DEVELOPMENT OF AN ALGINATE-BASED ANTIMICROBIAL EDIBLE COATING TO EXTEND THE SHELF-LIFE OF FRESH-CUT PINEAPPLE

A Thesis

by

NATALIA VANESSA MANTILLA

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

May 2012

Major Subject: Food Science & Technology

DEVELOPMENT OF AN ALGINATE-BASED ANTIMICROBIAL EDIBLE COATING TO EXTEND THE SHELF-LIFE OF FRESH-CUT PINEAPPLE

A Thesis

by

NATALIA VANESSA MANTILLA

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

Approved by:

Chair of Committee,	Elena Castell-Perez
Committee Members,	Rosana Moreira
	Carmen Gomes
	Alejandro Castillo
Chair of Intercollegiate Faculty,	Alejandro Castillo

May 2012

Major Subject: Food Science & Technology

ABSTRACT

Development of an Alginate-based Antimicrobial Edible Coating

to Extend the Shelf-life of Fresh-cut Pineapple. (May 2012)

Natalia Vanessa Mantilla, B.S., Universidad San Francisco de Quito (USFQ)

Chair of Advisory Committee: Dr. Elena Castell-Perez

In the last few years, especially in the developed countries, an increment in demand for fresh-cut fruit by the consumers of all ages has occurred. This increase is mainly due to the importance that people are giving to the consumption of fresh, healthy, and low-calorie food products. Fresh-cut pineapple (*Ananas comosus*) is one of the fruits that consumers can eat quickly and still enjoy its benefits; however, its shelf-life is very short (7 days).

A means to preserve all the natural and beneficial components of fresh-cut pineapple is coating the fruit with an edible material, a coating. This coating acts as a barrier against moisture loss and gas exchanges and can be a carrier of other components like antimicrobials, which can help to extend the shelf-life of the fresh-cut fruit.

The main objective of this study was to develop an edible coating with an antimicrobial agent for fresh-cut pineapple and to determine its effectiveness in extending shelf-life and preserving fruit quality attributes.

Different treatments consisted of several concentrations of sodium alginate (0.5%, 1%, and 2%); beta-cyclodextrin, *trans*-cinnamaldehyde (antimicrobial), pectin, and calcium chloride were tested for formulation of the edible coating. The layer-by-layer technique with a dipping method was used to coat the fruits. Pineapples were properly cleaned with a chlorine solution

(300 ppm) and triangular prisms (3.6 cm per side) were cut using a triangular cutter. The length of the triangular prisms was adjusted to 2.54 cm using a small knife measured with a ruler.

Color, texture, pH, ºBrix (total soluble solids), acidity, vitamin C, moisture content, and weight loss, were monitored every 3 to 4 days for 15 days. Microbiological tests (aerobic plate counts, psychrotrophic counts, and yeast and molds counts) were performed to determine the effectiveness of the antimicrobial compound.

In terms of microbiological and physicochemical quality attributes, the coating improved the shelf-life of the fresh-cut pineapple up to 12 days compared to the control (fresh-cut pineapple without the coating) which only lasted 7 days at 4 ºC. Color, texture and pH, were better preserved in the treated (coated) fruit compared to controls (uncoated). Different concentrations of the solutions in the formation of the coating had different results in terms of the preservation of the quality attributes of the fruit. Antimicrobial coatings with a concentration of alginate of 1% and 2% (w/w), pectin 2% (w/w) and calcium chloride 2% (w/w) presented a satisfactory formulation to preserve fruit quality attributes like moisture content, help to control juice leakage, and avoid microbial growth. Antimicrobial coating with 1% of alginate (w/w), 2% of pectin (w/w), 2% of calcium chloride (w/w) and 2% of antimicrobial compound (w/w) was the best formulation.

This research demonstrates the feasibility of an alginate-based antimicrobial edible coating, which acts as a carrier of antimicrobial compounds for fresh-cut pineapple.

DEDICATION

To my parents, Luisa and Enrique, my best and unconditional friends that always inculcate me the wish of studying and learning, who gave me all the personal values and who gave me the wings to fly and fight with effort for my dreams, for which this is possible and who I owe everything that I am.

To my brothers, Enrique and Cristian, who have always been with me in good and bad moments, the two people that have filled my life with games, fights, fun and smiles.

To my love, my husband Alex, who has been my strength, my company in the dawns, my happiness and quiet in the moments of distress; because without him this would not have been possible.

To my little angel, my son Martin, who is my engine to wake up every day and continue working and dreaming with effort and faith, who keep my hope that all the dreams become true.

ACKNOWLEDGEMENTS

I would like to express all my gratitude and appreciation to my major professor and Chair of the Advisory Committee, Dr. Elena Castell-Perez, for her guidance since the beginning and during my entire graduate program. I want to thank her for her patience, support and help during the time of this thesis preparation and for all the opportunities she gave me to learn new aspects of research and to work in this fascinating area. Thanks to Dr. Rosana G. Moreira, member of my committee, for her imparted knowledge during my graduate program and for her encouragement of always do the things right.

I would like to thank Dr. Carmen Gomes, my committee member, for her unconditional help and support during the whole process of my research and all the aspects of my time as a graduate student, and to Dr. Alejandro Castillo for his advices, time and kindness of being part of this as my committee member as well.

I would like to give special thanks to Paulo Da Silva, a good friend that always had the willingness of give me some of his time to help me and to share with me his experience about research and life. To all the friends that I have made during this time, for their big help in the Laboratory, in the classes, and why not, in the free time that we shared. Thank you Rabia Sipahi, Mauricio Martinon, Laura Hill, Alex Puerta, Isin Karagoz and all the other friends that always helped me in many different ways and that always had a smile and a nice word for me. Without them, this would not have been possible.

Thanks to all the faculty, staff and personnel in the department, for making my experience at Texas A&M University very valuable.

Finally, thanks to my family, my parents, for their unconditional love, for believing in me and for making my bad days, good days, for always know what to say to make me feel better.

TABLE OF CONTENTS

CHAPTER Page

Page

LIST OF FIGURES

FIGURE Page

LIST OF TABLES

TABLE Page

CHAPTER I

INTRODUCTION

The relevance of minimally processed commodities in the grocery stores of most developed countries has been increasing continuously during the last few years. Fresh-cut fruits and vegetables represent a rapidly growing segment of the produce industry as more consumers demand fresh, convenient, and nutritious foods. This is due to the lifestyles of modern consumers that prefer a fresh product that is easier and faster to eat, and desire natural products that can promote health benefits. This growing demand for minimally processed products has stimulated research on new ways to extend fresh-cut product's shelf life and preserving the quality attributes.

Fresh-cut pineapple is available in restaurants, schools, and food service establishments. In 2009, the annual production of pineapple in the United States was estimated at 200,000 Ton (FAO 2009).

In spite of the increased demand for fresh-cut fruits, there are some limitations in the production of these commodities, due to the difficulty of preserving their freshness during storage. The short shelf-life of fresh-cut fruits is due to the cutting operations, which damage and wound the fruit's cell membrane. As a result, the plant tissues increase the respiration rate, ethylene production, and tissue softening, which decrease the fruit's quality. In addition, the fruit becomes more susceptible to microbial attack due to the leakage of vesicular juice, and the absence of the protective peel. Therefore, it is necessary to find a means to prevent or slow down the deterioration process of the fresh-cut fruits.

This thesis follows the style of the Journal of Food Science.

One alternative is the use of edible coatings to control moisture transfer, gas exchange, or oxidation processes. One major advantage of using edible coatings is that several active ingredients can be incorporated into the polymer matrix and consumed with the food. Traditionally, edible coatings have been used in the fresh-cut industry as a strategy to reduce the undesired effects that minimal processing produces on intact fruit tissues (Giacalone and others 2010).

The growth of microorganisms on the cut surfaces is also an important cause of food spoilage for fresh-cut produce (Beuchat 2007). The application of antimicrobial substances directly to the food has some limitations because these active substances can be neutralized, evaporated, or they may inadequately diffuse into the bulk of the food. Hence, edible coatings could be a feasible option; however, many consumers have concerns about the addition of chemical additives to food and it is essential to use coating materials with a natural antimicrobial compound that is GRAS (generally recognized as safe) (FDA 2009).

Whenever applying a new technology, research on the microbiological, physico-chemical, nutritional, and sensory aspects needs to be undertaken to determine the effectiveness of the technology in improving and somewhat guarantying the preservation of the fruit and to ensure its safety (Rojas-Grau and others 2009). For the fresh produce, research and development of antimicrobial edible coatings to be applied onto fresh-cut fruits have the potential to increase the shelf-life of their products (Sangsuwam and others 2008).

The main goal of this research was to determine the best alginate-based antimicrobial edible coating that extends the shelf life of fresh-cut pineapple pieces. Specific objectives were to:

1. Optimize preparation of the alginate-based antimicrobial edible coating by testing several concentrations of alginate with antimicrobial compound, pectin, and calcium chloride.
2. Evaluate the effectiveness of the coating to extend the physical, chemical, microbiological, and sensory shelf-life of fresh-cut pineapple.

CHAPTER II

LITERATURE REVIEW

2.1. Fresh produce

2.1.1. Pineapple

Pineapple, (*Ananas comosus*), belongs to the order *Bromeliales,* family Bromeliaceae, and subfamily Bromelioideae (d'Eeckenbrugge and others 2003). It is the third most important tropical fruit in world production after banana and citrus. Around 70% of the pineapple produced in the world is consumed as fresh fruit in the country of origin. The most famous variety in the world trade is Cayenne Lisse ("Smooth Cayenne"), which was introduced to Europe from French Guiana. In the United States, the production and commercial processing of pineapple started in Hawaii at the end of the 19th century when Hawaii was a world leader in pineapple research and processing (Rohrbach and others 2003).

The processing of pineapple has made this fruit well known throughout the developed countries. Most of the pineapple products of international trade are canned slices, chunks, crush (solid pack), juice, and especially fresh fruit. International trade is dominated by a few multinational companies that have developed the infrastructure to process and commercialize pineapple. Thailand and Indonesia are exceptions with small local processing operations (Loeillet 1997).

Important countries that produce the fruit primarily for their own fresh-fruit markets are Brazil, India, China, Nigeria, Mexico, and Colombia. Statistics on world production collected by the Food and Agriculture Organization of the United Nations (FAO) revealed that total pineapple production was approximately constant in the 1999-2001 periods, with a mean world production for these three years of 13,527,149 metric tons. World production has more than tripled during

the past 30 years (3,833,137 tons in 1961 to 13,738,735 tons in 2001). The leading pineapple producing countries are Thailand with 2,311,332 tons, the Philippines with 1,520,175 tons and Brazil with 1,504,493 tons (means 1999-2001). The international fresh-pineapple market (about 670,000 tons) is dominated by Costa Rica, and the Philippines. The North American market is primarily supplied by Costa Rica and Hawaii. In the USA, annual per capita consumption of fresh pineapple fruit has gone from 0.3 to 0.9 kg. This is still very low compared with the approximately 5 kg of processed pineapple that has been consumed over the past 25 years, and with the consumption of other fruits such as bananas, apples, and oranges, but it has been growing during the last few years and it is expected to continue growing (Rohrbach and others 2003). Pineapple fruit is demanded by the consumers and highly appreciated for its aroma, flavor, and juiciness, but its consumption, is also related to its nutritional components (Table 1.1).

In some studies, it is shown that pineapple fruit being rich in phenolics may provide a good source of antioxidants (Hossain and Rahman 2011). Pineapple pulp and core possess low concentrations of epicatechin and ferulic acid (Yi and others 2006). In addition, many different volatile compounds were found in pineapple pulp. Some other bioactive components, which are important in the field of medicine, such as flavonoids, saponins, tannins, cardenolides, and bufadienolides were found in the exocarp of pineapple (Gundran and others 2001). Pineapple also is part of a big group of fruits that are important due to their antioxidant capacity according to the ascorbic acid equivalent antioxidant capacity (AEAC). Some of the fruits that are part of this group are: chiku, with the highest antioxidant capacity, followed by strawberry, plum, star fruit, guava, seedless grape, avocado, orange, solo papaya, mango, kiwifruit, pomelo, lemon, pineapple, apple, foot long papaya, rambutan, banana, coconut pulp, tomato, rockmelon, honeydew, watermelon and coconut water (Dembitsky and others 2011). Pineapple is also

important because of its ascorbic acid content as a source of vitamin C. The amount of ascorbic acid present on pineapple is approximately 22.4 ± 0.9 mg/100 g of fruit. (Beserra- Almeida and others 2011).

Table 1.1 Nutritional composition of pineapple (USDA 2010).

Nutrients	Units	Value per 100g pineapple
Water	g	87.24
Protein	g	0.55
Total lipid (fat)	g	0.13
Ash	g	0.27
Carbohydrate	g	11.82
Calcium, Ca	mg	13
Potassium, K	mg	125
Sodium, Na	mg	1
Vitamin C, total ascorbic acid	mg	16.9
Carotene, beta	mcg	31

2.1.2. Fresh-cut fruits

The production of fresh-cut fruit and vegetables is an emerging category, because consumers look for healthy food with quality, freshness, and convenience. Furthermore, today's busy consumers have no time to prepare their meals and healthy ready-to-eat products are interesting alternatives (Garret 2002, Oms-Oliu 2010). The International Fresh-cut Produce Association (IFPA) defines fresh-cut products as "any fruit or vegetable or combination thereof that has been physically altered from its original form, but remains in a fresh state". Fresh-cut fruit and vegetables generally consist in washed, cut, treated with sanitizing agents and packaged products and stored under refrigerated conditions (Bierhals and others 2010).

According to a past report by the International Fresh-cut Produce Association (IFPA), sales of fresh-cut produce in the United States were projected to increase from their $5.8 billion market in 1994 to $19 billion by 1999, with the majority of this increase in fresh-cut fruits. Fresh-cut produce has become so popular with consumers because they represent a value-added, ready-to-use commodity that satisfies their requirement for nutritional value, and naturalness (no preservatives added) (Spanier, 1998).

Fresh-cut pineapple is already found in many supermarkets and food service chains. Fresh-cut pineapple fruit is appreciated for its taste, flavor and juiciness (Gonzalez-Aguilar and others 2005, Montero-Calderon and others 2008).

Quality of fresh-cut fruit products determines their value to consumers and is a combination of attributes, properties, or characteristics including appearance, texture, flavor, and nutritional value. A major challenge faced by the produce industry is to manipulate the quality of fresh-cut produce that the shelf-life is long enough to ensure efficient marketing. Fresh-cut produce deteriorates faster than intact produce because of internal and external browning of the cut surfaces (Gonzalez-Aguilar and others 2005).

The requirement of consumer satisfaction imposes a challenge for the producer and packager who must physically transform the fruit into an entirely new product, yet maintain the fruits original flavor characteristics, and principally safety (Spanier 1998).

Fruits are living tissues that undergo enzymatic browning, texture decay, microbial contamination and undesirable volatile production, highly reducing their shelf-life, if they are in any way wounded (Tapia 2008). Wounding promotes the production of ethylene that leads the oxidation of fatty acids and produces some degradation (Watada and others 1990).

Because the tissue integrity of these products has been altered during processing, fresh-cut fruit are more perishable than the original raw materials. Fresh-cut processing increases

metabolic activities and delocalization of enzymes and substrates. This may lead to deterioration such as browning, softening, decay, and off-flavor development. These manipulations result also in increased rates of respiration and ethylene production and may reduce the shelf-life of fresh-cut fruit commodities (Di Egidio and others 2009). The manipulation of the fruit result in the production of ethylene within minutes (Abe & Watada,1991) and may reduce the shelf-life from 1 to 2 weeks to only 1 to 3 days even at optimal temperatures (Ahvenainen 1996, Gonzalez Aguilar and others 2004). The post-cutting life of pineapple has been reported to be very dependent on temperature, from a few hours at 20 ºC to several weeks at 1 ºC (Marrero and Kader 2005).

Fresh-cut processing may increase also spoilage of fruit through transfer of peel micro flora to fruit flesh where microorganisms can grow rapidly upon exposure to nutrient rich juices. This has been a particular problem observed when pineapples were processed for fresh-cut purposes. In the case of fresh-cut pineapple, one of the principal problems with this fruit and its minimal process was browning after 6 days of storage at 4 º C, not microbial decay (Gonzalez Aguilar, 2004).

In the minimal process of the fresh-cut fruits can also be present an enzymatic discoloration. The enzyme polyphenol oxidase (PPO) is able to catalyze the hydroxylation of monophenols to o-diphenols, and dehydrogenate o-diphenols to quinones. The resulting quinone products can then polymerize and react with amino acid groups of cellular proteins, resulting in brown or black pigmented deposits in many fruits and vegetables (Mayer 1987). In the case of pineapple, the discoloration process is present when the fruit is peeled and processed and could be present as a physiological disorder called Blackheart when it is a whole fruit. This is induced by exposure of the fruit to low temperature either in the field or during post-harvest storage (Zhou 2003).

Browning in fresh-cut fruits is a major concern because is related to the extension of the shelf-life of the final product, and in the case of fresh-cut pineapple could strongly affects the consumer's purchase decision (Oms-Oliu, 2010).

2.1.3. Quality attributes

Color, flavor, texture, and nutritive value are generally recognized as the four quality factors of fruits and vegetables. The natural pigments, chlorophylls, carotenoids, and anthocyanins, form the chemical basis of color. Enzymatic and non-enzymatic browning contributes to coloring of certain processed fruits and vegetables. Various volatile aroma and nonvolatile compounds give fruits and vegetables special flavors. Cell wall components and turgor pressure are two entities that provide the texture of fruits and vegetables. Pectic substances and pectic enzymes are closely related to firmness and softening of many fruits and vegetables. Celluloses and lignins are associated with toughness and woody texture. Vitamin C and minerals are the major nutrients of fruits and vegetables. Processing often alters the quality of fruits and vegetables but does not change the chemical basis (Jen, 1989).

Quality of fresh-cut fruit products determines their value and acceptability to consumers, and is a combination of attributes, properties or characteristics including appearance, flavor, texture and nutritional value (Di Egidio and others 2009).

Depending on the specific fruit or vegetable, the importance of each quality factor will vary. Flavor is one of the most difficult quality factors to maintain in fruit and vegetable products. Flavor change may result from the loss of compounds that provide good flavor or from the accumulation of compounds that produce off-flavors. Aroma components of flavor are most important for some products, especially fruits. Aroma quality may be lost by either the disappearance of good aromas or by the development of bad aromas. Light-processing systems

should supply products, which maintain the original flavor quality of the material from which they are made, and the systems must avoid conditions that lead the off-flavor development (Huxsoll and others 1989).

Texture is a quality factor that differentiates fresh from processed foods. Fresh fruits and vegetables have textures that are described as “crisp” or “firm” which are considered desirable. The cells of high quality fresh fruits and vegetables have a high “turgor”. Processing stress results in loss of that turgor (Huxsoll and others 1989). Texture also depends on geometrical, surface and mechanical attributes of the sample, tissue composition, and how the structure responds to physical stresses. It is perceived by a combination of tactile, visual and hearing senses and its determination is complex and influenced by assessment methods, instruments, and operation conditions (Dan and others 2007).

Color, antioxidant characteristics, vitamin C content and other quality properties have been reported as important values for product quality. However, these values may vary from the whole fruit to fresh-cut fruit product. It is suggested that vitamin C, for example, can be larger near the surface of the fruit than close to its core (Paull and Chen 2003).

Considering all these quality factors, fresh-cut fruits are still under study because of the difficulties in preserving them over prolonged periods. Temperature, atmosphere, relative humidity and hygiene must be regulated to maintain all those attributes (Giacalone and others 2010).

Pineapple quality attributes are very well appreciated all around the world. They combine good flavor, aroma, juiciness, sweetness, and texture together with nutritional content, as it is a source of vitamin C, fiber, and minerals (Brat and others 2004). Fresh-cut pineapples have a good potential as a value-added product, for which homogeneity is a key attribute, and thus, it is important to determine raw fruit characteristics and their variability throughout the

fruit for proper selection, processing, and quality assessment (Montero-Calderon and others 2010)

2.1.4. Microbial contamination

Foodborne illnesses are a major area of research and analysis in many countries and in the United States. Outbreaks of human diseases are, in an important percentage (33%), associated with consumption of raw fruits and vegetables contaminated with pathogenic bacteria and viruses. In fact, the number of outbreaks and cases of illness caused by consumption of fresh-cut fruits and unpasteurized juice has increased in the last years (Harris and others 2003). This increased frequency has occurred in many countries and in the United States in the past decade (Ayers and others 2009).

The development of sophisticated epidemiologic and surveillance techniques, together with changes in agronomic, harvesting, processing, distribution, consumption patterns and practices have undoubtedly contributed to this documented increase (Beuchat 2007).

Current practices associated with production, processing, and preparation of fruit and vegetables provide many opportunities for transfer of pathogens to humans. Therefore, because of inappropriate manipulation and storage conditions, both pathogenic and/or deteriorative microorganisms may contaminate a product, thus increasing the risk of microbial diseases and spoilage (Raybaudi-Massilia and others 2009).

Microbial contamination of the fruit flesh can occur due to the release of substrates increasing the fruit spoilage and the risk to consumer due to pathogenic microorganism's presence (Raybaudi-Massilia 2008, Moreira 2011). Microbial growth can seriously limit the safety and shelf-life of fresh-cut fruits and vegetables. The high content of organic acids and sugars present in the fruit tissue can be available after peeling and cutting and be a good source

of nutrients for bacteria, yeast, and mold growth. Among the deteriorative micro flora fungi are the most important microorganisms causing wastage of fresh-cut fruit, where the relatively acid conditions tend to suppress bacterial growth (Ayala-Zavala and others 2009).

Fruits and vegetables have heterogeneous characteristics regarding to their compositions. Consequently, the micro biota in these products may substantially differ depending on medium pH, nutrient availability, water activity, among other factors (Kalia and Gupta 2006). Fruits may become contaminated with pathogenic and spoilage microorganisms either during their growing in fields, orchards, vineyards, or greenhouses, or during harvesting, postharvest handling, and distribution (Beuchat 2002). As a whole, fresh fruits have a natural protective barrier (skin) that acts effectively against most plant spoilage and pathogenic microorganisms; however, this protection is eliminated during the processing, thus exposing the fruit flesh to unfavorable environmental conditions as well as to a possible contamination with pathogenic microorganisms including bacteria, viruses, and parasites during the handling, cutting, shredding, and maintenance of the fresh-cut fruit ambient temperature (Balla and Farkas 2006).

The causal agents of microbiological spoilage in fruits and derivatives can be bacteria, as well as yeast and molds. The latter are considered the main spoilage agents due to the low pH of most fruits. Certain common molds such as Penicillium spp., and Botrytis spp., have been shown to be involved in the spoilage of fresh-cut fruits and some processed fruit derivatives including thermal processed. 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 and others 2009).

Quality losses in fresh-cut fruits may occur because of microbiological, enzymatic, chemical, or physical changes. Safety and quality losses by microbiological causes are very important

because they constitute a hazard for consumers by the possible presence of microbial toxins or pathogenic microorganisms in the product. For this reason, many food preservation strategies such as chilling, freezing, acidification, or the use of antimicrobials have been traditionally applied to control microbial growth (Davidson 2001).

The human epidemics of enteric illness and gastrointestinal infections associated with fresh produce can be reduced by preventing contamination at all points from the field to the time of consumption. Decontamination procedures may be applied to fresh produce but it is known that current processes cannot eliminate the contaminating microorganisms (Gomes and others 2011).

Ways of handling produce that will prevent the growth of pathogens, as well as removing pathogens by washing or treating with sanitizers, are extremely important efforts by the fresh-cut industry to manage safety risks. The effectiveness of sanitizers is often minimal, however, because pathogens on and in plant tissues may be protected against exposure to the lethal components during treatment, thereby posing unique challenges to management of factors affecting safety. The challenge is to develop and apply treatments that will reach pathogens on the surface and in subsurface areas of fresh-cut produce in an active form without compromising sensory quality throughout subsequent shelf-life (Burnett and Beuchat 2001).

Management of safety risks associated with fresh and fresh-cut fruits and vegetables requires good agricultural practices (GAPs) and application of hazard analysis critical control point (HACCP) programs throughout various stages of growing, harvesting, processing, packaging, distribution, and preparing fresh and fresh-cut produce for consumption (Beuchat, 2007).

Despite a recent effort by industry, academia, and government to reduce the incidence of foodborne diseases associated with fruit and vegetables, infections continue to occur. This has

result in great interest in identifying and promoting adoption of practices that will absolutely result in a safer food supply and a more robust US agricultural economy (Wilson and others 2009).

2.2 Preservation alternatives

Fresh-cut fruit and vegetables industry is a relative new industry that has been looking for new alternatives to preserve quality and extend the shelf-life of their products. Fresh-cut fruit is still under study because of the difficulties in preserving their fresh quality over prolonged periods. Temperature, relative humidity, and hygiene must be regulated to maintain fresh-cut quality (Giacalone and others 2010).

Some of the alternatives that have been investigated as alternatives to preserve quality attributes of fresh-cut fruits are mentioned as follows.

Recently, the use of natural products and their derivatives have been found to be effective in reducing browning and decay of many fresh-cut fruits and vegetables. These antibrowning agents and their derivatives such as 4-hexylresorcinol, N-acetylcysteine (AC), ascorbic acid (AA), isoascorbic propionate, alone or in combination at different concentrations, have been found to be effective in retarding browning and reducing decay of fresh-cut produces. The effects of such treatments have not been reported in maintaining quality of fresh-cut pineapple (Gonzalez-Aguilar and others 2003).

Traditionally, sulfites have been used for browning prevention. However, their use on fresh-cut fruit and vegetables was banned in 1986 by the FDA owing to their potential hazards to health. Thus, various alternative approaches have been studied to minimize visual deterioration in fresh-cut fruit (Oms-Oliu and others 2010).

The most frequent alternative to sulfites is ascorbic acid (AA), which is recognized as a GRAS substance by the U.S. Food and Drug Administration (FDA) for its use to prevent browning of fruit and vegetables. Ascorbic acid is used to control PPO enzyme activity through its ability to reduce the o-quinones back to their phenolic substrates. Dips of ascorbic acid have long been applied in combination with organic acids and calcium salts to prevent enzymatic browning of fruit (Ahvenainen 1996).

Reducing agents such as citric acid, ascorbic acid, isoascorbic acid, and sodium erythorbate, have been investigated to prevent browning. Calcium treatments can maintain or improve tissue firmness and crispness of fresh-cut fruit. Calcium chloride has been one of the most frequently used salts of calcium although it is reported to impart residual taste to the product. Thus, other calcium salts such as calcium lactate, calcium propionate, or calcium ascorbate have been investigated as alternative sources of calcium. Surface treatments involving dipping fruit pieces into aqueous solutions containing antimicrobial agents, antioxidants, calcium salts, or functional ingredients such as minerals and vitamins are widely practiced to improve quality of fresh-cut fruit (Oms-Oliu and others 2010).

Because minimally processed fruit and vegetables are not heat treated, regardless of the use of additives or packaging, another alternative is that they must be handled and stored at refrigeration temperatures (< 5 ºC) to achieve a sufficient shelf-life and ensure microbiological safety (Ahvenainen 1996).

For most packaged salads and fresh-cut produce, modified atmosphere packaging (MAP) is a technology that is currently used with temperatures of storage from 5 ºC to 10 ºC. However, the benefits of MAP are used only to a lesser extent with fresh-cut such as cantaloupe, pineapple, and apple (Gonzalez-Aguilar and others 2004). This technology uses the principle that a modified atmosphere can be created either passively by using properly permeable packaging

materials, or actively by using a specified gas mixture together with permeable packaging materials. This principle is the most difficult to accomplish of all the tasks involved in manufacturing raw ready-to-use or ready-to-eat fruit and vegetable products of good quality and with a shelf-life of several days (Day 1994).

One interesting modified-atmosphere packaging method is moderate-vacuum packaging (MVP). In this system, respiring produce is packed in a rigid, airtight container under 40 kPa of atmospheric pressure and stored at refrigeration temperature (4-7 ºC). It was found that MVP improved the microbial quality of red bell pepper, chicory (endive), sliced apple, and sliced tomato; the sensory quality of apricot and cucumber; and both the microbial and sensory quality of mung-bean sprouts and a mixture of cut vegetables (Gorris and others 1994).

Another technology is what it is known as active packaging, that is, packaging that includes various gas absorbents and emitters. It appears that it is possible to affect respiration activity, microbial activity and plant hormone activity by correct active packaging. There are still more investigations and more research to do around this active packaging but it is one of the alternatives that are available for fresh-cut fruit and vegetables (Ahvenainen 1996).

2.3. Edible coatings

Based on the more basic concepts of the physiology of minimally processed produce and the reactions that occur and affect the quality of them, including the environments or processing mechanical operations (peeling, coring, cutting, and/or slicing) where microbial growth can occur or can be inhibited, many solutions have been studied in the last years, being the edible coatings the more recent and a cost effective alternative to modified atmosphere packaging (Tapia and others 2008, Vargas and others 2008, Oms-Oliu and others 2010, Gonzalez-Aguilar and others 2005, Brasil and others 2012).

The application of edible coatings to deliver active substances is one of the recent major advances made in order to increase the shelf-life of fresh-cut produce. Thus, the effectiveness of the different chemical treatments and compounds used to preserve the quality attributes of fresh-cut fruit and vegetables could be very much improved with their incorporation into edible coatings (Oms-Oliu and others 2010).

Any type of material used for wrapping various foods to extend the shelf-life of the product that may be eaten together with food with or without further removal is considered an edible film or coating. A film is occasionally differentiated from a coating by the notion that it is a stand-alone wrapping material, whereas a coating is applied and formed directly on food surface itself. Edible films provide replacement and/or fortification of natural layers to prevent moisture losses, while selectively allowing for controlled exchange of important gases, such as oxygen, carbon dioxide, and ethylene, which are involved in respiration processes. A film or coating can also provide surface sterility and prevent loss of other important components. Generally, its thickness is less than 0.3 mm (Pavlath and Orts 2009).

Items, which are edible or are in contact with food should be generally recognized by qualified experts as being safe under conditions of its intended use, with amounts applied in accordance with good manufacturing practices. These food-safe materials must typically have approval of the Food and Drug Administration (FDA) that recognizes they are safe for the human consumption (Pavlath and Orts 2009, Brasil and others 2012, FDA 2011).

Extending the shelf-life of the product is one of the objectives of the edible coating development but also trying to reduce and avoid the microbial growth on the final product, being the latest a big problem in the food safety area and causing human diseases and foodborne illness to many people in the United States and other countries (Beuchat 2007).

Edible coatings have been used to preserve whole or fresh-cut produce since they may act as barrier to water loss and oxygen access. The basic composition of edible coatings for fresh-cut fruits may include hydrocolloids and lipids. These hydrocolloids (proteins and carbohydrates) tend to form hydrophilic networks, usually being a good barrier to oxygen and carbon dioxide, but a poor barrier to water. Some polysaccharides that have been successfully used to coat fresh-cut fruits include carrageenan, maltodextrin, methylcellulose, carboxymethyl cellulose (CMC), pectin, alginate, chitosan, starch, and microcrystalline cellulose (Sadili-Bico and others 2010). It is important to consider that the effectiveness of edible coatings depends on the type of material with which they are elaborated, such as polysaccharides, protein, and lipids (Martinez-Ferrer and others 2005, Aguilar-Mendez and others 2008, Brasil and others 2012).

Some proteins that have been commonly used for making films and coatings are casein, whey protein, meat proteins, and egg albumen, from animal origin; wheat proteins, soy proteins, and corn zein from plant origin. Plasticizers are also used in the formulation of films and coatings. Plasticizers are small molecular-weight compounds that can be added to an edible film or coating solution to improve the flexibility and mechanical properties of the film matrix. Most protein-based films and coatings are very strong, but very brittle when not plasticized; thus, a plasticizer is necessary to improve the application potential of protein-based films. While plasticizers can improve the flexibility and elongation of protein films, they also affect the permeability of the films and coatings. As a rule, the addition of a plasticizer increases the permeability of a film or coating (Dangaran and others 2009).

Whichever be the material or ingredient that the edible coating is made of, it may contribute to extend the shelf-life on fresh-cut fruits, in this case pineapple, by reducing moisture and solute migration, gas exchange, respiration and oxidative reaction rates, as well as by

reducing microbial growth and extending the shelf-life of the product (Giacalone and others 2010).

2.3.1. Sodium alginate

Alginate is extracted from brown seaweed of the family *Phaeophyceae*. Commercial sources include *Laminaria sp.*, *Macroscystis pyrifera*, *Ascophyllum nodosum*, *Eclonia sp*., *Lessonia nigrescens*, *Durvillae antarctica* and *Sargassum spp*. (Draget 2000) Alginate is present in seaweed as a salt of sodium, calcium, magnesium, strontium and barium in gelled form; hence, the first step in the extraction process is to apply an acid treatment to convert alginate into alginic acid, followed by an alkali treatment (Na_2CO_3 or NaOH) to produce water-soluble sodium alginate (Nieto 2009).

Alginates are linear, unbranched polymers and highly anionic (-). Alginates are not strictly random copolymers, but are instead block copolymers. Alginates may also be prepared with a wide range of average chain lengths (Nieto 2009).

Gelation of alginate, with calcium or a bivalent ion, is instantaneous. The alginate responds to calcium cross-linking very fast, and the structure accommodates Ca^{2+} ions to form salt bridges, corresponding to junction zones between adjacent polymer chains (Donati and others 2005).

Sodium alginate forms a decent strong film, despite the negative charge on the molecule. The carboxylate groups make alginate very soluble in water, and the concentration of calcium salts and the change in pH affect the firmness and gel strength (Nieto 2009, Roopa and Bhattacharya 2009).

2.3.2. Edible coating formation

Typical methods for forming a coating include panning, fluidized-bed processing, spray-coating, and dipping.

Panning is a method used by both pharmaceutical and confectionery industries, and entails putting the product to be coated into a large, rotating bowl, referred to as the pan. The coating solution is ladled or sprayed into the rotating pan, and the product is tumbled within the pan to evenly distribute the coating solution over the surface of the food or pharmaceutical material. Forced air, either ambient or of elevated temperature, is used to dry the coating (Minifie 1989).

Fluidized-bed coating, a method used commonly by the pharmaceutical industry to coat tablets, has been studied for formation of whey protein edible coatings to protect nuts and peanuts. It was found that alternating the spraying of coating solutions with periods of drying allowed for the gradual formation of the coating. The action of the fluidized-bed during drying of the coating appeared to reduce the formation of clusters of coated product, a problem commonly encountered with formation of coatings via panning (Dangaran and others 2009, Lin and Krochta 2006).

Spray-coating is used to apply a uniform coating over a food surface, and is potentially a more controllable method of coating application than pan- or fluidized-bed-coating. However, spray-coating requires that the bottom surface of the product be coated in a separate operation after application of the initial coating and drying. In this scenario, the product must then be turned to expose the bottom for subsequent coating application. Spray-coating is preferred for items possessing a large surface area (Dangaran and others 2009).

Dipping, the other possible method of forming edible coatings on the surfaces of food is best-suited for irregularly-shaped food objects. Final formed coatings may be less uniform than

coatings applied by other methods, and multiple dipping (with draining and drying steps between dipping operations) may be necessary to ensure full coverage (Krochta and others 1994).

2.3.3. Application on fresh-cut fruits

There are very few published studies about edible coatings in fresh-cut pineapple. Montero-Calderon and others (2008) studied the application of sodium alginate coatings and modified atmosphere packaging in fresh-cut pineapple slices and found that a shelf-life of 14 days (at 5 ºC) was reached, reducing juice leakage and preserving chemical characteristics, color, and mechanical properties (texture). No sensory testing was conducted in this study. In a related study, Montero-Calderon and others (2008) reported that 2% sodium alginate edible coating applied to fresh-cut pineapple pieces was effective in preventing juice leakage. According to the researchers, the juice leakage of coated samples was 75% lower than uncoated samples, which indicates that the coating used in the study helped to preserve the freshness characteristic of the fruit.

Bierhals and others (2011) investigated the effect of cassava starch film on pineapple. They found that the pineapples treated with 1%, 2% and 3% of cassava starch and stored at 5 ºC did not present significant changes ($P>0.05$) in mechanical properties (stress and failure) and superficial color (L*and H*) when compared with the uncoated control sample, indicating that the edible coating did not affect the natural mechanical properties and maintained the fruit's natural color. The coating application did not influence the sensory attributes of the product and all treatments had good sensory acceptance, with all scores above 6.5 out of a 9-point hedonic scale. Cassava edible coating showed a beneficial effect on reducing the weight loss, juice leakage, respiration rate, and other parameters of minimally processed pineapples but with this

treatment a short shelf-life of 7 days was obtained, therefore the coating was not able to increase the shelf-life of fresh-cut pineapple.

In more recent investigations, Brazilian scientists tried to see how minimally processed strawberries, stored for 15 days at 5 ºC, would be affected by cassava starch (CS) as an edible coating, with and without the antimicrobial potassium sorbate (PS). They found that strawberries coated with cassava starch without the antimicrobial were appealing to consumers. They were acceptable for up to 12 days of storage (Hubinger 2010).

Another study found that edible coatings could allow for the development of "ready-to-eat" fresh blueberries with no reduction in shelf life. The results differed depending on the packaging system and the coatings used. Non-vented containers provided better barrier protection against water evaporation and gas exchange, thus delaying ripening and dehydration. However, the water accumulation allowed by this packaging system could also promote mold growth, which means an anti-fungal coating would be needed (Zhao 2011).

In the same trend, Marrero and others (2006) pointed the importance of an edible coating as an alternative to help improve quality factors on fresh-cut pineapple such as juice leakage that is not possible to control neither with refrigeration nor with modified atmosphere packaging.

After considering the different studies, it is possible to think about an alginate coating as a good option to preserve the quality of fresh cut pineapple and extend its shelf-life. This material presents some properties that create a good and homogenous coating on the fruit and it does not add any color or odor or extra flavor to it. A study by Tapia and others (2008) in papaya showed that this material helps to prevent water losses and firmness of the fresh-cut fruit so it is a good option to use in the future analyses with fresh-cut pineapple to extend its shelf-life.

2.4. Antimicrobial compounds

Edible coatings can lead to a substantial decrease in physiological disorders of products. The incorporation of additional nutrients, the enhancement of sensory characteristics, or the inclusions of antimicrobials are among the potential benefits that an edible coating has to offer. One major advantage of using edible films and coatings is that several active ingredients can be incorporated into the polymer matrix and consumed with the food, thus enhancing safety or even nutritional and sensory attributes. The development of edible films and coatings as carriers of active ingredients is considered a promising packaging system that would maintain the freshness of fresh-cut fruits and vegetables (Rojas-Grau and others 2009).

Edible coatings have been recognized for innovative applications beyond their current uses. Polysaccharide coatings can carry active ingredients such as antibrowning agents, colorants, flavors, nutrients, spices, and antimicrobial compounds that can extend product shelf-life and reduce the risk of pathogen growth on food surfaces. Coatings based on sodium alginate, pectins, and gellan gum have been shown to be effective, not only in retarding water loss, but also in incorporating different active agents such as probiotic microorganisms or natural antioxidants and antimicrobials. Despite the good results achieved by incorporating active compounds into edible films and coatings, the use of certain ingredients in formulations may have a detrimental impact on the flavor of products. For example, the incorporation of antimicrobial agents, especially essential oils, could impart undesirable sensory modifications in fresh-cut fruits. Future research must involve exploring alternatives for controlling the release of incorporated compounds, thus improving their effectiveness. This might be done by using nano-technological techniques (Soliva-Fortuny, 2010).

Dipping of aqueous solutions containing antimicrobials is the most practical way to extend the shelf-life of the product. However, application of antimicrobial agents directly on the

food surface may have limited benefits because the active substances are rapidly neutralized or diffuse from the surface into the food product, thus limiting the effect of the antimicrobial compound. In this sense, antimicrobial edible films and coatings may provide increased inhibitory effects against spoilage and pathogenic bacteria by maintaining effective concentrations of the active compounds on the food surfaces. There are several categories of antimicrobials that can be potentially incorporated into edible films and coatings, including organic acids (acetic, benzoic, lactic, propionic, sorbic), fatty acid esters (glyceryl monolaurate), polypeptides (lysozyme, peroxidase, lactoferrin, nisin), plant essential oils (EOs) (cinnamon, oregano, lemongrass), nitrites and sulphites, among others. Within these categories, plant essential oils are outstanding alternatives to chemical preservatives, and their use in foods meets consumer demands for minimally processed natural products. Essential oils are designated as "Generally Recognized as Safe" (GRAS), and are used as flavoring agents in various foods. These compounds can also be added to edible films and coatings to modify food flavor, aroma, and odor (Martin-Belloso and others 2009, Rojas-Grau and others 2009, Gomes and others 2011, Brasil and other 2012).

For the selection of an antimicrobial, it must be considered the effectiveness against the target microorganism and also the possible interactions among the antimicrobial, the film-forming biopolymer, and other food components present. These interactions can modify the antimicrobial activity and the characteristics of the film being these key factors for the development of antimicrobial films and coatings (Campos and others 2010).

Many factors must be considered in developing an antimicrobial edible coating, including the properties of the food, the coating and the effectiveness of the antimicrobial agents incorporated into the coating. Because of this, basic preliminary studies must be carried out to evaluate the antimicrobial effect of a compound incorporated into an edible film matrix before it

is applied on the surface of a real food system. Edible coatings are created to be consumed with the coated products. Therefore, the incorporation of compounds such as antimicrobials, antioxidants, and nutraceuticals should not affect consumer acceptance. Some authors have indicated that the incorporation of antimicrobial agents into edible coatings could impart undesirable sensorial modifications in foods, especially when essential oils are used (Rojas-Grau and others 2009).

2.5. Essential oils

In the last years, there has been a considerable pressure by consumers to reduce or eliminate chemically synthesized additives in foods. Essential oils represent an alternative to chemical preservatives and their use in foods meets the demands of consumers for natural products (Rojas-Grau and others 2009).

Essential oils are natural, volatile, complex plant compounds, oily or lipid-like in nature and frequently characterized by a strong fragrance (Bakkali and others 2008, Burt 2004). They have a low solubility in water but are soluble in fats, alcohol, organic solvents and other hydrophobic substances and are generally liquid at room temperature. They are stored in specialized plant cells, usually oil cells or ducts, resin ducts, glands or trichomes (glandular hairs) and may be extracted from the leaves, flowers, buds, seeds, fruits, roots, wood, or bark of plants by a variety of methods, including solvent and supercritical fluid extraction (Pengelly 2004, Carson and others 2011).

Essential oils are often described as secondary plant metabolites. Traditionally, secondary plant metabolites have been all those compounds synthesized by the plant, which do not appear to be essential for plant growth and development, and/or those compounds without an

obvious function. They are also not universally synthesized in all plants (Croteau and others 2000).

Essential oils are not simple compounds or even simple mixtures of several individual compounds. They may contain up to approximately 100 components, although many contain about 20 to 60 (Langenheim 1994, Dung and others 2008). The compounds found in essential oils are from a variety of chemical classes, predominantly terpenes, but phenylpropanoids and other compounds also occur although at lesser frequency and often, but not always, in smaller proportions (Friedrich 1976). They are all hydrocarbons and their oxygenated derivatives, and they may also contain nitrogen or sulfur. They are generally low-molecular-weight compounds with limited solubility in water (Griffin and others 1999).

Despite their history of being regarded as secondary, non-essential plant metabolites, it is becoming clear that essential oils and their components have specific biological functions, many of which lend themselves to commercial exploitation (Pichersky and others 2006, Gershenzon and Dudareva 2007). Given the range and complexity of the compounds present in essential oils, it is hardly surprising that they have the capacity to affect many biological systems. The biological activities of greatest interest center on applications in health, agriculture, and the cosmetic and food industries (Ballabeni and others 2004, Liao and others 2008).

In the arena of health and medicine, the diverse array of biological properties now being characterized includes antimicrobial, anticancer, analgesic, antioxidant, anti-inflammatory, and other immune-modulatory and antiplatelet, and antithrombotic activities. Along with fragrance and solvent properties, several of these activities also find application in the cosmetic and food industries. Of greatest interest in agriculture is the antimicrobial and insecticidal potential of essential oils and their components (Carson and others 2011).

The activity of essential oils and their active constituents have been widely studied against many microorganisms, including several pathogens, although their mechanisms of action have not been studied in great detail. In this sense, it has been reported that hydrophobicity is an important characteristic of essential oils, which makes them able to pass through cell membranes and enter mitochondria, disturbing the internal structures and rendering the membranes more permeable (Rojas-Grau and others 2009).

Whatever the reasons for the apparently renewed interest, there are now hundreds of reports of the *in vitro* antimicrobial activity of essential oils in the scientific and medical literature, including reviews of the medicinal properties of some of the more popular oils such as clove (Chaieb and others 2007), lavender (Cavanagh and Wilkinson 2002), *Lippia* spp. (Pascual and others 2001) and tea tree (Carson and others 2006). This antimicrobial activity includes activity against bacteria, viruses, and protozoa. Essential oils and components also exhibit activity against fungi, activity that is becoming increasingly well described. A wide range of human, animal, and agricultural fungal pathogens have been shown *in vitro* to be inhibited and/or killed by essential oils, heightening interest in their therapeutic or industrial application (Carson and others 2011).

Essential oils and their constituents are widely used in many foods and beverages, primarily as flavoring agents (Taylor 2005). Citrus-peel essential oils are amongst the most important of these, including orange, lemon, mandarin, tangerine, and grapefruit oils. Peppermint, corn mint, eucalyptus, and citronella oils are other leading oils in terms of volume (Schwab and others 2008). Amongst single constituents, one of the most important to the flavor industry is menthol (Serra and others 2005).

The concentrations used in foods and beverages are generally low; in beverages levels are typically at or below 0.1% (Taylor 2005). In foods in Europe, for example, eucalyptus oil is

approved for use as a flavoring agent at 5 mg/kg or less and in confectionery at 15 mg/kg (Batish and others 2008). As discussed previously, the levels of essential oils that are used in foods are governed largely by their effect on the organoleptic properties of the food. Their presence in food may also contribute to preservation of the products, depending on the concentrations used and the interaction they have with other ingredients and preservation factors in the product (Holley and Patel 2005, Carson and others 2011).

The application of essential oils in foods is yet limited due to their impact on organoleptic food properties, variability of their composition, and their variable activity in foods due to interactions with food components. Nevertheless, the use of essential oils to control microbial growth in foods has been proposed for several products including fresh-cut fruits and vegetables (Rojas-Grau and others 2009).

2.5.1. *trans*-cinnamaldehyde

In the antimicrobial action of essential oil components, the lipophilic character of their hydrocarbon skeleton and the hydrophilic character of their functional groups are of great importance. The activity rank of essential oil components is as follows: phenols > aldehydes > ketones > alcohols > hydrocarbons. The phenols include thyme, savory, and oregano oils containing thymol and carvacrol as well as clove oil containing eugenol. Cinnamon oil with cinnamaldehyde as the main component is also a member of this group (Kalemba and Kunicka 2003).

Cinnamaldehyde (cinnamic aldehyde or 3-phenyl-2-propenal) is the main component in cassia oil as well as cinnamon bark oil, and is a GRAS for food use (CFR 2009). It has been shown to be the major antimicrobial compound in cinnamon. In addition to exhibiting

antibacterial activity, cinnamic aldehyde also inhibits mold growth and mycotoxin production (Beuchat 1994).

Delivery of trans-cinnamaldehyde entrapped in polymeric nanoparticles has definite advantages over the delivery of non-entrapped antimicrobials. The release rate of these compounds can be controlled and the dose frequency reduced. Furthermore, the bioactivity and stability of the active substance entrapped in the nanoparticles is protected by encapsulation. In addition, it will prevent the loss of volatiles and improve their solubility in a hydrophilic medium (Gomes and others 2011).

CHAPTER III

MATERIALS AND METHODS

3.1. Raw material

Twenty five pineapples (*Ananas comosus*) were purchased at Farm Patch Produce Market (College Station, Texas), and stored in a refrigerator at 10°C and 50% relative humidity for one day until processing (Figure 3.1). Total soluble solids (°Brix) readings were used as an indicator of ripeness in the fruit.

Figure 3.1 Selected pineapples.

3.2. Sample preparation

Pineapples were sanitized by immersion in chlorine solution (300 ppm) for one minute; then rinsed with distilled water and finally dried at air conditions. All the utensils, equipment and surfaces in contact with food materials were sanitized as well with chlorine solution (200 ppm) for one minute. Pineapples were then cut into 3 cm wide slices with a knife (High carbon/ no stain, antimicrobial handle; Mundial, Brazil) and then the slices were cut into small triangular prisms of 3.6 cm each side, using a triangular cutter; the length of the triangular prisms were

adjusted to 2.54 cm using a small knife and measured with a ruler (Figure 3.2). Some samples of the fruit were taken at this point and ºBrix was measured to check that all the fruit was equally ripen. Pineapples with at least 11 ºBrix were considered as commercially ripe (Paull and Ching-Cheng 2003).

Figure 3.2 Slicing and cutting of pineapple samples.

3.3. Preparation of antimicrobial powder and coating solutions

3.3.1. Antimicrobial powder

An inclusion complex of *trans*-cinnamaldehyde (99+%, Sigma-Aldrich, St. Louis, MO) in beta-cyclodextrin (hydrate Alfa Aesar Johnson Matthey, Lancashire, UK) was prepared by freeze-drying. *trans*-Cinnamaldehyde 99% (2.11 g) and beta-cyclodextrin (18.16 g) were dispersed in one liter of distilled water to have a molecular ratio 1:1 and mixed in a laboratory stirrer (Fisher stirrer 60 Hz, Fisher Scientific, USA) for 24 h at room temperature. The suspension was filtered through a 0.45 μm nylon filter (VWR vacuum filtration systems, VWR international, West Chester, PA, USA), and the filtrate frozen at –20 ºC and freeze-dried at -50 ºC under 5 mtorr (9.67 x 10^{-5} psi) vacuum for 48 hours in a Labconco Freeze Dry-5 equipment

(Labconco, Kansas City, MO, USA). The freeze-dried compound was stored in a glass container in a desiccator placed inside a freezer (-20 ºC) until further use (Gomes, 2010).

3.3.2. Calcium chloride solution

Calcium chloride (food grade, Mallinckrodt Baker Inc., Phillipsburg, NJ) at 2% w/w was dissolved in sterile distilled water at room temperature.

3.3.3. Pectin solution

Pectin (citrus USP, Spectrum Chemical Mfg. Corp., Gardena, CA) at 2% w/w was added to sterile distilled water previously heated at 45°C on a stirring hot plate (Laboratory stirrer/ hot plate, Corning, model PC-220, USA) until it was completely dissolved.

3.3.4. Sodium-alginate + antimicrobial solution

Glycerol (Glycerin USP, J.T Baker, GMP, Phillipsburg, NJ, USA, 500 ml multi-compendia) at 2% w/w was weighted and dissolved in sterile distilled water. Upon that, sodium-alginate was added to the solution in three different concentrations (0.5%, 1% and 2% w/w) while heating on a stirring hot plate at 45°C until total dissolution of the components was reached. The antimicrobial agent (*trans*-cinnamaldehyde encapsulate powder at 2% w/w) was then added to the solution while it continued to be stirred (200 rpm) until the solution reached total homogeneity.

3.4. Coating procedure

A five-step procedure was used to ensure the proper coating of the fruit pieces. The samples were dipped into each coating solution for two minutes and then the excess of coating

material was allowed to drip off for 2 minutes before submerging the samples into the next solution. The order of the coating solutions (Figure 3.3) was as follows: calcium chloride solution, alginate + antimicrobial solution (Figure 3.4), calcium chloride solution, pectin solution and finally a third dipping onto calcium chloride solution.

Control samples were only dipped into sterile distilled water for 2 min and then allowed to drip off for 2 more minutes (Figure 3.5).

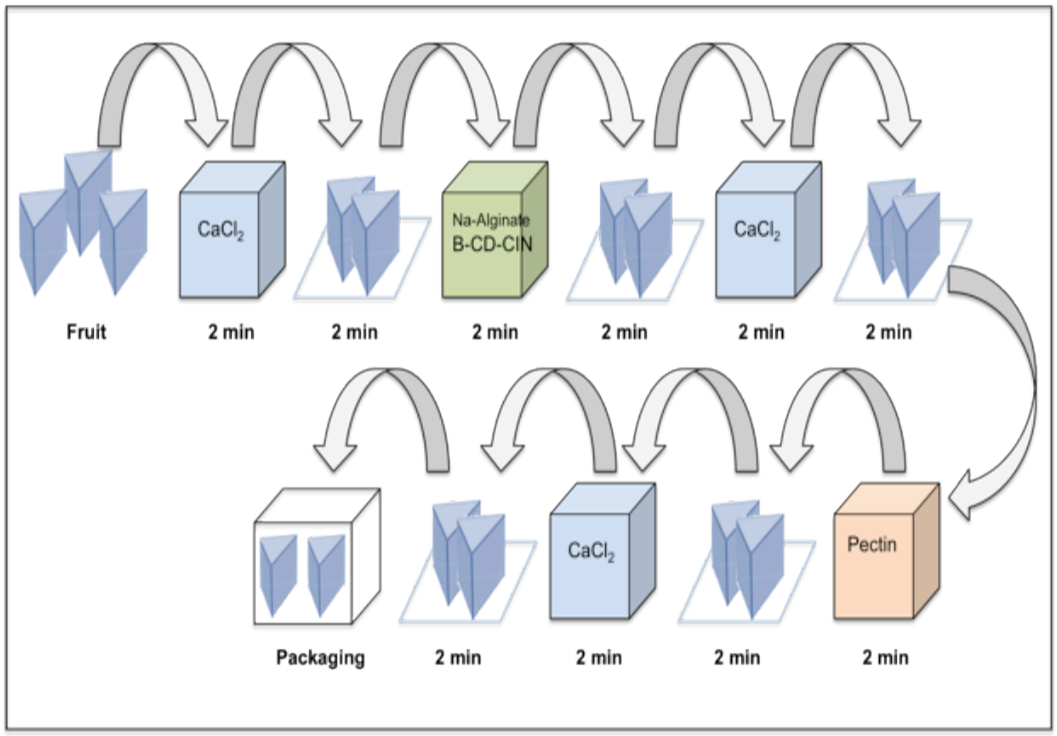


Figure 3.3 Schematic of coating procedure.

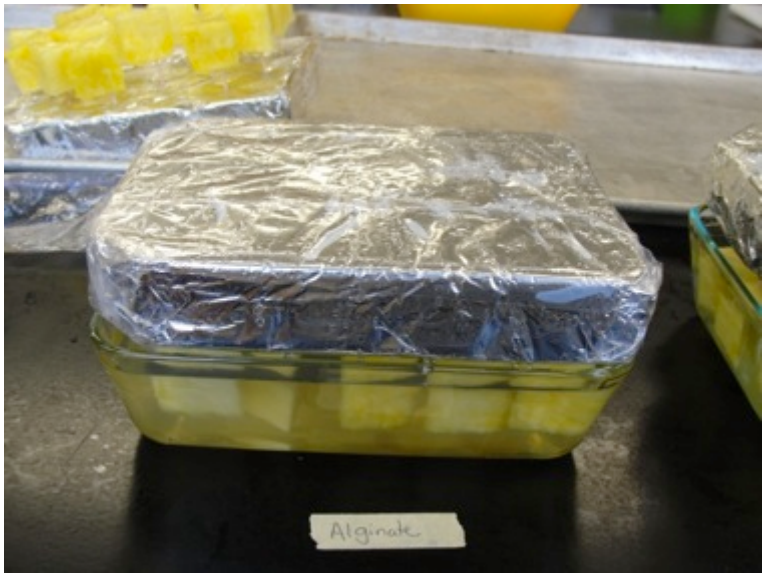


Figure 3.4 Na-Alginate dipping step-coating procedure.

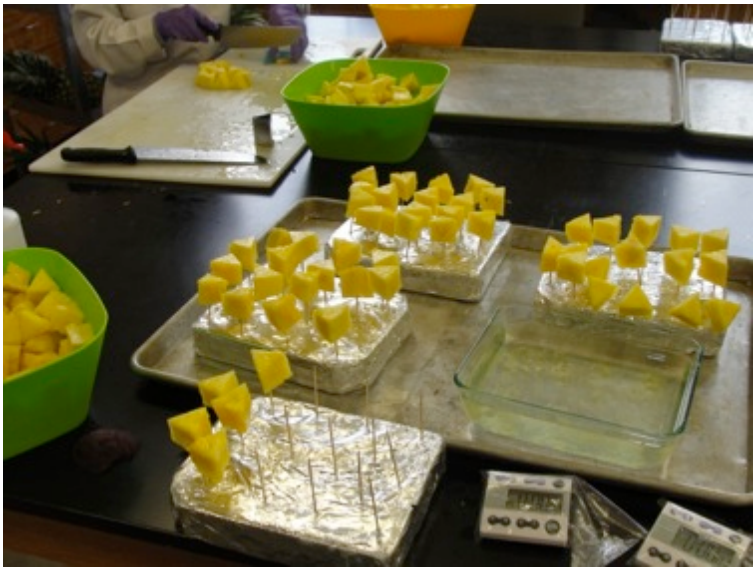


Figure 3.5 Control distilled water dipping procedure.

3.5. Packaging

After 8 minutes of drying at room temperature, ten sample pieces from each treatment (see section 3.6 below) were placed into plastic containers (Ziploc® Brand with Smart Snap™ Seal, 591-ml) with polyethylene lid, and stored at 4°C for 15 days (Figure 3.6).

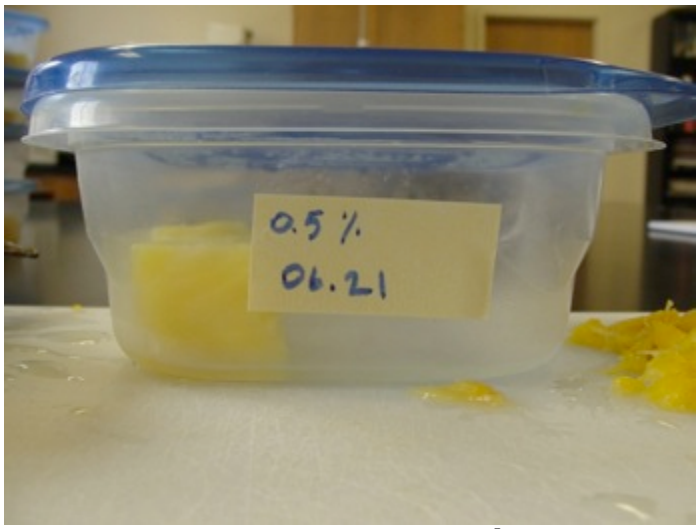


Figure 3.6 Samples packed in Ziploc® containers.

3.6. Experimental design

Based on a previous study (Gomes 2010) the addition of sodium alginate (w/w) may have an effect on 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 concentration of the encapsulated *trans*-cinnamaldehyde (2% w/w). Uncoated samples served as controls.

3.7. Product quality parameters

3.7.1 pH determination

The pH value of fresh-cut pineapple was measured using a digital pH meter (Cole Parmer, Ph 500 series, model #59003-20, Singapore) (AOAC method 981.12). The pH meter was first calibrated with different standard solutions for pH 4, 7 and 10. Then, the juice of two pieces of treated pineapples was squeezed and the pH meter immersed in the juice to record the value. The test was performed by triplicate per each treatment (coated samples and controls) at room temperature.

3.7.2 Total soluble solids (ºBrix)

Soluble solids concentration in the samples was measured using a refractometer (Reichert Analytical Instruments, 2003 Brix 15HP, NY 1404, USA) and expressed in °Brix scale. A couple of drops of pineapple juice used for pH readings were used for the coated and uncoated samples. For this test, three readings per treatment (coated samples and controls) were recorded at room temperature.

3.7.3. Titratable acidity

Titratable acidity was measured using the AOAC method for Acidity (Titratable) of fruit products (942.15) (AOAC, 1990). The test was performed in triplicate at room temperature. Three pieces per treatment (coated samples and controls) were used. Results were expressed as mg of ascorbic acid per gram of sample.

3.7.4. Vitamin C content

Vitamin C determination of the coated and uncoated samples was carried out following the AOAC method for Vitamin C (Ascorbic Acid) in vitamin preparations and juices (967.21) (AOAC, 1990). For this test, approximately 30 g of pineapple juice (2 pieces) were mixed with 30 ml of extracting solution (metaphosphoric acid-acetic acid solution). The homogenate was filtered with qualitative paper (Whatman No. 4) and 7 mL of the filtered solution was titrated with 2,6-dichloroindophenol standard solution. The titration volume was recorded and used to quantify vitamin C content of the sample (milligrams of ascorbic acid/g of sample, wet basis). The indophenol solution was standardized by titrating an ascorbic acid standard solution (1 mg/mL) and sample blanks. Three repetitions for each treatment (coated samples and controls) were performed throughout the study.

3.7.5. Moisture content

Moisture content was determined by weight loss after drying in a vacuum oven at 60 °C for 10 hours. Each sample's weight, approximately 15g, was recorded before and after drying (AOAC method 920.151). The samples were first chopped into small pieces and placed in aluminum canisters prior starting the drying process. The weight of canisters was also recorded for measurements that are more accurate. After removing the samples from the vacuum oven, the samples were placed in a desiccator to cool down before recording the final weight. Two pieces of pineapple for each repetition per treatment (coated samples and controls) were used for the analysis. The test was performed in triplicate and the moisture content (MC) in wet basis (w.b.) was calculated as follows:

$$MC_{(wb)} = (M_{wet} - M_{dry})/ M_{wet} \qquad [3\text{-}1]$$

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

3.7.6. Juice leakage

The percentage of juice leakage (weight loss) was determined by measuring the weight of four samples packed in the Ziploc® containers during the 15 days of storage (4 samples per treatment and control). The weight of each sample was recorded initially and during storage using a digital balance. 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. The procedure was done by triplicate at room temperature.

For each day, new samples were tested to avoid cross-contamination. The percentage of juice leakage was calculated as follows:

% juice of fruit = (initial sample weight) – (final sample weight)/ (initial sample weight) [3-2]

3.7.7. 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, 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 3.7). Four pieces of pineapple 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 opened to avoid gas losses. The concentrations of oxygen and carbon dioxide (%) inside the container were measured at room temperature (Figure 3.8) (Moreno and others 2006).

3.7.8. Color analysis

Samples were analyzed using a Labscan XE colorimeter (HunterLab, 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) from five samples per treatment (coated samples and controls) were recorded at room temperature.

3.7.9. Texture (Firmness) determination

Texture (Firmness) of samples was evaluated in ten samples (coated and controls) using a CT3 Brookfield Texture Analyzer (Brookfield Engineering Laboratories, Middleboro, MA, USA). Samples were triangular prisms 2.54 cm height and 3.6 cm wide. Samples were uniaxially compressed at a speed of 0.5 mm/s with a cylindrical probe (TA3/100, diameter 5.2 cm) (Figure 3.9). The maximum force (N) to compress the sample down to 50% of its original height (50% strain) was recorded (Figure 3.10). The 50% strain was sufficient to measure the maximum force at which the sample fails (Figure 3.11).

Fig 3.7 Glass containers with septum and pineapple samples.

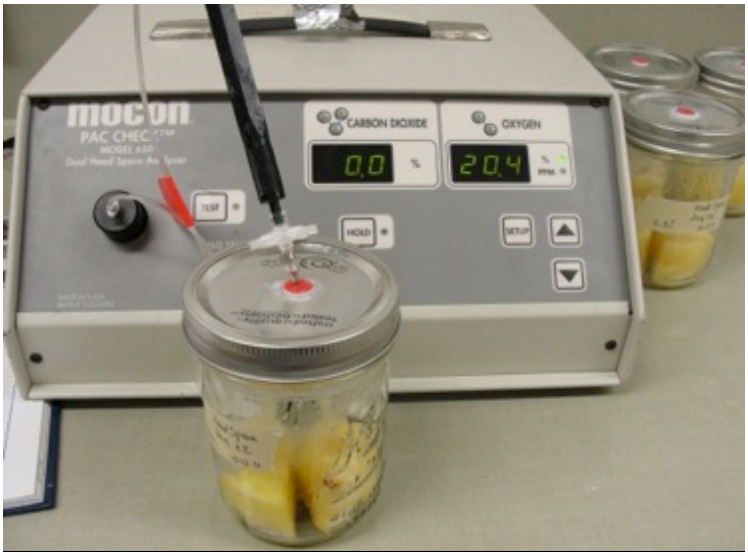


Fig 3.8 Head space analysis using the MOCON analyzer.

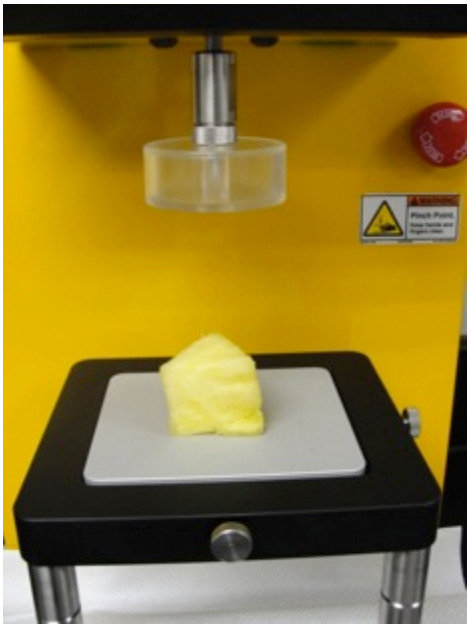


Fig 3.9 Brookfield CT3 with pineapple sample before compression test.

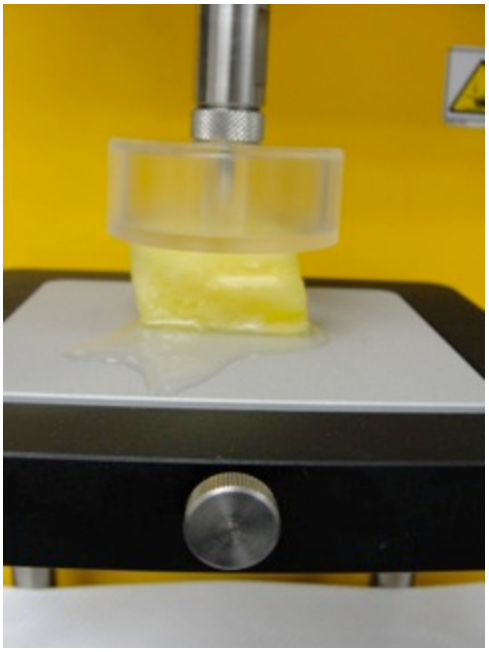


Fig 3.10 Brookfield CT3 with sample during compression test.

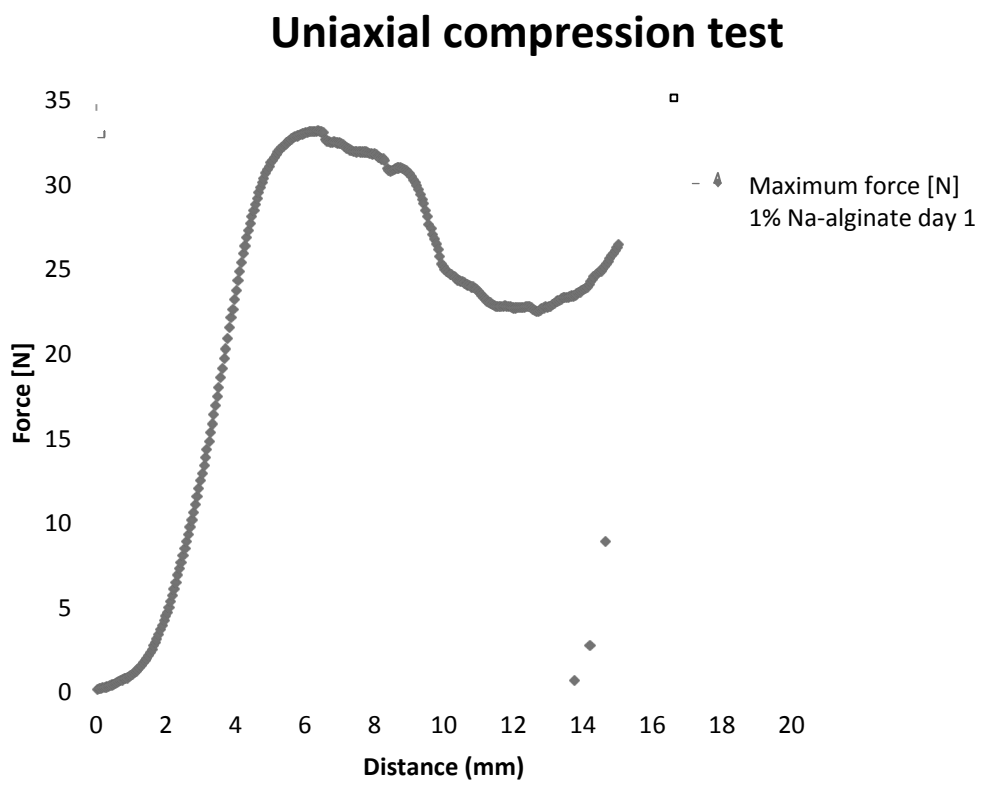


Figure 3.11 Force [N] versus Distance (mm) diagram showing the maximum force when sample fails. Maximum force: highest value of force 32.98 [N] at 6.13 mm distance.

3.7.10. Sensory evaluation

At least thirty people formed the consumer test panel. Panelists were asked to evaluate the samples by visual inspection of color, odor, texture, and overall quality for days 2, 6, 9 and 13 of storage at 4 ºC. Panelists scored the samples using a nine-point hedonic scale, where a score of 1 represents attributes most disliked and a score of 9 represents attributes most liked. The panelists evaluated one randomized sample per each treatment (3 samples in total). One sample was assigned as the control (uncoated) and the other two samples were fruits with 1% (w/w) and 2% (w/w) alginate coating, which were the best two options (in terms of microbiological shelf-life and other quality parameters). Scores higher or equal to 5 were considered acceptable.

3.7.11. 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. Four pineapple samples from each treatment were stomached inside a sterile stomacher bag. A 10 g aliquot of the blended material was transfer to another stomacher bag and mixed with 90 ml buffered peptone water and homogenized for 1 min, subsequently, 10-fold dilutions were made in this diluent. 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 and others 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 (Gomes, 2010).

3.8. Coating microscopy examination

Microscopic observations of the coating were made to assess the differences (if any) on coating thickness and coating homogeneity when using different concentrations of sodium alginate. Coating thickness was measured based on blue dye color illumination (Assorted food color & egg dye, McCormick & Co. Inc, Huntvalley, MD, USA) that was first introduced in the pectin component of the edible coating formulation at a concentration of 7.25 ml (175 drops) of blue dye in one litter of pectin (2% w/w) solution. 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 (cylinders) of the surface of the coated pineapple fruit were excised with a cork borer (#7, 4-mm diameter) and a transversal cut made using a stainless steel blade. Sample surfaces of coated pineapple pieces 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).

3.9. Statistical analysis

Data analysis was performed using SPSS software v. 19.0 (SPSS, 2007) for Windows. The effect of presence of antimicrobial edible coating and storage time was evaluated. Differences between variables were tested for significance by ANOVA and Tukey's tests with a randomized block experimental design with four treatments for all the quality analysis, three treatments for the sensory analysis and three repetitions respectively. The tests were conducted with a 95% significance level ($P<0.05$).

CHAPTER IV

RESULTS AND DISCUSSION

4.1. Effect of sodium alginate concentration on the chemical properties of fresh-cut pineapple

Three concentrations of sodium alginate (0.5, 1 and 2%) with 2% pectin and 2% 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), acidity, vitamin C, moisture content, juice leakage), quality attributes (color, texture, and microbiological quality), composition of the atmosphere inside the containers (headspace analysis), and sensory attributes (color, odor, texture, and overall quality). Uncoated fresh-cut pieces served as control.

4.1.1. pH determination

As time passes, the fruit goes through the process of senescence during which the pH increases. The application of the coating resulted in samples with lower values of pH (Table 4.1) by day 15. At day 12 of storage, the pH values for all the coated samples (0.5% (w/w), 1% (w/w), and 2% (w/w)) were significantly ($P < 0.05$) lower than the control. Throughout the time of storage the pH values of coated and uncoated samples were not significantly ($P > 0.05$) different. The concentration of sodium alginate in the coating did not ($P > 0.05$) have an impact on the pH of the coated fruits (See appendix B pp. 101).

4.1.2 Total soluble solids (ºBrix)

The application of the coating did not affect ($P > 0.05$) the total soluble solids of the fresh-cut pineapple (Table 4.2). Although the fruits coated with the 1% alginate concentration had lower ºBrix by the end of storage, the overall effect of the coating was negligible (See appendix B pp. 93). The results obtained were expected taking in consideration that pineapple is a non-climacteric fruit thus, sugar content was not expected to change drastically during its shelf-life (Paull and Chen 2003).

4.1.3 Titratable acidity

Total titratable acidity (TTA) of coated fruits remained constant throughout storage compared to the controls, which had decreasing ($P < 0.05$) values (Table 4.3) (See appendix B pp. 92). Similar results were obtained for a study on fresh-cut papaya (Brasil and others 2012). Thus, the multilayered edible coating helped control the metabolic reactions in the fruits and delayed the respiration process by serving as a gas barrier. Surface coating has been reported to increase resistance of fruit surface to gas permeability, creating a modified internal atmosphere and reducing the respiration rate (Marpudi and others 2011).

Table 4.1 Effect of multilayered antimicrobial coating on pH values of fresh-cut pineapple samples with different sodium alginate concentrations during 15 days of storage at 4°C.

pH				
Time (days)	**Control***	**0.5% Na-alginate**	**1% Na-alginate**	**2% Na-alginate**
1	$_{w}3.5^{a}$ [0.17][1]	$_{w,x}3.1^{a,b}$ [0.05]	$_{w,x}3.19^{a}$ [0.08]	$_{w,x}3.19^{a}$ [0.05]
3	$_{w}3.23^{a}$ [0.16]	$_{w}3.05^{a}$ [0.05]	$_{w,x}3.17^{a}$ [0.06]	$_{w}3.11^{a}$ [0.04]
7	$_{w}3.28^{a}$ [0.23]	$_{w,x}3.10^{a}$ [0.07]	$_{w}3.15^{a}$ [0.09]	$_{w}3.15^{a}$ [0.08]
12	$_{w}3.50^{a}$ [0.11]	$_{x}3.20^{b}$ [0.02]	$_{w,x}3.26^{b}$ [0.03]	$_{x}3.31^{b}$ [0.04]
15	$_{w}3.58^{a}$ [0.17]	$_{x}3.22^{a,b}$ [0.03]	$_{x}3.32^{a}$ [0.03]	$_{x}3.34^{a}$ [0.07]

*****Controls were uncoated fruits stored at 4°C up to 15 days.

[1] standard deviation

$_{a,b}$ Means within a row, which are not followed by a common superscript letter, are significantly different ($P<0.05$)

$_{w,x}$Means within a column, which are not followed by a common superscript letter, are significantly different ($P < 0.05$).

Table 4.2 Effect of multilayered antimicrobial coating on total soluble solids (°Brix) values of fresh-cut pineapple samples with different sodium alginate concentrations during 15 days of storage at 4°C.

°Brix				
Time (days)	**Control***	**0.5% Na-alginate**	**1% Na-alginate**	**2% Na-alginate**
1	$_{w}$12.87 ^{a} [0.49][1]	$_{w,x}$10.77 ^{a,b} [0.59]	$_{w}$12.47 ^{a} [1.42]	$_{w}$12.20 ^{a} [0.53]
3	$_{w}$12.47 ^{a} [0.12]	$_{w,x}$10.60 ^{a} [0.72]	$_{w}$11.53 ^{a} [1.86]	$_{w,x}$11.47 ^{a} [0.23]
7	$_{w}$11.77 ^{a} [1.07]	$_{w}$10.40 ^{a,b} [0.53]	$_{w}$12.40 ^{a} [0.35]	$_{x}$10.27 ^{a,b} [0.31]
12	$_{w}$12.67 ^{a} [1.33]	$_{w,x}$11.87 ^{a} [0.61]	$_{w}$11.87 ^{a} [0.31]	$_{w,x}$11.87 ^{a} [1.03]
15	$_{w}$13.13 ^{a} [1.17]	$_{x}$12.13 ^{a} [0.42]	$_{w}$10.40 ^{a,b} [0.69]	$_{w,x}$11.53 ^{a} [1.01]

*****Controls were uncoated fruits stored at 4°C up to 15 days.

[1] standard deviation

$_{a,b}$ Means within a row, which are not followed by a common superscript letter, are significantly different ($P<0.05$)

$_{w,x}$Means within a column, which are not followed by a common superscript letter, are significantly different ($P < 0.05$).

4.1.4 Vitamin C content

All samples showed a decreasing trend in vitamin C content during the storage (Table 4.4). Furthermore, the coating did not help ($P > 0.05$) to retain the vitamin C in the pineapple throughout storage (See appendix B pp. 103). Similar results were found in a study on fresh-cut pineapple (Bierhals and others 2011) where the vitamin C content also decreased significantly during the time of evaluation.

4.1.5 Moisture content

Moisture content of coated samples values ranged between 0.80 and 0.92% (w.b.), with the samples with the 2% (w/w) alginate having the highest values by day one of storage (Table 4.5). The increased moisture content was due to the application of the coating. Moisture content of the control significantly ($P < 0.05$) decreased during storage while the moisture content of all coated samples remained constant (See appendix B pp. 100). This result shows a benefit of the coating, which reduces loss of moisture, and consequently weight losses.

Table 4.3 Effect of multilayered antimicrobial coating on total titratable acidity values of fresh-cut pineapple samples with different sodium alginate concentrations during 15 days of storage at 4 ^{o}C.

Total Titratable Acidity (g citric acid/100 ml)				
Time (days)	**Control***	**0.5% Na-alginate**	**1% Na-alginate**	**2% Na-alginate**
1	$_{w}$0.46 ^{a} [0.26][1]	$_{w}$0.56 ^{a} [0.04]	$_{w,x}$0.50 ^{a} [0.06]	$_{w}$0.45 ^{a} [0.03]
3	$_{w}$0.43 ^{a} [0.18]	$_{w}$0.60^{a} [0.02]	$_{w}$0.44 ^{a} [0.07]	$_{w}$0.45 ^{a} [0.03]
7	$_{w}$0.51 ^{a} [0.21]	$_{w}$0.60 ^{a} [0.03]	$_{w,x}$0.47 ^{a} [0.05]	$_{w}$0.46 ^{a} [0.04]
12	$_{w}$0.49 ^{a} [0.15]	$_{w}$0.62 ^{a} [0.02]	$_{x}$0.60 ^{a} [0.04]	$_{w}$0.45 ^{a} [0.02]
15	$_{w}$0.34 ^{b} [0.07]	$_{w}$0.58 ^{a} [0.04]	$_{w,x}$0.47 ^{a,b} [0.06]	$_{w}$0.42 ^{b} [0.01]

*****Controls were uncoated fruits stored at 4^{o}C up to 15 days.

[1] standard deviation

$_{a,b}$ Means within a row, which are not followed by a common superscript letter, are significantly different ($P<0.05$)

$_{w,x}$Means within a column, which are not followed by a common superscript letter, are significantly different ($P < 0.05$).

Table 4.4 Effect of multilayered antimicrobial coating on Vitamin C content (mg ascorbic acid/ g) values of fresh-cut pineapple samples with different sodium alginate concentrations during 15 days of storage at 4 ^{o}C.

Vitamin C (mg ascorbic acid/g)				
Time (days)	**Control***	**0.5% Na-alginate**	**1% Na-alginate**	**2% Na-alginate**
1	$_{w}$0.58 ^{a} [0.11][1]	$_{w,x}$0.67 ^{a} [0.06]	$_{w}$0.59 ^{a} [0.13]	$_{w}$0.59 ^{a} [0.02]
3	$_{w}$0.54 ^{a,b} [0.11]	$_{w}$0.81 ^{a} [0.12]	$_{w}$0.64 ^{a} [0.05]	$_{w}$0.61 ^{a} [0.03]
7	$_{w}$0.42 ^{a} [0.10]	$_{x,y}$0.51 ^{a} [0.07]	$_{w,x}$0.46 ^{a} [0.15]	$_{w}$0.54 ^{a} [0.05]
12	$_{w}$0.34 ^{a,b} [0.08]	$_{w,x,y}$0.62 ^{a} [0.15]	$_{x}$0.51 ^{a} [0.02]	$_{w}$0.57 ^{a} [0.05]
15	$_{w}$0.32 ^{a} [0.09]	$_{y}$0.36 ^{a} [0.08]	$_{x}$0.23 ^{a} [0.04]	$_{x}$0.32 ^{a} [0.07]

*****Controls were uncoated fruits stored at 4^{o}C up to 15 days.

[1] standard deviation

$_{a,b}$ Means within a row, which are not followed by a common superscript letter, are significantly different ($P < 0.05$)

$_{w,x}$Means within a column, which are not followed by a common superscript letter, are significantly different ($P < 0.05$).

Table 4.5 Effect of multilayered antimicrobial coating on moisture content (w.b.) of fresh-cut pineapple samples with different sodium alginate concentrations during 15 days of storage at 4 ºC.

Moisture Content [w.b.]				
Time (days)	**Control***	**0.5% Na-alginate**	**1% Na-alginate**	**2% Na-alginate**
1	$_{w}0.87^{a}$ [0.03][1]	$_{w}0.88^{a}$ [0.02]	$_{w}0.89^{a}$ [0.02]	$_{w}0.91^{a}$ [0.01]
3	$_{w}0.87^{b}$ [0.01]	$_{w}0.89^{a,b}$ [0.01]	$_{w}0.9^{a}$ [0.01]	$_{w}0.9^{a}$ [0.00]
7	$_{w}0.87^{a}$ [0.00]	$_{w}0.90^{a}$ [0.00]	$_{w}0.89^{a}$ [0.01]	$_{w}0.9^{a}$ [0.01]
12	$_{w}0.86^{b}$ [0.01]	$_{w}0.89^{a,b}$ [0.01]	$_{w}0.89^{a}$ [0.01]	$_{w}0.9^{a}$ [0.01]
15	$_{w}0.84^{b}$ [0.00]	$_{w}0.88^{a}$ [0.01]	$_{w}0.89^{a}$ [0.01]	$_{w}0.91^{a}$ [0.01]

*****Controls were uncoated fruits stored at 4^{o}C up to 15 days.

[1] standard deviation

$_{a,b}$ Means within a row, which are not followed by a common superscript letter, are significantly different ($P < 0.05$)

$_{w,x}$Means within a column, which are not followed by a common superscript letter, are significantly different ($P < 0.05$).

4.1.6 Juice leakage

Based on the moisture content results, it was expected that the coating would be effective in preventing juice leakage (Table 4.6). Control samples had significantly ($P < 0.05$) higher losses than the coated fruits. Juice leakage of coated samples increased throughout storage from 0.31% to 6.41%, while the controls had an increase from 1.37% to 8.3%. The concentration of alginate did not have a significant ($P < 0.05$) effect on juice leakage until day 15 when the samples with 2% sodium alginate had the lowest percentage of juice leakage (See appendix B pp. 99). Similar results were found with a study on fresh-cut papaya (Brasil and others 2012). This results shows the benefit of applying the multilayered edible coating to fresh-cut pineapple.

4.2 Headspace analysis

Application of the coating to fresh-cut pineapple significantly ($P < 0.05$) delays the respiration process. The headspace in jars containing the uncoated samples had increased ($P < 0.05$) concentration of CO_2 (Table 4.7) by the end of storage (day 15). The concentration of sodium alginate in the coating also had an effect. The samples with 1% and 2% sodium alginate in the coating had the lowest ($P < 0.05$) concentrations of CO_2 in the jar's headspace while the 0.5% treatment did not perform as well.

Similarly, as part of the respiration process, the concentration of O_2 decreased during storage time (Table 4.8), with the O_2 concentration values for control samples being significantly ($P < 0.05$) lower. There was a significant ($P < 0.05$) difference between samples with 1% and 2% alginate coating and the uncoated samples (See appendix B pp. 97-98). Thus, the multilayered edible coating delayed the respiration process by serving as a gas barrier.

Table 4.6 Effect of multilayered antimicrobial coating on juice leakage (%) values of fresh-cut pineapple samples with different sodium alginate concentrations during 15 days of storage at 4 ºC.

Juice Leakage (%)				
Time (days)	**Control***	**0.5% Na-alginate**	**1% Na-alginate**	**2% Na-alginate**
1	$_{w}1.37^{a}$ [0.27][1]	$_{w}0.95^{a}$ [0.11]	$_{w}0.45^{b}$ [0.02]	$_{w}0.31^{b}$ [0.03]
3	$_{x}3.54^{a}$ [0.44]	$_{x}2.51^{b}$ [0.16]	$_{x}2.6^{b}$ [0.02]	$_{x}2.22^{b}$ [0.17]
7	$_{y}6.19^{a}$ [0.3]	$_{y}3.91^{b}$ [0.37]	$_{y}4.16^{b}$ [0.06]	$_{y}3.8^{b}$ [0.07]
12	$_{y,z}7.01^{a}$ [0.86]	$_{z}5.86^{a}$ [0.09]	$_{z}5.46^{a}$ [0.64]	$_{z}4.34^{a,b}$ [0.16]
15	$_{z}8.3^{a}$ [0.14]	$_{z}6.41^{b}$ [0.08]	$_{y,z}5.19^{c}$ [0.13]	$_{y,z}4.22^{d}$ [0.13]

*****Controls were uncoated fruits stored at 4^{o}C up to 15 days.

[1] standard deviation

$_{a,b}$ Means within a row, which are not followed by a common superscript letter, are significantly different ($P < 0.05$)

$_{w,x}$Means within a column, which are not followed by a common superscript letter, are significantly different ($P < 0.05$).

Table 4.7 Effect of multilayered antimicrobial coating on headspace CO_2 concentration (%) values of fresh-cut pineapple samples with different sodium alginate concentrations during 15 days of storage at 4 ºC.

Headspace CO_2 concentration (%)				
Time (days)	**Control***	**0.5% Na-alginate**	**1% Na-alginate**	**2% Na-alginate**
1	$_{v}4.2^{a}$ [0.14][1]	$_{v}3.00^{c}$ [0.14]	$_{v}3.05^{c}$ [0.07]	$_{v}3.23^{b}$ [0.11]
3	$_{w}6.4^{a}$ [0.14]	$_{w}6.36^{a}$ [0.07]	$_{w}5.6^{b}$ [0.14]	$_{w}5.3^{b}$ [0.14]
7	$_{x}10.7^{a}$ [0.14]	$_{x}10.55^{a}$ [0.07]	$_{x}9.55^{b}$ [0.07]	$_{x}9.2^{b}$ [0.14]
12	$_{y}13.2^{a}$ [0.14]	$_{y}12.3^{a}$ [0.14]	$_{x}9.7^{b}$ [0.14]	$_{x}9.5^{b}$ [0.14]
15	$_{z}15.2^{a}$ [0.14]	$_{z}14.8^{b}$ [0.14]	$_{y}11.75^{c}$ [0.21]	$_{y}10.25^{d}$ [0.42]

*****Controls were uncoated fruits stored at 4^{o}C up to 15 days.

[1] standard deviation

$_{a,b}$ Means within a row, which are not followed by a common superscript letter, are significantly different ($P < 0.05$)

$_{w,x}$Means within a column, which are not followed by a common superscript letter, are significantly different ($P < 0.05$).

Table 4.8 Effect of multilayered antimicrobial coating on headspace O_2 concentration (%) values of fresh-cut pineapple samples with different sodium alginate concentrations during 15 days of storage at 4 ºC.

Headspace O_2 concentration (%)				
Time (days)	**Control***	**0.5% Na-alginate**	**1% Na-alginate**	**2% Na-alginate**
1	$_{v}18.45^{c}$ [0.21][1]	$_{v}18.85^{b}$ [0.21]	$_{v}19.4^{a}$ [0.28]	$_{v}19.0^{b}$ [0.14]
3	$_{w}15.75^{d}$ [0.21]	$_{w}16.2^{c}$ [0.14]	$_{w}17.2^{b}$ [0.14]	$_{w}17.4^{a}$ [0.14]
7	$_{x}10.2^{c}$ [0.14]	$_{x}10.15^{c}$ [0.07]	$_{x}11.7^{b}$ [0.14]	$_{x}12.6^{a}$ [0.14]
12	$_{y}8.04^{d}$ [0.01]	$_{y}9.3^{c}$ [0.08]	$_{x}11.6^{b}$ [0.14]	$_{x}12.4^{a}$ [0.14]
15	$_{y}8.47^{c}$ [0.01]	$_{z}8.17^{c}$ [0.09]	$_{x}11.2^{b}$ [0.14]	$_{x}12.7^{a}$ [0.42]

*****Controls were uncoated fruits stored at 4^{o}C up to 15 days.

[1] standard deviation

$_{a,b}$ Means within a row, which are not followed by a common superscript letter, are significantly different ($P < 0.05$)

$_{w,x}$Means within a column, which are not followed by a common superscript letter, are significantly different ($P < 0.05$).

4.3 Effect of sodium alginate concentration on the quality of fresh-cut pineapple

4.3.1 Color analysis

Tables 4.9 to 4.11 present the values for lightness (*L**), redness-greenness (*a**), and yellowness-blueness (*b**) for coated and uncoated fresh-cut pineapple samples for 15 days of storage at 4 ºC.

The coating did not cause differences ($P > 0.05$) in lightness (*L**). However, control samples had higher values for lightness (lighter samples) in comparison with the coated samples. Throughout storage, control and samples with 0.5% and 1% alginate coating did not have significantly ($P > 0.05$) different lightness values (See appendix B pp. 94). On day 7, samples with 2% alginate coating had significant ($P < 0.05$) lower values of lightness (darker samples) compared with day 1 of evaluation.

The *a** values (redness-greenness) of the fresh-cut pineapple did not vary significantly ($P > 0.05$) throughout storage (See appendix B pp. 95). In terms of trends, control samples had higher values of redness when compared with the coated samples throughout storage. By day 12, the coated samples were significantly ($P < 0.05$) different from the control, which had higher values of redness. Overall, application of the coating had a positive effect on the degree of redness of fresh-cut pineapple.

The *b** values (yellowness-blueness) present a significantly ($P < 0.05$) decreased through time with the control samples showing higher values of yellowness in comparison with the coated fruits (See appendix B pp. 96). As the concentration of sodium alginate in the coating increased, the values of **b* were significantly ($P < 0.05$) lower. The coating helped to prevent drastic color changes on the fresh-cut fruit (See appendix B pp. 109 -122).

Table 4.9 Effect of multilayered antimicrobial coating on (*L**) color parameter values of fresh-cut pineapple samples with different sodium alginate concentrations during 15 days of storage at 4 ºC.

Color parameter - *L**				
Time (days)	**Control***	**0.5% Na-alginate**	**1% Na-alginate**	**2% Na-alginate**
1	$_{w}$59.03 ^{a} [3.04][1]	$_{w}$52.48 ^{a,b} [2.51]	$_{w}$54.46 ^{a} [4.87]	$_{w}$57.59 ^{a} [4.06]
3	$_{w}$54.72 ^{a} [2.44]	$_{w}$49.33 ^{a} [5.57]	$_{w}$49.27 ^{a} [2.69]	$_{w,x}$52.06 ^{a} [4.89]
7	$_{w}$55.51 ^{a} [5.79]	$_{w}$54.36 ^{a} [6.80]	$_{w}$52.35 ^{a} [4.89]	$_{x}$49.49 ^{a} [3.09]
12	$_{w}$53.88 ^{a} [6.55]	$_{w}$55.68 ^{a} [3.80]	$_{w}$54.12 ^{a} [4.50]	$_{x}$48.61 ^{a} [2.44]
15	$_{w}$55.49 ^{a} [9.66]	$_{w}$53.68 ^{a} [7.52]	$_{w}$48.92 ^{a} [3.09]	$_{x}$47.18 ^{a} [2.06]

*****Controls were uncoated fruits stored at 4^{o}C up to 15 days.
[1] standard deviation
$_{a,b}$ Means within a row, which are not followed by a common superscript letter, are significantly different (P<0.05)
$_{w,x}$Means within a column, which are not followed by a common superscript letter, are significantly different (P < 0.05).

Table 4.10 Effect of multilayered antimicrobial coating on (*a**) color parameter values of fresh-cut pineapple samples with different sodium alginate concentrations during 15 days of storage at 4 ºC.

Color parameter - *a**				
Time (days)	**Control***	**0.5% Na-alginate**	**1% Na-alginate**	**2% Na-alginate**
1	$_{w}$1.96 ^{a} [0.95][1]	$_{w}$1.47 ^{a} [0.81]	$_{w}$1.21 ^{a} [0.62]	$_{w}$1.22 ^{a} [1.10]
3	$_{w}$2.38 ^{a} [0.63]	$_{w}$1.27 ^{a} [0.70]	$_{w}$1.14 ^{a} [0.46]	$_{w}$0.97 ^{a,b} [0.61]
7	$_{w}$2.56 ^{a} [1.74]	$_{w}$1.31 ^{a} [0.30]	$_{w,x}$0.84 ^{a} [0.38]	$_{w1}$.13 ^{a} [0.86]
12	$_{w}$3.2 ^{a} [1.64]	$_{w}$0.67 ^{b} [0.29]	$_{x}$0.27 ^{b} [0.11]	$_{w}$0.98 ^{b} [0.52]
15	$_{w}$2.33 ^{a} [1.34]	$_{w}$0.77 ^{b} [0.69]	$_{w,x}$0.63 ^{b} [0.36]	$_{w}$0.58 ^{b} [0.31]

*****Controls were uncoated fruits stored at 4^{o}C up to 15 days.

[1] standard deviation

$_{a,b}$ Means within a row, which are not followed by a common superscript letter, are significantly different (P<0.05)

$_{w,x}$Means within a column, which are not followed by a common superscript letter, are significantly different (P < 0.05).

Table 4.11 Effect of multilayered antimicrobial coating on (*b**) color parameter values of fresh-cut pineapple samples with different sodium alginate concentrations during 15 days of storage at 4 ºC.

Color parameter - *b**				
Time (days)	**Control***	**0.5% Na-alginate**	**1% Na-alginate**	**2% Na-alginate**
1	$_{w}$26.00 ^{a} [0.65][1]	$_{w}$22.50 ^{b} [0.75]	$_{w}$21.39 ^{b} [1.10]	$_{w}$20.28 ^{b,c} [0.96]
3	$_{w}$24.83 ^{a} [0.66]	$_{w,x}$19.73 ^{b} [1.67]	$_{x}$18.12 ^{c} [1.02]	$_{x}$17.30 ^{c} [1.56]
7	$_{x}$22.62 ^{a} [1.11]	$_{x,y}$17.96 ^{b} [2.51]	$_{x,y}$16.68 ^{b} [1.7]	$_{y}$14.00 ^{b,c} [1.30]
12	$_{x,y}$21.55 ^{a} [1.42]	$_{x,y}$18.65 ^{a} [0.81]	$_{x,y}$16.73 ^{a,b} [2.83]	$_{y}$12.74 ^{b} [0.73]
15	$_{y}$20.35 ^{a} [1.19]	$_{y}$15.81 ^{b} [1.78]	$_{y}$13.71 ^{b} [0.82]	$_{y}$12.84 ^{b,c} [1.25]

*****Controls were uncoated fruits stored at 4^{o}C up to 15 days.
[1] standard deviation
$_{a,b}$ Means within a row, which are not followed by a common superscript letter, are significantly different (P<0.05)
$_{w,x}$Means within a column, which are not followed by a common superscript letter, are significantly different (P < 0.05).

4.3.2 Texture (Firmness) determination

As expected, all fruit samples got softer with time (Table 4.12). However, the uncoated control had the lowest values of firmness during storage time. The coated samples had higher firmness values since the first day of the study, especially those samples coated with 1% and 2% alginate-based coating. The 2 % sodium alginate-based coating yielded firmer fruits with higher values of force (30-36 N), followed by the samples coated with 1% sodium alginate (24.5-30.3 N). Samples coated samples with 0.5% sodium were not different from the uncoated controls.

By the end of storage, only the fruits with the 2% sodium alginate-based coating remained very firm and there was no significant difference ($P < 0.05$) among the other treatments; however, all the coatings demonstrated effectiveness in preserving the natural crispy and juicy texture of fresh-cut pineapple during the time of evaluation, while the uncoated samples were considerably softer (See appendix B pp. 102).

4.3.3 Sensory evaluation

Results for sensory analysis are presented in Tables 4.13 to 4.17 (See appendix B pp. 104-108). Sensory test was performed to determine whether the coating had an effect on the acceptability of the fruits in terms of five parameters (color, odor, texture, flavor, and overall quality) based on a 9-point hedonic scale (See appendix A pp. 91). Control samples and controls were evaluated at days 2, 6, 9 and 13 of storage.

Color scores for coated and uncoated controls decreased throughout storage (Table 4.13). There was a significant ($P < 0.05$) difference between the control and the samples coated with 1% and 2% sodium alginate.

Control samples were ranked higher than the coated samples; with the samples with 2% sodium alginate in the coating having a score lower than 5.0 by day 9 of storage. Since the coating gave the pineapple an appearance different (opaque) from the uncoated control, panelists gave the coated samples lower scores. Overall, the panelists found both the controls and the samples with the 1% sodium alginate coating acceptable (scores greater than or equal to 5). The samples with the 2% sodium alginate coating were ranked as unacceptable due to the opaque (lower values of lightness (*L) color parameters (Table 4.9)) and less yellowish color (lower values of *b color parameter (Table 4.11)).

In terms of odor (Table 4.14), consumers showed a significant ($P < 0.05$) preference for the uncoated samples. This was mainly due to the particular odor imparted by the *trans*-cinnamaldehyde in the coated samples, since the panelists did not associate the cinnamon odor with fresh-cut pineapple pieces. All the coated samples received scores under 5, making them unacceptable. Similar results were obtained for texture parameter (Table 4.15). Control samples received the highest values for texture until day 9 of storage. After that, controls were ranked significantly ($P < 0.05$) under the acceptability score.

Panelists scored the samples with 1% and 2% alginate coating as acceptable until day 6 and, for those fruits coated with 1% alginate coating, until day 13. As it was shown in Table 4.12, control samples got significantly softer throughout storage and the panelists ranked them as unacceptable in terms of their texture attribute.

In terms of flavor, there was a significant ($P < 0.05$) difference between control and coated samples (Table 4.16). At days 2 and 6 of storage, control samples were scored higher than coated samples. This was due to the presence of trans-cinnamaldehyde and calcium chloride in the coating that gave the fresh-cut fruit a slight bitterness.

However, by day 9, there was no significant difference ($P > 0.05$) between control and coated samples. The lower scores for the control sample at days 9 and 13 indicatet that the fruits were showing signs of decay with a consequent change in flavor. A similar trend was observed for the overall quality of the samples (Table 4.17), mostly a consequence of the flavor and odor problems with the coated samples. The coated samples were acceptable to the consumers by day 6 for samples with the 2% sodium alginate coating and until day 13 for the samples with the 1% sodium alginate coating.

These results are a strong indication that the calcium chloride should be replaced with an alternative which will not bring out the bitterness in the coated fruits. Although the sensory scores of the coated fruits were lower than those for the uncoated controls, the coating still shows benefits in terms of the microbiological quality (shelf-life) of the fruit (see section 4.3.4).

Table 4.12 Effect of multilayered antimicrobial coating on firmness (N) values of fresh-cut pineapple samples with different sodium alginate concentrations during 15 days of storage at 4 ºC.

Firmness - Maximum force [N]				
Time (days)	**Control***	**0.5% Na-alginate**	**1% Na-alginate**	**2% Na-alginate**
1	$_{w}$28.66 ^{b} [2.61][1]	$_{w}$24.80 ^{b,c} [1.47]	$_{w}$30.31 ^{b} [3.43]	$_{w}$36.44 ^{a} [2.40]
3	$_{w}$27.85 ^{a} [2.34]	$_{w}$27.69 ^{a} [7.29]	$_{w}$29.38 ^{a} [1.88]	$_{w,x}$33.26 ^{a} [3.24]
7	$_{w,x}$27.42 ^{a} [1.76]	$_{w}$27.67 ^{a} [7.14]	$_{w}$29.24 ^{a} [5.99]	$_{w,x}$32.38 ^{a} [1.41]
12	$_{w,x}$24.86 ^{a,b} [3.60]	$_{w}$25.03 ^{a,b} [1.23]	$_{w}$26.17 ^{a} [3.90]	$_{w,x}$31.07 ^{a} [2.20]
15	$_{x}$22.74 ^{a,b} [3.96]	$_{w}$25.52 ^{a} [4.10]	$_{w}$24.49 ^{a} [3.54]	$_{x}$30.15 ^{a} [5.71]

*****Controls were uncoated fruits stored at 4^{o}C up to 15 days.

[1] standard deviation

$_{a,b}$ Means within a row, which are not followed by a common superscript letter, are significantly different ($P<0.05$)

$_{w,x}$Means within a column, which are not followed by a common superscript letter, are significantly different ($P < 0.05$).

Table 4.13 Effect of multilayered antimicrobial coating on color sensory attribute values of fresh-cut pineapple samples with different sodium alginate concentrations during 15 days of storage at 4 ºC.

Color sensory attribute [hedonic scale]			
Time (days)	**Control***	**1% Na-alginate**	**2% Na-Alginate**
2	$_{w}7.6^{a}$ [1.00][1]	$_{w}6.00^{b}$ [1.46]	$_{w,x}5.67^{b}$ [1.65]
6	$_{w,x}7.3^{a}$ [1.47]	$_{w}5.9^{b}$ [1.49]	$_{x}6.3^{b}$ [1.21]
9	$_{x}6.63^{a}$ [1.97]	$_{w}5.93^{a}$ [1.6]	$_{w}4.83^{b}$ [1.46]
13	$_{y}4.60^{a}$ [1.04]	$_{w}6.63^{b}$ [1.00]	$_{x}6.37^{b}$ [1.22]

*****Controls were uncoated fruits stored at 4^{o}C up to 15 days.
[1] standard deviation
$_{a,b}$ Means within a row, which are not followed by a common superscript letter, are significantly different (P<0.05)
$_{w,x}$Means within a column, which are not followed by a common superscript letter, are significantly different (P < 0.05).

Table 4.14 Effect of multilayered antimicrobial coating on odor sensory attribute values of fresh-cut pineapple samples with different sodium alginate concentrations during 15 days of storage at 4 ºC.

Odor sensory attribute [hedonic scale]			
Time (days)	**Control***	**1% Na-alginate**	**2% Na-alginate**
2	$_{w}7.07^{a}$ [1.74][1]	$_{w}5.6^{b}$ [1.90]	$_{w}5.3^{b}$ [1.42]
6	$_{w}7.63^{a}$ [1.00]	$_{w}4.93^{b}$ [1.72]	$_{w}4.57^{b}$ [1.7]
9	$_{w}6.97^{a}$ [1.71]	$_{w}4.53^{b}$ [1.46]	$_{w}4.53^{b}$ [1.43]
13	$_{x}4.87^{a}$ [0.86]	$_{w}5.37^{a}$ [1.73]	$_{w}4.93^{a}$ [1.55]

*****Controls were uncoated fruits stored at 4^{o}C up to 15 days.
[1] standard deviation
$_{a,b}$ Means within a row, which are not followed by a common superscript letter, are significantly different (P<0.05)
$_{w,x}$Means within a column, which are not followed by a common superscript letter, are significantly different (P < 0.05).

Table 4.15 Effect of multilayered antimicrobial coating on texture sensory attribute values of fresh-cut pineapple samples with different sodium alginate concentrations during 15 days of storage at 4 ºC.

Texture sensory attribute [hedonic scale]			
Time (days)	**Control***	**1% Na-alginate**	**2% Na-alginate**
2	$_{w}7.67^{a}$ [1.35][1]	$_{w}6.23^{b}$ [1.74]	$_{w,x}5.33^{b}$ [2.04]
6	$_{w}7.73^{a}$ [1.20]	$_{w}5.67^{b}$ [1.71]	$_{w,x}5.8^{b}$ [2.09]
9	$_{w}7.17^{a}$ [1.64]	$_{w}5.63^{b}$ [1.76]	$_{w}4.7^{b}$ [2.15]
13	$_{x}4.40^{a}$ [0.89]	$_{w}6.70^{b}$ [1.09]	$_{x}6.50^{b}$ [1.04]

*****Controls were uncoated fruits stored at 4^{o}C up to 15 days.
[1] standard deviation
$_{a,b}$ Means within a row, which are not followed by a common superscript letter, are significantly different (P<0.05)
$_{w,x}$Means within a column, which are not followed by a common superscript letter, are significantly different (P < 0.05).

Table 4.16 Effect of multilayered antimicrobial coating on flavor sensory attribute values of fresh-cut pineapple samples with different sodium alginate concentrations during 15 days of storage at 4 ºC.

Flavor sensory attribute [hedonic scale]			
Time (days)	**Control***	**1% Na-alginate**	**2% Na-alginate**
2	$_{w}$7.8 ^{a} [1.56][1]	$_{w}$6.57 ^{b} [1.63]	$_{w}$5.13 ^{c} [1.63]
6	$_{w}$7.13 ^{a} [1.61]	$_{x}$4.93 ^{b} [1.41]	$_{w}$4.83 ^{b} [1.37]
9	$_{x}$5.9 ^{a} [1.60]	$_{x}$5.17 ^{a,b} [1.12]	$_{w}$5.03 ^{b} [1.30]
13	$_{y}$4.93 ^{a} [0.45]	$_{x}$5.57 ^{b} [0.63]	$_{w}$5.17 ^{a} [0.38]

*****Controls were uncoated fruits stored at 4^{o}C up to 15 days.
[1] standard deviation
$_{a,b}$ Means within a row, which are not followed by a common superscript letter, are significantly different (P<0.05)
$_{w,x}$Means within a column, which are not followed by a common superscript letter, are significantly different (P < 0.05).

Table 4.17 Effect of multilayered antimicrobial coating on overall quality sensory attribute values of fresh-cut pineapple samples with different sodium alginate concentrations during 15 days of storage at 4 ºC.

Overall quality sensory attribute [hedonic scale]			
Time (days)	**Control***	**1% Na-alginate**	**2% Na-alginate**
2	$_{w,x}$7.57 ^{a} [1.3][1]	$_{w}$6.37 ^{b} [1.45]	$_{w}$5.47 ^{c} [1.55]
6	$_{w}$8.00 ^{a} [0.64]	$_{x}$5.33 ^{b} [1.32]	$_{w,x}$5.17 ^{b} [1.58]
9	$_{x}$7.23 ^{a} [1.55]	$_{x}$5.43 ^{b} [1.59]	$_{w}$4.43 ^{b} [1.77]
13	$_{y}$5.03 ^{a} [0.61]	$_{w}$6.47 ^{c} [0.90]	$_{x}$5.83 ^{b} [1.18]

*****Controls were uncoated fruits stored at 4^{o}C up to 15 days.

[1] standard deviation

$_{a,b}$ Means within a row, which are not followed by a common superscript letter, are significantly different ($P<0.05$)

$_{w,x}$Means within a column, which are not followed by a common superscript letter, are significantly different ($P < 0.05$).

4.3.4 Microbiological analysis

Aerobic microorganisms were evaluated by aerobic plate counts. All the antimicrobial coatings demonstrated to be highly effective in the reduction of the microbial population throughout the 15 days of storage (Figure 4.1). All the three coatings significantly ($P < 0.05$) reduced the growth of aerobic microorganisms by approximately 2.7log CFU/g compared to the uncoated control (~ 5 log CFU/g) by the end of storage. The use of 1% sodium alginate in coating preparation seems to be a suitable choice in terms of aerobics growth while maintaining their sensory attributes.

Similar results were obtained for psychrotrophic plate counts (Figure 4.2). The samples with sodium alginate at 2% had the lowest counts (2.7 log reductions), followed by those with the 0.5% sodium alginate with 2.28 log reduction and then 1% sodium alginate with a reduction of 1.23 log cycles. Control samples had the highest counts (3.4 log cycles) by day 15 of storage.

In the case of yeast and molds results (Figure 4.3), a difference in growth was found after day 7 of storage, and control samples had significantly ($P < 0.05$) higher counts (6.89 log cycles) by day 15. All the coated samples had considerably lower counts by the end of storage, with a reduction of almost 3 log cycles for samples with 2% and 0.5% alginate, and 1.76 log cycles for 1% alginate, in comparison with the control samples.

Samples coated with 0.5% and 2% sodium alginate coating showed higher reduction in the microbial growth of psychrotrophic and yeast and molds due to the difference in the amount of antimicrobial compound that was present in the coating (higher amount of antimicrobial in samples coated with 2% sodium alginate).

Another factor for the results obtained was the variation in homogeneity that the coating with 1% sodium alginate presented around the entire samples in comparison with samples with 0.5% sodium alginate coating.

The results confirm that the antimicrobial compound is effective in reducing the microbial growth in the case of aerobic and psychrotrophic microorganisms, where the growth in coated samples consistently decreased throughout storage; while the control samples had increased counts. This antimicrobial effect is not just due to the action of the antimicrobial compound but also to the difference in pH between the control (higher pH values) and the coated samples (lower pH values). In the case of yeasts and molds, there was a difference in growth compared to the controls, though all samples had increased counts. This difference in trends of growth may be due to the difference in pH as well. Yeast and molds are microorganisms that are capable of living in low-pH environments (Jay and others 2005).

The results of microbiological analyses show the effectiveness of the multilayered edible coating as a carrier of an antimicrobial compound that helps to control microbial growth, thus extending the shelf-life.

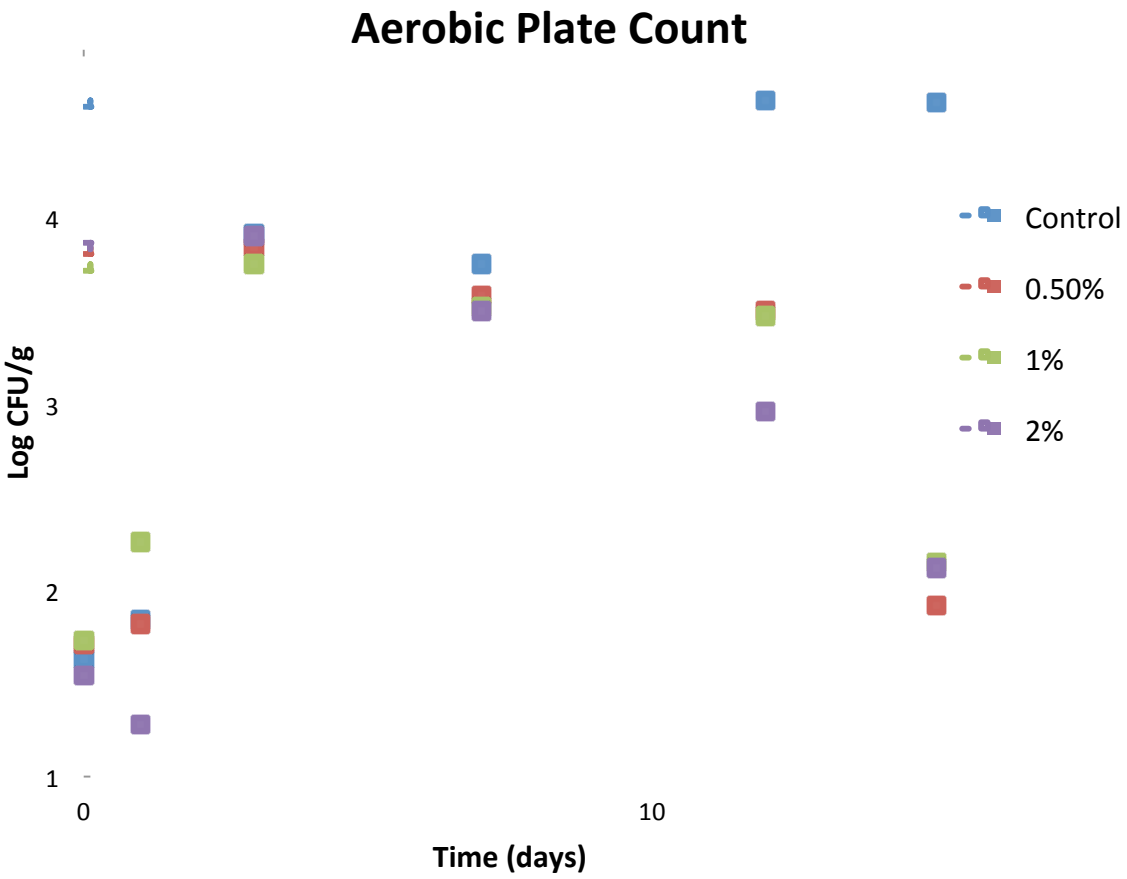


Figure 4.1 Effect of multilayered antimicrobial coating on aerobic plate counts of fresh-cut pineapple samples with different sodium alginate concentrations during 15 days of storage at 4 ºC.

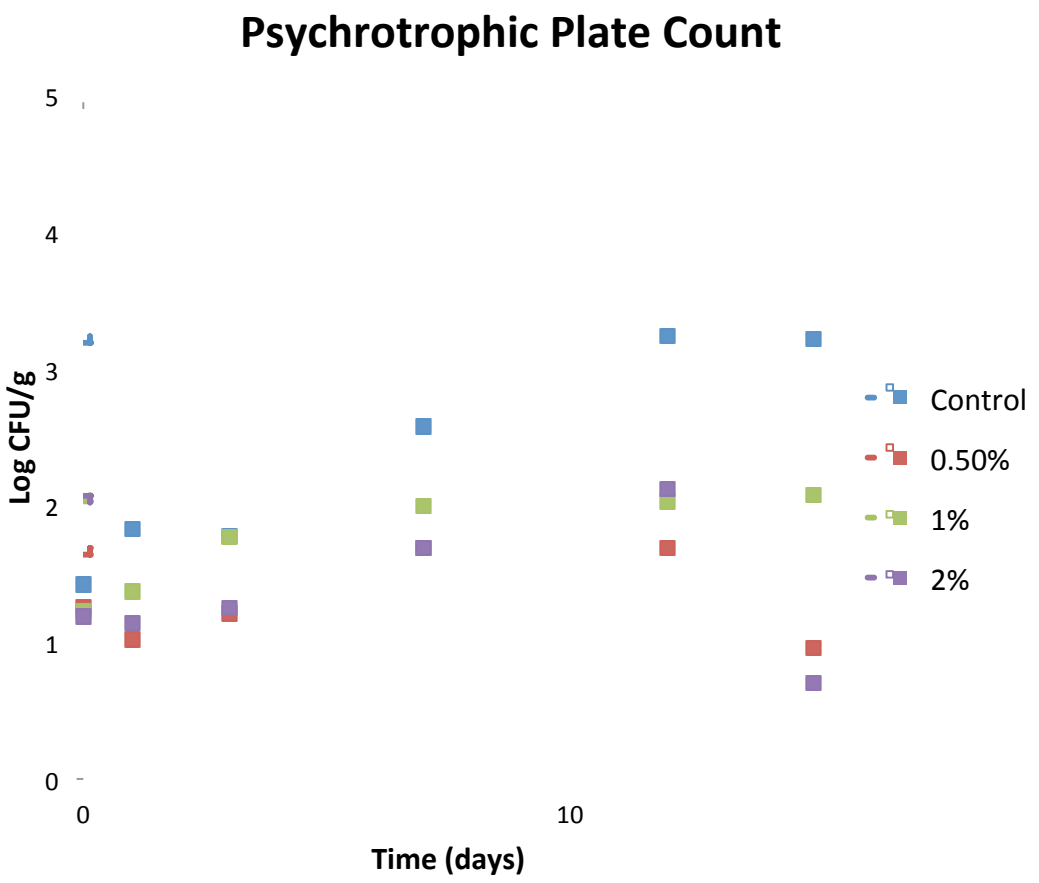


Figure 4.2 Effect of multilayered antimicrobial coating on psychrotrophic plate counts of fresh-cut pineapple samples with different sodium alginate concentrations during 15 days of storage at 4 ºC.

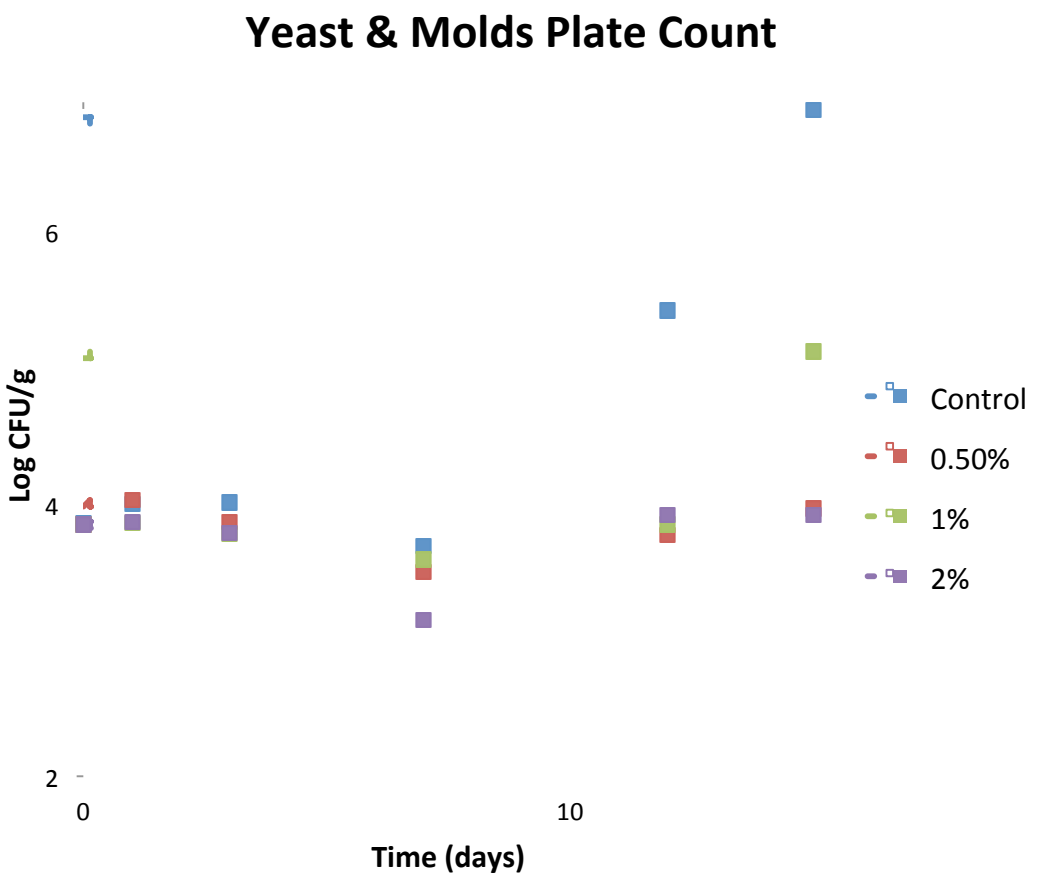


Figure 4.3 Effect of multilayered antimicrobial coating on yeast and molds plate counts of fresh-cut pineapple samples with different sodium alginate concentrations during 15 days of storage at 4 ºC.

4.3.5 Microscopic examination of the multilayered edible coating

Figure 4.4 shows the thickness for the coating with 0.5% sodium alginate, 2% pectin and 2% antimicrobial compound, showing that the coating was homogenous throughout the entire surface of the fruit. The mean value for the coating thickness was 132 μm with a standard deviation of 1.0.

The coating with 1% sodium alginate, 2% pectin, and 2% antimicrobial compound (Figure 4.5), and the coating with 2% sodium alginate, 2% pectin and 2% antimicrobial compound (Figure 4.6) showed more variability in its thickness. The coating was homogenously extended through the fruit sample, this implicate that it is possible for the coating to preserve the quality of the fruit sample homogenously as well as to promote an homogenously antimicrobial activity from the coating around all the sample. The mean values for thickness was 180 μm with a standard deviation of 2.0, and 412 μm with a standard deviation of 0.9 respectively.

Coating thickness may be affected by the rheological properties of the polymers used on the coating (Tapia and others 2008); for instance, coating thickness is directly proportionally affected by alginate and pectin concentrations. All of the coating samples had the same antimicrobial concentration (2% (w/w)) in the coating; however, the amount of antimicrobial present in the coatings varied slightly due to the difference in thickness, the homogeneity of the coating around the entire fresh-cut pineapple sample and, the size of it. Samples coated with 0.5% sodium alginate coating presented a mass of antimicrobial compound of 0.037 grams with a standard deviation equal to 0.16. Samples coated with 1% sodium alginate coating presented a mass of antimicrobial compound of 0.044 grams with a standard deviation of 1, and samples coated with 2% sodium alginate coating showed a mass of antimicrobial of 0.045 grams with a standard deviation of 0.16. Definitively, the samples showed how the thickness of the coating affected the amount of antimicrobial that was present in the coating.

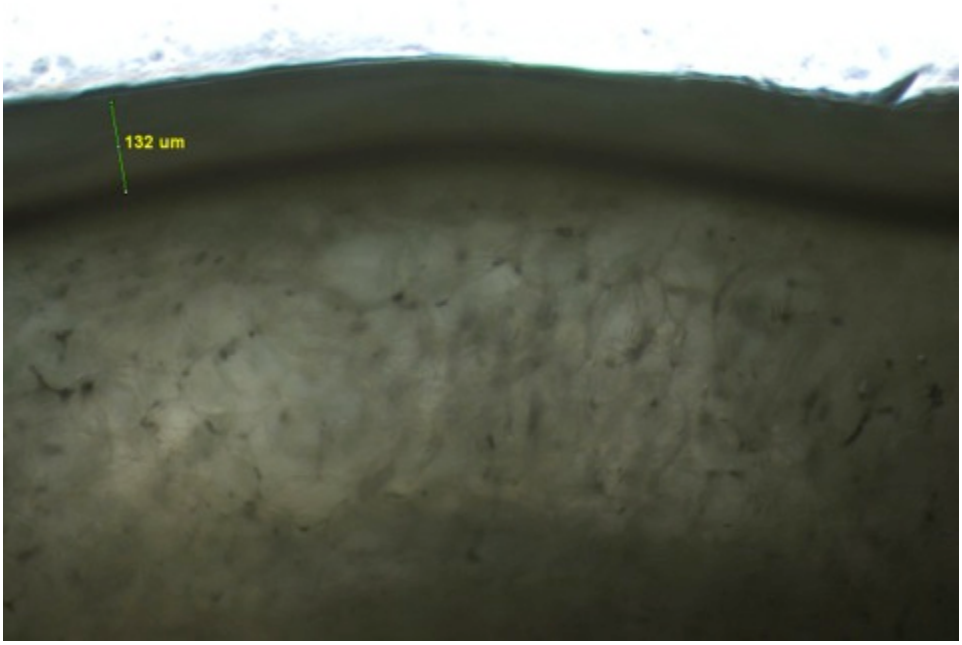


Figure 4.4 Microscopic examination of cross-section of multilayered antimicrobial coating on fresh-cut pineapple samples with 0.5% sodium alginate concentration, 2% pectin concentration and 2% antimicrobial compound concentration.

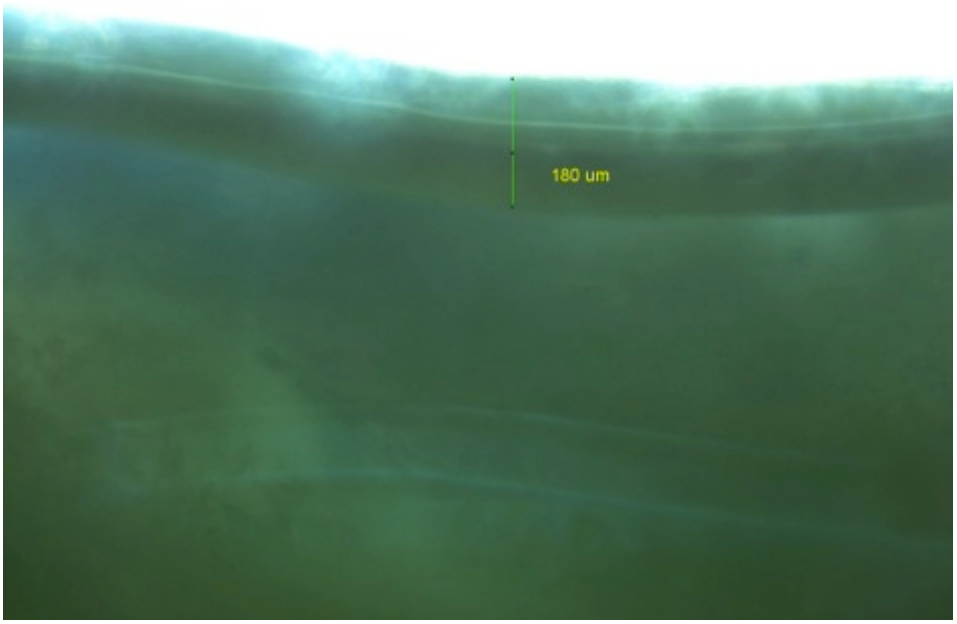


Figure 4.5 Microscopic examination of cross-section of multilayered antimicrobial coating on fresh-cut pineapple samples with 1% sodium alginate concentration, 2% pectin concentration and 2% antimicrobial compound concentration.

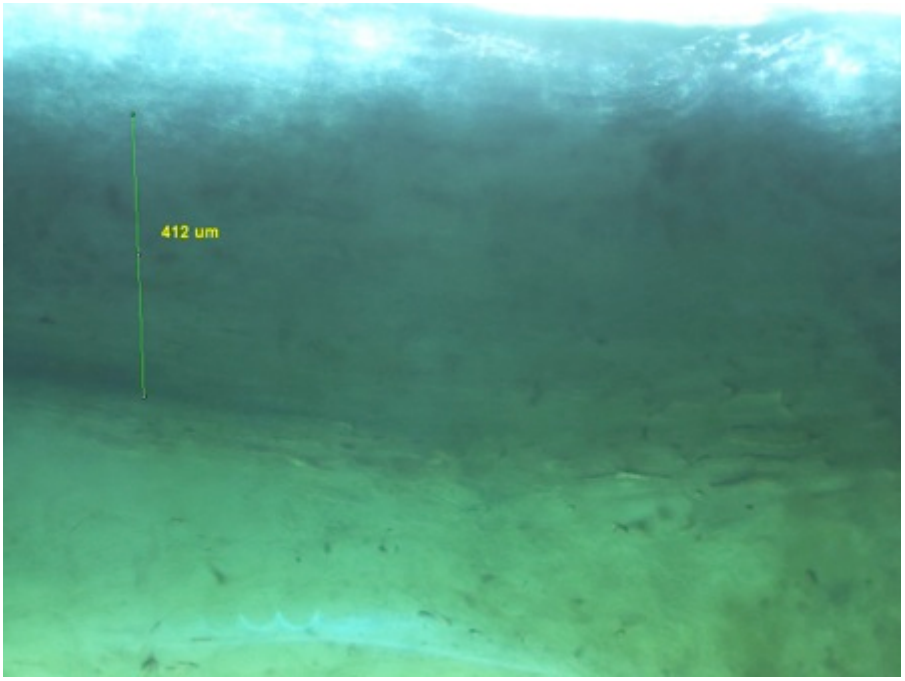


Figure 4.6 Microscopic examination of cross-section of multilayered antimicrobial coating on fresh-cut pineapple samples with 2% sodium alginate concentration, 2% pectin concentration and 2% antimicrobial compound concentration.

CHAPTER V

CONCLUSIONS

The development and application of a multilayered edible antimicrobial coating and its effect on the quality and shelf-life of fresh-cut pineapple were evaluated. Formulation of the coating included sodium alginate, pectin, antimicrobial compound and calcium chloride. For sodium alginate, three different concentrations (0.5% (w/w), 1% (w/w), and 2% (w/w)) were tested. The concentrations of pectin, calcium chloride, and antimicrobial compound were kept constant for all treatments (2% (w/w)).

Pineapple chemical properties like pH, ºBrix, acidity, juice leakage, vitamin C, and moisture content; and product quality attributes (color, texture, and sensory attributes) of coated and uncoated controls were evaluated for 15 days stored at 4 ºC. In addition, microbiological analyses were performed to determine the effectiveness of the antimicrobial compound in preventing microbial growth.

The main results and conclusions obtained from this research are mentioned below:

- The presence of the alginate-based multilayered antimicrobial coating did not affect the pH and ºBrix of fresh-cut pineapple during storage. Moreover, moisture content retention was enhanced during storage time due to the application of the coating. Coated samples retained moisture, which translated into less juice leakage.
- There was only a slight difference in the color of coated samples as compared to the controls. However, the difference was just due to the antimicrobial coating application. The color quality of coated samples was not affected during the evaluation time.
- Texture of the fresh-cut fruit was well preserved by application of antimicrobial coating. This factor is very important considering that texture is one of the main sensory

attributes that people look for in a final product. During the shelf-life study, differences in texture were easily detectable. Control samples were significantly softer and their surface was drier just by day 7 of storage.

- The headspace concentration analysis demonstrated that the coating was effective in delaying the respiration process of the fruits. The concentration of CO_2 in the jars holding the coated samples was lower in comparison with those containing the control samples during the 15 days of evaluation. Lower concentration of CO_2 in the headspace translates into longer preservation of the product (shelf-life).
- A consumer acceptance test using thirty panelists confirmed that consumers can accept the presence of the coating on the fresh-cut fruit as long as the color, odor and flavor parameters are not affected by it. The flavor of 1% alginate-based coated samples was acceptable to panelists up to day 13, when the control sample began to show signs of deterioration.
- The coated samples had the lowest scores for odor throughout the study. Panelists commented on the fact that they did not like to eat pineapple that had some cinnamon-like taste. This sensory attribute is one of the principal factors that would be important to improve in future studies.
- Microbiological analyses demonstrated the effectiveness of the coating as a carrier of antimicrobial compounds and the effectiveness of this compound against microbial growth as well. The effectiveness against psychrothrophics and yeast and molds was particularly significant.
- The best formulation of the coating in terms of the preservation of quality attributes of fresh-cut pineapple is the one made with 1% (w/w) of alginate, 2% (w/w) of antimicrobial compound (trans-cinnamaldehyde) and 2% (w/w) of pectin. This particular

treatment showed to be an effective alternative to maintain pineapple original quality and to preserve it for longer (15 days), making the shelf-life extension a fact.

- The use of a multilayered edible coating with the combination of an antimicrobial compound (trans-cinnamaldehyde), presents a huge potential as a means to extend the shelf-life of fresh-cut pineapple and giving the consumer a natural, healthy, and tasty final product.

CHAPTER VI

RECOMMENDATIONS FOR FURTHER STUDY

Some recommendations for further study on multilayered antimicrobial coatings, based on the results and conclusions already obtained by the present research include:

- To develop a set of experiments with concentrations of alginate between 1% and 2% in the coating (1.25%, 1.5%, 1.75%) to determine the best concentration in terms principally of physical appearance and preservation of quality attributes.
- To develop a set of experiments that assess the effect of different concentrations of the antimicrobial compound on the quality attributes of the fruit, and enhance the reduction or control of microbial growth.
- To investigate whether there is an alternative antimicrobial compound that does not impart undesirable color, flavor, or aroma characteristics to the coated fruit.
- To develop a procedure to optimize the dipping process to avoid sample cross-contamination and to improve the homogeneity of the coating over the whole surface of the cut fruit.

REFERENCES

Abe K, Watada AE. 1990. Ethylene absorbent to maintain quality of lightly processed fruits and vegetables. Journal of Food Science 56: 1589-1592.

Ahvenainen R. 1996. New approaches in improving the shelf-life of minimally processed fruit and vegetables. Trends in Food Science and Technol. 7: 179-187.

Ayala-Zavala JF, Gonzalez-Aguilar GA, Del toro-Sanchez L. 2009. Enhancing safety and aroma appealing of fresh-cut fruits and vegetables using the antimicrobial and aromatic power of essential oils. Journal of Food Science 74: R84-R90.

Ayers LT, Williams IT, Gray S, Griffin PM, Hall AJ. 2009. Surveillance for foodborne disease outbreaks United States 2006. Morbidity and Mortality Weekly Report. 58: 609-615.

Bakkali F, Averbeck S, Averbeck D and Idaomar M. 2008. Biological effects of essential oils: a review. Food Chem Toxicol 46(2): 446-475.

Balla C, Farkas J. 2006. Minimally processed fruits and fruit products and their microbiological safety. In: Handbook of fruits and fruit processing. Hui YH, Barta J, Cano MP, Gusek T, Sidhu JS, Sinha N (eds). Ames, Iowa. Blackwell Publishing. pp: 115-128.

Ballabeni V, Tognolini M, Chiavarini M, Impicciatore M, Bruni R, Bianchi A, Barocelli E. 2004. Novel antiplatelet and antithrombotic activities of essential oil from *Lavandula hybrid* Reverchon "grosso". Phytomedicine 11: 596-601.

Batish DR, Slingh HP, Kohli RK, Kaur S. 2008. Eucalyptus essential oil as a natural pesticide. Forest Ecol Management 256: 2166-2174.

Beserra Almeida MM, Machado de Sousa PH, Campos Arriaga AM, Matias do Prado GM, Carvalho Magalhaes CE, Arraes Maia G, Gomes de Lemos TL. 2011. Bioactive compounds and antioxidant activity of fresh exotic fruits from northeastern Brazil. Food Research International 44: 2155-2159.

Beuchat LR. 1994. Antimicrobial properties of spices and their essential oils. In: Natural antimicrobial systems and food preservation. Dillon VM, Board RG (eds). Wallingford, UK. CABI. p: 328.

Beuchat LR. 2002. Ecological factors influencing survival and growth of human pathogens on raw fruits and vegetables. Microbes and Infection 4: 413-423.

Beuchat LR. 2007. Managing Food Safety risks in the fresh-cut industry. Proceedings of the International Conference on Quality Management of Fresh Cut Produce. 746: 103-114.

Bierhals VS, Chiumarelli M, Hubinger MD. 2011. Effect of Cassava starch coating on quality and shelf-life of fresh-cut pineapple (*Ananas comosus* L. Merril cv "Perola"). Journal of Food Science 76: E62-E71.

Brasil IM, Gomes C, Puerta-Gomez A, Castell-Perez ME, Moreira RG. 2012. Polysaccharide-based multilayered antimicrobial edible coating enhances quality of fresh-cut papaya. LWT Food Science and Technol. 47(1): 39-45.

Brat P, Thi-Hoang W, Soler A, Reynes M, Brillouet JM. 2004. Physicochemical characterization of a new pineapple hybrid (FLHORAN 41 Cv.) Journal of Agricultural and Food Chemistry 52(20): 6170-6177.

Burnett SL and Beuchat LR. 2001. Human pathogens associated with raw produce and unpasteurized juices, and difficulties in decontamination. Journal of Industrial Microbiology and Biotechnology. 27: 104-110.

Burt S. 2004. Essential oils: their antibacterial properties and potential applications in foods: a review. International Journal of Food Microbiology 94: 223-253.

Campos CA, Gerschenson LN, Flores S. 2010. Development of edible films and coatings with antimicrobial activity. Food and Bioprocess Technol 4(6): 849-875.

Carson CF, Hammer KA, Riley TV. 2006. *Melaleuca alternifolia* (tea tree) oil: a review of antimicrobial and other properties. Clin Microbiol Rev 19(1):50-62.

Carson CF, Hammer KA. 2011. Chemistry and bioactivity of essential oils. In: Lipids and essential oils as antimicrobial agents. Thormar H (ed). Chichester, UK. John Wiley & Sons, Ltd. ch9 pp: 204-223.

Cavanagh HMA and Wilkinson JM. 2002. Biological activities of lavender essential oil. Phytotherapy Research 16(4): 301-308.

CFR, Code of Federal Regulations. 2009a. Tittle 21 part 172.515. Food additives permitted for direct addition to food for human consumption: synthetic flavoring substances and adjuvants. 3: 56-63.

Chaieb K, Hajlaoui H, Zmantar T,Kahla-Nakbi AB, Rouabhia M, Mahdouani K,Bakhrouf A. 2007. The chemical composition and biological activity of clove essential oil, *Eugenia caryophyllata* (*Syzigium aromaticum* L. Myrtaceae) a short review. Phytotherapy Research 21(6): 501-506.

Coppens d' Eeckenbrugge G and Leal F. 2003. Morphology, Anatomy and Taxonomy. In: The pineapple: Botany, Production and uses. Bartholomew DP, Paull RE, Rohrbach KG (eds). Wallingford, UK. CAB International. ch2. pp 13-29.

Croteau R, Kutchan TM, Lewis NG. 2000. Natural products (secondary metabolites). In: Biochemistry and molecular biology of plants. Buchanan B, Gruissen W, Jones R (eds). Rockville, MD, USA. Society of Plant Biologists. pp: 1250-1268.

Dan H. 2007. Characterization of cucumber cultivars by mechanical stress distributions during the compression process. Jarq-Japan Agricultural Research Quarterly. 41(2): 115-121.

Dangaran K, Tomasula P, Qi P. 2009. Structure and function of protein-based edible films and coatings. In: Edible films and coatings for food applications. Embuscado ME, Huber KC (eds). Dordrecht, NY, USA. Springer. pp: 25-51.

Davidson PM. 2001. Chemical preservatives and natural antimicrobial compounds. In: Food Microbiology fundamentals and frontiers. Doyle HP, Beuchat LR, Montville TJ (eds). 2nd ed. Washington DC. ASM Press. pp: 593-627.

Day BPF. 1994. Modified atmosphere packaging and active packaging of fruits and vegetables in minimal processing of foods. In: VTT symposium series 142. Ahvenainen R, Mantilla-Sandholm T, Ohlsson T (eds). Espoo, Finland. Technical Research Centre of Finland (VTT). pp: 173-207.

Dembitsky VM, Poovarodom S, Leontowicz H, Leontowicz M, Vearasilp S, Trakhtenberg S, Gorinstein S. 2011. The multiple nutrition properties of some exotic fruits: biological activity and active metabolites. Food Research International 44: 1671-1701.

Di Egidio V, Sinelli N, Limboa S, Torri L, Franzetti L, Casiraghia E. 2009. Evaluation of shelf-life of fresh-cut pineapple using FT-NIR and FT-IR spectroscopy. Postharvest Bio and Technol 54: 87-92.

Donati I, Holtan S, Morch YA, Borgogna M, Dentini M, Skjak-Brae K. 2005. New hypothesis on the role of alternating sequences in calcium-alginate gels. Biomacromolecules 6: 1031-1040.

Draget KI. 2000. Alginates. In: Handbook of hydrocolloids. Phillips GO, Williams PA (eds). Boca Raton, FL. CRC Press. pp: 379-395.

Dung NT, Kim JM, Kang SC. 2008. Chemical composition, antimicrobial and antioxidant activities of the essential oil and the ethanol extract of *Cleistocalyx operculatus* (Roxb.) Merr and Perry buds. Food and Chemical Toxicology 46(12): 3632-3639.

FDA. 2011. CFR. Code of Federal Regulations. Tittle 21 Part 172. Food Additives permitted for direct addition to food for human consumption. CFR 21 (3), 21 CFR 172.515. http://www.accesdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?fr=172.515. Retrieved on 02/03/2012.

Friedrich H. 1976. Phenylpropanoid constituents of essential oils. Lloydia-The Journal of Natural Products 39(1): 1-7.

Garret H. 2002. Fresh-cut produce: tracks and trends. In: Fresh-cut fruits and vegetables: science, technology, and market. Lamikanra O. (ed). Boca Raton, FL. CRC Press. pp: 21-29.

Gershenzon J and Dudareva N. 2007. The function of terpene natural products in the natural world. Nature Chemical Biology 3(7): 408-414.

Giacalone G, Chiabrando V, Bardi L. 2010. Changes in nutritional properties on minimally processed fresh fruit during storage. International Journal of Food Science 22: 305-311.

Gomes C, Moreira RG, Castell-Perez E. 2011. Microencapsulated antimicrobial compounds as a means to enhance electrobeam irradiation treatment for inactivation of pathogens on fresh spinach leaves. Journal of Food Science 76: E479-E488.

Gomes C, Moreira RG, Castell-Perez E. 2011. Poly (DL-lactide-co-glycolide) (PLGA) nanoparticles with entrapped trans-cinnamaldehyde and eugenol for antimicrobial delivery applications. Journal of Food Science 76: N16-N24.

Gomes C. 2010. Personal Comunication. Department of Biological and Agricultural Engineering, Texas A&M University, College Station, Texas.

Gonzalez-Aguilar GA, Ruiz-Cruz S, Cruz-Valenzuela R, Rodriguez-Felix A, Wang CV. 2004. Physiological and quality changes of fresh-cut pineapple treated with antibrowning agents. Lebensm-Wiss. Technol 37: 369-376.

Gonzalez-Aguilar GA, Ruiz-Cruz S, Soto-Valdez H, Vazquez-Ortiz F, Pacheco-Aguilar R, Yi Wang Ch. 2005. Biochemical changes of fresh-cut pineapple slices treated with antibrowning agents. International Journal of Food Science and Technol. 40: 377-383.

Gorris LGM, de Witte Y, Bennik MJH. 1994. Refrigerated storage under moderate vacuum. Focus International 45(6): 63-66.

Griffin SG, Wyllie SG, Markham JL, Leach DN. 1999. The role of structure and molecular properties of terpenoides in determining their antimicrobial activity. Flavour and Fragance Journal 14: 322-332.

Gundran AL. 2001. Pythochemical screening of the exocarp of papaya (*Carica papaya*), pineapple (*Ananas comosus*), and starapple (*Chrysophylium cainito*). Abstracts of papers of the American Chemical Society 222: 135-Medi Part 1.

Harris LJ, Farber JN, Beuchat LR, Parish ME, Suslow TV, Garnet EH, Busta FF. 2003. Outbreaks associated with fresh produce: incidence, growth, and survival of pathogens in fresh and fresh-cut produce. Comprehensive Reviews in Food Science and Food Safety (CRFSFS). Institute of Food Technologists. 2(1): 78-141.

Holley RA and Patel D. 2005. Improvement in the shelf-life and safety of perishable foods by plant essential oils and smoke antimicrobials. Food Microbiology 22(4): 273-292.

Hossain MA, Rahman SMM. 2011. Total phenolics flavonoids and antioxidant activity of tropical fruit pineapple. Food Research International. 44(3): 672-676.

Hubinger MD. 2010. Cassava starch edible coatings improve quality of minimally processed strawberries. Emerging Food Research and Development Report. 21(3): 3.

Huxsoll C, Bolin HR, King D Jr. 1989. Physicochemical changes and treatments for lightly processed fruit and vegetables. In: Quality factors of fruits and vegetables. Jen J (ed). Washington, DC. American Chemical Society. pp: 203-215.

Jay JM, Loessner MJ, Golden DA. 2005. Modern Food Microbiology. 7th Ed. New York, USA. Springer Science & Business Media Inc.

Jen J. 1989. Chemical Basis of quality factors in fruit and vegetables. An overview. In: Quality factors of fruits and vegetables. Jen J (ed). Washington, DC. American Chemical Society. pp: 1-9.

Kalemba D and Kunicka A. 2003. Antibacterial and antifungal properties of essential oils. Current Medicinal Chemistry 10(10):813-829.

Kalia A, Gupta RP. 2006. Fruit microbiology. In: Handbook of fruits and fruit processing. Hui YH (ed). Ames, Iowa. Blackwell publishing. pp: 3-28.

Krochta JM, Baldwin EA, Nisperos-Carriedo MO. 1994. Edible coatings and films to improve food quality. Boca Raton, FL. CRC press. Lancaster, Pa. Technomic publishing. ch8.

Langenheim JH. 1994. Higher plant terpenoids: a phytocentric overview of their ecological roles. Journal of Chemical Ecology 20(6):1223-1280.

Liao BC, Hsieh CW, Liu YC, Tzeng T, Sun YW, Wung BS. 2008. Cinnamaldehyde inhibits the tumor necrosis factor –a- induced expression of cell adhesion molecules in endothelial cells by suppressing NF-KB activation: effects upon IKB and Nrf2. Toxicology and Applied Pharmacology 229: 161-171.

Lin SY and Krochta JM. 2006. Whey protein coating efficiency on mechanically roughened hydrophobic peanut surfaces. Journal Food Science 71(6): E270-E275.

Loeillet D. 1997. Panorama du marche mundial d ananas: Importance de l'Europe (The world pineapple market: the importance of Europe). Second International Pineapple Symposium. 425: 37-48.

Marpudi SL, Abirami LSS, Pushkala R, Srividya N. 2011. Enhancement of storage life and quality maintenance of papaya fruits using Aloe Vera based edible coating. Indian Journal of Biotechnology 10: 83-89.

Marrero A, Kader A. 2006. Optimal temperature and modified atmosphere for keeping quality of fresh-cut pineapples. Postharvest Bio and Technol 39: 163-168.

Martin-Belloso O, Rojas-Grau MA, Soliva-Fortuny R. 2009. Delivery of flavor and active ingredients using edible films and coatings. In: Edible films and coatings for food applications. Embuscado ME, Huber KC (eds). Dordrecht, NY, USA. Springer. pp: 295-309.

Mayer AM. 1987. Polyphenol oxidase and peroxidase in plants. Recent progress. Phytochemistry 26: 11-20.

Minifie BW. 1989. Chocolate, cocoa, and confectionary: science and technology. 3rd ed. Van Nostrand Reinold. New York, USA. Springer Publishing.

Montero-Calderon M, Rojas-Grau MA, Martin-Belloso O. 2010. Mechanical and chemical properties of Gold cultivar pineapple flesh (*Ananas comosus*). European Food Research and Technology 230(4): 675-686.

Montero-Calderon M, Rojas-Grau MA, Martin-Belloso O. 2008. Effect of packaging conditions on quality and shelf-life of fresh-cut pineapple (*Ananas comosus*) Postharvest Bio and Technol 50: 182-189.

Moreira MR, Roura SI, Ponce A. 2011. Effectiveness of chitosan edible coatings to improve microbiological and sensory quality of fresh-cut broccoli. LWT Food Science and Technol 44: 2335-2341.

Moreno M, Castell-Perez ME, Gomes C, Da Silva P, Moreira RG. 2006. Effects of electron beam irradiation on physical, textural, and microstructural properties of "Tommy Atkins" mangoes. Journal Food Science 71(2): E80-E86.

Nieto M. 2009. Structure and function of polysaccharide gum-based edible films and coatings. In: Edible films and coatings for food applications. Embuscado ME, Huber KC (eds). Dordrecht, NY, USA. Springer. pp: 57-107.

Oms-Oliu G, Rojas-Grau MA, Alandes Gonzalez L, Varela P, Soliva-Fortuny G, Hernando Hernando MA, Perez Munuera I, Fiszman S, Martin-Belloso O. 2010. Recent approaches using chemical treatments to preserve quality of fresh-cut fruit: A review. Postharvest Bio and Technol 57: 139-148.

Pascual ME, Slowing K, Carretero E and others. 2001. *Lippia*: traditional uses, chemistry and pharmacology: a review. Journal of Ethnopharmacology 76(3): 201-214.

Paull RE, Chen CC. 2003. Postharvest physiology, handling and storage of pineapple. In: The pineapple: botany, production and uses. Bartholomew DP, Paull RE, Rohrbach KG (eds). Wallingford, UK. CAB International. pp: 253-279.

Pavlath AE, Orts W. 2009. Edible films and coatings: why, what and how?. In: Edible films and coatings for food applications. Embuscado ME, Huber KC (eds). Dordrecht, NY, USA. Springer. pp: 1-23.

Pengelly A. 2004. Essential oils and resins. In: The constituents of medicinal plants. 2nd ed. Sydney, Australia. Crows Nest, NSW. Allen & Unwin. ch 7.

Pichersky E, Sharkey TD, Gershenzon J. 2006. Plant volatiles: a lack of function or a lack of knowledge?. Trends in Plant Science 11(9): 421.

Raybaudi-Massilia R, Mosqueda-Melgar J, Martin-Belloso O. 2008. Edible alginate-based coating as carrier of antimicrobials to improve shelf-life and safety of fresh-cut melon. International Journal of Food Microbiology 121: 313-327.

Raybaudi-Massilia RM, Mosqueda-Melgar J, Soliva-Fortuny S, Martin-Belloso O. 2009. Control of pathogenic and spoilage microorganisms in fresh-cut fruits and fruit juices by traditional and alternative natural antimicrobials. Comprehensive reviews in food science and food safety. Comprehensive Reviews in Food Science and Food Safety 8(3): 157-180.

Rohrbach KG, Leal F, Coppens d'Eeckenbrugge G. 2003. History, distribution, and crop production. In: The pineapple: botany, production and uses. Bartholomew DP, Paull RE, Rohrbach KG (eds). Wallingford, UK. CAB International. pp: 1-10.

Rojas-Grau MA, Soliva-Fortuny R, Martin-Belloso O. 2009. Edible coatings to incorporate active ingredients to fresh-cut fruits: a review. Trends in Food Science and Technol 20: 438-447.

Roopa BS and Bhattacharya S. 2009. Alginate gels: rupture characteristics as a function of the conditions of gel formation. Journal of Food Engineering 91: 448-454.

Sadili-Bico S, Raposo M, Santos Costa de Morais R, Miranda Bernardo A. 2009. Chemical dips and edible coatings to retard softening and browning of fresh-cut banana. International Journal of Postharvest Technology and Innovation 2: 13-22.

Schwab W, Davidovich-Rikanati R, Lewinsohn E. 2008. Biosynthesis of plant-derived flavor compounds. Plant Journal 54(4): 712-732.

Serra S, Fuganti C, Brenna E. 2005. Biocatalytic preparation of natural flavors and fragances. Trends in Biotechnology 23(4): 193-198.

Soliva-Fortuny R. 2010. Polysaccharide coatings extend fresh-cut fruit shelf-life. Emerging Food Research and Development Report. 21: 1-2.

Spanier AM, Flores M, James C, Lasater J, Lloyd S, Miller JA. 1998. Fresh-cut pineapple (*Ananas* sp.) flavor. Effect of storage. In: Food Flavors: Formation, analysis and packaging influences. Contis ET (ed). New York, USA. Elsevier Science B.V. pp: 331-341.

Tapia MS, Rojas-Grau MA, Carmonac A, Rodriguez FJ, Soliva-Fortuny R, Martin-Belloso O. 2008. Use of alginate- and gellan-based coatings for improving barrier, texture and nutritional properties of fresh-cut papaya. Food Hydrocolloids 22: 1493-1503.

Taylor B. 2005. Other beverages ingredients. In: Chemistry and technology of soft drinks and fruit juices. Ashurst PR (ed). Oxford, UK. Wiley-Blackwell. pp: 90-128.

USDA. 2010. National Database for standard reference, release 23.

Watada AE. 1990. Physiological activities of partially processed fruits and vegetables. Food Technology. 44: 116-122.

Wilson R, Parker J, Kovacs D, Doohan D, Lejeune J. 2009. Contamination prevention and response related to fresh and fresh-cut produce: an expert perspective on the farmer decision making process. Food Protection Trends 29: 488-492.

Yi. 2006. Determination of the phenolic compounds in pineapple by high-performance liquid chromatography. Shipin Yu Fajiao Gongye. 32: 99-101.

Zhao Y, 2011. Edible coatings enhance shelf-life of ready-to-eat blueberries. Emerging Food Research and Development Report. p: 5.

Zhou Y, O'Hare Tim J, Jobin-Décor M, Underhill SJR, Wills RBH, Graham MW. 2003. Transcriptional regulation of a pineapple polyphenol oxidase gene and its relationship to blackheart. Plant Biotechnology Journal. 1: 463-478.

APPENDIX A

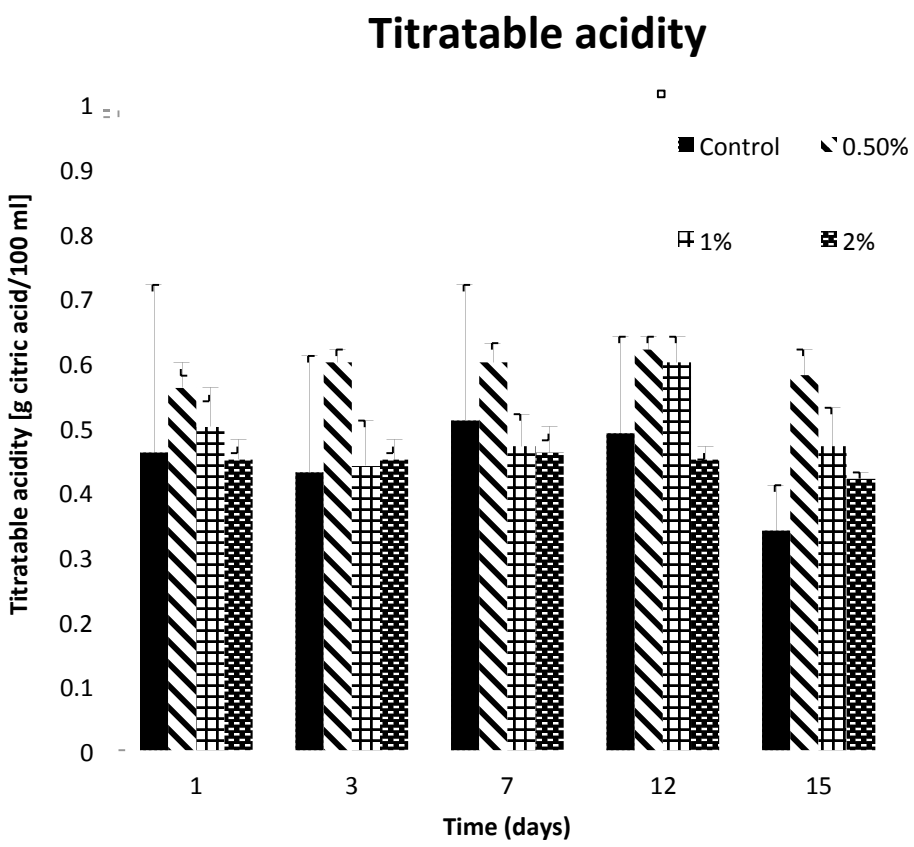
Acceptance Test: 117

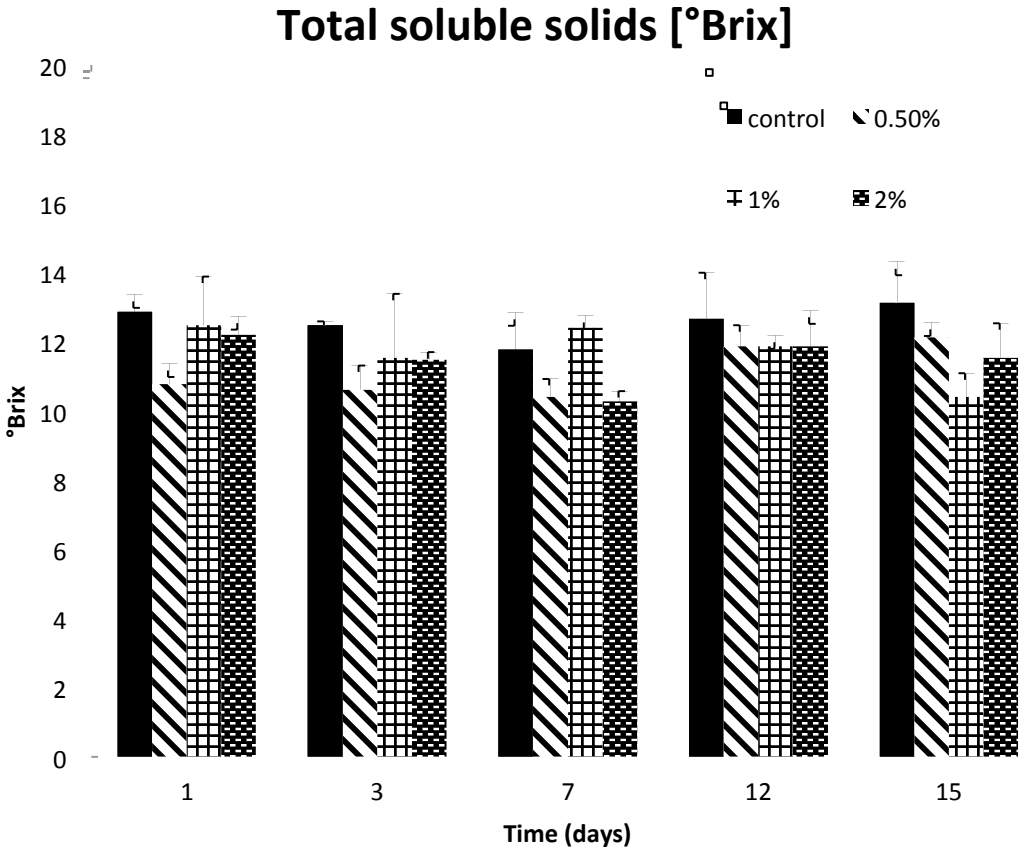
Place a mark in the box which you feel best describes how you like the sample.
An honest expression of your personal feelings will help us. Thank you.

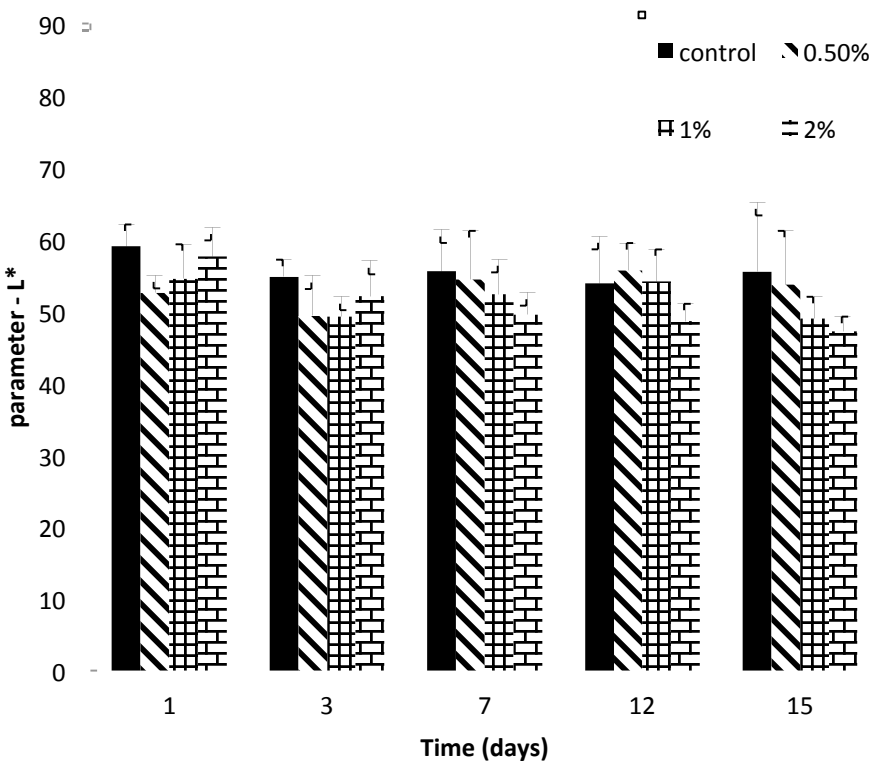
Color	☐ Like extremely	☐ Like Very much	☐ Like moderately	☐ Like slightly	☐ Neither like nor dislike	☐ Dislike slightly	☐ Dislike moderately	☐ Dislike very much	☐ Dislike extremely
Odor	☐ Like extremely	☐ Like Very much	☐ Like moderately	☐ Like slightly	☐ Neither like nor dislike	☐ Dislike slightly	☐ 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

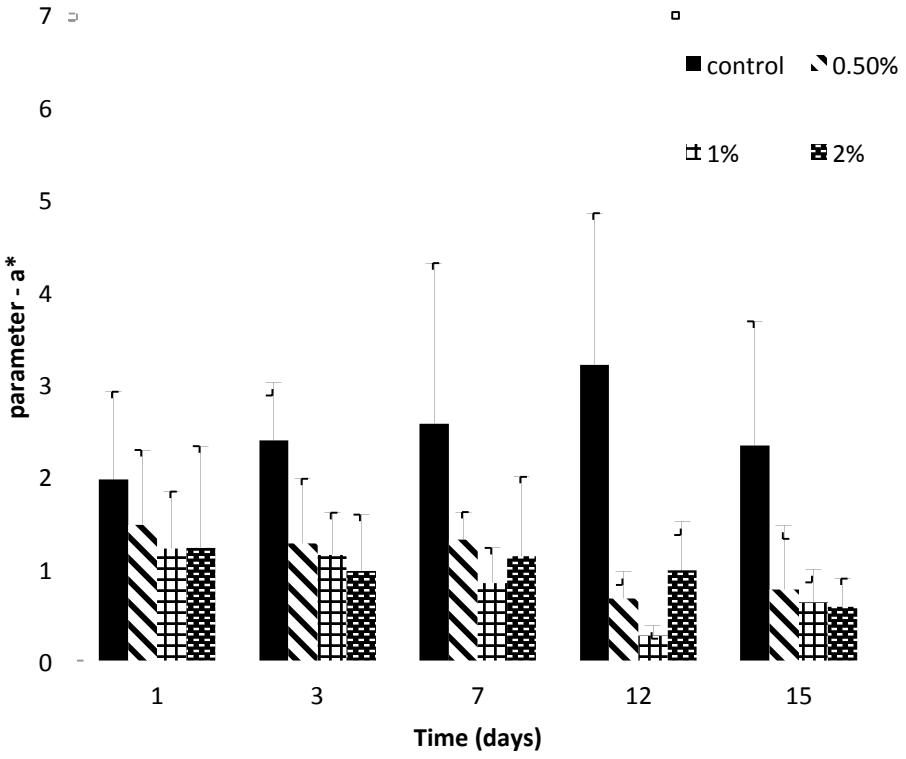
Comments:

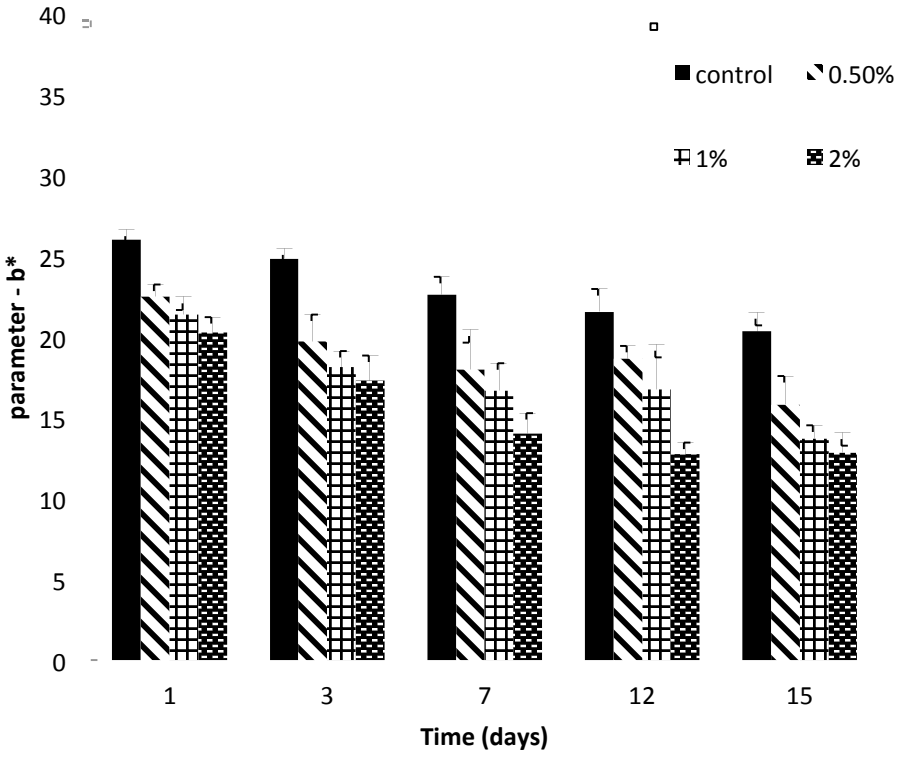
APPENDIX B

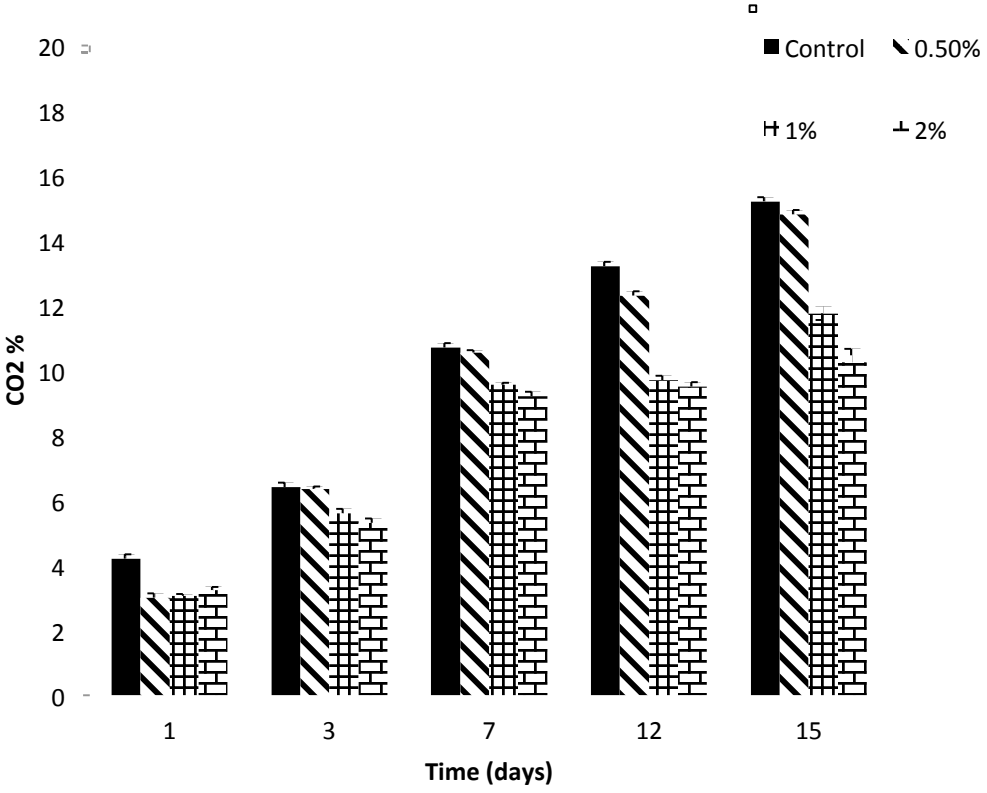

Titratable acidity
Control
0.50%
1%
2%
Titratable acidity [g citric acid/100 ml]
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
1
3
7
12
15
Time (days)

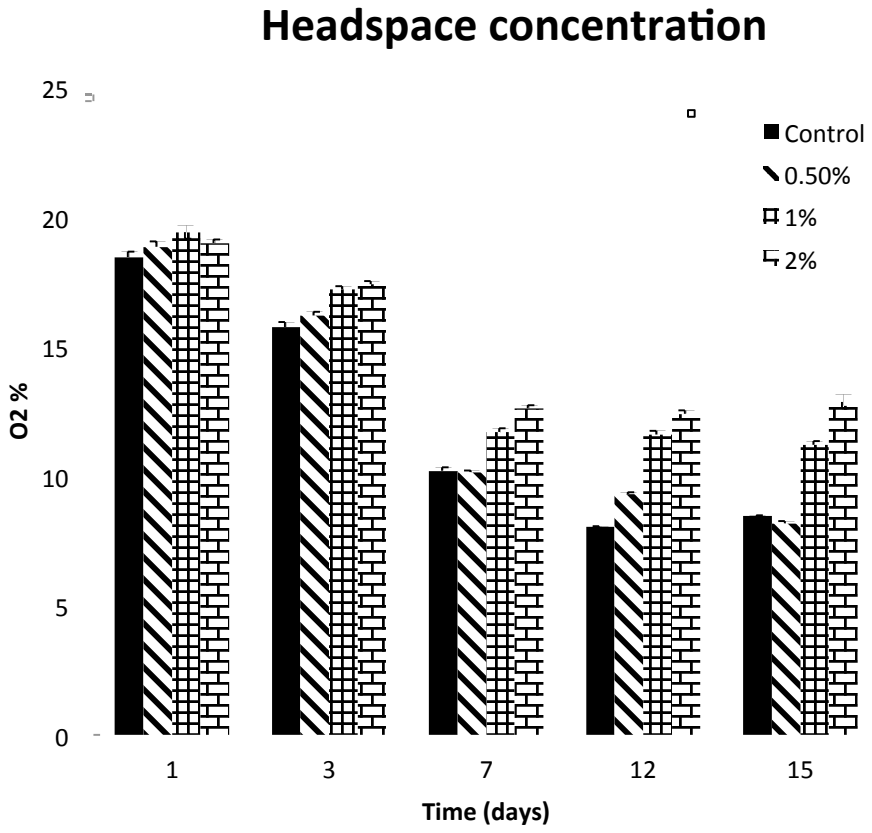

Total soluble solids [°Brix]
control
0.50%
1%
2%
°Brix
20
18
16
14
12
10
8
6
4
2
0
1
3
7
12
15
Time (days)

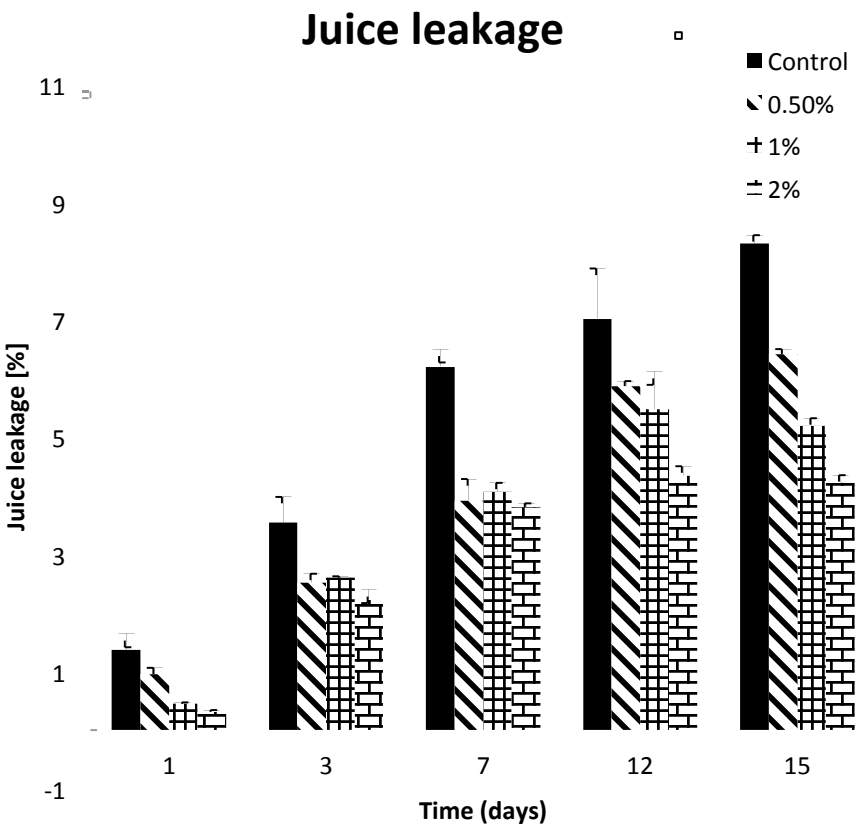

Color parameter L*
control
0.50%
1%
2%
parameter - L*
90
80
70
60
50
40
30
20
10
0
1
3
7
12
15
Time (days)

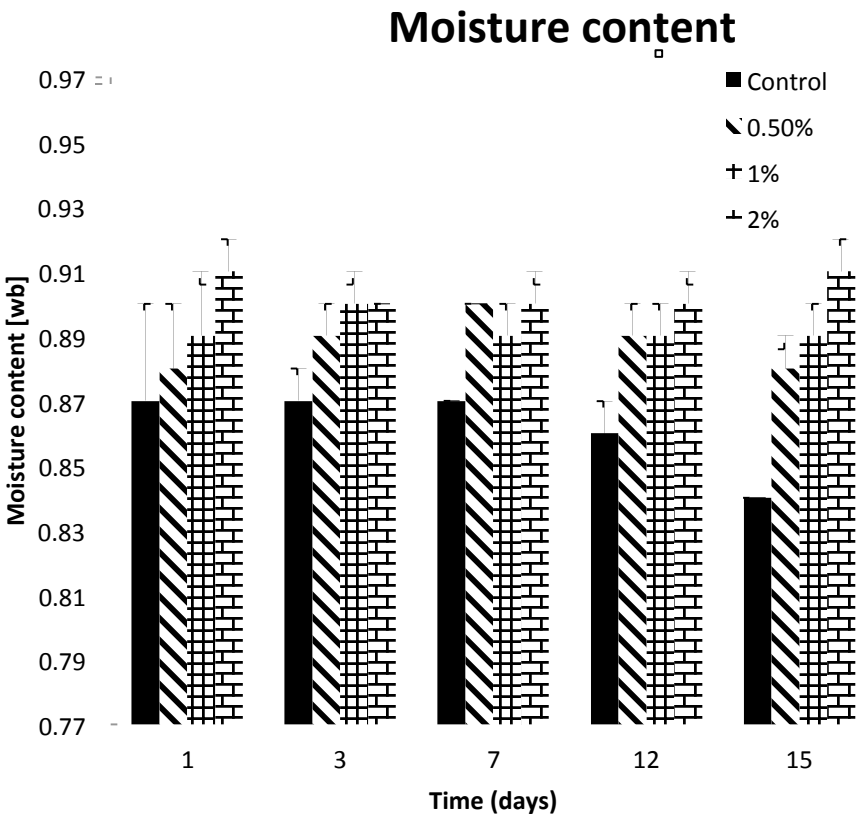

Color parameter a*
control
0.50%
1%
2%
parameter - a*
0
1
2
3
4
5
6
7
1
3
7
12
15
Time (days)

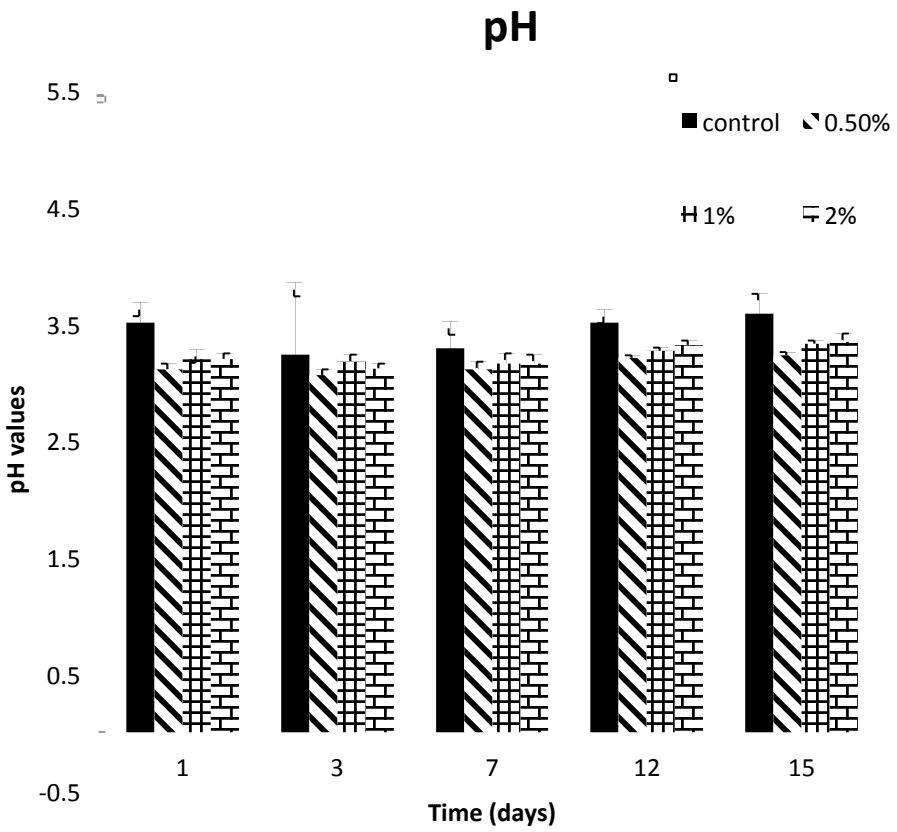

Color parameter b*
control
0.50%
1%
2%
parameter - b*
40
35
30
25
20
15
10
5
0
1
3
7
12
15
Time (days)

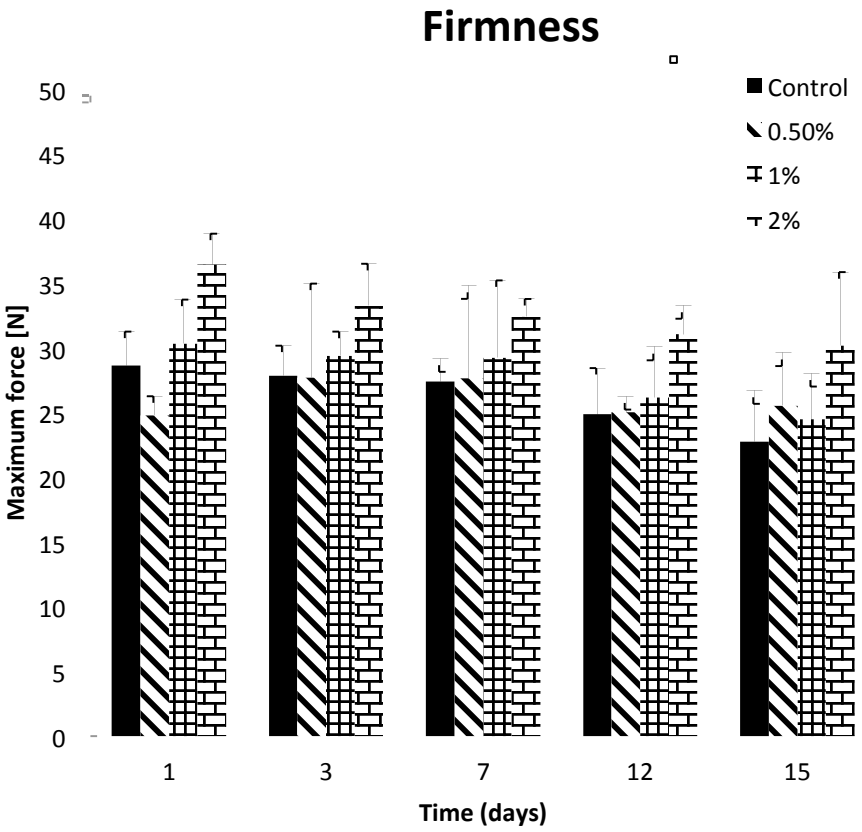

Headspace concentration
Control
0.50%
1%
2%
CO2 %
20
18
16
14
12
10
8
6
4
2
0
1
3
7
12
15
Time (days)

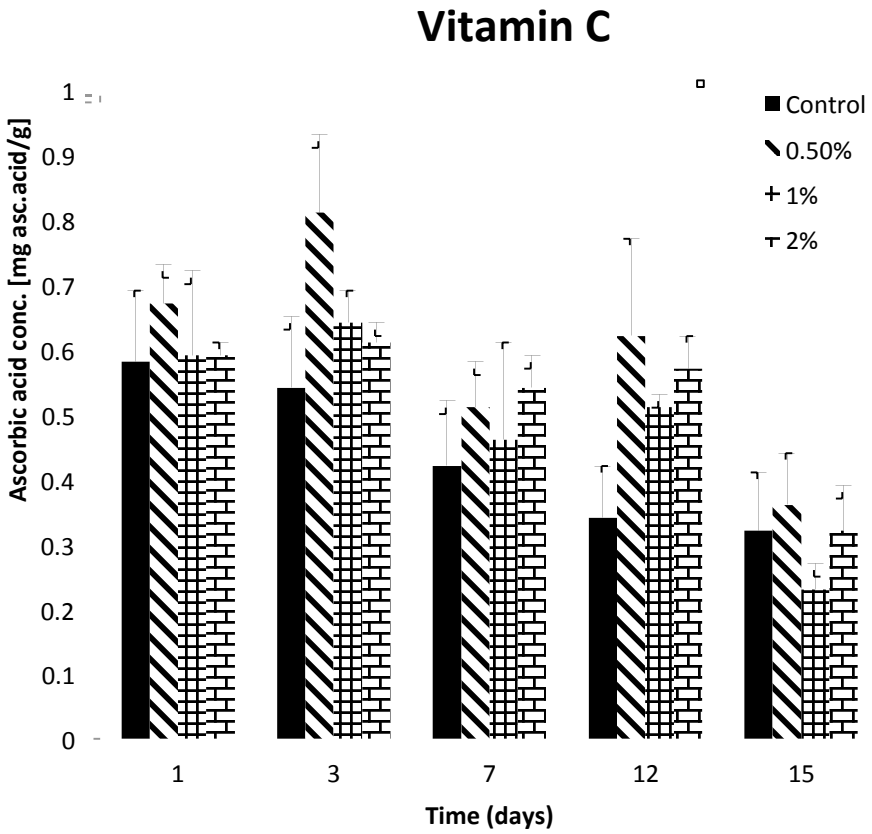

Headspace concentration
25
20
15
10
5
0
O2 %
1
3
7
12
15
Time (days)
Control
0.50%
1%
2%

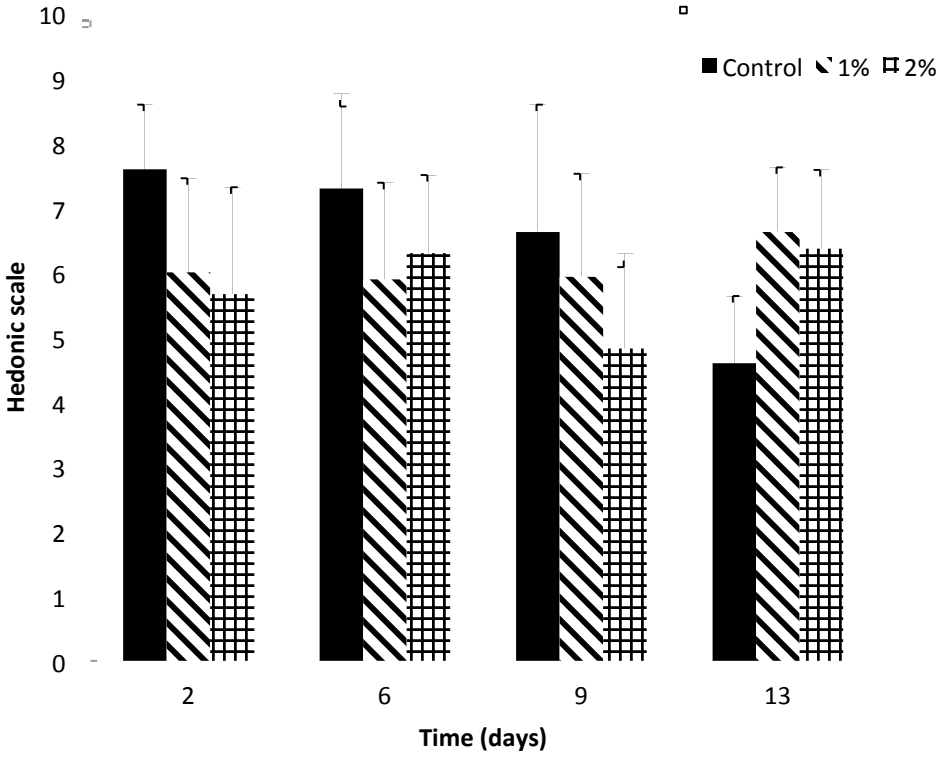

Juice leakage
Control
0.50%
1%
2%
11
9
7
5
3
1
-1
Juice leakage [%]
1
3
7
12
15
Time (days)

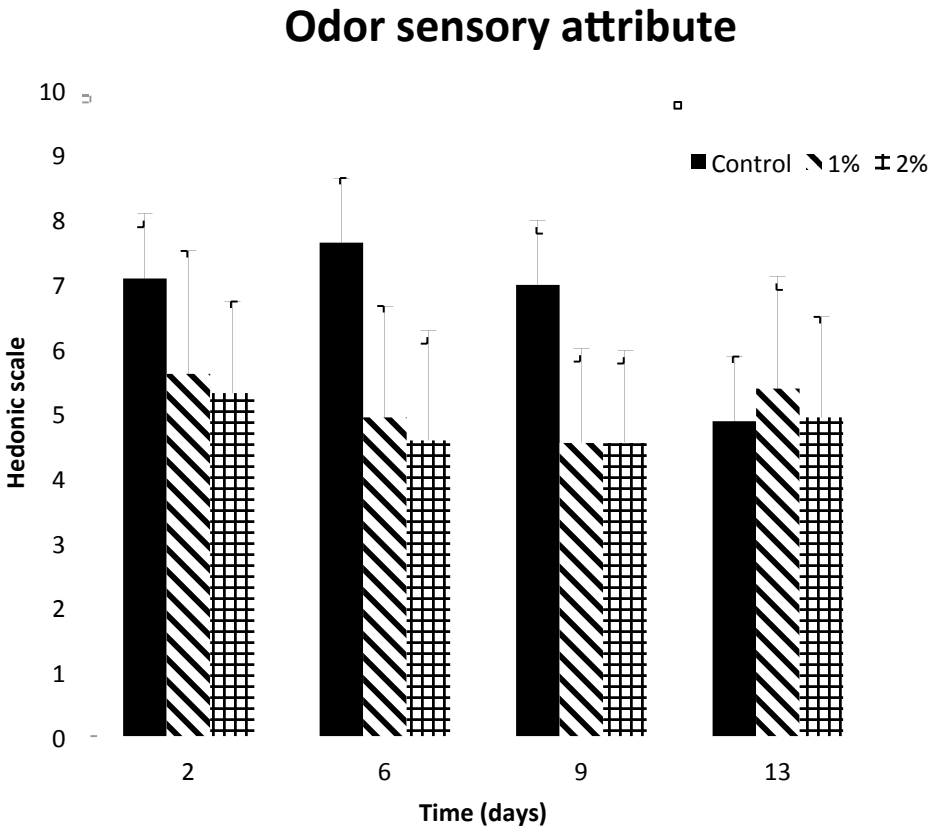

Moisture content
Control
0.50%
1%
2%
0.97
0.95
0.93
0.91
0.89
0.87
0.85
0.83
0.81
0.79
0.77
Moisture content [wb]
1
3
7
12
15
Time (days)

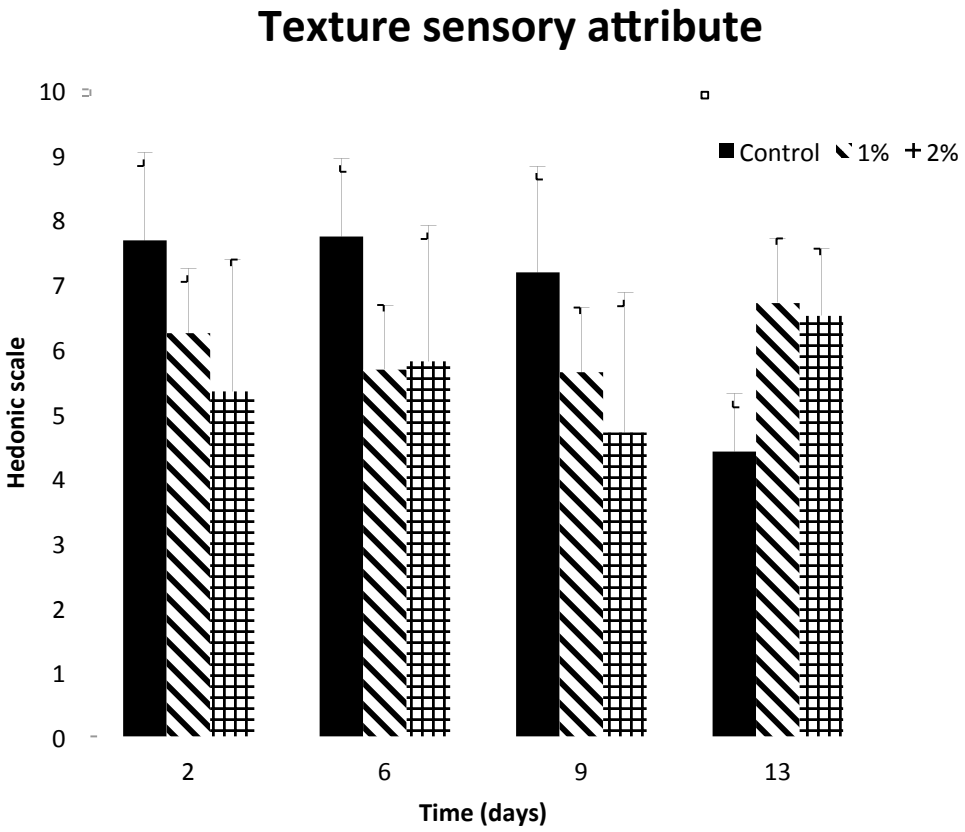

pH
control
0.50%
1%
2%
5.5
4.5
3.5
2.5
1.5
0.5
-0.5
pH values
1
3
7
12
15
Time (days)

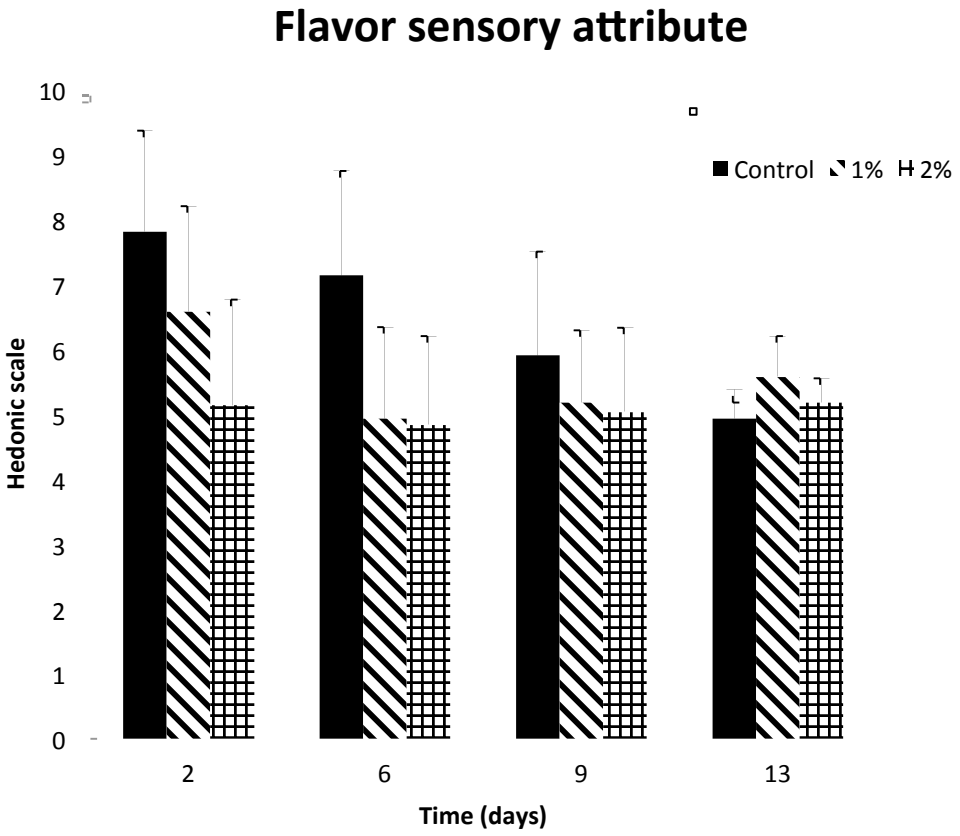

Firmness
Control
0.50%
1%
2%
Maximum force [N]
50
45
40
35
30
25
20
15
10
5
0
1
3
7
12
15
Time (days)

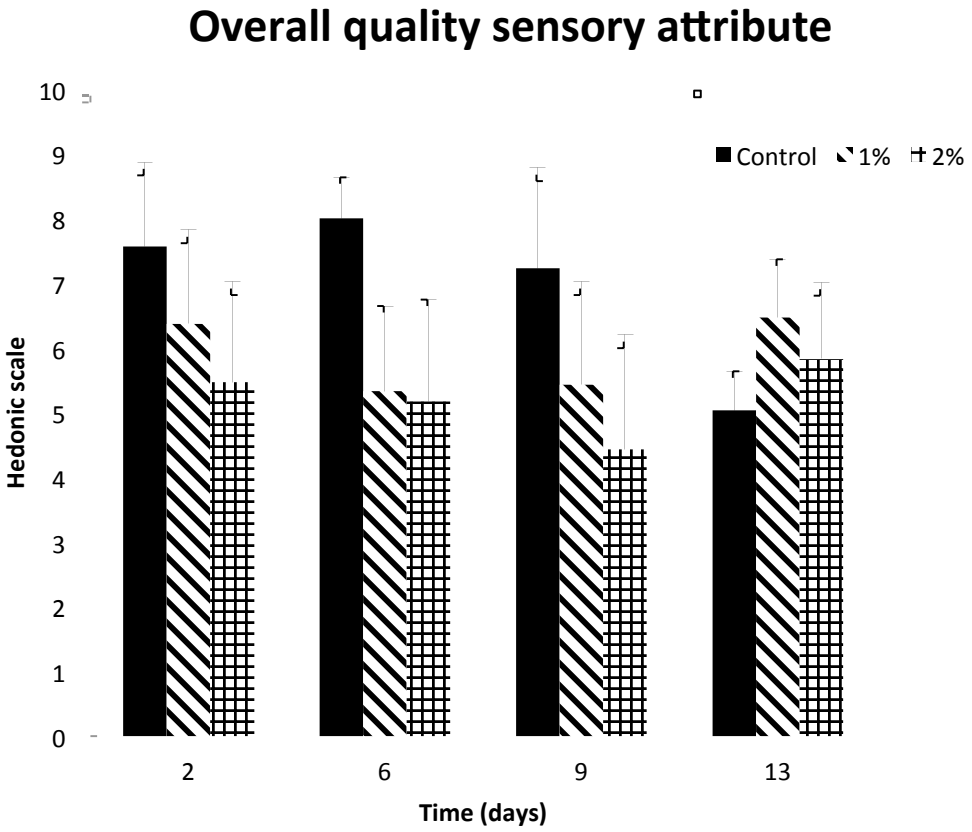

Vitamin C
Ascorbic acid conc. [mg asc.acid/g]
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
1
3
7
12
15
Time (days)
Control
0.50%
1%
2%

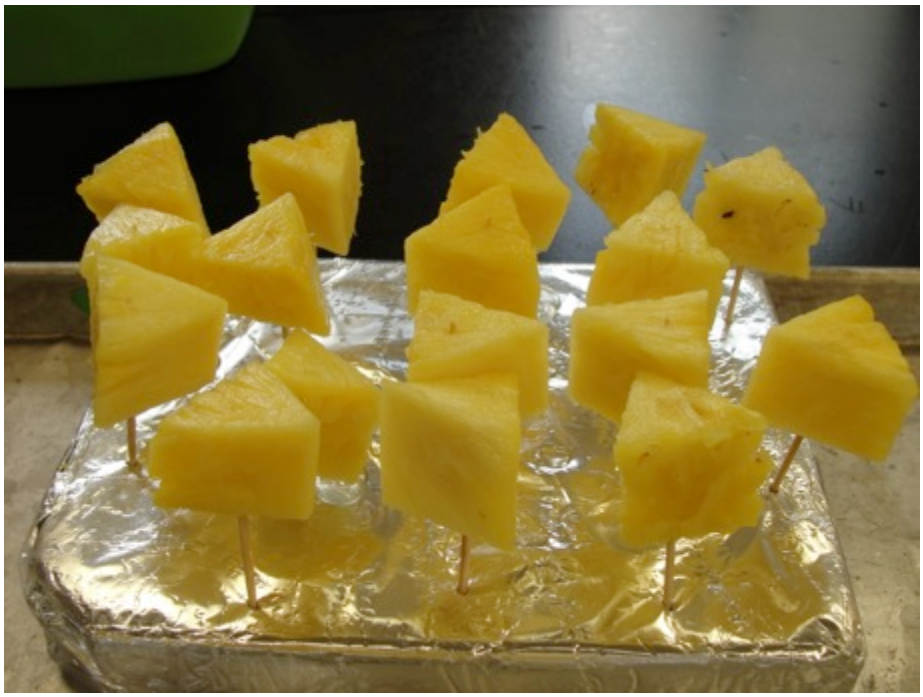

Color sensory attribute
Control
1%
2%
Hedonic scale
10
9
8
7
6
5
4
3
2
1
0
2
6
9
13
Time (days)


Odor sensory attribute
Control
1%
2%
Hedonic scale
10
9
8
7
6
5
4
3
2
1
0
2
6
9
13
Time (days)

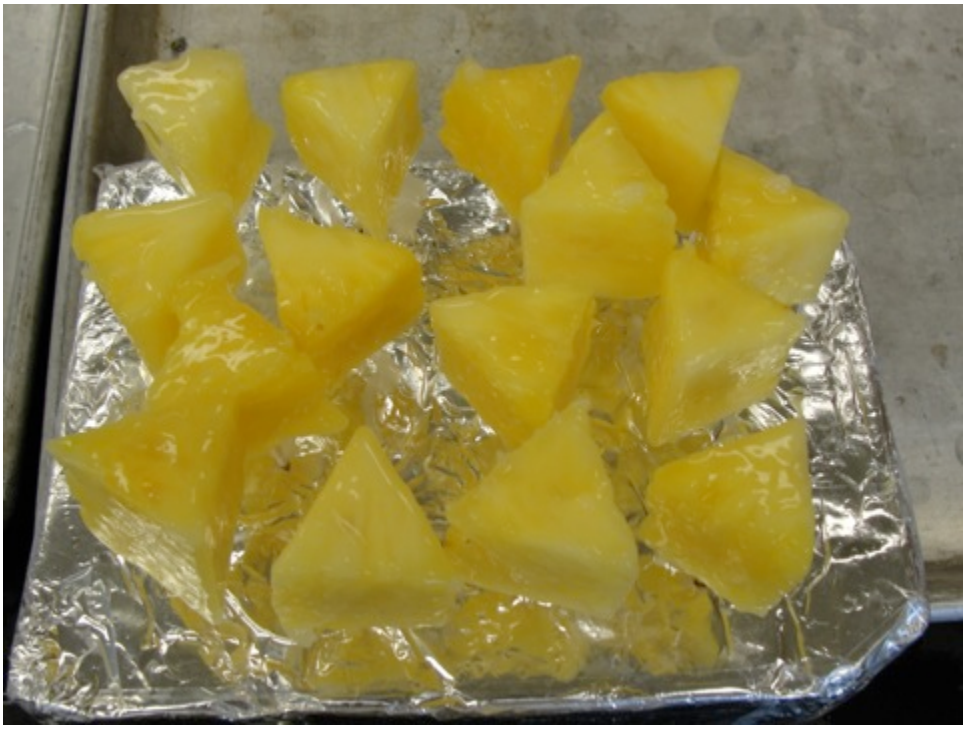

Texture sensory attribute
Control
1%
2%
Hedonic scale
10
9
8
7
6
5
4
3
2
1
0
2
6
9
13
Time (days)


Flavor sensory attribute
Control
1%
2%
Hedonic scale
10
9
8
7
6
5
4
3
2
1
0
2
6
9
13
Time (days)


Overall quality sensory attribute
Control
1%
2%
Hedonic scale
10
9
8
7
6
5
4
3
2
1
0
2
6
9
13
Time (days)

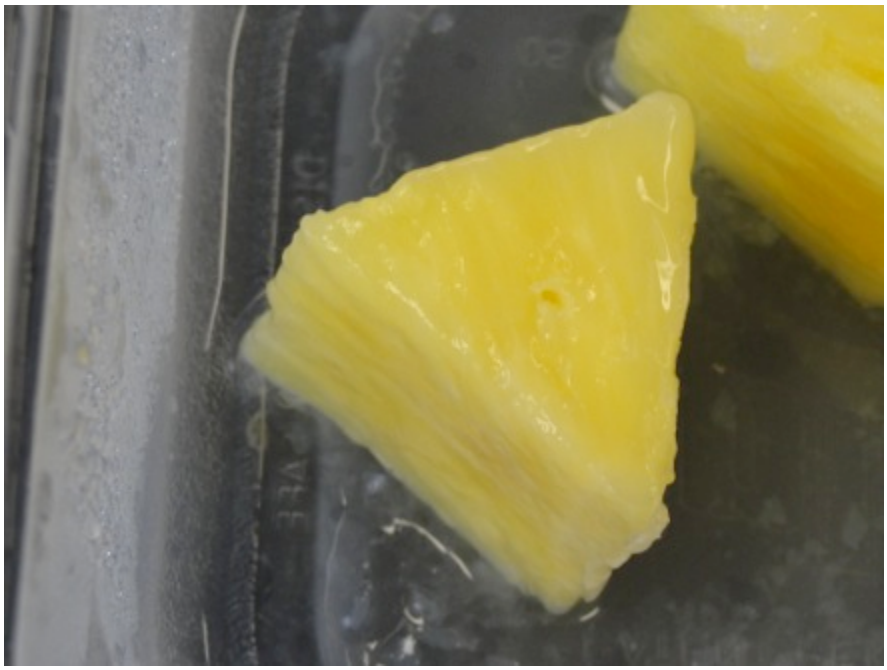


Day 0. Control samples.



Day 0. 0.5% sodium alginate coated samples.

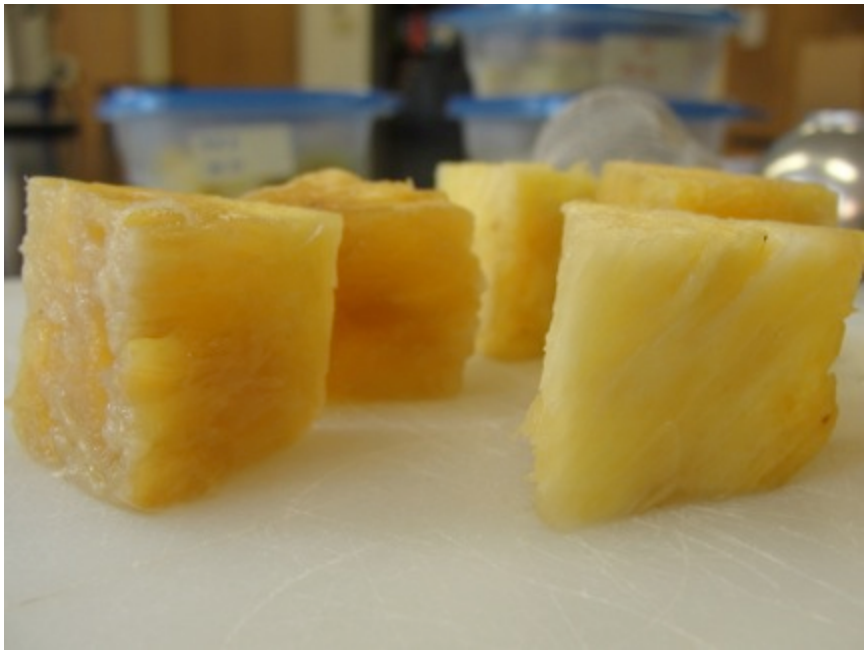
Day 3. Control samples.



Day 3. 0.5% sodium alginate coated samples.

Day 3. 1% sodium alginate coated samples.

Day 3. 2% sodium alginate coated samples.

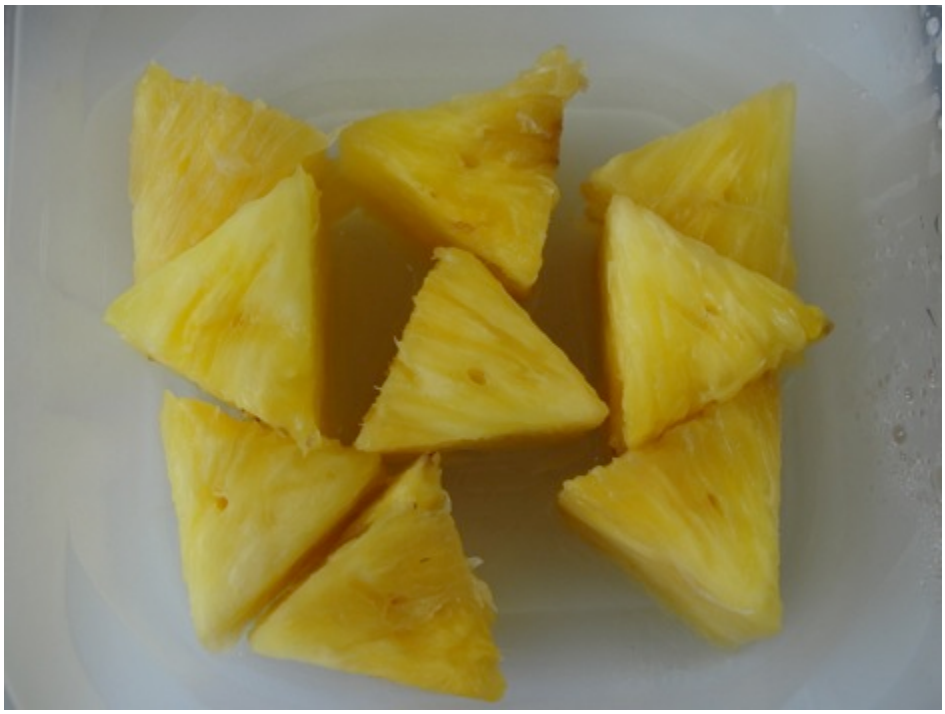


Day 12. Control samples.

Day 12. 0.5% sodium alginate coated samples.

Day 12. 1% sodium alginate coated samples.

Day 12. 2% sodium alginate coated samples.



Day 15. Control samples.

Day 15. 0.5% sodium alginate coated samples.

Day 15. 1% sodium alginate coated samples.

Day 15. 2% sodium alginate coated samples.

VITA

Natalia Vanessa Mantilla received her Bachelor of Science degree in agribusiness engineering from San Francisco de Quito University, Ecuador in 2009. In 2010, she joined the graduate program at Texas A&M University in the Food Science and Technology Department to pursue her Master of Science degree.

Her permanent address is:

Natalia Vanessa Mantilla

Texas A&M University

Biological & Agricultural Engineering Department

2117 TAMU

College Station, TX – 77843-2117

email: nvmanti16@tamu.edu