ALGINATE-BASED EDIBLE COATING TO ENHANCE QUALITY AND EXTEND SHELF LIFE OF FRESH-CUT WATERMELON (*CITRULLUS LANATUS*)

A Thesis

by

RABIA ESMA SIPAHI

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

August 2012

Biological and Agricultural Engineering

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ABSTRACT

Alginate-based Edible Coating to Enhance Quality and Extend Shelf life of Fresh-Cut Watermelon (Citrullus Lanatus). (August 2012)

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Chair of Advisory Committee: Dr. Elena Castell-Perez

Fresh-cut watermelon is appreciated for its taste, flavor, and juiciness. However, there are challenges in maintaining the freshness since fresh-cut processing of fruits promotes faster deterioration. Our objective was to determine the effectiveness of multilayered antimicrobial edible coating on the shelf-life of fresh-cut watermelon while keeping its original attributes for longer, without affecting its sensory properties. A set of solutions containing sodium alginate (0.5, 1, 2% w/w), beta-cyclodextrin, *trans*-cinnamaldehyde (antimicrobial agent), pectin, and calcium lactate were used as coating systems for fresh-cut watermelon cylinders. The samples were coated by the layer-by-layer dipping technique and stored at 4°C for 15 days. Results were analyzed individually for each quality attribute evaluated, and the best concentration among the solutions to improve each attribute was found. Watermelon quality was measured in terms of texture, color, juice leakage (weight loss), ^{o}Brix, and pH. Microbiological analysis consisted of total coliforms, yeasts and molds, aerobics, and psychrotrophs. A consumer test was carried out (~ 40 panelists)

to support the objective quality data. Panelists scored the samples using a 9-point hedonic scale. Uncoated samples served as controls. Quality tests were conducted at days 1, 3, 7, 12, and 15 of storage. Sensory tests showed high acceptance ($P < 0.05$) of the coated samples when compared to the controls. Quality attributes, particularly texture (firmness) and juice leakage (weight loss) were enhanced ($P < 0.05$) by the coating. Microbiological analysis demonstrated that alginate-based edible coating has a huge effect against microbial growth. 1% sodium alginate coating provided better preservation in terms of quality parameters, microbiological growth, and sensory acceptance. These results indicate that different ratios between solutions present a significant variation for each quality attribute measured in this study; and the thickness of the coating as well as the amount of antimicrobial are critical for shelf-life extension of fresh-cut watermelon. Hence, application of an alginate based multilayered edible coating has tremendous potential to enhance microbial quality and extend the shelf-life of fresh-cut watermelon.

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CHAPTER I

INTRODUCTION

Fresh-cut fruits are becoming more attractive to consumers who are aware of the importance of healthy eating habits, but have less time available for food preparation. Therefore, the economical importance of the fresh-cut fruit industry is becoming progressively more significant (Tapia et. al., 2008). Fresh-cut fruit is a fruit that has been washed, cut, peeled, and sliced before the packaging process. Then, it is ready to eat. Thus, these products are very popular among people who do not have much time to prepare healthy food but have the economic power to consume.

Among these fresh-cut fruits, watermelon sales have increased steadily in the last decade. According to National Watermelon Promotion Board, over the last decade fresh-cut watermelon accounts for more than 10% of all watermelon sales. Fresh-cut watermelons are marketed as quarters, halves with rind, or as cubes without rind. Similar to other fresh-cut fruits, loss of quality of fresh-cut watermelon can be described as loss of texture, color, and sweetness (Rushing et al., 2001). Therefore improving the shelf-life of fresh cut watermelon is important as there is an increasing demand and market share for them.

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However, there are some challenges in processing of fresh-cut fruits and maintaining their freshness to increase their economic value. Fresh-cut processing of fruit and vegetables promotes faster deterioration. Since fruits are living tissues, they could be wounded and undergo enzymatic browning, off-flavor development, texture breakdown, and microbial contamination. The shelf-life of fresh-cut products tends to be very short if they are wounded. Especially, microbial growth and biochemical reactions on wounded and deformed plant tissues are common. Thus, these tissues are responsible for decreasing the safety, shelf-life, and quality of the product in terms of off-flavor, food spoilage, and foodborn illness. In addition, microbial and enzymatic deterioration in fresh-cut products mainly occurs on their wounded surfaces, while the inner part is generally considered sterile. For this reason, a surface treatment, even if not effective on the whole product, could be sufficient to extend the product shelf-life (Manzocco et. al., 2011).

The enhancement of shelf-life of these products is very important as even a few days extension of shelf-life could represent a significant economic advantage for the companies (Manzocco et al., 2011). By providing barrier to moisture, edible coatings are potential systems for the improvement of quality and shelf life of fresh-cut commodities. One major advantage of coatings is that a variety of ingredients can be used and consumed with the food, thus enhancing safety or even nutritional, and sensory attributes. In addition, edible films and coatings can be used as an unique method for including natural or chemical antimicrobial agents, antioxidants, enzymes or probiotics, minerals, and vitamins (Tapia et al., 2007).

The main objective of this study was to evaluate the effectiveness of an antimicrobial agent incorporated into a multilayered edible coating in preventing fresh-cut watermelon from spoilage thus extending its shelf-life. The main goal was achieved after accomplishing the following specific objectives:

1. Identify the optimum alginate-based edible coating composition including β-cyclodextrin-cinnamaldehyde as an antimicrobial agent. Parameters evaluated include layer-by-layer steps using sodium alginate and antimicrobial, calcium lactate and pectin.

2. Evaluate the effect of the coating on the physical, chemical, sensory quality, and microbiological quality of fresh-cut watermelon.

CHAPTER II

LITERATURE REVIEW

2.1. Fresh Produce

2.1.1. Watermelon

Watermelon [*Citrullus lanatus* (Thunb. Matsum. and Nakai] is native to tropical Africa and is a popular thirst-quencher fruit and is mainly available during summer time (Quek et al., 2007). Being low in calories and highly nutritious, it is enjoyed by many people across the world as a fresh-fruit (Tarazona-Diaz et al., 2011). Watermelon is highly cultivated around the world and has a huge economic importance (Artes-Hernandez et al., 2010). The consumption of fresh-cut watermelon has increased at a rate of 20% to 30% annually (Fonseca et al., 2004). The total estimated production of watermelon is approximately 79.2 thousand million tons in the world and China is the largest producer (56.6 thousand million tons) while United States ranked 5th in the world (FAO, 2010). Watermelons are available in different forms, such as sliced, quarters, halves or chunks with rind, or as cubes in plastic containers without rind. They are widely distributed and account approximately 10% of all watermelon sales in the USA (Perkins-Veazie and Collins, 2004; Artes-Hernandez et al., 2010).

Watermelon is rich in some of the major antioxidants, vitamin C, and a good source of lycopene which is responsible for the red color (Oms-Oliu et al., 2009).

According to Perkins-Veazie et al., (2001) and Quek et al., (2007), watermelon has one of the highest lycopene content among all fruits and vegetables.

The lycopene content of watermelon has become very important for consumers as recently lycopene has stimulated attention as a health-promoting antioxidant, associated with lowered risk of coronary heart disease (Oms-Oliu et al., 2009). It has been show that lycopene acts as a free radical scavenger and the highest single oxygen-quenching. It also deactivates DNA-breaking agents related to some cancers (Artes-Hernandez et al., 2010). According to Tarazona-Diaz et al., (2011), consuming fruits and vegetables rich in lycopene significantly decrease incidence of coronary heart disease and provides protection against prostate, kidney (Tarazona-Diaz et al., 2011), breast, digestive-tract, and lung cancers (Perkins-Veazie and Collins, 2004; Quek et al., 2007).

In addition to containing high levels of lycopene, watermelon is also an excellent source of vitamin C and a good source of vitamin A. A 100 g of watermelon provides 8.1 mg of vitamin C and 569 IU vitamin A, corresponding to 13.5% of the daily value for vitamin C and 11.38% of the daily value for vitamin A (Quek et al., 2007). Additionally, it is also a good source of vitamins B, especially B1 and B6, as well as minerals such as potassium and magnesium (USDA, food composition database).

2.1.2. Fresh-Cut Fruits

A fresh-cut fruit is defined as fresh fruit that have been trimmed, peeled, washed and cut into usable form and then bagged and pre-packed (Opara and Al-Ani, 2010).

They are considered minimally processed fruits which refer to any type of fruit that has been physically altered from its original state but remains in a fresh, unprocessed form. They are presented to the consumer in a state that allows for direct and immediate consumption without need for previous preparation or transformation. Ease of consumption is a desirable feature in a fruit like melon that is too large to provide a single portion and requires preparation prior to eating (Amaro et al., 2012).

In the past decade, fresh-cut fruit is one of the most popular and rapidly growing food categories in U.S. supermarkets. They are of great importance in the food industry not only for direct consumption, but also for addition to desserts and use in fruit salads (Quiles et al., 2007). Also the increase in consumers' concern about consuming healthy products, pressure to be in a good physical condition and the awareness of importance of more natural ingredient foods have increased the demand for minimally processed or fresh-cut fruits and vegetables (Marcos et al., 2010; Tarazona-Diaz et al., 2011). Watermelon consumption is associated with their fresh-like quality, convenience and practicality as ready to eat product as well as health benefits due to vitamins, minerals and dietary fiber as well as reducing the risk of cancer and heart disease (Gonzales-Aguilar et al., 2004; Gil et al., 2006; Alandes et al., 2009). Beside health concerns, nowadays consumers have less time available for food preparation which also increases demand of fresh-cut fruits (Olivas and Barbosa Canovas, 2005; Tapia et al., 2008).

According to Beaulieu and Lea, (2007) total fresh cut fruit sales are expected to be $1 billion per year by 2008.

A major drawback of these products is being highly perishable and having shorter shelf-life compared to fresh fruits as they deteriorate faster than their intact counterpart mainly due to the mechanical stress and damage, such as cutting, trimming or removal of their natural protective skin which cause tissue deformation and makes the shelf-life of fresh-cut products shorter (Quiles et al., 2005, 2007; Bizura-Hasid et al., 2011). Additionally, fresh-cut fruit processing brings previously separated enzymes and substrates together and activate enzymes, and enhance microbial attack as a consequence of increase respiration rate. These changes turn out flavor, water, vitamin, and color loss, rapid softening and short shelf-life (Beaulieu et al., 2006). Preserving and maintaining the quality and prolonging the shelf-life are the main problems about fresh-cut fruits which are still under research. Even a few days of shelf-life extension could represent a remarkable advantage for the companies operating in this sector (Manzocco et al., 2011).

Among all fresh-cut fruits, watermelons rapidly gained popularity and a large share of produce market across the United States (Bareuther, 2000). Consumer acceptance of watermelon mostly relies on color, texture, and flavor since good looking is highly accepted as a sign of good taste. Processed fresh-cut watermelons deteriorate rapidly due to the injuries during processing compared with the intact fruits and the shelf-life of fresh-cut watermelon is limited as well as other fresh-cut fruits due to juice leakage, loss of lycopene, and soluble solids. In particular, the most detrimental problems for fresh-cut watermelon are loss of texture, sweetness and color (Mao et al., 2006). Therefore, development of new treatments to overcome

these problems would enhance the quality and safety of fresh-cut watermelon and allow being widely marketed (Mao et al., 2006). Uncut watermelon can be stored at room temperature for a few days but fresh-cut watermelons should be refrigerated and the approximate shelf life is 3 to 5 days.

2.1.3. Quality Attributes

Fruits are living tissues that undergo enzymatic browning, texture decay, microbial contamination and undesirable volatile production if they are wounded (Tapia et al., 2008). The shelf-life of fresh-cut fruit and vegetable products are shortened by mainly two problems namely, browning and texture deterioration.

Color is a critical quality property of many fresh-cut fruit such as pears, apple, bananas, and pineapples (Oms-Oliu et al., 2010). Polyphenol oxidase is an enzyme that naturally found in many fruit cells and responsible for enzymatic browning on the wounded tissues (Alandes et al., 2009). During peeling and cutting process, an undesirable brown color is produced as a result of enzymatic browning reaction due to intermixing of polyphenol oxidase with phenolics compounds. Also, a high respiration rate, induced by tissue wounding, causes faster texture deterioration compared to intact tissues (Dong et al., 2000).

Texture is another important attribute for fresh-cut fruits that determines the acceptance or rejection by the consumers and generally changes over time as a result of tissue stresses during processing (Montero-Calderon et al., 2008). Fruit ripening also causes tissue softening which is a consequence of depolymerization and deesterification of the components in the cell that results in the loss of textural

quality. Textural changes can occur during processing and storage as well. Accordingly, decrease in firmness affect the consumer acceptance of the product, commercial and nutritive value, flavor, taste, smell as well as its shelf-life (Quiles et al., 2007). One of the significant determinants of softening rate is calcium (Ca) levels in the fruit tissue. Calcium application often results in reduced respiration rates and ethylene production, increased firmness and reduced incidence of physiological disorder and decay (Silveria et al., 2011).

In general, the acceptability and quality of fresh-cut fruits limited by water soaking, juice leakage, ethylene production and respiration rate, off odor development, increased microbial growth and spoilage (Saftner et al., 2007). Among these quality attributes, flavor and sugar content are major determinants of watermelon quality and consumer preference (Amaro et al., 2012).

The possibility of using natural compounds to prevent quality loss of these products has been increased since consumers wish to reduce or eliminate chemical additives in food products (Alandes et al., 2009).

2.1.4. Microbial Contamination

There has been a rapid increase in foodborne illness outbreaks linked to fresh produce. One of the largest foodborne outbreaks associated with consumption of fresh produce occurred primarily within the southern states of the U.S. in 2008. The initial cause of the *Salmonella* outbreak was identified as tomatoes (Warriner et al., 2009). Approximately 48 million cases of foodborne illness occur annually and 25%

of these are associated with fresh-cut fruits. Each year these illnesses result in an estimated 128,000 hospitalizations and 3,000 deaths (USFDA).

Microbial load of fresh-cut products depend on several factors including raw material, agricultural practices and conditions of harvesting, and processing. As argued in Corbo et al. (2004) during the minimal processing, skin micro flora could be transferred to fruit flesh. Fresh-cut fruits provide suitable environment for microbial spoilage since microorganisms can grow rapidly upon exposure to nutrients and nutrient rich juices. In addition, cross contamination may occur during cutting and create an environment conductive to growth of microorganism (Gonzales-Aguilar et al., 2004). Intrinsic factors of raw material (water activity, pH, redox potential, nutrients, structures, and antimicrobial agents), as well as extrinsic factors or environmental conditions (temperature, relative humidity, and atmosphere) are very important microbial quality of fresh-cut fruits (Raybaudi-Massilia et al., 2009).

As discussed by Castell-Perez et al.(2004), one of the most serious problems challenging the fresh-cut industry is microbial invasion during marketing. Since fresh cut products contain unprotected cut surfaces, a variety of microorganisms easily find a way to grow rapidly which in turn causes infection and limits the shelf-life.

Microbial quality of fresh products refers to the overall effects of microbial growth, enzymatic and metabolic activity, and also visual quality of foods. The quality of food highly depends on harvesting, handling, transporting, storage, and

marketing conditions. Physical factors including temperature, pH, and moisture also affect metabolic activity of microorganism (Sela and Fallik, 2009). Both the type and the number of pathogenic and spoilage bacteria present on the food surface determine quality, safety, and shelf life of ready-to-eat (RTE) foods. Therefore processing steps such as slicing and packaging operations are major points at which both pathogenic and spoilage organisms can be introduced into RTE foods, such as fresh-cut fruits (Cagri et al., 2004).

The major agents of microbiological spoilage in fruits can be bacteria, as well as yeasts and molds. Although both molds and yeasts are able to grow in fruit tissue, the latter are more often associated with spoilage of cut fruits due to their ability to grow faster than molds (Raybaudi-Massilia et al., 2009).

United States and most European countries have regulations which limit the counts of aerobic microorganisms to 10^6 CFU/g. In particular, some pathogenic microorganisms are not allowed (i.e. *Salmonella*) or are greatly restricted (*E.coli*, *L. monocytogenes*) in ready-to eat foods prepared from raw material. In general, pathogens including *Salmonella* spp. and *Shigella* spp. may often be able to grow on some fruits surfaces such as melon, watermelon, papaya, avocado due of the high pH of the fruit products (Oms-Oliu et al., 2010; Martin Belloso et al., 2006). As discussed in Soliva-Fortuny et al., (2003) acidification of the product surface using citric acid has been widely accepted as effective method in reducing pH. On the other hand, naturally occurring compounds with antimicrobial capacity such as phenols, aldehydes, organic acids, and essential oils have been tested to prove their

effectiveness in fresh-cut fruits. However, since they have strong odors and tastes, their usage is limited (Soliva-Fortuny and Martin-Belloso, 2003).

2.2. Preservation Alternatives

Fresh-cut fruits processing techniques are still under development and nowadays limited methods are available to prolong the shelf-life of fresh-cut products. Most of them are based on the use of modified atmosphere packaging and refrigeration. Rapid cooling treatment after harvest and then storage at low temperatures (0-4°C) also is widely used to maintain the fruit quality. For example modified atmosphere storage (MAP) with low concentration of O^2 (1–5%) and high in CO^2 (5–10%) have been shown to be effective inhibiting growth of most aerobic microorganisims and extend the shelf-life of fresh-cut fruits and vegetables by reducing respiration and ethylene production (Soliva-Fortuny Martin-Belloso, 2003; Fan et al., 2009; Rojas-Grau et al., 2009). Moreover, besides all preservative effects, modified atmosphere packaging (MAP) often results in surface dehydration, or contrarily promotes the formation of water condensates.

As argued in Rojas-Grau et al. (2009), edible coating can also be used either as a compliment or an alternative to MAP. In accordance, development and use of bio-based packaging materials to prolong shelf-life and improve quality of fresh, frozen and formulated food products has been a growing area of interest.

Over the last decade, interest has been rapidly growing in the development and use of bio-based film and coating materilas to improve quality of fresh-cut products. A new concept, in which antimicrobial compounds can be incorporated into

packaging films to maintain high concentrations of preservatives on the surface of foods and to improve storage stability, has been developed (Fan et al., 2009, Campos et al., 2011).

As mentioned before, browning is another major concern to the extension of shelf-life of fresh-cut fruits as it highly affects the consumer's purchase decision. Sulfites had been used for browning prevention. However, their use on fresh-cut fruit and vegetables was banned in 1986 by U.S. Food and Drug Administration (FDA) because of their potential hazard to health (Oms-Oliu et al., 2010). Dipping in anti-browning solutions is one of the alternatives to preserve the initial color of fresh-cut fruits. The most frequent alternative to sulfites is ascorbic acids (AA) which is recognized as a GRAS substance by FDA for its use to prevent browning of fruits and vegetables. The anti-browning effect of AA has been widely demonstrated in several fresh-cut fruits under a wide range of conditions (Soliva-Fortuny and Martin-Belloso, 2003).

Surface treatments involving dipping fruit pieces into aqueous solutions containing antimicrobial agents, antioxidants, calcium salts, or functional ingredients such as vitamins, minerals are widely practiced to improve quality of fresh-cut fruits. A dip treatment after peeling or cutting is the most common way to control browning phenomena in fresh-cut fruit (Oms-Oliu et al., 2010).

In order to preserve the firmness of a fruit, calcium dips have been used as firming agents to extend postharvest life of several products. Addition of calcium can help stabilization of membrane systems and formation of calcium pectate, which

increases rigidity of cell wall and increases resistance to turgor pressure. The correct treatment of the fruit with calcium salts can increase its shelf-life and lenghten storage times and would preserve the quality and firmness of the fruit for longer period (Quiles et al., 2007, Silveria et al., 2011).

Besides all these preservation techniques, recently, ultraviolet-C (UV-C) light has raised large attention. It is a powerful surface germicidal method, safe to apply, easy to use and characterized by favorable costs of equipments, energy and maintenance. It has a great ability to damage microbial DNA and have been used to reduce deterioration, prolong shelf-life and retain the nutritional value of fresh fruits (Duan et al., 2011; Manzocco et al., 2011).

2.3. Edible Coating

Edible films and coatings are defined as a thin layer, which can be consumed, coated on a food or placed as barrier between the food and the surrounding environment. For the past 10 years, research on edible films and coatings in foods is driven by food engineers due to the high demand of consumers for longer shelf–life and better quality of fresh foods as well as environmentally friendly packaging (Siracusa et al., 2008). They are commonly used to protect perishable food products from deterioration by slowing dehydration, providing a selective barrier to moisture, oxygen and carbon dioxide, improving textural quality, reducing microbial growth (Fan et. al., 2009). They provide selective barriers to moisture transfer, oxygen uptake, respiration, lipid oxidation, and the loss of volatile aromas and flavors (Rojas-Grau et al., 2007 and Tapia et al., 2008).

According to their components, edible films and coatings can be divided into three categories: hydrocolloids, lipids, and composites. Hydrocolloids include proteins and polysaccharides or alginates, lipids are constituted by fatty acids, acylglycerols or waxes, and composites are made by combining substances from the two categories (Skurtys et al., 2010; Valencia-Chamorro et al., 2011).

Polysaccharides generally present a good barrier to oxygen at low relative humidity (RH) due to their tightly packed structure and low solubility. Polysaccharide-based coatings have been used to extend the shelf-life of fresh-cut fruits and vegetables by reducing respiration rate and gas exchange due to selective permeabilities to O_2 and CO_2 (Rojas-Grau et al., 2009). One shortcoming of polysaccharides is they provide poor moisture barrier due to their hydrophilic character. Polysaccharide-based edible coatings may include cellulose derivatives; starch and its derivatives, alginate, pectin, and gellan gum (Olivas et al., 2005; Valencia-Chamorro et al., 2011).

The use of edible coatings with antimicrobial properties or with incorporation of antimicrobial compounds is another potential alternative to enhance the safety of fresh-cut produce. The protective function of edible films and coatings may be enhanced with the addition of antioxidants, antimicrobials, nutrients, flavoring agents, coloring agents, and growth regulators that will improve food quality and safety (Tapia et al., 2007; Valencia-Chamorro et al., 2011). Edible films and coatings as carrier of antimicrobial compounds, provide an innovative way to improve safety and extend the shelf-life of fresh-cut fruits.

2.3.1. Sodium Alginate

Alginate is an indigestible biomaterial and highly hydrophilic polysaccharides which is derived from a marine brown seaweed (*Phaeophyceae*). It has unique colloidal and good film-forming properties including thickening, stabilizing, suspending, film forming, gel producing, and emulsion stabilizing. These properties make it useful in food applications as a potential coating component (Oms-Oliu et al., 2008).

Alginate edible-films are appropriate to load additives and antibacterial compounds. Good results are obtained by applying probiotics and oil compounds such as garlic oil and oregano oil (Skurtys et al., 2010). An attractive feature of alginate solutions is the gelling capacity in presence of calcium salts (Olivas et al. 2008). Edible films prepared from alginate can form strong films and exhibit poor water resistance because of their hydrophilic nature (Skurtys et al., 2010).

A wide range of studies demonstrated that alginate-based edible coatings with antimicrobial compounds can reduce bacterial levels and prolong shelf life of fresh-cut apples (Rojas-Grau et al., 2009). Another study by Tapia et al. (2008) suggested that alginate-based edible films seem to be efficient in supporting *Bifidobacterium lactis* on fresh-cut apple and papaya. The use of edible films and coatings with these substances showed a beneficial effect on the human digestive system.

On the other hand, among others, Martin-Belloso et al. (2008) document that an alginate-based coating improved the shelf life of fresh-cut melon in respect to microbiological growth compared to non-coated fruits. Surface treatments by

spraying antimicrobials or dipping fruits into antimicrobial solutions are widely used to prevent microbial growth (Oms-Oliu et al., 2010). In particular, dipping treatments into aqueous solutions containing antimicrobial agents after peeling or cutting has shown to be effective. Thus, edible coatings with antimicrobial agents may provide inhibitory effects against spoilage and pathogenic bacteria (Rojas–Grau et. al., 2009). Coatings improved some quality attributes of fresh-cut melon. Pectin or alginate increased water vapor resistance and prevented desiccation in comparison with uncoated fresh-cut melon. Also, wounding stress of fresh-cut melon can be reduced by coating and best quality attributes maintained (Oms-Oliu et al. 2008).

2.3.2. Pectin

Pectins are common polysaccharides used as a gelling agent in food industry that widely occurs in fruits and vegetables and mainly extracted from apple waste or the peel of citrus fruits. These polysaccharides are potential coating components because of their unique colloidal properties (Oms-Oliu et al., 2008).

Pectic substances are a heterogeneous grouping of acidic structural polysaccharides. This complex anionic polysaccharide is composed of β-1, 4-linked d-galacturonic acid residues naturally esterified with methanol. Mechanical measurements demonstrated that edible films become stiff and not much flexible as pectin amount increased (Skurtys et al., 2010). In another recent work, the results revealed that fresh-cut fruits coated with pectin sustained a significantly slower rate of respiration rate. In addition, quality changes (texture and color) were much lower in pectin coated fruits as compared with the control. Thus, the pectin-based coating

seems to be effective in controlling the spread and severity of stem and rot in many fresh-cut fruits (Campos et al., 2011).

It is also interesting to point out the application of low methoxy pectin (LMP) based edible coatings as a pre-treatment in osmotic dehydration for obtaining better dehydration efficiency. Finally, recently investigations demonstrated the prevention of crumb aging of dietetic sucrose-free sponge cake when a pectin-containing edible film was used. This sponge cake had better preserved freshness, especially up to the fifth day of storage (Skurtys et al., 2010).

2.3.3. Calcium Lactate

Calcium levels in the fruit tissue is related to the softening and firmness of fresh-cut fruits. Calcium treatments can maintain or improve tissue firmness and crispiness of fresh-cut fruit. Calcium salts have been used as firming agents to maintain and improve firmness and crispiness and decrease respiration rate and ethylene production. The most common method to apply calcium salts is immersion and impregnation (Oms-Oliu et al., 2010; Silveria et al., 2011).

According to Oms-Oliu et al. (2010), application of calcium chloride minimized softening of fresh-cut apples and melon. Also the firmness of a wide variety of fruits and vegetables including fresh-cut honeydew melon, lettuce, cantaloupe, and carrots were maintained with calcium salts treatment (Silveria et al., 2011). Moreveor calcium chloride has been widely used to improve or maintain textural attributes of either whole or sliced fresh fruits, and it is commonly used at industrial scale as a firming agent during the canning process of many products (Manganaris et al.,

2005). Also increasing the calcium content in the cell wall of fruit tissue can help to delay softening and mold growth and decrease the incidence of physiological disorders (Hernandez-Munoz et al., 2006; Dong et al., 2000).

Although there are beneficial effects of calcium chloride for the texture of fresh-cut fruits, the drawback of these calcium salts is it may impart to a bitter taste in the product (Alandes et al., 2009; Manganaris et al., 2005). Therefore, other calcium salts such as, calcium lactate, propionate or calcium ascorbate have been investigated as alternative sources of calcium (Oms-Oliu et al., 2010). For example in recent years, calcium lactate has been used as an alternative source of other calcium derivatives in terms of antibrowning agents and acidity regulator, since it does not leave any residual taste in the product. Calcium lactate extended the shelf life of fresh-cut pears, reduced softening during storage, preserved the texture, and improved the mechanical properties of fresh-cut apples. Similarly, peaches treated with calcium lactate were firmer than untreated ones (Oms-Oliu et al., 2010, Alandes et al., 2009).

2.3.4. Plasticizers

Plasticizers are typically small molecules of low molecular weight agents that are incorporated into film-forming materials to decrease the intermolecular forces between polymer chains, enhance properties of the final film and modify the flexibility of edible films, which results in greater film flexibility, decrease film permeability to gases, and toughness (Cagri et al., 2004). Generally, plasticizers are required for polysaccharides (or proteins) based edible films. Their amount added

into hydrocolloid film-forming preparations varies between 10% and 60% by weight of the hydrocolloid. Common food-grade plasticizers used for edible films and coatings include sorbitol, glycerol, mannitol, sucrose, and polyethylene glycol that is important in packaging applications. Also, propylene glycol, polyethylene glycol, fatty acids, and monoglycerides are used as plasticizers in edible films (Cagri et al., 2004; Valencia-Chamorro et al., 2011).

2.4. Antimicrobial Agents

During the fresh-cut processing, physical and chemical barriers of fruits which prevent the growth of microorganisims on the fruit surface are removed. Thus, the fresh-cut fruits are more perishable and vulnerable than their corresponding whole uncut commodities due to physical stress during processing. Edible films and coatings can host nutrients that are lacking or present in low quantities in fruits and vegetables (Cagri et. al., 2004), and as well as are enhanced with incorporation of antimicrobial compounds to provide inhibitory effect against spoilage and pathogenic bacteria (Rojas-Grau et al., 2009).

Antimicrobial packaging is a new potential application of biodegradable materials. Edible films containing antimicrobial agents provide an inhibiting effect for both pathogenic and spoilage organisms (Marcos et al., 2010) on a variety of ready-to-eat (RTE) foods. The overall advantage of antimicrobial edible films and coatings is that the inhibitory agents in these films and coatings can be targeted to post-processing contaminations on the food surface (Cagri et. al., 2004).

There are various types of antimicrobial compounds. Some antimicrobials are the chemical compounds that may delay microbial growth or cause microbial death when they are incorporated into a food matrix. Also other antimicrobial compounds include inorganic (carbonates, bicarbonates, etc.) or organic acids (acetic, benzoic, lactic) and their salts (propionates, sorbates, benzoates, etc.), parabens, chitosan, enzymes, bacteriocins, polypeptides, and plant essential oils (cinnamon, oregano, etc), nitrites and sulphites or other natural extracts (Rojas-Grau et al., 2009; Skurtys et al., 2010; Valencia-Chamorro et al., 2011; Campos et al., 2011).

2.5. Essential Oils

Essential oils (EOs) are aromatic oily liquids obtained from individual or integrated plant material such as: flowers, buds, seeds, leaves, twigs, bark, herbs, wood, fruits, and roots (Campos et al., 2011). They give the odor , aroma, and flavor of spices and herbs. These compounds can be added to edible films in order to enhance the flavor, aroma, odor, and antimicrobial properties. For example essential oils of angelica, anise, carrot, cardamom, cinnamon, cloves, coriander, dill weed, fennel, garlic, nutmeg, oregano, parsley, rosemary, sage, and thymol are inhibitory to various spoilage or pathogenic bacteria, as well as yeasts and molds. Plant essential oils have been studied for their antimicrobial activity against microorganisms including several pathogens. A recent study by Valencia-Chamorro et al., (2011) suggests that shelf-life of fresh-cut melons, from both the microbiological (up to 9.6 days) and physicochemical (more than 14 days) directions, had increased by usage of essential oils of cinnamon, lemongrass and their main active compounds in an

alginate-based edible coating. Also the usage of natural compounds to prevent microbial growth in response to consumer awareness of the use of chemically synthesized additives in foods (Oms-Oliu et al., 2010).

Even though several types of essential oils can be added into edible coatings and films to extend the shelf-life of fresh commodities as an antimicrobial agent, their use in fresh-cut fruits is limited. Currently, organic acids and plant essential oils are the main antimicrobial agents incorporated into edible coatings for fresh-cut fruits. In spite of some good results, their strong flavor could change the original taste of the fresh-cut fruits. As argued in Rojas-Grau et al. (2008), this is the major handicap of using essential oils into edible films and coatings.

2.5.1. Cinnamaldehyde

The use of essential oils to control microbial growth in food has been applied for several products including fresh-cut fruits and vegetables. According to Rojas-Grau et al. (2009), some researches proved that cinnamaldehyde is very effective for reducing the natural flora on kiwi fruit.

The lipophilic character of essential oils and the hydrophilic character of their functional groups are very important in the action of antimicrobial.The rate of activity of essential oils as follows : phenols > aldehydes > ketones > alcohols > hydrocarbons. Cinnamon oil is also a member of phenols (Kalemba et al., 2003).

In this sense, the antimicrobial activities against *E. coli* O157:H7 of several essential oils (oregano, cinnamon, and lemongrass) and oil compounds (carvacrol,

cinnamaldehyde, and citral) incorporated in alginate-apple puree edible film has been shown in a number of study (Campos et al., 2011).

2.6. Application of Edible Coating on Fresh-Cut Fruits

Edible coating process has a remarkable effect on the properties of resulting film structure. Uniformity is one of the significant physical properties of edible coating. Non-uniformity, air bubble and mechanical damages of the food product are some of the defects that should be avoided during coating process (Skuryts et al., 2010).

Dipping and spraying are the most common methods that have been widely used as surface treatments. Spraying can be applied to less viscous coating solutions while it is inconvenient for highly viscous solutions. For this reason dipping techniques can be convenient to supply desired high thickness to the coating. Drying time, drying temperature and drying methods are other different factors that are critical in formation of polymeric films by spraying systems (Skurtys et al., 2010)

According to Campos et al. (2011), solvent casting is one of the most used techniques for edible coatings. The first step for solvent preparation is dissolving and mixing all macromolecules in water, diluted acid or alcohol solution. In some cases heating and stirring are required to dissolve macromolecules and have homogenous solutions. Glycerol, is the most commonly used plasticizer addition which provides the films flexibility and stability for this method. After hydrocolloids are dispersed, some other substances and enhancers, such as antimicrobials, antioxidants, flavorings, colorants can be added to film solution to provide desired properties. According to Ouattara et al. (2000), direct application of antimicrobial agents onto

food surfaces by dipping has proven to be less effective as there is loss of activity because of leaching onto the food, enzymatic activity, and reaction with other food components.

Dipping is also a common and convenient method to apply coating on fruits and vegetables and different theoretical approaches can be used to estimate film thickness from coating solution properties (Snoeijer et al., 2008). In many cases, fruits and vegetables are dipped into film solutions between 1 to 5 minutes. Sometimes it is challenging to have the coating stick to the hydrophilic surface of the cut fruit since hydrophilic surface of fresh-cut fruits do not allow to obtain a good adhesion of coating (Soliva-Fortuny and Martin-Belloso, 2003). This makes the application of edible coatings to minimally processed fruits is a challenging. Multilayer edible coating technique has been used as a good alternative to overcome this problem. More than two layers of film material with nanometer dimensions are used in the layer-by-layer technique to bond physically and chemically to each other (Caruso and Mohwald, 1999; Weiss et al., 2006; Skurtys et al., 2010). In this method, fruits and vegetables are dipped into different solutions prior to peeling and cutting into slices. The excess of coating material from the fruits' surface is allowed to be removed by drying between each dipping steps.

CHAPTER III

MATERIALS AND METHODS

3.1. Raw Material

Watermelons (*Citrullus Lanatus*) were purchased at Farm Patch Produce Market (College Station, Texas), and stored in a refrigerator (10°C, 50% relative humidity) until processing. Total soluble solids (°Brix) readings were used as an indicator of ripeness in the fruit.

3.2. Sample Preparation

Watermelons were sanitized by immersion in chlorine solution (300 ppm). Then rinsed with distilled water and dried at room temperature. All utensils and surfaces used during cutting were also sanitized with 300 ppm chlorine solution. The cleaned fruits were cut into slices with a knife and then cut with a cylindrical instrument into small cylinders of approximately 3 cm in diameter. The length of the cylinders was adjusted to 2.54 cm using a small kitchen knife. Dimensions were measured with a ruler (Figure 3.1).

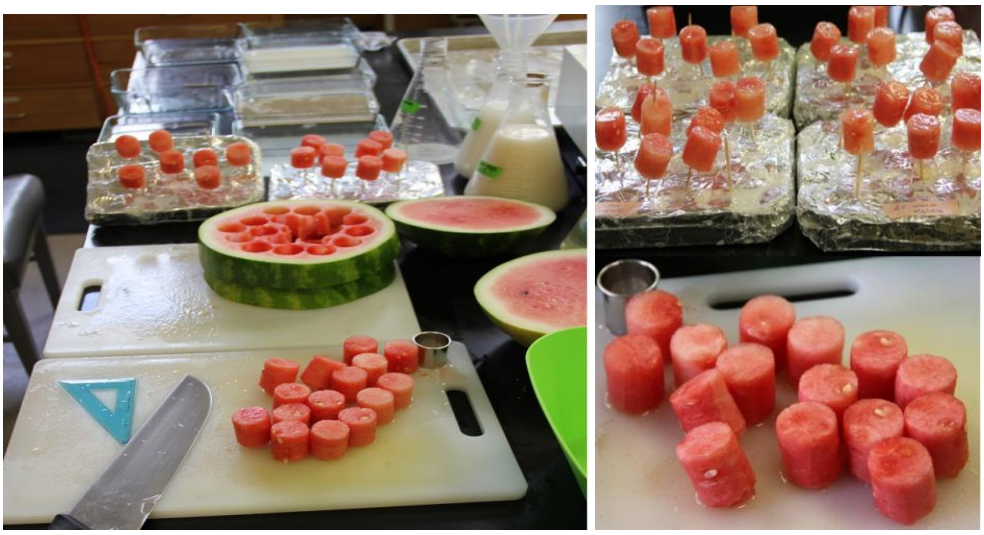

Figure 3.1 Sample Preparation

3.3. Experimental Design

Based on a previous study (Gomes, 2010) the addition of sodium alginate (w/w) may have an effect on the quality of fresh-cut fruit. This study evaluated three different concentrations of sodium alginate (0.5% (w/w), 1% (w/w), and 2% (w/w)) as part of the coating formulation. All treatments had the same concentrations of the encapsulated β-cyclodextrin *trans*-cinnamaldehyde (2% w/w). Uncoated samples served as controls.

3.4. Coating Solutions and Antimicrobial Compound Preparation

3.4.1. Antimicrobial Compound

The inclusion complex of *trans*-cinnamaldehyde in beta-cyclodextrin was used as an antimicrobial agent and prepared by freeze drying. Approximately 2 grams of *trans*-cinnamaldehyde (Food grade, Sigma-Aldrich, St, Louis, MO., USA) and 18.16 g of beta-cyclodextrin hydrate (Alfa-Aesar Johnson Mathey, Lanch, UK) were dispersed in 1 L of aqueous solution and mixed in a laboratory stirrer for 24 h at room temperature. The suspension was filtered through a 0.45 μm nylon filter (VWR vacuum filtration system, VWR international, West Chester, PA, USA) and the filtrate frozen at -20°C and freeze dried at -50°C under 5 mtorr (9.67×10^{-5} psi) vacuum for 48 hours in a Labconco Freeze Dry-5 equipment (Labconco, Kansas City, MO, USA). The lyophilized compound was stored in a freezer at -20°C until further use.

3.4.2. Calcium Lactate Solution

Calcium lactate pentahydrate (USP/NF, Sigma-Aldrich, St. Louis, MO., USA) at 2% w/w was dissolved in sterile distilled water at room temperature.

3.4.3. Pectin Solution

Pectin solution was prepared by adding pectin (USP, Citrus, Spectrum NJ., USA) at 2% w/w in sterile distilled water previously heated at 45°C on a stirring hot plate (Laboratory stirrer/hot plate, Corning, model PC-220, USA) until pectin was completely dissolved.

3.4.4. Sodium-Alginate + Antimicrobial Solution

Film forming solutions were prepared by dissolving alginate (Food grade sodium-alginate, SAFC, St. Louis, MO., USA) at three different concentrations (0.5%, 1%, 2% w/w), and glycerol at 2% w/w (USP, Mallinckrodt Baker, Phillipsburg, NJ., USA). Sodium alginate and glycerol were weighted and dissolved into sterile distilled water heated previously at 45°C and stirred until total dissolution of the components. The antimicrobial compound (2% w/w) was then added to the solution while it continued to be stirred until all components were completely homogenous are suspended.

3.5. Coating Procedure

A five-step procedure (layer-by-layer) was used to ensure the proper coating of the fruit pieces. Watermelon pieces at room temperature (~20°C) were dipped into each coating solution for 2 minutes. Afterwards, the excess coating solution was allowed to drip off for 2 minutes, just before submerging the watermelon pieces for

additional 2 min in the solution of calcium lactate. The subsequent dipping was required to form a solid polymeric matrix around the fruit pieces through the crosslinking of carbohydrate polymers with calcium ions (Oms-Oliu et. al., 2008). The order of the coating solutions was as follows: calcium lactate, sodium alginate + antimicrobial, calcium lactate, pectin, and calcium lactate (Figure 3.2). Control samples (uncoated) were only dipped into sterile distilled water for 2 minutes and then allowed to drip off 2 more minutes before further analysis.

3.6. Shelf-Life Study

Eight coated samples from each treatment were placed into plastic containers (Ziploc®, capacity 591 ml), and stored at 4°C for 15 days (Figure 3.3). Similarly, controls (uncoated samples) were packed and stored at the same conditions. Physical, chemical, microbiological, and sensory quality attributes of coated and uncoated samples were evaluated on days 1, 3, 7, 12, and 15 of storage. These results were used to determine the feasibility of the coating to increase the shelf-life and safety of fresh-cut watermelon.

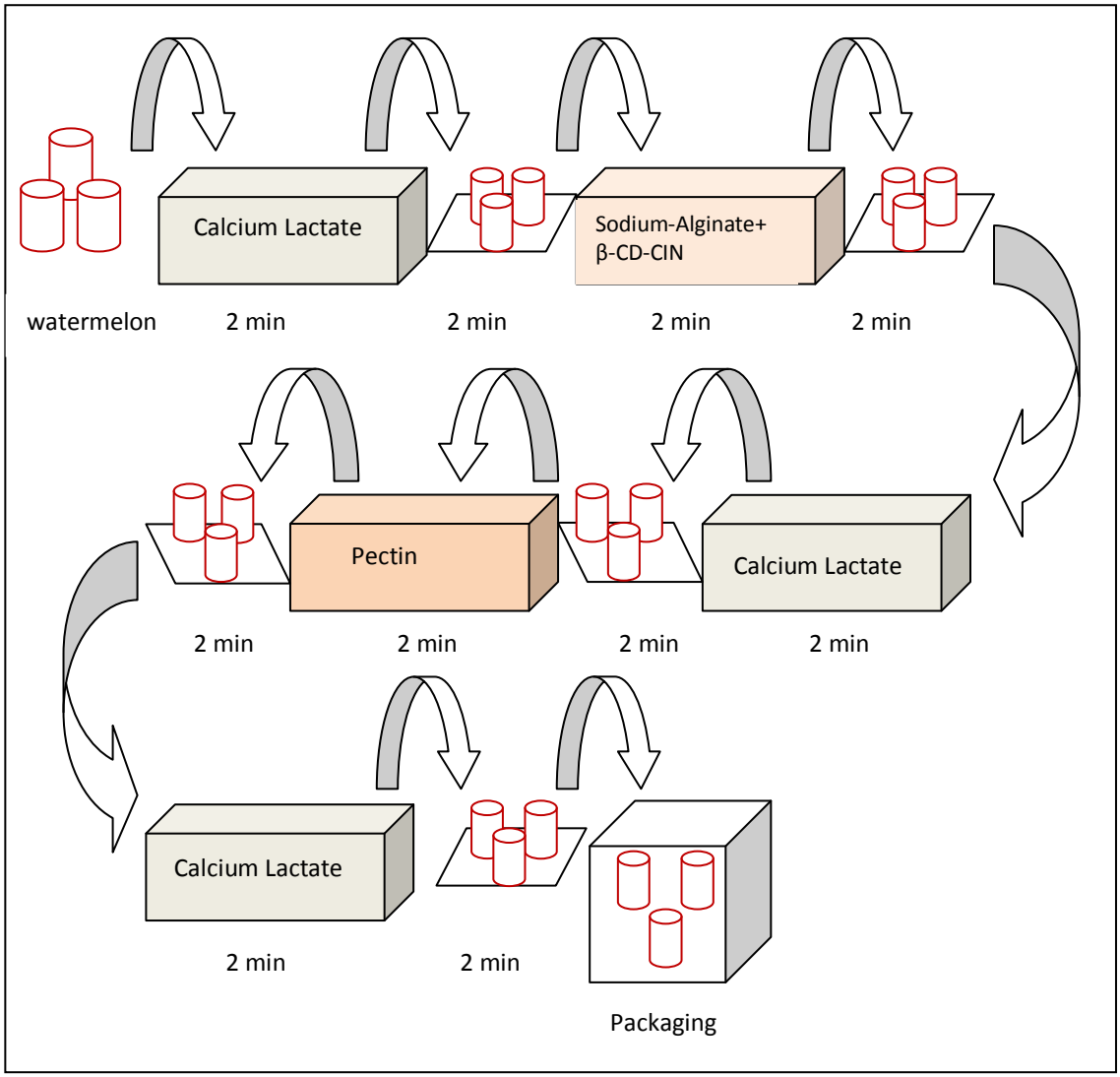

Figure 3.2 Schematic representation of multilayered edible coating

Figure 3.3 Samples packed in Ziploc® container for shelf-life study

3.7. Product Quality Attributes

3.7.1. pH

The pH of watermelon samples was measured using a digital pH meter (Cole Parmer pH meter, pH 500 series, Singapore) previously calibrated with standard

solutions, pH 4, 7, and 10 (AOAC method 981.12). The juice of watermelon cylinders was squeezed to avoid any solid particles from the samples, and the electrode was immersed to record pH readings. The test was carried out in triplicate all treatments (coated samples and controls) at room temperature.

3.7.2. Total Soluble Solids (°Brix)

Total soluble solids concentration in the samples was measured using a refractometer (Reichert Analytical Instrument, 2003 Brix 35HP, Inc., NY., USA) and expressed in °Brix scale. A few drops of watermelon juice were randomly taken and three readings per treatments (coated samples and controls) were recorded at room temperature.

3.7.3. Moisture Content

Moisture content was determined by weight loss after drying in a vacuum oven (Lab-Line Instruments, Inc, IL., USA) at 60°C for approximately 10 hours (AOAC method 920.151). The control and coated samples were randomly taken and analyzed in triplicate at each sampling interval. The samples were first chopped into small pieces and placed in aluminum canisters prior to the drying (Figure 3.4). After removing the samples from the vacuum oven the samples were placed in a desiccator to cool down before recording the final weight. The weight of the canisters was also recorded. The moisture content (MC) in wet basis (w.b.) was calculated as follows:

$$MC\ (w.b.) = \frac{(Mwet - Mdry)}{Mwet} \quad \text{x } 100 \qquad [3\text{-}1]$$

where, M_{wet} (g) is weight of the wet sample and M_{dry} (g) is weight of the dry sample

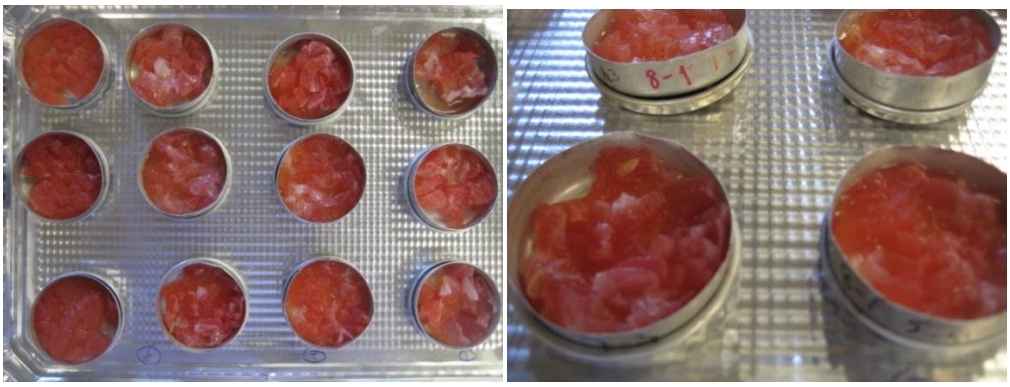

Figure 3.4 Chopped samples were placed in aluminum canisters inside the oven

3.7.4. Juice Leakage

The percentage of juice leakage (weight loss) was determined by recording the weight of three samples per treatment (0.5%, 1%, 2% of sodium alginate, and the uncoated controls) throughout the shelf-life study (15 days). The weight of the containers before and after being filled with the samples was also recorded in addition to the weight of each sample (with and without coating) using a Mettler Toledo Laboratory Balance (PG5002-S, Switzerland). For each day, new samples were tested to avoid cross-contamination. The percentage of juice leakage was calculated as follows:

$$\%\, Juice\ leakage = \frac{(initial\ sample\ weight) - final\ sample\ weight)}{(initial\ sample\ weight)} \qquad [3\text{-}2]$$

3.7.5. Water Activity (a_w)

Water activity measures the availability of free water in a food system that is closely related to physical, chemical, and biological properties of foods (Chirife and Fontan, 1982). Moisture content is different from water activity since it only represents the water composition in a food system. Biochemical reactions occur more in food with high level of water activity. Hence, high level of water activity is an indicator of more free water available for biochemical reactions and thus the shorter the shelf life. A water activity < 0.6 means that the food product is microbiologically stable and spoilage is induced by chemical reactions (Quek et al., 2007).

Measurement of water activity was carried out using a water activity meter (Rotronic intrument corp model: HygroLab 3) at room temperature. The samples were first sliced into thin pieces and placed in a small chamber (Figure 3.5). The water in the chamber air was measured after reaching equilibrium conditions (steady-state) (Aktas and Polat, 2007). Triplicate samples were anlyzed for the coated samples and the uncoated controls. The means were recorded at each sampling interval.

Figure 3.5 Sliced samples placed in chambers to measure water activity

3.7.6. Texture (Firmness)

Texture (firmness) of watermelon samples was evaluated using a CT3 Brookfield Texture Analyzer (Brookfield Engineering Laboratories, Middleboro, MA, USA). Ten samples per treatment coated and uncoated controls were cylindrical in shape with 2.54 cm in height and 3.0 cm in diameter. The samples were subjected to uniaxial compression at a speed of 0.5 mm/s with a cylindrical probe (TA3/100, diameter 5.2 cm) (Figure 3.6). The maximum force to compress the sample down to 50% of its original height (50% strain) was recorded (Figure 3.7). Previous tests confirmed that the 50% strain was sufficient to measure the maximum force at which the sample failed (Figure 3.8).

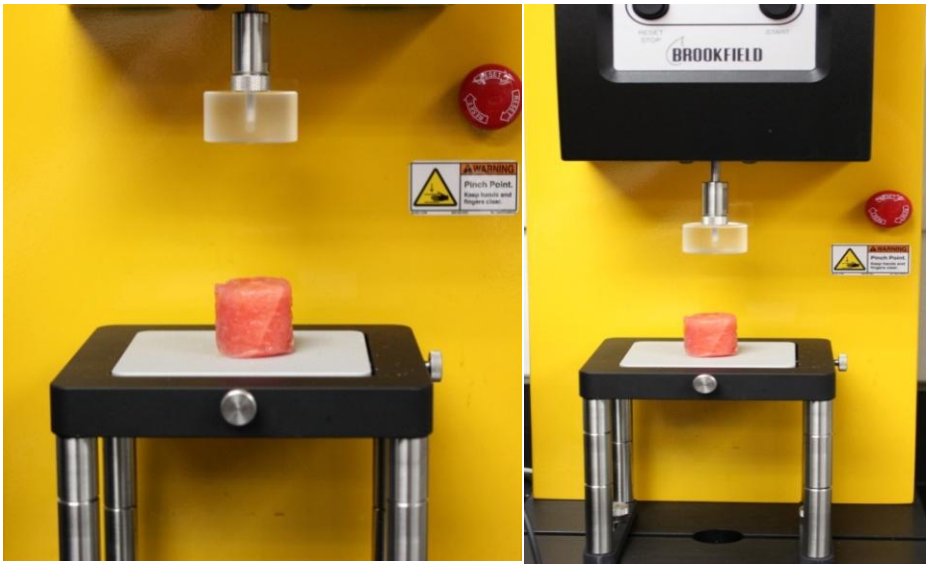

Figure 3.6 Brookfield CT3 with watermelon sample before compression test

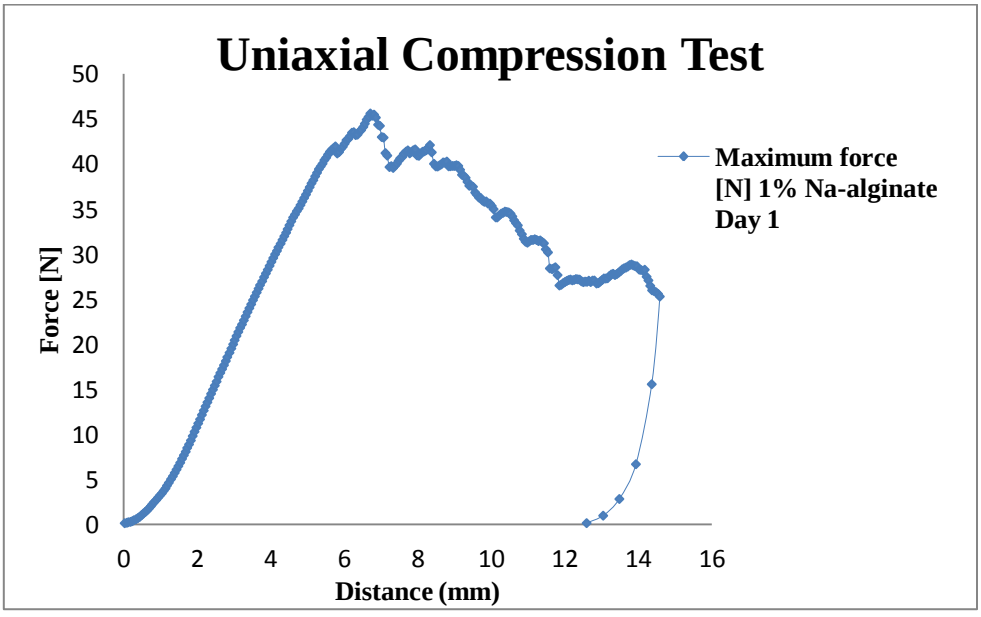

Figure 3.7 Force [N] versus Distance (mm) diagram showing the maximum force when sample fails. Maximum force: highest value of force 45.64 [N] at 6.71 mm distance

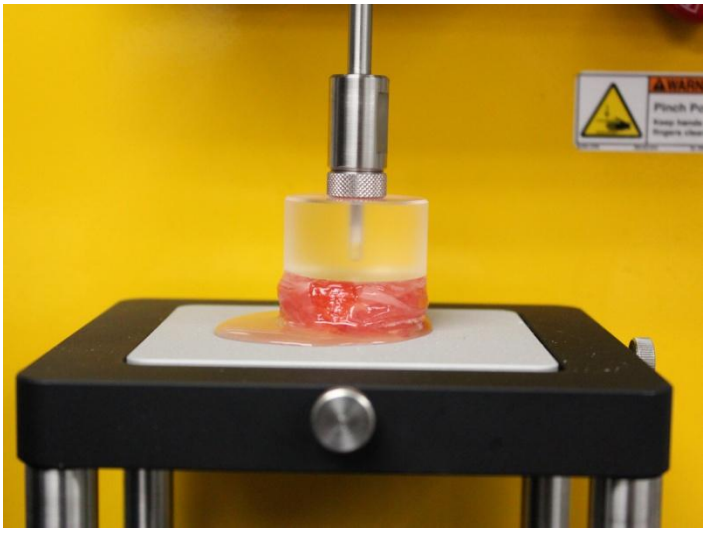

Figure 3.8 Brookfield CT3 with watermelon sample during compression test

3.7.7. Color

The effect of application of the multilayered antimicrobial coating on the color of the fruits throughout the shelf-life study was evaluated using a Labscan XE colorimeter (Hunter Lab, Inc., Reston, VA, USA) calibrated with a standard plate (Y=94.00, x=0.3578, y=0.4567). Readings of L^* (lightness), a^*(green-red chromaticity) and b^* (yellow-blue chromaticity). Five samples per treatment (coated samples and uncoated controls) were evaluated at room temperature.

3.7.8. Microbiological Analysis

Total aerobic plates, psychrotrophic, yeast and molds counts were determined on days 1, 3, 7, 12 and 15 of storage at 4 ºC in triplicate. Under sterile conditions, two watermelon pieces from each treatment were stomached inside a sterile stomacher

bag. A 10 g aliquot of the blended material was transfer to another stomacher bag, mixed with 90 ml of 0.1% buffered peptone water, and homogenized for 1 min; subsequently, 10-fold dilutions were made in these diluents. All counts were performed using petrifilms (3M aerobic plate count and 3M yeast and mold count plates, 3M microbiology, St. Paul, MN). All inoculated 3M aerobic plate count plates (APC) were incubated at 37 ºC for 48h (AOAC official method 990.12); for the psychrotrophics count the APC plates were incubated at 4ºC for 7 days (Brasil et al., 2012), and all 3M yeast and mold count plates were incubated at 20 ºC for 7 days (AOAC official method 997.02). After incubation, colonies were enumerated and results reported as log CFU/g of sample (AOAC, 2010).

3.7.9. Sensory Evaluation

Sensory analysis of coated watermelons is very important and gives a credible idea to show the acceptability of new products (Olivas et al., 2005). Samples were presented to at least forty panelists inside white plastic cups labeled with 3 random digits (Bierhals et al., 2011). Color, odor, flavor, texture, and overall quality were evaluated by the panelists for days 1, 3, 7, 13, and 15 of storage in terms of consumer acceptability. Panelists scored the samples using a nine-point hedonic scale where a score of 1 represents “dislike extremely” and a score of 9 represents “like extremely” (Artes-Hernandez, 2010). Scores higher or equal to 5 were considered acceptable. The panelists evaluated one randomized sample per treatment (4 samples in total). One sample was assigned as the control (uncoated) and the other samples were fruits with alginate-based coating.

3.8. Headspace Analysis

Headspace composition was quantified using a MOCON headspace analyzer (PAC CHECK ™, Model 650, Dual Head Space Analyzer, Minneapolis, Minnesota, USA). It was necessary to create a hermetic package to avoid any gas leakage. For that purpose, glass jars (Mason Jars, 350 ml, USA) with hermetic metal lids were used. The lids were perforated to have a very small hole where the needle of the analyzer could pass through. To seal that hole, a septum was applied to the lid to maintain the system hermetically sealed (Figure 3.9). Three pieces of watermelon fruit per treatment (coated samples and controls) were placed inside the glass containers. The test was performed throughout the 15 days of storage (at days 1, 3, 7, 12 and 15) in duplicate, and for each sampling day, a new container was used to avoid gas losses. The concentrations of oxygen and carbon dioxide (%) inside the container were measured at room temperature.

Figure 3.9 Headspace analysis

3.9. Multilayered Antimicrobial Coating Microscopic Examination

Microscopic observations were performed to evaluate coating uniformity and adherence to the watermelon's surface. Samples from each treatment (0.5%, 1%, and 2 % of sodium alginate) and uncoated controls were analyzed in triplicate at each sampling interval at room temperature. Thickness measurements were randomly taken in different sections of the fruit. Coating thickness was measured based on green color (Assorted food color & egg dye McCormick &Co., Inc., MD-USA) illumination that was first introduced in the pectin solution of the edible coating formulation. The coating thickness was imaged using an Inverted Microscope (Nikon Eclipse TS100**,** Nikon Instruments Inc., NY, USA), illuminated with a pre-centered 6V-30W halogen lamp. Small pieces of the surface were excised with a cork borer (#7, 4-mm diameter) and a transversal cut made using a stainless steel blade. Sample surfaces were observed with 10X magnification lens (numerical aperture 0.25) and

the images were analyzed and recorded with software NIS-Elements BR 3.2 (Nikon Instruments Inc., NY-USA).

3.10. Statistical Analysis

Data analysis was performed using SAS® software (SAS 9.2. for Windows, 2010; SAS Institute Inc., Cary, NC, USA). The effect of presence of the antimicrobial coating on watermelon quality and shelf-life was evaluated. Differences between variables were tested for significance by one-way analysis of variance (ANOVA) using Tukey's test with a randomized block experimental design with four treatments for all the quality and sensory analysis, and three repetitions. The statistical significance was expressed at the 95% significance level ($P < 0.05$).

CHAPTER IV

RESULTS AND DISCUSSION

Three concentrations of sodium alginate (0.5, 1 and 2% w/w) with 2% w/w pectin and 2% w/w antimicrobial were tested to determine whether the concentration of alginate would have any significant effect on the fruit's chemical properties (pH, total soluble solids (ºBrix), moisture content, juice leakage, and water activity), quality attributes (color, texture, and microbiological quality), composition of the atmosphere inside the containers (headspace analysis), and sensory attributes (color, odor, texture, flavor, and overall quality). Uncoated fresh-cut pieces served as control.

4.1. Effect of Multilayered Edible Coating Sodium Alginate Concentration On the Chemical Properties of Fresh-Cut Watermelon

4.1.1. pH

Saftner et al. (2007) states that pH and total soluble solids in watermelon tissue are generally considered as indicators of fruit ripening and tend to increase upon ripening. In this study, the pH of the fruit remained constant throughout the shelf-life study (Table 4.1) and there was no significance ($P > 0.05$) among treatments (See Figure B.1 in Appendix B). Hence, the different formulations of antimicrobial edible coating did not ($P > 0.05$) affect the pH value of the samples by through shelf-life.

4.1.2. Total Soluble Solids (ºBrix)

All the samples, including the (uncoated) controls, had slightly higher values of total soluble solids by day 15 (Table 4.2) (See Figure B.2 in Appendix B). Although the uncoated fruits had higher °Brix by the end of storage, the overall effect of the coating was negligible ($P > 0.05$). Thus, application of multilayered antimicrobial coating did not aid in delaying the ripening process (lower °Brix). These results were expected taking into consideration that watermelon is a non-climacteric fruit; and its sugar content will not change drastically during the fruit's shelf-life (Paul and Chen, 2003).

4.1.3. Moisture Content

Moisture content of fresh-cut coated samples and uncoated controls ranged between 0.88 and 0.92 % (w.b.) by day 15 (Table 4.3). Moisture content of the control significantly ($P <0.05$) decreased during storage while the moisture content of all coated samples remained constant (See Figure B.3 in Appendix B). Thus, the application of the multilayered antimicrobial coating showed benefits reducing loss of moisture and weight.

Table 4.1.Effect of multilayered antimicrobial coating on pH of fresh-cut watermelon stored at 4°C for 15 days.

pH				
Time (days)	Control	0.5% Sodium Alginate	1% Sodium Alginate	2% Sodium Alginate
1	$_{x}5.23^{a}$ $^{*}(0.17)$	$_{x}5.25^{a}$ (0.14)	$_{x}5.13^{a}$ (0.28)	$_{x}5.13^{a}$ (0.24)
3	$_{x}5.13^{a}$ (0.18)	$_{x}5.26^{a}$ (0.23)	$_{x}5.19^{a}$ (0.22)	$_{x}5.19^{a}$ (0.11)
7	$_{x}5.42^{a}$ (0.10)	$_{x}5.24^{a}$ (0.07)	$_{x}5.29^{a}$ (0.07)	$_{x}5.23^{a}$ (0.15)
12	$_{x}5.49^{a}$ (0.12)	$_{x}5.44^{a}$ (0.07)	$_{x}5.33^{a}$ (0.34)	$_{x}5.80^{a}$ (0.35)
15	$_{x}5.22^{a}$ (0.38)	$_{x}5.50^{a}$ (0.14)	$_{x}5.30^{a}$ (0.18)	$_{x}5.74^{a}$ (0.57)

Values are means of 3 replications.
*Standard deviation
^{a,b}Values within a column followed by a common superscript letter indicate that mean values are not significantly different ($P < 0.05$).
^{x,y}Values within a row followed by a common subscript letter indicate that mean values are not significantly different ($P < 0.05$).

Table 4.2.Effect of multilayered antimicrobial coating on total soluble solids (◦Brix) of fresh-cut watermelon stored at 4°C for 15 days.

Total Soluble Solids (◦Brix)				
Time (days)	Control	0.5% Sodium Alginate	1% Sodium Alginate	2% Sodium Alginate
1	$_{x}7.53^{a}$	$_{x}6.67^{a}$	$_{x}6.77^{a}$	$_{x}7.27^{a}$
	*(1.18)	(1.15)	(1.16)	(0.64)
3	$_{x}7.33^{a}$	$_{x}6.93^{a}$	$_{x}6.93^{a}$	$_{x}6.27^{a}$
	(1.81)	(1.01)	(0.50)	(0.12)
7	$_{x}10.40^{a}$	$_{y}6.73^{a}$	$_{y}6.87^{a}$	$_{y}5.93^{a}$
	(1.56)	(1.10)	(1.03)	(0.12)
12	$_{x}9.00^{a}$	$_{x}6.20^{a}$	$_{x}7.00^{a}$	$_{x}7.80^{a}$
	(0.40)	(1.00)	(1.73)	(1.93)
15	$_{x}8.80^{a}$	$_{x}7.87^{a}$	$_{x}7.13^{a}$	$_{x}7.80^{a}$
	(2.03)	(0.76)	(0.83)	(1.59)

Values are means of 3 replications.
*Standard deviation
^{a,b}Values within a column followed by a common superscript letter indicate that mean values are not significantly different($P < 0.05$).
^{x,y}Values within a row followed by a common subscript letter indicate that mean values are not significantly different ($P < 0.05$).

4.1.4. Juice Leakage (Weight Loss)

Application of the multilayered antimicrobial coating was effective in preventing juice leakage (Table 4.4). The uncoated control samples had significantly ($P < 0.05$) higher losses than the coated watermelons. Juice leakage of the control and the samples coated with 0.5% w/w sodium alginate increased significantly ($P < 0.05$) throughout storage (See Figure B.4 in Appendix B). The concentration of alginate had a significant effect on juice leakage on day 15 with the samples with 2% sodium alginate-based coating having the lowest percentage of juice leakage; on the other hand, application of coating with 1% yielded similar results.

4.1.5. Water Activity (a_w)

Water activity of (uncoated) controls and coated watermelons ranged between 0.96 and 0.97 through the storage period (Table 4.5). Water activity of controls and samples coated with the 2% sodium alginate formulation were not significantly ($P > 0.05$) different throughout storage whereas the samples coated with the 0.5% and 1% sodium alginate formulations had significantly lower ($P < 0.05$) values by day 15 (See Figure B.5 in appendix B).

Table 4.3.Effect of multilayered antimicrobial coating on moisture content of fresh-cut watermelon stored at 4°C for 15 day

Moisture Content (w.b.)				
Time (days)	Control	0.5% Sodium Alginate	1% Sodium Alginate	2% Sodium Alginate
1	$_{x}0.92^{a}$ *(0.01)	$_{x}0.92^{a}$ (0.01)	$_{x}0.94^{a}$ (0.01)	$_{x}0.93^{a}$ (0.02)
3	$_{x}0.92^{a}$ (0.01)	$_{x}0.93^{a}$ (0.01)	$_{x}0.93^{a,b}$ (0.01)	$_{x}0.93^{a}$ (0.02)
7	$_{x}0.89^{b}$ (0.02)	$_{x,y}0.92^{a}$ (0.02)	$_{y}0.93^{a,b}$ (0.01)	$_{y}0.93^{a}$ (0.01)
12	$_{x}0.88^{b}$ (0.01)	$_{x,y}0.92^{a}$ (0.02)	$_{y}0.93^{a}$ (0.02)	$_{y}0.92^{a}$ (0.02)
15	$_{x}0.88^{b}$ (0.01)	$_{x,y}0.90^{a,b}$ (0.01)	$_{y}0.91^{a}$ (0.01)	$_{y}0.92^{a}$ (0.01)

Values are means of 3 replications.
*Standard deviation
[a,b]Values within a column followed by a common superscript letter indicate that mean values are not significantly different ($P < 0.05$).
[x,y]Values within a row followed by a common subscript letter indicate that mean values are not significantly different ($P < 0.05$).

Table 4.4.Effect of multilayered antimicrobial coating on percentage juice leakage of fresh-cut watermelon stored at 4°C for 15 days.

Juice Leakage (%)				
Time (days)	Control	0.5% Sodium Alginate	1% Sodium Alginate	2% Sodium Alginate
1	$_{x}4.12^{a}$ (2.40)	$_{x}3.01^{a}$ (1.39)	$_{x}2.20^{a}$ (0.74)	$_{x}2.79^{a}$ (0.59)
3	$_{x}8.42^{a,b,c}$ (2.49)	$_{x,y}5.6^{a}$ (0.41)	$_{y}3.76^{a}$ (0.51)	$_{y}2.49^{a}$ (0.94)
7	$_{x}7.08^{a,b}$ (1.48)	$_{x,y}3.75^{a}$ (1.19)	$_{x,y}3.15^{a}$ (2.16)	$_{y}1.92^{a}$ (0.94)
12	$_{x}12.31^{b,c}$ (4.09)	$_{y}3.48^{a}$ (0.31)	$_{y}3.95^{a}$ (1.86)	$_{y}3.36^{a}$ (0.98)
15	$_{x}14.29^{c}$ (1.06)	$_{y}10.00^{b}$ (1.14)	$_{z}5.53^{a}$ (1.04)	$_{z}3.37^{a}$ (1.95)

Values are means of 3 replications.
*Standard deviation
[a,c]Values within a column followed by a common superscript letter indicate that mean values are not significantly different ($P < 0.05$).
[x,z]Values within a row followed by a common subscript letter indicate that mean values are not significantly different ($P < 0.05$).

Table 4.5.Effect of multilayered antimicrobial coating on water activity of fresh-cut watermelon stored at 4°C for 15 days.

Water Activity (a_w)				
Time (days)	Control	0.50% Sodium Alginate	1% Sodium Alginate	2% Sodium Alginate
1	$_{x}0.97^{a}$ $^{*}(0.01)$	$_{x}0.98^{a}$ (0.00)	$_{x}0.97^{a}$ (0.01)	$_{x}0.98^{a}$ (0.01)
3	$_{x}0.97^{a}$ (0.02)	$_{x}0.96^{c}$ (0.00)	$_{x}0.98^{a}$ (0.00)	$_{x}0.98^{a}$ (0.00)
7	$_{x}0.98^{a}$ (0.00)	$_{y}0.97^{b}$ (0.00)	$_{x}0.98^{a}$ (0.01)	$_{x}0.98^{a}$ (0.00)
12	$_{x}0.97^{a}$ (0.01)	$_{x}0.97^{b}$ (0.00)	$_{x}0.98^{a}$ (0.00)	$_{x}0.98^{a}$ (0.00)
15	$_{x}0.99^{a}$ (0.00)	$_{y}0.97^{b}$ (0.00)	$_{y}0.97^{a}$ (0.01)	$_{x}0.98^{a}$ (0.00)

Values are means of 3 replications.
*Standard deviation
$^{a, b}$Values within a column followed by a common superscript letter indicate that mean values are not significantly different ($P < 0.05$).
$^{x, y}$Values within a row followed by a common subscript letter indicate that mean values are not significantly different ($P < 0.05$).

4.2. Effect of Multilayered Edible Coating Sodium Alginate Concentration On the Physical Properties of Fresh-Cut Watermelon

4.2.1. Texture (Firmness)

Although all the fruits lost firmness throughout the shelf-life study, the maximum force required to compress the uncoated controls significantly ($P < 0.05$) decreased with time (Table 4.6) while all the coated samples retained their firmness better. On the first day of storage, the average firmness of fruits coated with the 1% and 2% w/w sodium alginate formulations was significantly ($P < 0.05$) preserved when compared with the uncoated controls. In general, although all samples got softer with time (as expected), coated samples were significantly ($P < 0.05$) firmer than the uncoated controls by day 15 (See Figure B.6 in appendix B). The application of the multilayered antimicrobial coating helped preserve the fruits' firmness due to the action of calcium lactate, among other factors.

Regression analysis was also performed to understand the changes in firmness across time among the different treatments. A quadratic model (See Figure B.7 in appendix B) fits best the decrease in firmness with time. According to the results, the quadratic model explains 90%, 97%, and 93% of variation in firmness over time for control, 0.5%, 1%, and 2% coated groups, respectively (Table 4.7). Among the coated samples the results suggest that, over time, firmness decreases while the decrease is most pronounced among the samples coated with 2% alginate. The rate of softness in samples coated with 1%, and 2% decreased in as time passed.

Table 4.6.Effect of multilayered antimicrobial coating on firmness [N] values of fresh-cut watermelon stored at 4°C for 15.

Maximum force (N)				
Time (days)	Control	0.5% Sodium Alginate	1% Sodium Alginate	2% Sodium Alginate
1	$_{x,y}31.28^{a}$ *(7.52)	$_{x}31.09^{a}$ (6.27)	$_{y,z}41.90^{a}$ (4.52)	$_{z}42.32^{a}$ (4.88)
3	$_{x}27.74^{a}$ (3.62)	$_{x}28.46^{a}$ (4.87)	$_{y}41.52^{a}$ (2.49)	$_{y}41.55^{a}$ (6.67)
7	$_{x}23.51^{a,b}$ (3.00)	$_{x}26.19^{a}$ (2.28)	$_{y}37.24^{a}$ (4.55)	$_{y}36.00^{a}$ (3.89)
12	$_{x}23.18^{a,b}$ (4.70)	$_{x}27.44^{a}$ (6.53)	$_{y}35.88^{a}$ (1.23)	$_{y}35.97^{a}$ (3.24)
15	$_{x}16.65^{b}$ (1.66)	$_{x}22.23^{a}$ (4.48)	$_{y}34.92^{a}$ (5.09)	$_{y}36.03^{a}$ (1.72)

Values are means of 10 replications.
*Standard deviation
[a,b]Values within a column followed by a common superscript letter indicate that mean values are not significantly different ($P < 0.05$).
[x,y]Values within a row followed by a common subscript letter indicate that mean values are not significantly different ($P < 0.05$).

Table 4.7. Change in firmness as a function of time (days)

Firmness	Model	R^2
Control	$y = 0.003x^2 - 0.9309x + 31.291$	0.899
0.5% sodium alginate	$y = -0.0028x^2 - 0.4339x + 30.617$	0.750
1% sodium alginate	$y = 0.028x^2 - 0.975x + 43.306$	0.966
2% sodium alginate	$y = 0.0647x^2 - 1.5152x + 44.349$	0.935

y= Average Firmness in N, x = time (day), x^2 =time^2 $(day)^2$

4.2.2. Color

Tables 4.8-4.10 show the values for redness-greenness (a^*), yellowness-blueness (b^*), and lightness (L^*) for coated and uncoated fresh-cut watermelon pieces stored for 15 days at 4°C. The (uncoated) controls had significantly ($P < 0.05$) higher values of a^*(redness-greenness) indicating that they were more reddish in color compared to the coated samples (Table 4.8). Across time within all coated groups, there was a significant ($P < 0.05$) decrease in the values of a^* (See Figure B.8 in Appendix B). This may be due to the fact that the sodium alginate coating formulation was white in color and the red color of watermelon was "diluted" as the more concentrated coating was used. The values of b^* (yellowness-blueness) decreased significantly ($P < 0.05$) across the storage time for 0.5%, 1% and 2% coated samples (Table 4.9). However, there was no significant ($P > 0.05$) change in this parameter for the control group (See Figure B.9 in Appendix B). Between groups, the average b^* values were significantly ($P < 0.05$) higher for controls throughout storage. No significant ($P > 0.05$) differences in L^* (lightness) were observed for (uncoated) controls and sodium alginate coated watermelons by day 15 (Table 4.10) (See Figure B.10 in Appendix B).

Table 4.8. Effect of multilayered antimicrobial coating on (*a**) color parameter values of fresh-cut watermelon stored at 4°C for 15 days.

Color parameter a^*				
Time (days)	Control	0.5% Sodium Alginate	1% Sodium Alginate	2% Sodium Alginate
1	$_{x}19.49^{a}$	$_{x,y}16.54^{a}$	$_{x,y}16.11^{a}$	$_{y}14.38^{a}$
	*(2.01)	(1.82)	(1.38)	(2.29)
3	$_{x}19.33^{a,b}$	$_{y}14.89^{a,b}$	$_{y}14.80^{a}$	$_{y}12.22^{a,b}$
	(2.48)	(1.60)	(0.53)	(1.54)
7	$_{x}16.42^{a,b}$	$_{y,z}11.26^{b}$	$_{y}13.28^{a,b}$	$_{z}9.61^{b}$
	(1.44)	(2.22)	(1.29)	(0.26)
12	$_{x}15.83^{b}$	$_{y}11.50^{b}$	$_{x,y}13.34^{a,b}$	$_{y}9.84^{b}$
	(1.49)	(1.92)	(2.51)	(2.33)
15	$_{x}18.10^{a,b}$	$_{y}12.76^{b}$	$_{y,z}11.28^{b}$	$_{z}9.29^{b}$
	(1.71)	(2.20)	(1.78)	(1.55)

Values are means of 5 replications.
*Standard deviation
^{a,b}Values within a column followed by a common superscript letter indicate that mean values are not significantly different ($P < 0.05$).
^{x,z}Values within a row followed by a common subscript letter indicate that mean values are not significantly different ($P < 0.05$).

Table 4.9.Effect of multilayered antimicrobial coating on (*b**) color parameter values of fresh-cut watermelon stored at 4°C for 15 days.

Color parameter b^*				
Time (days)	Control	0.5% Sodium Alginate	1% Sodium Alginate	2% Sodium Alginate
1	$_{x}10.41^{a}$ $^{*}(0.61)$	$_{y}7.10^{a}$ (1.16)	$_{y}7.15^{a}$ (0.39)	$_{y}7.02^{a}$ (0.84)
3	$_{x}10.51^{a}$ (0.71)	$_{y}6.36^{a,b}$ (0.78)	$_{y}6.21^{a}$ (0.91)	$_{y}5.04^{a,b}$ (1.11)
7	$_{x}9.65^{a}$ (0.61)	$_{y}3.77^{c}$ (1.71)	$_{y}5.68^{a,b}$ (0.62)	$_{y}3.95^{b}$ (1.07)
12	$_{x}8.16^{a}$ (1.57)	$_{y}4.15^{c}$ (0.65)	$_{x,y}6.34^{a}$ (1.80)	$_{y}4.33^{b}$ (1.71)
15	$_{x}9.49^{a}$ (2.03)	$_{y}4.68^{b,c}$ (0.84)	$_{y}4.30^{b}$ (0.48)	$_{y}3.47^{b}$ (0.24)

Values are means of 5 replications.
*Standard deviation
^{a,c}Values within a column followed by a common superscript letter indicate that mean values are not significantly different ($P < 0.05$).
^{x,y}Values within a row followed by a common subscript letter indicate that mean values are not significantly different ($P < 0.05$).

Table 4.10. Effect of multilayered antimicrobial coating on (L*) color parameter values of fresh-cut watermelon stored at 4°C for 15 days.

Color parameter L^*				
Time (days)	Control	0.5% Sodium Alginate	1% Sodium Alginate	2% Sodium Alginate
1	$_{x}30.53^{a}$ *(4.41)	$_{x}30.99^{a,b}$ (2.25)	$_{x}29.90^{a}$ (0.88)	$_{x}31.33^{a}$ (2.90)
3	$_{x}32.28^{a}$ (2.60)	$_{x}33.74^{a}$ (4.24)	$_{x}29.43^{a}$ (2.17)	$_{x}28.75^{a,b}$ (3.36)
7	$_{x}29.91^{a}$ (3.03)	$_{x}29.53^{a,b,c}$ (3.38)	$_{x}29.47^{a}$ (3.98)	$_{x}29.97^{a,b}$ (4.24)
12	$_{x}23.90^{a}$ (5.14)	$_{x}26.19^{b,c}$ (2.61)	$_{x}29.41^{a}$ (4.97)	$_{x}27.56^{a,b}$ (2.75)
15	$_{x}26.10^{a}$ (6.62)	$_{x}24.83^{c}$ (2.01)	$_{x}25.69^{a}$ (1.84)	$_{x}25.60^{b}$ (0.69)

Values are means of 5 replications.
*Standard deviation
^{a,c}Values within a column followed by a common superscript letter indicate that mean values are not significantly different ($P < 0.05$).
^{x,y}Values within a row followed by a common subscript letter indicate that mean values are not significantly different ($P < 0.05$).

4.3. Effect of Multilayered Edible Coating Sodium Alginate Concentration On the Sensory Attributes of Fresh-Cut Watermelon

Results for sensory analysis are presented in Tables 4.11 to 4.15. Color values for coated and uncoated controls decreased throughout storage (Table 4.11). There was a significant ($P < 0.05$) difference between controls and the samples coated with 0.5% and 1% sodium alginate formulations. Samples coated with 2% sodium alginate were ranked lower than the controls and had a score lower than 5.0 by day 13 of storage (See Figure B.11 in Appendix B). A probable cause for this result is that the application of the coating gave the watermelon a different appearance when compared with the control, which may have affected the panelists' perception of good watermelon quality in terms of reddish color. On the other hand, the panelists found samples with both 1% and 2% sodium alginate coating acceptable. This finding is supported by the objective color measurements since the samples with the 2% sodium alginate coating were significantly less reddish in color (lower values of *a color parameter (Table 4.8)).

In terms of odor, there was a significant ($P < 0.05$) difference between uncoated control and coated samples (Table 4.12). Consumers showed a significant preference for the uncoated samples and those coated with 1% sodium alginate formulations until day 7 of storage. Samples coated with 2% sodium alginate formulation received scores under 5.0 making them unacceptable. This result is explained by the fact that the coated samples had a particular odor imparted by *trans*-cinnamaldehyde. The panelists found both the samples with 0.5% and 1% sodium alginate coating

acceptable (scores greater than 5.0) by day 15 of storage (See Figure B.12 in Appendix B).

Similar results were obtained for flavor (Table 4.13). Control samples received the highest values for flavor until day 7 of storage. However, there was no significant ($P > 0.05$) difference between control and coated samples by day 13 of storage (See Figure B.13 in Appendix B). At day 15 of storage, samples with 0.5% and 1% sodium alginate coating were scored as acceptable with scores significantly ($P < 0.05$) higher than those for the uncoated controls.

In terms of texture (Table 4.14), control samples received the highest values for texture until day 7 of storage. After day 7, controls were ranked significantly ($P < 0.05$) under the acceptable score (See Figure B.14 in Appendix B). Panelists scored as acceptable all the samples coated until day 15 of storage. As it was shown in the measurement of firmness (Table 4.6), control samples were significantly ($P < 0.05$) softer throughout storage period and the panelists were able to notice this difference.

In terms of overall quality (Table 4.15), samples coated with 0.5% and 1% sodium alginate formulations were acceptable to the consumers by day 15 of storage (See Figure B.15 in Appendix B). In the case of the 2% sodium alginate coating, the main problems with acceptance were color and odor. Sensory scores of coated watermelons showed benefits in terms of texture and flavor.

Table 4.11. Effect of multilayered antimicrobial coating on color sensory attribute values of fresh-cut watermelon stored at 4°C for 15 days.

Color Sensory Attribute (Hedonic Scale)				
Time (days)	Control	0.5% Sodium Alginate	1% Sodium Alginate	2% Sodium Alginate
1	$_{y}7.28^{a}$ *(1.45)	$_{y}6.45^{a}$ (1.55)	$_{y}6.63^{a}$ (1.67)	$_{x}5.08^{a}$ (1.79)
3	$_{y}7.10^{a}$ (1.71)	$_{y}6.20^{a}$ (1.71)	$_{y}6.63^{a}$ (1.66)	$_{x}4.90^{a}$ (1.77)
7	$_{y,z}6.70^{a}$ (1.49)	$_{y}6.03^{a}$ (1.07)	$_{z}7.00^{a}$ (1.11)	$_{x}4.57^{a,b}$ (1.51)
13	$_{y}5.09^{b}$ (1.29)	$_{z}6.34^{a}$ (1.51)	$_{z}6.28^{a}$ (1.73)	$_{x}3.84^{b}$ (1.50)
15	$_{x}4.77^{b}$ (1.37)	$_{y}6.35^{a}$ (1.37)	$_{y}6.16^{a}$ (1.16)	$_{x}4.26^{a,b}$ (1.48)

Values are means of 40 panelists
*Standard deviation
[a,b]Values within a column followed by a common superscript letter indicate that mean values are not significantly different ($P < 0.05$).
[x,z]Values within a row followed by a common subscript letter indicate that mean values are not significantly different ($P < 0.05$).

Table 4.12. Effect of multilayered antimicrobial coating on odor sensory attribute values of fresh-cut watermelon stored at 4°C for 15 days.

Odor Sensory Attribute (Hedonic Scale)				
Time (days)	Control	0.5% Sodium Alginate	1% Sodium Alginate	2% Sodium Alginate
1	$_{y}7.00^{a}$ *(1.24)	$_{x}5.98^{a}$ (1.58)	$_{x}5.90^{a}$ (1.52)	$_{x}5.55^{a}$ (1.78)
3	$_{y}6.95^{a}$ (1.50)	$_{x}5.83^{a}$ (1.58)	$_{x,y}6.20^{a}$ (1.56)	$_{x}5.35^{a}$ (1.64)
7	$_{z}6.23^{a}$ (1.25)	$_{y}5.37^{a}$ (1.29)	$_{y,z}5.93^{a}$ (1.48)	$_{x}4.33^{b}$ (1.51)
13	$_{x,y}5.06^{b}$ (1.28)	$_{y}5.84^{a}$ (1.40)	$_{y}5.69^{a}$ (1.79)	$_{x}4.75^{a,b}$ (1.36)
15	$_{x}4.84^{b}$ (1.02)	$_{z}5.87^{a}$ (1.15)	$_{y,z}5.55^{a}$ (0.81)	$_{x,y}5.03^{a,b}$ (1.33)

Values are means of 40 panelists
*Standard deviation
[a,b]Values within a column followed by a common superscript letter indicate that mean values are not significantly different ($P < 0.05$).
[x,z]Values within a row followed by a common subscript letter indicate that mean values are not significantly different ($P < 0.05$).

Table 4.13. Effect of multilayered antimicrobial coating on flavor sensory attribute values of fresh-cut watermelon stored at 4°C for 15 days.

Flavor Sensory Attribute (Hedonic Scale)				
Time (days)	Control	0.5% Sodium Alginate	1% Sodium Alginate	2% Sodium Alginate
1	$_{y}8.15^{a}$ *(0.70)	$_{x}6.13^{a}$ (1.64)	$_{x}6.08^{a}$ (1.49)	$_{x}5.75^{a}$ (1.43)
3	$_{z}8.13^{a}$ (0.99)	$_{x,y}5.70^{a}$ (1.76)	$_{y}6.25^{a}$ (1.51)	$_{x}5.30^{a}$ (1.98)
7	$_{y}7.27^{b}$ (0.99)	$_{x}5.60^{a}$ (1.27)	$_{x}5.90^{a}$ (1.71)	$_{x}5.27^{a,b}$ (1.65)
13	$_{x,y}4.88^{c}$ (1.33)	$_{y}5.84^{a}$ (1.63)	$_{y}5.25^{a}$ (2.03)	$_{x}4.22^{b}$ (1.74)
15	$_{x}4.65^{c}$ (1.39)	$_{y}5.71^{a}$ (1.56)	$_{y}5.71^{a}$ (1.02)	$_{x,y}4.90^{a,b}$ (1.64)

Values are means of 40 panelists
*Standard deviation
[a,c]Values within a column followed by a common superscript letter indicate that mean values are not significantly different ($P < 0.05$).
[x,z]Values within a row followed by a common subscript letter indicate that mean values are not significantly different ($P < 0.05$).

Table 4.14. Effect of multilayered antimicrobial coating on texture sensory attribute values of fresh-cut watermelon stored at 4°C for 15 days.

Texture Sensory Attribute (Hedonic Scale)				
Time (days)	Control	0.5% Sodium Alginate	1% Sodium Alginate	2% Sodium Alginate
1	$_{y}7.80^{a}$ *(0.99)	$_{x}6.80^{a}$ (1.30)	$_{x}6.18^{a}$ (1.39)	$_{x}6.13^{a}$ (2.11)
3	$_{y}7.95^{a}$ (0.88)	$_{x}6.23^{a}$ (1.78)	$_{x}6.03^{a}$ (1.54)	$_{x}5.83^{a}$ (1.63)
7	$_{x}6.27^{b}$ (1.18)	$_{x}6.13^{a}$ (1.48)	$_{x}6.63^{a}$ (1.25)	$_{x}5.87^{a}$ (1.35)
13	$_{x}4.94^{c}$ (1.71)	$_{y}6.00^{a}$ (1.71)	$_{y}5.97^{a}$ (1.83)	$_{x,y}5.75^{a}$ (1.28)
15	$_{x}4.42^{c}$ (1.37)	$_{y}6.16^{a}$ (1.42)	$_{y}6.29^{a}$ (1.20)	$_{y}5.68^{a}$ (1.65)

Values are means of 40 panelists
*Standard deviation
[a,c]Values within a column followed by a common superscript letter indicate that mean values are not significantly different ($P < 0.05$).
[x,y]Values within a row followed by a common subscript letter indicate that mean values are not significantly different ($P < 0.05$).

Table 4.15. Effect of multilayered antimicrobial coating on overall quality sensory attribute values of fresh-cut watermelon stored at 4°C for 15 days.

Overall Quality Sensory Attribute (Hedonic Scale)				
Time (days)	Control	0.5% Sodium Alginate	1% Sodium Alginate	2% Sodium Alginate
1	$_{y}7.85^{a}$ *(0.80)	$_{x}6.35^{a}$ (1.55)	$_{x}6.10^{a,b}$ (1.32)	$_{x}5.68^{a}$ (1.42)
3	$_{y}7.78^{a}$ (0.95)	$_{x}5.95^{a}$ (1.54)	$_{x}6.20^{a,b}$ (1.57)	$_{x}5.35^{a}$ (1.72)
7	$_{z}6.97^{b}$ (0.97)	$_{y}6.17^{a}$ (1.13)	$_{y,z}6.37^{a}$ (1.37)	$_{x}5.20^{a,b}$ (1.44)
13	$_{x,y}4.88^{c}$ (1.37)	$_{z}5.94^{a}$ (1.60)	$_{y,z}5.34^{b}$ (1.93)	$_{x}4.28^{b}$ (1.62)
15	$_{x}4.45^{c}$ (1.28)	$_{y}5.77^{a}$ (1.30)	$_{y}5.81^{a,b}$ (0.97)	$_{x}4.77^{a,b}$ (1.33)

Values are means of 40 panelists
*Standard deviation
[a,c]Values within a column followed by a common superscript letter indicate that mean values are not significantly different ($P < 0.05$).
[x,z]Values within a row followed by a common subscript letter indicate that mean values are not significantly different ($P < 0.05$).

4.4. Effect of Multilayered Edible Coating Sodium Alginate Concentration On the Microbiological Quality of Fresh-Cut Watermelon

Figures 4.1 to 4.4 present the results of microbiological analysis for aerobics, psychrotrophics, coliforms, and yeast and mold counts, respectively.

4.4.1. Aerobic Microorganisms

The population of aerobic microorganisms increased significantly ($P < 0.05$) for both controls and coated samples throughout storage since the high moisture content of watermelon provides a suitable environment for their growth during the shelf life study (Figure 4.1). A significant ($P < 0.05$) inhibition was observed on day 7 of storage for all coated samples, and the population of aerobics in the control samples was higher than in the coated samples by day 15 (See Table B.1 in Appendix B). Since the pH of controls and coated samples remained constant during the shelf-life study, there must be growth of another type of microorganisms such as Pseudomonas, a gram negative microorganism that does not affect the pH of samples while growing.

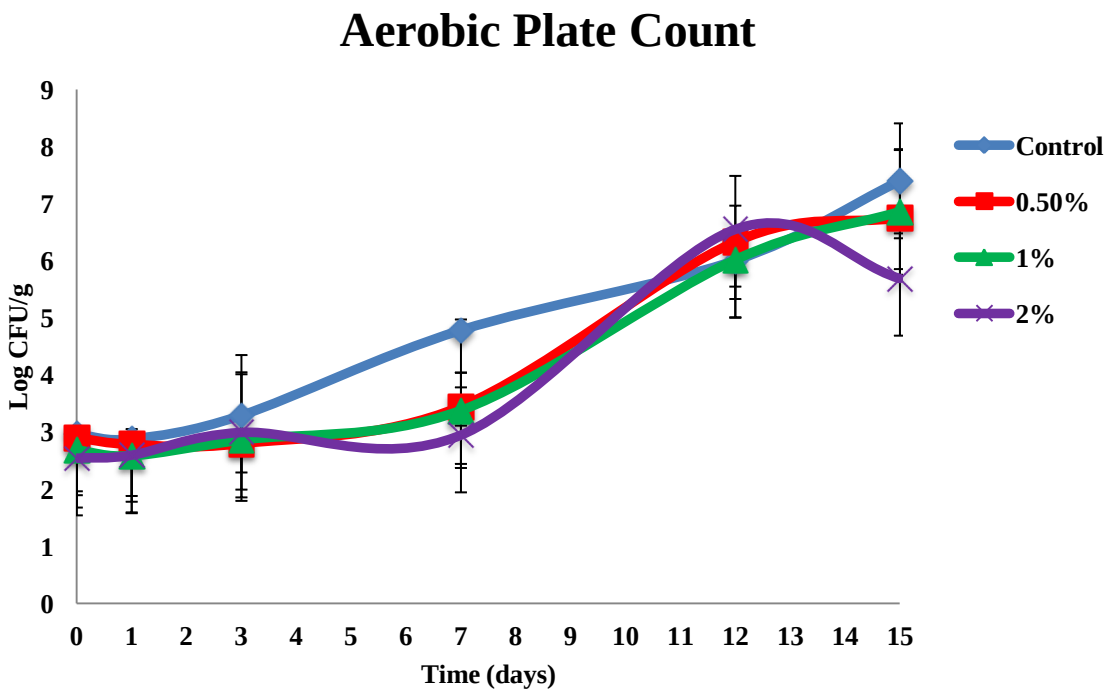

Figure 4.1 Effect of multilayered antimicrobial coating on aerobic plate counts of fresh-cut watermelon stored at 4°C for 15 days.

4.4.2. Psychrotrophic Microorganisms

Similar to aerobic plate counts, the alginate-based coating showed the same effect in the inhibition of psychrotrophic microorganisms (Figure 4.2). Uncoated controls had the highest counts (7.33 log cycles) by day 15 of storage compared to coated samples. The samples with 2% sodium alginate coating had the lowest counts with an approximate decrease of 1.30 log. Significant inhibition was observed on day 7 of storage for samples coated with 1% and 2% sodium alginate formulations. However, no significant ($P > 0.05$) differences were observed among uncoated controls and coated samples by day 15 of storage (See Table B.2 in appendix B).

4.4.3. Coliform Plate Count

The 1% and 2% sodium alginate coating formulations were significantly ($P < 0.05$) more effective in inhibiting the initial population of coliforms (Figure 4.3). By day 12 of storage, application of 1% sodium alginate coatings significantly ($P < 0.05$) inhibited the growth of coliforms by approximately 1.5 log CFU/g compared to the control (5.85 log CFU/g). No significant differences ($P > 0.05$) were found in the final counts between the uncoated control and the coated samples (See Table B.3 in Appendix B). The 1% sodium alginate coating was the more effective treatment to inhibit total coliforms throughout the 15 days of storage.

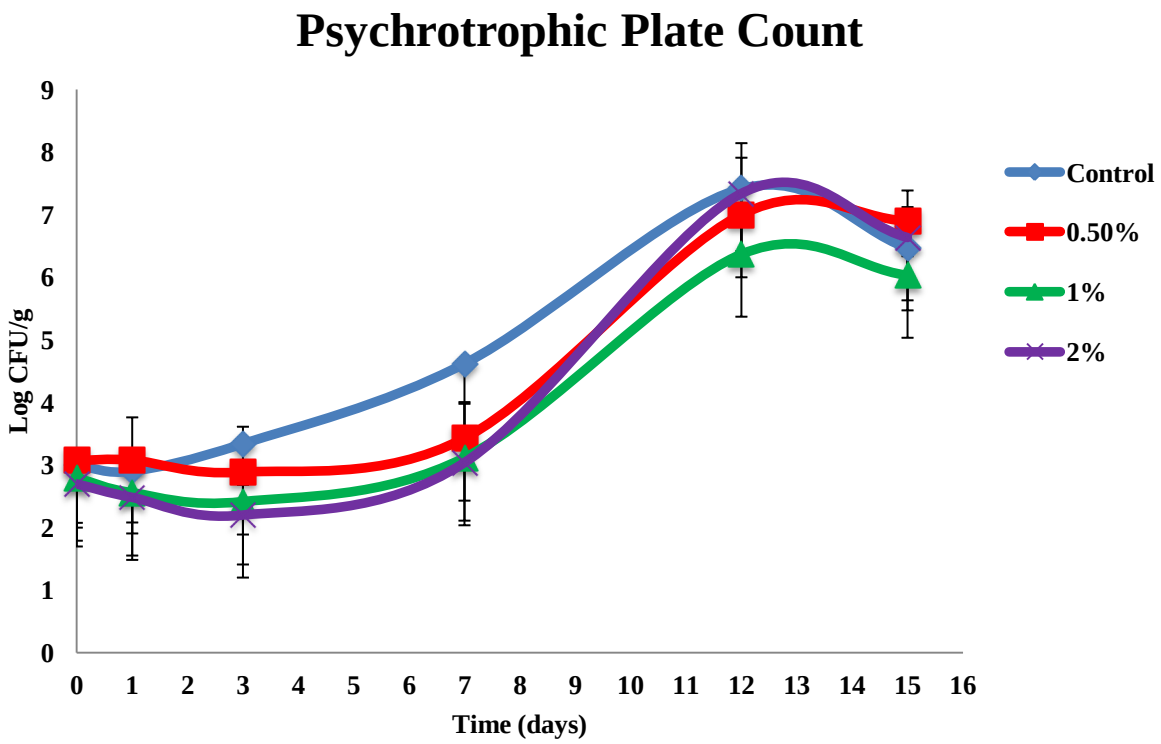

Figure 4.2 Effect of multilayered antimicrobial coating on psychrotrophic plate counts of fresh-cut watermelon stored at 4°C for 15 days.

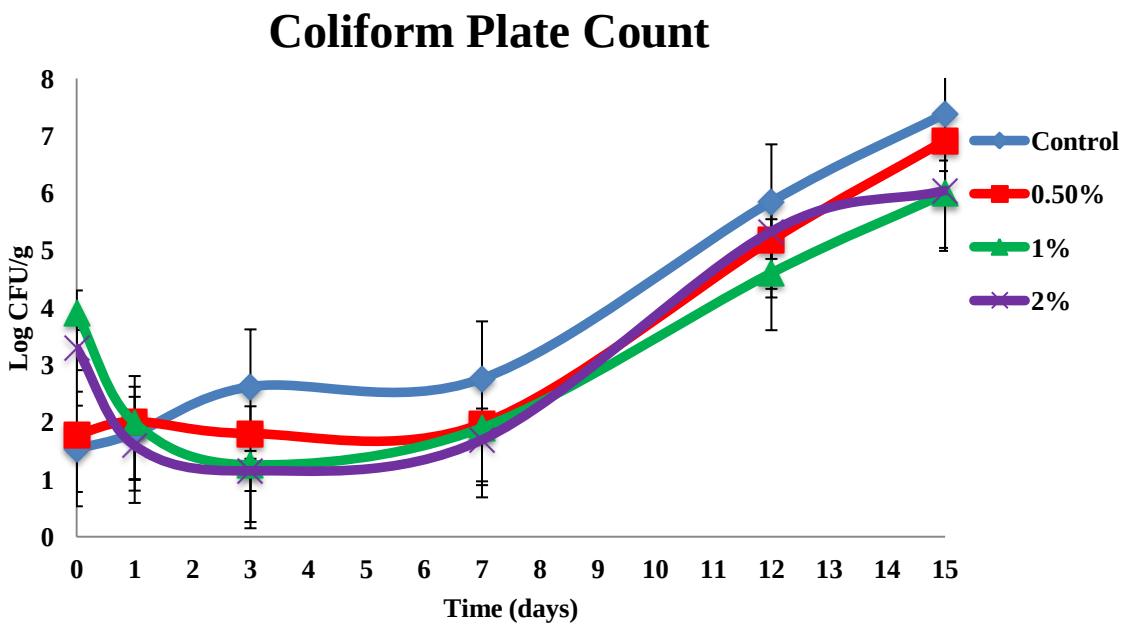

Figure 4.3 Effect of multilayered antimicrobial coating on coliform plate counts of fresh-cut watermelon stored at 4°C for 15 days.

4.4.4. Yeast and Mold Plate Count

No significant ($P > 0.05$) differences were found in the population of yeast and molds in the coated samples by day 15 of storage (Figure 4.4) while the control samples had significantly ($P < 0.05$) higher counts (5.45 log CFU/g). In particular, samples coated with 2% sodium alginate formulation had the lowest counts by the end of the storage, with a reduction of almost 4.0 log CFU/g in comparison with the controls. However, no significant ($P > 0.05$) differences were found between watermelons coated with 1% and 2% sodium alginate formulations (See Table B.4 in Appendix B). These results suggest that application of coating with 1% and 2% concentration of sodium alginate were effective in inhibiting the population of yeast and molds during the storage period of this study.

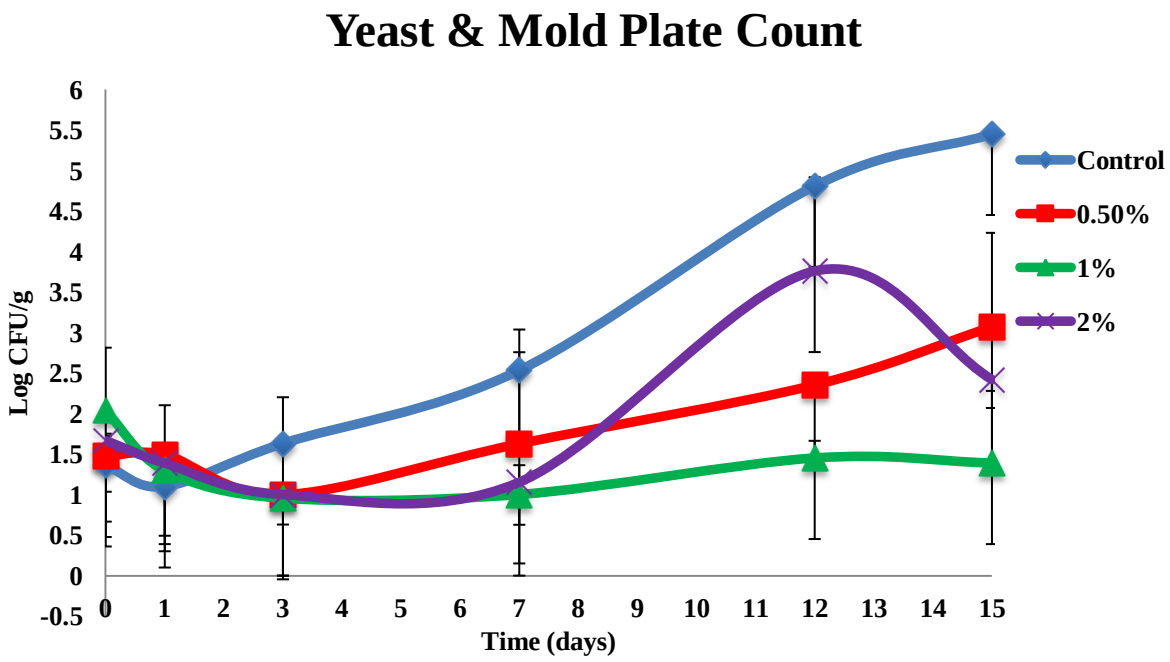

Figure 4.4 Effect of multilayered antimicrobial coating on yeast and mold plate counts of fresh-cut watermelon stored at 4°C for 15 days.

Table 4.16. Shelf-life prediction of coated watermelon

Shelf-Life Prediction (Days)				
	Aerobic Microorganisims	Psychrotrophic Microorganisims	Coliforms	Yeast&Molds
Control	7	7	11	12
0.5% Sodium Alginate	10	9	12	> 15
1% Sodium Alginate	11	10	13	> 15
2% Sodium Alginate	10	9	12	> 15

In addition to the statistical analysis, the number of days to reach the maximum tolerable microorganisms level, i.e. 5 Log CFU/g of aerobics, psychrotrophic, coliform, and yeast and mold plate counts (Brasil et al., 2012) were presented in table 4.16. The results in table 4.16 suggest that expected shelf- life of watermelon extended for coated groups since the shelf-life of controls was lower than coated watermelons through the shelf-life study. In other words, the maximum tolerable microorganisms' level was reached earlier for control groups compared to the coated groups and multilayered edible coating extended the microbial shelf-life of fresh-cut watermelon.

Overall, the results confirm that the multilayered antimicrobial coating is effective in reducing the microbial growth of aerobics, psychrotrophics, coliforms, and yeast and molds in fresh-cut watermelon stored at 4°C for 15 days. Samples

coated with 1% and 2% sodium alginate formulation showed higher reduction in the microbial growth of coliforms, yeast and molds, and aerobic microorganisms. The use of both 1% and 2% sodium alginate in coating preparation is a suitable choice in terms of the growth of microorganisms.

4.5. Effect of Multilayered Edible Coating Sodium Alginate Concentration On the Headspace of Containers With Fresh-Cut Watermelon Samples

The concentration of CO_2 (Table 4.17) in jars containing uncoated controls increased significantly ($P < 0.05$) by the end of storage (day 15) and the coated samples had lower ($P < 0.05$) concentrations of CO_2 in the jar's headspace (See Figure B.18 in Appendix B). This finding supports the hypothesis that application of the multilayered antimicrobial edible coating to fresh-cut watermelon has a beneficial effect on the amount of CO_2 produced during the respiration process.

The concentration of O_2 in the jars of both uncoated controls and sodium alginate coated samples decreased during storage time (Table 4.18). However, the concentrations in jars with uncoated controls were significantly ($P < 0.05$) lower than in jars holding the coated samples (See Figure B.16 in Appendix B). Regarding the effect of the % sodium alginate in the coating, no significant ($P > 0.05$) differences were found.

Low O_2 level and high CO_2 accumulation in the internal atmosphere enhances softening of fruits (Rojas-Grau et al., 2009). In other words, the multilayered antimicrobial edible coating effectively delays the softening of fresh-cut watermelon

since CO_2 concentration was lower while O_2 concentration was higher in the headspace in jars.

Regression analysis was also conducted to understand the evolution of O_2 and CO_2 concentrations across time among different groups (Table 4.19). Among different model specifications, including a linear, exponential, power and quadratic, the highest R^2 values were obtained with a quadratic model (Also see Figures B.17 and B.19 in Appendix B). These results suggest that, over time in all groups, the decrease in O_2 concentration and increase in CO_2 concentration is most significant among samples coated with the 2% sodium alginate-based coating. Hence, the quadratic model explains the variation in O_2 concentration over time well for all groups as the R^2 are higher than 85%. Similar results were obtained for the CO_2 data. Overall, the results confirm the findings in tables 4.17 and 4.18.

Table 4.17. Effect of multilayered antimicrobial coating on headspace CO_2 concentration of fresh-cut watermelon stored at 4°C for 15 days.

Headspace CO_2 Concentration (%)				
Time (days)	Control	0.5% Sodium Alginate	1% Sodium Alginate	2% Sodium Alginate
1	$_{x}0.06^{d}$ *(0.01)	$_{x}0.08^{d}$ (0.01)	$_{x}0.05^{c}$ (0.0)	$_{x}0.06^{d}$ (0.0)
3	$_{x}0.13^{d}$ (0.01)	$_{x}0.13^{d,c}$ (0.0)	$_{y}0.09^{b,c}$ (0.0)	$_{x}0.12^{c}$ (0.01)
7	$_{x}0.23^{c}$ (0.02)	$_{x}0.21^{b,c}$ (0.03)	$_{x}0.17^{a,b}$ (0.01)	$_{x}0.18^{b}$ (0.01)
12	$_{x}0.38^{b}$ (0.01)	$_{y}0.27^{a,b}$ (0.02)	$_{y}0.24^{a}$ (0.04)	$_{y}0.25^{a}$ (0.02)
15	$_{x}0.56^{a}$ (0.05)	$_{y}0.35^{a}$ (0.04)	$_{y}0.26^{a}$ (0.04)	$_{y}0.28^{a}$ (0.0)

Values are means of 2 replications.
*Standard deviation
[a,d]Values within a column followed by a common superscript letter indicate that mean values are not significantly different ($P < 0.05$).
[x,y]Values within a row followed by a common subscript letter indicate that mean values are not significantly different ($P < 0.05$).

Table 4.18.Effect of multilayered antimicrobial coating on headspace O_2 concentration of fresh-cut watermelon stored at 4°C for 15 days.

Headspace O_2 Concentration (%)				
Time (days)	Control	0.5% Sodium Alginate	1% Sodium Alginate	2% Sodium Alginate
1	$_{x}0.91^{a}$ $^{*}(0.05)$	$_{x}0.87^{a}$ (0.0)	$_{x}0.88^{a}$ (0.0)	$_{x}0.87^{a}$ (0.0)
3	$_{x}0.82^{a,b}$ (0.03)	$_{x}0.82^{a}$ (0.02)	$_{x}0.84^{a}$ (0.03)	$_{x}0.81^{a,b}$ (0.03)
7	$_{x}0.73^{b}$ (0.02)	$_{x}0.79^{a}$ (0.01)	$_{x}0.76^{a,b}$ (0.03)	$_{x}0.77^{a,b}$ (0.05)
12	$_{y}0.52^{c}$ (0.04)	$_{x,y}0.68^{b}$ (0.04)	$_{x}0.74^{a,b}$ (0.04)	$_{x}0.71^{a,b}$ (0.05)
15	$_{y}0.40^{c}$ (0.01)	$_{x}0.62^{b}$ (0.04)	$_{x}0.68^{b}$ (0.05)	$_{x}0.70^{b}$ (0.06)

Values are means of 2 replications.
*Standard deviation
^{a,c}Values within a column followed by a common superscript letter indicate that mean values are not significantly different ($P < 0.05$).
^{x,y}Values within a row followed by a common subscript letter indicate that mean values are not significantly different ($P < 0.05$).

Table 4.19. Change in headspace composition as a function of time (days)

O_2 concentration	**Model**	**R^2**
Control	$y = -0.0158x^2 - 0.5348x + 20.479$	0.994
0.5% sodium alginate	$y = -0.011x^2 - 0.2078x + 19.391$	0.986
1% sodium alginate	$y = 0.0064x^2 - 0.3907x + 19.83$	0.959
2% sodium alginate	$y = 0.0168x^2 - 0.5308x + 19.806$	0.979
O_2 concentration	**Model**	**R^2**
Control	$y = 0.0267x^2 + 0.3203x + 1.3206$	0.991
0.5% sodium alginate	$y = -7E\text{-}05x^2 + 0.4046x + 1.5317$	0.986
1% sodium alginate	$y = -0.0165x^2 + 0.5983x + 0.4925$	0.996
2% sodium alginate	$y = -0.0118x^2 + 0.5283x + 0.9423$	0.997

y= Average CO_2 or O_2 Concentration , x=time , x^2 =time^2 (day)

4.6. Microscopic Examination of the Multilayered Edible Coating

Different cross-sections of the multilayered edible coating on fresh-cut watermelon are presented in Figures 4.5 to 4.7. The coating manufactured with 0.5% sodium alginate, 2% antimicrobial, and 2% pectin showed uniform surface thickness and good adhering throughout the surface. The mean thickness value was 101.0±3.0 μm for multi-layer edible film containing 0.5% sodium alginate (Figure 4.5).

The mean values for thickness of the coating with 1% and 2% sodium alginate were 405.67±12.22 μm (Figure 4.6) and 500.33±49.60 μm (Figure 4.7), respectively. Hence, the coating formulations produced coatings with very different thicknesses. This result indicates that further work must be done to produce coatings with similar thickness when changing its formulation. While all coatings covered the surface of fresh-cut watermelon in a homogenous fashion, as expected, 2% sodium alginate-based coating was thicker than the other formulations. For example during the sensory tests, it is observed that the panelists noticed the coatings for all coated groups and in particular for 2 % sodium alginate-based coating as it was ticker. Also while the coating was acceptable for 0.5% and 1% groups in terms of appearance, 2% grouped seemed to be unacceptable to the panelists

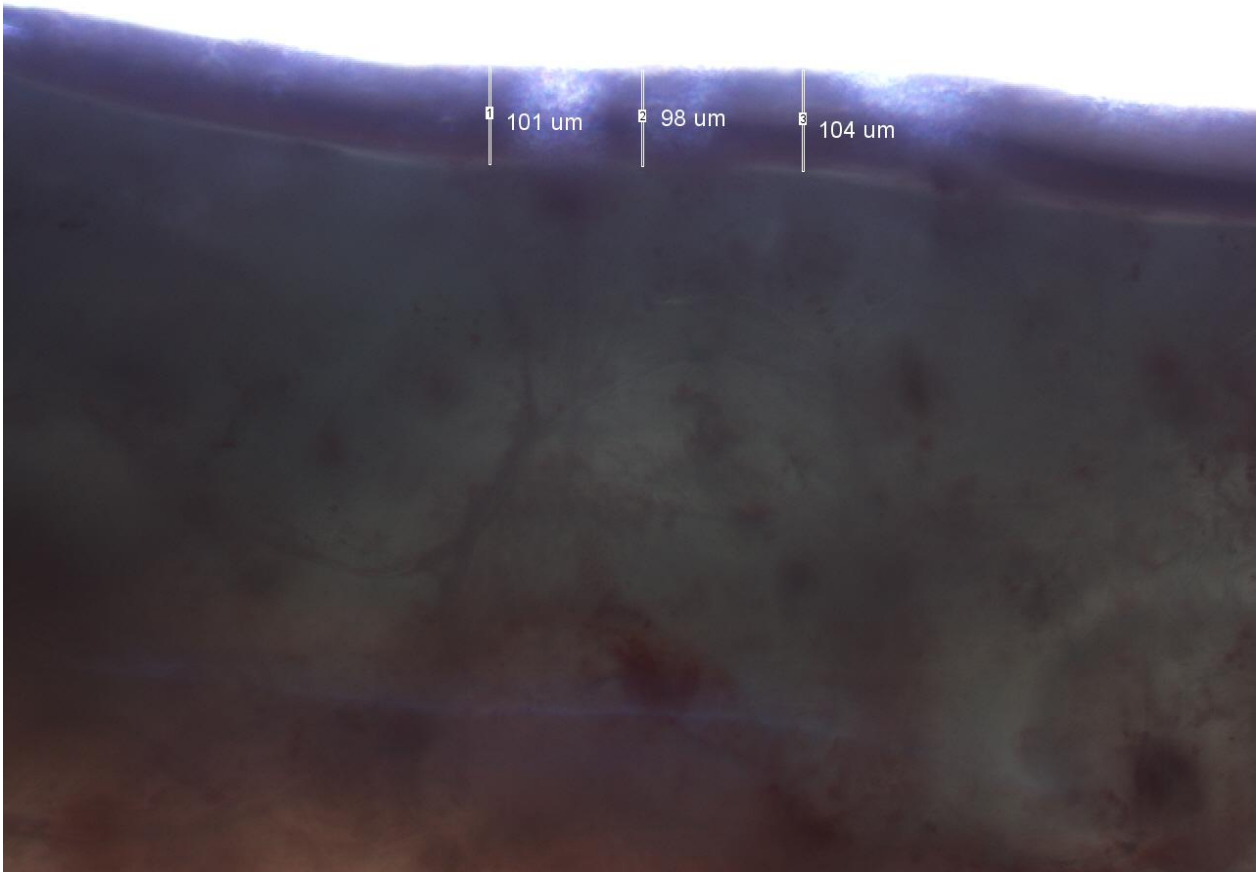

Figure 4.5 Microscopic observations of cross-section of alginate-based multilayer edible coated (0.5% sodium alginate, 2% antimicrobial, 2% pectin) fresh-cut watermelon showing thickness of coating.

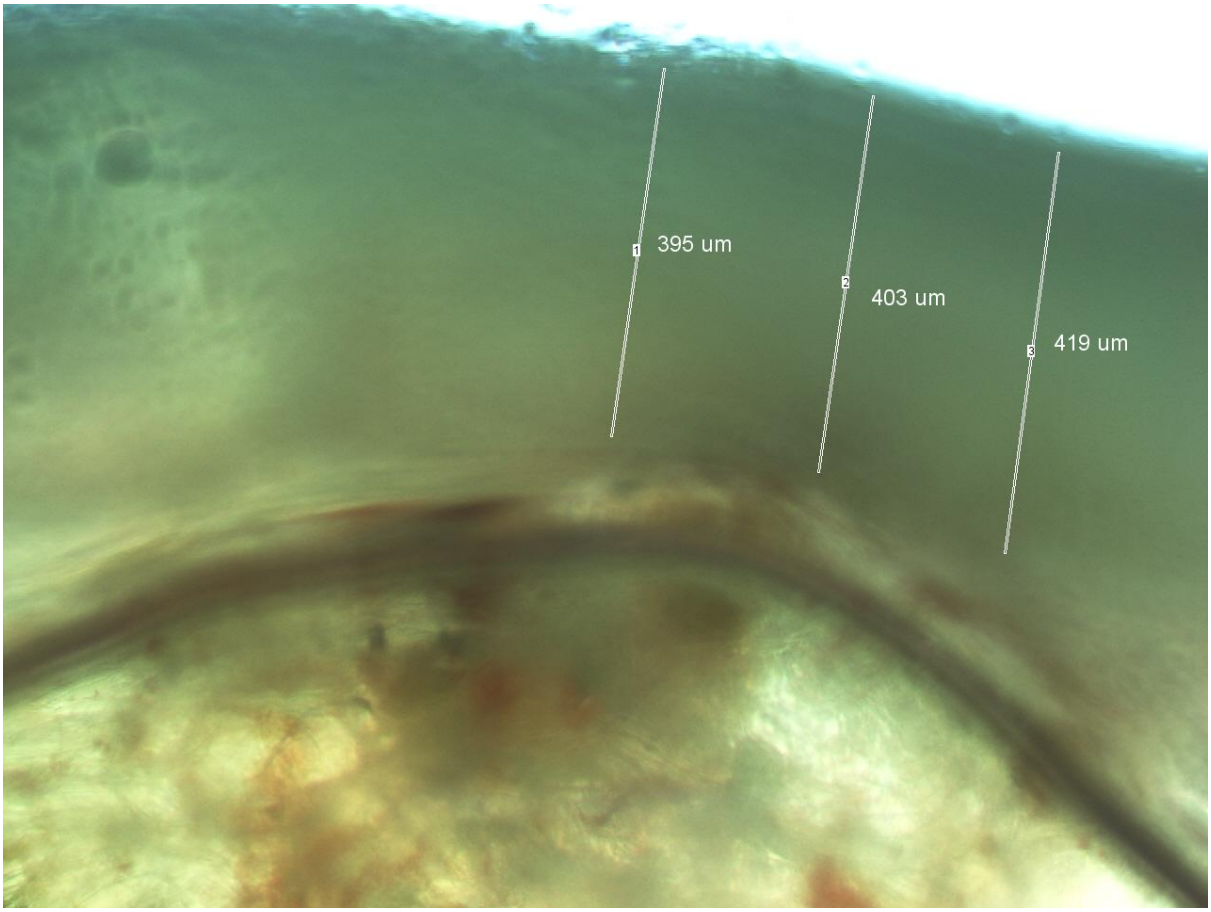

Figure 4.6 Microscopic observations of cross-section of alginate-based multilayer edible coated (1% sodium alginate, 2% antimicrobial, 2% pectin,) fresh-cut watermelon showing thickness of coating.

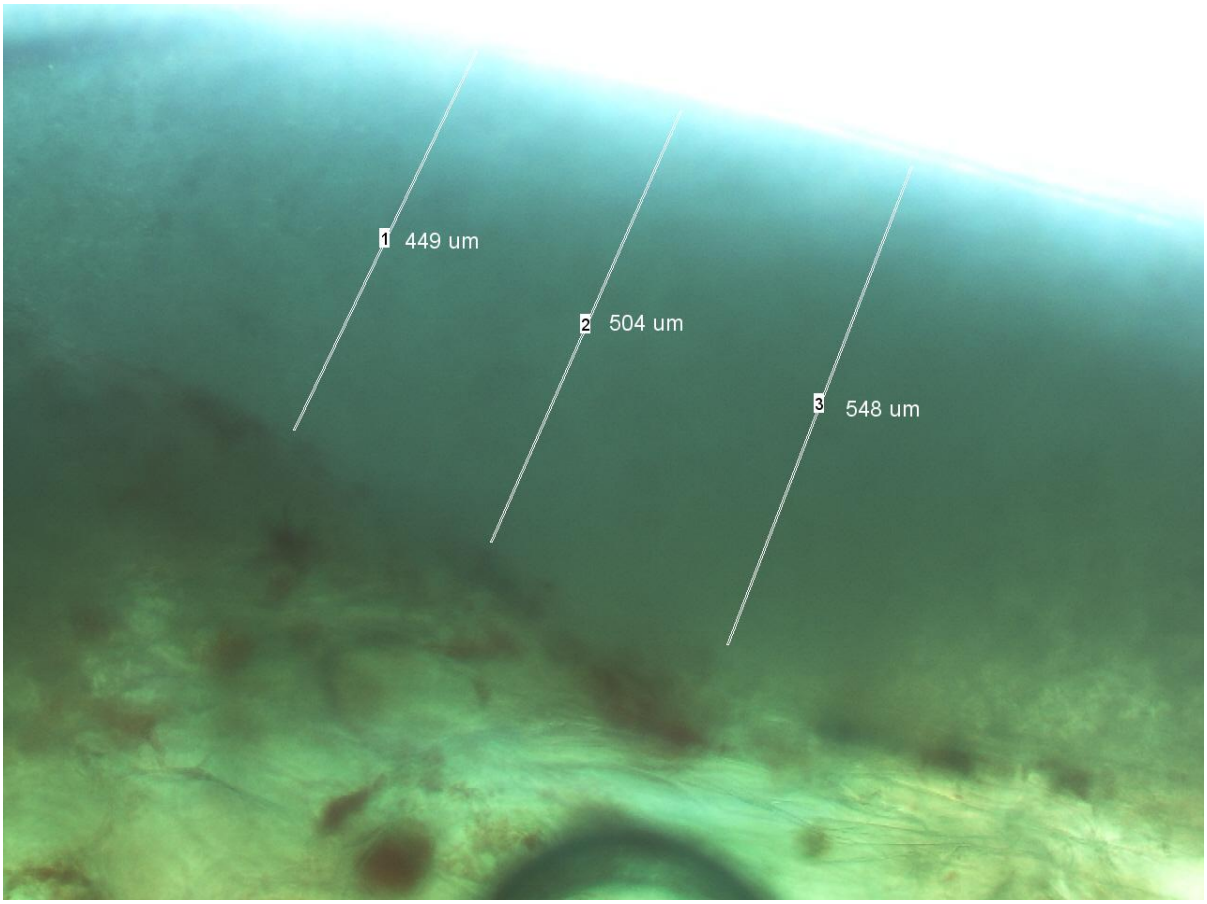

Figure 4.7 Microscopic observations of cross-section of alginate-based multilayer edible coated (2% sodium alginate, 2% antimicrobial, 2% pectin,) fresh-cut watermelon showing thickness of coating.

CHAPTER V

RECOMMENDATIONS FOR FUTURE STUDY

Based on the results and conclusions of the present study, recommendations for future research on edible coatings of fresh-cut watermelon produce include:

- Evaluate whether sodium alginate concentrations between 1% and 2% are suitable for quality maintenance and shelf life extension of fresh-cut watermelon.
- Determine the most effective concentration of antimicrobial compound to ensure optimum antimicrobial effectiveness.
- Evaluate alternative antimicrobial compounds which will not impart undesirable color, flavor or odor characteristics to the coated fruits, as it was the case of *trans*-cinnamaldehyde.
- Study the effect of the edible coating on the preservation of lycopene in fresh-cut watermelon.
- Optimize the layer- by-layer coating manufacture method to ensure uniform thickness of the coating.
- Develop a peelable coating using the same formulation of the coating that can be removed.

CHAPTER VI

CONCLUSIONS

The effect of alginate-based multilayered antimicrobial edible coating on the quality attributes and shelf-life of fresh-cut watermelon were evaluated. Coating formulation included sodium alginate, pectin, calcium lactate, and antimicrobial compound. The concentrations of pectin, calcium lactate, and antimicrobial compound were kept constant (2% w/w), while three different concentrations (0.5%, 1%, and 2% w/w) of sodium alginate were tested.

The chemical properties of watermelon (pH, °Brix, juice leakage, water activity, and moisture content) and product quality attributes (color, texture, and sensory attributes) as well as composition of the atmosphere inside the containers (headspace analysis) of coated fresh-cut watermelons and uncoated controls were evaluated in a shelf-life study at 4°C for 15 days. Additionally, microbiological analysis was carried out to evaluate the effectiveness of antimicrobial compound against microbial spoilage and growth.

The main results and conclusions obtained from this work are as follows:

- The alginate-based multilayered antimicrobial coating did not affect the original chemical properties of fresh-cut watermelon during storage. Only few differences were observed for water activity and moisture in comparison to uncoated controls. However, juice leakage was considerably reduced due to the application of the multilayered coating.

- The firmness of fresh-cut watermelon was preserved by application of the alginate-based multilayered coating throughout storage. Coated samples were significantly ($P < 0.05$) firmer while uncoated controls started to lose firmness and getting softer faster. Moreover, higher concentration of sodium alginate (1% and 2% w/w) in the coating formulation ensured a firmer texture. Texture is an important attribute for consumer acceptance of the product which is a sign of freshness of fresh-cut fruits.
- The objective color values of multilayered antimicrobial coated watermelon samples were significantly ($P < 0.05$) different than those of the uncoated controls because the antimicrobial compound is opaque. However, despite the whitish color of the coated samples, consumer acceptance of the product was high by the end of shelf life (day 15).
- Sensory evaluation results demonstrated that consumers were concerned about the color and odor of the samples. The coated samples were given lower scores than the controls during the shelf-life study, which confirms the results obtained with the objective measurements. Since consumers first evaluate the appearance of the product and want to eat a red watermelon, the whiter appearance of the coated samples was less accepted. On the other hand, the coated samples were given the highest scores in terms of texture.
- Even though some parameters were affected by the application of the coating, the watermelon samples coated with the 1% alginate-based formulation were still acceptable in terms of sensory attributes (color, flavor, odor, and texture).

- Overall, sensory scores of coated watermelons showed some beneficial aspects of the coating in terms of texture and flavor.
- The application of the multilayered antimicrobial edible coating has a beneficial effect on the amount of CO_2 production during the respiration process and effectively delays the softening of fresh-cut watermelon.
- Microscopic observations showed that as the concentration of alginate in the multilayered coating formulation increased, the thickness of the coating increased. The sensory test results proved that a very thick film is not desirable since it can affect the appearance of product and acceptability among consumers, in terms of its appearance.
- Application of the multilayered edible coating carrying an antimicrobial agent was effective in reducing the microbial growth of aerobics, psychrotrophics, coliforms, and yeast and molds in fresh-cut watermelon stored at 4°C for 15 days. This effect was particularly obvious for aerobics and coliforms.
- The multilayered edible coating with 1% sodium alginate was the most effective in reducing microbial growth.

In summary, the results from this study indicate that the multilayered alginate-based edible coating as carrier of an antimicrobial agent is an effective means to extend the shelf-life of fresh-cut watermelons in terms of quality attributes as well as microbiological safety.

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APPENDIX A

Acceptance Test: 304

Place a mark in the box which you feel best describes how you like the sample.
An honest expression of your personal feelings will help us. Thank you.

Color	Like extremely	Like Very much	Like moderately	Like slightly	Neither like nor dislike	Dislike slightly	Dislike moderately	Dislike very much	Dislike extremely
Odor	Like extremely	Like Very much	Like moderately	Like slightly	Neither like nor dislike	Dislike slightly	Dislike moderately	Dislike very much	Dislike extremely
Texture	Like extremely	Like Very much	Like moderately	Like slightly	Neither like nor dislike	Dislike slightly	Dislike moderately	Dislike very much	Dislike extremely
Flavor	Like extremely	Like Very much	Like moderately	Like slightly	Neither like nor dislike	Dislike slightly	Dislike moderately	Dislike very much	Dislike extremely
Overall Quality	Like extremely	Like Very much	Like moderately	Like slightly	Neither like nor dislike	Dislike slightly	Dislike moderately	Dislike very much	Dislike extremely

Comments:……

APPENDIX B

Table B.1.Effect of multilayered antimicrobial coating on aerobic plate counts of fresh-cut watermelon stored at 4°C for 15 days.

Aerobic Plate Count				
Time (days)	Control	0.5% Sodium Alginate	1% Sodium Alginate	2% Sodium Alginate
0	$_{x}2.97^{c}$ (0.06)	$_{x}2.9^{b}$ (0.03)	$_{y}2.68^{b}$ (0.04)	$_{z}2.55^{b}$ (0.05)
1	$_{x}2.88^{c}$ (0.06)	$_{x}2.79^{b}$ (0.27)	$_{x}2.58^{b}$ (0.37)	$_{x}2.60^{b}$ (0.32)
3	$_{x}3.29^{c}$ (1.06)	$_{x}2.80^{b}$ (1.25)	$_{x}2.86^{b}$ (1.16)	$_{x}3.00^{b}$ (0.29)
7	$_{x}4.79^{b}$ (0.19)	$_{y}3.45^{b}$ (0.60)	$_{y}3.37^{b}$ (0.67)	$_{y}2.95^{b}$ (0.17)
12	$_{x}6.00^{b}$ (0.00)	$_{x}6.33^{a}$ (1.15)	$_{x}6.01^{a}$ (0.40)	$_{x}6.55^{a}$ (0.30)
15	$_{x}7.40^{a}$ (0.40)	$_{x}6.76^{a}$ (1.17)	$_{x}6.86^{a}$ (0.77)	$_{x}5.69^{a}$ (0.79)

Values are means of 3 replications.
*Standard deviation
[a,c]Values within a column followed by a superscript letter indicate that mean values are not significantly different ($P < 0.05$).
[x,y]Values within a row followed by a subscript letter indicate that mean values are not significantly different ($P < 0.05$).

Table B.2.Effect of sodium alginate concentration in the multilayered antimicrobial coating on psychrotrophic plate counts of fresh-cut watermelon stored at 4°C for 15 days.

Psychrotrophic Plate Count				
Time (days)	Control	0.5% Sodium Alginate	1% Sodium Alginate	2% Sodium Alginate
0	$_{x,y}3.0^{c}$ (0.0)	$_{x}3.08^{b}$ (0.13)	$_{y,z}2.79^{b}$ (0.14)	$_{z}2.70^{b,c}$ (0.02)
1	$_{x}2.91^{c}$ (0.15)	$_{x}3.08^{b}$ (0.68)	$_{x}2.55^{b}$ (0.26)	$_{x}2.48^{b,c}$ (0.41)
3	$_{x}3.34^{c}$ (0.27)	$_{x,y}2.89^{b}$ (0.07)	$_{y,z}2.41^{b}$ (0.27)	$_{z}2.20^{c}$ (0.12)
7	$_{x}4.62^{b}$ (0.01)	$_{x,y}3.43^{b}$ (0.55)	$_{y}3.11^{b}$ (0.89)	$_{y}3.04^{b}$ (0.30)
12	$_{x}7.42^{a}$ (0.73)	$_{x}7.00^{a}$ (0.00)	$_{x}6.37^{a}$ (0.72)	$_{x}6.63^{a}$ (0.49)
15	$_{x}7.33^{a}$ (0.58)	$_{x}6.47^{a}$ (0.92)	$_{x}6.90^{a}$ (0.17)	$_{x}6.03^{a}$ (0.22)

Values are means of 3 replications.
*Standard deviation
[a,c] Values within a column followed by a common superscript letter indicate that mean values are not significantly different ($P<0.05$).
[x,z]Values within a row followed by a common subscript letter indicate that mean values are not significantly different ($P<0.05$).

Table B.3.Effect of sodium alginate concentration in the multilayered antimicrobial coating on coliform plate counts of fresh-cut watermelon stored at 4°C for 15 days.

Coliform Plate Count				
Time (days)	Control	0.5% Sodium Alginate	1% Sodium Alginate	2% Sodium Alginate
0	$_{x}1.53^{d}$ (0.47)	$_{x}1.78^{b}$ (0.14)	$_{y}3.91^{b}$ (0.39)	$_{y}3.29^{b}$ (0.32)
1	$_{x}1.81^{c,d}$ (0.22)	$_{x}2.01^{b}$ (0.61)	$_{x}1.99^{c}$ (0.45)	$_{x}1.59^{c}$ (0.53)
3	$_{x}2.62^{c,d}$ (0.56)	$_{x,y}1.80^{b}$ (0.48)	$_{y}1.26^{c}$ (0.24)	$_{y}1.15^{c}$ (0.15)
7	$_{x}2.76^{c}$ (0.15)	$_{x}1.97^{b}$ (0.20)	$_{x}1.90^{c}$ (0.00)	$_{x}1.69^{c}$ (0.39)
12	$_{x}5.85^{b}$ (0.00)	$_{x,y}5.18^{a}$ (0.37)	$_{y}4.61^{b,c}$ (0.52)	$_{x,y}5.33^{a}$ (0.42)
15	$_{x}7.38^{a}$ (0.66)	$_{x}6.91^{a}$ (1.51)	$_{x}5.99^{a}$ (1.12)	$_{x}6.04^{a}$ (0.37)

Values are means of 3 replications.
*Standard deviation
[a,d]Values within a column followed by a common superscript letter indicate that mean values are not significantly different ($P < 0.05$).
[x,y]Values within a row followed by a common subscript letter indicate that mean values are not significantly different ($P < 0.05$).

Table B.4.Effect of sodium alginate concentration in the multilayered antimicrobial coating on yeast & molds plate counts of fresh-cut watermelon stored at 4°C for 15 days.

Yeast & Molds Plate Count				
Time (days)	Control	0.5% Sodium Alginate	1% Sodium Alginate	2% Sodium Alginate
0	$_{x}1.36^{c}$ (0.39)	$_{x}1.48^{a,b}$ (0.0)	$_{x}2.03^{a}$ (0.78)	$_{x}1.67^{b,c}$ (0.06)
1	$_{x}1.10^{c}$ (0.17)	$_{x}1.49^{a,b}$ (0.61)	$_{x}1.30^{a,b}$ (0.00)	$_{x}1.38^{b,c}$ (0.09)
3	$_{x}1.63^{b,c}$ (0.57)	$_{x}1.00^{b}$ (0.00)	$_{x}0.95^{b}$ (0.00)	$_{x}1.00^{c}$ (0.00)
7	$_{x}2.53^{a}$ (0.51)	$_{x,y}1.63^{a,b}$ (0.63)	$_{y}1.00^{b}$ (0.00)	$_{y}1.15^{c}$ (0.15)
12	$_{x}4.81^{a}$ (0.11)	$_{x,y}2.35^{a,b}$ (0.01)	$_{y}1.45^{a,b}$ (0.15)	$_{x}3.33^{a}$ (1.06)
15	$_{x}5.45^{a}$ (0.00)	$_{y}3.07^{a}$ (1.16)	$_{y,z}1.39^{a,b}$ (0.39)	$_{z}2.42^{a,b}$ (0.03)

Values are means of 3 replications.
*Standard deviation
[a,b]Values within a column followed by a common superscript letter indicate that mean values are not significantly different ($P<0.05$).
[x,y]Values within a row followed by a common subscript letter indicate that mean values are not significantly different ($P<0.05$).

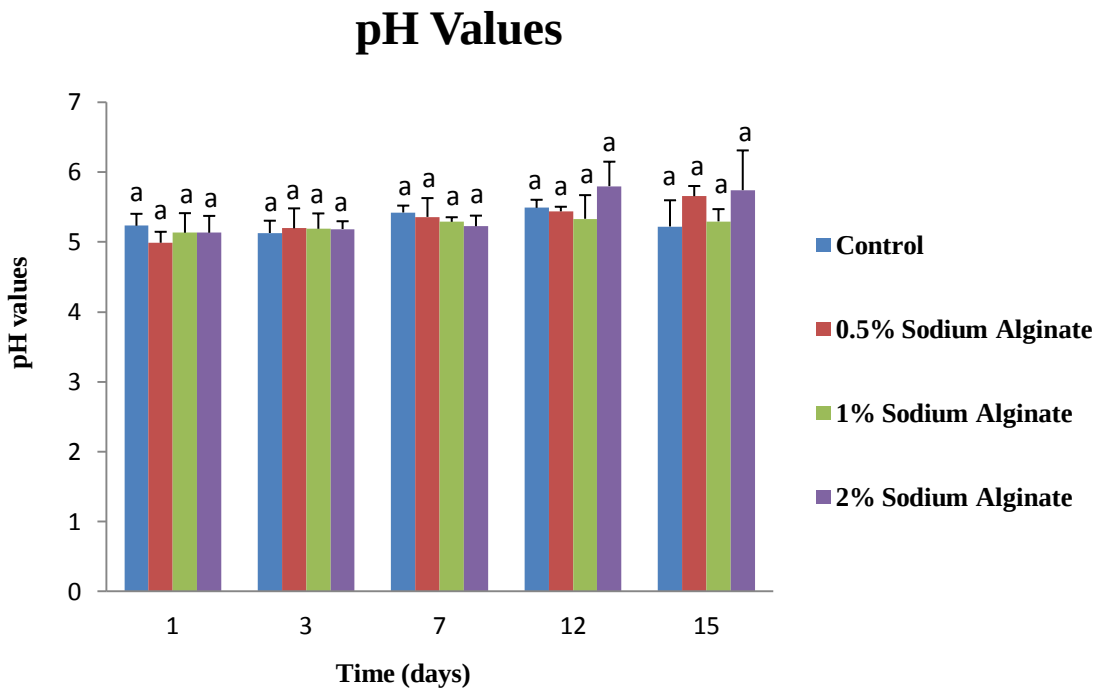

Figure B.1.Effect of sodium alginate concentration in the multilayered antimicrobial coating on pH values of fresh-cut watermelon stored at 4°C for 15 days.

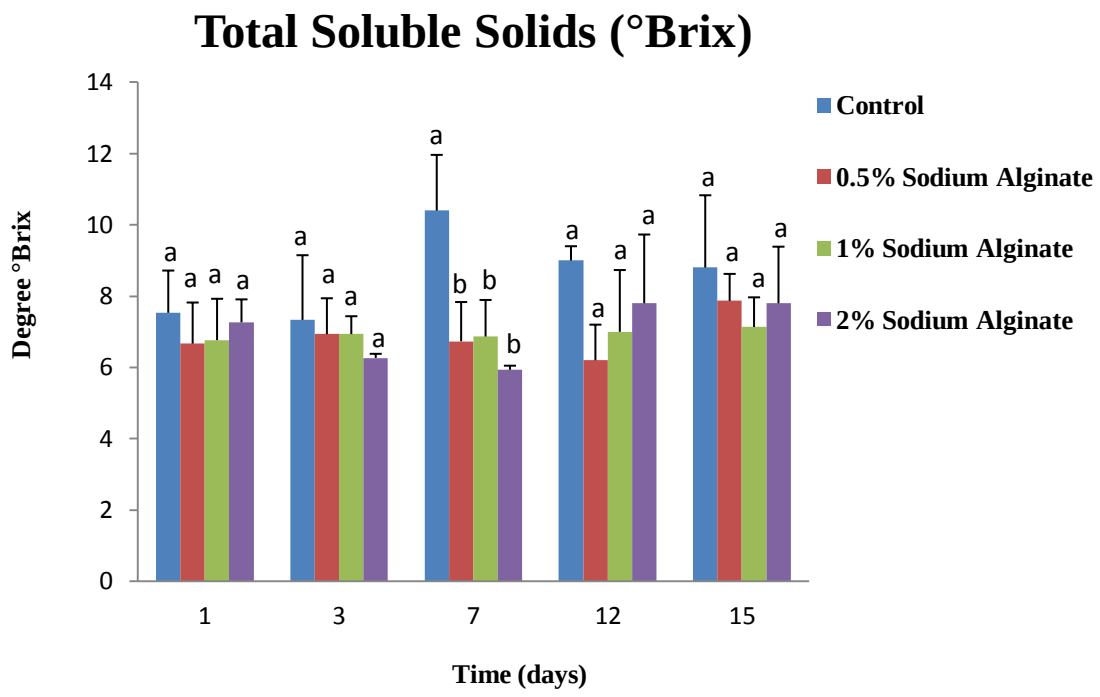

Figure B.2.Effect of sodium alginate concentration in the multilayered antimicrobial coating on total soluble solids (°Brix) of fresh-cut watermelon stored at 4°C for 15 days.

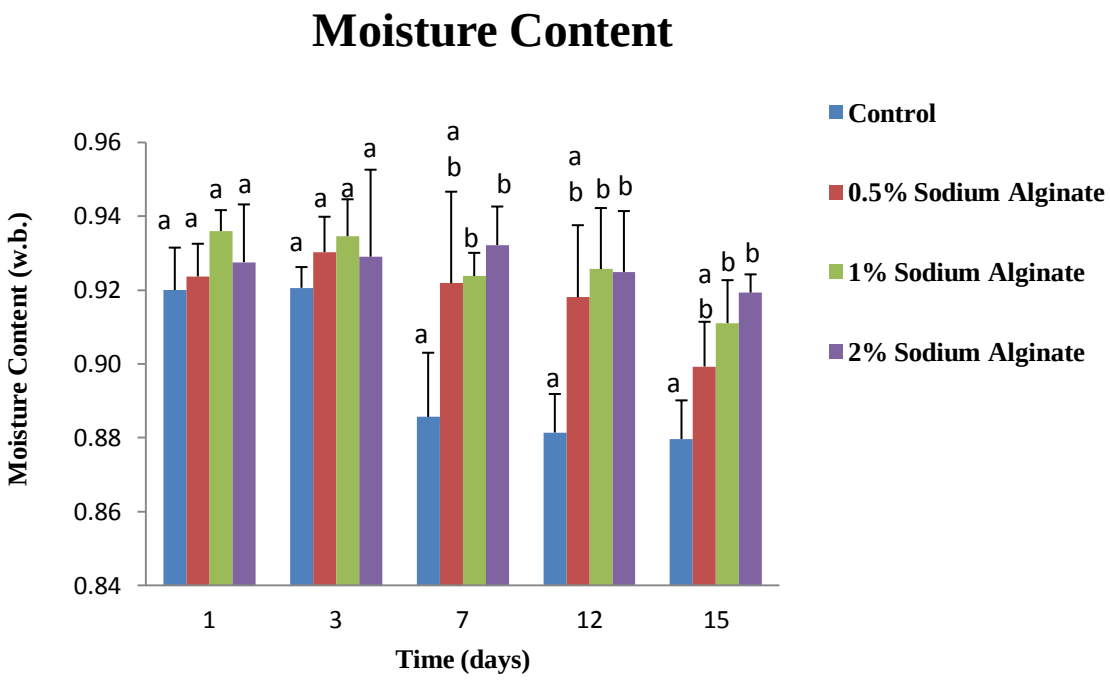

Figure B.3.Effect of sodium alginate concentration in the multilayered antimicrobial coating on moisture content of fresh-cut watermelon stored at 4°C for 15 days.

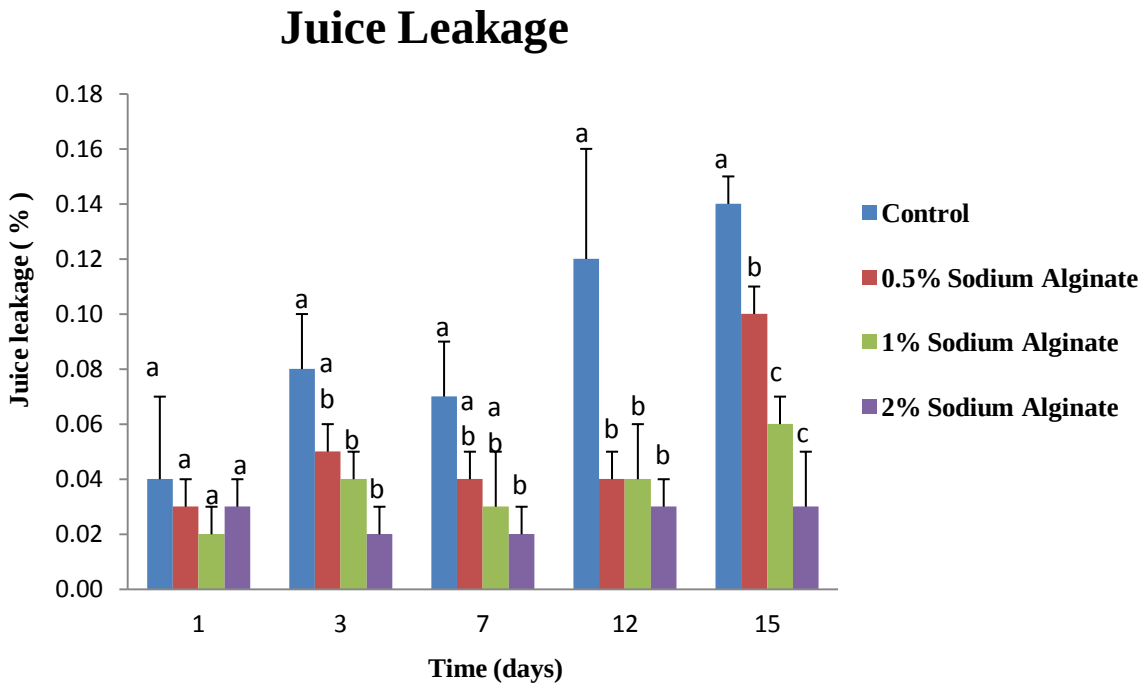

Figure B.4.Effect of sodium alginate concentration in the multilayered antimicrobial coating on percentage juice leakage of fresh-cut watermelon stored at 4°C for 15 days.

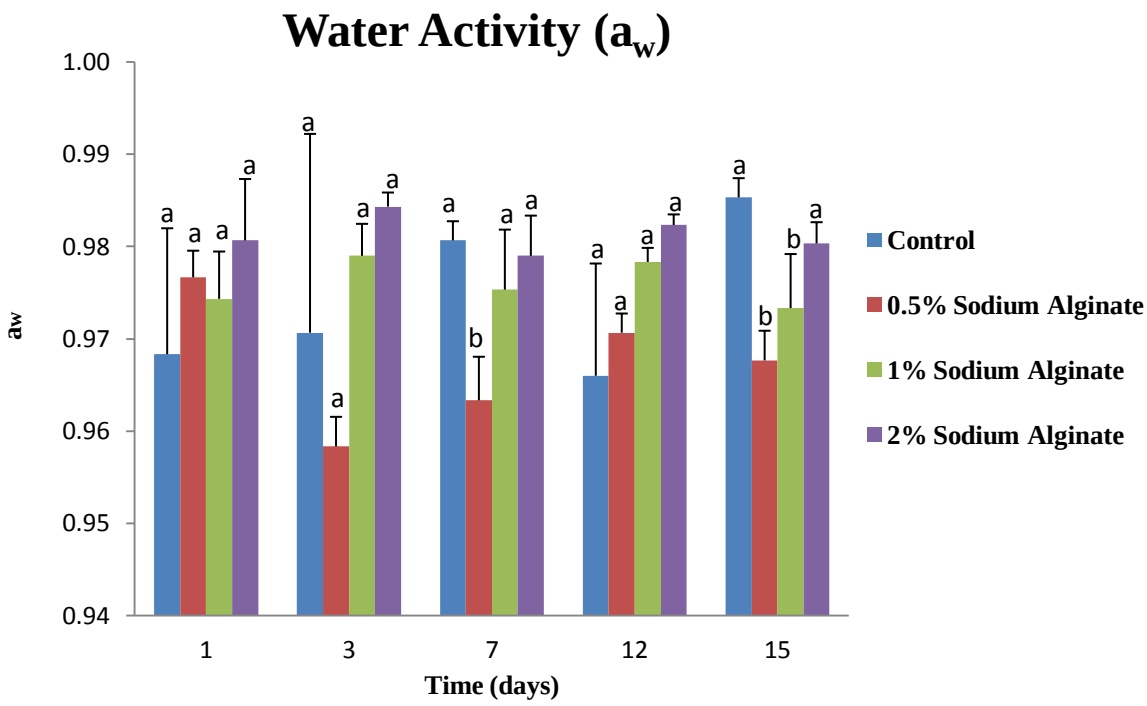

Figure B.5.Effect of sodium alginate concentration in the multilayered antimicrobial coating on water activity (a_w) of fresh-cut watermelon stored at 4°C for 15.

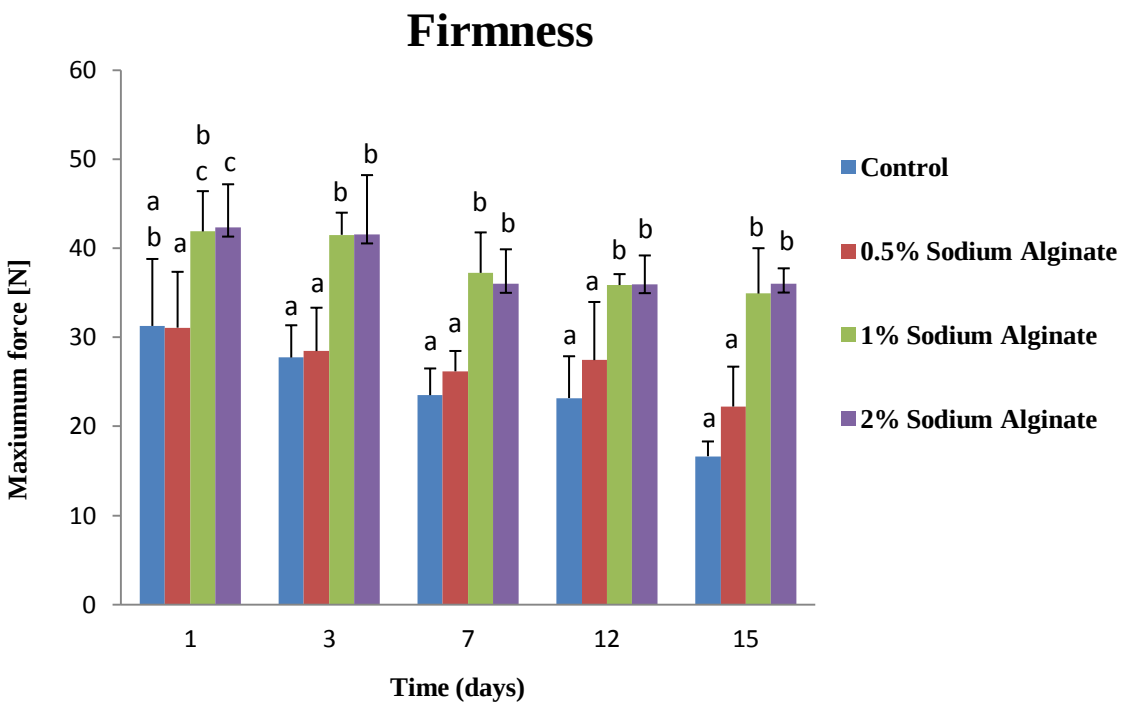

Figure B.6.Effect of sodium alginate concentration in the multilayered antimicrobial coating on firmness [N] values of fresh-cut watermelon stored at 4°C for 15.

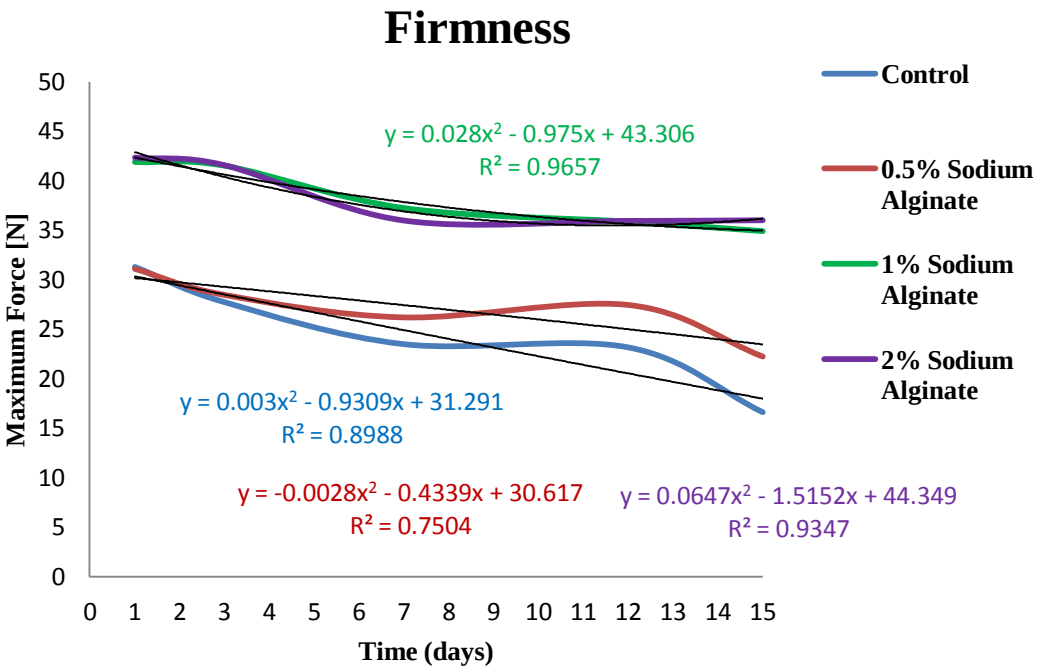

Figure B.7 Quadratic models for regression analysis of firmness data for multilayered antimicrobial coated watermelon and uncoated controls

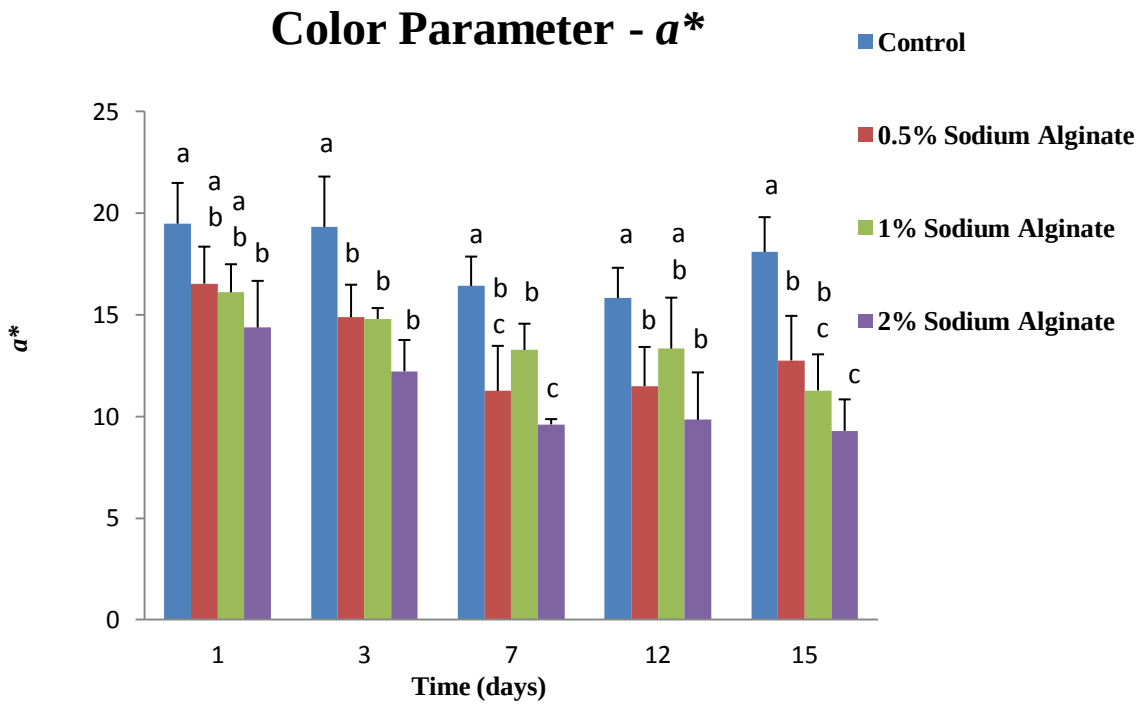

Figure B.8 Effect of sodium alginate concentration in the multilayered antimicrobial coating on (*a**) color parameter values of fresh-cut watermelon stored at 4°C for 15 days.

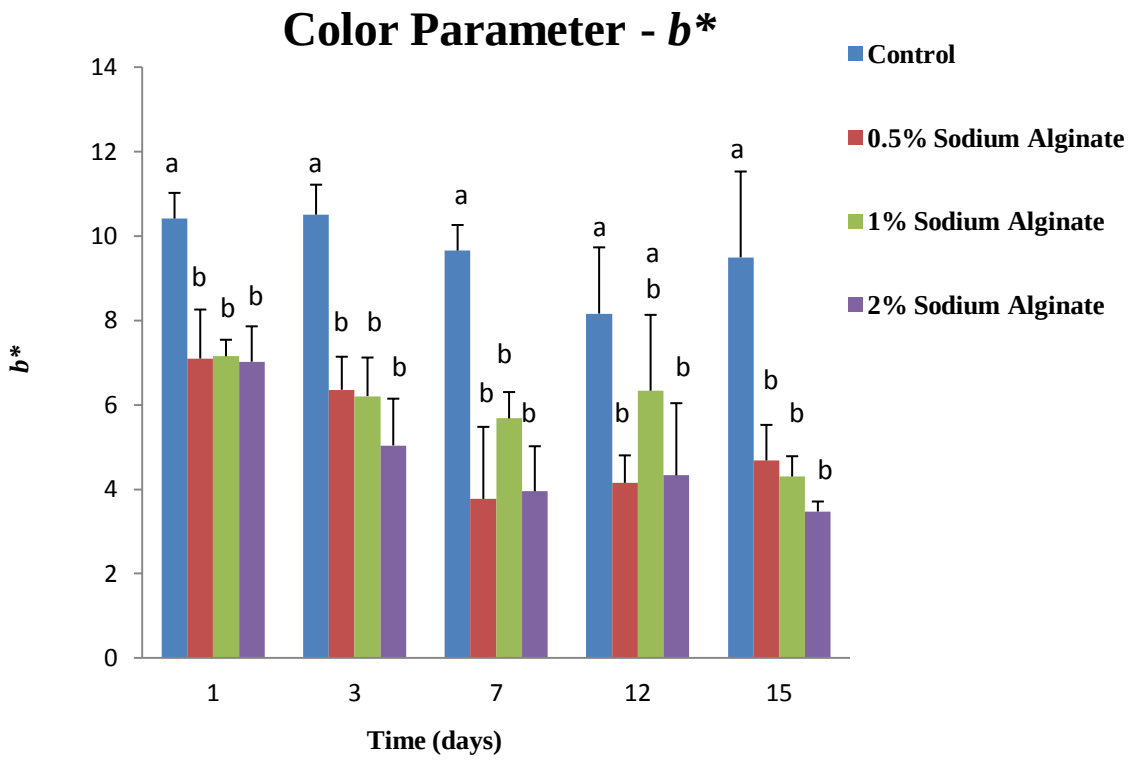

Figure B.9 Effect of sodium alginate concentration in the multilayered antimicrobial coating on (*b**) color parameter values of fresh-cut watermelon stored at 4°C for 15 days.

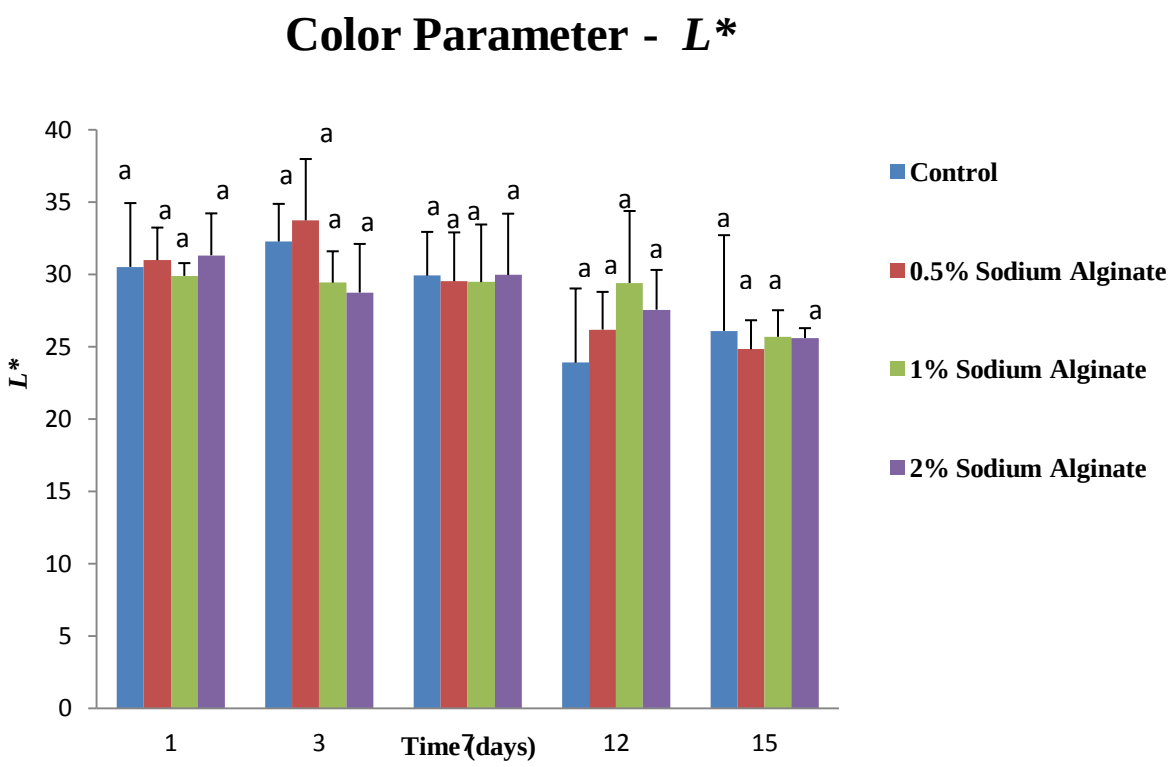

Figure B.10 Effect of sodium alginate concentration in the multilayered antimicrobial coating on (*L**) color parameter values of fresh-cut watermelon stored at 4°C for 15 days.

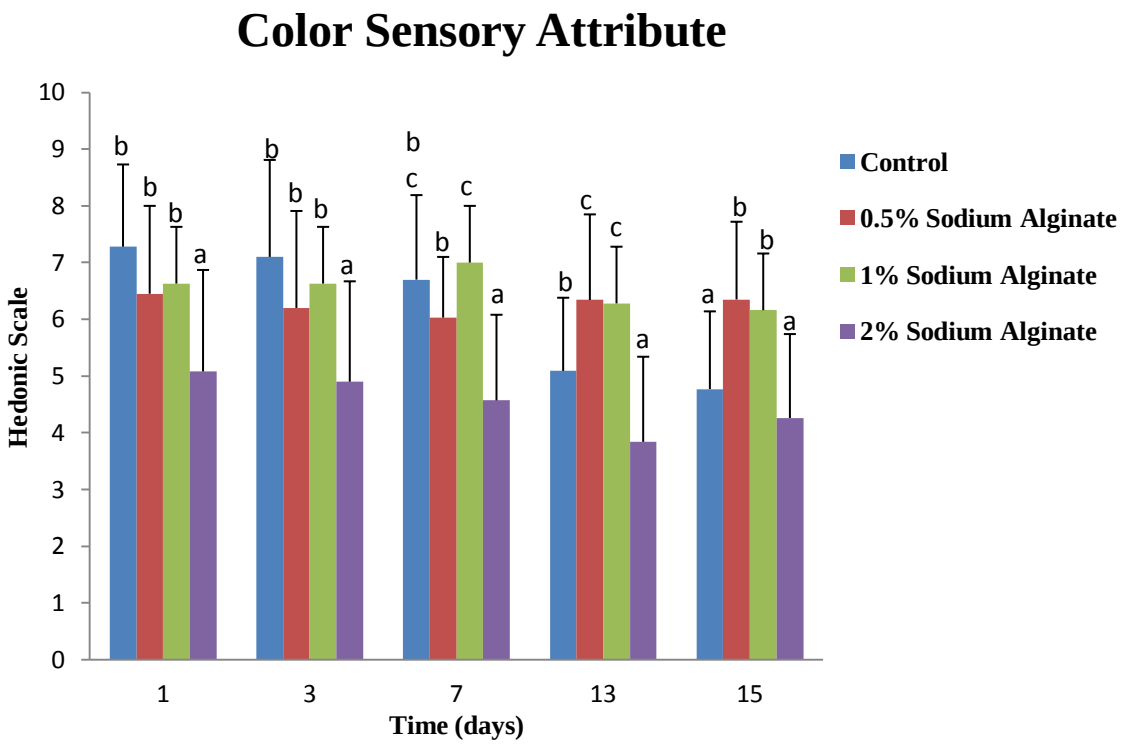

Figure B.11 Effect of sodium alginate concentration in the multilayered antimicrobial coating on color sensory attribute values of fresh-cut watermelon stored at 4°C for 15 days.

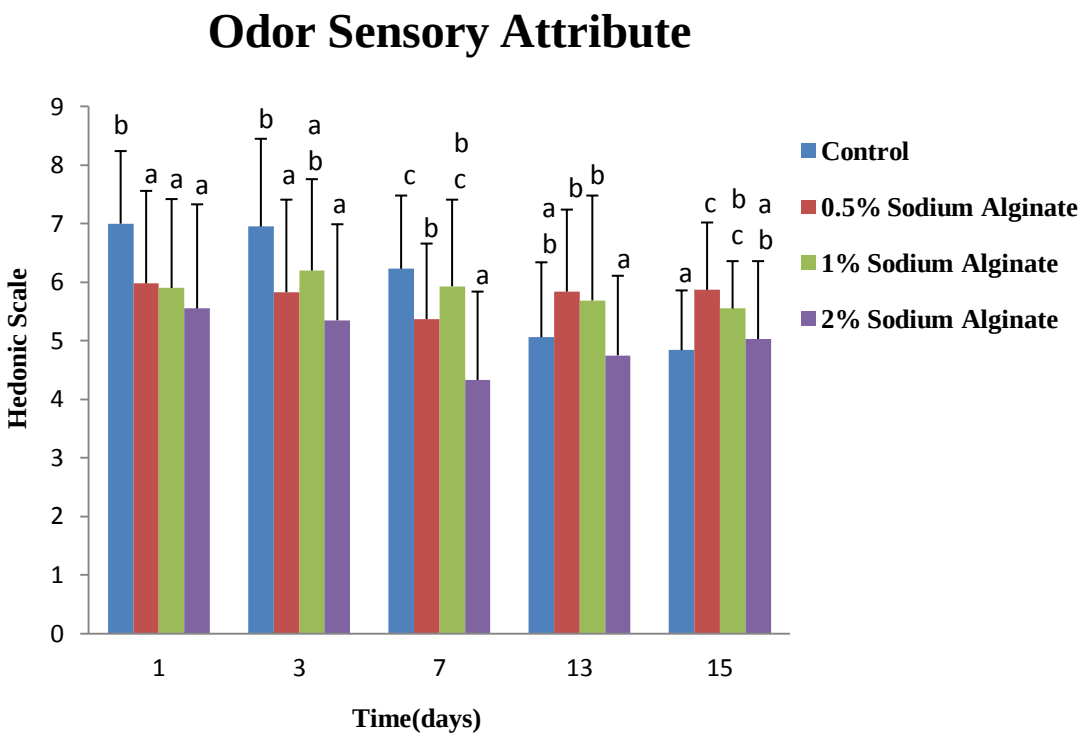

Figure B.12 Effect of sodium alginate concentration in the multilayered antimicrobial coating on odor sensory attribute values of fresh-cut watermelon stored at 4°C for 15 days.

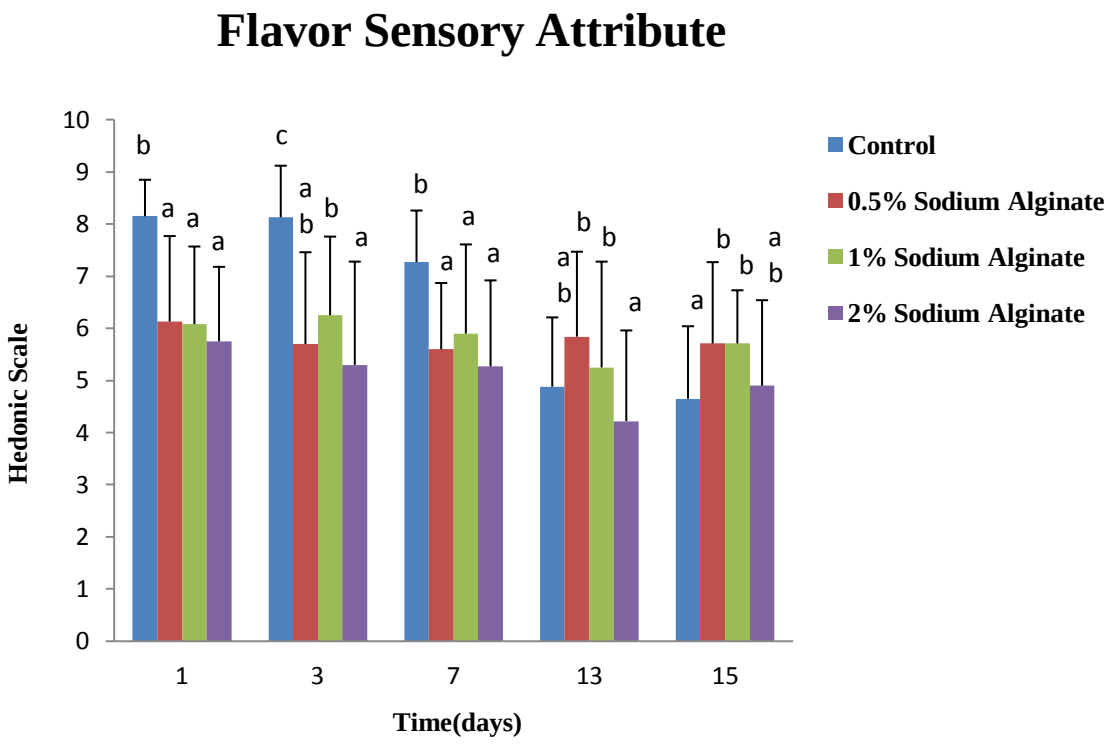

Figure B.13 Effect of sodium alginate concentration in the multilayered antimicrobial coating on flavor sensory attribute values of fresh-cut watermelon stored at 4°C for 15 days.

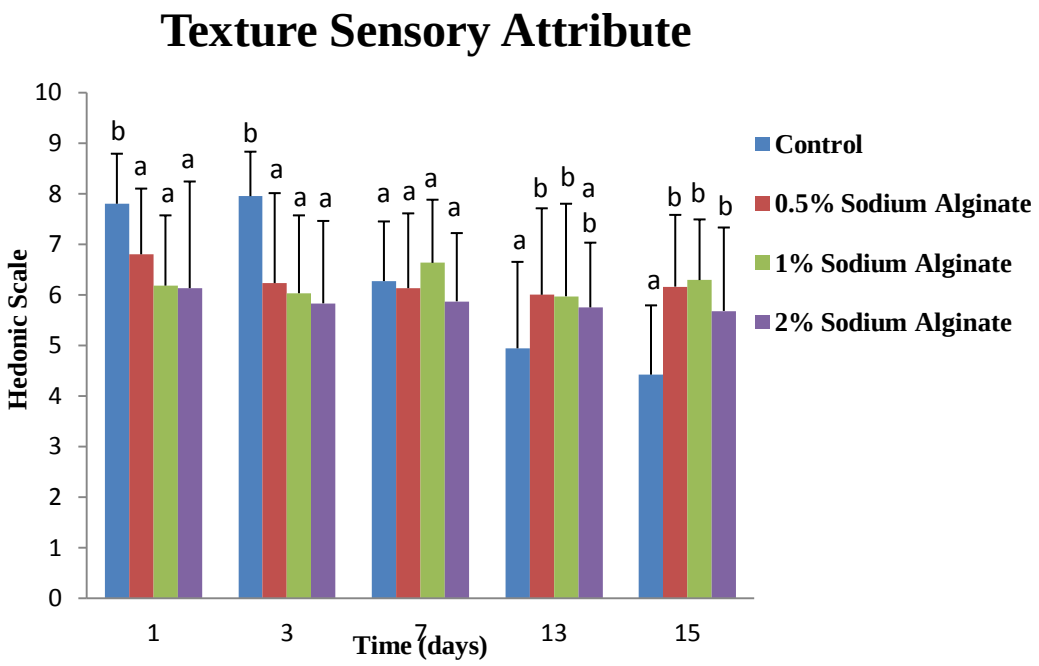

Figure B.14 Effect of sodium alginate concentration in the multilayered antimicrobial coating on texture sensory attribute values of fresh-cut watermelon stored at 4°C for 15 days.

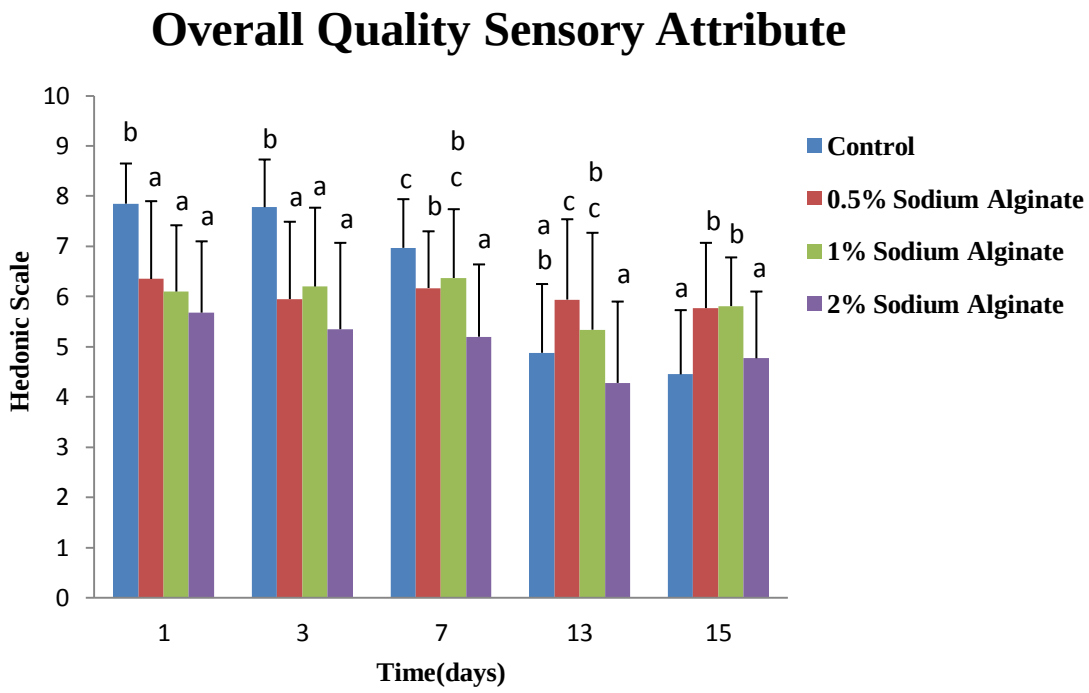

Figure B.15 Effect of sodium alginate concentration in the multilayered antimicrobial coating on overall quality sensory attribute values of fresh-cut watermelon stored at 4°C for 15 days.

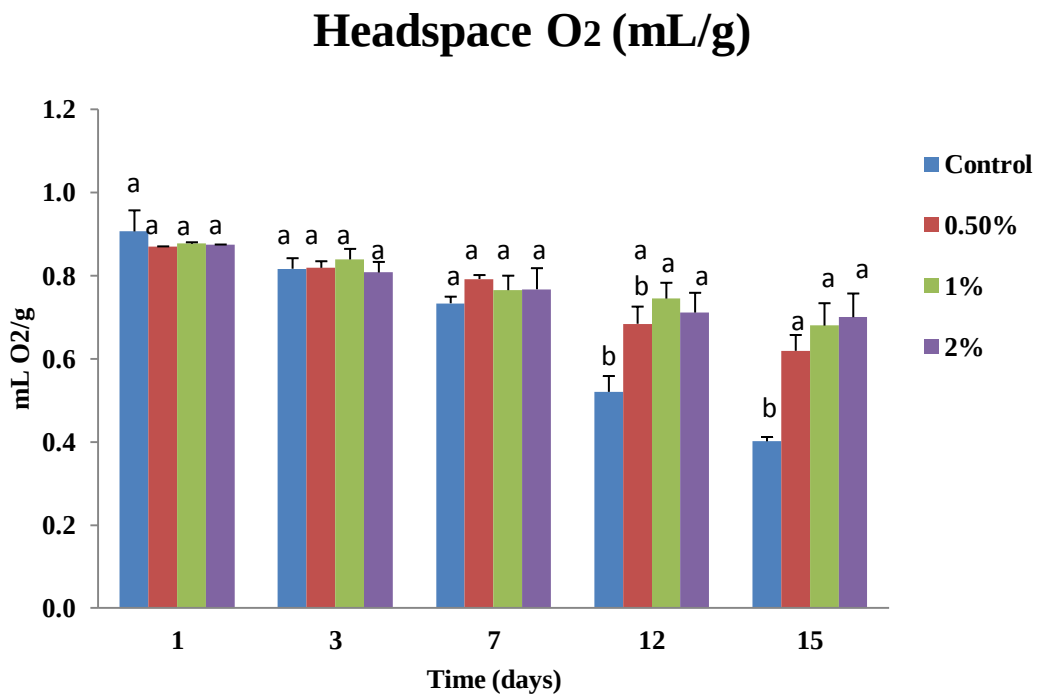

Figure B.16 Effect of sodium alginate concentration in the multilayered antimicrobial coating on headspace O_2 concentration of fresh-cut watermelon stored at 4°C for 15 days.

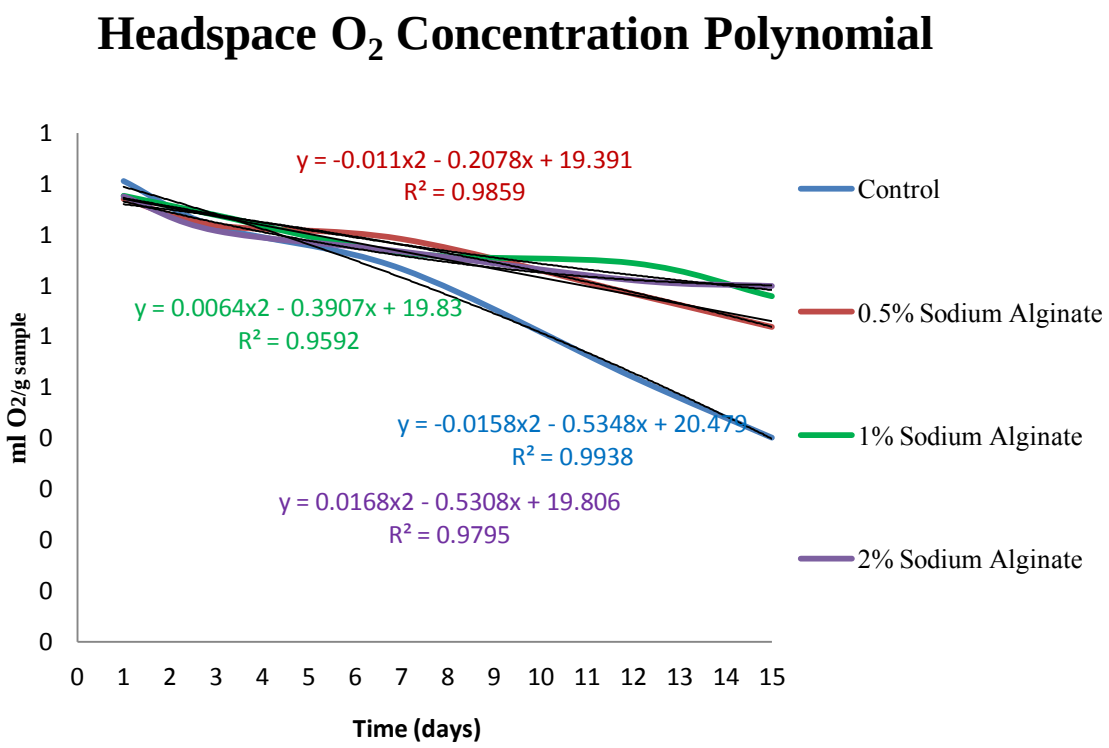

Figure B.17 Regression analyses of headspace O_2 concentration data for multilayered antimicrobial coated watermelon and uncoated controls

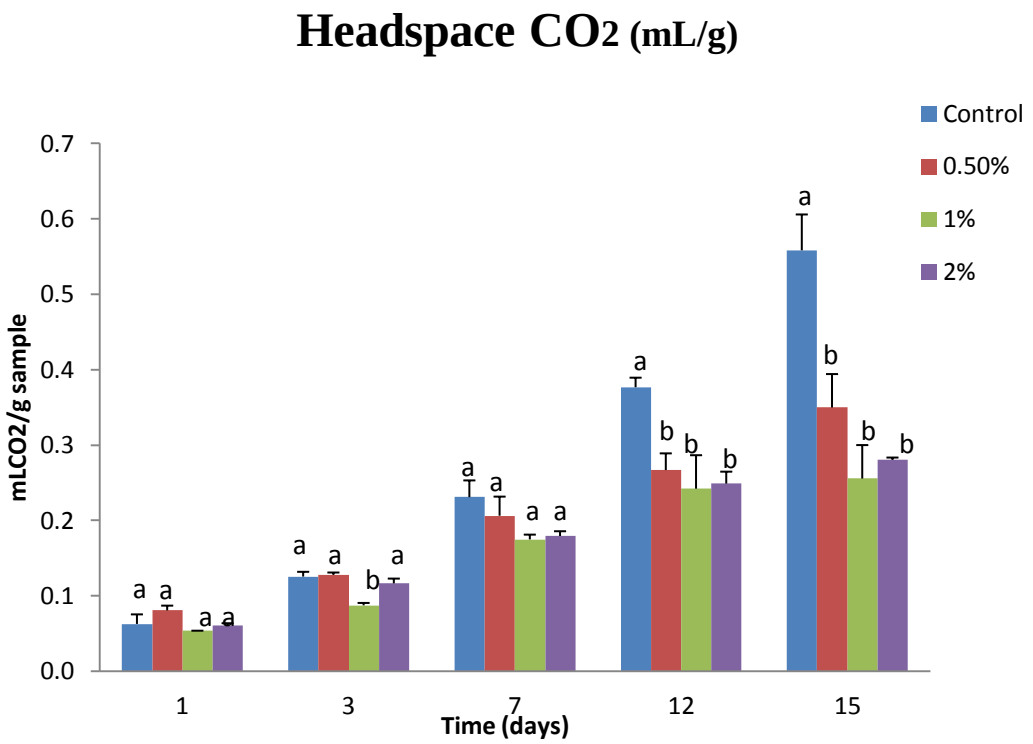

Figure B.18 Effect of sodium alginate concentration in the multilayered antimicrobial coating on headspace CO_2 concentration of fresh-cut watermelon stored at 4°C for 15 days.

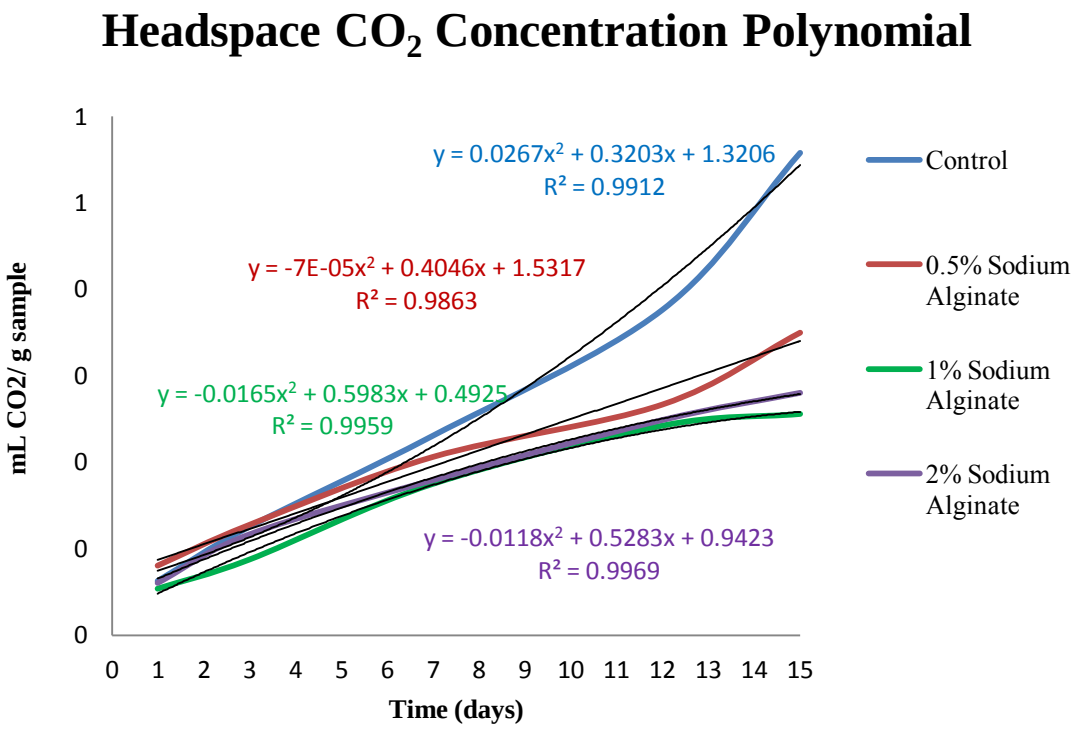

Figure B.19 Regression analyses of headspace CO_2 concentration data for multilayered antimicrobial coated watermelon and uncoated controls.

VITA

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