UNIVERSITY OF CENTRAL OKLAHOMA

Edmond, Oklahoma

Jackson College of Graduate Studies

Application of Cinnamon oil Nanoemulsion to Control Foodborne Bacteria such as *Listeria* Sp. and *Salmonella* Sp. On Melons

A THESIS

SUBMITTED TO THE GRADUATE FACULTY

in partial fulfillment of the requirements

for the degree of

MASTER OF SCIENCE IN NUTRITION AND FOOD SCIENCE

By

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Edmond, Oklahoma

June 2017

Application of Cinnamon oil Nanoemulsion to Control Foodborne Bacteria such as *Listeria* Sp. and *Salmonella* Sp. On Melons

A THESIS APPROVED FOR

THE DEPARTMENT OF HUMAN ENVIRONMENTAL SCIENCES

July 2017

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ACKNOWLEDGEMENTS

I would like to thank Dr. Kanika Bhargava for her support, advice, and being patient with me throughout of this project. Without her extraordinary support and guidance this would not have been possible. She has guided me through the entire process and I am indebted to her.

I also express my gratitude to my academic adviser and committee member Dr. Tawni Holmes. She has helped me in my journey since day one when I joined this university. She has always been there for me in my hour of need and advised me throughout and I express my endless gratitude.

I would also like to thank my committee member Dr. Hari Kotturi for guiding me and helping me through the problems that I faced in the microbiology lab and for always being available.

I would also like to thank my parents and family for their continuous support for whatever endeavors I have pursued. I could not have done this without the support of my friends. Also thanks to the members of the Micro-prep lab for helping me whenever I needed help. Thank you all.

ABSTRACT

Listeria and *Salmonella* related recalls and outbreaks are of major concern to the melon industry. Cinnamon oil has shown its usefulness in food treatment due to strong antifungal, antiviral, and antibacterial activities. However, its applications are limited due to poor solubility of cinnamon oil in water. Utilization of Cinnamon oil nanoemulsion may offer effective antimicrobial washing treatment to melon industry. The purpose of this study was to test the antimicrobial efficacy of cinnamon oil nanoemulsion on melons against major food borne pathogens such as *Listeria monocytogenes* and *Salmonella enterica*. Different formulations of cinnamon oil nanoemulsion were made by ultrasonication using Tween 80 as an emulsifier. Nanoemulsion exhibiting the smallest oil droplets was applied. Oil droplets were characterized for particle size by dynamic light scattering. Microbroth dilution assay was performed on three strains each of *Listeria monocytogenes* and *Salmonella enterica* to find out the antimicrobial efficacy of cinnamon oil nanoemulsion. Honeydew and cantaloupe were artificially inoculated with the strains mentioned above followed by treatment in nanoemulsion (control, 0.1%, 0.25%, and 0.5%) for one minute. Samples were dried and enumerated after one hour of treatment on selective media (PALCAM and XLD agar). The average diameter of nanoemulsion was 9.63±0.3nm. Minimum inhibitory concentration (MIC) of cinnamon oil nanoemulsion for both *Listeria* and *Salmonella* strains was 0.078% v/v and 0.039% v/v, respectively and the minimum bactericidal concentration was 0.078125% v/v for both. Compared to the water control, 0.5% nanoemulsion showed up to 7.7 and 5.5 log CFU/gm reductions in *L. monocytogenes* and *S. enterica*, respectively. The data suggests that cinnamon oil nanoemulsion can be used as an effective natural microbial control agent for melons.

Keywords: Nanoemulsion, ultrasonication, antimicrobial

1 INTRODUCTION

1.1 Introduction and Statement of the Problem

The safety of melons has been critical to the food industry in the United States. In recent years' melons, such as cantaloupe, honeydew, and water melons have been infected by dangerous pathogens. Such outbreaks have been increasing. Recent estimates indicate more than 81 million instances of food borne illnesses in the United States annually, which costs around $152 billion dollars per year out of which 46% were due to contaminated produce (J. A. Painter, 2013). In comparison to outbreaks originating from other sources one of the fastest growing 'trends' in foodborne illness is produce-related outbreaks (M. Lynch, R. Tauxe, & C. Hedberg, 2009).

Melons belong to the family *Cucurbitaceae* which have sweet edible, fleshy fruit. The most important varieties of melons are watermelons, cantaloupe, and honey dew. They had a total value of production in 2013 at $393.5 million. Cantaloupe production was at $319 million and honeydew production was at $74.5 million which in combination made the third highest ranked vegetable and melon crop behind lettuce and onions in the United States (Boriss, Brunke, & Kreith, 2006; USDA-NASS, 2014). Melons can be eaten alone; however, they are often combined into fruit and vegetable salads. Despite the manner in which they are prepared, melons are commonly consumed raw without a processing step which would eliminate pathogenic bacteria. The safety of melons has been a critical food safety issue on the United States as of recent. They may be contaminated with pathogens during harvest, shipping, or preparation for consumption. One of the main cause can be melons being in contact with soil during their

production which is a major source of potential contamination in melon varieties (Richards & Beuchat, 2005).

Essential oils (EOs) are natural compounds that have been shown to be promising treatment for food application because of their potent antifungal, antiviral, and antibacterial activities (Burt, 2004; Ferreira et al., 2010; Giatrakou, Ntzimani, & Savvaidis, 2010). Cinnamon is a spice obtained from the inner bark of the tree species of the genus *Cinnamomum*. Cinnamon oil contains two important compounds, cinnamaldehyde and eugenol, which are good inhibitors of microbial growth (Burt, 2004; Lee & Ahn, 1998; Ooi et al., 2006). Also, the cinnamon oil contains broad spectrum antimicrobial effect which makes it ideal for utilization in various produce products. These properties provide an alternative natural and safe antimicrobial to standard antimicrobial products.

Essential oils dissolve the cytoplasmic membrane of bacterial cells in the hydrophobic domain which explains their antimicrobial properties(Ghosh, Mukherjee, & Chandrasekaran, 2014). There are many studies that have been done on the properties of essential oils as antimicrobial agent to treat against pathogens. Studies showed that essential oils have antimicrobial properties or they were able to halt *Bacillus cereus* (Ghosh et al., 2014), *Zygosaccharomyces bailli* (Chang, McLandsborough, & McClements, 2012), and *Listeria monocytogenes*, and *Staphylococcus aureus* (Liang et al., 2012). There were positive results from the use of carvacrol and cinnamaldehyde in kiwifruit and melon which showed that the flora of the product was significantly reduced after the application of essential oils (Roller & Seedhar, 2002). One recent study by Bhargava, Conti, da Rocha, and Zhang (2015) studied the effect of oregano oil nanoemulsion to disinfect lettuce and found the application of oregano oil nanoemulsion to be an effective antimicrobial agent.

The application of cinnamon oil is limited due to its lipophilic behavior and insolubility in water as is any essential oil (Donsì, Annunziata, Vincensi, & Ferrari, 2012). Due to this fact the application of cinnamon oil emulsion as an antimicrobial agent is limited due to high minimum inhibitory concentration (MIC) and lack of solubility in water. One of the strategies in dealing with such hydrophobic compounds is by dispersing them in nanoemulsion delivery system (Shah, Davidson, & Zhong, 2012). In this study, we focus on the utilization of cinnamon oil as a nanoemulsion through the novel process of ultrasonication in cinnamon oil and study the minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC), and the efficacy of the prepared cinnamon oil nanoemulsion against *Listeria* and *Salmonella* artificially inoculated in melons.

1.2 Hypothesis and Objectives of the Study

1.2.1 Hypothesis

Cinnamon oil nanoemulsion (CONE) possesses antimicrobial properties and will decontaminate melons from common foodborne pathogens such as *Listeria monocytogenes*, *Salmonella spp*.

1.2.2 Objectives and Specific Aims

The main objective of this study is to utilize cinnamon oil nanoemulsion against pathogenic bacteria such as *Listeria* and *Salmonella* which are of major concern in melons. This study also aims to do the following things:

1. Design, Fabricate & Characterize Cinnamon Oil Nanoemulsions.
2. Demonstrate Practical Utility of Cinnamon Oil Nanoemulsions on Melons.

2 REVIEW OF THE LITERATURE

2.1 Melons

The Webster's Dictionary describes melons as 'large round fruit of various plants of the gourd family, with sweet pulpy flesh and many seeds (honeydew, cantaloupe, muskmelon).' They can be consumed raw, cooked and used in many types of other foods. Human have consumed melons for more than 4,000 years. Melons presumed to be originated in southwest Asia and brought to the Americas by early settlers in the 1600s (Barash, 2005). The oldest records or mention of melons can be seen in the Egyptian mural paintings (Stepansky, Kovalski, & Perl-Treves, 1999). They are one of the most important horticulture crops in the world (Stepansky et al., 1999). Melons are an important food in the United States and is one of the leading consumers of melons in the world. The total value of melon production in 2013 was at $393.5 million. The cantaloupe production was at $319 million. Honeydew production was at $74.5 million. The total cantaloupe and honeydew production value was the third highest ranked vegetable and melon crop behind lettuce and onions in the United States (Boriss, Brunke, & Kreith, 2006; USDA-NASS, 2014).

Honeydew melon is the food of cultivar group of muskmelons also known as *Cucumis melo*. It is either round or oval. The honeydew has sweet aromatic flavor and is grown for the fruit (Nunez-Palenius et al., 2008). They are also one of the most cultivated crops on the world and are abundant in provitamin A and vitamin C (Laur & Tian, 2011; Nunez-Palenius et al., 2008).

Cantaloupe is also a variety of the *Cucumus melo*. It has a net-like skin covering and is round, somewhat orange and has a thin grey rind (Ensminger & Ensminger, 1993). It is normally

eaten as fresh fruit or salads. It should be washed properly and consumed in less than three days to prevent risk of pathogens particularly *Salmonella (Mun*noch et al., 2009).

Melons contain β-carotene and C, and other nutrients such as vitamin E and folic acid which are strong antioxidants and are important in human metabolic reactions (Li, Yao, Yang, & Li, 2006).

Composition	**Cantaloupe**	**Honeydew**
Overall composition		
Water (g)	89.78	89.66
Minerals (g)	0.36	0.34
Proteins (g)	0.88	0.46
Total lipid (g)	0.28	0.1
Carbohydrate (g)	8.36	9.18
Fiber, total dietary (g)	0.8	0.6
Ash (g)	0.71	0.6
Vitamins		
Vitamin A (IU)	3224	40
Vitamin C (mg)	42.2	24.8
Thiamin (mg)	0.036	0.077
Riboflavin (mg)	0.021	0.018
Niacin (mg)	0.574	0.6
Pantothenic acid (mg)	0.128	0.207
Vitamin B6 (mg)	0.115	0.059
Vitamin E (tocopherol<comma> alpha) (mg)	0.14	0.14

Table 1. Nutritional compositions of cantaloupe and honeydew melons (value per 100 g of edible portion). USDA Nutrient Database, July 2001 as cited in (Li et al., 2006)

2.2 Overview of food borne illness in the US

Fruits and vegetables are recognized as an important source of nutrients, fibers, and vitamins to human. The production of fruits and vegetables increased by 94% between 1994-2004 in the world and the import of fresh fruits and vegetables was doubled from 1994-2004 (Olaimat & Holley, 2012). Due to the increased consumption, there has been concern over the safety of fresh produce due to the outbreaks and illnesses caused or related to fresh fruits and vegetables. Fruits and vegetables have been a growing source of outbreaks in recent history. There has been a of increasing reported outbreaks in the United States, Australia, Europe and the rest of the world (M. F. Lynch, R. V. Tauxe, & C. W. Hedberg, 2009). According to Rangel, Sparling, Crowe, Griffin, and Swerdlow (2005) outbreaks from produce related sources were reported in 1991 for the first time and would usually peak in the summer and fall months. They also mentioned that the occurrence of produce related outbreaks were most common in restaurants.

From 1990-2005 13 % of outbreaks and 21% of illness were associated with produce (DEWAAL & BHUIYA, 2007). Other outbreaks were associated with ground beef where *E. coli* was the main culprit, other meat products such as roast beef, steak, sirloin tips and salami, and dairy products such as raw milk, cheese curds, butter and some commercial ice-cream bars (Rangel et al., 2005). The increased rate of outbreaks may be due to improvement in surveillance. It may also be due to the increase in consumption, distribution systems and increase in consumer habits, increased intensity of livestock production near produce production area, greater availibilty of produce, and increased numbers of immune-compromised consumers (Larry R Beuchat, 2002; Harris et al., 2003; Warriner, Huber, Namvar, Fan, & Dunfield, 2009). Between 1998-2008 46% of outbreaks were linked to or were a direct consequence of produce

related outbreaks out of more than 9 million estimated food borne illnesses each year in the United states between (J. A. Painter, R. M. Hoekstra, T. Ayers, R. V. Tauxe, C. R. Braden, F. J. Angulo, and P. M. Griffin, 2013). Painter et al. (2013) also attributed contamination of produce to 38% hospitalizations and 23% of the deaths associated to food borne outbreaks between 1998-2008. The food borne pathogens usually associated with produce are *Cyclospora cayetanesis*, *Camphylobactor*, Coliforms, *Enterococcus, Escheria coli* 0157:H7, Hepatitis A, *Listeria monocytogenes*, *Norovirus*, *Salmonella* spp., and *Shihella* spp (L. Beuchat, 1998; L.R. Beuchat, 1996; De Roever, 1998; Ebel et al., 2016; FDA, 2008; Newman et al., 2017; Taormina, Beuchat, & Slutsker, 1999). FDA has also reported that there was higher number of reported incidence of *Salmonella*, *Shigella* in imported melons (FDA, 2001).

2.2.1 Melon Contamination

Of the outbreaks reported in 1991, 11% of them were related to melons (Rangel et al., 2005). Melons specially, cantaloupe and honeydew which are popular, and grown and consumed all over the world have more chances of causing food borne outbreaks. According to CDC data, US melon industry has observed major recalls due to *Listeria monocytogenes* (Cantaloupes and melons, Burch Farms, 2012; Whole Cantaloupes, Jensen Farms, 2011) and *Salmonella Typhimurium* and *Salmonella* Newport (Multistate outbreak, 2012). Richards and Beuchat (2005) mentioned that since during production. Melons may be in direct contact with the soil, which is a potential source of contamination, even if preventive measures such as plastic much is used. Also, there may be contamination during harvesting, packaging, shipping, and/or preparation for consumption. Mechanical damage during these processes cause puncture, cracks and bruising which may be a point of pathogen contamination (Fleming, Pool, & Gorny, 2005; Richards & Beuchat, 2005). The surface of melons can also be a cause for concern for the protection of

melons from pathogenic bacteria. Other things such as maturity of the melons can also play a vital role on the presence of pathogenic bacteria on melons and their surfaces. Ripe melons may have better conditions for pathogens to grow and survive on melon surfaces (Suslow, 2013). Ukuku and Fett (2002) noted that the surface of melon, especially cantaloupe which has a netted surface, can be a supportive environment for pathogens to grow and even more strenuous to remove those pathogens.

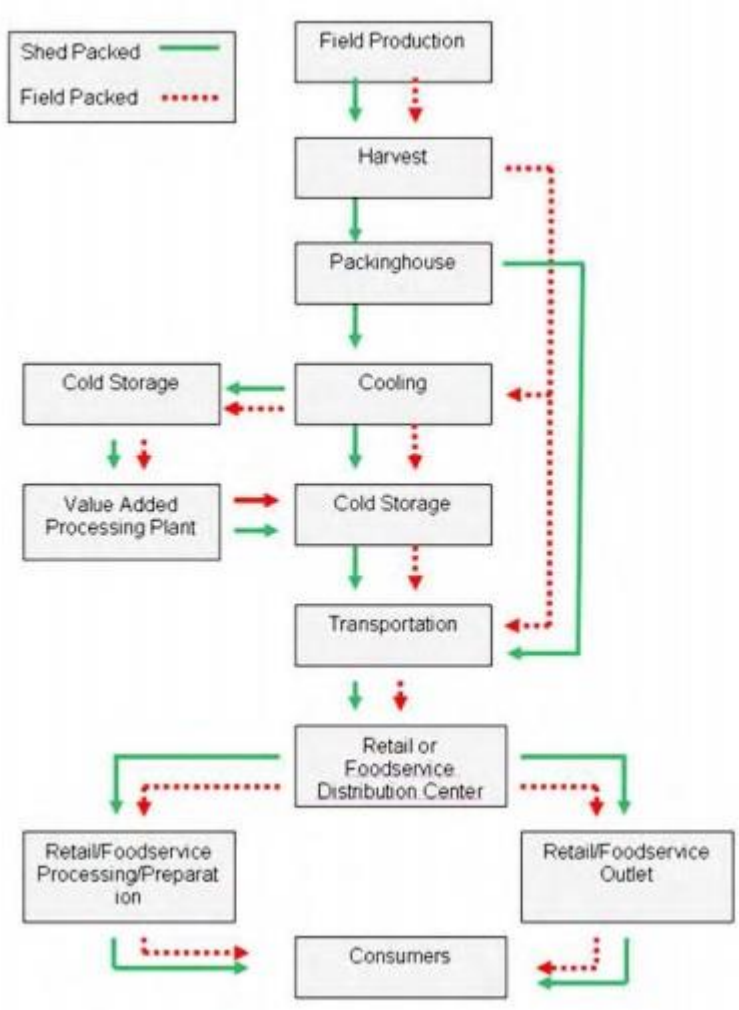

Figure 1: General Supply chain flow for melons (Fleming et al., 2005).

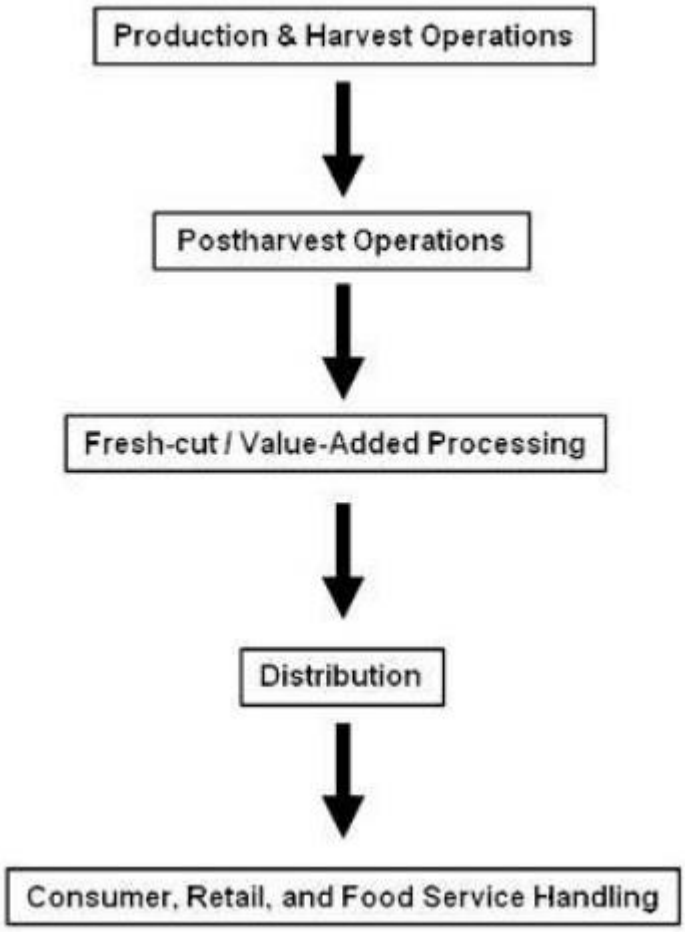

Figure 2: Melon unit operations (Fleming et al., 2005).

Since the surface of honeydew and cantaloupe melons are different in structure, they attract a wide variety of bacterial pathogens which could potentially be transformed into a major outbreak. Fresh produce such as tomatoes, cabbage, and melons have been prone to infection with pathogens such as *Salmonella*, *Escherichia coli* O157:H7, *Shigella*, and *Listeria monocytogenes* according to the FDA. *Listeria* (McCollum et al., 2013), and *Salmonella* (Castillo et al., 2004; EFSA, 2014) have been associated with melon outbreaks in recent times and have been a great cause of concern for the food industry as a whole in recent times.

2.2.2 Salmonella

Salmonella are facultative anaerobic Gram negative, rod shaped bacteria. They can be found in the intestines of warm and cold-blooded animals. They are generally 2-5 microns long and 0.5-1.5 microns wide. They move with the help of a peritrichous flagella and belong to the family Enterobacteriaceae and are very important pathogenic organisms medically to both animals and humans (Andino & Hanning, 2015; Farrar et al., 2013; Sorensen et al., 2002; Wells, Fedorka-Cray, Dargatz, Ferris, & Green, 2001). They consist of two species and six subspecies. The two species are *S. enterica* and *S. bongori* and and the subspecies are *enterica, aruzonae, diarizonae, houtenae,* and *indica* and consists of more than 2,579 serovars or serotypes which are all capable of causing human diseases (Andino & Hanning, 2015; Yaun, 2002).

According to the Center for Disease Control and Prevention (CDC) (2016) *Salmonella* causes an estimated one million foodborne illnesses in the United States. There are 19,000 hospitalizations and 380 deaths related to *Salmonella*, however there are more suspected mild cases that may not be reported so the actual number of contamination or illness may be way higher than reported. Symptoms of *Salmonella* infection may be diarrhea, fever, and abdominal cramps 12 to 72 hours after the infection. It may last between 4 to 7 days and usually most people recover without treatment but some individuals might have severe diarrhea and may require hospitalization (CDC, 2016). Long term effect of *Salmonella* infection may cause reactive arthritis, and painful bowel movements and urine passage according to the CDC. Salmonellosis is seen more in the summer than the winter and children are more likely to get infected than adults. Treatment is usually related to relieving the symptoms and the antibiotics are not generally used. This is because *Salmonella* has been known to be resistant to antibiotics. The *Salmonella* Typhimurium DT104, which emerged in the 80's and 90's in the United States,

has been known to be resistant to ampicillin, chloramphenicol, streptomycin, sulfisoxazole, and tetracycline (CDC, 2001; Wedel et al., 2005).

Salmonella is generally associated with dairy, meat, and poultry but there have been cases of recent outbreaks in produce as well. *Salmonella* has been known to survive in a large variety of produce such as melons. There has been contamination of *Salmonella* in 1989 and in 1991 and others attributed to watermelons (Hanning, Nutt, & Ricke, 2009). Recent outbreaks related to melons happened in 2012 related to *Salmonella* Typhimurium and *Salmonella* Newport according to the CDC. Other outbreaks associated were in Alfalfa sprouts in 2016 which were contaminated with *Salmonella* Reading, *Salmonella* Abony, *Salmonella* München, and *Salmonella* Kentucky and there were outbreaks related to cucumbers in 2014 and 2015 related to *Salmonella* Newport and *Salmonella* Poona respectively (CDC, 2016).

Year	Genus Species	Serotype or Genotype	Illnesses	Hospitalizations	Deaths	Food Vehicle
2010	*Salmonella enterica*	Saintpaul	17	11	0	watermelon
2011	*Salmonella enterica*	Panama	20	3	0	cantaloupe
2011	*Salmonella enterica*	Uganda	25	4	0	cantaloupe
2011	*Salmonella enterica*	Typhimurium	6	0		cantaloupe & strawberry mix
2011	*Salmonella enterica*	Typhimurium	15	2	0	watermelon
2012	*Salmonella enterica*	Newport	24	5	0	cantaloupe
2012	*Salmonella enterica; Salmonella enterica*	Typhimurium; Newport	261	94	3	cantaloupe
2012	*Salmonella enterica*	Newport	33	11	1	cantaloupe
2012	*Salmonella enterica*	Typhimurium	14	1	0	cantaloupe
2013	*Salmonella enterica; Salmonella enterica*	Typhimurium; Typhimurium	14	3	0	cantaloupe
2013	*Salmonella enterica*		7	1	0	watermelon
2014	*Salmonella enterica*	Baildon	20	6	0	cantaloupe

Table 2: Recent Outbreaks related to *Salmonella* (CDC, 2016)

2.2.3 Listeria

Listeria is a Gram positive, non-spore forming facultative anaerobic rod-shaped bacteria. It is closely related *to Bacillus, Clostridium, Enterococcus, Streptococcus, and Staphylococcus*. They have a growth temperature range from -0.4 to 50 ºC and is the causative agent of listeriosis which is highly fatal foodborne illness (Farber & Peterkin, 1991; Junttila, Niemelä, & Hirn, 1988; Vázquez-Boland et al., 2001; Walker, 1987). The genus Listeria currently includes six species: *L. monocytogenes, L. ivanovii, L. seeligeri, L. innocua, L. welshimeri, and L. grayi* but only *L. monocytogenes* and *L. ivanovii* which are known to be pathogenic and could potentially cause listeriosis *(Vá*zquez-Boland et al., 2001). They can be found in a variety of sources such as soil, water, foods and human and animal waste.

More than 1,600 people get serious infection related to *listeria* each year and approximately 206 deaths associated with listeriosis and are usually caused by the consumption of food contaminated with the bacteria *Listeria monocytogenes* (Center for Disease Control and Prevention, 2016). There may be various symptoms pertaining to listeriosis depending on the person and the part of the body infected. The symptoms can include headache, stiff neck, confusion, loss of balance, fever and muscle ache but pregnant women may experience only fever and other flu-like symptoms but may result in miscarriage, stillbirth, premature delivery, or infection of the newborn which may be life threatening (CDC, 2016). Older adults and pregnant women are more likely to get *Listeria* infection. But in most of the cases according to Farber and Peterkin (1991) most cases of listeriosis can be found in or are at more risk for in people who have an underlying medical conditions such as cancer, diabetes, liver or kidney disease, alcoholism, and HIV or AIDS (CDC, 2016) which will cause suppression in their T-cell count and immunity power. It is usually diagnosed when a bacterial culture is grown and confirmed as *Listeria monocytogenes*.

The sample may be taken from body tissue or fluid, such as blood, spinal fluid, or the placenta (CDC, 2016). Listerosis can be generally treated with the help of antibiotics.

The foodborne *Listeria* outbreaks have been in different variety of food products such as turkey meat (Olsen et al., 2005), in milk (Dalton et al., 1997), in corn (Aureli et al., 2000), cheese (CDC, 2013), bean sprouts, frozen vegetables, apples, cantaloupe (CDC, 2016).

Year	Genus Species	Illnesses	Hospitalizations	Deaths	Food Vehicle
2008	Listeria monocytogenes	20	16	0	sprouts
2011	Listeria monocytogenes	147	143	33	cantaloupe
2014	Listeria monocytogenes	5	3	2	mung bean sprouts
2014	Listeria monocytogenes	2	2	0	sprouts

Table 3: Recent outbreaks related to *Listeria* (CDC, 2016)

2.3 Antimicrobial strategy currently utilized

Usually most produce is minimally processed and are usually consumed raw without any cooking or heat treatment. Therefore, there is always a risk of pathogenic contamination. There is a huge chance of microbial contamination in every or any step from production to the handling at home (Olaimat & Holley, 2012). To combat the said problem FDA (1997) introduced a guide which helps to reduce microbial hazards for fresh fruits and vegetables. Use of chlorine can be a useful technique to reduce the pathogenic load or contamination (Parnell, Harris, & Suslow, 2005; Warriner et al., 2009). Although chlorine has often been used for its convenience and cost (as hypochlorite ranging from 50-200ppm concentration) there is evidence to demonstrate that chlorine has limitations due to its loss of impact in the presence of organic matter, particularly

pertaining to leafy greens. This is because of the fact that washing of produce with chlorine or other solutions usually doesn't reduce the attached pathogens (Gandhi, Golding, Yaron, & Matthews, 2001; Kondo, Murata, & Isshiki, 2006) which may be due to the fact that the efficacy of such solutions are affected by the internalization of pathogens with plant tissue and biofilm formation by the pathogen (Whipps, Hand, Pink, & Bending, 2008). In recent years, new sanitizers have been used/considered for managing produce wash water including: peroxyacetic acid, ozone, organic acids, hydrogen peroxide, and electrolyzed water. Previous research using these sanitizers has given variable results ranging from a 1.4 – 6.6 log reduction depending on produce type, treatment method, and treatment concentration (Joshi, Mahendran, Alagusundaram, Norton, & Tiwari, 2013). Other methods for the control of pathogens in produce used are irradiation (Gomes et al., 2009), (Selma, Beltrán, Allende, Chacón-Vera, & Gil, 2007), antagonistic bacteria (Cooley, Chao, & Mandrell, 2006), and bacteriophages (Kocharunchitt, Ross, & McNeil, 2009).

One of the most recent development is the use of essential oil nanoemulsions. Essential oils are natural compounds which have strong antifungal, antiviral, and antibacterial properties (Burt, 2004; Ferreira et al., 2010; Giatrakou et al., 2010). Essential oils contain photo-chemicals, such as 1,8-cineole, carvacrol, eugenol, cinnamaldehyde, carvone, citral, estragole, geraniol, perillaldehyde, terpineol, thymol, and vanillin which can extend shelf life of processed food products by preventing lipid oxidation and antimicrobial properties (Burt, 2004; Singh, Maurya, & Catalan, 2007; Wang, Wang, & Yang, 2009). Previously done research has shown that essential oils were able to inhibit *Bacillus cereus* (Ghosh et al., 2014), *Listeria monocytogenes* (Bhargava et al., 2015; Liang et al., 2012), *Zygosaccharomyces baili* (Chang et al., 2012), and *Staphylococcus aureus* (Liang et al., 2012). Bhargava et al. (2015) demonstrated one use of

essential oil nanoemulsion (oregano oil) on fresh lettuce and found that it had up to 3.44-3.57, 2.31-3.26, and 3.05-3.35 log CFU/gm reductions for *L. monocytogenes, S. Typhimurium, and E. coli* O157:H7, respectively for different concentration of oregano oil nanoemulsion. Another study evaluated the use of carvacrol and cinnamaldehyde in kiwifruit and melon by dipping the food products in solution. The study showed that the natural flora of the product was reduced significantly after the essential oil application (Roller & Seedhar, 2002).

2.3.1 Nanoemulsion technology

There is a great need for new and innovative food preservative techniques. The processes such as pulse electric field processing (PEF), plasma processing and, food irradiation have shown some degree of success but have several limitations such as high cost, reduction of nutritive value and also may cause non-desirable reactions (Fu, Sarkar, Bhunia, & Yao, 2016). One of the strategies to combat this is the use of natural essential oils and successful application of this may reduce or nullify the limitations. But even though essential oils are universally accepted to have antimicrobial properties, their utilization on food have been hindered as result of their lipophilic behavior and insolubility in water (Donsì et al., 2012). Because of their limited water solubility, the undissolved essential oils applied at a concentration above the solubility affects their antimicrobial efficacy which is because of uneven dispersion and tendency to bind with fats and proteins (Shah et al., 2012). Therefore, to solve this very problem the dispersion of such hydrophobic compounds in a nano-dispersion delivery system (Shah et al., 2012) has been studied. For nanoemulsion systems, oil droplets can be kinetically stabilized in the continuous aqueous phase by utilizing appropriate surfactants.

A majority of the studies evaluate their efficacy in broth or agar by dissolving them in ethanol or Dimethyl sulfoxide (DMSO) and there are limited efforts to address this issue

(Gutiérrez-Larraínzar et al., 2012; Nostro et al., 2004).There have been recent interest on this nanoemulsion technology and more research has been going on to try to find out the efficacy of the nanoemulsion based delivery system. There have been some studies that have applied essential oils in different food systems and showing more promise for nanoemulsion technology.

Antimicrobial	Target Microorganism	Food System	Reference
Oregano oil	*Listeria monocytogenes*, *Salmonella Typhimurium*, *E. coli* O157:H7	Lettuce	Bhargava et al. (2015)
Carvacrol	*Salmonella enteric Enteritidis* *E. coli*	Broccoli and Radish seed	Landry, Micheli, McClements, and McLandsborough (2015)
Carvacrol	*Salmonella enteric Enteritidis* *E. coli*	Mung bean and Alfalfa seeds	Landry, Chang, McClements, and McLandsborough (2014)
Mandarin oil	*Listeria innocua*	Green beans	Severino et al. (2014)
Mandarin oil	*Listeria innocua*	Green beans	Donsì et al. (2015)
Lemongrass oil	*Salmonella Typhimurium* *E. coli*	Plums	Kim et al. (2013)
Cinnamaldehyde	*Lactobacillus delbrueckii* *Saccharomyces cerevisiae* *Escherichia coli*	Apple and Pear Juice	Donsì et al. (2012)
Eugenol	*Escherichia coli* O157:H7 *Listeria monocytogenes*	Fruit Juice	Ghosh et al. (2014)

Table 4: Food model research studies on delivery system for natural antimicrobials (Amaral & Bhargava, 2015).

Shah et al. (2012) demonstrated that the nano dispersed thymol showed promising results against *E. coli* and *Listeria monocytogenes*. Bhargava et al. (2015) utilized oregano oil nanoemulsion against food borne pathogens, *Listeria monocytogenes, Salmonella Typhimurium and Escherichia coli* O157:H7, and showed that the nanoemulsion distrupted bacterial membranes on fresh lettuce. Ultrasonic nanoemulsified basil oil (*Ocimum basilicum)* was found to show antimicrobial activity against *E. coli* (Vijayalakshmi Ghosh, Amitava Mukherjee, & Natarajan

Chandrasekaran, 2013) and a similar action was shown by *Thymus daenesis* nanoemusion (Moghimi, Ghaderi, Rafati, Aliahmadi, & McClements, 2016).

2.4 Cinnamon

Cinnamon is a spice obtained from the bark of the tree from the species *Cinnamomum* but nowadays the species is referred to as cassia (Santich, Toussaint-Samat, & Bell, 2009). Few species of *Cinnamomun* are grown commercially for spice. It has been known and used from ancient times and has been known to be brought to Egypt as early as 2000 BCE (Santich et al., 2009) but the species *Cinnamomun* is indigenous to Srilanka and India (Paranagama et al., 2010). Every part of the cinnamon tree including bark, leaves, flowers, fruits and roots can be used in some way (Ranasinghe et al., 2013). Cinnamon contains different hydrocarbons, the main constituents being cinnamaldehyde, eugenol and camphor (Gruenwald, Freder, & Armbruester, 2010).

Cinnamon oil is mainly derived from the leaf or the bark. The bark contains a higher amount of cinnamaldehyde and the leaf contains eugenol (Gruenwald et al., 2010). Cinnamon oil is an essential oil. Plant essential oils have been used to preserve food, alternative medicine and pharmaceutical therapies (Jones, 1996; Ranasinghe et al., 2013), and many of them have antimicrobial properties against a range of bacteria (Bassyouni et al., 2016). Like other plant derived oils, cinnamon oil has been shown to have antibacterial, antiviral, antifungal, and insecticidal properties. The main reason for these properties is thought to be cinnamaldehyde (Shan, Cai, Brooks, & Corke, 2007). Cinnamaldehyde is thought to cause inhibition of the proton motive force, respiratory chain, electron transfer, and substrate oxidation. (Nuryastuti et al., 2009). The result of these inhibitions causes uncoupling of oxidative phosphorylation, inhibition

of active transport, loss of pool metabolites, and disruption of DNA, RNA, protein, lipid, and polysaccharide synthesis (Denyer, 1995; Farag, Daw, Hewedi, & El-Baroty, 1989; Nychas, 1995).

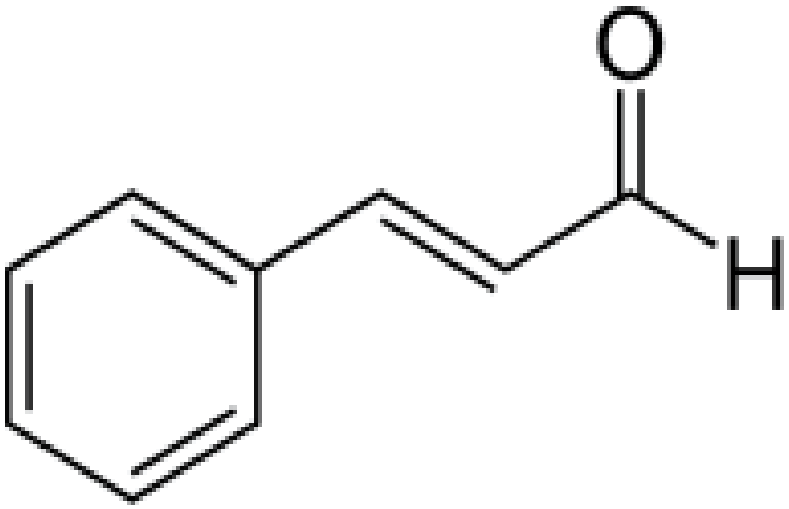

Figure 3: Structure of cinnamaldehyde (Inuzuka, 1961)

After cinnamaldehyde, eugenol is one other important compound found in cinnamon. It is a colorless to pale yellow phenolic compound found in essential oils (Mallavarapu et al., 1995; Pavithra, 1981). Eugenol has been shown to be effective against *Sitophilus zeamais* and *Tribolium castaneum* in a research conducted by Huang, Ho, Lee, and Yap (2002). It possesses antioxidant properties (Gordon, 1996), anti-inflammatory action (Wargovich, Woods, Hollis, & Zander, 2001) but is also known to have antibacterial properties against *Bacillus subtilis* (ATCC 6633), *Escherichia coli* (ATCC 10536), and *Saccharomyces cerevisiae* (ATCC 9763) according to a research done by De, Krishna De, and Banerjee (1999).

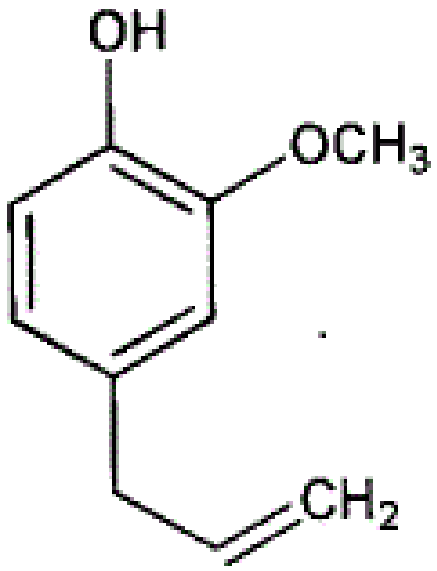

Figure 4: Structure of eugenol (Ito, Murakami, & Yoshino, 2005)

3 METHODOLOGY

3.1 Materials

The experiments were carried out in the laboratory of the Department of Human Environmental Science and the laboratory of the Department of Biology, the University of Central Oklahoma, Edmond, OK. The microbial experiments were done in the Biosafety Level 2 (BSL2) hood in the microbiology lab at the Department of Biology, the University of Central Oklahoma, Edmond, OK. The particle size of the nanoemulsion was measured by the process of dynamic light scattering technology by DynaPro Plate Reader II, Wyatt Technology provided by Stanton Young Biomedical Research Center, University of Oklahoma Health Sciences Center, Oklahoma City, OK. Honeydew and Cantaloupe melons were purchased from the local Walmart. The cinnamon oil was purchased from FisherChemical, Inc. The materials used in this experiment were Thermo Scientific™ Oxoid™ PALCAM Agar Base (FisherChemical, Inc.), BD Difco™ Dehydrated Culture Media: Xylose Lysine Deoxycholate (XLD) Agar (FisherChemical, Inc.), TWEEN®-80 (Sigma-Aldrich, Inc.), pH/ORP Meter (HI 9125, HANNA instruments Co., Ltd), ultra sonicator (QSONICA, Q700, Hudson Fusion LLC.), magnet stirrer (MS-H280-Pro, Scilogex, LLC.), PBS, distilled water, Thermo Scientific™ alamarBlue™ Dye (FisherChemical, Inc.), 96-well plate. Pure bacterial cultures of *Salmonella entrica* (strain- S11975), *Salmonella entrica subspecies enterica serovar newport* (strain- E2002001708), *Salmonella choleraesuis subsp. choleraesuis, Listeria monocytogenes* (strain- FSL N1-017), *Listeria monocytogenes* (strain- FSL J2-064) and *Listeria monocytogenes* (strain- FSL N3-165) were provided by Dr. Hari Kotturi, Department of Biology, the University of Central Oklahoma, Edmond, OK.

3.2 Bacterial strains used

Table 5: Bacterial Strains

Bacteria	Strain	Letter Designation
Salmonella enterica	S11975	SalI
Salmonella enterica subs. enterica	E2002001708	SalII
Salmonella enterica subs. choleraesius	Lot- 3807133	SalIII
Listeria monocytogenes	FSL N1-017	LMI
Listeria monocytogenes	FSL J2-064	LMII
Listeria monocytogenes	FSL N3-165	LMIII

Three different strains of *Salmonella* and three different strains of *Listeria* were used with the name of the strains and their letter designation for the experiment listed in the table above. The end results are the averages of the effect of cinnamon oil nanoemulsion against all the strains listed above.

3.3 Preparation of nanoemulsion

5% cinnamon oil was crudely mixed with a magnetic stir plate for 30 minutes at a constant speed (700 rpm). 46.25 ml of sterile DI water was measured using a pipette, 1.25 ml of Tween 80 was pipetted and 2.5 ml of cinnamon oil was mixed and stirred. Ultra sonicator (QSONICA, Q700, Hudson Fusion LLC.) was used to mix the nanoemulsion through sonification. The probe depth was maintained at $3/4^{th}$ of an inch (the probe is 0.5 inch in diameter). The amplitude was set to 60. If there was splashing or foaming at this amplitude, the amplitude was lowered and the probe depth was checked. The watts used was in range from 50-70. The emulsion pulse was: start for 5 seconds and stop for 3 seconds. The experiment was done in a water bath to keep the solution from heating.

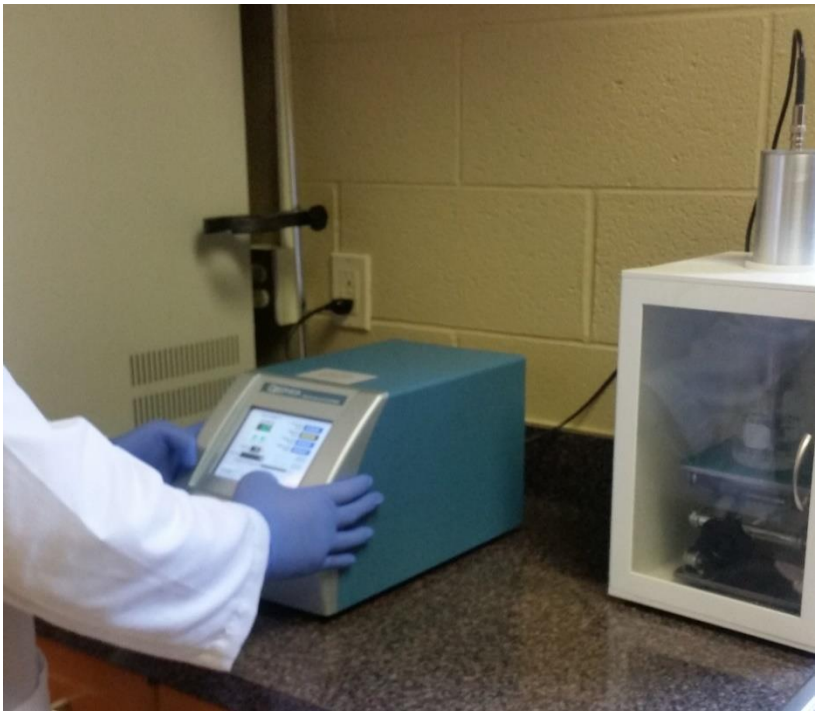

Figure 5: Ultra sonicator (QSONICA, Q700, Hfffudson Fusion LLC.)

Different formulation of nanoemulsion was made with variation in sonication time and the percentage of Tween 80. 2.5% and 5% Tween 80 concenration was used for every 5% of cinnamon oil with the ultrasonication time of 10 min and 20 min. Controls were also made without the use of cinnamon oil in the same concentration as former.

3.4 Emulsion Characterization

Particle size of emulsions was measured by using dynamic light scattering (DynaPro Plate Reader II, Wyatt Technology). The smallest particle size formulation was used for melon experiments.

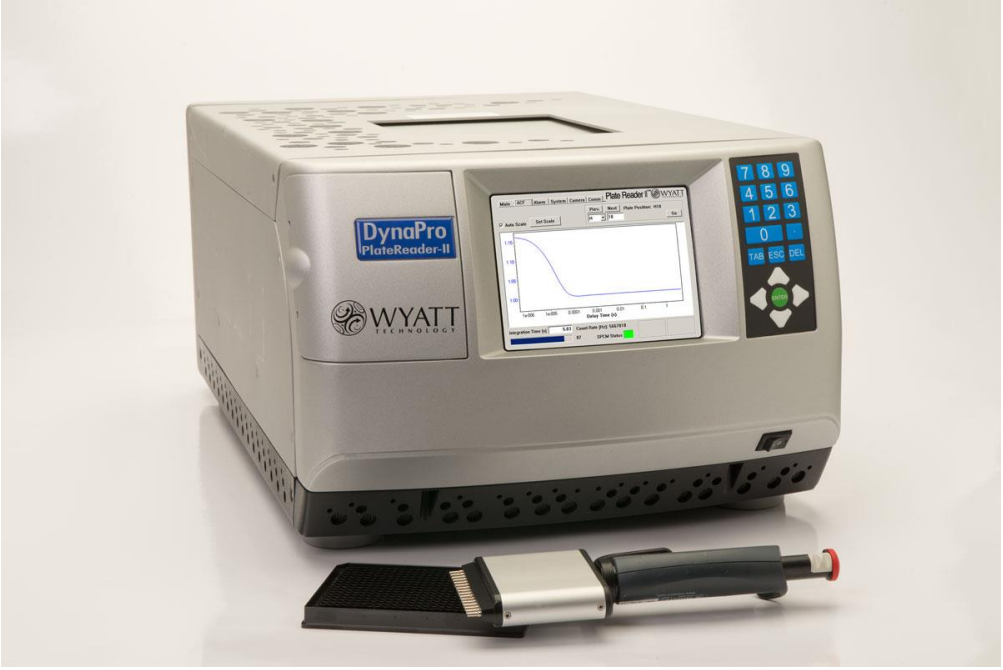

Figure 6: Dynamic Light Scattering (DynaPro Plate Reader II, Wyatt Technology)

3.5 Antimicrobial Susceptibility Testing

3.5.1 Broth Micro-Dilution Method

Minimum Bactericidal Concentration (MBC) and Minimum Inhibitory Concentration (MIC) values were determined by broth micro-dilution method as per National Committee for Clinical Laboratory Standards (NCCLS) guidelines for both the *Salmonella* and *Listeria* spp. Prepared stock solutions of individual essential oils (50µl/L) were serially diluted in 96-well plate and fifty microliters of the inoculum (0.5 McFarland) were added to wells to obtain final concentrations of 2.5%, 1.25%, 0.625%, 0.3125%, 0.15625%, 0.078125%, 0.0390625%, 0.01953125%, 0.009765625%, 0.004882813%, 0.002441406%, and 0.001220703% v/v. Negative controls without the respective organism and tested oils were included to detect any cross contamination from one well to the other during handling of plates. Plates were incubated

at 37ºC for 24 hrs and streaked on PALCAM and XLD agar for growth. Experiments were performed in triplicate.

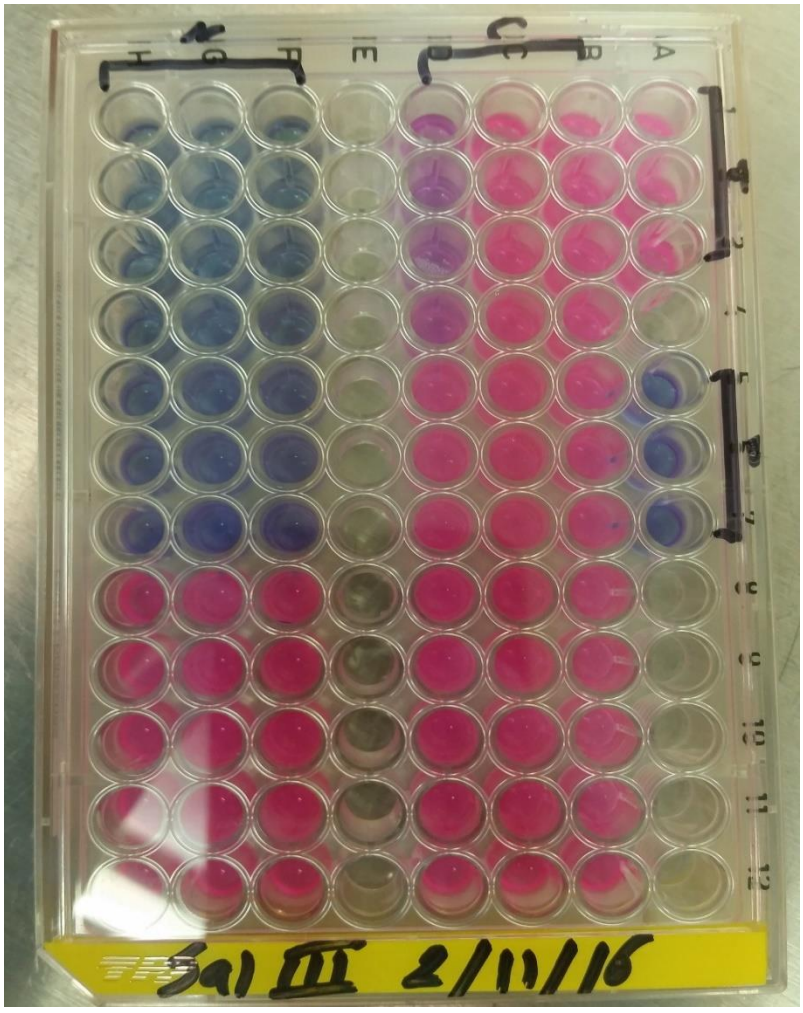

Figure 7: MIC on 96-well plate

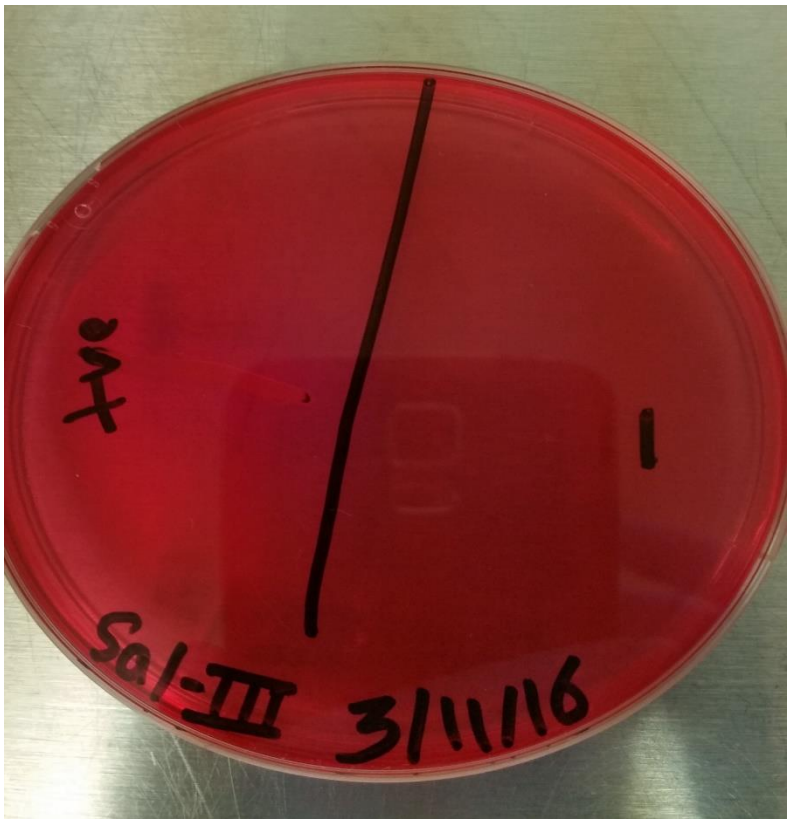

Figure 8: MBC reading after MIC count

3.6 Preparation of nanoemulsion to different cinnamon concentration

The smallest particle size formulation was then diluted to make three different cinnamon oil concentration of 0.1%, 0.25% and 0.5%. Sterile DI water was used to dilute the nanoemulsion formulations.

3.7 Practical Utility of Cinnamon Oil Nanoemulsions on Melons

The potency of cinnamon oil nanoemulsion was tested against pathogens (*Listeria monocytogenes* and *Salmonella* sp.) on melons. A mixture (cocktail) of bacterial strains (*Listeria monocytogenes* and *Salmonella* sp.) was used to assure that the antimicrobial effectiveness is against both Gram positive and Gram negative representative foodborne pathogens.

Honeydew and cantaloupe melons were first marked in a 1x1 cm space with a permanent marker. The marked area of the fruits were removed from fruit with a sterile scalpel. Then the marked spaces were inoculated by spotting several locations within the melons with a 5µl of each pathogen cocktail. The spots were left to dry for 15 mins. After spots have dried, the melons were submerged in antimicrobial emulsions and negative control buffers for 1 minutes for different concentration of cinnamon oil nanoemulsion (0.1%, 0.25% and 0.5%). Again, the melon cuts were dried for half hour. The dried melon cuts were then incubated and plate counts were done at 24 hrs, 48 hrs, 72 hrs and 7days. The surviving cell numbers were determined by plating on selective agars for *Listeria monocytogenes* and *Salmonella* sp. Decrease in overall bacterial load was also determined.

Figure 9: Preparation of melon before spotting with bacterial cocktail

3.8 Microbial counts

The bacterial counts were processed in triplicate immediately after culture inoculation or after 24 hrs., 48 hrs., 72hrs storage. Every procedure was carried out in sterile conditions. 5gm of melon cuts were macerated in 45 ml of 1% PBS in a stomacher (400 Circulator, Seward) for 5 minutes. Samples were then diluted and plated into PALCAM and XLD agars for the enumeration of *Listeria spp.* and *Salmonella* species respectively. All the experiments were performed in triplicate.

3.9 Statistical Analyses

All tests were conducted in triplicate. An analysis of variance (ANOVA) was performed using general linear model procedure to identify significant differences ($p<0.05$) among the samples, followed by Tukey's test. All statistical analyses were carried out using SPSS (SPSS 20.0, IBM Crop, Armonk, NY).

4 Results and Discussion

4.1 Nanoemulsion formulation

Table 6: Radius and polydispersity of different nanoemulsion formulations based on percentage of tween and ultrasonication time

Formulation	Radius nm	% Polydispersity
2.5%T 10min S control	1279.16±196.3	347.07±29.1
2.5%T 10min S nanoemulsion	9.63±0.3	10.43±0.8
2.5%T 20min S control	33.57±2.3	60.20±9.5
2.5%T 20min S nanoemulsion	10.60±0.4	10.87±0.8
5% T 10 min S control	1729.23±201.8	300.27±12.4
5% T 10 min S nanoemulsion	38.23±3.8	8.27±0.9
5% T 20 min S control	62.27±3.4	35.03±5.0
5% T 20 min S nanoemulsion	9.30±2.5	25.73±5.0

Legend: T= tween 80, S= ultrasonication, nm= nanometer

All samples contain 5% cinnamon oil

The nanoemulsion was made by the method of ultrasonic emulsification. It is a high energy method to develop nanoemulsion (Vijayalakshmi Ghosh, Amitava Mukherjee, & N Chandrasekaran, 2013). Emulsion created by using ultrasonication has smaller dispersed water droplets, larger emulsion stability and small droplet size (Lin & Chen, 2008). The particle size or the radius and the polydispersity date were calculated using dynamic light scattering method. Polydispersity deals with the size distribution or homogeneity of the particle. The smaller the value the more homogenous the emulsion. Here we decided that the formulation with 2.5% tween 80, 5% cinnamon oil with an ultrasonication time of 10 mins. It has a radius of 9.6±0.3 nm and a polydispersity percentage of 10.4±0.8%. The standard deviations for radius and polydispersity of the selected sample was calculated as 0.5 and 1.4 respectively.

Polydispersity index or the heterogeneity ratio is the ratio of molecular weight averages and used as a measure of molecular weight distributions (MWD) (Rogošić, Mencer, & Gomzi, 1996). Therefore a polydispersity index near to 1 or 100% implies that there is a heterogenous distribution between the oil droplet size of a nanoemulsion (Salvia-Trujillo, Rojas-Graü, Soliva-Fortuny, & Martín-Belloso, 2013). Also polydispersity gives us the extent consistency and cohesion of the droplet size in the emulsion (Sugumar, Ghosh, Nirmala, Mukherjee, & Chandrasekaran, 2014). Our polydispersity index was 10.4±0.8% or 0.104±0.008 which is a pretty good number to describe it being as homogeneous as according to Flores et al. (2011) the polydispersity index below 0.25 indicates adequate homogeneity. A similar study done while formulating essential oil based nanoemulsion of basil oil yielded a droplet size of 30 nm and a polydispersity index of 0.234. Similarly in another experiment done in a blended cloves/cinnamon essential oil nanoemulsion, the investigators reported a polydispersity index in the range of 0.22-0.29 (Zhang, Zhang, Fang, & Liu, 2017). Our droplet size and polydispersity index of the nanoemulsion used were lower than these values.

4.2 Minimum inhibitory concentration (MIC) and Minimum bactericidal concentration

The MICs and MBCs for cinnamon oil nanoemulsion against all six pathogenic strains are given below.

Table 7: Broth microdilution assay

Strain	MIC (v/v)	MBC (v/v)
SalI	.039%	.078%
SalII	.039%	.078%
SalIII	.039%	.078%
LMI	.078%	.078%
LMII	.078%	.078%
LMIII	.078%	.078%

The average MIC of cinnamon oil nanoemulsion for *Salmonella* and *Listeria* strains were 0.039% v/v and 0.785% v/v respectively and the average MBCs for *Salmonella* and *Listeria* strains were 0.78% for both the bacteria. On a similar study done by Bhargava et al. (2015) the MIC value of oregano oil nanoemulsion against *L. monocytogenes*, *S. Typhimurium,* and *E. coli* was 0.0625% which is greater than what we got for cinnamon oil nanoemulsion against *Salmonella* but less than what we got for *Listeria*. Similarly, Bhargava et al. (2015) reported the MBC values for *L. monocytogenes* and *S. Typhimurium* at 0.625 % which is higher than what we have for both for *Salmonella* and *Listeria*, but reported a lower MBC of 0.125% for *E. Coli* at 0.125% which is lower than ours. Both of our studies confirm than tween 80 only had no effect on bacterial growth.

It is known that Gram-positive bacteria are more affected by essential oil or are more susceptible to harm by essential oils than Gram-negative bacteria. Gram-positive bacteria do not have lipopolysaccharide (LPS) protection from hydrophobic compounds as discussed by M. Hyldgaard, T. Mygind, and R. L. Meyer (2012). Due to the process of emulsification through sonication the cinnamon oil nanoemulsion has reduced hydrophobic property and will increase the antibacterial effect on Gram-negative bacteria. Cinnamaldehyde and eugenol are the major

compounds found in cinnamon oil. Cinnamaldehyde inhibits ATPase and perturbs cell membrane as well as inhibit cytokinesis (Morten Hyldgaard, Tina Mygind, & Rikke Louise Meyer, 2012; Kwon, Yu, & Park, 2003). Eugenol permeabilizes the cell membrane interacting with proteins (Morten Hyldgaard et al., 2012) by altering the cell membrane which results in the release of cellular content (Bennis, Chami, Chami, Bouchikhi, & Remmal, 2004). Gill and Holley (2004) suggested that eugenol was stronger than cinnamaldehyde against *Listeria monocytogenes*. This would explain our good MIC data of cinnamon oil nanoemulsion against both Gram-positive and Gram-negative bacteria. Our MIC against Gram-negative bacteria were lower than against Gram-positive bacteria. Bhargava et al. (2015) observed an equal MIC for both Gram-positive and Gram-negative bacteria. Cinnamaldehyde was found to be more potent against *Salmonella typh*imurium in a study done by (Helander et al., 1998) and it appeared to be less potent than eugenol against *L. monocytogenes* (Gill & Holley, 2004). Cinnamon oil is known to contain higher amounts of cinnamaldehyde than eugenol. This may explain lower MIC for Gram-negative bacteria in this study

4.3 Effect of Cinnamon oil nanoemulsion on Salmonella and Listeria on cantaloupe and honeydew melon

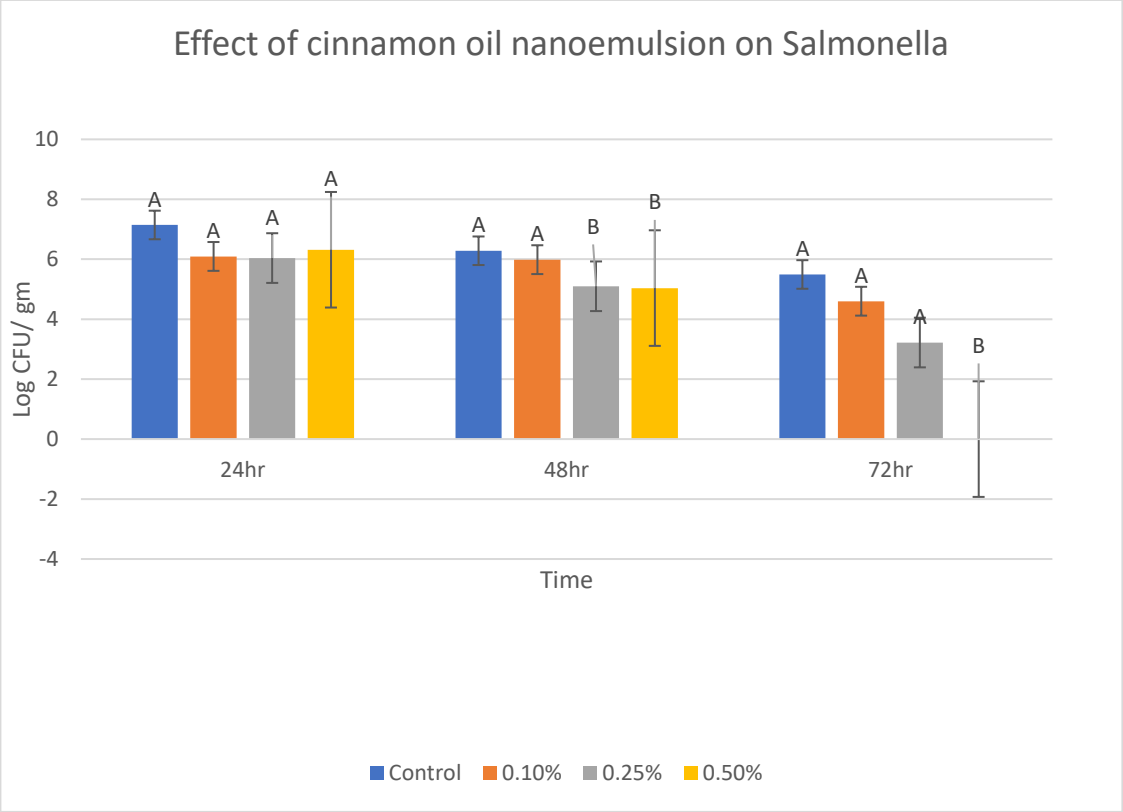

Figure 10: *Salmonella* colony count from cinnamon oil nanoemulsion treated cantaloupe melon at different time intervals with different formulations

Control= the mixture of Tween 80 and water only with ultrasonication; 0.10%= 0.10% of cinnamon oil nanoemulsion; 0.25%= 0.25% of cinnamon oil nanoemulsion; 0.50%= 0.50% of cinnamon oil nanoemulsion. Readings were taken at 24hrs, 48hrs and 72hrs.
Means capped by the same letter (ABC) in the same column are not significantly different between each concentration of cinnamon oil nanoemulsion on the same colony count hour, according to Tukey test ($p<0.05$).

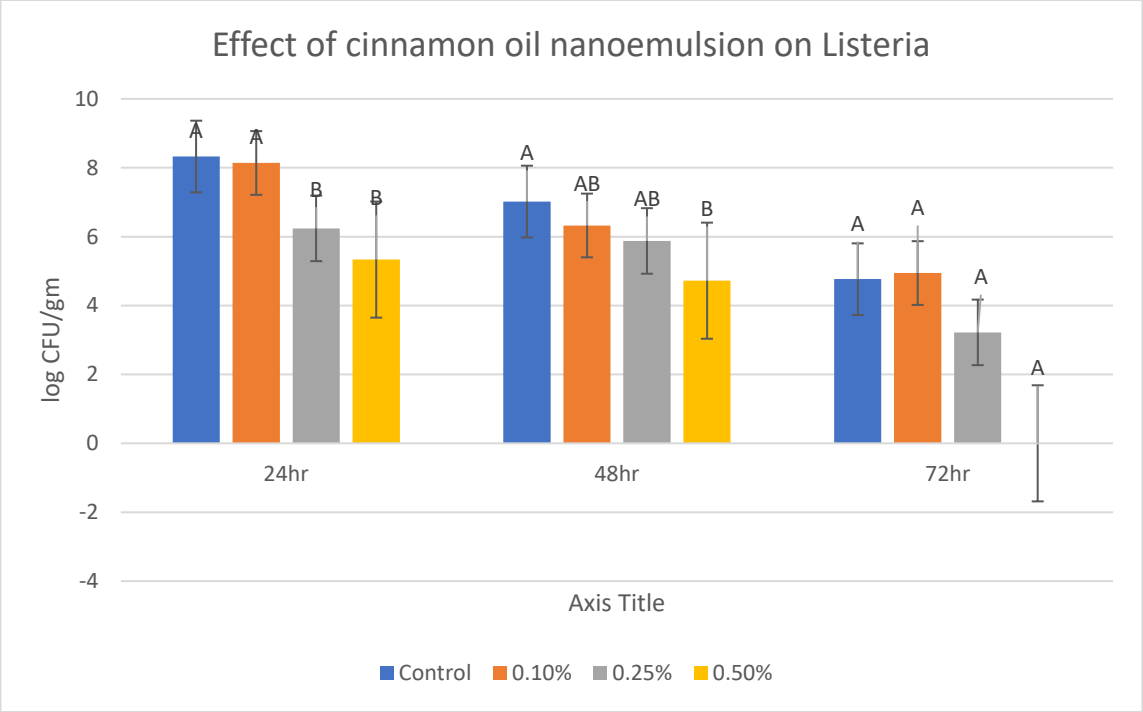

Figure 11: Listeria colony count from cinnamon oil nanoemulsion treated cantaloupe melon at different time intervals with different formulations.

Control= the mixture of Tween 80 and water only with ultrasonication; 0.10%= 0.10% of cinnamon oil nanoemulsion; 0.25%= 0.25% of cinnamon oil nanoemulsion; 0.50%= 0.50% of cinnamon oil nanoemulsion. Readings were taken at 24hrs, 48hrs and 72hrs.
Means capped by the same letter (ABC) in the same column are not significantly different between each concentration of cinnamon oil nanoemulsion on the same colony count hour, according to Tukey test ($p<0.05$).

The initial concentration of *Salmonella* and *Listeria* on cantaloupe melons was 5.6 and 7.8 log CFU/gm, respectively for cantaloupe. Both the bacteria were sensitive to treatment on cantaloupe but a higher microbial load reduction was observed for *Listeria*. Microbial populations were calculated at three different time intervals (24hrs, 48hrs, and 72hrs) after treatment (Fig. 10 and Fig. 11). All three concentrations of cinnamon oil nanoemulsion were able to inhibit microbial growth. The treatments 0.25% and 0.50% at 48hrs showed significant difference ($p<0.05$) compared to control for *Salmonella.* The treatment 0.50% at 48h exhibited significant difference ($p<0.05$) compared to control in *Listeria.* However, there was no significant difference ($p>0.05$) between all three treatments and controls at 72hrs. There was a

final log reduction of 5.5 log reduction for *Salmonella* and 7.7 log reduction for *Listeria* by 78hrs in cantaloupe for 0.05% treatment. After 48h there was a log reduction of 1.15, 2.04, and 2.10 log CFU/gm for 0.10%, 0.25%, and 0.50% treatments respectively for *Salmonella.* Similarly, after 48hrs there was a log reduction of 2.01, 2.46, and 3.61 for 0.10%, 0.25%, and 0.50% treatments respectively for *Listeria.*

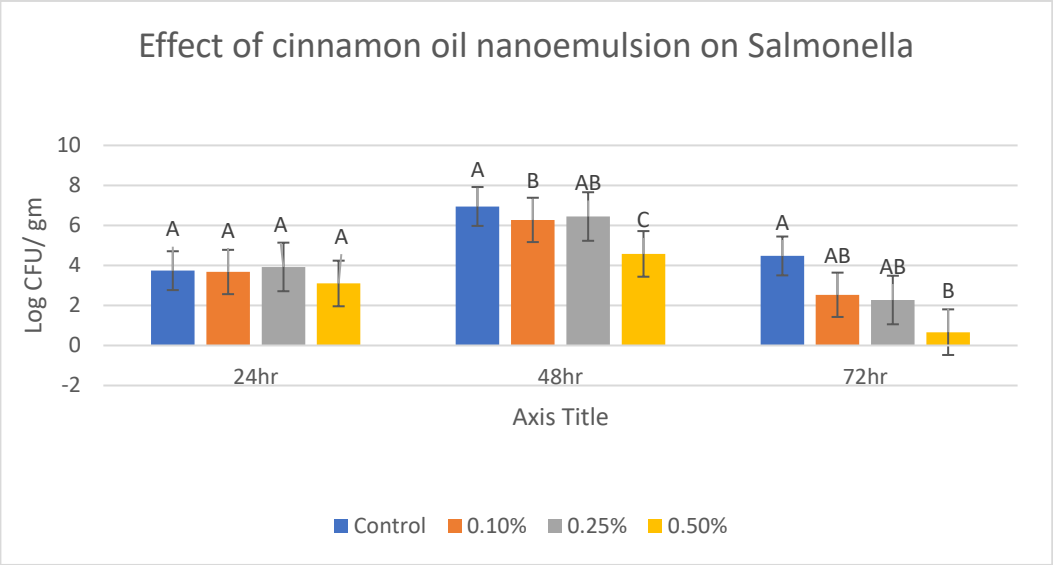

Figure 12: Salmonella colony count from cinnamon oil nanoemulsion treated honeydew melon at different time intervals with different formulations.

Control= the mixture of Tween 80 and water only with ultrasonication; 0.10%= 0.10% of cinnamon oil nanoemulsion; 0.25%= 0.25% of cinnamon oil nanoemulsion; 0.50%= 0.50% of cinnamon oil nanoemulsion. Readings were taken at 24hrs, 48hrs and 72hrs.
Means capped by the same letter (ABC) in the same column are not significantly different between each concentration of cinnamon oil nanoemulsion on the same colony count hour, according to Tukey test ($p<0.05$).

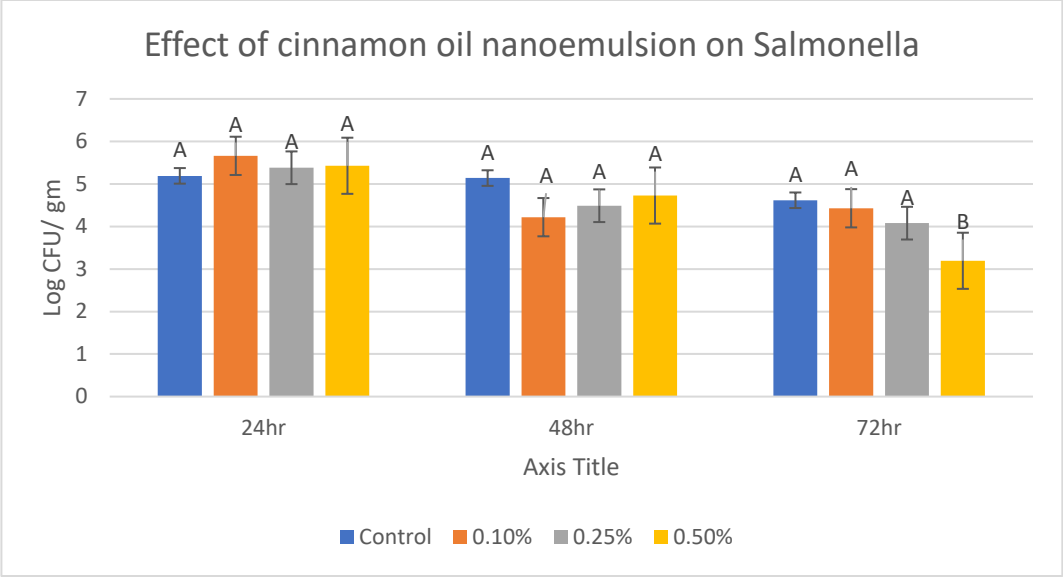

Figure 13: Listeria colony count from cinnamon oil nanoemulsion treated honeydew melon at different time intervals with different formulations.

Control= the mixture of Tween 80 and water only with ultrasonication; 0.10%= 0.10% of cinnamon oil nanoemulsion; 0.25%= 0.25% of cinnamon oil nanoemulsion; 0.50%= 0.50% of cinnamon oil nanoemulsion. Readings were taken at 24hrs, 48hrs and 72hrs.
Means capped by the same letter (ABC) in the same column are not significantly different between each concentration of cinnamon oil nanoemulsion on the same colony count hour, according to Tukey test ($p<0.05$).

The initial concentration of *Salmonella* and *Listeria* on honeydew melons was 5.5 and 5.6 log CFU/gm, respectively for honeydew. Both the bacteria were sensitive to treatment on honeydew. Microbial populations were calculated at three different testing points (24hrs, 48hrs, and 72hrs) after treatment (Fig. 12 and Fig. 13). All three concentrations of cinnamon oil nanoemulsion were able to inhibit microbial growth in honeydew. There was significance difference ($p<0.05$) between 0.10% and 0.50% formulation with control at 42hrs but no significance difference ($p>0.05$) between 0.25% and control at the same time. There was only significant difference between the control and 0.50% formulation at 72hrs. The final log reductions for *Salmonella* and *Listeria* after treatment at 72hrs were 3.5 log reduction and 3 log reductions respectively in honeydew melons.

Our results show a gradual decrease in both the bacterial samples in the control over time but there was increase in *Salmonella* count at 48hrs. The increase in the bacterial count may be due to the fruit pulp providing essential energy for the bacterium to survive, hence, increase the bacterial load. The gradual decrease in the rest of them may be because the number of viable counts decreased as the number of colonies treated decreased over time (Davis, Joseph, & Janssen, 2005). Bhargava et al. (2015) also observed a slight decrease in microbial load with time. This explains why there is no significant difference ($p>0.05$) between the most treatments and control except for 0.05% treatment for honeydew melons at 72hrs.

Studies on oregano oil nanoemulsion have reported up to 3.57 log reductions (Bhargava et al., 2015). Our study ranges form 3.5-7.7 log reduction. This may entertain the possibility of cinnamon oil having stronger antimicrobial activity. In a research done on antimicrobial activity of cinnamon oil, allspice, and clove bud oils in apple puree edible films against *E. coli* O157:H7, *S. enterica*, and *L. monocytogenes*, the antimicrobial effect of cinnamon oil was found to be compellingly outstanding than allspice and clove bud oil (Du et al., 2009). There was higher log reduction value in cantaloupe than honeydew melons. This may be due to the surface of melons affecting the initial bacterial growth as noted by Ukuku and Fett (2002). They further suggested that cantaloupe having a netted surface, have more supportive environment for pathogen growth. This explains the higher initial bacterial load and higher log reduction in cantaloupe compared to honeydew.

Similar study on nanoemulsion of clove/cinnamon mixture reported antimicrobial activity against bacterial strains of *E. coli, B. subtilis, S. typhimurium*, and *S. aureus* even though 4% of the essential oil was used to make the nanoemulsion (Zhang et al., 2017). Our concentration used was very low (0.10%, 0.25%, and 0.50%) compared to this experiment.

5 Conclusion and Future Directions

This study optimized the formulation of nanoemulsion by using different time and emulsifier combinations. We used radius size and polydispersity index as the basis for the optimal nanoemulsion. Based on this research, we concluded that the nanoemulsion preparation procedure which created the smallest radius size and the lowest polydispersity index was the best formulation process which will be our default procedure for preparing cinnamon oil nanoemulsion. We were able to achieve a radius size of 9.63±0.3 nm and a polydispersity index of 10.43±0.8 % which is our best radius and polydispersity index.

We tested the antimicrobial properties of our cinnamon oil nanoemulsion against 3 strains of *Salmonella* and 3 strains of *Listeria.* Our results showed an average MIC value of 0.39% and 0.78% v/v for *Salmonella* and *Listeria* respectively. Our MBC values were the same for both which was 0.78% v/v. These are the lowest concentrations of nanoemulsion that were effective.

We had prepared four different formulation of cinnamon oil nanoemulsion to test against pathogens on melons which were control, 0.10%, 0.25%, and 0.50%. 0.50% cinnamon oil nanoemulsion showed significant reduction on the log CFU/gm count in both honeydew melons and cantaloupe melons against both the pathogenic bacteria. 0.50% had a reduction of 3.5-5.5 log CFU/gm for *Salmonella* and a reduction of 3-7.7 log CFU/gm for *Listeria* sp. Both 0.10% and 0.25% were effective in reducing the bacterial load but 0.50% concentration was the most effective.

To conclude we effectively optimized a process of producing cinnamon oil nanoemulsion with a small radius and low polydispersity index. The cinnamon oil nanoemulsion was able to significantly reduce different strains of *Salmonella sp.* and *Listeria sp.* on honeydew and

cantaloupe melons. The initial bacterial load was reduced by cinnamon oil emulsions and maintained during treatment confirming that the application of antimicrobial emulsion of cinnamon oil. It is a simple and effective preservation method for melons.

Future studies on the process or the mechanism by which the main compounds in cinnamon oil, cinnamaldehyde and eugenol, act against pathogens should be studied to realize the full potential of cinnamon oil nanoemulsion. The potency of cinnamon oil nanoemulsion on a range of different pathogenic bacterium can be an important study to further the knowledge on this area. Studies against potential risk produce types such as sprouts and tomatoes can be done to expand the horizon of impact of cinnamon oil nanoemulsion. Further sensory and texture study should be done to confirm no harm to the produce is done by the nanoemulsion.

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Table 8: Tukey test for Salmonella in cantaloupe

Tukey HSD							
Dependent Variable	(I) Formulation	(J) Formulation	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
LogCFUa	0.1%	0.25%	.1901	.75993	.994	-2.2435	2.6237
		0.5%	.2593	.75993	.985	-2.1742	2.6929
		Control	-.3744	.75993	.959	-2.8080	2.0591
	0.25%	0.1%	-.1901	.75993	.994	-2.6237	2.2435
		0.5%	.0692	.75993	1.000	-2.3643	2.5028
		Control	-.5646	.75993	.877	-2.9981	1.8690
	0.5%	0.1%	-.2593	.75993	.985	-2.6929	2.1742
		0.25%	-.0692	.75993	1.000	-2.5028	2.3643
		Control	-.6338	.75993	.837	-3.0674	1.7998
	Control	0.1%	.3744	.75993	.959	-2.0591	2.8080
		0.25%	.5646	.75993	.877	-1.8690	2.9981
		0.5%	.6338	.75993	.837	-1.7998	3.0674
LogCFUb	0.1%	0.25%	.8563*	.19255	.009	.2397	1.4729
		0.5%	.9950*	.19255	.004	.3784	1.6116
		Control	-.3218	.19255	.396	-.9384	.2948
	0.25%	0.1%	-.8563*	.19255	.009	-1.4729	-.2397
		0.5%	.1387	.19255	.886	-.4779	.7553
		Control	-1.1781*	.19255	.001	-1.7947	-.5615
	0.5%	0.1%	-.9950*	.19255	.004	-1.6116	-.3784
		0.25%	-.1387	.19255	.886	-.7553	.4779
		Control	-1.3168*	.19255	.001	-1.9334	-.7002
	Control	0.1%	.3218	.19255	.396	-.2948	.9384
		0.25%	1.1781*	.19255	.001	.5615	1.7947
		0.5%	1.3168*	.19255	.001	.7002	1.9334
LogCFUc	0.1%	0.25%	1.9501	1.48404	.580	-2.8023	6.7025
		0.5%	3.1831	1.48404	.218	-1.5693	7.9355
		Control	-1.5946	1.48404	.714	-6.3470	3.1578
	0.25%	0.1%	-1.9501	1.48404	.580	-6.7025	2.8023
		0.5%	1.2330	1.48404	.839	-3.5194	5.9854
		Control	-3.5447	1.48404	.157	-8.2971	1.2077
	0.5%	0.1%	-3.1831	1.48404	.218	-7.9355	1.5693

		0.25%	-1.2330	1.48404	.839	-5.9854	3.5194
		Control	-4.7777*	1.48404	.049	-9.5301	-.0253
	Control	0.1%	1.5946	1.48404	.714	-3.1578	6.3470
		0.25%	3.5447	1.48404	.157	-1.2077	8.2971
		0.5%	4.7777*	1.48404	.049	.0253	9.5301
Based on observed means. The error term is Mean Square(Error) = 3.304.							
*. The mean difference is significant at the .05 level. LogCFUa, LogCFUb, LogCFUc are the plate count readings at 24hrs, 48hrs and 72hrs respectively for *Salmonella*.							

Table 9: Tukey test for Listeria in cantaloupe

Tukey HSD							
Dependent Variable	(I) Formulation	(J) Formulation	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
LogCFUa	0.1%	0.25%	1.7715	.58327	.063	-.0963	3.6394
		0.5%	2.3937*	.58327	.014	.5259	4.2616
		Control	-.1339	.58327	.995	-2.0018	1.7339
	0.25%	0.1%	-1.7715	.58327	.063	-3.6394	.0963
		0.5%	.6222	.58327	.718	-1.2456	2.4901
		Control	-1.9054*	.58327	.046	-3.7733	-.0376
	0.5%	0.1%	-2.3937*	.58327	.014	-4.2616	-.5259
		0.25%	-.6222	.58327	.718	-2.4901	1.2456
		Control	-2.5277*	.58327	.011	-4.3955	-.6598
	Control	0.1%	.1339	.58327	.995	-1.7339	2.0018
		0.25%	1.9054*	.58327	.046	.0376	3.7733
		0.5%	2.5277*	.58327	.011	.6598	4.3955
LogCFUb	0.1%	0.25%	.8413	1.21021	.896	-3.0342	4.7168
		0.5%	3.0475	1.21021	.131	-.8280	6.9230
		Control	-.5098	1.21021	.973	-4.3854	3.3657
	0.25%	0.1%	-.8413	1.21021	.896	-4.7168	3.0342
		0.5%	2.2062	1.21021	.330	-1.6693	6.0817
		Control	-1.3511	1.21021	.690	-5.2267	2.5244
	0.5%	0.1%	-3.0475	1.21021	.131	-6.9230	.8280
		0.25%	-2.2062	1.21021	.330	-6.0817	1.6693
		Control	-3.5573	1.21021	.072	-7.4328	.3182
	Control	0.1%	.5098	1.21021	.973	-3.3657	4.3854

		0.25%	1.3511	1.21021	.690	-2.5244	5.2267
		0.5%	3.5573	1.21021	.072	-.3182	7.4328
LogCFUc	0.1%	0.25%	2.1793	1.84961	.656	-3.7438	8.1024
		0.5%	3.4123	1.84961	.321	-2.5108	9.3354
		Control	.4358	1.84961	.995	-5.4873	6.3589
	0.25%	0.1%	-2.1793	1.84961	.656	-8.1024	3.7438
		0.5%	1.2330	1.84961	.907	-4.6901	7.1561
		Control	-1.7435	1.84961	.784	-7.6666	4.1796
	0.5%	0.1%	-3.4123	1.84961	.321	-9.3354	2.5108
		0.25%	-1.2330	1.84961	.907	-7.1561	4.6901
		Control	-2.9765	1.84961	.425	-8.8996	2.9466
	Control	0.1%	-.4358	1.84961	.995	-6.3589	5.4873
		0.25%	1.7435	1.84961	.784	-4.1796	7.6666
		0.5%	2.9765	1.84961	.425	-2.9466	8.8996

Based on observed means.

The error term is Mean Square(Error) = 5.132.

*. The mean difference is significant at the .05 level.

LogCFUa, LogCFUb, LogCFUc are the plate count readings at 24hrs, 48hrs and 72hrs respectively for *Listeria.*

Table 10: Tukey test for Salmonella in honeydew

Tukey HSD

Dependent Variable	(I) Formulation	(J) Formulation	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
LogCFUa	0.1%	0.25%	-.2523	2.57160	1.000	-8.4875	7.9829
		0.5%	.5734	2.57160	.996	-7.6618	8.8086
		Control	-.0654	2.57160	1.000	-8.3006	8.1697
	0.25%	0.1%	.2523	2.57160	1.000	-7.9829	8.4875
		0.5%	.8257	2.57160	.988	-7.4095	9.0609
		Control	.1869	2.57160	1.000	-8.0483	8.4221
	0.5%	0.1%	-.5734	2.57160	.996	-8.8086	7.6618
		0.25%	-.8257	2.57160	.988	-9.0609	7.4095
		Control	-.6388	2.57160	.994	-8.8740	7.5964
	Control	0.1%	.0654	2.57160	1.000	-8.1697	8.3006
		0.25%	-.1869	2.57160	1.000	-8.4221	8.0483
		0.5%	.6388	2.57160	.994	-7.5964	8.8740
LogCFUb	0.1%	0.25%	-.1707	.19923	.826	-.8087	.4673

		0.5%	1.6984*	.19923	.000	1.0604	2.3364
		Control	-.6725*	.19923	.039	-1.3105	-.0345
	0.25%	0.1%	.1707	.19923	.826	-.4673	.8087
		0.5%	1.8691*	.19923	.000	1.2311	2.5071
		Control	-.5018	.19923	.131	-1.1398	.1362
	0.5%	0.1%	-1.6984*	.19923	.000	-2.3364	-1.0604
		0.25%	-1.8691*	.19923	.000	-2.5071	-1.2311
		Control	-2.3709*	.19923	.000	-3.0089	-1.7329
	Control	0.1%	.6725*	.19923	.039	.0345	1.3105
		0.25%	.5018	.19923	.131	-.1362	1.1398
		0.5%	2.3709*	.19923	.000	1.7329	3.0089
LogCFUc	0.1%	0.25%	.2608	1.32074	.997	-3.9686	4.4903
		0.5%	1.8674	1.32074	.525	-2.3621	6.0968
		Control	-1.9409	1.32074	.496	-6.1704	2.2885
	0.25%	0.1%	-.2608	1.32074	.997	-4.4903	3.9686
		0.5%	1.6065	1.32074	.634	-2.6230	5.8360
		Control	-2.2018	1.32074	.398	-6.4313	2.0277
	0.5%	0.1%	-1.8674	1.32074	.525	-6.0968	2.3621
		0.25%	-1.6065	1.32074	.634	-5.8360	2.6230
		Control	-3.8083	1.32074	.078	-8.0378	.4212
	Control	0.1%	1.9409	1.32074	.496	-2.2885	6.1704
		0.25%	2.2018	1.32074	.398	-2.0277	6.4313
		0.5%	3.8083	1.32074	.078	-.4212	8.0378

Based on observed means.

The error term is Mean Square(Error) = 2.617.

*. The mean difference is significant at the .05 level.

LogCFUa, LogCFUb, LogCFUc are the plate count readings at 24hrs, 48hrs and 72hrs respectively for *Salmonella.*

Table 11: Tukey test for Listeria in cantaloupe

Tukey HSD

Dependent Variable	(I) Formulation	(J) Formulation	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
LogCFUa	0.1%	0.25%	.2801	.51333	.945	-1.3638	1.9239
		0.5%	.2332	.51333	.967	-1.4107	1.8771
		Control	.4707	.51333	.797	-1.1732	2.1145
	0.25%	0.1%	-.2801	.51333	.945	-1.9239	1.3638
		0.5%	-.0469	.51333	1.000	-1.6907	1.5970

		Control	.1906	.51333	.981	-1.4533	1.8345
	0.5%	0.1%	-.2332	.51333	.967	-1.8771	1.4107
		0.25%	.0469	.51333	1.000	-1.5970	1.6907
		Control	.2375	.51333	.965	-1.4064	1.8813
	Control	0.1%	-.4707	.51333	.797	-2.1145	1.1732
		0.25%	-.1906	.51333	.981	-1.8345	1.4533
		0.5%	-.2375	.51333	.965	-1.8813	1.4064
LogCFUb	0.1%	0.25%	-.2696	.41461	.913	-1.5973	1.0581
		0.5%	-.5088	.41461	.628	-1.8366	.8189
		Control	-.9202	.41461	.198	-2.2479	.4076
	0.25%	0.1%	.2696	.41461	.913	-1.0581	1.5973
		0.5%	-.2392	.41461	.936	-1.5669	1.0885
		Control	-.6505	.41461	.445	-1.9783	.6772
	0.5%	0.1%	.5088	.41461	.628	-.8189	1.8366
		0.25%	.2392	.41461	.936	-1.0885	1.5669
		Control	-.4113	.41461	.758	-1.7390	.9164
	Control	0.1%	.9202	.41461	.198	-.4076	2.2479
		0.25%	.6505	.41461	.445	-.6772	1.9783
		0.5%	.4113	.41461	.758	-.9164	1.7390
LogCFUc	0.1%	0.25%	.3500	.32199	.707	-.6811	1.3812
		0.5%	1.2350*	.32199	.021	.2038	2.2661
		Control	-.1885	.32199	.934	-1.2197	.8426
	0.25%	0.1%	-.3500	.32199	.707	-1.3812	.6811
		0.5%	.8849	.32199	.095	-.1462	1.9160
		Control	-.5386	.32199	.396	-1.5697	.4925
	0.5%	0.1%	-1.2350*	.32199	.021	-2.2661	-.2038
		0.25%	-.8849	.32199	.095	-1.9160	.1462
		Control	-1.4235*	.32199	.010	-2.4546	-.3924
	Control	0.1%	.1885	.32199	.934	-.8426	1.2197
		0.25%	.5386	.32199	.396	-.4925	1.5697
		0.5%	1.4235*	.32199	.010	.3924	2.4546

Based on observed means.

The error term is Mean Square(Error) = .156.

*. The mean difference is significant at the .05 level.

LogCFUa, LogCFUb, LogCFUc are the plate count readings at 24hrs, 48hrs and 72hrs respectively for *Listeria.*