Effective pack practices to control postharvest diseases in small berries

by

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Abstract

Postharvest losses can occur anywhere from harvesting to handling and shipping. In 2014, approximately $30 billion of fresh produce were lost in the United States food supply chain. In particular, small fruits shelf-life can be reduced by weight loss, stem scar injury, gray mold and ripe rot. It is important therefore to find sustainable packing alternatives to protect produce, increase shelf-life, minimize waste, and preserve resources. The objectives of this study were to: 1) evaluate the mechanical, physical and anti-fungal properties of pullulan packaging films loaded with essential oil (EO) nanoemulsions; 2) investigate the effectiveness of the formulated active packaging systems to extend shelf-life and enhance strawberries' quality.

Food-grade emulsions with sub-micron droplets were used to encapsulate cinnamaldehyde (CIN), eugenol (EUG) and thymol (THY) within either a refined coconut oil (carrier) or palm stearin in pullulan packaging systems. Different film combinations loaded with EO nanoemulsions were studied. Tensile strength, moisture content and antifungal activity against *Rhizopus stolonifer, Alternaria* spp., and *Aspergillus niger* were measured and compared to control pullulan systems. A lower tensile strength ($P > 0.05$) was measured for combinations loaded with EO nanoemulsions as compared to films without active lipid solutions. Nevertheless, the active combinations presented good elasticity and ductility. The control formulations showed no antifungal activity. Conversely, active combinations exhibited significant inhibition zones against postharvest fungi ($P < 0.05$). Film containing CIN had the biggest inhibition halos: 13.7, 15.7 and 14.5 mm were measured against *R. stolonifer, Alternaria* and *A. niger*, respectively.

Pullulan films incorporated with CIN nanoemulsions were selected for the field study. Fresh strawberries were harvested from a local farm in Kansas and separated into two groups (control and treatment). For each group, 10 strawberries were placed into a molded fiber berry

basket. A pullulan film was added at the bottom of each container for the treated group. Samples were stored for up to 10 days at 3°C and 12°C. Every 2 days microbial, visual, and physiological quality parameters were evaluated. For treated strawberries stored at 3°C, a reduction of 2 log CFU/g in yeast and mold population was observed over the 10-day period ($P < 0.05$), as compared to the control. An improvement in visual quality was noted for treated berries at both storage temperatures as compared to the control group. Overall, a reduction of fungal and quality decay in strawberries packaged with pullulan films incorporated with nanoemulsions was observed. This study demonstrates the potential application of pullulan active packaging systems as a mean of controlling and reducing postharvest disease in small fruits during shipping and storage.

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Acknowledgements

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Dedication

I dedicate this to my family who are always there for me no matter what, my friends who kept my spirits lifted, and my professors and fellow graduate students (Olathe and Manhattan) who never stopped encouraging me to succeed even when I did not think it was possible.

Chapter 1 - Review of the Literature

1. Postharvest Losses

Postharvest loss is defined as the amount of edible food available for human consumption but is not consumed for any reason. This includes moisture loss, fungal spoilage, pests, inadequate temperature control, and consumer food waste (Buzby, Farah-Wells, & Hyman, 2014). Not only does food loss result in lost nutrients for the population and loss of profit for producers but it is estimated that 2.5 percent of United States energy consumption is attributed to food loss (Webber, 2012). In 2010, the amount of postharvest losses in fresh produce from retail and consumers was 31.9 billion pounds (35%): 38.89% ($34.8 billion) of the total food lost in the United States (Buzby et al., 2014). Extending produce shelf life might help limit the amount of postharvest losses.

In order to comprehend how to manage postharvest losses, it is foremost important to understand how to properly use pre-harvest techniques to give the best chance for healthy, nutritional, and vibrant looking produce. Proper growth conditions and fertilization techniques are two examples of pre-harvest factors that affect postharvest loss.

Produce respiration is directly related to decay rate (Seymour, 2002; Wills, McGlasson, Graham, & Joyce, 2007). The faster the produce respires the shorter their nutritional reserves will last, leading to a shorter shelf-life. One strategy to reduce respiration rate is to store products at low temperatures: the rate of respiration increases 2 to 2.5 folds for every 10°C rise in environmental temperature for most produce commodities after harvest (Wu, 2010). Another strategy is to slow ripening in fruit by reducing the rate of respiration and ethylene production. Ethylene is a hormone released by produce that can hasten the process of decay and senescence. Ethylene plays a major role in several postharvest factors, such as the ripening of fruits and

degradation of chlorophyll (Seymour, 2002; Wills et al., 2007). Ripening of fruit is a natural occurence where the fruit begins the process of senescence and is necessary in certain produce for consumers' willingness to buy. It begins in the last stage of maturation until the early stage of senescence and can be sped up after the process of harvesting (Kader AA; Saltveit ME, 2003; Wills et al., 2007). Climacteric fruit display a drastic increase in respiration rate and ethylene production during the ripening process. This causes most climacteric produce to be harvested before ripening has occurred. Therefore, harvesting of climacteric fruit before being fully ripe is important to reducing postharvest loss as they will naturally ripen after separation from the mother plant. If picked when ripe there is a high possibility the climacteric produce will be non-salable by the time it reaches consumers. Nonclimacteric fruit are harvested when fully ripe and display only a slight decline in respiratory pattern and no significant ethylene production (Wu, 2010). Even though nonclimacteric fruit do not display a significant increase in ethylene production after harvest; they can still be affected heavily by environmental ethylene presence. Chen et al. (2018) found that grapes exposed to exogenous ethylene had a reduced titratable acidity and an increased amount of anthocyanin accumulation. Therefore, it is important to control ethylene for both climacteric and nonclimacteric fruit to increase shelf life of these products (Kader AA; Saltveit ME, 2003; Saltveit, 1999; Wills et al., 2007).

Water content is another critical factor in controlling postharvest loss. Fruits and vegetables typically contain 80-90% water on a fresh weight basis (Wills et al., 2007). Negative effects on appearance, sale weight, and textural properties are starting to be observed during postharvest shipping and handling for most commodities with only a 2-5% water loss (Wu, 2010). Water can be lost due to transpiration, and damage from mechanical injury (A. Kader, 2002; Wills & Golding, 2016) and it mainly affects produce with large surface to volume ratios

such as watermelon or head lettuce. Furthermore, the vapor pressure deficit (VPD), defined as the difference between the amount of relative humidity in the air and how much humidity the air can hold when saturated, also influences the rate of water loss. A high VPD can lead to large amounts of water loss, while a low VPD can help maintain moisture in the produce (A. Kader, 2002; Wills & Golding, 2016). Nevertheless, decreasing the VPD can lead to increased rate of spoilage in produce due to increased moisture in the environment. Surface waxing, coating, and plastic film wrapping can help with water loss control (Wills et al., 2007).

A significant portion of postharvest loss is attributed to spoilage (Buzby et al., 2014): produce are high in water and organic contents and they have a pH slightly lower than neutral. Some of the most common spoilage fungi are: *Alternaria, Aspergillus, Botrytis, Diplodia, Penicillium, Rhizopus,* and *Sclerotinia,* while two of the most common spoilage bacteria are *Erwinia* and *Pseudomonas* (Wills et al., 2007). Postharvest pathogens can use damaged produce tissues in order to invade the commodity and proliferate. Infected produce often displays no sign of infection at the time of harvest. Therefore, they are not typically sorted out and this can cause widespread infections amongst healthy produce. Infections caused by an organism like *Alternaria* spp. or *Aspergillus* spp., which are mycotoxin producers, are hard to detect in large production and packing plants (Sivakumar & Bautista-Baños, 2014). The presence of mycotoxins is considered a critical risk factor and The Food and Agricultural Organization (FAO) has indicated that 25% of global commodities are affected by mycotoxins every year (Schatzmayr et al., 2006). Mycotoxins are a health hazard for consumers. These molecules are usually resistant to processing techniques (Sanzani, Reverberi, & Geisen, 2016) and they represent a large postharvest issue both in developing and developed countries.

Implementing postharvest technologies is often complicated, since produce have different stages of maturity at harvest, physiology, and perishability (Wills et al., 2007). Some commonly used strategies are temperature management, proper packaging practices, minimizing respiration rate, and reducing microbiological activity. As mentioned previously, temperature control is the most effective tool to maintain quality and extend shelf life of fresh produce after harvest (A. Kader, 2002; Wills & Golding, 2016). When harvesting produce, it is recommended to harvest at the coolest point of the day and to protect the harvested produce from direct exposure to sunlight. Products are then quickly transferred to a precooling area (removal of field heat immediately after harvest) in order to decrease metabolic activities (Kader, 2002; Wills et al., 2007). The most common precooling methods are room cooling, forced-air cooling, hydrocooling, packaging-icing, and vacuum cooling. Produce are then delivered to refrigerated storage areas.

Another possible postharvest strategy is the use of heat. This treatment can be used for specific produce (potato, tomato, carrot, etc.) and it is considered natural and environmentally friendly. It involves the immersion of products in hot water or forced-hot air treatment and/or vapor heat treatment (Lurie, 1998). Hot water immersion is often used for fungal control. Vapor heat treatment has been specifically developed for insect and pest control, while forced-hot air treatment is used to control fungi, insects and pests (Lurie, 1998). Since heat treatment involves a high level of process control and risk of damaging produce there has not been much interest to implement this technology in large scale operations (Lurie, 1998; Wills et al., 2007).

Overall, postharvest loss is a complex subject. One of the main goals of studying postharvest physiology is to minimize loss. Creating effective preservation methods as well as

using technologies that are economical, consumer friendly, and sustainable represent the primary goal of postharvest handling (A. Kader, 2002; Wills & Golding, 2016).

2. Active packaging as postharvest technology for produce

Active packaging is defined as a packaging system that interacts with the packaged food in a desirable way, other than the passive role of advertising and protecting the food products from the outside environment (Otoni, Espitia, Avena-Bustillos, & McHugh, 2016). Since traditional preservation methods, such as refrigeration and careful handling procedures, do not always prevent spoilage microorganism growth (Sung et al., 2013), their combination with active packaging might represent an effective way to extend produce shelf-life. Multiple active packaging systems are already used in the produce market. Oxygen scavengers can help control residual oxygen in the food packages which can limit the presence of oxygen gas in the headspace down to 0.01%, although not typically useful in fresh produce (de Abreu, Cruz, & Losada, 2012). Ethylene scavengers can be used to limit the amount of ethylene gas present, slowing the rate of ripening and limiting the amount of chlorophyll degradation (yellowing of bananas), thus leading to healthier looking vegetables. Ethylene scavengers however can be toxic if in direct contact with the food (de Abreu et al., 2012). Moisture scavengers are often used in products that require a dry environment to maintain quality as well as safety. By limiting the amount of free moisture in the package the rate of microbial growth is greatly suppressed and can be used in virtually any food system where low moisture content is desired (Ozdemir & Floros, 2004). These are often implemented in food products like beef jerky in the form of absorbent silica pouches and are not typically used for fresh produce, where retention of water in the produce is necessary. Carbon dioxide scavengers and emitters are commonly used in fruit

and vegetable packaging to control the amount of carbon dioxide in order to inhibit microbial growth and decrease the ripening rate (Ozdemir & Floros, 2004).

Antimicrobial packaging is a form of active packaging in which the package acts to reduce, inhibit or retard the growth of microorganisms present in the food or the material itself (Appendini & Hotchkiss, 2002). Antimicrobial substances, such as essential oils, can be incorporated in or coated onto food packaging material to control undesired microorganisms. The active compounds are released from the film matrix to allow for continual migration into the product (Jung & Zhao, 2016): antimicrobial films can therefore be used during shipping and transportation. Since single layer packaging films can have poor stability against water and oxygen, as observed by Elbarbary & Mostafa (2014) when trying to apply a chitosan based film coating onto peaches, a multilayer film approach might provide a better solution. Arnon, Granit, Porat, & Poverenov (2015) used a bi-layer system on citrus fruit: the first layer was intended to provide a uniform and stable coating matrix, while the second layer provided a glossy appearance and antimicrobial function. Nevertheless, antimicrobial films are not typically mechanically acceptable and economically advantageous to make up for the entire package. For these reasons their applications have been used as inserts or sachets into already existing packaging systems (Otoni et al., 2016). Absorbent pads, which are like sachets, have already been developed to include active compounds that exhibit antimicrobial activity. These pads have been implemented to keep excess moisture from increasing spoilage of fresh fruits and vegetables that arises from respiration (De Azeredo, 2013).

As described above, antimicrobial packaging is a versatile technology and a combination of different systems can be used to create an ideal environment for a specific food product. Some examples are the use of ethylene scavenger in combination with antimicrobial packaging systems

to extend shelf-life and quality of bananas or the application of film coatings directly on produce (e.g. like a wax) and the incorporation of Essential Oils (EOs) to provide protection from water loss and pathogens growth. Active packaging needs should be considered based upon the food product and the desired internal environment for a specific food, as every fresh produce commodity is different.

3. The use of Essential Oils in active packaging

There are many antimicrobial compounds that can be incorporated for use into antimicrobial active packaging. Metal compounds such as silver incorporated into polyethylene and chitosan based packaging material (Nobile et al., 2004; Rhim, Hong, Park, & Ng, 2006) and titanium using an oriented polypropylene material (Chawengkijwanich & Hayata, 2008) have been proven effective as antimicrobials. Chemical compounds like potassium sorbate have been incorporated into a chitosan based active packaging system and proven effective against multiple bacterial species (Pranoto, Rakshit, & Salokhe, 2005). Rocha, Loiko, Tondo, & Prentice (2013) incorporated sorbic acid and benzoic acid into active packaging using fish proteins to control multiple bacterial species as well. Another type of antimicrobial compound that can be used in active packaging is bacteriocins. Nisin was incorporated into a chitosan based active packaging system and proven effective against multiple bacterial species (Pranoto et al., 2005). Lee, Park, & Lee (2004) used nisin incorporated into a paper packaging material to show effectiveness of the compound against aerobic bacteria and different types of yeasts.

Among the studied antimicrobial molecules for active packaging applications, EOs have been investigated for their ability to control and/or inhibit microbial contamination and reduce the phenomenon of lipid oxidation (Bevilacqua, Corbo, & Sinigaglia, 2010; Burt, 2004; Regiane

Ribeiro-Santos, Andrade, Sanches-Silva, & de Melo, 2018; Valentina Trinetta, Morgan, Coupland, & Yucel, 2017). EOs are fragrant liquid oils extracted from plants, especially herbs and spices (Shah, Davidson, & Zhong, 2012). Several EOs have broad antimicrobial properties (Bevilacqua et al., 2010) and can be used to control microbial contamination, ensure safety, quality and extend shelf-life (Burt, 2004). EOs are generally recognized as safe (GRAS) by the FDA and accepted by consumers as natural alternatives to synthetic additives. EOs contain many different types of phytochemicals leading to multiple modes of action against microorganisms. These include the interference of the phospholipid bilayer of the cellular membrane, the impairment of enzymatic mechanisms for metabolism and energy production, the destruction of genetic material, or proton motive force (Ultee, Bennik, & Moezelaar, 2002). The mode of action of EOs against postharvest pathogens is towards mycelial growth and spore metabolism (Regnier, Combrinck, Plooy, & Botha, 2010; Serrano, Martínez-Romero, Castillo, Guillén, & Valero, 2005): the hydrophobic nature of these molecules allows the oil to partition in the lipid layers of the cell membranes and disrupts the structure and integrity. This allows for H^+ and K cation exchanges at a higher rate. Leading to changing the flow of protons which can affect the cells pH, the chemical composition, and metabolic processes which will result in death of the cell (Beckman, 2000). Essential oils that contain more phenolic compounds (hydroxyl groups in the benzene ring) in their chemical structure have been found to have the strongest antimicrobial properties against fungal pathogens, such as eugenol and thymol. They have also been found to have the highest antioxidant properties (Lambert et al., 2001). This property can be attributed to the fact that phenolic compounds can interact with membrane proteins leading to deformations in the structure and function (Fung et al., 1977). Essential oils that function without phenolic compounds, such as lemongrass oil, are likely attributed to membrane disfunction by

lipophilic compounds (Mendoza et al., 1997). The mechanisms of action for each essential oil is varied, yet they exhibit deeply complex but effective antimicrobial properties against fungal pathogens. While not every detail is understood completely for essential oils pathway to disrupt fungal growth, there are many differing and debated theories about what is going on at the cellular level (Sivakumar & Bautista-Baños, 2014). As reported by Plotto, Roberts, & Roberts (2003), thyme oil was able to completely inhibit the growth of *Botrytis cinerea, Rhizopus stolonifer,* and *Alternaria alternate* in tomatoes. Arrebola, Sivakumar, Bacigalupo, & Korsten (2010) found that lemongrass oil was effective in controlling *R. stolonifer* and *B. cinerea* on contaminated peaches. Similarly, Vitoratos, Bilalis, Karkanis, & Efthimiadou (2013) found that lemongrass oil was able to control *Botrytis cinerea* on inoculated strawberries. When oregano was infused in chitosan and applied as a coating on table grapes, morphological changes in *R. stolonifer* and *A. niger* spores were observed. These changes to the spores include loss of cellular material, mycelia thinning and wrinkling, and loss of cytoplasmic material (dos Santos et al., 2012). Controlling spore development is important as generally spores are known as the inoculum able to spread diseases and thus an antimicrobial that can destroy or limit spore germination can be used to play a major part in controlling decay of postharvest crops (Sivakumar & Bautista-Baños, 2014).

However, there are practical challenges in using EOs in food and in food packaging. These molecules are often readily oxidized or hydrolyzed to give off flavors. Moreover, due to their relative hydrophobicity and insolubility in water, EOs can be difficult to incorporate in aqueous food matrixes (Liang, Qiao, Bi, Zou, & Zheng, 2012). Several researchers (Jamshidian, Tehrany, & Desobry, 2012; Lagarón, Ocio, & López-Rubio, 2011) reported a change in physical, structural and mechanical characteristics when these active ingredients were incorporated into

film-forming solutions: decreased Young's modulus and tensile strength, and increased elongation at break. Conversely, other studies described improved gas barrier properties of fruit-base films when carvacrol was added to the formulation (Mancini & McHugh, 2000; Rojas-Graü, Tapia, Rodríguez, Carmona, & Martin-Belloso, 2007). Differences were attributed to the interaction between active ingredients and the differing polymer networks.

These challenges can be overcome by encapsulating EOs in emulsions, therefore, obtaining a controlled release of the molecules from the package to the food. By encapsulating the antimicrobials, a controlled release packaging could be made. Oil-in-water (o/w) emulsions are widely used to encapsulate and effectively deliver hydrophobic additives into aqueous food matrices (Yucel, Elias, & Coupland, 2012, 2013). The emulsion is readily diluted in the food, where the droplets serve as a reservoir for the lipophilic additive to maintain a constant concentration in the aqueous phase. They can be structured by changing the lipid composition or interfacial properties to allow controlled release of the encapsulated molecules. The diffusion of migrant compounds into the headspace and mass transference in the interface from headspace to the food product (Bhunia, Sablani, Tang, & Rasco, 2013). The two main factors involved in both processes are the diffusion coefficient and partition coefficient. The diffusion coefficient is defined as "mass transfer due to random movement of molecules from regions of high concentrations to regions of low concentration until equilibrium is reached" and determines the diffusion process. Partition coefficient is defined as "the ratio of concentrations of migrant in the food and the polymer at equilibrium" and describes distribution of migrant compounds in the different phases (Nerin et al., 2016). These are affected by many factors such as packaging material, food product, migrant properties and much more. Migration of compounds, specifically essential oils, can affect the sensory characteristics of a food product. Nerín (2010) states that

sensorial changes on a food product could be solved by using antimicrobial encapsulation of the active compounds. Emulsions with fine sub-micron droplets, sometimes referred as nanoemulsions, can be used to encapsulate hydrophobic antimicrobial compounds in foods and enhance their antimicrobial activity (Donsì, Annunziata, Sessa, & Ferrari, 2011; Ziani, Fang, & McClements, 2012). The increased surface area and the more homogenous distribution of droplets can provide higher contact possibilities for bacteria and therefore improve antimicrobial effectiveness (Weiss, Gaysinsky, Davidson, & McClements, 2009). Other researchers argued that the enhancement of EO's antimicrobial activity is affected by the surfactant type, and surface charge as well as the size of the droplets (Donsì et al., 2011; Liang et al., 2012; Ziani et al., 2012). Solid lipid nanoparticles (SLN), fine o/w emulsions with crystalline droplets, have been used as drug delivery systems (Bonacucina, Cespi, & Palmieri, 2009; Malmsten, 2006; Velikov & Pelan, 2008). SLN are typically formed by dissolving the active ingredient in a hot molten lipid, homogenizing, and then cooling to crystallize the droplets. If the additives can be entrapped within the solid fat matrix then their diffusion to the aqueous phase may be limited, providing controlled release properties and enhanced protection from chemical degradation (Müller, Mäder, & Gohla, 2000; Üner, Wissing, Yener, & Müller, 2004). However, crystallization of the droplets often results in expulsion of the encapsulated material from the lipid droplets into the aqueous phase, however the excluded compound tends to accumulate at the droplets' surface (Tikekar & Nitin, 2011) where the behavior of the molecules is largely affected by the interfacial interactions (Yucel et al., 2012). Such interactions may improve the antimicrobial activity of water insoluble EOs.

Finally, it is imperative that the active packaging be effective enough that the losses that are saved outweigh the cost of the system. While reducing food loss is an important goal, there will be little use if too costly for a company to justify.

4. Pullulan and its applications as antimicrobial packaging film

Biopolymers represent a novel form for use in the food industry as packaging materials, as they are bio-based, disposable, biodegradable, and recyclable materials (Valentina Trinetta, Cutter, & Floros, 2011). There are many approaches to active packaging development. Derivatives of cellulose, such as methyl cellulose and carboxymethyl cellulose, have been studied as a polysaccharide-based edible coatings against such fresh products as pears (Olivas & Barbosa-Cánovas, 2005). Many studies have also used chitosan as their film forming material. Gol, Patel, & Rao (2013) showed that edible coatings enriched with chitosan were shown to have a beneficial impact on strawberries quality retention ability. Chitosan is a polysaccharide derived from the hard outer skeleton of shellfish and can be incorporated into films or coatings due to its natural emulsifying properties and has been reported to have natural antimicrobial properties (Sánchez-González, González-Martínez, Chiralt, & Cháfer, 2010). A novel study by Del-Valle, Hernández-Muñoz, Guarda, & Galotto (2005) used a cactus-mucilage edible coating to extend strawberry shelf-life. Mucilage is typically a hetero-polysaccharide that can be obtained using the stems of plants. Cactus mucilage has been shown to be able to absorb large amounts of water and to dissolve and disperse itself into aqueous matrices (A. D. López, 1995). Cellulose derivatives, chitosan, and mucilage are all similar to pullulan as they can be used to form films and coatings, are polysaccharides, and are derived from natural and renewable sources.

Among the available biopolymers, pullulan shows excellent high film formability, considerable mechanical strength, the ability to form thin and flexible layers, and unique antimicrobial activity. Pullulan is highly soluble in water and provides good flexibility. It is a white powder, colorless, and tasteless as well. It is nontoxic, noncarcinogenic, biodegradable, and edible (Kumar, Saini, Pandit, & Ali, 2012; Oğuzhan & Yangılar, 2013). A fungus called *Aureobasidium pullulans* is responsible for the production of pullulan via fermentation of the fungi (Valentina Trinetta, Floros, & Cutter, 2010). All these exclusive characteristics make pullulan an ideal alternative candidate to petroleum-based polymers. The Japanese company, Hayashibara, has been producing pullulan since 1976. In 1982, the polysaccharide was introduced to the market commercially in the form of films. The industrial production of pullulan is usually conducted by the fungi fermentation. Pullulan films and coatings are usually prepared by dissolving the dry powder in water. The solution is then cast and allowed to dry on smooth surfaces. The film can be shaped with heat and pressure by the addition of an appropriate amount of plasticizer (Mitrus, Wojtowicz, & Moscicki, 2010). Films can be as thin as 5 µm, are usually clear, with excellent mechanical properties and oxygen-barrier characteristics (Singh, Saini, & Kennedy, 2008). When combined with glycerol, xanthan gum, and locust bean gum it can produce a film that can keep its integrity during handling and storage, while also presenting color characteristics preferable by consumers (Ozdemir & Floros, 2008; Trinetta et al., 2011). Pullulan has been reported to have inherent antimicrobial properties (Farris, Unalan, Introzzi, Fuentes-Alventosa, & Cozzolino, 2014). Gniewosz & Synowiec (2011) observed that when thymol was incorporated into pullulan film there was a greater reduction in *Bacillus subtilis, Staphylococcus aureus, Salmonella enteritidis,* and *Escherichia coli* growth. Increased quality characteristics, brighter appearance and improved shelf-life were also observed when pullulan-based edible

coatings were applied to strawberries and white asparagus under refrigerated storage conditions (Eroglu, Torun, Dincer, & Topuz, 2014; Tzoumaki, Biliaderis, & Vasilakakis, 2009). Acting as a barrier against gas transport, coated strawberries weighed more, were firmer, and exhibited less degradation of ascorbic acid, carotenoids, and decreased fungal decay. Similar positive effects were observed on the quality of coated asparagus, including less moisture loss, reduced hardening, decreased purple color development, and a significantly brighter appearance (Tzoumaki et al., 2009) .The development of active pullulan films and coatings, obtained by adding antimicrobial compounds to the film-forming solutions, demonstrated enhanced antimicrobial effects against specific bacteria and the ability to extend the overall shelf-life of treated produce. Despite all these positive suitable characteristics, the cost of pullulan production is relatively high, as compared to other biopolymers-(Oğuzhan & Yangılar, 2013). Continued improvements in the technical aspects of pullulan production could be made to lower the cost of production and propel pullulan into more marketable products.

Chapter 2 - Research needs

The development of packaging systems that assure safety and protect products against foodborne and spoilage microorganisms, maintaining quality and extending shelf-life, would undoubtedly provide a means of minimizing food loss, even more if the solution is economical and sustainable. Improving productivity, manufacturing new materials, and implementing sustainable and cost-effective technologies while maintaining product quality and safety are all imperative needs for the food industry in today's market. Companies and researchers have been striving to streamline effective schemes to help fulfill these requirements (Mahalik & Nambiar, 2010). Packaging not only needs to maintain and protect the original characteristics of the product, but needs to be functional, sustainable, and enhance safety, quality and shelf-life (Otoni et al., 2016). According to Gustavsson, Cederberg, Sonesson, Van Otterdijk, & Meybeck (2011)every year 40% of the food items intended for human consumption never reach the table. Wasted product translates to loss of money for the food industry. With better preservation techniques and extended product shelf-life, food waste can be greatly reduced (Galanakis, 2012; Ribeiro-Santos et al., (2018). Extending produce shelf-life might help limit the amount of postharvest losses by reducing spoilage microorganisms and decreasing metabolic activity.

Therefore, there is a need to look for new packaging solutions to allow consumers to keep products for a longer time without being concerned about spoilage and for producers to deliver produce with acceptable organoleptic characteristics and appearance. In order to help with some of these gaps, the objectives of this research were the following:

Evaluate the mechanical, physical, and anti-fungal properties of pullulan packaging films loaded with cinnamaldehyde (CIN), eugenol (EUG), and thymol (THY) nanoemulsions;

Determine the efficacy of the formulated active pullulan films to increase strawberries shelf-life during a 10-day storage trial;

Assess the willingness of Kansas and Missouri growers to pay for anti-fungal packaging and estimate the impact of relevant farm and producer traits on willingness to pay.

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Chapter 3 - Formulation and Development of Lipid Nanoparticle Antifungal Packaging Films to Control Postharvest Disease in Small Berries

(Article submitted to *Food Control* Journal)

Abstract

Postharvest losses are still a major problem in the produce industry, and they can occur anywhere along the food supply chain. Therefore, the objective of this study was to evaluate the mechanical, physical, and antifungal properties of packaging films loaded with essential oil (EO) liquid lipid nanodroplets and solid lipid nanoparticles. Food-grade emulsions with sub-micron droplets were used to encapsulate cinnamaldehyde, eugenol, and thymol using liquid (refined coconut oil) and solid (hydrogenated palm oil) carrier oils and incorporated into pullulan packaging systems. The emulsion-doped films were characterized for their tensile strength, moisture content, color, and antifungal activity against *Rhizopus stolonifer*, *Alternaria* spp., and *Aspergillus niger*. The tensile strength of films was not significantly affected ($P > 0.05$) by the presence of EO nanodroplets and nanoparticles. The active combinations presented good elasticity and ductility. Eugenol active film moisture contents were higher as compared to film without EO ($P < 0.05$). Conversely there was no significant difference between cinnamaldehyde, and thymol films as compared to film without EO ($P< 0.05$). The control formulations showed no antifungal activity. Active combinations instead exhibited significant inhibition zones against postharvest fungi ($P < 0.05$). Films containing cinnamaldehyde showed the biggest inhibition halos against *R. stolonifer*, *Alternaria,* and *A. niger*: 18.2, 20.3, and 15.9 mm respectively. This study demonstrates the potential application of pullulan packaging films loaded with EO

nanoemulsions as a means of controlling and reducing postharvest disease in produce during shipping and storage.

1. Introduction

Fresh produce demand from consumers coupled with high product quality expectations is continuously growing. Furthermore, there are significant postharvest losses in the produce industry annually. Food loss is defined as the "amount of edible food that is available for human consumption but is not consumed for any reason" (Buzby et al., 2014). Postharvest loss is a more specific form of food loss that can occur anywhere along the food supply chain: during harvesting, packing, transportation, and storage in retail facilities, but does not include wasted yet viable food products (Sivakumar & Bautista-Baños, 2014). It is estimated that 12% of all fresh produce goes uneaten during shipping alone, from production sites to retailers, and can reach up to 23% for more perishable commodities (Buzby et al., 2014). In 2010, the amount of postharvest losses in fresh produce were 91.1 billion pounds (21.1%), for a total of 32.9% (49.8 billion) of the total food lost in the United States (Buzby et al., 2014). Packaging technologies can be designed to protect, increase shelf-life, and maintain appearance of produce, and subsequently to make them more accessible, affordable, and attractive to consumers. Packaging makes up approximately 2% of the gross national product in all developed countries and half of this is used for food packaging (Robertson, 2006). In addition, packaging should be economically viable and preferentially designed from sustainable and GRAS materials.

Food packaging serves multiple purposes and must not sacrifice the integrity of the product in order to maintain its characteristics. Extension of shelf life, assured safety, and acceptable quality are aspects of packaging that could serve as a solution to reduce postharvest loss. Active antimicrobial packaging is one of the strategies that interacts with the food to further reduce, retard, and inhibit the growth of foodborne pathogens and spoilage microorganisms in addition to traditional packaging purposes (Otoni et al., 2016; Galanakis, 2012; Ribeiro-Santos et

al., 2018). Our previous work on active packaging focused on the use of pullulan, a microbially-derived and biodegradable polysaccharide with excellent film-forming characteristics (Trinetta et al., 2010; Trinetta et al., 2011).

Essential oils (EOs) have been one of the attractive antimicrobial molecules for active packaging applications due to their effectiveness in controlling and/or inhibiting microbial contamination (Bevilacqua et al., 2010; Burt, 2004; Trinetta et al., 2017). EOs are Generally Recognized as Safe (GRAS) as well. EOs have known antimicrobial properties that can inhibit viruses, fungi, and bacteria (Pei et al., 2009). They also exhibit insect and pest repellent properties that can help reduce contamination (Nerio et al., 2010). The use of food-grade ingredients and natural antimicrobial compounds can provide a huge benefit for the industry adopting this technology, since these ingredients are considered "label-friendly". On the other hand, EOs are highly volatile molecules, hydrophobic in nature, and often readily oxidized or hydrolyzed to give off flavors and can be difficult to incorporate in aqueous food matrixes (Liang et al., 2012). Nevertheless, the encapsulation of active ingredients, such as EOs, in nanoemulsions might represent a way to overcome all these challenges.

Based on previous studies (Trinetta et al., 2017; Yucel et al., 2012, 2013), where enhanced antimicrobial activity of encapsulated active ingredients was demonstrated, the objective of this study was to evaluate the mechanical, physical, and anti-fungal properties of pullulan packaging films loaded with cinnamaldehyde (CIN), eugenol (EUG), and thymol (THY) nanoemulsions.

2. Materials and Methods

2.1. Experimental design

A full factorial design with three variables was applied to optimize film formulations. The independent variables were: carrier lipid (refined coconut oil composed of 1:1 caprylic and capric acids as liquid oil, and hydrogenated palm oil as solid lipid), essential oil (cinnamaldehyde, eugenol, thymol), and emulsion dispersed phase fraction (1% and 2%, named as set 1 and set 2, respectively). For each film combination the following responses were recorded: thickness, ultimate tensile strength, color (L*, a*, b*), moisture content, and antifungal activity.

2.2. Materials and cultures

Pullulan was supplied by Hayashibara Company (Okayama, Japan). Glycerol was obtained from VWR Company (Batavia IL, USA), xanthan gum was purchased from TCI America (Portland OR, USA), and locust bean from CP Kelco (Lille Skensved, Denmark). Sodium-caseinate powder was purchased from Alfa Aesar (Ward Hill, MA, USA). Cinnamaldehyde (CIN), eugenol (EUG), and thymol (THY) were purchased from Sigma-Aldrich Company (Milwaukee, WI, USA). Carrier lipids used were liquid coconut oil (refined coconut oil composed of composed of 1:1caprylic and capric acids, Better Body Foods, Lindon, Utah, USA), and hydrogenated palm oil (solid lipid mainly composed of stearic acid and palmitic acids with a melting point between 55-58 °C, obtained from Cargill, Minneapolis, MN, USA). *Alternaria* spp. ATCC 20084, *Aspergillus niger* ATCC 16888, and *Rhizopus stolonifer* ATCC 62276 were purchased from Microbiologics (St. Cloud, MN., USA). Cultures were grown on PDA (Potato Dextrose Agar, Difco Laboratories, Spark, Md., USA) at room temperatures (~22°

C) for up to 4 days. The cultures were chosen based upon their prevalence in produce postharvest disease (Van de Perre et al., 2014).

2.3. Nanoemulsions preparation

Emulsions with Liquid Lipid Nanodroplets (LLN) and Solid Lipid Nanoparticles (SLN) were prepared using a hot-homogenization technique (Yucel et al., 2013). LLN and SLN were formulated to encapsulate active compounds using liquid oil (refined coconut oil), and solid oil (hydrogenated palm oil). Emulsion premixes were prepared by mixing the two different carrier lipids (8-9 wt%) with a 2% casein sodium salt solution mixed using deionized water, (90 wt%) as an emulsifier, and the active compound (CIN, EUG, THY) as 1% or 2% of final emulsion volume, using a high-shear mixer (Brinkmann Polytron, Brinkmann Instruments Inc., Westbury, NY). The coarse emulsion was then homogenized using a 2-stage valve homogenizer (Panda Plus 2000, GEA) at 500 bars and 60-65 °C for 3 passes. The particle size of the emulsions was characterized using DelsaMax Pro (Beckman Coulter, Indianapolis, IN, USA) equipped with a DelsaMax Assist unit (Beckman Coulter, Indianapolis, IN, USA).

All emulsion samples were sterilized in a water bath (90 °C for 15 min) and then used for film preparation. The crystallization and melting profiles of SLN were analyzed and verified by using a modulated differential scanning calorimeter (Q2000, TA Instruments - Waters L.L.C., New Castle, DE, USA).

2.4 Film Formulation

Pullulan films were made following the protocol developed by Trinetta et al. (2011). Two film formulations were used in this study. The first formulation included pullulan (50 g/l), glycerol (5 g/l), xanthan gum (5 g/l), and locust bean gum (5 g/l) that were mixed with deionized water (500 ml) at 70-80 °C for 20 min and then autoclaved at 121 ºC for 5 minutes. Film

solutions were kept in a water bath (at 90 °C) and within 5-10 minutes, either LLN or SLN emulsions, containing 2% EO were added (500 ml) to the film solution and stirred for at least 2 minutes until complete dissolution. The second film formulation included pullulan (50 g/l), glycerol (5 g/l), xanthan gum (5 g/l), and locust bean gum (5 g/l) directly added to the LLN or SLN emulsions containing 1% EO (1 L). This mixture was stirred for 5 minutes or until complete dissolution. Films were then cast by pouring solutions onto an ultraviolet light-sterilized aluminum sheet pan (17 x 11 inch) and allowed to dry for 24 h at room temperature and 40% relative humidity. LLN films were stored at room temperature (~ 22 °C) in airtight containers. SLN films were stored at freezer temperatures (-20°C) to allow complete crystallization.

2.5. Mechanical properties

The film thickness was evaluated using a micrometer at 3 random locations and an average measurement for each replicate was reported. The ultimate tensile strength (UTS) of the films was measured using a texture analyzer (Texture Technologies Corporation, Hamilton, MA, USA) with TA-96 double clamp attachments following the Standard Test Methods for tensile properties of thin plastic sheeting (ASTM D882-02). UTS is defined as a capacity of material to withstand stretching force, and evaluated using the Equation 1:

$$\text{UTS} = \frac{\text{maximum force}}{(\text{Film thickness} * \text{Film width})} \qquad \text{Equation (1)}$$

The film sheets were cut to be 1 cm in width and 5 cm in length (Han & Floros, 1997). Test speed was set at 10 mm/sec, and the pulling force was recorded until film breaks at the approximate mid-section.

2.6. Moisture content

Moisture content of the films was calculated using the oven-dry method (ASTM C566-13) by placing films (weight) in an oven at 100 °C for 24 h. Verification of complete desiccation of the films was done by measuring moisture content after 24 and 48 h. Moisture was measured as a percent of the total film weight using Equation 2:

$$\text{Moisture \%} = \frac{(\text{Initial Weight} - \text{Dry Weight})}{\text{Initial Weight}} \times 100 \qquad \text{Equation (2)}$$

2.7. Color Measurement

The color of the films was evaluated using a Hunter MiniScan EZ spectrophotometer (Model: MSEZ-4500L, 45°/0° geometry, Large viewing area; HunterLab Reston, VA). Each film was sampled three times and averages were reported for L*, a*, and b*. The total color difference (ΔE) was calculated using Equation 3:

$$\Delta E^*_{ab} = \sqrt{(L^*_2 - L^*_1)^2 + (a^*_2 - a^*_1)^2 + (b^*_2 - b^*_1)^2} \qquad \text{Equation (3)}$$

2.8. Antifungal Activity

The antifungal activity of the obtained films was evaluated against *Alternaria* spp., *Aspergillus niger,* and *Rhizopus stolonifer.* Cultures were obtained using a sterilized coring tool (10 mm diameter) to get a disc of inoculated sample grown previously. This sample was transferred to a new PDA plate and grown for up to 4 days. The plates were then harvested using 0.1% peptone water and a sterile spreader to gently scrape the fungi from the agar. This fungi solution was harvested into a sterile tube. A sterilized coring tool was used to produce cores of 10 mm in diameter for each film sample. The PDA plates were inoculated with 1 mL of the harvested fungal solution and allowed to dry for 1 hour. The 10 mm film discs were then placed onto inoculated PDA following a modified Zone of Inhibition Kirby-Bauer test setup (Hudzicki, 2009). The plates were then left to grow for up to 4 days at room temperatures and the zones of

inhibition were recorded (mm). The tests were performed in duplicate and averaged to give the reported inhibition zone.

3. Results and discussion

3.1. Emulsion Particle Size

EO addition showed no difference in particle size of LLN emulsions compared to control emulsions (no emulsion added, d_{32} ~172 nm) as shown in Figure 1. All samples showed monodisperse behavior, where a large population of droplets have similar diameters after homogenization. Trinetta et al., (2017) mentioned that EO loaded emulsions had similar particle sizes with non-loaded emulsions. CIN loaded emulsions showed the significantly largest particle size (d_{32} ~200 nm) while THY loaded emulsions had the significantly smallest particle size (d_{32} ~168 nm) for SLN emulsions shown in Figure 2. Acevedo-Fani et al., (2015) observed the type of EO significantly affected the particle size of emulsions. They discussed the difference in particle size of thyme oil, lemongrass oil, and sage oil emulsions might be due to their chemical composition and affinity. The melting temperatures of palm fat and coconut are around 55 and 24 °C, respectively. Palm fat carrier oil was heated above 65 °C to make sure that the lipid particles were liquid. EO loaded SLN emulsions and films showed a melting peak around 52 °C, while LLN emulsions and films showed a melting peak around 22 °C. The crystallization of SLN and LLN start below 25 and 10 °C, respectively.

3.2 Mechanical Properties

A full factorial design was used for film formulation optimization, p-values for each response measured are presented in Table 1. No significant difference was reported for UTS in both LLN or SLN film samples. All other responses showed significant differences between the

formulations ($P \leq 0.05$). The physical and mechanical measurements were obtained for 16 different formulations (Table 2 and Table 3).

UTS was measured, as reported in Table 2 and Table 3 for each film formulation. Thickness measurements (mm) were used to calculate UTS (MPa). Variability in thickness of films is mainly attributed to glycerol and pullulan concentrations (Trinetta et al., 2011). In this study, concentrations of glycerol and pullulan were kept constant for all film samples, thus leading to no significant differences for thickness. UTS is an important mechanical property as it determines the ability to resist a certain load and gives an understanding of the energy a package can absorb before mechanical failure (DuPont, 2015). UTS, defined as the amount of force required to break an object, As highlighted in Table 1**,** were similar ($P>0.05$) for all film formulations containing EO and the control. This can be attributed to pullulan's versatile film forming abilities. In addition, the inclusion of a plasticizing material (glycerol) in pullulan film mixture reduces internal hydrogen bonding and increases intermolecular spaces when interacting with biopolymer chains, as previously reported by Trinetta et al (2011). In comparison, previous research showed that chitosan-based film loaded with cinnamaldehyde showed a significant difference in tensile strength as a function of increased EOs % incorporated (Ojagh et al., 2010). Chitosan is another polysaccharide widely studied for active packaging application. The value of UTS reported for LDPE packaging films is >7.85 MPa, which is slightly higher than the reported UTS of the pullulan packaging films (MatWeb, 2019). Despite this difference, the pullulan films displayed acceptable workability and flexibility.

3.3 Color Measurements

When produce starts to lose its freshness and therefore its color attributes, it heavily impacts consumers' willingness to purchase the product (Pathare, Opara, & Al-Said, 2013). In

our study, no difference was observed between control and EUG/THY formulations for set 1 and set 2 LLN, while a significant difference was observed for films containing CIN (Table 2). Similar observations were reported with SLN films containing CIN and THY as compared to the control (Table 3). CIN films appeared as light yellow. The control films were translucent while the films with EO were not (Figure 3). Our proposed solution is targeting the improvement of small fruit shelf-life: these products are usually packaged in transparent clamshells with an absorbent pad. The antifungal films developed in this study can be used in the same type of packaging systems (clamshell pad with antifungal pullulan film insert) and offer a convenient way to ensure the postharvest quality of produce.

3.4 Moisture Content

Moisture content of a food is critical for the growth of microorganisms. Adding extra moisture to a packaging system can increase the rate of spoilage (Pitt & Hocking, 2009). As reported in Tables 2 and 3, control films had a lower moisture content as compared to LLN and SLN samples containing EOs. The boiling point of CIN, THY, and EUG is approximately 253 ℃, 233 ℃, and 225 ℃, respectively (O'Neil, 2006; Yalkowsk & He, 2003). The temperature of the boiling point also follows a similar pattern to our moisture content, where the higher the boiling point temperature, the lower the moisture content tended to be for the respective EO films. The EO compounds can have an increased rate of evaporation as the temperature increases, which may lead to the EO compounds being evaporated as well as water. This could skew the results, showing a higher moisture content of the films than there is present due to high amounts of EO compounds being lost in the oven drying process. Contrary to our results, chitosan film incorporated with cinnamon essential oil showed a significant decrease in moisture

content compared to chitosan films with no essential oil added using the oven dry method at 110 ℃ (Ojagh et al., 2010).

3.5 Antifungal Results

The use of essential oils as an antimicrobial compounds has been well studied and proven effective against human pathogens (Trinetta et al., 2017). However, spoilage in produce is most often caused by fungi (Buzby et al., 2014). Antifungal agents, such as EO, can deactivate fungi by inhibiting structural and functional properties of cell membranes, organelles, and nuclear material or protein synthesis (Jager, 2014). In this study, the antifungal activity of LLN and SLN containing CIN, EUG, and THY were measured against postharvest fungi. Minimum Inhibitory Concentrations (MICs) values, determined in preliminary experiments were used to select susceptible microorganisms and the essential oils encapsulation conditions. Our data demonstrated an enhanced antimicrobial activity when active compounds were incorporated in emulsions. All films loaded with EOs showed antifungal activity (Table 4 and Table 5). Control films exhibited no antifungal properties which shows that the addition of EO is necessary to inhibit the growth of fungi. Set 1 films that contain 2% nanoemulsion, exhibited, on average, a slightly higher inhibition, than set 2. Moreover, SLN, prepared with the 2% nanoemulsion, CIN films showed the highest inhibition zones: 20.3, 15.9, and 18.2 mm against *Alternaria spp.*, *Aspergillus niger*, and *Rhizopus stolonifer*, respectively (Figure 4). These results are similar to our CIN SLN preliminary tests which showed this formulation as the most effective combination. We selected this formulation for microscopy investigation. As shown in Figure 3 structural changes to the polymer structure can be seen upon the addition of EOs, enhancing antifungal activity, but not influencing other mechanical properties. Bernardos et al. (2015)

reported results analogous to ours: an enhanced antifungal activity against *Aspergillus niger* was observed when CIN was loaded into silica mesoporous supports. However, they reported thymol was the most effective antifungal EO when compared to cinnamaldehyde and eugenol, which contrasts with results from our study with pullulan films. This inconsistency may be attributed to the difference in encapsulation material (silica vs pullulan film) or that Bernardos et al. (2015) studied fungal inhibition over 30 days. The study reported also how encapsulation helped increase release behavior and antifungal activity of EOs.

4. Conclusion

Our study establishes the potential application of pullulan packaging films loaded with EOs against postharvest disease. Overall, our results showed that: (a) addition of EOs into packaging films can increase moisture content; (b) EOs addition into film formulation does not significantly affect UTS strength for both sets; (c) pullulan films containing active nanoemulsions exhibit significant antifungal properties; (d) CIN films showed the most effective antifungal activity.

Further studies will be needed to assess flavor and olfactory profile of food in contact with the formulated EOs packaging films, since EOs have the ability to impart flavors of food due to the high volatility (Tongnuanchan & Benjakul, 2014). A field trial experiment will also need to be conducted in order to verify the feasibility of the formulated packaging systems to control and reduce postharvest disease in small fruits during shipping and storage.

Acknowledgement

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Table 3-1. P-values for each response (UTS: Ultimate Tensile Strength; Color; Moisture content; Antifungal activity against *Alternaria* spp., *Aspergillus niger*, *Rhizopus stolonifer*; Thickness) measured during the study.

Responses	P-values	
	LLN [#]	**SLN** [$]
UTS (Mpa);	0.6546[A]	0.0811
Color (ΔE)	0.0072	0.0002
Moisture (%)	<0.0001	<0.0001
Alternaria spp. (mm)	<0.0001	<0.0001
Aspergillus niger (mm)	<0.0001[A]	<0.0001
Rhizopus stolonifer (mm)	<0.0001	<0.0001
Thickness (mm)	0.0859	0.4338

A= Logarithmically transformed data

LLN= Liquid Lipid Nanodroplets

[$] SLN= Solid Lipid Nanoparticles

Table 3-2 Ultimate Tensile Strength (UTS), Moisture content and Color responses measured for LLN (Liquid Lipid Nanodroplets) Set 1 and Set 2.

	Set 1 [#]				**Set 2** [$]			
	CIN	EUG	THY	CONTROL	CIN	EUG	THY	CONTROL
UTS (MPa)	5.67 ± 0.41^{A}	4.38 ± 1.32^{A}	6.79 ± 2.62^{A}	5.43 ± 0.57^{A}	3.93 ± 2.40^{A}	5.90 ± 2.79^{A}	4.50 ± 0.51^{A}	3.34 ± 0.30^{A}
Moisture (%)	4.09 ± 0.68^{BCD}	5.33 ± 0.61^{AB}	6.23 ± 1.46^{A}	3.39 ± 0.14^{CD}	4.12 ± 0.20^{BCD}	5.46 ± 0.83^{AB}	6.11 ± 0.33^{A}	2.91 ± 0.12^{D}
Color (ΔE)	22.45 ± 6.23^{AB}	16.67 ± 6.71^{ABC}	14.35 ± 1.82^{ABC}	11.15 ± 4.14^{C}	24.02 ± 3.09^{A}	15.7 ± 0.85^{ABC}	15.12 ± 1.42^{ABC}	11.96 ± 0.93^{BC}

Data are presented as mean values of three replicates ± standard deviation. Values with different letters in the same row are significantly different ($p < .05$).

#: Set 1 consists of 1:1 DI water and 2% EO nanoemulsion

$: Set 2 consists of no DI water and a 1% EO nanoemulsion.

Table 3-3 Ultimate Tensile Strength (UTS), Moisture content and Color responses measured for SLN (Solid Lipid Nanoparticles) Set 1 and Set 2.

	Set 1[#]				**Set 2**[$]			
	CIN	EUG	THY	CONTROL	CIN	EUG	THY	CONTROL
UTS (MPa)	3.12± 2.10[A]	5.09± 1.0o[A]	6.29± 1.47[A]	6.75± 0.83[A]	3.27± 1.00[A]	6.08± 1.46[A]	7.20± 3.76[A]	4.28± 1.85[A]
Moisture (%)	5.84±0.65[BCD]	9.50± 1.72[AB]	6.34± 0.68[BC]	4.11± 0.86[CD]	5.45± 0.36[BCD]	7.96± 1.30[AB]	6.27± 0.64[BC]	3.68± 0.58[D]
Color (Δe)	34.89±6.59[A]	27.19±5.15[ABC]	12.81±0.57[D]	14.38±1.40[CD]	29.06±0.83[AB]	23.85±8.39[ABCD]	17.18±0.43[BCD]	16.80±2.34[BCD]

Data are presented as mean values of three replicates ± standard deviation. Values with different letters in the same row are significantly different ($p < .05$).

#: Set 1 consists of 1:1 DI water and 2% EO nanoemulsion

$: Set 2 consists of no DI water and a 1% EO nanoemulsion.

Table 3-4 Antifungal activity (mm) measured for LLN (Liquid Lipid Nanodroplets) Set 1 and Set 2.

	Set 1[#]				**Set 2[$]**			
	CIN	EUG	THY	CONTROL	CIN	EUG	THY	CONTROL
Alternaria spp.	15.7± 2.3^{A}	14.3± 2.4^{A}	12.7± 1.2^{A}	0±0^{B}	14± 4.4^{A}	12.2± 1.8^{A}	11±0^{A}	0±0^{B}
Aspergillus niger	14.5± 2.2^{A}	13±1.3^{AB}	11.7± 1.2^{AB}	0±0^{C}	12±1^{AB}	10.7± 0.3^{B}	10.8± 0.3^{B}	0± 0^{C}
Rhizopus stolonifer	13.7± 0.8^{A}	13.5± 1.3^{A}	12± 0^{A}	0± 0^{B}	12.5± 0.9^{A}	11.8± 1.4^{A}	11.3± 0.6^{A}	0±0^{B}

Data are presented as mean values of three replicates ± standard deviation. Values with different letters in the same row are significantly different ($p < .05$).

#: Set 1 consists of 1:1 DI water and 2% EO nanoemulsion

$: Set 2 consists of no DI water and a 1% EO nanoemulsion.

Table 3-5 Antifungal activity (mm) measured for SLN (Solid Lipid Nanoparticles) Set 1 and Set 2.

	Set 1				**Set 2**			
	CIN	EUG	THY	CONTROL	CIN	EUG	THY	CONTROL
Alternaria spp.	20.3± 6.9^{A}	12.5± 0.7^{A}	13.3± 1.1^{A}	0±0^{B}	16.8± 6.8^{A}	12.3± 1.3^{A}	10.6± 0.8^{AB}	0±0^{B}
Aspergillus niger	15.9± 2.3^{A}	12.6± 0.8^{ABC}	11.9± 1.2^{BC}	0±0^{D}	14.9± 0.6^{AB}	11.1± 0.3^{C}	10.4± 0.2^{C}	0± 0^{D}
Rhizopus stolonifer	18.2± 2.3^{A}	13.5± 2.1^{AB}	11.6± 1.6^{B}	0±0^{C}	13.8± 1.2^{AB}	10.8± 1.2^{B}	12.2± 2.9^{B}	0±0^{C}

Data are presented as mean values of three replicates ± standard deviation. Values with different letters in the same row are significantly different ($p < .05$).

#: Set 1 consists of 1:1 DI water and 2% EO nanoemulsion

$: Set 2 consists of no DI water and a 1% EO nanoemulsion.

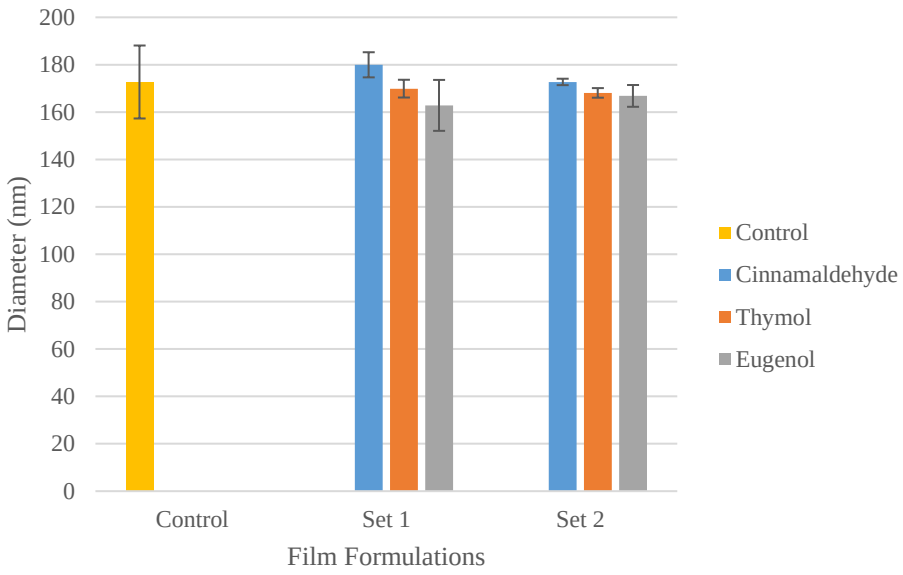

Figure 3-1. Particle size results of Set 1 and 2 LLN emulsions without (control) and with EO. The results show the average values (n=3).

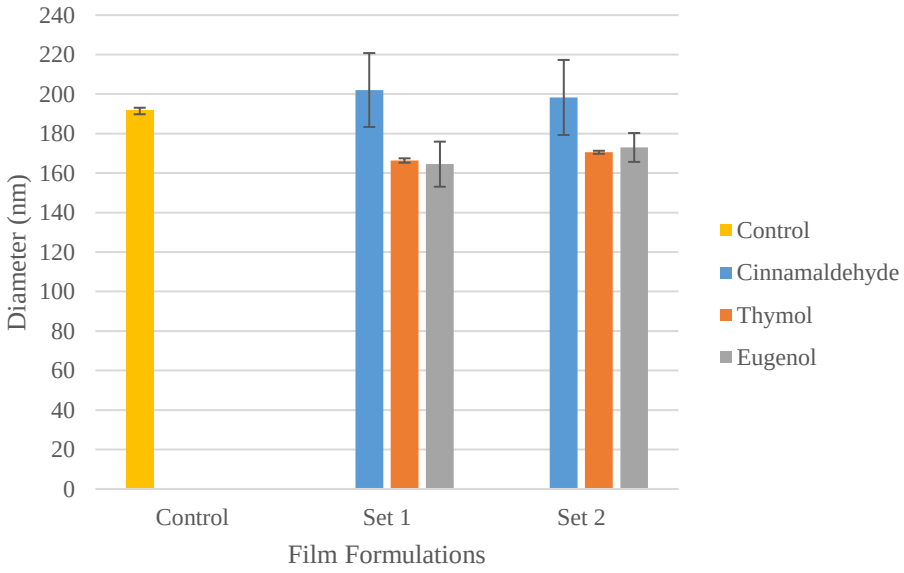

Figure 3-2. Particle size results of Set 1 and 2 SLN emulsions without (control) and with EO. The results show the average values (n=3).

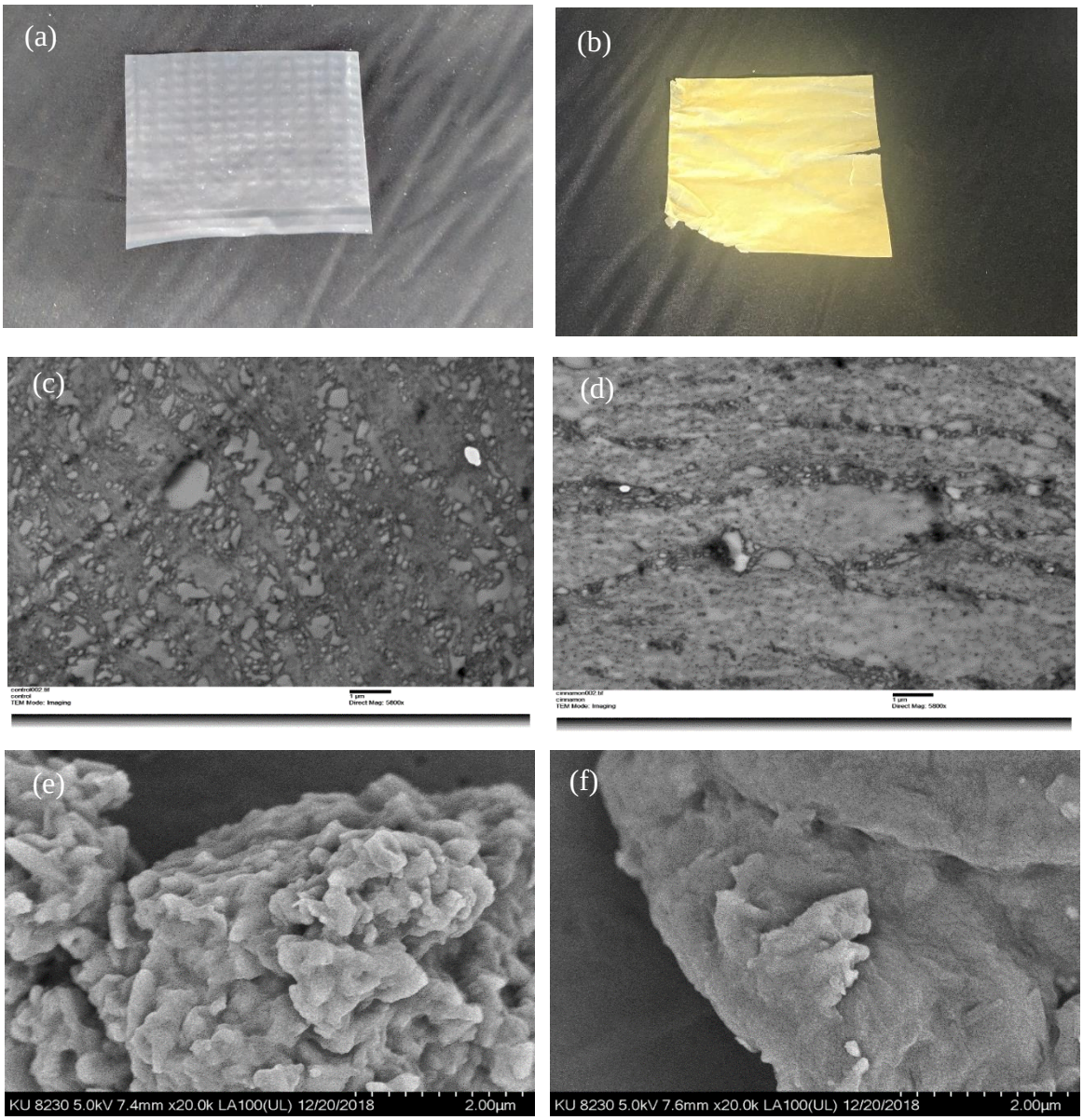

Figure 3-3. Images of (a) pullulan control films and pullulan films containing CIN (b); Transmission Electron Microscopy (TEM) images of (a) Control SLN films showing homogenous and random distribution of nanoparticles and (b) CIN SLN films showing localized nanoparticles oriented through film fibers. Scanning Electron Microscopy (SEM) images of (c) Control SLN films showing a porous surface and (d) CIN SLN films showing a smooth surface filled with EO nanoparticles.

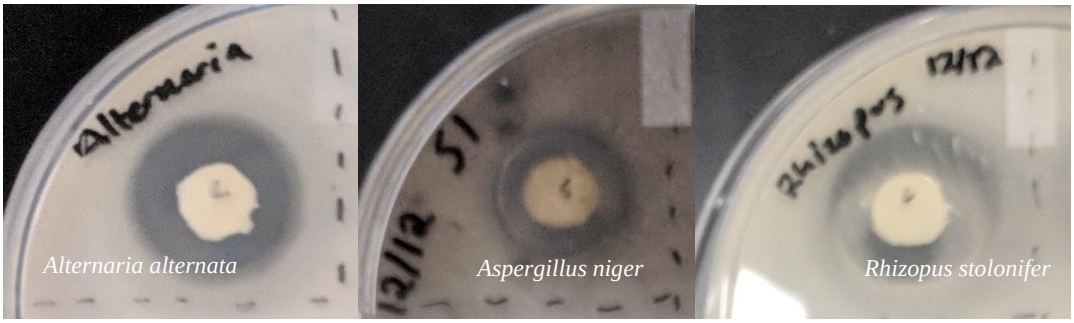

Figure 3-4. SLN Set 1 films containing cinnamaldehyde (CIN) exhibiting zones of inhibition against *Alternaria alternata*, *Aspergillus niger*, and *Rhizopus stolonifer*.

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Chapter 4 - Antifungal packaging films to control postharvest diseases in strawberries

1. Abstract

Every year 40 % of the food items intended for human consumption never reach the table. Produce can be lost due to inadequate storage conditions, package failure, spoilage, or degradation. Fruits account for 12.3% of food loss in the United States and berries tend to be the most susceptible to waste. Produce growers need cost-effective technologies to help decrease loss and maintain quality and safety until reaching consumers' tables. The objective of this research was to evaluate the effectiveness of previously formulated active packaging systems on strawberry shelf-life and quality. Pullulan films incorporated with cinnamaldehyde nanoemulsions were selected for this study. Fresh strawberries were harvested from a local farm in Kansas and separated into two groups (control and treatment). For each group, 10 strawberries were placed into a molded fiber berry basket containing a pullulan film layer (7x8 cm) for the treated group. Samples were stored up to 10 days at 3°C and 12°C. Every 2 days microbial, visual, and physiological quality parameters were evaluated. The experiment was run in triplicate. For treated strawberries stored at 3°C, a reduction of 2 log CFU/g in yeast and mold population was observed (P <0.05), as compared to the control. Yeast and molds count were significantly lower on days 2 and 4 at 12°C (P< 0.05) for treated strawberries. Significant reduction was also observed in aerobic plate count on treated strawberries at both storage temperatures. A visual difference between strawberries stored at 3°C was observed as well, where treated strawberries appeared more visually attractive. This study demonstrates the effectiveness of pullulan films incorporated with cinnamaldehyde nanoemulsions to reduce fungal decay in strawberries and promote desired quality during storage.

2. Introduction

The last Census of Agriculture estimated that the total farms in the United States producing strawberries represent 54% and that the total acres producing strawberries had increased 67% (Gude, Rivard, Chiebao, Swaney-Stueve, & Pliakoni, 2018). Fresh strawberries represent a 2.2-billion-dollar industry in the U.S. Strawberries are a very nutritious fruit but at the same time delicate, perishable, and susceptible to mechanical injury, desiccation, fungal decay, and physiological disorders during storage (Vu, Hollingsworth, Leroux, Salmieri, & Lacroix, 2011). Therefore, strawberries usually have a short shelf-life. Postharvest losses during strawberries storage can be as high as 40% (Duran et al., 2016). Rapid cooling can usually help extend shelf-life for a few days, reducing physiological deterioration but not fungal decay. Careful handling of strawberries during harvest and shipping is crucial for a healthy fruit. Mechanical damage can impact the attractiveness for consumers' purchase reducing willingness to buy. Damage to the fruit flesh also creates sites for potential pathogenic infections and localized areas of increased ethylene production and respiration (Wu, 2010). These negative factors from mechanical damage can drastically reduce potential shelf life for strawberries.

Alternaria alternata, *Aspergillus niger*, and *Rhizopus stolonifer* are a few of the fungal diseases observed in strawberries. *Alternaria* is a saprophytic pathogen that causes early blight and necrosis on stem and tissue (V. Trinetta, Linton, & Morgan, 2013). This black spot occurs in fields where strawberries have been damaged by hail, excess mite injury, natural cracking, or other physical or biological source of wounding. *Alternaria* rot is considered a secondary infection, since the fungus does not cause direct injury but grows on injured fruit. *A. niger* is also considered a saprophyte and it causes a disease called black mold. This pathogen is ubiquitous in soil and frequently reported from indoor environment. The fruit infected by *A. niger* starts

leaking juices from the tissues, thus reducing moisture content and nutrient reserves. Strawberries become so soft that they cannot be picked up without falling apart. Affected fruit are usually covered with a wispy, fuzzy black and white growth of the fungus (Moss, 2008). *R. stolonifer* is the causal agent of *Rhizopus* rot disease, infecting mainly ripe fruit only after harvest (Naureen, Humaira, Viqar, Ara, & Ehteshamul-Haque, 2009).This pathogen is considered one of the fastest-growing fungi, and it has a wide array of hosts that make it one of the most destructive fungi. During the first day of soft rot infection, a strawberries surface can be covered with thin, fluffy, cotton-like fungal structures. Sporulation forms a dark mass with black sporangia at their tips, which can cover the entire surface of the fruit (Feliziani & Romanazzi, 2016).

The lower pH of strawberries, the optimal water activity for fungi growth, the high level of sugars and nutrients coupled with soft texture make strawberries a perfect environment for microbial growth. Therefore, finding suitable methods for preserving the quality of strawberries during storage is important to prevent serious economic losses and reducing postharvest losses. Industry have sought alternative approaches to maintain quality of strawberries. Consumers demand natural, fresh and healthy produce without the use of technology that has a potential risk for health and environment safety (Gol et al., 2013). Thus, natural and environmentally friendly preservation techniques could fulfill consumers' needs and prevent postharvest losses in strawberries. In a previous study we reported the evaluation of the mechanical and physical properties of pullulan packaging films loaded with essential oil (EO) nanoemulsions and their anti-fungal activity against post-harvest disease (McDaniel, Tonyali, Yucel, & Trinetta, 2019). Thus, the objective of this study was to conduct a pilot experiment in collaboration with Kansas

strawberry growers to evaluate the benefit of using the formulated active packaging technology in terms of microbial safety, shelf-life extension, and quality of crops.

3. Materials and Methods

3.1. Experimental Design

This study implemented a generalized randomized block design. The treatment structure is 2-way factorial combinations. Fixed effects of the linear model were harvest, day, treatment. Interaction between treatment and day were also evaluated. Least Square Means (LSmeans) and their standard errors were reported for each treatment group. Treatment effects were evaluated against control within a given day based on a 2-sided difference test. LSmeans and their standard errors were plotted by treatment over time. Statistical Analysis System version 9.4 was used for analysis of data.

3.2. Lipid Nanoemulsion-Doped Antifungal Packaging Film

Pullulan packaging films loaded with cinnamaldehyde lipid nanoemulsions were selected for this research, based on their mechanical, physical and antifungal properties, as reported in our previous study (McDaniel et al., 2019).

3.3. Strawberry Harvest

Strawberries were harvested the day of the experiment from a local farm near Olathe, KS at the stage of commercial ripeness. The strawberries were hand harvested by trained workers in the morning and transported immediately to the Kansas State Urban Food Production and Postharvest Handling Laboratory. Upon arrival strawberries were randomly assigned to treatment (active packaging films of the dimension of 7x8cm were placed at the bottom of the

container) or control group (no active packaging). Ten strawberries were transferred in each molded fiber berry box (Pactiv corporations, Lake Forest, IL). Samples were stored at 3°C or 12°C with 95% relative humidity up to 10 days. Evaluations were conducted every 2 days for respiration, texture, color, microbiological counts, and visual quality. At each sampling day, remaining strawberries were frozen using liquid nitrogen and stored at -20°C for further analysis of Ferric Reducing Ability of Plasma (FRAP), Oxygen Radical Absorbance Capacity (ORAC), total phenolics, percent of titratable acidity (TA), and Soluble Solid Content (SSC) using the hydrophilic fraction.

3.4. Respiration measurement

Respiration rate (mg CO_2/kg-hr) was determined with a static system by measuring the CO_2 production (Kader, 1987) with a portable gas analyzer (model 900141; Bridge Analyzers, Alameda, CA). At each sampling day, 10 strawberries were stored in this system at their respective storage temperature for 1 hour.

3.5. Color Measurements

An A5 Chroma-Meter Minolta CR-400 (Minolta Co. Ltd., Osaka, Japan) was used to determine color. The instrument was calibrated using a white standard calibration plate. Readings were taken at 3 different locations for each strawberry (10 strawberries for each treatment group and storage temperature for every sampling day). Color results were expressed following the CIELAB color system: L* (-darkness to +lightness), a* (-greenness to +redness) and Hue (h), expressed as an angular measure.

3.6. Firmness Measurement

The flesh firmness (g) of strawberries was determined using a texture analyzer with an 8-mm probe (TA-XT. plus Texture Technologies Corp., Scarsdale, NY, USA). Pretest speed was set to 5 mm/s, test speed was 1 mm/s, posttest speed was 8 mm/s, and compression distance was 4 mm. Probe measurements were taken in duplicate for each fruit, in diametrically opposed sides (10 strawberries for each treatment group and storage temperature for every sampling day).

3.7. Microbiological Shelf-life

At day 0, 2, 4, 6 and when possible 8 and 10, 10 grams of strawberries were aseptically transferred in 90 mL of 0.1% peptone water (Becton, Dickinson and Company, Sparks, MD, USA) and gently hand massaged for 1 minute. Appropriate dilutions were made, and 1 mL of samples was plated onto Rapid Yeast and Mold (RYM) petrifilm and Rapid Aerobic Count (RAC) petrifilm (3M, St. Paul, Minnesota, USA). RYM petrifilms were incubated for 48 hours at room temperature (21 ± 2 ℃) before enumeration, while RAC plates for 18-24 hours at 37℃.

3.8. Visual Quality

Also for overall quality 10 strawberries for each treatment group and storage temperature were examined every two days using a visual scale with scores from 5 (excellent) to 1 (very poor) (Nunes, 2015).

3.9. Nutritional Quality Analysis

20 grams of frozen strawberry samples were homogenized using a mill with liquid nitrogen and 20 g were centrifuged at 17600x*g* for 20 minutes at 4 °C. The precipitate was then

used to obtain the hydrophilic fraction and analyzed for ferric reducing ability of plasma (FRAP), oxygen radical absorbance capacity (ORAC), phenolic content, titratable acidity, and SSC as described below. A synergy H1 Multi-Mode reader (BioTek Instruments, Inc. Winooski, VT, USA) was used to perform the antioxidant capacity readings.

Total antioxidant capacity was measured with the FRAP method by (Benzie & Strain, 1996) and expressed as micromolar Trolox equivalent in 100 g fresh weight basis (μM TE 100 g-1 FW). ORAC was evaluated using a method described by Cao, Alessio, & Cutler (1993) and modified by Ou, Hampsch-Woodill, & Prior (2001), and Prior et al. (2003). Results were expressed as micromolar Trolox equivalent in kg fresh weight basis (μM TE 100 g-1 FW). Total phenolic content was measured according to the Singleton & Rossi (1965) procedure. The results were expressed as mg gallic acid equivalent in kg fresh weight basis (GAE kg-1 FW). TA was measured with an automatic titrometer (Compact Titrosampler 862, Metrohm USA Inc. Riverview, FL.) and the results were expressed as % of citric acid equivalency (Garner, Crisosto, Wiley, & Crisosto, 2005). Finally, SSC was measured with a refractometer (Reichert Technologies, Depew, New York) and expressed in Brix.

4. Results

4.1. Respiration Measurements

Respiration measurements are important to understand the metabolic activity of plant tissues (Saltveit, 2000). Table 1 shows respiration rates (mg CO_2/kg-hr) for strawberries stored at 3 ℃. At day 2 there was no significant difference ($P>0.05$) between treatment and control. However, for days 4,6,8, and 10 there was a significantly reduced respiration rate for treated strawberries ($P<0.05$). Similar results were observed at 12 ℃ as well after 4 days of storage (Table 2).

4.2. Color Analysis

Our study revealed significant effects on color measurements for both 3 ℃ and 12 ℃ storage temperatures (Table 1 and 2 respectively). Our study had significantly different L* and hue measurements at days 2,4,6, and 8, while the a* measurement was only significantly different from the control on days 2 and 4 at storage temperature. of 3 ℃. At 12 ℃ storage temperature, the treated berries displayed a significant difference for L* and a* at days 4 and 6, while hue angle was only significantly different for day 4. An overall positive effect was observed on treated strawberries with the formulated antifungal packaging.

4.3. Texture Results

Strawberries treated with the formulated films stored at 3℃ had a significantly higher reported firmness at days 2 and 8 (Table 1). There were no differences observed for strawberries stored at 12 ℃ (Table 2).

4.4. Microbiological Shelf-life

Treated strawberries displayed lower microbial counts than untreated strawberries. Yeast and mold (Y&M) counts were significantly different across all days for 3 ℃, and significantly different for days 2 and 4 for 12 ℃ (Table 1 and 2, respectively). Aerobic plate counts (APC) were significantly different for strawberries stored at 3 ℃ on day 8 (Table 1), while no difference was observed during the first six days. The 12 ℃ stored strawberries were only significantly different on day 4 with both storage temperatures showing less APC for treated strawberries than untreated (Table 2).

4.5. Visual Quality

Visual quality is a subjective measurement based upon a quality scale developed by Nunes (2015) (Figure 1). Visual quality was used as an indicator of salability, defined as the capability of an item to be sold, of the strawberries with a cutoff of 30% of the berries being below the saleable point of a 1.5 on the rating scale. Overall quality pictures for 3 ℃ and 12 ℃ were taken on each sampling day and are presented in Figure 2 and Figure 3, respectively.

4.6. Antioxidant Analysis Results

In our study the only significant difference for phenolic content was observed at 3 ℃ storage temperature on day 10 (Table 1). The treatment had a lower level of phenolics as compared to the control but there were no significant differences at 12 ℃ (Table 2).

No significant differences between ORAC values of strawberries stored at 3 ℃ (Table 1). were observed in our study. However, after 6 days of storage at 12 ℃ the treated strawberries had a significantly lower ORAC value when compared to control strawberries (Table 2).

In this study, strawberries stored at 3 ℃ had significantly lower FRAP measurements at days 6 and 10 (Table 1). There were no significant differences observed in strawberries stored at 12 ℃ (Table 2).

4.7. Titratable Acidity and SSC

Our study revealed that there was no significant effect of the treatment on titratable acidity of the strawberries for 3 ℃ or 12 ℃ storage temperatures (Tables 1 and 2, respectively). Similar results were observed for SSC of treated and untreated strawberries for 3 ℃ or 12 ℃ storage temperatures (Tables 1 and 2, respectively). No detrimental effect on titratable acidity and SSC was observed when using the formulated active packaging technology.

5. Discussion

A reduced respiration rate means reduced metabolic activity within a cell, this phenomenon could translate to an extension of shelf-life and reduced postharvest loss of treated strawberries. Diab, Biliaderis, Gerasopoulos, & Sfakiotakis (2001) found that application of pullulan edible film coatings had a beneficial effect on shelf-life of strawberries. The researchers attribute this to the reduced respiration rates, which is similar to the results of our study. However, they also note that the edible coating had an internal atmosphere modification around the strawberry which could have been a critical factor for this result as well. Our study found that pullulan films impact respiration rates of strawberries in a similar manner. Treated strawberries for days 4,6,8, and 10 are all significantly lower than the control strawberries. Based upon the fact that respiration rate directly influences the shelf-life capabilities of strawberries it can be inferred that this is a positive result for possibly extending shelf-life.

Color is an important factor for consumers when purchasing produce. A bright red color, mainly caused by anthocyanins (antioxidant properties), is one of the most important quality characteristics of strawberries (Crecente-Campo, Nunes-Damaceno, Romero-Rodríguez, & Vázquez-Odériz, 2012). In a study conducted by Eroglu, Torun, Dincer, & Topuz (2014) they found that an 8% and 10% pullulan based edible film coating did not significantly impact the color values. The researchers did not use any active compounds, such as EOs, which is likely why this is not similar to our results where we found a significantly better color value for the treated berries than the control berries for both storage temperatures. However, Diab et al. (2001) did report an improvement in external color. The researchers used the pullulan edible coating to show that the color attributes of lightness (L*) and redness (a*) were both significantly higher

than that of the control. This study did not report whether it affected the hue angle measurements.

Loss of firmness is one of the most prolific changes that can occur in fruits and vegetables during extended storage and relates to changes in water content and metabolism (Garcia, Pereira, De Luca Sarantópoulos, & Hubinger, 2012). The differences observed on days 2 and 8 for 3 ℃ could be linked to the reduced microbial activity observed during storage as the antimicrobial essential oil compounds begin to release and build up in the headspace surrounding the strawberries. Plant pathogens can have a severe impact on textural properties of infected produce as they break down plant tissues during proliferation (Pitt & Hocking, 2009), thus providing inhibition of yeast and mold growth there could be improvements in firmness. Gude et al. (2018) conducted a study examining different pre-harvest factors on quality attributes of fresh strawberries. Firmness for six different cultivars of strawberries were reported with only two cultivars being significantly lower. This shows that perhaps the strawberries used in this study were a cultivar with an inherently lower flesh firmness, and thus not the highest quality. Eroglu et al. (2014) reported that a 10% pullulan coating showed significantly higher firmness values than that of the control and 8% pullulan coated strawberries. They report a loss of firmness for the control and 8% as 54.2 and 41.6% respectively, while the 10% pullulan coating only showed an 8.3% decline. Over a 10-day storage period, our study found a reduction of firmness for treated strawberries stored at 3 ℃ as a loss of 13.5%, while the control had a loss of 31.6%. At 12 ℃, our study showed a loss of firmness for treated strawberries was 24.3% and 38.9% for control strawberries. However, after 10 days of storage there were no significant differences in firmness between treatment or control.

Visual quality results for 3 ℃ (figure 2) show a visual progression of the strawberries over 10 days. The strawberries at day 0 seem firm and brightly colored and progressively degrade to have lower firmness and less acceptable color properties. However, the treatment strawberries display a much slower rate of degradation than the control strawberries. At day 10, the control strawberries have multiple locations of noticeable fungal growth with a wilted calyx and a dark red color. The treatment strawberries have very few areas of fungal growth and the calyx are a brighter green color with the strawberries showing a more bright and vivid red color. Visual quality results for 12 ℃ (figure 3) show a much more rapid rate of deterioration than those stored at 3 ℃. There are not many visually noticeable differences between treatment and control when stored at the higher temperature. However, days 2 and 4 did show significantly lower Y&M counts but was not observed on a visual scale.

At 12 ℃, strawberries are considered under temperature abuse and can have loss of antioxidant capacity, texture, and flavor, as well as providing a more optimal growth temperature and a more susceptible strawberry to grow upon for plant pathogens (Feliziani & Romanazzi, 2016). At 3 ℃, treated strawberries had consistently lower microbial counts than untreated strawberries. Proper storage temperatures might have enhanced the antifungal activity of the developed packaging systems. López, Sánchez, Batlle, & Nerín, (2007) found that polypropylene and polyethylene/ethylene films made with cinnamon and cinnamon fortified with cinnamaldehyde, were effective for up to two months after production at preserving foodstuffs and inhibiting spoilage fungi growth. Gol, Patel, & Rao (2013) found that when using edible coatings enriched with chitosan that the coatings significantly reduced decay percentage compared to controls. They also noted that both control and treatment strawberries showed an overall increase in amount of decay over time. Our study found a similar result at 12 ℃, where

there was an increase over time in the microbial counts. However, at 3 ℃ we saw an initial decrease in Y&M and APC for the treated strawberries while the control strawberries began proliferating after just 2 days in storage.

Phenolic compounds are phytochemicals that exhibit antioxidative activity and have been linked with reduced oxidative damage to DNA (Djuric et al., 1998). They represent a large group of secondary metabolites that contain one or more aromatic rings. These compounds contribute to fruit color, astringency, and bitterness (Crozier et al., 2006). Therefore, phenolic compounds are a desirable component in a berry. Storage temperature is important in preserving antioxidant levels, when storage temperature is elevated this can result in a loss of some antioxidants (Manganaris, Goulas, Vicente, & Terry, 2014). It has been reported that EO components had an ability to increase antioxidant levels in plant tissues. Thyme oil was found to increase the total phenol content in inoculated avocado fruit (Assis, Maldonado, Muñoz, Escribano, & Merodio, 2001). Our study showed no differences in phenolics except for strawberries stored at 3 ℃ after 10 days. The treated strawberries showed a significantly lower phenolic content than the control strawberries. Gol et al. (2013) reported an overall decrease in phenolics for both treatment and control strawberries using a chitosan-based coating. However, there was a significantly more rapid decrease of phenols for the control group. Our study showed that at 12 ℃ there was an overall decline with treatment and control however no differences in the rate of decline. ORAC analysis relies on free radical damage to a fluorescent probe, in this case fluorescein, where an oxidizing reagent causes loss of fluorescent intensity over time (Brescia, 2012). A significantly lower ORAC value for day 10 at 12 ℃ was observed, however there were no statistically significant differences at 3 ℃. Since the strawberries exhibited quality that was no longer saleable at 12 ℃, further analysis for ORAC of the strawberries following day

6 was not performed. FRAP analysis is a novel method that assesses antioxidant power where ferric to ferrous ion reduction causes a colored complex to form. Values are obtained by comparing the absorbance change at 593 nm to a known standard (Benzie & Strain, 1996). While there were no significant differences in strawberries stored at 12 ℃, strawberries stored at 3 ℃ showed interesting results at day 6, and 10. The treated strawberries had significantly lower FRAP measurements than the control.

Titratable acidity (TA) is directly related to the amount of organic acids that are present in the fruit. Therefore, reduction in TA is a natural part of the process of metabolic activity during storage. Our research supports this claim that TA does naturally reduce over time, however there were no significant differences between treatment or control strawberries for either storage temperature across all days. Garcia M., Martino M., & Zaritzky N. (1998) and Velickova, Winkelhausen, Kuzmanova, Alves, & Moldão-Martins (2013) both reported retention of TA in treated strawberries when using a starch-based coating and chitosan-beeswax coating, respectively. Total soluble solids (TSS) showed no difference between treatment or controls for either storage temperature across all days. There was an observed decline over time in TSS which is logical as it is linked with metabolic activity. As strawberries degrade, their natural metabolic activity will lead to a reduction in TSS over time (Gol et al., 2013). However, Gol & Ramana Rao (2011) found that using chitosan based edible coatings on bananas, that an accumulation of TSS in bananas was observed. A similar result was found by Ali, Muhammad, Sijam, & Siddiqui (2011) when using chitosan based coatings on papaya fruits. Both researchers claim this is likely due to the respiration rate being lowered in the respective commodities. Our study found no difference in TSS for strawberries however, even though there was a reduced respiration rate of the treated strawberries compared to the control.

6. Conclusion

In this study, the effect of pullulan packaging films loaded with cinnamaldehyde lipid nanoemulsions has been shown to reduce respiration rate and inhibit yeast and mold growth in strawberries. Overall a better quality was maintained in strawberries packaged with the active formulation at refrigerated temperature (3 ℃). Furthermore, a reduction in metabolic activity of strawberries stored in these systems was reported. Further studies investigating the impact of the active ingredient on the sensory characteristics of the packaged products might be necessary, since the selected compound is a volatile essential oil.

Table 4-1. Response measurements for strawberries stored at 3°C over 10 days.

Day	0	2	4	6	8	10
Respiration Rate (mg CO_2/kg-h)						
Treatment	4.10± 0.56	2.64± 0.21^{A}	2.81± 0.21^{B}	2.61± 0.21^{B}	2.85± 0.27^{B}	1.43± 0.38^{B}
Control	4.10± 0.56	2.75± 0.21^{A}	3.56± 0.21^{A}	3.61± 0.21^{A}	4.18± 0.27^{A}	3.23± 0.38^{A}
Color (L)						
Treatment	31.6± 3.3	31.2± 0.5^{B}	29.8± 0.5^{B}	29.7± 0.5^{B}	30.3± 0.6^{B}	29.2± 0.9^{A}
Control	31.6± 3.3	29.4± 0.5^{A}	27.1± 0.5^{A}	27.2± 0.5^{A}	28.0± 0.6^{A}	27.6± 0.9^{A}
Color (a)						
Treatment	30.9± 2.1	30.4± 0.7^{B}	30.8± 0.7^{B}	30.1± 0.7^{A}	30.9± 0.9^{A}	28.6± 1.3^{A}
Control	30.9± 2.1	28.2± 0.7^{A}	28.7± 0.7^{A}	28.4± 0.7^{A}	28.6± 0.9^{A}	28.7± 1.3^{A}
Color (hue)						
Treatment	29.9± 2.4	30.0± 0.6^{B}	30.6± 0.6^{B}	30.7± 0.6^{B}	30.7± 0.8^{B}	29.7± 1.1^{A}
Control	29.9± 2.4	27.7± 0.6^{A}	27.0± 0.6^{A}	27.8± 0.6^{A}	27.7± 0.8^{A}	27.1± 1.1^{A}
Firmness (g)						
Treatment	288± 20	293± 12^{B}	307± 12^{A}	282± 12^{A}	336± 15^{B}	249± 22^{A}
Control	288± 20	250± 12^{A}	283± 12^{A}	283± 12^{A}	226± 15^{A}	197± 22^{A}
Yeast and Mold (Log CFU/g)						
Treatment	2.11± 0.81	1.53± 0.27^{B}	1.87± 0.81^{B}	2.04± 0.27^{B}	1.56± 0.88^{B}	2.34± 0.27^{B}
Control	2.11± 0.81	2.20± 0.44^{A}	3.30± 0.81^{A}	3.27± 0.44^{A}	3.62± 0.81^{A}	3.67± 0.44^{A}
Aerobic Plate Count (Log CFU/g)						
Treatment	2.96± 1.47	2.18± 1.03^{A}	2.30± 0.79^{A}	3.00± 1.47^{A}	2.26± 1.03^{B}	3.72± 0.79^{A}
Control	2.96± 1.47	3.38± 1.09^{A}	3.20± 1.47^{A}	3.86± 1.09^{A}	4.80± 1.47^{A}	5.15± 1.09^{A}
Phenolics (mg gallic acid)						
Treatment	14.6± 1.8	14.8± 0.6^{A}	13.1± 0.6^{A}	10.3± 0.6^{A}	11.3± 0.6^{A}	8.2± 1.2^{B}
Control	14.6± 1.8	14.6± 0.6^{A}	14.3± 0.6^{A}	11.8± 0.6^{A}	11.6± 0.6^{A}	14.5± 1.2^{A}
Oxygen Radical Absorbance Capacity (ORAC) (umol TE/100g)						
Treatment	316± 55	347± 18^{A}	315± 18^{A}	289± 18^{A}	344± 22^{A}	294± 32^{A}
Control	316± 55	356± 18^{A}	320± 18^{A}	317± 18^{A}	321± 22^{A}	353± 32^{A}
Ferric Reducing Ability of Plasma (FRAP) (umol TE/100g)						

Treatment	335± 66	370± 38^{A}	322± 38^{A}	262± 38^{B}	360± 52^{A}	112± 69^{B}
Control	335± 66	397± 38^{A}	400± 38^{A}	397± 38^{A}	345± 48^{A}	305± 69^{A}
% Titratable Acidity						
Treatment	65± 12	69± 3^{A}	69± 3^{A}	70± 3^{A}	58± 4^{A}	66± 6^{A}
Control	65± 12	68± 3^{A}	68± 3^{A}	68± 3^{A}	51± 4^{A}	63± 6^{A}
Soluble Solid Content (°$_{Brix}$)						
Treatment	7.33± 1.27	7.41± 0.55^{A}	8.03± 0.55^{A}	6.57± 0.55^{A}	6.37± 0.69^{A}	6.61± 1.0^{A}
Control	7.33± 1.27	7.78± 0.55^{A}	8.66± 0.55^{A}	7.13± 0.55^{A}	5.17± 0.69^{A}	7.11± 1.0^{A}

*Different superscript letter for each day denotes a significant difference ($P<0.05$) between treatment and control

Table 4-2. Response measurements for strawberries stored at 12℃ over 6 days

Day	0	2	4	6
Respiration (mg CO_2/kg-h)				
Treatment	4.10± 0.56	4.53± 0.36^{A}	5.88± 0.36^{B}	6.24± 0.46^{B}
Control	4.10± 0.56	5.21± 0.36^{A}	7.71± 0.36^{A}	9.34± 0.46^{A}
Color (L)				
Treatment	31.6± 3.3	31.1± 0.5^{A}	31.8± 0.5^{B}	29.8± 0.7^{B}
Control	31.6± 3.3	30.5± 0.5^{A}	29.6± 0.5^{A}	27.9± 0.7^{A}
Color (a)				
Treatment	30.9± 2.1	30.2± 0.6^{A}	30.5± 0.6^{B}	28.9± 0.8^{B}
Control	30.9± 2.1	29.2± 0.6^{A}	28.4± 0.6^{A}	26.5± 0.8^{A}
Color (hue)				
Treatment	29.9± 2.4	29.6± 0.7^{A}	31.2± 0.7^{B}	30.1± 0.8^{A}
Control	29.9± 2.4	29.0± 0.7^{A}	28.6± 0.7^{A}	28.9± 0.8^{A}
Firmness (g)				
Treatment	288± 20	271± 13^{A}	248± 13^{A}	218± 16^{A}
Control	288± 20	276± 13^{A}	229± 13^{A}	176± 16^{A}
Yeast and Mold (Log CFU/g)				
Treatment	2.11± 0.81	2.04± 0.94^{B}	2.00± 1.54^{B}	3.72± 2.65^{A}
Control	2.11± 0.81	2.66± 0.84^{A}	2.68± 0.75^{A}	3.79± 2.14^{A}
Aerobic Plate Count (Log CFU/g)				
Treatment	2.96± 1.47	3.04± 1.03^{A}	2.34± 0.79^{B}	3.51± 3.48^{A}
Control	2.96± 1.47	2.73± 1.09^{A}	4.00± 3.49^{A}	4.57± 1.93^{A}
Phenolics (mg gallic acid)				
Treatment	14.6± 1.8	13.6± 0.5^{A}	11.5± 0.5^{A}	10.5± 0.7^{A}
Control	14.6± 1.8	14.6± 0.5^{A}	11.8± 0.5^{A}	11.4± 0.7^{A}
Oxygen Radical Absorbance Capacity (ORAC) (umol TE/100g)				
Treatment	316± 55	349± 17^{A}	298± 17^{A}	286± 21^{B}
Control	316± 55	351± 17^{A}	297± 17^{A}	347± 21^{A}

	Ferric Reducing Ability of Plasma (FRAP) (umol TE/100g)			
Treatment	335± 66	372± 30^{A}	278± 30^{A}	247± 41^{A}
Control	335± 66	434± 30^{A}	350± 30^{A}	273± 38^{A}
	% Titratable Acidity			
Treatment	65± 12	64± 3^{A}	64± 3^{A}	50± 4^{A}
Control	65± 12	68± 3^{A}	66± 3^{A}	54± 4^{A}
	Soluble Solid Content (°Brix)			
Treatment	7.33± 1.27	6.77± 0.51^{A}	6.97± 0.51^{A}	5.91± 0.65^{A}
Control	7.33± 1.27	7.27± 0.51^{A}	6.63± 0.51^{A}	6.73± 0.65^{A}

*Different superscript letter for each day denotes a significant difference ($P<0.05$) between treatment and control

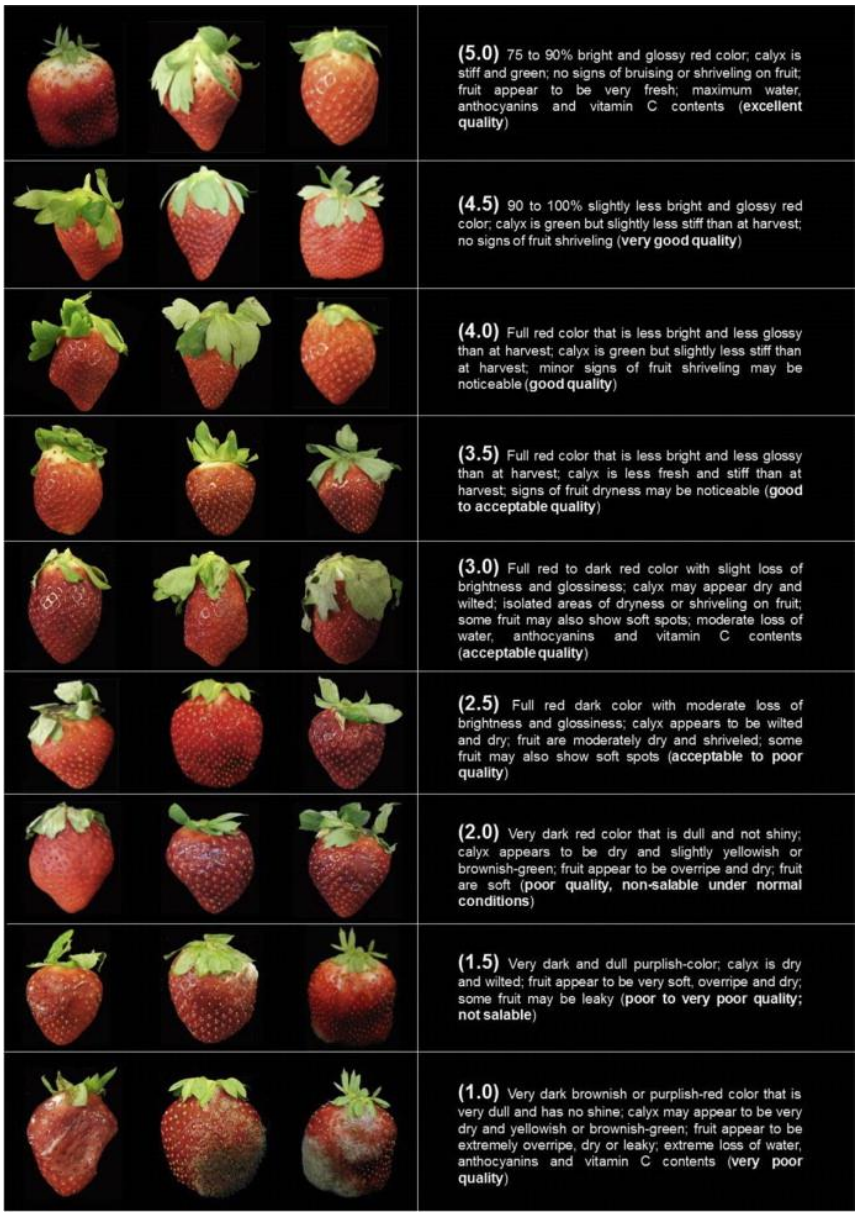

Figure 4-1. Visual quality scale for strawberry showing photographs with subjective quality ratings (1= very poor, 3= acceptable, 5= excellent).

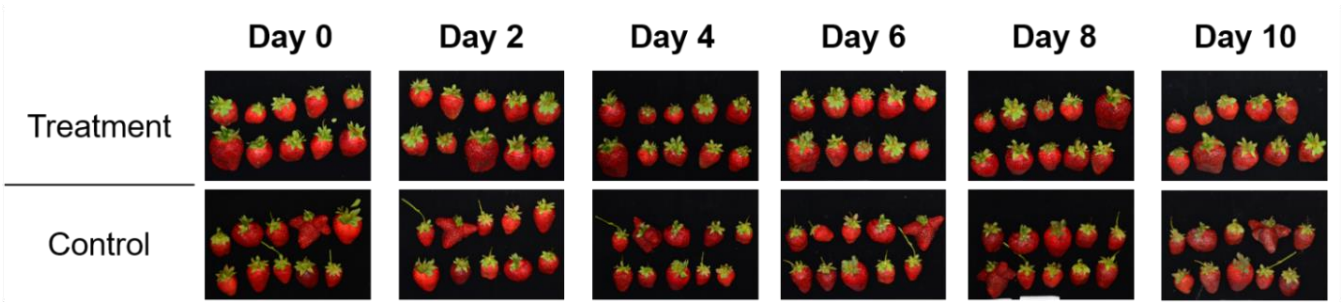

Figure 4-2. Strawberries stored for 10 days at 3℃, treatment (top) shows noticeably less visual degradation than the control sample (bottom).

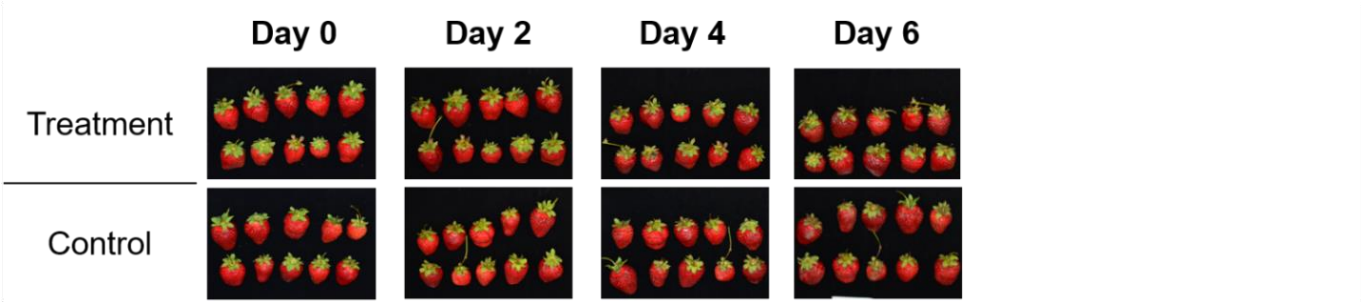

Figure 4-3. Strawberries stored for 6 days at 12°C, treatment (top) shows noticeably less visual degradation than the control sample (bottom).

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Chapter 5 - Willingness of producer to pay for Active Packaging Films to Reduce Postharvest Decay of Strawberries

This chapter was adapted with the permission of the authors from the manuscript of Coffey et al. (2019).

1. Abstract

Specialty crop producers in Kansas and Missouri were surveyed to determine their willingness to pay for new active packaging technology that reduces postharvest loss and potentially extends shelf life. The 52 respondents were asked demographic questions in order to understand production and operation traits. Sixty percent of respondents were under 50 years of age and less than half were male. Smaller operations tend to utilize direct marketing and social media activity more than larger operations. Willingness to pay estimates indicated that producers are willing to pay between $0.24 to $0.36 per cardboard flat to purchase the active film that increases shelf life of strawberries. The average cost of implementing the technology is $0.28 per cardboard flat. There was no statistical relationship between producer or operation characteristics and/or willingness to pay.

2. Introduction

Kansas agriculture is traditionally known for grain and livestock production. However, there is increased interest in specialty crops. The legal USDA definition of specialty crop is "fruits and vegetables, tree nuts, dried fruits and horticulture and nursery crops, including floriculture" that are cultivated for food, medicinal purposes or aesthetic gratification. In 2015 the Kansas Department of Agriculture surveyed specialty crop producers in Kansas and found that 42% had plans to expand. Of those surveyed, 35% have produced some variety of berries in the last three years. Nationally, strawberries are the second largest fresh produce commodity for total sales, after apples (Hinson & Bruchhaus, 2008). Consumption of fresh strawberries in the United States has grown considerably over the past decade, with 2017 per capita annual consumption equal to 8.0 pounds (USDA, 2017).

Berry producers need ways to circumvent the issues of fragility and rapid postharvest decay associated with these popular fruits (Aday & Caner, 2013). It was estimated that in 2010, the amount of postharvest losses in fresh produce from retail and consumers was 31.9 billion pounds (35%). This represented 38.89% ($34.8 billion) of the total food lost in the United States during that year (Buzby et al., 2014). Produce loss can occur during harvest, transportation, production, and in retail facilities. Therefore, the use of intervention technologies during shipping and storage might help reduce produce postharvest loss.

Microbial spoilage can cause significant detrimental issues in produce: appearance, taste, and nutritional quality decline due to postharvest decay, reducing consumers' willingness to purchase berries (X. Wang, Glawe, Kramer, Weller, & Okubara, 2018). Storability and appearance of strawberries directly impact many producers' ability to sell their product, which is directly affected by rates of microbial spoilage, and therefore the profitability of their operations.

One method of mitigating microbial decay in berries is to apply active packaging technologies that protect produce and extend shelf life and appearance, making them more accessible and attractive to consumers. One successful solution includes the combination of edible coatings with storage of freshly picked berries at appropriate storage conditions: the coating can extend the shelf life and freshness of the berries as they are stored and transported (C. Y. Wang, Wang, Yin, Parry, & Yu, 2007; S. Y. Wang, Chen, & Yin, 2010; S. Y. Wang & Gao, 2013). A recent innovation in the area of active packaging allows antifungal packaging films to be placed into cardboard flats for berry harvest. In a previous study we reported the evaluation of the mechanical, physical and anti-fungal properties of packaging films loaded with essential oil (EO) nanoemulsions and their effectiveness to extend shelf-life and enhance strawberries' quality.

The objective of the present survey was to determine the willingness of Kansas and Missouri area produce growers to pay for newly developed antifungal packaging and estimate the impact of relevant farm and producer traits on willingness to pay. Surveys were conducted during regional producer meetings in 2018. Auxiliary data were also collected with the experiment. These data were used to achieve a second objective of providing insight into the characteristics of Kansas and Missouri produce growers, as little is known about this emerging group.

3. Antifungal Film Technology

As above reported, our laboratory previously has evaluated the mechanical, physical and anti-fungal properties of packaging pullulan films loaded with essential oil (EO) nanoemulsions and their effectiveness to extend shelf-life and enhance strawberries' quality (McDaniel et al., 2019). We demonstrated the ability to incorporate active EO nanovesicles into pullulan, a

microbially-derived polysaccharide with excellent film-forming characteristics, to control microbial growth. The formulated packaging films exhibited antimicrobial effectiveness against microorganisms commonly associated with strawberry decay (*Alternaria* spp., *Aspergillus niger* and *Rhizopus stolonifer).* Moreover, when the active systems were used in a field trial where freshly picked strawberries were stored in refrigerated conditions for ten days, respiration and microbial counts were reduced, which indicate potential for shelf life extension, as shown in Figure 1.

4. Methodology

Understanding how much producers will pay for the active film technology is crucial in evaluating the potential effectiveness of antifungal films. If the revenue generated from implementing the technology is greater than the cost to implement, then there is potential for success. However, since the film is not commercially available, it is not possible to directly observe this willingness to pay (WTP). In such cases, some form of the contingent valuation method can be used for non-market valuation of products, product attributes, or labels attributes (Hong, Gallardo, Silva, & Orozco, 2018; McCluskey & Loureiro, 2003). The double bounded dichotomous choice contingent evaluation is one possible method (Hanemann, Loomis, & Kanninen, 1991; Tonsor, Schroeder, & Lusk, 2013) which is appropriate for application to novel foods (Lusk & Hudson, 2004). In this method, 52 survey participants are asked if they would purchase a specific product at a given price. If the answer is *yes*, the question is asked again at a higher price. If *no*, the question is asked again at a lower price. Levels of initial price and product attributes can be varied across respondents to determine how sensitive willingness to pay is to

these factors. In our case, the possible extension of shelf life was varied across survey participants. Figure 2 contains our survey questions.

5. Results

Kansas and Missouri specialty crop producers were surveyed during the summer of 2018 at a series of educational meetings. Fifty-two usable surveys were collected. Sixty percent of the producers were under 50 years of age and 56% were female. Twenty-three % of respondents represented larger operations, indicating an annual revenue of more than $250,000. Direct sales and internet usage were relatively common among respondents. Sixty three percent of the producers reported marketing more than a quarter of their produce via direct sales and 55% said they use social media or the internet for business purposes. Only 25% were certified organic but another 23% reported being in process to achieve this or other certifications (Table 1). Smaller operations attempt to capitalize on using social media and direct marketing more than larger operations (Table 1).

Since proper storage conditions of postharvest produce is the most effective way to mitigate postharvest loss and decay (Gustavsson, Cederberg, Sonesson, van Otterdijk, & Meybeck, 2011), we asked survey participants that were currently growing strawberries to choose among a list of common refrigeration regimes to identify their current practice.

Therefore, results from this question are reported in Table 2. Half (n=26) of the survey participants reported that they currently were growing strawberries, with an additional five respondents saying they did not currently grow strawberries but planned to in the future. Twenty-three survey participants who reported growing strawberries responded to the question about refrigeration. Eleven of those producers engage in the most desirable regime choice offered:

refrigerating at 32 to 37 degrees (F) within an hour of harvest. Only one strawberry producer indicated no refrigeration. Most of those responding to this survey understood the key benefits of storing strawberries at refrigeration temperatures.

In the final section of the survey, we presented producers with a double bounded dichotomous question regarding their willingness to pay for the antifungal film to be used in cardboard flats at harvest (Figure 2). Responses were used, to estimate WTP. Mean WTP for the film is estimated to be $0.39 per cardboard flat. This indicates that implementing this technology in a commercial scale could be feasible as the WTP price is greater than estimated cost of production (the average cost of implementing the technology is $0.28 per cardboard flat).

6. Discussion

This research surveyed Kansas and Missouri produce growers regarding operation characteristics, producer traits, and willingness to pay for a new antifungal packaging film technology that has the potential to extend shelf life of strawberries and decrease loss due to postharvest decay. Results show that many produce farmers are young, with 60% of the sample below 50 years of age. The majority (56%) are also female. Direct marketing and social media are strategies mainly used by smaller operations.

Engaging with this growing population of local specialty crop producers can shape future education and extension efforts. For example, with more than half the producers using social media for business purposes, instruction on how to manage risks and be effective in that space would be useful. Organic certification, which might grant access to niche markets, is not utilized by smaller operations. Of the 22 farms that reported revenue less than $25,000, only 1 was certified organic. The reason for this might be that the cost and initial investment into

certification is too high for such operations. However, a detailed examination of why these smaller operations are hesitant to try for an organic label is needed. This would include estimating the costs and benefits that a farm experiences with organic certification of differing production sizes.

Our estimates of willingness to pay for antifungal packaging film are encouraging, in that they are higher than the cost of production of the film. This indicates at least the potential for commercializing the product. These results should be treated with caution as the cost of production does not factor the initial investment that a producer of the antifungal films will have to pay in order to begin production. Nevertheless, there was no relationship that could be established between days of shelf-life improvement and willingness to pay. This result could be an indicator that survey respondents did not understand the implication of using the antifungal film on their respective operations. However, the fact many of the producers surveyed use aggressive refrigeration regimes (Table 2) suggests that postharvest decay is a risk that they recognize and attempt to manage. Likewise, the high proportion of producers who answered yes to both willingness to pay questions (29 of 38) indicates an interest in mitigating the effects of postharvest loss. Our willingness to pay estimates, ranging from $0.24 to $0.39, are likely biased upward but are indicative of interest in this type of technology.

In summary, this study offers a starting point for understanding Midwestern producers, since it suggests that education regarding marketing methods and calculating costs of production would be helpful. Further, results specific to the antifungal packaging film are promising in that producers are interested but field tests and improved survey methods are needed to help producers understand potential financial benefits of using the technology and, thus, arrive at more precise willingness to pay measures.

Table 5-1. Marketing Practices of Surveyed Produce Growers by Size of Operation**.**

Annual Revenue	Certified Organic	GAP Certified	Uses Social Media for Business	Markets > 25% of Produce Directly
$0 - $25,000	1	0	12	14
$25,000 - $250,000	10	1	11	17
$250,000 - $500,000	0	2	2	1
> $500,000	2	2	1	0
Not Currently Farming	0	0	2	0
Total	13	5	28	32

Notes: GAP (Good Agricultural Practices) certification requires a voluntary audit to verify fruits or vegetables are harvested, handled, and stored in a way to minimize microbial food safety risk. N=51

Table 5-2. Postharvest Storage Practices of Strawberry Producers.

Place strawberries in refrigeration:	Number of Producers
At 32-37 °F, within 1 hour of harvest	11
At 38-45 °F, within 1 hour of harvest	2
At 46-70 °F, within 1 hour of harvest	7
Longer than 1 hour after harvest	2
Never	1

Note: This question specified that only respondents currently raising strawberries should respond. Twenty-three of the 26 farmers reporting that they currently raised strawberries responded.

Figure 5-1. Strawberries with antifungal film (right) and without antifungal film(right) after 8 days of storage at 12°C.

Hello,

We would like to invite you to complete a short research survey. Completing the survey will take about 5 minutes.

Kansas State University researchers have developed a packaging film that can be used in carboard flats to increase shelf-life of strawberries and other produce. The goal of this research survey is to determine how likely it is that producers will use this new packaging technology. We will use information from the survey to help inform future research and development on this film and related technologies to increase the shelf life of strawberries and other produce.

Your identity will be kept completely anonymous in the survey results. Only group comparisons will be made and reported in summary form. Your participation is entirely voluntary, and you may withdraw from participation at any time without penalty

The project is funded by a Kansas Department of Agriculture 2017 Specialty Crop Block Grant.

1. What is your age?

- ☐ 18-29
- ☐ 30-39
- ☐ 40-49
- ☐ 50-59
- ☐ 60+ years

2. What is your gender?

- ☐ Male
- ☐ Female

3. What is your average annual revenue from produce sales?

- ☐ $0-$25,000
- ☐ $25,000-$250,000
- ☐ $250,000-$500,000
- ☐ More than $500,000
- ☐ Not currently farming

4. **Are you currently certified USDA organic?**
   - ☐ Yes
   - ☐ No
   - ☐ No, but working towards being organic certified in the future

5. **Are you currently GAP certified?**
   - ☐ Yes
   - ☐ No
   - ☐ No, but working towards being GAP certified in the future

6. **Do you currently use the internet and/or social media in your personal life?**
   - ☐ Yes, very often
   - ☐ Yes, some
   - ☐ Yes, occasionally
   - ☐ No

7. **Do you currently use the internet and/or social media specifically to promote your business?**
   - ☐ Yes, very often
   - ☐ Yes, some
   - ☐ Yes, occasionally
   - ☐ No

8. **What percentage of your produce do you typically sell directly to consumers through farmers markets, Community Supported Agriculture (CSA), farm stand, U-pick, etc.?**
   - ☐ 100% (all)
   - ☐ Between 25%-75%
   - ☐ 0% (none)
   - ☐ More than 75%
   - ☐ Some, but less than 25%

9. **If you currently raise and sell strawberries, do you refrigerate them? If you do not currently raise strawberries, you may skip this question.**
   - ☐ Yes, we refrigerate them at 32-37°F within 1 hour of harvest
   - ☐ Yes, we refrigerate them at 38-45°F within 1 hour of harvest
   - ☐ We get them into cool storage conditions (between 46-70°F) within 1 hour of harvest
   - ☐ Yes, we do refrigerate them, but it is longer than 1 hour after harvest
   - ☐ No, we do not refrigerate them.
   - ☐ If you use other storage practices, please list them below

10. **Do you currently raise strawberries?**
    - ☐ Yes
    - ☐ No
    - ☐ No, but plan to in the future

Instructions

Please answer the following questions regarding whether you would purchase the packaging film described earlier, given the conditions in the question. You should only answer two questions.

Notes: We estimate the shelf life of strawberries (without the film) to be 6-7 days when stored under optimum conditions (stored at 40°F or less within 1 hour of harvest). Note also that we plan to test this film on other types of produce in the future, but have currently only tested it on strawberries so have asked these questions related to strawberries. If you do not currently raise strawberries or sell all your strawberries through U-pick (and thus do not pick the strawberries that you sell), please complete these questions as if you did sell strawberries and pick them into a flat to sell them.

Would you pay an additional $0.30 per cardboard flat to add a packaging film that is expected to improve the shelf life of strawberries stored in the flat by 2 days when they are stored at 40°F or less?

☐ **Yes** ☐ **No**

*If you answered **Yes**, answer the following:*

Would you pay an additional $0.33 per cardboard flat for a packaging film which is expected to improve the shelf life of strawberries stored in the flat (at 40°F or less) by 2 days?

☐ Yes ☐ No

*If you answered **No**, answer the following:*

Would you pay an additional $0.24 per cardboard flat for a packaging film which is expected to improve the shelf life of strawberries stored in the flat (at 40°F or less) by 2 days?

☐ Yes ☐ No

Thank you for your time. We appreciate your participation in the survey and value your input.

Figure 5-2.: Example of Survey Instructions and Question.

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Chapter 6 - Overall Conclusions

Study 1 showed that adding essential oils (CIN, EUG, and THY) to a biopolymer matrix does not degrade the quality of the pullulan films in any significant way. Study 1 also shows that pullulan films incorporated with essential oils does exhibit antifungal properties against *Alternaria* spp., *Aspergillus niger,* and *Rhizopus stolonifer.* Study 2 provided research in a practical setting where we showed that a popular fresh fruit commodity (strawberries) were exhibiting reduced respiration rates and reduced microbiological counts. There were no significant negative effects of the films on the strawberry's physiology or antioxidant capacity. Organoleptic properties have yet to be researched however and may infer a taste or smell difference for consumers due to the volatile nature of the essential oils. In study 3 we found that Kansas and Missouri farmers are willing to spend up to $0.39 for the active packaging technology per cardboard flat. The estimated cost of the film per cardboard flat is $0.28, which shows that this technology in its current state would be feasible economically for farmers. However, implementing a facility that can mass produce these films at a cost-effective rate will need to be studied further. Pullulan active packaging films are still a new technology, but this research has shown there are many promising aspects that need to be investigated further.

Appendix A - Supplementary Information

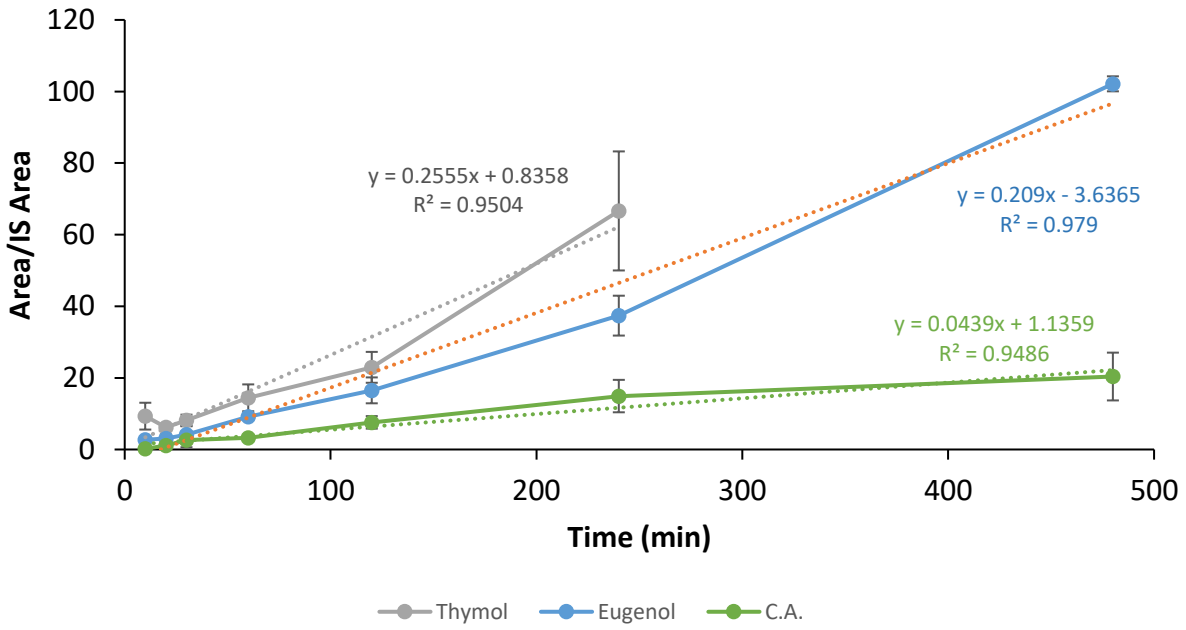

Figure A.1. Cinnamaldehyde, thymol, and eugenol release kinetics from SLN films formulated with 1% EO (Set 2).

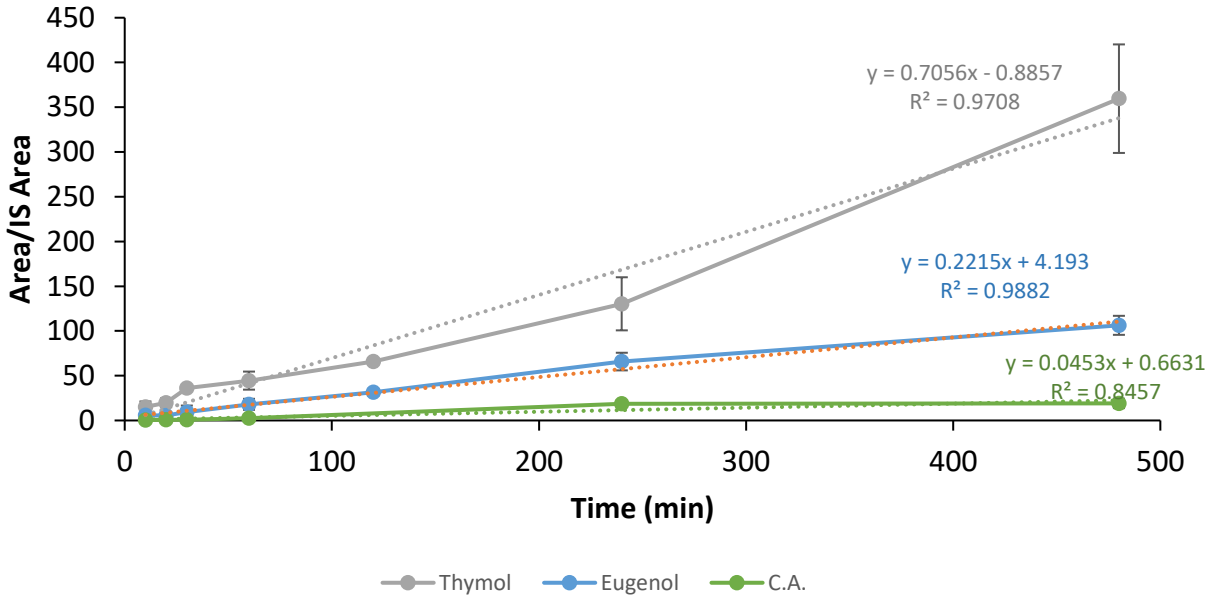

Figure A.2. Cinnamaldehyde, thymol, and eugenol release kinetics from SLN films formulated with 2% EO (Set 1).

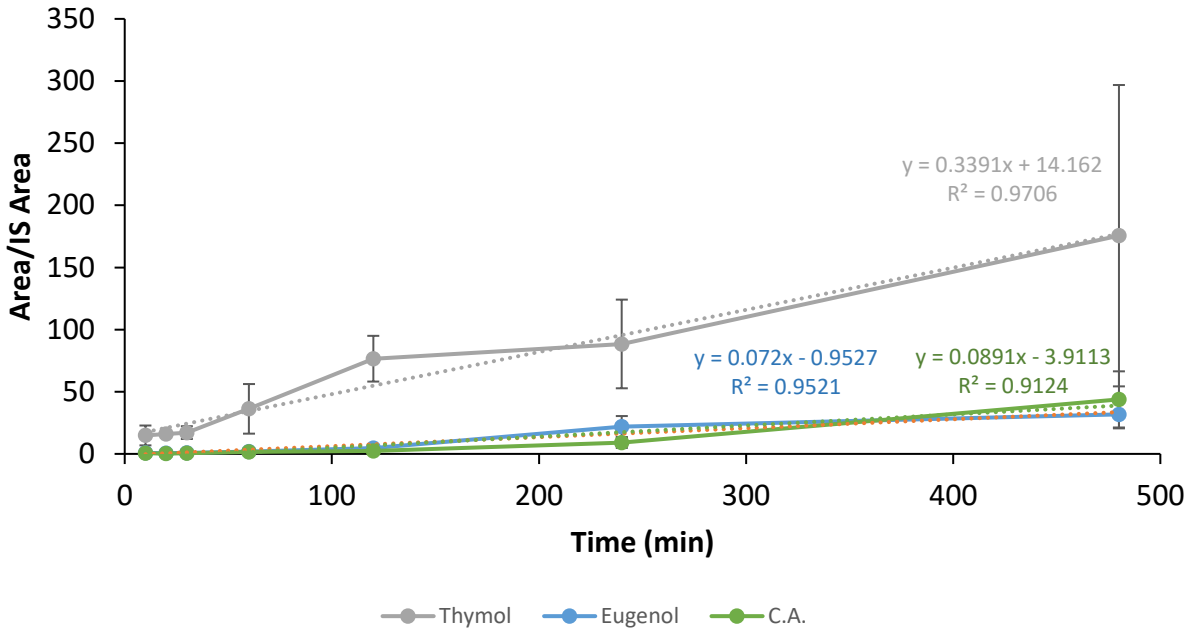

Figure A.3. Cinnamaldehyde, thymol, and eugenol release kinetics from LLN films formulated with 1% EO (Set 2).

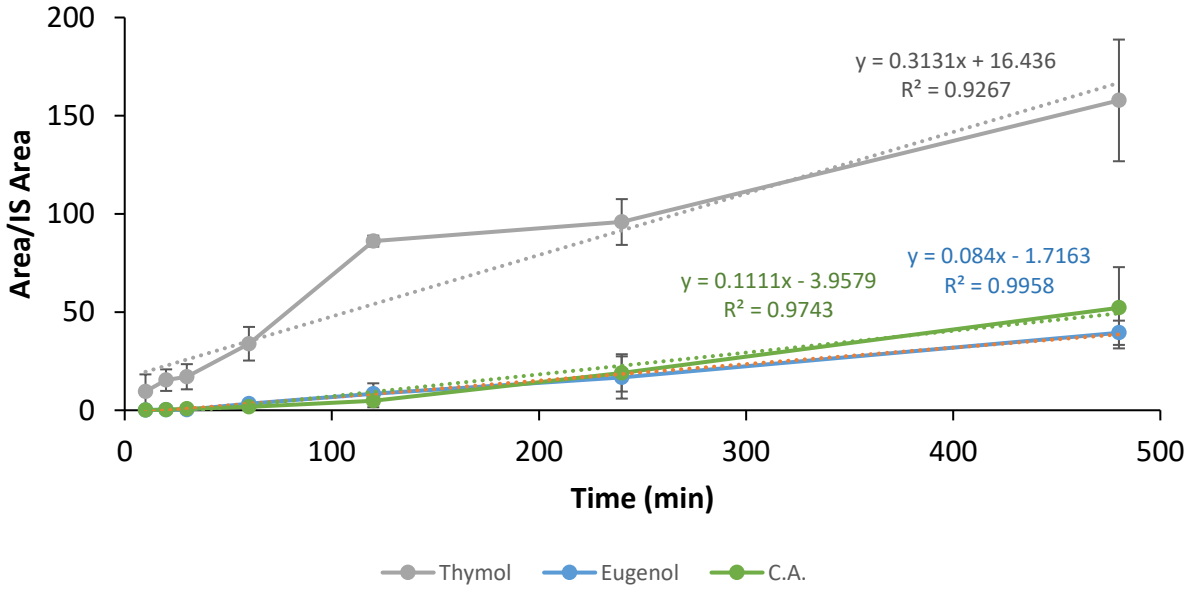

Figure A.3. Cinnamaldehyde, thymol, and eugenol release kinetics from LLN films formulated with 2% EO (Set 1).