene – retention time: 9.20 min ($p = 0.000$); (d) Sabinene – retention time: 9.67 min ($p = 0.000$). $N=3$.

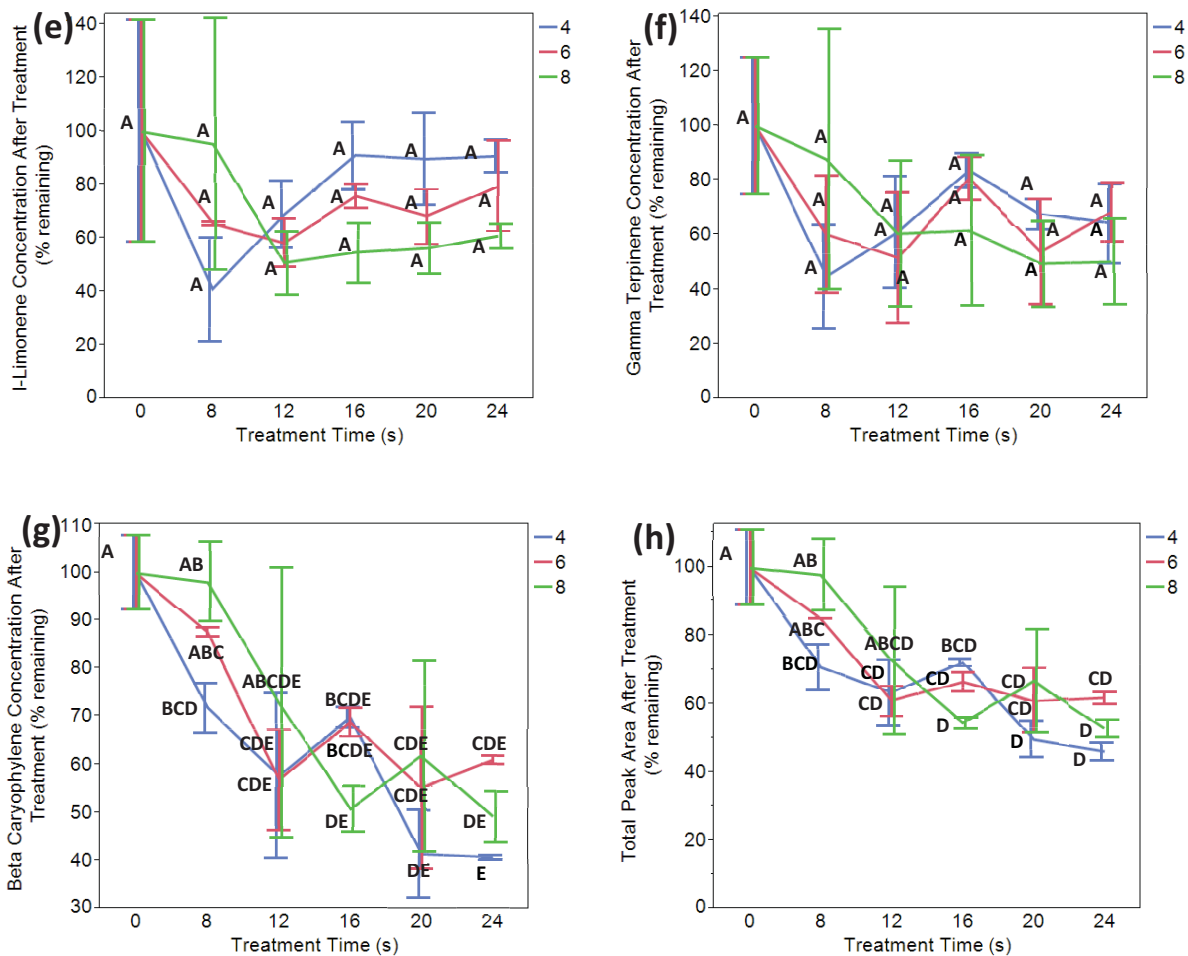


Figure 4.17 cont. (e-h). Peak area for volatile measurements for black pepper when treated for various lengths of time with FIR, showing standard deviations. Varying letters indicate statistically different treatment means. (e) l-Limonene – retention time: 12.07 min ($p = 0.000$); (f) γ -Terpinene – retention time: 13.44 min ($p = 0.002$); (g) β -Caryophyllene – retention time: 22.32 min ($p = 0.000$); (h) Total peak area ($p = 0.000$). N=3.

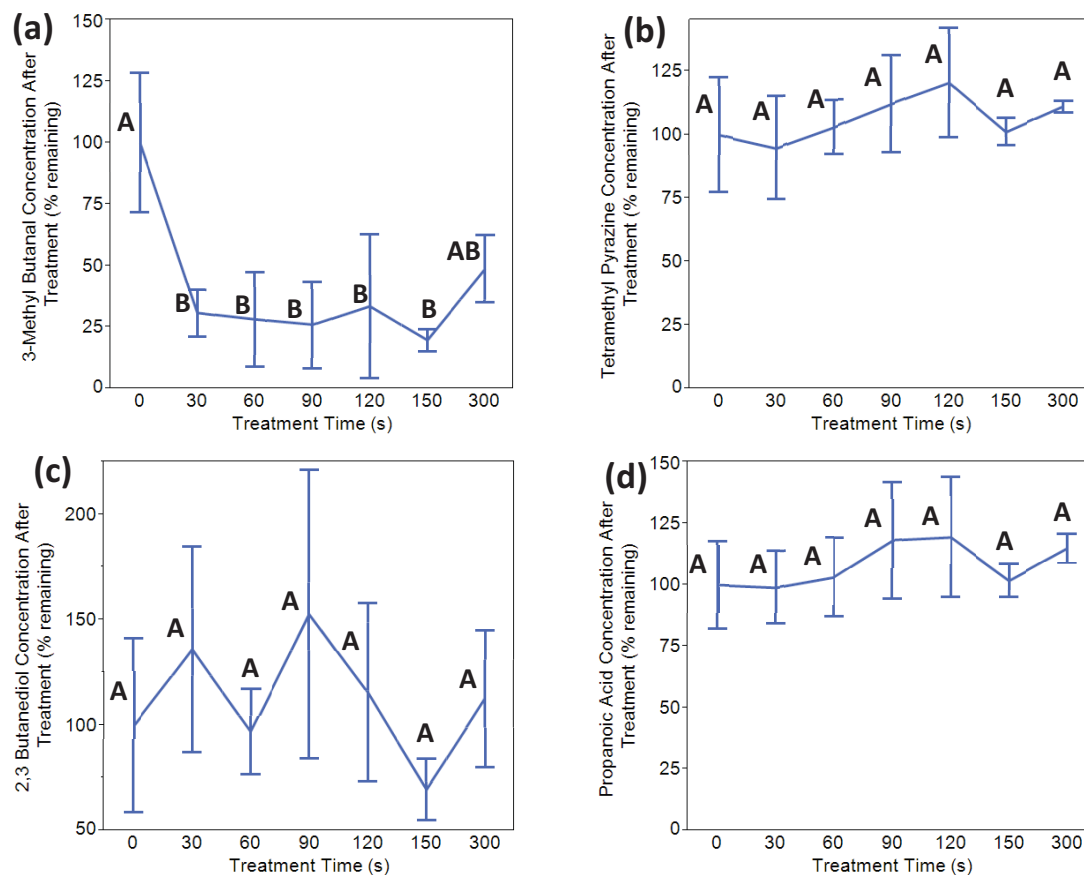


Figure 4.18. (a-d). Integrated peak area for volatiles of paprika when treated for various lengths with ethanol vapor, presented with standard deviations. Varying letters indicate statistically different treatment means. (a) 3-Methyl Butanal – retention time: 3.53 min ($p = 0.51$); (b) Tetramethyl Pyrazine – retention time: 21.37 min ($p = 0.55$); (c) 2,3 Butanediol – retention time: 24.3 min ($p = 0.33$); (d) Propanoic Acid – retention time: 24.56 min ($p = 0.55$). $N=3$.

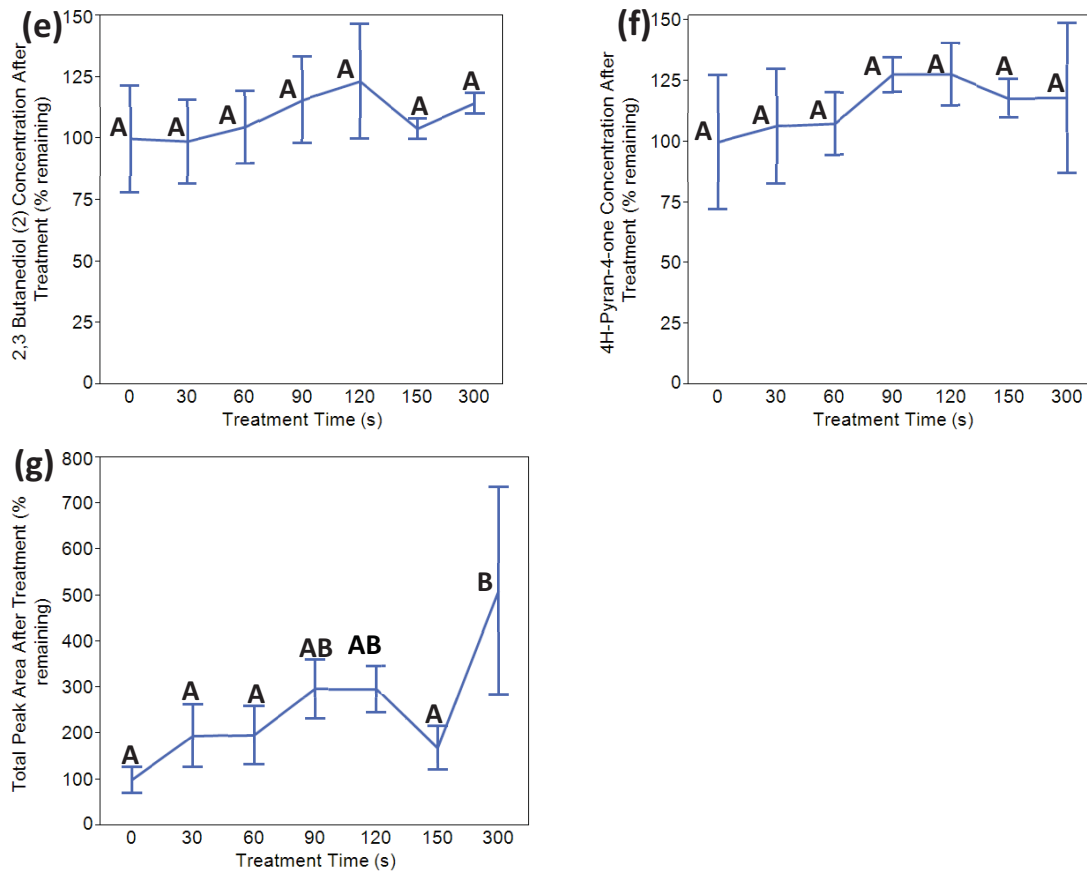


Figure 4.18 cont. (e-g). Integrated peak area for volatiles of paprika when treated for various lengths with ethanol vapor, presented with standard deviations. Varying letters indicate statistically different treatment means. (e) 2,3 Butanediol (2) – retention time: 24.7 min ($p = 0.49$); (f) 3 Dihydro-3,5 Dihydroxy-6 Methyl-4H-Pyran-4-one – retention time: 42.06 min ($p = 0.52$); (g) Total peak area. $N=3$.

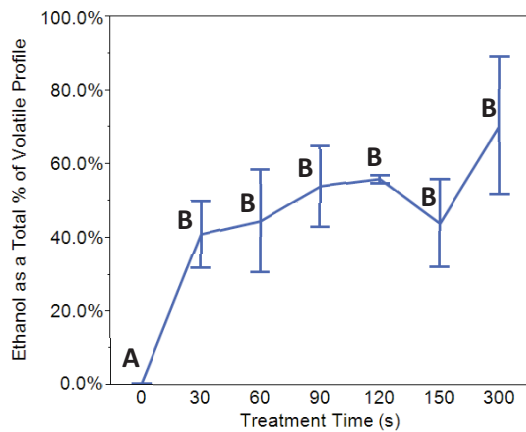


Figure 4.19. Ethanol concentration in paprika treated with heated ethanol vapor. Varying letters indicate statistically different treatment means. A large increase in residual ethanol was observed after treatment. (a) Peak Area of Ethanol - retention time: 3.93 min ($p = 0.007$); (b) Ethanol as a percentage of total volatiles recovered from samples ($p < 0.0001$). $N=3$.

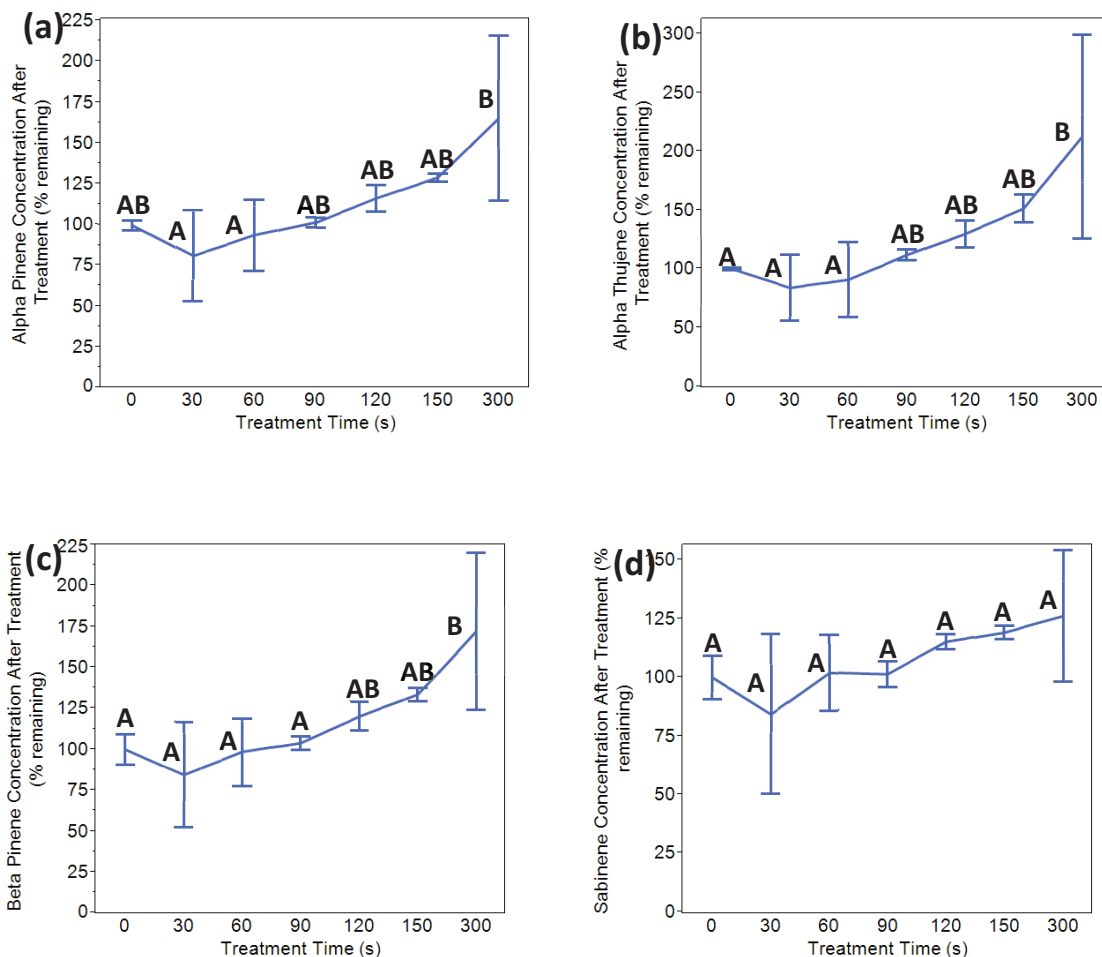


Figure 4.20 (a-d). Peak area for volatile measurements for black pepper when treated for various lengths of time with ethanol vapor. Varying letters indicate statistically different treatment means. No significant differences were found in the aroma profiles of selected volatiles up to 150 seconds of treatment at the 0.05 level. (a) α -Pinene – retention time: 6.93 min ($p = 0.012$); (b) α -Thujene – retention time: 7.13 min ($p = 0.011$); (c) β -Pinene – retention time: 9.20 min ($p = 0.009$); (d) Sabinene – retention time: 9.67 min ($p = 0.17$). $N=3$.

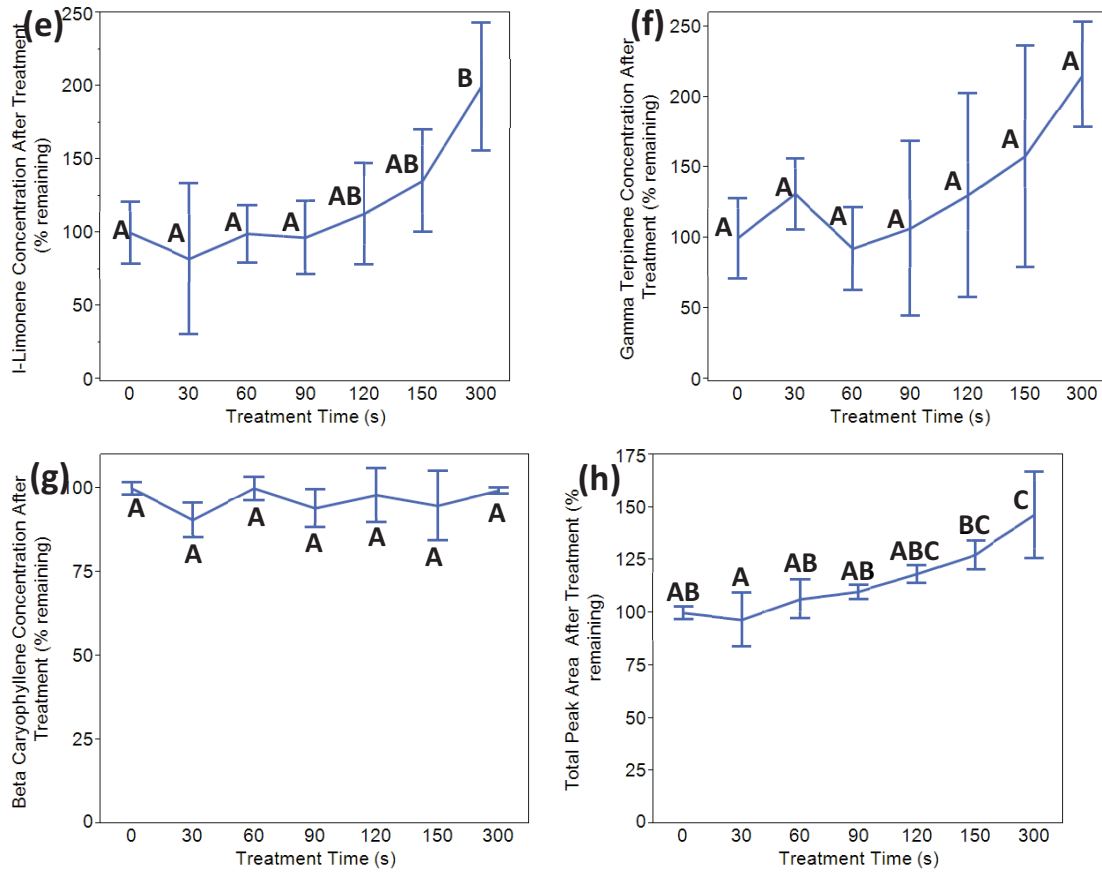


Figure 4.20 cont. (e-h). Peak area for volatile measurements for black pepper when treated for various lengths of time with ethanol vapor. Varying letters indicate statistically different treatment means. No significant differences were found in the aroma profiles of selected volatiles up to 150 seconds of treatment at the 0.05 level. (e) l-Limonene – retention time: 12.07 min ($p = 0.016$); (f) γ -Terpinene – retention time: min ($p = 0.13$); (g) β -Caryophyllene – retention time: min ($p = 0.41$); (h) Total Peak Area. N=3.

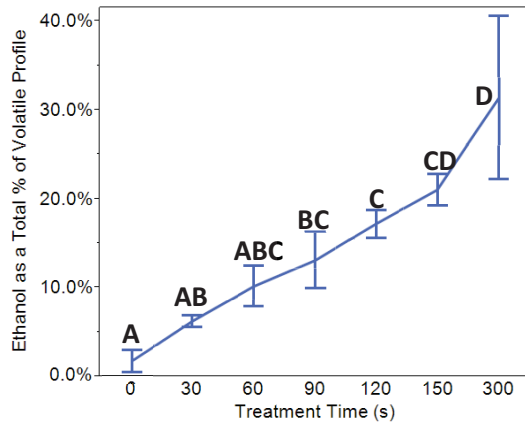


Figure 4.21. Ethanol concentration in black pepper treated with heated ethanol vapor. A large increase in residual ethanol was observed after treatment. Varying letters indicate statistically different treatment means. Ethanol as a percentage of total volatiles recovered from samples ($p < 0.0001$). $N=3$.

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CHAPTER 5

SUMMARY AND CONCLUSIONS

This research project investigated alternative methods for disinfection of dried, ground spices. Two potential methods were evaluated: far infrared radiation (FIR) and ethanol vapor (EV). One method is a thermal method employing high temperature (~350°C) – short time (8-24s) treatment. The other is a non-thermal, chemical method. The efficacy of the ability of these methods on inactivation of *Salmonella enterica* Tennessee in paprika, black pepper, and sage was evaluated. The Bigelow and Weibull models were used to calculate pathogen inactivation times. Both treatment methods were found to be effective in reducing *Salmonella* Tennessee 3-5 log CFU/g. Inactivation curves showed significant tailing. Weibull modeling proved to be an acceptable technique to calculate the thermal death times in spices. FIR was able to achieve a greater than three log CFU/g reduction in less than 45s for all spices, while EV required up to 97s for equivalent reduction in black pepper. Paprika samples were more rapidly disinfected than either sage or black pepper.

To measure the quality aspect, the spices were tested for post-treatment alteration of color, water activity, and headspace volatiles. It was found that many of the FIR samples were severely altered by treatment, while EV showed a better retention of quality parameters. Water activity (a_w) values for FIR samples showed a significant decrease after all treatments, while EV showed no alteration. Color values showed minimal change and values which were statistically different showed little real world significance as color changes could not be viewed by the naked eye.

Head space analysis of treated samples provided volatile profiles. A low degree of volatile recovery was noticed for FIR samples. A 40% loss of total volatiles was noted for paprika and black paprika after FIR treatment. 3-Methyl butanal and 2,3 butanediol suffered almost total losses in paprika. This may be attributable to the low boiling points of each compound. Black pepper saw rapid degradation of β -pinene and sabinene, with 60% losses occurring as early as

12s for each respective compound. β -caryophyllene, the most abundant volatile in black pepper, showed a steady decline during FIR treatment. Ethanol treatment succeeded in maintaining most volatiles. 3-methyl butanal concentration was also impaired after EV treatment, but not to the same degree as FIR samples (75% loss versus 100%). Ethanol levels drastically increased during EV treatment. The impact of the residual ethanol needs to be investigated. At worst case, the ethanol was found to constitute up to 1% of each sample on a weight basis.

Despite the treatments times being much shorter (seconds versus minutes) than other reported studies, infrared radiation was found to alter spice characteristics, while others report such results (Staack, Ahrné et al. 2008, Staack, Ahrné et al. 2008, Erdoğdu and Ekiz 2011, Erdoğdu and Ekiz 2013). The treatment of spices in their whole, unground form may assist in reducing the impact from the intensity of FIR due to a decrease in surface area. In order to prevent evaporation of water during treatment, Stack et al (2008) successfully used NIR on paprika with a specially designed heat shield. Other possible future work should include a sensory study for investigating the subjective quality impact of the volatile losses. Members of the research team could not tell the difference in color of the samples after treatment, but more verification is needed. Ethanol vapor shows great promise as a possible treatment for spices. FIR shows potential, but in its current state is not recommended as a treatment method.