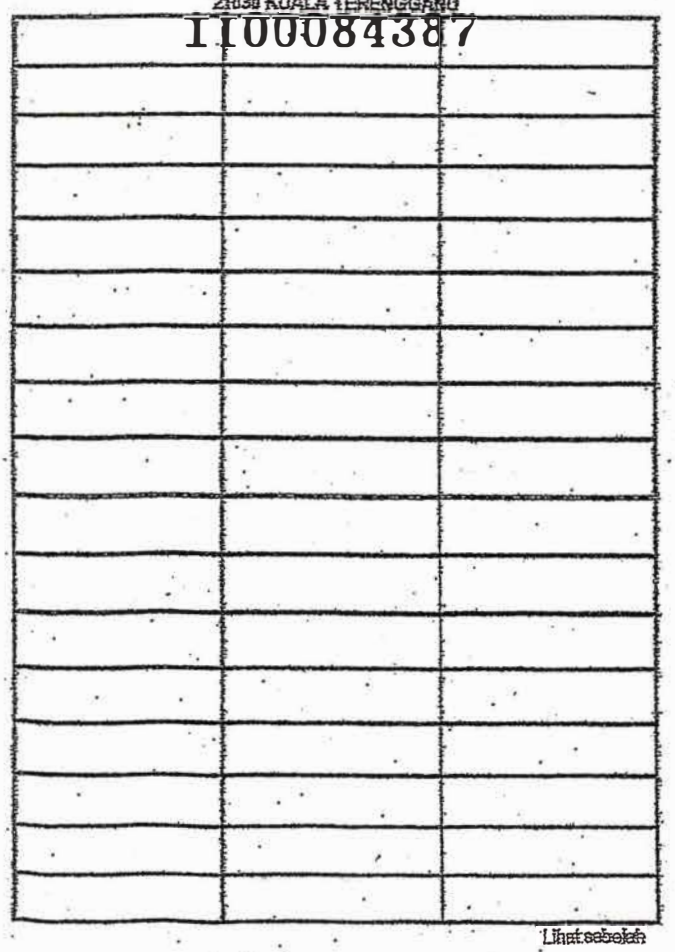
EFFECT OF [illegible] COMBINED WITH VANILLIN ON POST-HARVEST QUALITY OF GUAVA FRUIT (*Psidium guajava* L.)

DOREEN YONG SHENG YUEN

FACULTY OF AGROTECHNOLOGY AND FOOD SCIENCE
UNIVERSITI MALAYSIA TERENGGANU

2010

Effect of sodium alginate combined with vanillin on postharvest quality of guava fruit / Doreen Yong Sheng Yuen.



EFFECT OF SODIUM ALGINATE COMBINED WITH VANILLIN ON POSTHARVEST QUALITY OF GUAVA FRUIT (*Psidium guajava* L.)

By
Doreen Yong Sheng Yuen

Research Report submitted in partial fulfilment of
the requirements for the degree of
Bachelor of Science in Agrotechnology (Post Harvest Technology)

Department of Agrotechnology
FACULTY OF AGROTECHNOLOGY AND FOOD SCIENCE
UNIVERSITI MALAYSIA TERENGGANU
2010

ENDORSEMENT

The project report entitled **Effect of sodium alginate combined with vanillin on postharvest quality of guava fruit (*Psidium guajava* L.)** by **Doreen Yong Sheng Yuen**, Matric No. **UK 15689** has been reviewed and corrections have been made according to the the recommendations by examiners. This report is submitted to the Department of Agrotechnology in partial fulfilment of the requirements for the degree of Bachelor of Science in Agrotechnology (Post Harvest Technology), Faculty of Agrotechnology and Food Science, Universiti Malaysia Terengganu.

.....

(DR. ADZEMI MAT ARSHAD)
Main supervisor

DR. ADZEMI MAT ARSHAD
Ketua
Jabatan Agroteknologi
Fakulti Agroteknologi dan Sains Makanan
Universiti Malaysia Terengganu
21030 Kuala Terengganu.

Date:

.....

(DR. CHUAH TSE SENG)
Co-supervisor

Date:

DECLARATION

I hereby declare that the work in this thesis is my own except for quotations and summaries which have been duly acknowledge.

Signature : 

Name : DOREEN YONG SHENG YUEN

Matric No : UK 15689

Date : 28 MARCH 2009

ACKNOWLEDGEMENT

First of all, I would like to express my endless gratitude to my final year project supervisor, Dr Adzemi Mat Arshad for his guidance and supervision throughout the whole process of performing and completion of my final year project and my appreciation to Dr. Chuah Tse Seng as co-supervisor for his suggestion and advice especially in the manuscript writings. Sincere thanks to Pn. Maizatul, Pn Rafidah, En. Shahrul, En. Fauzi, En. Ruzairi for providing assistance and facilitating throughout the conduction of this project. Lastly, I would like to thank all my coursemates for their help and moral support in accomplishing this research. Finally, thanks to all that have not been mentioned above but have contributed to the success of this project.

ABSTRACT

This study was conducted to determine combine effects of sodium alginate plus vanillin coating on shelf life of Thai seedless guava fruits when stored under ambient temperature of 28°C for twelve days. Total soluble solids, firmness of fruits, colour changes, weight loss, disease severity and disease incidence of the fruits were assessed every two days. Green chlorophyll loss, fruit softening, disease severity and disease incidence of coated fruits were significantly reduced but total soluble solids were not affected by any treatments. Only treatments 5mM vanillin combined with 2 or 4% sodium alginate were able to significantly reduced weight loss as compared to control. The result of this study suggests that 4% sodium alginate combined with 5mM vanillin is the best treatment in extending shelf life of Thai seedless guava due to its advantages over other treatments in retention of fruit firmness, reduced weight loss and ability to maintain disease-free for an extended period of two days.

ABSTRAK

Kajian ini dijalankan untuk mengkaji kesan kombinasi penyalutan natrium alginat dan vanillin ke atas jangka hayat buah jambu tidak berbiji Thai yang disimpan pada suhu bilik (28°C) selama dua belas hari. Kandungan pepejal terlarut, tekstur buah, kadar kehilangan berat , perubahan warna buah, insiden penyakit dan tahap jangkitan buah dinilai setiap dua hari. Kadar kehilangan klorofil, perlembutan buah, insiden penyakit dan tahap jangkitan bagi buah dapat dikurangkan dengan penyalutan sodium alginat dan vanillin tetapi kandungan pepejal terlarut tidak dipengaruhi oleh penyalutan. Hanya rawatan 5mM vanilin digabungkan dengan 2 atau 4% natrium alginat dapat mengurangkan kadar kehilangan berat buah berbanding dengan kawalan. Keputusan kajian ini mencadangkan bahawa kombinasi 4% sodium alginat dan 5mM vanilin merupakan rawatan yang terbaik dalam pemanjangan tempoh penyimpanan buah jambu tidak berbiji Thai kerana rawatan ini mempunyai kelebihan dalam mengekalkan keteguhan buah, mengurangkan kadar kehilangan berat buah di samping melambatkan insiden penyakit selama dua hari berbanding rawatan lain.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF SYMBOLS / ABBREVIATION

ANOVA	-	Analysis of Variance
SA	-	Sodium alginate
VNL	-	Vanillin
%	-	Percentage
g	-	Gram
mM	-	Milimolar
w/w	-	Weight per weight
w/v	-	Weight per volume
°C	-	Degree Celsius
+	-	Plus
±	-	More or less
<	-	Less than
>	-	More than
≤	-	Equal or less than

LIST OF APPENDICES

CHAPTER 1

INTRODUCTION

1.1 Background of study

Guava (*Psidium guajava* L.) is one of the most commercial fruit crops of the dicotyledon family Myrtaceae. It was originated from Central America, but due to the growing demand, today it is commercially grown in both tropical and subtropical regions. According to Jagtiani et al. (1998), India is the major world producer of guava, with an estimated production of 165,000 metric tonnes (MT) of fresh guava fruits annually.

In Malaysia, commercial guava production had begun in the mid 80s. Guavas are mainly produced for domestic use as well as export purposes. Currently, important guava growing areas of Malaysia are located in the states of Perak, Johore, Selangor and Negeri Sembilan, with Perak as the largest area for guava plantation (Kwee & Chong, 1990). The main importing countries of Malaysia's guava fruits include Singapore, Brunei, Hong Kong and Saudi Arabia with export value of RM 3 million in the year 2000 (Milan, 2008).

Besides consuming it fresh, guava can be process into many other important secondary products. In the processing line, a variety of produces such as pulp, puree, jam, cheese, toffee, ketchup, juice, beverage, squash, syrup, nectar, concentrate, jelly,

wine, dehydrated as well as canned products can be produced. The content of the processed products served as criteria for the selection of guava variety, as pulp, seeds, sugar and pectin content, acids and tannins of the fruit varies among cultivars (Adsule and Kadam, 1995).

Guava being a highly perishable fruit due to rapid ripening has a relatively short postharvest life of a few days under ambient temperature (Singh and Pal, 2008b). Besides restricted shelf-life of guavas, high susceptibility to mechanical damage and post-harvest disease also greatly limits the commercialization of the fruit. Therefore new post-harvest handlings techniques were continuously developed and adopted to reduce post-harvest loses of the guava fruits. In Malaysia, common post-harvest techniques applied include low temperature storage and application of fungicides.

Recently, edible coating is increasingly used as coating material for fresh or minimally processed commodities due to its potential to form a semi-permeable barrier to gasses, retard water loss and suppress physiological order. This causes a modified atmosphere to be generated on coated fruits, thus extending the shelf life of commodities. Alginate, a polysaccharide derived from various species of brown seaweeds (*Phaeophyceae*) is increasingly used as an edible film that serves as a carrier for various food additives including antibrowning agents, colorants, flavors, nutrients, spices and various antimicrobials.

1.2 Problem statement

In Malaysia, common post-harvest techniques applied include low temperature storage and application of fungicides. Although low temperature storage is effective to delay ripening and fungal deterioration, tropical fruits such as guavas are very susceptible to chilling injury. Chilling injury phenomena was reported to occur when fruits are stored below 10 °C, causing severe symptoms such as skin and flesh browning or discolouration, surface pitting (González-Aguilar et al., 2004), abnormal ripening, and increased incidence of decay, particularly anthracnose which is caused by *Colletrotrichum gloeosporiodes* (Lim and Khoo, 1990). As for fungicides, trifloxystrobin, tebuconazole and cyprodinil along with fludioxonil were widely used to control the incidence of post-harvest disease. Though effective, it raises health concerns among consumers.

1.3 Significance of study

Vanillin, a phenolic compounds derived from vanilla beans having earned the generally recognized as safe (GRAS) status is extensively used in food industry as its organoleptic feature is well accepted by consumers. Extensive studies have shown that vanillin are capable of exhibiting strong antimicrobial properties (López-Malo et al., 1995, Cerrutti and Alzamora, 1996, López-Malo et al., 1997, Fitzgerald et al., 2003, Moon et al.,2006). Hence it is increasingly used as a food additive to enhance antimicrobial property of food (Lopez-Malo et al., 1995, Cerrutti and Alzamora, 1996, Ngarmsak, 2007). With rising concerns on safety level of food being consumed,

incorporation of such antimicrobial properties into edible coating can serve as an alternative to extend shelf life of fresh fruits.

The retailing of guava fruit can be carried out without refrigeration and therefore, the preservation of fruit at room temperature is highly desirable. Increased shelf-life period could help long-distance transportation and improve its commercialization. Besides, rising concerns of widely used conventional fungicides on postharvest commodities has also given an impetus for the development of edible coatings as carriers of preservatives and antimicrobial properties. Therefore, this study is necessary to evaluate the quality and shelf life of guavas coated with different combinations of alginate and vanillin under ambient temperature.

1.4 Objective

The objective of this project was to determine the suitable concentration of sodium alginate combined with vanillin on extending shelf life of guava fruit (*Psidium guajava* L.).

CHAPTER 2

LITERATURE REVIEW

2.1 Guava (*Psidium guajava* L.)

2.1.1 Botany and chemical composition

Guava (*Psidium guajava* L.) is a tropical fruit that consist of a fleshy pericarp and seed cavity with fleshy pulp and numerous small seeds within it. The seeds are kidney shaped and hard. For seedless varieties, they have a sandy granular pulp containing very few seeds. These fruits have a distinctive sweet aromatic flavor and emit a strong musky smell when overripe. In Malaysia, the skin colours of commonly planted varieties were mainly green in colour, but some are yellow at the point of maturity stage. Fruits are normally harvested before full maturity, which has skin colour of slight dark green that gradually turns to pale yellow-green color as it ripen. The flesh can be white, yellowish, pink or red, depending upon the variety.

Guava fruits are mainly composed of carbohydrates with sugar constituting about 6-11% of fresh weight of guava (Chan et al., 1974). Guava fruits are not only rich in pectin but also have a good source of vitamin C. It has a relatively high dietary antioxidant value as the fruit contains both major classes of antioxidant pigments, which are carotenoids and polyphenols (Mahattanatawee et al., 2006, Hassimotto et al., 2005 and Jiménez-Escrig et al., 2001).

In Malaysia, seedless varieties that were commercially planted are "Thai seedless" and "Bangkok Apple". Thai seedless clones possessed a round but asymmetrical fruit shape. It has a green skin that is smooth and less thick compared to seed varieties. The flesh of the fruit is white, sweet and crunchy. Within the fruit, there is a hollow cavity that can reach one third the length of the fruit. The fruits weigh between 150-300 g. Due to the high percentage of flowers and fruit drop (more than 50%), Thai seedless have a very low yield of guavas of 20-25 t/ha/year (Milan, 2008). With the increasing high demand and low supply, seedless varieties are generally sold at a higher price compared to seed varieties.

2.1.2 Post-harvest storage

Guava is a climacteric fruit (Akamine & Goo, 1979, Brown and Wills, 1983, Bashir and Abu-Goukh, 2003) which exhibits respiratory and ethylene peaks during ripening. However there are reports of some cultivars which behaved in a non-climacteric fruit manner (Biale and Barcus, 1970 and Azzolini et al., 2005). The nature of guava is thus suspected to vary among cultivars (Azzolini et al., 2005).

Guava has a relatively short shelf life of only 2-3 days at room temperature. For guavas with a climacteric nature, an ethylene and carbon dioxide production peak occurs after 5-6 days after harvest resulting in rapid ripening. Due to the highly perishable nature of guavas, low temperature storage is commonly used to prolong storage life of guavas.

Low temperature storage has been proven capable in delaying ripening and suppressing fungal development (Reyes and Paull, 1995). However, most tropical

fruits, such as guavas, are very susceptible to chilling injury. Little is known with respect to the sensitivity and the responses of guava fruits toward low temperature. But it is proposed that the critical temperature which induces chilling injury varied among species. This is evident as there are several dissimilarities between the studies on the exact temperature for chilling injury induction. According to Adsule and Kadam (1995), 5°C is considered the optimum storage temperature of guavas as chilling injury only occurred at 0°C. On the other hand, González-Aguilar et al. (2004) have reported that guavas stored under temperature below 10°C had manifested chilling injury symptoms.

In responds to this problem, methyl jasmonate (MJ) treatment on guavas has been proposed to reduce the chilling injury caused during low temperature storage of 8°C. The effects of methyl jasmonate treatment on guavas under storage temperature of 5°C were assessed by González-Aguilar et al. (2004). Methyl jasmonate was reported to be able to decrease deterioration through the improvement of fruit defence mechanism towards chilling injury. Improved fruit defence respond was evaluated through the increase of sugar content and lipoxygenase (LOX) and phenylalanine-ammonia lyase (PAL) activities. Methyl jasmonate treatment was also reported to show no effects on the degradation of vitamin C, chlorophyll and total phenols, implying that MJ can be used as a solution towards chilling injury occurrence.

Besides low temperature storage, the use of 1-methycyclopropene (MCP) had been studied on its ability to extend guava storage life. Concentration of 1-MCP ranging between 300 nLL^{-1} and 900 nLL^{-1} under a range of exposure length of 3 hours to 24 hours were studied (Bassetto et al., 2005, Singh and Pal, 2008a). Best results were shown in guavas treated with 300nLL^{-1} for 12 hours for both Pedro Sato and

Allahabad Safeda guavas (Bassetto et al., 2005, Singh and Pal, 2008). High concentration treatment of 1-MCP (900 nl l^{-1}) under long hours of exposure (6 hr and 9 hrs) were reported to cause guava fruits unable to ripen (Bassetto et al., 2005). According to Singh and Pal (2008a), a combination of 1-methycyclopropene (MCP) and low storage temperature (10°C) were able to further extend the shelf life of guavas.

Controlled atmosphere which alters the atmosphere with elevated carbon dioxide and reduced oxygen levels is also one of the alternatives to increase storage life of guava fruits. In the studies of Singh and Pal (2008b), the effects of controlled atmospheres (CA) on respiration, ethylene production, firmness, weight loss, quality, chilling injury, and decay incidence of three guava cultivars were studied during storage in atmospheres containing 2.5, 5, 8, and 10 kPa O_2 with 2.5, 5, and 10 kPa CO_2 at 8 °C. It is reported, while, climacteric event can be delayed with greater suppressing effect of increasingly low oxygen level (≤5 kPa), an over elevated carbon dioxide of more than 5 kPa can result in ascorbic acid degradation. Atmosphere with oxygen level which is too low (2.5kPa) is also reported to reduce organoleptic qualities of the fruit as it encourages the build up of fermentative metabolites. The optimal atmosphere to improve storage quality of guavas varies among cultivars. Controlled atmosphere is proven to be successful in reducing weight loss and fruit firmness while retarding the biochemical changes of the fruit.

Using the latest technology to improve storage life of guavas, Singh and Pal (2009) investigated on the potential of ionizing radiation to improve physiological responses, quality, and storage time of fresh guavas. Although ionizing radiation treatment on guavas with 0.25 kGy dose was able to maintain fruit quality, reduce decay incidence and extend storage life of guava fruits by 3 to 4 days, it is reported that the positive

effects of ionizing radiation treatment were not long lasting, limiting to only 22 days under cold storage of 10°C. Hence a combination of low temperature storage and ionizing radiation treatment were unable to produce a synergistic effect in prolonging shelf life and quality of guava fruits.

2.2 Edible Coating

Edible coating is increasingly used as coating material for fresh or minimally processed commodities due to its edibility, biocompatibility, aesthetic appearance, barrier properties, non-toxic and low cost. It has the potential to form a semi-permeable barrier to gasses, retard water loss and suppress physiological order. This causes a modified atmosphere to be generated on each coated fruit, which is able to extend the shelf life of commodities.

Edible films are mainly derived from natural monomers such as protein or polysaccharide due to their publicly recognized environmental compatibility and biodegradable properties. Despite possessing good mechanical strength, their hydrophilic nature makes them less effective in providing moisture barrier. As a result, many studies were carried out to improve film properties through the incorporation of hydrophobic components such as lipid (McHugh and Krochta, 1994 and Yang and Paulson, 2000), blending with less hydrophilic polymer (Wang et al., 2003 and Dong et al., 2006) and other methods including chemical modification and the use of various plasticizer in films (Tong et al., 2008).

Edible films also serves as a carrier for various food additives including antibrowning agents, colorants, flavors, nutrients, spices and various antimicrobials

that can extend product shelf-life and reduce the risk of pathogen growth on food surfaces (Rojas-Graü et al., 2007). The incorporation of antimicrobial compounds into edible coatings provides a novel way to improve the safety and shelf-life of ready-to-eat foods (Cagri et al., 2004).

2.2.1 Alginate

Alginate is a polysaccharide derived from various species of brown seaweeds (*Phaeophyceae*). It appears in forms of flavourless gum when extracted from the cell walls of brown algae. Alginate has several unique colloidal properties, which include thickening, stabilizing, suspending, film forming, gel producing, and emulsion stabilizing (Rhim, 2004). Also, due to alginate's biocompatibility and simple gelation with divalent cations such as Ca^{2+}, strong and insoluble polymers can be produced. This contributes to the food processing industry for producing restructured foods and biotechnology industry for producing beads for immobilization of cells or enzymes (Rhim, 2004).

Alginate is increasingly of interest as a potential coating component because it is the only polysaccharide, which possesses various abilities for functional materials (Rhim, 2004). However, most hydrophilic films (polysaccharide or protein based films) have good mechanical properties and are excellent gas, aroma and lipid barriers but poor moisture barriers which are not efficient in reducing weight loss of coated produce. Hence, plasticizers like glycerol are required for polysaccharide and protein-based edible films to bind water and also generally increase film permeability to oxygen (McHugh and Krochta, 1994a and McHugh and Krochta, 1994b). The

incorporation of lipids, either in an emulsion or as a layer coating into the films formulations, greatly improves their water vapour barrier properties (García et al., 2000, Yang & Paulson, 2000).

Several studies have been carried out using alginate and other polysaccharide-based edible coating to maintain quality of fruits. In the studies of Maftoonazad et al. (2006), sodium alginate and methyl cellulose edible coatings which was coated on peaches were successful in extending its shelf life for six and nine days respectively as compared to control under refrigeration storage of 15°C. The coating was reported to act as a physical barrier for the gaseous exchange between the fruit and the environment, retarding the respiration rate and reduces moisture loss as well as changes in quality parameter.

In another study by Rojas-Grau et al. (2008), the application of alginate and gellan edible coatings on fresh-cut apples were investigated. The study was found to successfully retard the microbiological deterioration, surface browning and tissue softening of the wedges. It effectively prolonged the fresh-cut apples shelf-life by two weeks of storage compared to control which has a shelf life less than four days.

In an earlier study of Henriette et al. (2003), alginate had been used in a combined method technology to extend shelf life of minimally processed mango cubes by reduction of colour and moisture loss as well as preserving its' flavour intensity. However, the results were less desirable as fungus development on the alginate-coated mango cubes were reported to increase with storage time despite remaining in the region of acceptability. The alginate coated mango cubes also fail to reduce moisture lost and retain fruit color.

In one of the latest studies, the effects of calcium alginate coating on the shelf life of strawberry under refrigeration condition of 5°C was investigated by Moayednia et al. (2009). Coated strawberries were reported to enhance fruit preservation and protection against fungal infection. However the calcium alginate coatings were not able to significantly reduce weight loss or improve the physicochemical properties of the coated strawberries.

Other than that, the effect of alginate serving as a carrier for antimicrobial agents in prolonging shelf life of processed food was also investigated. Rojas-Grau et al. (2007) has incorporated essential oil into apple puree-alginate coating in his studies on extending the shelf life of fresh-cut apples. All antimicrobial coatings were found to significantly inhibit growth of fungi, while lemongrass at concentration 1.0 and 1.5% w/w and oregano oil at concentration 0.5% w/w are found to exhibit the strongest antimicrobial activity against *Listeria innocua*.

One of the recent concepts being proposed was the incorporation of microorganism into edible films being applied on fresh fruits to increase its storability period. A study on the preservation of fresh strawberries quality using bio-films, developed through the incorporation of *Cryptococcus laurentii* into sodium alginate film was carried out by Fan et al. (2009). A positive result of reduced weight loss, microbiological decay incidence and maintained texture of strawberries were reported.

2.3 Vanillin

Vanillin (4-hydroxy-3-methoxybenzaldehyde) is the major constituent of vanilla beans which contributes to the principal flavor and aroma compound in vanilla. It is produced naturally via a multi-step curing process, however due to the scarcity and expense of natural vanilla extract, 90% of vanillin currently in use is synthetically produced from lignin, eugenol or guaiacol (Hocking, 1997, Roa and Ravishankar, 2000).

Vanillin has been generally recognized as safe (GRAS) status and is used widely as a flavouring compound in food industries such as ice cream, chocolate, confectionaries and desserts etc. (Hocking, 1997, and Roa and Ravishankar, 2000) and as an aroma compound in fragrance industries such as perfumes, livestock fodder and cleaning products. Synthetic vanillin is also used as an intermediate in the chemical and pharmaceutical industries for the synthesis of herbicides, antifoaming agents and drugs (Walton et al. 2003).

Many natural compounds, including phenolic compounds derived from plants, exhibit strong antimicrobial properties. However, only a limited number are applied to food products as food preservatives (Fitzgerald, 2003). Vanillin is one of the low-molecular weight phenolic compounds that has been used extensively in food industry as its' organoleptic feature is well accepted by consumers (Hocking, 1997, Ramachandra Roa and Ravishankar, 2000).

With a futuristic view of commercially using vanillin as a natural preservative, it is important to understand how vanillin functions as an antimicrobial compound. The mode of action of most of the antimicrobials can be classed into any of the three

groups; cell membrane reaction, inactivation of essential enzymes, or destruction or inactivation of genetic material (Davidson 1993). Phenolic antimicrobials are generally regarded as membrane active compounds due to their hydrophobicity in nature (Davidson, 1993, Davidson and Naidu, 2000). They primarily target the cytoplasmic membrane. Interactions between both lipids and membrane embedded proteins with the phenolic compound results in the destabilizing of the membrane and loss of integrity (Sikkema et al., 1994, Sikkema et al., 1995, Weber and de Bont, 1996).

The destabilizing effect of vanillin on the membrane is at a sublethal level for most of the microbial population. This provides an explanation for the inhibitory action of vanillin at minimum inhibitory concentration to be bacteriostatic compared to more potent phenolic antimicrobials such as carvacrol and thymol that are bactericidal (Ultee et al. 1998, Friedmanet al. 2002).

Extensive studies have demonstrated that vanillin exhibits strong antimicrobial activities against a number of yeast, moulds strains in laboratory media (Fitzgerald et al., 2003), fruit-based agar systems fruit purees and fruit juices (Cerrutti and Alzamora, 1996, López-Malo et al., 1995, Moon et al.,2006, López-Malo et al., 1997). Besides, vanillin was effectively used as preservative against yeast spoilage in fruit juice and soft drinks which contained low lipid and protein content (Fitzgerald et al., 2004).

The antimicrobial activity of vanillin depends on the duration of exposure, concentration (Cerrutti and Alzamora, 1996, Ngarmsak, 2007), the pH of the food (Sangsuwan et al., 2008b, Lopez-malo et al., 1998, Matamoros-Leon et al.,1999) and the target organism (Fitzgerald et al., 2004b, Rupasinghe et al., 2006, Sangsuwan et al., 2009).

Cerrutti and Alzamora (1996) reported that vanillin inhibited the growth of *Saccharomyces cerevisiae*, *Zygosaccharomyces bailii*, *Debaryomyces hansenii* and *Zygosaccharomyces rouxii* at a concentration of 13 mM in culture medium as well as in apple puree for 40 days. However, a high level of 20 mM vanillin was insufficient to inhibit the growth of *Z. bailii* in banana puree. According to Cerrutti and Alzamora (1996), vanillin is less effective on commodity which contains higher level of fat and protein, as these food components are known to bind and or solubilised phenolic compounds hence reducing its' antimicrobial activity.

In another study by Lopez-Malo et al. (1995), the incorporation of vanillin (3–7 mM) into fruit-based agars (apple, banana, mango, papaya and pineapple) inhibited the growth of *Aspergillus flavus*, *Aspergillus niger*, *Aspergillus ochraceus* and *Aspergillus parasiticus* for 2 months. Besides, Voda et al. (2003) also performed a study on the antifungal activities of 22 essential oil phenols, phenol ethers, and aromatic aldehydes. It is reported that vanillin at MIC value of 10mM was sufficient to inhibit wood-decaying fungi, *Trametes versicolor* and *Coniophora putean.*

Sykes and Hooper (1954) also found that phenolic compounds at acid pH values have greater antimicrobial effect. This is due to the increased solubility and stability of these phenolic compounds at low pH where they bind better to hydrophobic regions of the membrane proteins and dissolve better in the lipid phase of the membrane. When saturation of the sites of action occurs, there is gross damage and sudden collapse of the integrity of the microbial cell (Lopez-Malo, 1997).

Besides, there were also many other studies done using different concentration of vanillin as an antimicrobial property. Cerutti et al. (1997) found that strawberry puree treated with 3000mg/L vanillin and 500 mg/L ascorbic acid combined with mild heat

treatment were able to inhibit fungi growth and extend shelf life for sixty days under ambient temperature. In another study, Penny et al. (2004) were able to use vanillin concentration of 2000ppm to inhibit fungi development for yogurt for three weeks.

Furthermore, synergistic effects were observed when combination of essential oil extracts (thyme, lavender and peppermint) with vanillin was used. A marked antifungal activity for *Botrytis cinerea,* which causes gray mold rot in many agricultural products was exhibited. (Rattanapitigorn et al., 2006). While in another study, the combination of vanillin and potassium sorbate was examined by Matamoros-León et al. (1999) established that a slight reduction in pH and water activity (a_w), 3 mM vanillin in combination with 2 mM potassium sorbate could inhibit the growth of *Penicillium digitatum*, *Penicillium glabrum* and *Penicillium italicum* for 1 month.

In one of the recent studies, Ngarmsak (2007) verifies that vanillin exhibits antifungal activities against 18 fungi isolated from stored fresh-cut fruits in vitro in laboratory media with minimum inhibitory concentration (MIC) of 5.0-15.8mM depending on species. This MIC value range is in agreement with the previously reported MIC values (6–18 mM) for different microorganisms (Fitzgerald et al., 2004b, Rupasinghe et al. 2006). It is also found that concentration 80 mM, vanillin significantly delayed the growth of fungi populations in the fresh-cut fruits (Ngarmsak, 2007). Sangsuwan et al. (2008b) also found that vanillin incorporated into stretched films (methyl cellulose and chitosan) were found more effective in reducing the number of yeast in fresh-cut pineapple compared to stretched films.

CHAPTER 3

MATERIALS AND METHODS

3.1 Materials

3.1.1 Fruit

Full sized and mature light green colour fruit (Browns and Wills, 1983) of white-fleshed, Thai seedless variety guava were obtained from guava orchards in Bidor, Perak. Fruits were selected for uniformity of size, colour and freedom from blemishes.

3.1.2 Reagents

Vanillin (brand name ALDRICH) and Sodium Alginate (brand name ALDRICH) was purchased from Sigma Chemical Company. Commercial grade sodium hypochlorite and laboratory grade Calcium Chloride, $Cacl_2$ were purchased from R&M chemicals, Essex, United Kingdom while glycerol were purchased from Merck Schuchardt OHG, Hohenbrunn, Germany.

3.2 Methodology

3.2.1 Coating solution preparation

Film forming solution was prepared by dissolving sodium alginate (2 or 4% w/v) powders in distilled water and heating at 70 °C while the solution was stirred until the solution became clear. Glycerol was added as plasticizer at (3% w/w), followed by vanillin at 5mM or 15mM in the alginate solution. Calcium chloride (3% w/v) was prepared separately for the cross-linking of carbohydrate polymers.

3.2.2 Sterilization of fruits

All fruits were washed several times in running water and sterilized superficially by dipping in 2% Sodium hypochlorite for two minutes. Then, they were washed twice in distilled water, and dried on sterilized trays and air-dried.

3.2.3 Coating Treatment

The guavas were first dipped into coating solution for two minutes. Residual solutions of each polysaccharide were allowed to drip off for one minute, before submerging the coated fruits for two minutes in the solution of calcium chloride and drained. The treated fruits were dried for ten minutes to set a coat of the film on their surface. They were then stored along with control samples on trays at ambient temperature. This study comprises five treatments with three replicates. Each replicate

has three fruits. A total amount of 375 fruits were used in this study. The five treatments are listed in Table 3.1

Table 3.1 Coating treatments

Treatment	Type of coating
T_0	Control
T_1	2 % Sodium alginate + 5mM Vanillin
T_2	2 % Sodium alginate + 15mM Vanillin
T_3	4 % Sodium alginate + 5 mM Vanillin
T_4	4 % Sodium alginate + 15 mM Vanillin

3.2.4 Fruit Assessment

The maximum fruit shelf-life period was determined at the completely ripened stage or at the limit of acceptability. Fruits were considered totally ripened when their skins were completely yellow ($h° \leq 100$). The limit of acceptability was determined by fruit appearance with fruit showing visible wilting or fruit softening is considered unsuitable for consumption. Fruits were evaluated every two days for ten days for the parameter of total soluble solids, fruit firmness and fruit colour and twelve days for the parameter of decay incidence and disease severity. Only weight loss parameter was taken once, on the tenth day of storage.

3.2.4.1 Total soluble solids

The total soluble solids (Brix value) were determined by direct reading of blended juice samples in a handheld refractometer (Hand Refractometer Model 103) with results expressed in percentage (%).

3.2.4.2 Fruit firmness

Fruit firmness was determined with a texture analyzer (Stable Macro System, TA-XT Plus). Penetration was set at ten millimetre depth and three readings per fruit were carried out on the sides along the equatorial region. The fruit firmness results are expressed in force (g).

3.2.4.3 Fruit colour

Skin colour was determined with a chromameter (Minolta CR-300, Osaka, Japan). Colour measurement was done in terms of the Commision Internationale de L'Eclairage (CIE) 'Lab" colour space coordinate where 'L' measures the degree of lightness, 'a' from green to red and 'b' from blue to yellow. Hue angle is calculated using the formula: $h° = \tan^{-1}$ (b*/a*). The results were expressed as hue color angle ($h°$) for skin color. In addition to hue angle values, the total color change (ΔE) was calculated as the root mean square of the differences in individual L^*, a^* and b^* values using the formula: $\Delta E = [(\Delta L^2 + \Delta a^{*2} + \Delta b^{*2})^{1/2}]$. Skin color was evaluated by means of three readings on sides along the equatorial region of the fruit.

3.2.4.4 Weight loss

Weight loss was determined by the difference between the initial and final weights of each fruit. Initial weight of fruit was weigh at the beginning of the experiment just after coating and air-dried while final weight was collected at the end of storage. The formula below was used to calculate the percentage of weight loss:

$$\text{Percentage of weight loss} = \frac{(\text{initial weight} - \text{final weight}) \times 100\%}{\text{Initial weight}}$$

3.2.4.5 Decay incidence

Decay incidence was recorded throughout the twelve days storage period. Fruits showing surface fungus spots were considered decayed. It is expressed as a percentage of affected fruit. The formula bellow was used to calculate percentage of decay incidence:

$$\text{Percentage of affected fruit} = \frac{\text{Number of fruit with signs of decay} \times 100\%}{\text{Initial number of fruit}}$$

3.2.4.6 Disease severity

Pathogen disease severity was estimated by subjective method using a score of 0 to 6 based on percentage of fruit surface fungal (Reyes and Paull, 1995). The parameters for evaluation of disease severity are listed in Table 3.2

Table 3.2 Evaluation of disease severity

Score	Fruit surface fungal
0	0 %
1	1-10 %
2	11-30 %
3	31-60 %
4	61-90 %
5	91-99 %
6	100 %

Source: Reyes and Paull (1995)

3.2.5 Statistical Analysis

The experiment was laid out in a complete randomized design (CRD). Data were subjected to analysis of variance (one-way ANOVA), and the treatment means were compared using Tukey test at a significant level of 5%.

CHAPTER 4

RESULTS AND DISCUSSION

4.0 Effect of sodium alginate and vanillin on guava fruit

4.1 Effect on total soluble solids

Both treated fruit and non treated fruit showed an overall increasing trend of total soluble solids during ten days of ambient temperature storage (Figure 4.1). All fruit exhibited increased sugar content for the first six days of storage. This result is in line with the findings of Singh and Pal (2008b) and Bashir and Abu-Goukh (2003). This suggests that Thai seedless guava is a climacteric fruit that has typical climacteric pattern during ripening. The increase of TSS is due to the hydrolysis of starch to sugar through enzymes activities during fruit ripening. (Bashir and Abu-Goukh, 2003). The sugar components are mainly made up of fructose and sucrose (Bulk et al., 1997).

Total soluble solids and total sugar is expected to increase along with climacteric peak of respiration (Bashir and Abu-Goukh, 2003) and decline with storage time in overripe fruits (Le-Riche, 1951). According to Browns and Wills (1995), guavas exhibit climacteric peaks after four to six days after harvest. The continuous increase observed in total sugar after the climacteric peak is probably due to the increase activities in enzymes responsible for hydrolytic conversion of starch to

sugar and the decline of sugar breakdown through respiration (Bashir and Abu-Goukh, 2003).

For the first six days, guava fruits treated with 2% sodium alginate + 5mM vanillin, 2% sodium alginate + 15mM vanillin and 4% sodium alginate + 5mM vanillin had no significant difference in TSS values as compared to control, except on day four (Appendix A). All treated fruits successfully maintained Brix value ranging from 8 to 8.5 while untreated fruits had Brix value of 10 on day four.

Coating application indirectly creates a modified atmosphere around coated fruits, with selective gas diffusion, reduction in respiration rate can be resulted (Perez-Gago et al., 2003). However, this positive effect only lasted for one or two days, implying that the sodium alginate combined with vanillin coating were not able to significantly retard respiratory breakdown of substrate during the first six days of storage. Due to unacceptable organoleptic qualities and rot infestation of the fruit, untreated fruits were terminated on day six. No significant differences were observed between coated treatments.

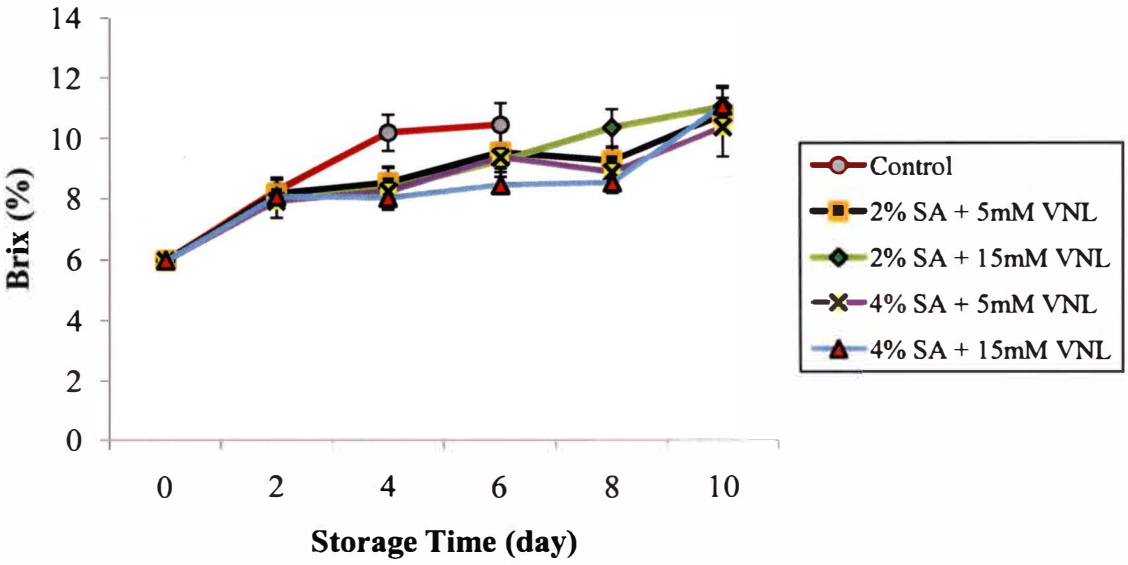


Figure 4.1: Total soluble solids of guava (*Psidium guajava* L.) throughout ten days of storage under ambient temperature after coated with combination of sodium alginate (SA) and vanillin (VNL) of various concentrations.

4.2 Effect on firmness

Firmness values of treated and non treated guava declines over storage time of ten days under ambient temperature (Figure 4.2). All treated fruit were able to delay fruit softening beginning on day four for six days as compared to untreated fruit. According to Reyes and Paull (1995), ethylene is the primary promoter of postharvest fruit softening which also indirectly induces the overall ripening of fruit. During climacteric peaks, a maximum value of carbon dioxide and ethylene gases are produced (Brown and Wills, 1983) and it is expected to cause a rapid decrease in fruit firmness. Fruit firmness started at the value of 2017.7g, after six day, untreated fruit exhibited a decrease in firmness to 269.4g while all treated fruits had firmness value ranging from 522.4g to 772.9g (Appendix B).

The rapid softening of fruit is proposed to be caused by the increase in pectin solubilization and disruption of the xyloglucan–cellulose microfibril networks of guava fruit moderated by increased in the activities of exo-polygalacturonase (PG), pectin methylesterase, β (1 → 4)-glucanase and β-galactosidase (Ali et al.,2004). Other factors that influence firmness include strength of cell wall, cell to cell contact and cellular turgor (Harker et al., 1997). Firmness retention of treated fruits can be explained by the retardation of activities of the pectin solubilising enzymes through low oxygen and high carbon dioxide concentrations which is created by the guava fruit coatings (Maftoonazad et al., 2007).

Figure 4.2 presents the firmness value of guava (*Psidium guajava* L.) treated with 2% sodium alginate + 5mM vanillin, 2% sodium alginate + 15mM vanillin, 4% sodium alginate + 5mM vanillin, 4% sodium alginate + 15mM vanillin and untreated fruit when stored under ambient temperature throughout ten days. Except the increase

in firmness value between day two and day four, both coated and uncoated fruits showed an overall trend of fruit softening.

Initially (beginning day zero till day two), fruit softening was attributed to the degradation of pectin and other cell wall components, such as hemicellulose, cellulose and lignin which occurred throughout ripening (Jain et al., 2003). By the end of day two, both treated and untreated fruit begin to increase abruptly in their firmness texture. According to Jain et al. (2003), the increase in firmness is due to an increased proportion of cell wall components with respect to moisture loss.

Moisture loss of fruit causes fruit to shrink in size, resulting in the compaction of tissue and cell wall components, which contributes to the increase in fruit firmness. A lesser increase in firmness of control compared to coated fruit is proposed to be due to the effect of coatings, which creates a modified atmosphere in delaying the degradation of cell wall components. In coated fruits, cell wall components degrade much slower than the rate of moisture loss, resulting in the greater increase in firmness as compared to control.

Assessment of untreated fruits was terminated after day six due to unacceptable organoleptic qualities and rot infestation. Among treatments, 4% sodium alginate + 5mM vanillin has the best effect on preventing rapid softening of fruit. Similarly, 2% sodium alginate + 5mM vanillin and 4% sodium alginate + 15mM vanillin also significantly help retain fruit firmness compared to treatment 2% sodium alginate + 15mM vanillin. Although all fruits softened during storage, softening of all treated guavas were remarkably delayed for four days as compared to untreated fruits on day six.

The results of this study shared that coating application using a combination of sodium alginate and vanillin can significantly reduce fruit softening. This agrees with the conclusion drawn by Carrilo-Lopez et al. (2000) in the studies of the effect of edible film coating on mango quality. Fruit softening which is initiated by ethylene production of fruit can be delayed by the micro-atmosphere created through coating. The other reason is the reduction of moisture loss by the film in preserving fruit firmness. (Fan et al., 2009)

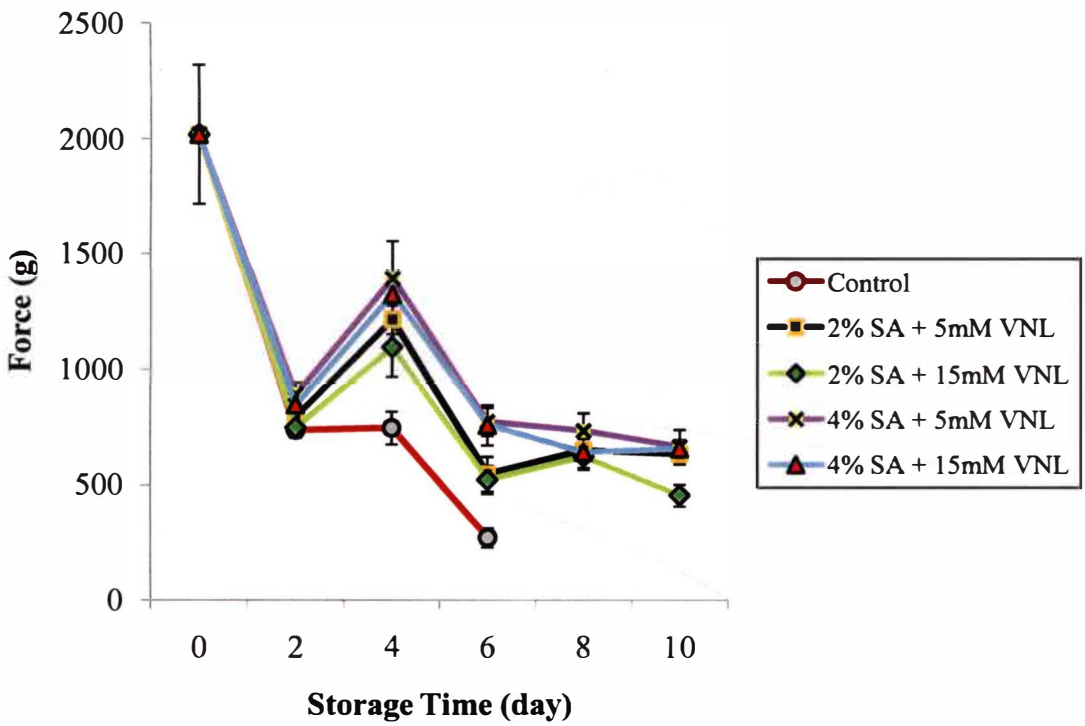


Figure 4.2: Firmness changes of guava (*Psidium guajava* L.) throughout ten days of storage under ambient temperature after coated with combination of sodium alginate (SA) and vanillin (VNL) of various concentrations.

4.3 Effect on color

Throughout ten days of storage, for all treated and untreated fruits, there were a gradual loss of green color as the fruits ripen, turning gradually yellowish. This is in agreement with the observations of Singh and Pal (2007) in air-stored guava fruits. As the fruit gradually ripens, hue angle of the guava fruit decreases. This is due to green chlorophyll pigment in the skin are gradually being degraded, unmasking the carotenoids to give a green-yellowish color to the guava fruit (Aked, 2000). Chlorophyll degradation during ripening is attributed to the increase in activities of chlorophyll degrading enzymes such as chlorophyllase, chlorophyll oxidase, and peroxidises (Jain et al., 2003). These changes in pigmentation aid the visual differentiation of fruits at various maturity stages.

The slight yellowing and browning of skin color of untreated guavas was visible on the fourth day after the beginning of the experiment compared to the treated ones. Throughout ten days of storage, all treated fruits manage to retain hue angle values that are higher than control beginning day four (Appendix C). This implies that all treatments had significantly delayed green color loss for an extended period of six days. However, further color retention might be possible if the fruits have been held for a longer time. Treated fruits were able to delay yellowing of skin color could be due to the low oxygen and high carbon dioxide concentration created by the coating, retarding the degradation rate of green chlorophyll pigment (Maftoonazad et al.,2007).

The total color change (ΔE) which uses a combination of parameter L*, a* and b* values to characterize the variation of color perception is also used to examine the color changes of guava fruits for a storage period of ten days under ambient temperature. Except guavas treated with 2% sodium alginate + 15mM have some

fluctuations of color change values that are neglible, generally, all treated and untreated fruits have an increasing pattern in total color change (Figure 4.3b).

Though no significant difference found between treatments, the observation of lower total color change of all treated fruits compared to untreated fruits implies that coating with a combination of sodium alginate and vanillin is able to reduce color changes in guava fruits throughout storage period.

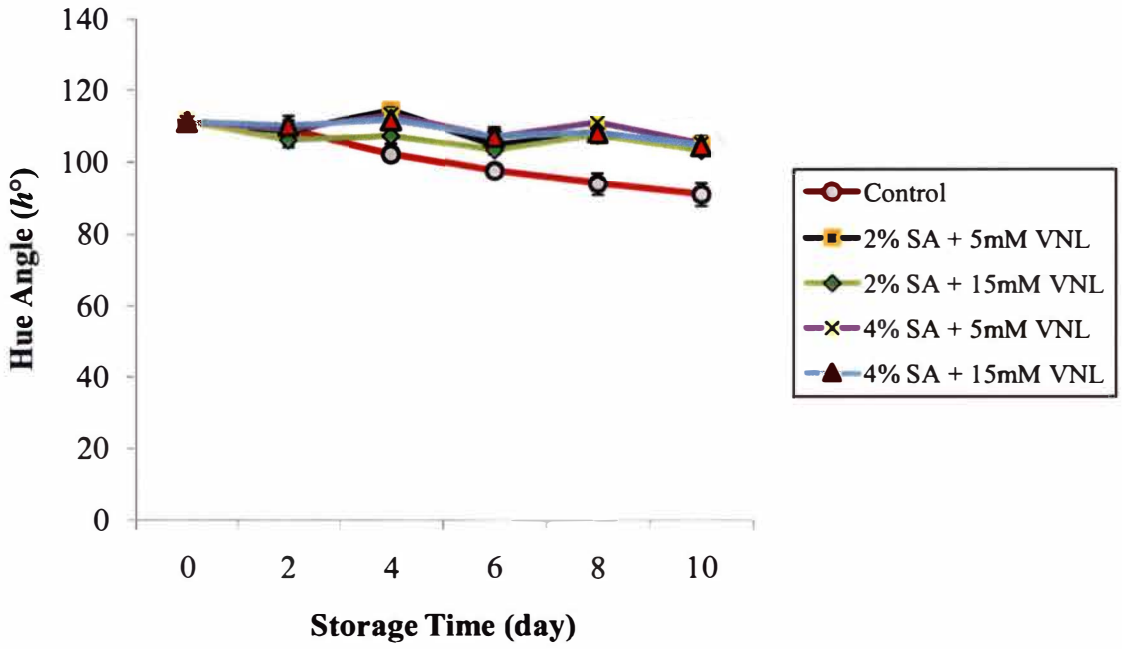


Figure 4.3a: Color change (hue angle, *h*°) of guava (*Psidium guajava* L.) throughout ten days of storage under ambient temperature after coated with combination of sodium alginate (SA) and vanillin (VNL) of various concentrations.

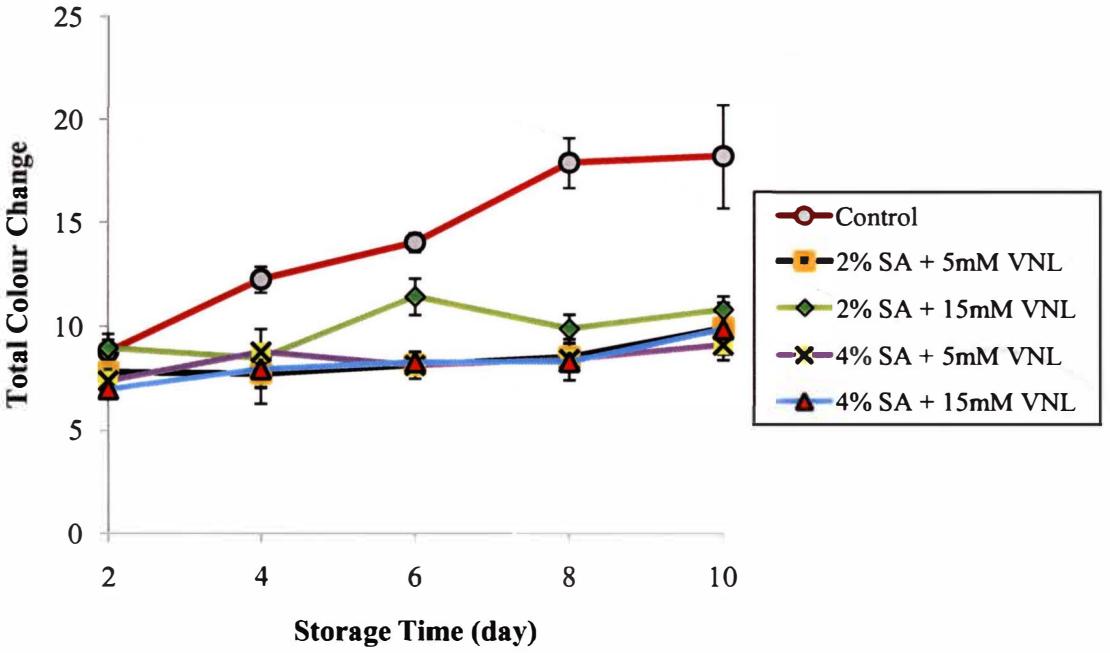


Figure 4.3b: Total color change of guava (*Psidium guajava* L.) throughout ten days of storage under ambient temperature after coated with combination of sodium alginate (SA) and vanillin (VNL) of various concentrations.

4.4 Effect on weight loss

Weight losses of fruits are generally associated with respiratory activity during fruit ripening and moisture evaporation to the atmosphere. As guava fruits are composed largely of moisture (74-87%) with little dry matter (13-26%) (Adsule and Kadam, 1995), moisture loss to the environment which results in wilting and shriveling of the fruit can greatly reduce fruit quality as well as its marketability. This is an important factor to be considered with respect to consumer acceptance.

Figure 4.7 presents the weight loss of guava (*Psidium guajava* L.) treated with 2% sodium alginate + 5mM vanillin, 2% sodium alginate + 15mM vanillin, 4% sodium alginate + 5mM vanillin, 4% sodium alginate + 15mM vanillin and untreated fruit when stored under ambient temperature after ten days. Guavas treated with 2% sodium alginate + 5mM vanillin and 4% sodium alginate + 5mM vanillin were able to significantly reduce weight loss (25.0% and 21.2% respectively) as compared to control and other treatments (29.9% to 32.3%).

In the study of Rhim (2004), sodium alginate films were proven to exhibit water resistant properties, which is in agreement to the studies of Fan et al. (2009) where sodium alginate coatings have effectively showed a reduction of moisture loss in coated strawberries compared to control. However, in contrast to the previous findings, guavas treated with 2% sodium alginate + 15mM vanillin and 4% sodium alginate + 15mM vanillin had weight loss value that is comparable to control. This implies that the effectiveness of a film in controlling weight loss is largely associated with the composition in the film.

Greater weight loss is observed as concentration of vanillin increases from 5mM to 15mM. According to Sangsuwan et al. (2008a) in their studies of the effects of vanillin and plasticizer on properties of chitosan-methyl cellulose based film, an increase in vanillin exhibits film properties of increased oxygen barrier but not water vapour barrier. Film flexibility was also reported to decrease with an increase of vanillin concentration. The large molecule of vanillin has been proposed to be the factor for the reduction of film crystallization. This implicates that by increasing vanillin concentration, water vapour permeability of the film also increases because poor film flexibility can result in microcracks to be formed in the film, resulting in poorer water barrier properties (Silva and Taylor, 2004).

Statistical analysis has shown that at the tenth day storage at ambient temperature, fruits treated with 2% sodium alginate + 5mM vanillin and 4% sodium alginate + 5mM vanillin were able to reduce weight loss of guava fruits by 30% and 17% respectively as compared to control. (Appendix E).

Although there is no significant differences between guavas treated with 2% sodium alginate + 5mM vanillin and 4% sodium alginate + 5mM vanillin, 4% sodium alginate + 5mM vanillin treatment showed better effect on moisture retention. It is proposed that this slight difference is due to the increment of sodium alginate concentration from 2% to 4%, resulting in increased water vapor barrier of the film due to reduced film porosity. With no significant difference, 4% sodium alginate + 5mM vanillin treatment showed least loss of weight (21.2%), implying it is the most effective concentration to retard moisture loss.

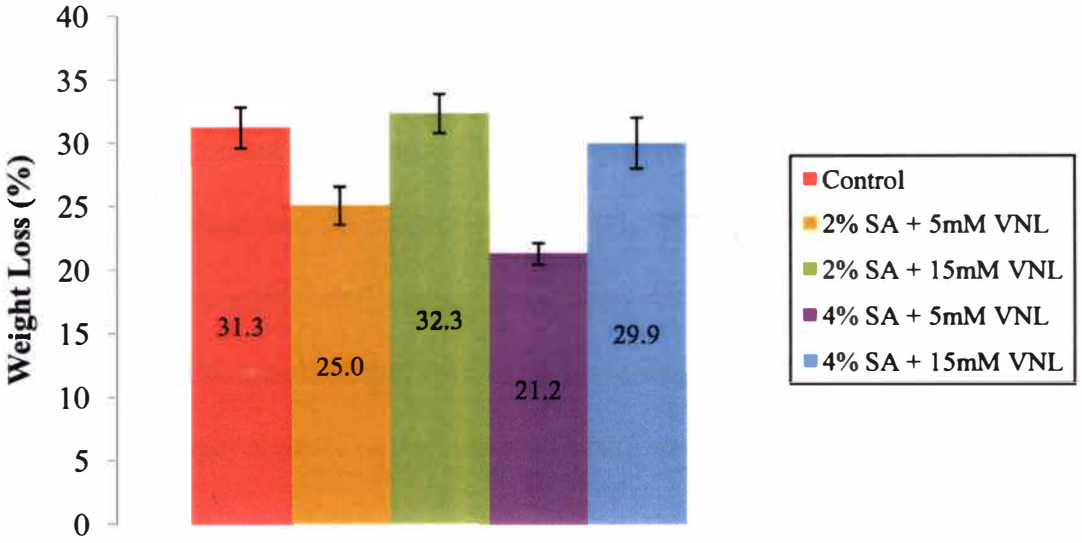


Figure 4.5: Weight loss of guava (*Psidium guajava* L.) after ten days of storage under ambient temperature after coated with combination of sodium alginate (SA) and vanillin (VNL) of various concentrations.

4.5 Effect on decay incidence and disease severity

Anthracnose and Rhizopus rot are common post-harvest disease of guavas that occurs during storage and ripening (Singh and Pal, 2008b, Reyes and Paull, 1995). All coated fruits have significantly lower decay incidence compared to untreated fruits (Appendix G). This result agrees with the previously reported work of reduced decay incidence in sodium alginate coated strawberries (Fan et al., 2009).

The early signs of fungal onset on untreated fruits appear after four days of storage under ambient temperature. Guavas treated with 4% sodium alginate + 5mM vanillin and 4% sodium alginate + 15mM vanillin were able to maintain guavas being disease-free for an extended period of four days compared to untreated fruits.

The numbers of infected fruits for guavas treated with 4% sodium alginate + 5mM vanillin and 4% sodium alginate + 15mM vanillin were comparable to guavas treated with 2% sodium alginate + 5mM vanillin and 2% sodium alginate + 15mM vanillin at day 8, but with slight lower disease severity score ($P>0.05$). This implies that the susceptibility of treated fruits towards fungal onset for all treatments were comparably high although guavas treated with 4% sodium alginate + 5mM vanillin and 4% sodium alginate + 15mM vanillin were able to maintain guavas from being disease-free for an extra of two days compared to other treatments.

The decay resistance of the treated fruits is dependent upon the ability of the coating in preventing fungal onsets. The sodium alginate properties of the film is suspected to be the main preventive agent as an increased in sodium alginate concentration showed delayed fungal onsets on fruits (two to four days compared to control). Addition of sodium alginate concentration was reported to improve film-

forming ability (Wang et al., 2007), creating a more effective layer of micro-atmosphere around the fruit in preventing the onsets of fungus.

Untreated fruits showed a marked increase of about three fold for both disease severity as well as numbers of incidence from day four till day six. The rapid increase in decay is proposed to be related to the loss of tissue integrity during ripening which increase disease susceptibility of the fruits. By the end of ten days of storage, a difference of approximately 20% or more was reduced in numbers of decay incidence by treated fruits compared to control. Assessment of untreated fruit is terminated by day ten due to severe mould development.

Figure 4.5b presents the disease severity of guava (*Psidium guajava* L.) treated with 2% sodium alginate + 5mM vanillin, 2% sodium alginate + 15mM vanillin, 4% sodium alginate + 5mM vanillin, 4% sodium alginate + 15mM vanillin and untreated fruit throughout twelve days stored under ambient temperature. The severity of fungal development for all treated fruits were significantly delayed as compared to control ($P<0.05$). By day twelve, all treated fruits showed an average severity score of 2 which can be comparable to the disease score of control at day 6, which is 2.66. This implies that the sodium alginate combined with vanillin coating had successfully slow down the growth of the fungal disease and reduce its damage on the guava fruits for an extended period of four days. Guava fruits with severity score between 2 to 3 are considered unmarketable.

Observing the results obtained, vanillin is suspected to be effective in exhibiting its antifungal property only under low concentration of sodium alginate (2%). With no significant difference, a lower decay incidence and severity was observed in guavas treated with 2% sodium alginate + 15mM as compared to 2%

sodium alginate + 5mM treated guavas throughout the first ten days of storage. This is attributed to the increase in vanillin concentration which increases the inhibitory action of vanillin. Phenolic compound such as vanillin is reported to retard microbial activity through the destabilization and integrity loss of the cytoplasmic membrane of targeted organism (Sikkema et al., 1994).

However, with an increase of sodium alginate from 2% to 4%, decay incidence was significantly delayed by two days and disease severities were found to be lower than 2% sodium alginate coated guavas. Sodium alginate coating is reported to successfully create a layer of micro-atmosphere with reduced level of oxygen and water vapour permeability (Wang et al., 2007) at the top of fruit skin. This unfavorable condition is the reason which discourages the onset of fungus and reduces disease severity of the guava fruits.

The different mode of fungal inhibition of sodium alginate and vanillin is suggested to be the cause to the result obtained. Sodium alginate inhibits fungal onset through creating an unfavorable condition for fungus to live in while vanillin inhibits fungal development through the disruption of cytoplasmic membrane of microorganism. Nonetheless, sodium alginate has a greater antifungal effect compared to vanillin. This can be noted from the significant delayed disease incidence of 4% sodium alginate coated fruits compared to 2% sodium alginate coated fruits, and the insignificant reduction in disease severity or decay incidence with increased vanillin concentration from 5mM to 15mM.

Four percent sodium alginate + 5mM vanillin is suggested to have the best effect in reducing fungal onset and development because it has the lowest disease severity as well as decay incidence among all treatments ($P>0.05$). As discussed above,

a high concentration of sodium alginate is preferable to low concentration, while higher vanillin concentration should be optimal compared to low concentration. In contrast, 4% sodium alginate + 15mM showed higher disease severity and decay incidence. This could be explained by the increase in film porosity and water vapour permeability due to high concentration of vanillin. The inhibitory action of vanillin is insignificant to control disease development despite the high concentration of vanillin.

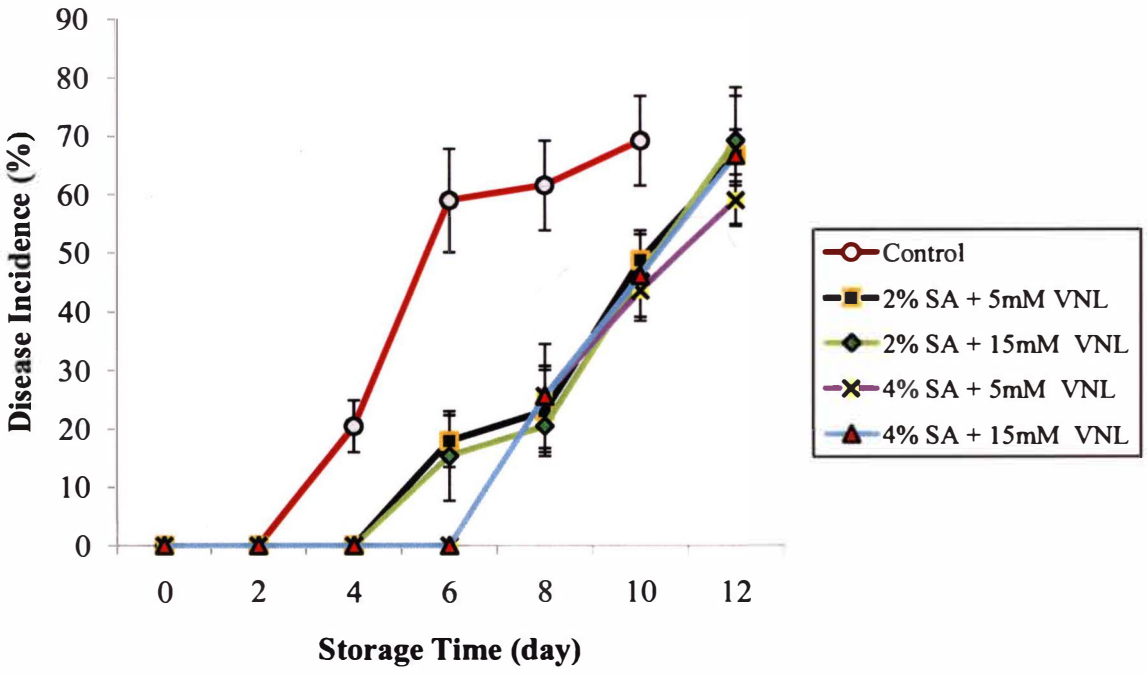


Figure 4.5a: Decay incidence of guava (*Psidium guajava* L.) throughout twelve days of storage under ambient temperature after coated with combination of sodium alginate (SA) and vanillin (VNL) of various concentrations.

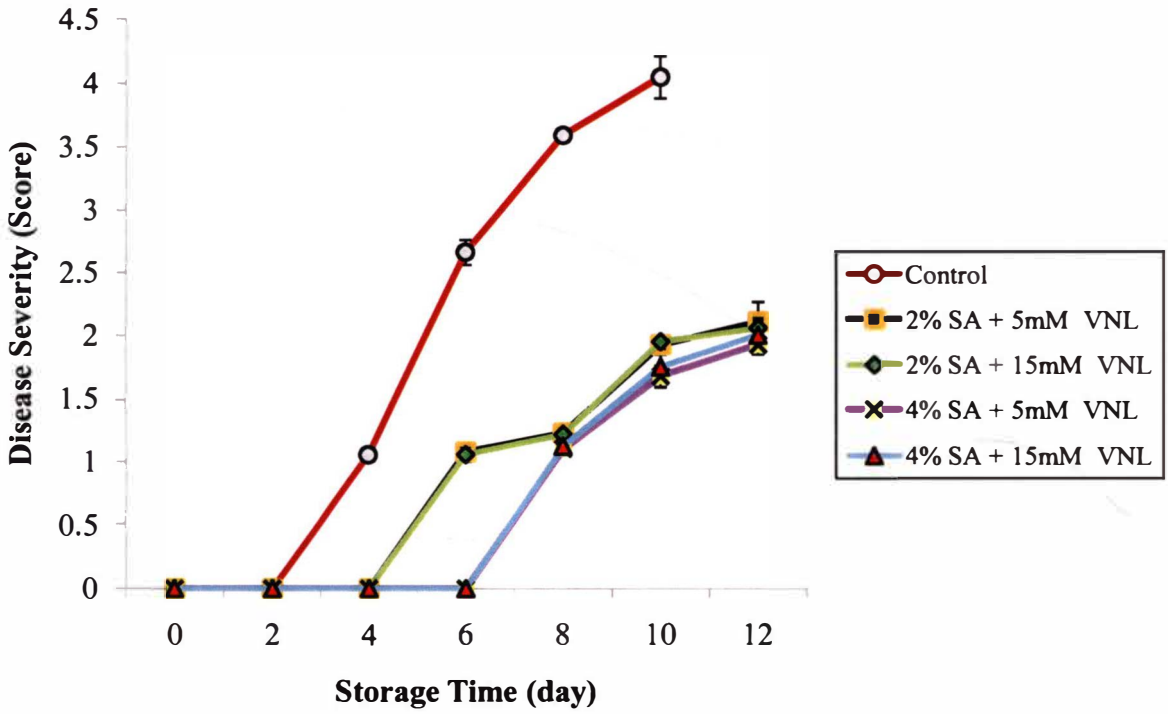


Figure 4.5b: Disease severity (score) of guava (*Psidium guajava* L.) throughout twelve days of storage under ambient temperature after coated with combination of sodium alginate (SA) and vanillin (VNL) of various concentrations.

CHAPTER 5

CONCLUSION AND SUGGESTION

5.1 Conclusion

Sodium alginate combined with vanillin coating is capable of extending shelf life of Thai seedless guavas under ambient temperature for four to six days. All treated fruits significantly delayed fruit softening, reduced colour change, disease severity and disease incidence as compared to untreated fruits. However, total soluble solids of fruits were not affected by any treatments. Treatment 15mM vanillin combined with 2 or 4% sodium alginate fails to reduce weight loss as compared to control. It is proposed that the high concentration of vanillin at 15mM is the contributing factor to the reduction of water barrier properties of the sodium alginate film. Disease incidence is significantly delayed with increased concentration of sodium alginate but not with increased concentration of vanillin. This implies that the antimicrobial properties of the film are not significantly enhanced with higher concentration of vanillin. 4% sodium alginate combined with 5mM vanillin was found to exert the best effect in extending shelf life of Thai seedless guava because this treatment has advantages over other treatments in retention of fruit firmness, reduced weight loss and maintained disease-free for an extended period of two days.

5.2 Suggestion for further study

A combination of sodium alginate and vanillin as an edible coating has a potential to extend the shelf life of guavas held at ambient temperature, especially in firmness retention of fruit and decay and moisture loss reduction. For further study, the best concentration of vanillin incorporated into edible coating should be further examined by using concentration less than 15mM. Coating fruits with edible coating without any antimicrobial agents could also be considered as sodium alginate alone is capable of modifying a micro-atmosphere which unfavor the growth of fungus. Besides, sensory analysis is needed to determine the combine coating effect on the organoleptic qualities of the fruits, in terms of crunchiness, juiciness, sweetness and potential development of off-flavor.

REFERENCES

Adsule, R.N. and Kadam, S.S. 1995. Handbook of fruit science and technology: *production, composition, storage and processing.* Salunkhe, D.K and Kadam, S.S. (Ed). New York: Marcel Dekker. Pp. 419-429

Akamine, E.K. and Goo, T. 1979. Respiration and ethylene production in fruits of species and cultivars of *Psidium* and *Eugenia* species. *Journal of the American Society for Horticultural Science.* 104(5): 632–636.

Aked, J. 2000. Fruits and vegetables. In: Kilcast, D. and Subramaniam, P., (Ed). The stability and shelf-life of food. New York: Woodhead Publishing Limited. Pp. 249-278

Ali, Z.M., Chin, L.H. and Lazan, H. 2004. A comparative study on wall degrading enzymes, pectin modifications and softening during ripening of selected tropical fruits. *Plant Science.* 167: 317–327

Azzolini, M., Jacomino, A.P., Bron, I.U., Kluge, R.A. and Schiavinato, A. 2005. Ripening of "Pedro Sato" guava: study on its climacteric or non-climacteric nature. *Brazilian Journal of Plant Physiology.* 17: 299–306.

Bashir, H.A. and Abu-Goukh, A.A. 2003. Compositional changes during guava fruit ripening. *Food Chemistry.* 80: 557–563.

Bassetto, E., Jacomino, A.P, Pinheiro, A.L and Kluge, R.A. 2005. Delay of ripening of 'Pedro Sato' guava with 1-methylcyclopropene. *Postharvest Biology and Technology.* 35: 303–308.

Biale, J.B. and Barcus, D.E. 1970. Respiratory patterns in tropical fruits of the Amazon basin, *Tropical Science* 12: 93–104.

Brown, B.I. and Wills, R.B.H. 1983. Postharvest changes in guava fruits of different maturity. *Scientia. Horticulturae.* 19: 237–243.

Bulk, R.E.E, Babiker, E.F.E. and Tinay, A.H.E. 1997. Changes in chemical composition of guava fruits during development and ripening. *Food Chemistry.* 59(3): 395-399

Cagri, A., Uspunol, Z. and Ryser, E. 2004. Antimicrobial edible films and coating. *Journal of Food Protection®.* 67: 833–848.

Carrilo-Lopez, A., Ramirez-Bustamante, F., Valdez-Torres, J.B., Rojas-Villegas, R. and Yahia, E.M. 2000. Ripening and quality changes in mango fruit as affected by coating with an edible film. *Journal of Food Quality.* 23: 479–486.

Cerruti, P. and Alzamora, S.M. 1996. Inhibitory effects of vanillin on some food spoilage yeasts in laboratory media and fruit purees. *International Journal of Food Microbiology* 29: 379–386.

Cerruti, P. and Alzamora, S.M. and Vidales, S.L. 1997. Vanillin as an antimicrobial for producing shelf-stable strawberry puree. *Journal of Food Science.* 62: 608–610.

Chan, H.T. and Kwok. S.C.M. 1974. Identification and determination of sugars in some tropical fruit products. *Journal of Food Science*. 40:419

Davidson, P.M. 1993. Parabens and phenolic compounds. In: P.M. Davidson and A.L. Branen, Editors, *Antimicrobials in Foods* (2nd ed.) Marcel Dekker, New York. Pp. 263–306.

Davidson, P.M. and Naidu, A.S. 2000. Phyto-phenols. In: A.S. Naidu, Editor, *Natural Food Antimicrobial Systems. CRC Press LLC*, Boca Raton, London. Pp. 265–294.

Dong, Z.F., Wang, Q. and Du, Y.M. 2006. Alginate/gelatin blend films and their properties for drug controlled release. *Journal of Membrane Science.* 280: 37–44

Fan, Y., Xu, Y., Wang , D., Zhang, L., Sun, J. Sun, L. and Zhang, B. 2009. Effect of Alginate coating combined with yeast antagonist on strawberry (*Fragaria* × *ananassa*) preservation quality. *Postharvest Biology and Technology.* 53(1-2): 84-90

Fitzgerald, D.J., Stratford, M. and Narbad, A. 2003. Analysis of the inhibition of food spoilage yeasts by vanillin. *International Journal of Food Microbiology* 86: 113–122.

Fitzgerald, D.J., Stratford, M., Gasson M.J. and Narbad, A. 2004. The potential application of vanillin in preventing yeast spoilage in soft drinks and fruit juices. *Journal of Food Protection®*. 67 (2): 391-395

Friedman, M., Henika, P.R. and Mandrell, R.E. 2002. Bactericidal activities of plant essential oils and some of their isolated constituents against *Campylobacter jejuni*, *Escherichia coli*, *Listeria monocytogenes*, and *Salmonella enteric*. *Journal of Food Protection®*. 65: 1545–1560.

García, M.A., Martinó, M.N. and Zaritzky, N.E. 2000. Lipid addition to improve barrier properties of edible starch-based films and coatings. *Journal of Food Science.* 65 (6): 941–947.

González-Aguilar, G.A., Tiznado-Hernández, M.E., Zavaleta-Gatica, M. and Martínez-Téllez, M.A. 2004. Methyl jasmonate treatments reduce chilling injury and activate the defense response of guava fruits. *Biochemical and Biophysical Research Communications.* 313(3): 694–701.

Harker, F.R., Redgwell, R.J., Hallett, I.C. and Murray, S.H., 1997. Texture of fresh fruit. *Horticultural Review.* 20: 121–224.

Hassimotto, N. M., Genovese, M. I. and Lajolo, F. M. 2005. Antioxidant activity of dietary fruits, vegetables, and commercial frozen fruit pulps, *Journal of Agriculture Food Chemistry.* 53(8): 2928-35.

Henriette, M.C.A, Fátima, B.S.A, Deborah, S.A, Ana, A.M.Q and Gustavo, A.S.P. 2003. Evaluation of Stability of Alginate-coated Mango Cubes Preserved by the Combined Methods Technology. *Proceedings of the Interamerican Society for Tropical Horticulture. 47:203-205*

Hocking, M.B. 1997. Vanillin: synthetic flavoring from spent sulfiteliquor. *Journal of Chemical Education* 74: 1055–1059.

Jagtiani, J., Chan, H.T. and Sakai, W.S., 1998. Guava. In: *Tropical fruit processing*, Academic Press, New York.

Jain, N., Dhawan, K., Malhotra, S. and Singh, R. 2003. Biochemistry of Fruit Ripening of Guava (*Psidium guajava* L.): Compositional and Enzymatic Changes. *Plant Foods for Human Nutrition* 58: 309–315

Jiménez-Escrig, A., Rincón, M., Pulido, R., and Saura-Calixto, F. 2001.Guava fruit (*Psidium guajava* L.) as a new source of antioxidant dietary fiber. *Journal of Agriculture Food Chemistry.* 49(11): 5489-93.

Kwee, L.T. and Chong, K.K., 1990. Guava in Malaysia: Production, pest and diseases. Tropical Press Sdn Bhd, Kuala Lumpur.

Le-Riche, F. 1951. Chemical changes during the development of guava varieties. *Union of South African Department of Agriculture.* Bulletin no. 21.

Lim, K.T. and Khoo, C.K. (Eds). 1990. Guava in Malaysia: *Production, Puts and Diseases* (first ed.), Tropical Press Pvt. Ltd, Kuala Lumpur, Malaysia.

López-Malo, A., Alzamora, S.M. and Argaiz, A. 1995. Effect of natural vanillin on germination time and radial growth of moulds in fruit-based agar systems. *Food Microbiology.* 12: 213–229.

López-Malo, A., Alzamora, S.M. and Argaiz, A. 1997. Effect of vanillin concentration, pH and incubation temperature on *Aspergillus flavus*, *Aspergillus niger*, *Aspergillus ochraceus* and *Aspergillus parasiticus* growth. *Food Microbiology.* 14: 117–124.

Lopez-malo, A., Alzamora, S.M. and Argaiz, A. 1998. Vanillin and pH synergistic effects on mold growth, *Journal of Food Science.* 63: 143–146.

Mahattanatawee, K., Manthey, J. A. Luzio, G., Talcott, S. T., Goodner, K., and Baldwin, E. A. 2006. Total antioxidant activity and fiber content of select Florida-grown tropical fruits, *Journal of Agriculture Food Chemistry.* 54(19):7355-63.

Maftoonazad, N., Ramaswamy H.S. and Marcotte.M. 2008. Shelf-life extension of peaches through sodium alginate and methyl cellulose edible coatings. *International Journal of Food Science and Technology.* 43(6): 951-957

Matamoros-Leon, B., Argaiz, A. and Lopez-Malo, A. 1999. Individual and combined effects of vanillin and potassium sorbate on *Penicillium digitatum*, *Penicillium glabrum*, and *Penicillium italicum* growth, *Journal of Food Protection.* 62: 540–542.

McHugh, T.H. and Krochta, J.M. 1994a. Water vapor permeability properties of edible whey protein-lipid emulsion films. *Journal of AOAC International.* 71: 307–312.

McHugh, T.H. and Krochta, J.M. 1994b. Sorbitol- vs. glycerol-plasticized whey protein edible films: Integrated oxygen permeability and tensile property evaluation, *Journal of Agricultural and Food Chemistry.* 42: 841–845.

Milan, A.R. 2008. Breeding horticultural crops: *Guava (Psidium guajava).* Chan, Y.K., Tan, S.L and Siti H.B.J (Eds). Serdang: Mardi. Pp. 99-108

Maftoonazad, N., Ramaswamy, H.S., Moalemiyan M. and Kushalappa, A.C. 2007. Effect of pectin-based edible emulsion coating on changes in quality of avocado exposed to *Lasiodiplodia theobromae* infection. *Carbohydrate Polymers.* 68(2): 341-349

Moayednia, N., Ehsani, M.R., Emamdjomeh, Z., Mazaheri, A.F. 2009. The effect of calcium alginate coating on the storability of strawberry at the refrigerator. *5th International Technical Symposium on Food Processing, Monitoring Technology in Bioprocesses and Food Quality Management*. pp. 498-506

Moon, K.D., Delaquis, P., Toivonen, P. and Stanich, K. 2006. Effect of vanillin on the fate of Listeria monocytogenes and *Escherichia coli* O157:H7 in a model apple juice medium and in apple juice. *Food Microbiology.* 23: 169–174.

Ngarmsak, M. 2007. Antifungal activity of vanillin on fresh-cut tropical fruits. *Acta Horculturae (ISHS.)* 746: 409-416.

Penney, V., Henderson, G., Blumb, C. and Johnson-Green, P. 2004. The potential of phytopreservatives and nisin to control microbial spoilage of minimally processed fruit yogurts. *Innovative Food Science Emergence Technology.* 5:69–375.

Perez-Gago, Rojas, C. and Rio, M.A.D. 2003. Effect of hydroxypropyl methylcellulose-lipid edible composite coating on plum (*cv. Autumn giant*) quality during storage. *Journal of Food Science.* 68 (3): 879–883.

Reyes, M.U. and Paull, R.E. 1995. Effect of storage temperature and ethylene treatment on guava (*Psidium guajava* L.) fruit ripening. *Postharvest Biology and Technology.* 6: 357–365.

Rhim, J.W. 2004. Physical and mechanical properties of water resistant sodium alginate films. *Lebensmittel-Wissenschaft und-Technologie* 37(3): 323-330

Roa, S.R. and Ravishankar, G.A. 2000. Vanilla flavor: production by conventional and biotechnological routes. *Journal of the Science of Food and Agriculture* 80: 289–304.

Rojas-Graü, M.A., Tapia, M.S., Rodríguez, F.J., Carmona, A.J. and Martín-Belloso, O. 2007. Alginate and gellan based edible coatings as support of antibrowning agents applied on fresh-cut Fuji apple. *Food Hydrocolloids* 21: 118–127.

Rojas-Graü, M.A., Tapia M.S. and Martín-Belloso, O. 2008. Using polysaccharide-based edible coatings to maintain quality of fresh-cut Fuji apples. *LWT - Food Science and Technology* 41(1): 139-147

Rupasinghe, H.P.V., Boulter-Bitzer, J., Ahn, T, and Odumeru, J.A. 2006. Vanillin inhibits pathogenic and spoilage microorganisms in vitro and aerobic microbial growth in fresh-cut apples, *Food Research International.* 39(5): 575-580

Sangsuwan, J., Rattanapanone, N., Rachtanapun, P. 2008a. Effects of vanillin and plasticizer on properties of chitosan-methyl cellulose based film. *Journal of Applied Polymer Science.* 109 (6): 3540-3545

Sangsuwan, J., Rattanapanone, N., Rachtanapun, P. 2008b. Effect of chitosan/methyl cellulose films on microbial and quality characteristics of fresh-cut cantaloupe and pineapple. *Postharvest Biology and Technology.* 49(3): 403-410

Sangsuwan, J., Rattanapanone, Auras, R.A., Harte, B.R. and N., Rachtanapun, P. 2009. Factors Affecting Migration of Vanillin from Chitosan/Methyl Cellulose Films. *Journal of Food Science.* 74(7): 549-555

Sikkema, J., de Bont, J.A.M. and Poolman, B. 1994. Interactions of cyclic hydrocarbons with biological membranes. *Journal of Biological Chemistry.* 269: 8022–8028.

Sikkema, J., de Bont, J.A.M. and Poolman, B. 1995. Mechanisms of membrane toxicity of hydrocarbons. *Microbiological Reviews.* 59: 201–222.

Silva, L.S and Taylor, J.R.N. 2004. Physical, mechanical, and barrier properties of kafirin films from red and white sorghum milling fractions. *Cereal Chemistry.* 82(1): 9–14

Singh, S.P. and Pal, R.K. 2008a. Controlled atmosphere storage of guava (*Psidium guajava* L.) fruit. *Postharvest Biology and Technology.* 47(3): 296-306

Singh, S.P. and Pal, R.K. 2008b. Response of climacteric-type guava (*Psidium guajava* L.) to postharvest treatment with 1-MCP. *Postharvest Biology and Technology.* 47(3): 307-314

Singh, S.P. and Pal, R.K. 2009. Ionizing radiation treatment to improve postharvest life and maintain quality of fresh guava fruit. *Radiation Physics and Chemistry.* 78(2): 135-140

Sykes, G. and Hooper, M. C. 1954. Phenol as the preservative in insulin injections. *Journal of Pharmacy and Pharmacology.* 6: 552–558.

Tong, Q., Xiao, Q. and Lim, L.T. 2008. Preparation and properties of pullulan–alginate–carboxymethylcellulose blend films. *Food Research International.* 41(10): 1007-1014

Ultee, A., Gorris, L.G.M. and Smid, E.J. 1998. Bactericidal activity of carvacrol towards the food-borne pathogen *Bacillus cereus*. *Journal of Applied Microbiology.* 85: 211–218.

Walton, N.J., Mayer, M.J. and Narbad, A. 2003. Vanillin. *Phytochemistry*, 63(5): 505-515

Wang, L., Liu, L., Holmes, J., Kerry, J.F. and Kerry, J.P. 2007. Assessment of film-forming potential and properties of protein and polysaccharide-based biopolymer films. *International Journal of Food Science and Technology*. 42: 1128–1138

Weber, F.J. and de Bont, J.A.M. 1996. Adaptation mechanisms of microorganisms to the toxic effects of organic solvents on membranes. *Biochimica et Biophysica Acta*. 1286: 225–245.

Yang, L. and Paulson, A.T. 2000. Effects of lipids on mechanical and moisture barrier properties of edible gellan film. *Food Research International* 33 (7): 571-578

APPENDIX A

Table : Effect of guavas coated with different concentrations and combinations of sodium alginate (SA) and vanillin (VNL) on total soluble solids (Brix %) throughout ten days of storage at ambient temperature.

	Total Soluble Solids (Brix %)					
Treatment	0 day	2nd Day	4th Day	6th Day	8th Day	10th Day
Control	5.98±076[a]	8.24±0.45[a]	10.18±0.60[b]	10.44±0.73[b]	-	-
2% SA + 5 mM VNL	5.98±076[a]	8.17±0.46[a]	8.52±0.54[a]	10.25±0.55[b]	9.25±0.44[a]	10.78±0.35[a]
2% SA + 15 mM VNL	5.98±076[a]	7.95±0.19[a]	8.33±0.69[a]	9.25±0.25[ab]	10.34±0.62[a]	11.03±0.70[a]
4% SA + 5 mM VNL	5.98±076[a]	7.92±0.53[a]	8.24±0.10[a]	9.93±0.20[b]	8.87±0.54[a]	10.37±0.97[a]
4% SA + 15 mM VNL	5.98±076[a]	8.08±0.18[a]	8.02±0.34[a]	8.44±0.27[a]	8.52±0.34[a]	11.07±0.60[a]

Mean within the same columns with different letters are significantly different ($P<0.05$)

APPENDIX B

Table : Effect of guavas coated with different concentrations and combinations of sodium alginate (SA) and vanillin (VNL) on firmness (force, g) throughout ten days of storage at ambient temperature.

Treatment	Firmness (force, g)					
	0 day	2nd Day	4th Day	6th Day	8th Day	10th Day
Control	2017.73±301.79^{a}	737.18±70.41^{a}	747.27±32.87^{a}	269.45±41.81^{a}	-	-
2% SA + 5 mM VNL	2017.73±301.79^{a}	793.70±65.59^{ab}	1214.05±61.80^{bc}	547.75±74.92^{b}	650.86±49.71^{a}	632.97±30.61^{b}
2% SA + 15 mM VNL	2017.73±301.79^{a}	747.94±6.15^{a}	1095.20±126.21^{b}	498.62±44.97^{ab}	622.52±47.42^{a}	454.30±47.04^{a}
4% SA + 5 mM VNL	2017.73±301.79^{a}	890.56±52.84^{b}	1391.51±163.33^{c}	773.93±60.40^{c}	734.42±76.99^{a}	665.52±74.83^{b}
4% SA + 15 mM VNL	2017.73±301.79^{a}	846.69±46.53^{ab}	1322.77±92.69^{bc}	758.85±86.51^{c}	641.76±75.46^{a}	657.42±38.71^{b}

Mean within the same columns with different letters are significantly different ($P<0.05$)

APPENDIX C

Table : Effect of guavas coated with different concentrations and combinations of sodium alginate (SA) and vanillin (VNL) on color (Hue angle, $h°$) throughout ten days of storage at ambient temperature.

Treatment	Hue angle ($h°$)					
	0 day	2nd Day	4th Day	6th Day	8th Day	10th Day
Control	111.17±1.26[a]	108.90±0.36[a]	102.33±1.92[a]	97.52±1.65[a]	94.12±3.10[a]	91.08±3.18[a]
2% SA + 5 mM VNL	111.17±1.26[a]	108.55±1.03[a]	114.25±0.88[c]	104.74±2.52[b]	107.54±0.96[b]	104.92±0.95[b]
2% SA + 15 mM VNL	111.17±1.26[a]	105.97±1.94[a]	107.27±2.05[b]	103.29±0.89[b]	107.50±0.28[b]	103.28±1.22[b]
4% SA + 5 mM VNL	111.17±1.26[a]	108.99±1.48[a]	113.18±1.87[c]	106.85±2.89[b]	111.08±1.44[b]	105.22±2.18[b]
4% SA + 15 mM VNL	111.17±1.26[a]	110.10±3.94[a]	111.64±0.99[c]	106.99±2.10[b]	108.27±1.10[b]	104.70±1.87[b]

Mean within the same columns with different letters are significantly different ($P<0.05$)

APPENDIX D

Table : Effect of guavas coated with different concentrations and combinations of sodium alginate (SA) and vanillin (VNL) on total color change throughout ten days of storage at ambient temperature.

	Color Change					
Treatment	0 day	2nd Day	4th Day	6th Day	8th Day	10th Day
Control	0.00±0.00[a]	8.79±0.58[b]	12.24±0.63[b]	14.02±0.46[c]	17.87±1.21[b]	18.19±2.51[b]
2% SA + 5 mM VNL	0.00±0.00[a]	7.81±0.68[ab]	7.73±1.42[a]	8.16±0.65[a]	8.54±0.68[a]	9.91±1.23[a]
2% SA + 15 mM VNL	0.00±0.00[a]	8.96±0.69[b]	8.47±1.41[a]	11.43±0.87[b]	9.89±0.68[a]	10.80±0.65[a]
4% SA + 5 mM VNL	0.00±0.00[a]	7.39±0.53[ab]	8.76±0.37[a]	8.13±0.40[a]	8.42±0.98[a]	9.11±0.71[a]
4% SA + 15 mM VNL	0.00±0.00[a]	6.99±0.47[b]	7.96±0.85[a]	8.29±0.52[a]	8.30±0.40[a]	9.87±0.27[a]

Mean within the same columns with different letters are significantly different ($P<0.05$)

APPENDIX E

Table : Effect of guavas coated with different concentrations and combinations of sodium alginate (SA) and vanillin (VNL) on weight loss after ten days of storage at ambient temperature.

Treatment	Weight Loss (%)
Control	31.24 ± 1.57^{b}
2% SA + 5mM VNL	25.03 ± 1.50^{a}
2% SA + 15mM VNL	32.33 ± 1.55^{b}
4% SA + 5mM VNL	21.24 ± 0.83^{a}
4% SA + 15mM VNL	29.97 ± 2.01^{b}

Mean within the same columns with different letters are significantly different ($P<0.05$)

APPENDIX F

Table : Effect of guavas coated with different concentrations and combinations of sodium alginate (SA) and vanillin (VNL) on disease severity changes throughout twelve days of storage at ambient temperature.

	Disease Severity (score)						
Treatment	0 day	2nd Day	4th Day	6th Day	8th Day	10th Day	12th day
Control	0.00±0.00[a]	0.00±0.00[a]	1.06±0.05[b]	2.66±0.09[c]	3.58±0.36[b]	4.04±0.16[b]	-
2% SA + 5 mM VNL	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]	1.08±0.07[b]	1.22±0.39[a]	1.92±0.08[a]	2.0±0.16[a]
2% SA + 15 mM VNL	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]	1.06±0.05[b]	1.21±0.07[a]	1.94±0.05[a]	2.06±0.12[a]
4% SA + 5 mM VNL	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]	1.10±0.43[a]	1.67±0.08[a]	1.92±0.08[a]
4% SA + 15 mM VNL	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]	1.12±0.05[a]	1.74±0.16[a]	2.0±0.08[a]

Mean within the same columns with different letters are significantly different ($P<0.05$)

APPENDIX G

Table : Effect of guavas coated with different concentrations and combinations of sodium alginate (SA) and vanillin (VNL) on decay incidence changes throughout twelve days of storage at ambient temperature.

	Decay Incidence (%)						
Treatment	0 day	2nd Day	4th Day	6th Day	8th Day	10th Day	12th day
Control	0.00±0.00[a]	0.00±0.00[a]	20.5 ± 4.4[b]	58.9 ± 8.8[c]	61.5 ± 7.7[b]	69.2 ± 7.7[b]	-
2% SA + 5 mM VNL	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]	17.9 ± 4.4[b]	23.1 ± 8.8[a]	48.7 ± 4.4[a]	66.7 ± 11.7[a]
2% SA + 15 mM VNL	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]	15.3 ± 7.7[b]	20.5 ± 4.4[a]	46.2 ± 7.7[a]	69.2 ± 7.7[a]
4% SA + 5 mM VNL	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]	25.6 ± 7.7[a]	43.6 ± 4.4[a]	58.9 ± 4.4[a]
4% SA + 15 mM VNL	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]	25.6 ± 4.4[a]	46.2 ± 7.7[a]	66.7 ± 4.4[a]

Mean within the same columns with different letters are significantly different ($P<0.05$)

CURRICULUM VITAE

Name	: Doreen Yong Sheng Yuen
Permanent Address	: No 13, Jalan SS1/17, Kampung Tunku, 47300 Petaling Jaya, Selangor.
Telephone Number	: 017-2745363
E-mail	: dolly_87113@hotmail.com
Date of birth	: 13 January 1987
Nationality	: Malaysian
Race	: Chinese
Gender	: Female
Religion	: Christian

Education Background:

2007-2010	Universiti Malaysia Terengganu (UMT)
2005-2006	Sekolah Menengah Kebangsaan TAMAN S.E.A
2000-2004	Sekolah Menengah Katholik
1994-1999	Sekolah Rendah Jenis Kebangsaan (Cina) Puay Chai

EFFECT OF SODIUM ALGINATE COMBINED WITH VANILLIN ON POSTHARVEST QUALITY OF GUAVA FRUIT (PSIDIUM GUAJAVA L.) - DOREEN YONG SHENG YUEN