Chapter 2

Literature Review

2.1 Pineapple

2.1.1 Introduction

The pineapple (*Ananas comosus* (L.) Merr.), a member of Bromeliaceae family, is the third most important tropical fruit in world after banana and citrus (Bartholomew *et al.*, 2002). The main pineapple-producing countries are Brazil, Thailand, Philippines, Vietnam, Mexico, China, Nigeria, Indonesia, and Columbia (Chen *et al.*, 2011). Statistics on world pineapple production are collected by the Food and Agriculture Organization of the United Nations (FAO). According to FAO statistics (Chen *et al.*, 2011; Othman *et al.*, 2011), in 2008, the leading pineapple production countries are Brazil with 2.5 million tons, Thailand with 2.3 million tons, the Philippines with 2.2 million tons, Malaysia with 1.2 million tons, Vietnam with 0.51 million tons, Mexico with 0.5 million tons, China with 0.45 million tons and USA with 0.29 million tons of fruits.

Pineapple can be divided into 5 groups according to morphological characteristics, including spination, length and shape of the leaves, and weight, shape, texture and taste of the fruits. These 5 groups are Abacaxi, Cayenne, Maipure or Perolera, Queen, and Spanish (Leal and Soule, 1977; Py *et al.*, 1987). The most famous variety in world trade is Cayenne Lisse (Smooth Cayenne) while Queen is known in small specific niches of high-quality, good flavor and expensive fresh fruit (Bartholomew *et al.*, 2002). The cultivar Queen is widely distributed, but it is more particularly cultivated in the southern hemisphere, in South Africa and Australia, for the fresh-fruit market. It has been called ‘Mauritius’, ‘Moris’, ‘Victoria’, Malacca’, ‘Red Caylon’, ‘Buitenzorg’, ‘Ripley Queen’ and ‘Alexandra’. The plant is small (60-80cm), with short and very spiny silvery leaves, and gives a small fruit with a full yellow shell and small prominent eyes.

In Vietnam, the pineapple is the second most important fruit in terms of area planted and production levels, after the banana, and is also considered one of the most important fruits in terms of processing (FAO, 2004). At present the main pineapple production areas in Vietnam are in Tien Giang, Kien Giang, Ninhbinh, Thanh Hoa, Nghe An and Quang Nam, with the total area grown in Vietnam having increased slightly from 365 km^2 in 2000 to 399 km^2 in 2010, and with production having increased dramatically from 291,000 to 502,700 tons over the same period (Vietnam General Statistics Office, 2010). Of the many pineapple cultivars in Vietnam, cv. Ninhbinh is one of the most attractive and has attracted much attention due to its excellent texture, taste and flavor. Moreover, the fruit is small - weighing around 750 to 1100 grams, has a sweet taste and a crispy texture and thick skin. The skin is usually yellow or greenish-yellow in color when it ripens.

The Queen pineapple has been cultivated for a long time in Vietnam with some popular names such as cv. TienGiang, cv. LongAn, cv. Ninhbinh, cv. BenLuc, cv. QuangNam, cv. KhomHoa, cv. KhomTay, cv. TanPhuoc. The 'pineapple' English name is translated into Vietnamese vernacular with 'Khom' or 'Dua' names. With cropping seasons, the pineapple is planted in 2 crops a year. The first crop (main crop) is harvested in summer from April until the end of June. The second crop (late crop) is harvested in winter from November to December (FAO, 2004).

2.1.2 Queen pineapples morphology

The Queen pineapple is small fruit, dry flesh, sweet taste and delicate mild flavor. Leaves are spiny, narrow and long, light green in color with a pink streak in the middle. Thorns line the edges of the leaf throughout its entire length. The pineapples have crisp texture, the skin will be yellow or greenish yellow when it ripens. Pineapple undergoes changes during maturation and ripening. As the fruit ripens, the "eyes" change from pointed to flat, with a slight hollowness at the centre (Figure 2.1). The fruit becomes enlarged, less firm and more aromatic. The shell color of pineapple is generally used to determine the various stages of maturity. Pineapples with slightly yellowed to one-half yellowed surface color and fruits with no yellowing may not be mature enough for optimum quality (Pantastico, 1975).

Figure 2.1 Queen pineapple

2.1.3 Chemical composition and nutrient values

The nutrient value of pineapple depends on many factors, including the nutritional status of pineapple plant, soil and water nutrient status, weather, pineapple cultivar, preharvest and postharvest technologies. Sugar content plays an important role in the flavor characteristics and commercial assessment of pineapple (Py *et al.*, 1987). The major sugars in mature fruit are sucrose, glucose and fructose and the peak in sucrose concentration is attained at full yellow stage and then declines (Gawler, 1962). Chen and Paull (2000) showed that total soluble content is low during fruit growth and composed mainly of glucose and fructose. Glucose is at a slightly higher concentration than fructose during the early stage and the glucose and fructose continue to increase in postharvest (Py *et al.*, 1987; Tay, 1977). Three sugar metabolism enzymes (sucrose synthase, sucrose phosphate synthase and invertase) are found in pineapple. The activity of sucrose synthase is high in younger pineapple and declines to very low levels 6 weeks before harvest while the activity of sucrose phosphate synthase is relatively low and constant throughout fruit development (Chen and Paull, 2000). Additionally, pineapple contains some other enzymes such as *peroxidase, polyphenol oxidase* and *proteinase bromelain* (Rowan *et al.*, 1990).

Three major organic acids are found in pineapple fruit, including malic acid, citric acid and ascorbic acid (Bartholomew *et al.*, 2003). Malic acid levels do not change after harvest or during and after storage. Citric acid (28-66% of total acid) increase uniformly, with fruit development peaking before malic acid and before full ripeness. Ascorbic acid content varies significantly with the clone and increases with increasing solar radiation and air temperature (Bartholomew *et al.*, 2002). The level of fruit ascorbic acid at harvest has been negatively related to the intensity of internal-browning symptoms associated with postharvest chilling injury. Internal browning is a minor problem if fruit ascorbic acid content is greater than 500 µM (Teisson *et al.*, 1979). Chemical composition, nutrient values per 100g flesh fruit is described as Table 2.1.

Table 2.1 Chemical composition and nutrient values of pineapple cv. Smooth Cayenne (per 100g) (USDA, 2009)

Component	Content	Component	Content
Non-edible proportion (core, crown, parings)	42%	Thiamine (B1)	0.078 mg
		Riboflavin (B2)	0.029 mg
Water	87g	Niacin (B3)	0.470 mg
Energy	190 kJ	Pantothenic acid (B5)	0.193 mg
Carbohydrate	11.82 g	Pyridoxine (B6)	0.106 mg
Sugars	8.29 g	Sodium	1 mg
Dietary fibre	1.4 g	Folate (B9)	11 µg
Fat	0.13 g	Vitamin C	16.9 mg
Protein	0.55 g	Iron	0.25 mg
Sucrose	4.59g	Phosphorus	9 mg
Glucose	1.76g	Potassium	115 mg
Fructose	1.94g	Zinc	0.08 mg
Calcium	13.0 mg	Magnesium	12 mg
Carotene, beta	31 µg	Vitamin K	0.7 µg

2.1.4 Pineapple harvesting

Pineapple is hand-harvested, with pickers being directed as to ripen stage and fruit quality. A definition of pineapple fruit quality often refers to the sum of those characteristics of a fruit that make it most palatable and therefore desirable to consumers (Paull, 1993). However, this definition does not allow us to measure as a quality standard; the standard varies with consumer's tastes, technical procedures and may be related to price paid. Normally, there are couple ways to evaluate the fruit quality for harvesting.

First of all, determination of fruit quality is based on the fruit maturity and harvest date. A 1.0% solution of CaC_2 or 0.15% ethephon are applied by growers for 48 h or more before harvest to accelerate shell degreening and foster ripening (Bartholomew *et al.*, 2002; Maruthasalam *et al.*, 2010) . Fruit maturity is evaluated on

the extent of fruit 'eye' flatness and shell color. Current estimate of harvest date ranges from 140 to 150 days after flowering (Deka *et al.*, 2005; Maruthasalam *et al.*, 2010). With Queen pineapples cv. Ninhbinh, the fruits were harvested during 141-145 days after flowering. Appropriate estimate of harvest date will reduce the postharvest losses as well as to maintain quality of the harvested produce.

Table 2.2 Subjective rating used for fresh pineapple fruit (Bartholomew *et al.*, 2002)

Criteria	Scale	Description
Shell color	0-6	Immature green to overripe. 1-12%, 13-37%, 38-62%, 63-87%, 88-100% yellow eyes
Shell appearance	1-3	Brown or black discoloration to normal flesh, glossy
Crown	1-3	Dry, limp, brown to turgid, normal color and fresh
Whole-fruit texture	1-3	Soft to firm: feel of fruit
Flesh texture	1-3	Limp to firm, crisp or fibrous texture
Translucency (slide)	1-5	Opaque to fully translucent
Translucency (1/3 from base)	1-4	Opaque to full translucent
Diseases and disorders		Number affected fruit and severity
Flavor	1-3	Fermented, bitter after-taste
		Very high acid, very high ester, very bland
		Normal

In another way, fruit harvesting is based on quality criteria. The quality criteria have been described throughout appearance (size, condition, and sharp), color (shell and flesh), taste (sugar, acid), aroma, flesh translucency, texture and fiber content (Table 2.2). Skin color is the most common measure of physiological maturity. Normally, the pineapples are harvested when more than 10% of the shell color changes from green to yellow. Moreover, minimum total soluble solids (TSS) content is required, which

ranges from 12 to 14% Brix which varies by country. A sugar-to-acid ratio of 20:40 is recommended in former literature, while Soler (1992) recommended 14:20. However, extremely high or low values can lead to an insipid flavor (Bartholomew *et al.*, 2002; Soler *et al.*, 2006).

2.1.5 Postharvest losses

The major factors reducing the storage life and marketability of pineapple are weight loss, fermentation, fruit rot (black rot) and fruitlet core rots. When pineapple is stored at low temperature, chilling injury and internal browning are also important factors that affect fruit quality.

2.1.5.1 Weight loss

Preharvest conditions largely affect fruit quality, chemical composition, texture, and postharvest moisture loss (Gomez-Gadindo *et al.*, 2004). At the time of harvest, the water status of the procedure is usually high but after harvesting there are two factors that lead to fruit weight loss. First, water can no longer be taken up from the soil due to the interruption of the plant's natural life cycle. Second, water transpiration, which is a physical process by which water vapor, can permeate the stomas and epidermis. Water is also lost through lenticels, which are gaps in the periderm formed to enable gas exchange for respiration. If the epidermis or periderm is damaged, water loss can be massively exacerbated (Daniel and Maria, 2010).

The rate of postharvest water loss is dependent primarily on the external vapor pressure deficit and other factors such as fruit cultivars, fruit peel, storage temperature conditions, relative humidity, modified atmosphere packaging, packaging materials and coating.

2.1.5.2 Black rot (fruit rot) and fruitlet core rots

Black rot of pineapples is a common postharvest problem in many countries. Black rot, also called Thielaviopsis fruit rot, water blister, soft rot or water rot, is caused by the fungus *Chalara paradoxa (Thielaviopsis paradoxa)* (Figure 2.2). The disease is a universal fresh-fruit problem but normally not a problem with processed fruit, because times from harvest to processing are too short for infection. The severity

of the problem is dependent on the degree of bruising or wounding during harvesting and packing, the level of inoculums on the fruit and the storage temperature during transportation and marketing. Black rot does not occur in the field unless fruit is overriped or injured (Bartholomew *et al.*, 2002; Reyes *et al.*, 2004).

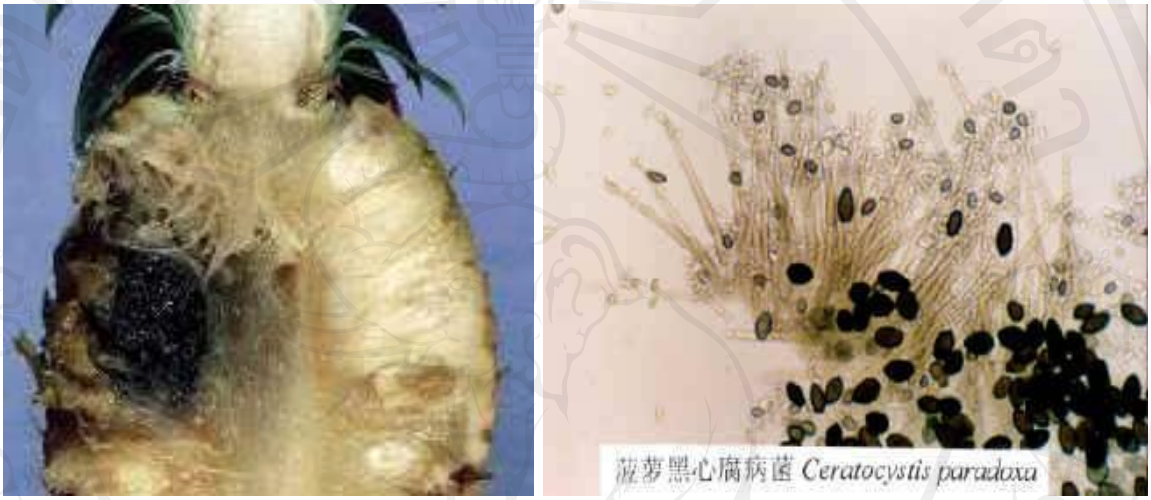


Figure 2.2 Black rot on pineapple (www.cd3wd.com/cd3wd_40/Biovision) by *Ceratocystis paradoxa* (www.plantwise.org)

Black rot (fruit rot) of the pineapple fruit is characterized by soft watery rot, which usually stars at the point of detachment of the fruit. Diseased tissue turns dark in the later stages of the disease (Figure 2.2) because of the dark colored mycelium and chlamydospores (Bartholomew *et al.*, 2002). Infection of the pineapple fruit occurs through wounds resulting from harvesting and postharvest handling. Refrigeration at 9°C during transportation will slow development of the disease, but, when fruit are returned to ambient temperatures, disease development will resume (Rohrbach and Schmitt, 2003). Black rot of pineapple is controlled by the application of specific fungicides (Cho *et al.*, 1977). Dipping pineapple in hot water treatment at 54°C for 3 minutes (Wilson-Wijeratnam *et al.*, 2005) delayed the development of this disease.

Fruitlet core rot, also called black spot or fruitlet brown rot, is a descriptive term for a brown to black color of the central part of an individual fruitlet (Figure 2.3). Fruitlet core rot is caused by an infection by a pathogen or, more commonly, a group

of pathogens. The *Penicillium* and *Fusarium* fungi, the round yeasts (*Candida guillermondi*) and bacteria have been associated with the fruitlet core rot symptom. In addition to the multiple pathogens, two mites have also been reported to be associated with the occurrence of fruitlet core rot epidemics (Rohrbach and Schmitt, 2003). Fruitlet core rot symptoms produced by *Penicillium* species are dark to medium brown in color, usually with a grey, water-soaked center while light brown color is found for the fruitlet rot symptom from yeast. The degree to which these symptoms develop appears to depend on the time of injection, the pathogen or mixture of pathogens present, the cultivar and environmental conditions.

Figure 2.3 Fruitlet core rot

(http://ucanr.org/sites/postharvest/PFfruits/PineapplePhotos)

2.1.5.3 Chilling injury and internal browning

Rapid produce cooling after harvest is essential in the preservation of most fresh fruits, since temperature has the single greatest effect on the respiration rate and thus in the deterioration rate of produce. However, some precooling methods are not suited for preservation. It must be also to taken account that some produce are sensitive to low temperature, suffering damage know as chilling injury.

Chilling injury (CI) has been called endogenous brown spot, physiological breakdown, black-heart and internal browning (Figure 2.4). CI is primarily a physiological disorder occurring in crops of pineapple when stored at low temperatures. CI is not the same as freezing injury, which is a result of damage from

ice crystals formed in tissues stored below their freezing point. However, CI occurs when the pineapples are stored at temperatures below 10-13°C. This physiological disorder develops faster and more intensely when sensitive fruits are stored at temperatures between 2 and 10°C (killing temperature zone) than when they are stored at 0°C or below, but above their freezing point. Therefore, fruit maximum storage life can be achieved near or below 0°C depending on the total soluble solids content of the fruit (Daniel and Maria, 2010). CI may occur in the field, in transit and distribution, and in retail and home refrigerators (Paull and Chen, 2003).

Figure 2.4 Chilling injury symptoms

Potential symptoms of CI include: wilting, drying and discoloration of crown leaves; failure of green-shelled fruit to yellow; browning and dulling of yellow fruit; water-soaking of tissues; fruit water loss; and internal flesh browning (Bartholomew *et al.*, 2002; Daniel and Maria, 2010). However, detection and diagnosis of CI is often difficult when removed from the chilling injury temperature. But the CI symptoms are more pronounced when the fruits are returned from chilling injury conditions to physiological temperatures (17.5-27.5°C) and the crown is also more susceptible to CI than the fruit itself (Paull and Chen, 2003). In addition, CI symptoms vary depending on a wide range of factors, such as fruit cultivars, ripening stage at harvest, and duration of storage and prestorage conditions (Sevillano *et al.*, 2009).

The chilling injury occurs from various causes. The primary cause of CI is thought to be damage to plant cell membranes, and the secondary causes include ethylene production, increased respiration, reduced photosynthesis and interference with energy production, accumulation of toxic compounds (ethanol and acetaldehyde) and altered cellular structure (Kratsch and Wise, 2000). Furthermore, it has been reported that low temperature induces changes in cell membrane lipids from a liquid-crystalline to a solid-gel state, which lead to an increase in membrane permeability and leakage of ions (Gomez-Gadindo *et al.*, 2004).

In order to extend the shelf life and quality retention in commodities, there are some postharvest techniques for reducing chilling injury. The obvious way to avoid chilling damage is to avoid chilling injury temperature. If chilling could be avoided, treatments should be developed either to increase the tolerance of the tissue before chilling or to reduce the development of injury symptoms (Bramlage, 1982), including chemical treatment, hot water treatment, growth bio-regulators application, controlled atmosphere storage, and waxing and packaging. In fact, lower incidence of CI was negatively correlated with total calcium concentration in fruits of pineapple varieties and even in different parts of a single fruit (Hewajulige *et al.*, 2003). However, calcium foliar sprays on peach and nectarines showed no effect on CI symptoms (Crisosto *et al.*, 2000). With respect to nitrogen fertilization during fruit growth, no relation with CI has been found in peach or nectarines cultivar (Lurie and Chrisosto, 2005), while in avocado excessive nitrogen concentration increased CI symptoms severity (Van and Bower, 2005). Additionally, application hot water treatment (38°C for 60 minutes) to induce cold tolerance in pineapple fruit (cv. Mauritius) to reduce the development of internal browning and chilling injury symptoms was reported (Darshani and Adikaram, 2005).

Waxing and polyethylene packing have been applied to the surface of fresh fruits such as pineapple (Paull and Rohrbach, 1985), papaya (Chen and Paull, 1986) and mango (Peris *et al.*, 2000). A wax, frequently containing polyethylene/paraffin or carnauba/paraffin-based, has also been applied for pineapple fruit with the fungicide. The major advance of waxing is the reduction of the internal browning symptoms of chilling injury (Bartholomew *et al.*, 2002). Waxing also reduces postharvest water loss and improves fruit appearance (Paull and Rohrbach, 1985).

2.1.5.4 Yeast fermentation

Yeast fermentation is caused by the yeast species *Hanseniaspors valbyensis, Saccaromyces cerevisiae, Candida intermedia* var. *alcoholophila*, can be a major problem in overripe fruits (Figure 2.5). Yeasts use sugar as an energy source in cellular respiration. Without oxygen, the process of glycolysis continues through fermentation, producing alcohol. The sugar in the fruit gets converted into alcohol, which eventually kills the yeast due to the high concentration. If the juice is exposed to the air, microbes will begin to use the alcohol as an energy source and change it into acetic acid. Occasionally, the disease will occur in green fruit, having severe interfruitlet corking symptoms with associated fruit cracking. The disease has also been associated with high incidences of fruit sunburn (Bartholomew *et al.*, 2002).

Figure 2.5 Yeast fermentation
(http://ucanr.org/sites/postharvest/PFfruits/PineapplePhotos)

2.1.6 Postharvest storage

2.1.6.1 Temperature and relative humidity management

Temperatures in the range 7.5-12.5°C are recommended for pineapple storage, with relative humidity 70-95%.; the higher relative humidities significantly reduce

water loss (Paull, 1997). The more recent recommendation is for 90-95% relative humidity. At a temperature of 0-4°C, the fruit fails to continue ripening and shows severe chilling injury symptoms (Bartholomew *et al.*, 2002).

Quarter-yellow fruit at harvest gain about 1 additional week's storage for every 6°C decrease in storage temperature. Haft-ripe 'Smooth Cayenne' fruit can be held for about 10 days at 7.5-12.5°C and still have about a week of shelf-life with no chilling-induced internal browning. The maximum storage life at 7°C is about 4 weeks (Paull and Chen, 2003); however, when removed to ambient conditions, chilling injury induced internal browning develops within 2-3 days. The CI symptoms development is more rapid at 20-25°C. Storage at 7.5-12.5°C does not generally lead to dramatic changes in acidity, though both sugars and ascorbic acid may decline (Paull and Rohrbach, 1982). Recently, temperature preconditioning has been applied. In fact, pineapple cv. N36 in Malaysia was held for 24 h at 15°C before storage at 5°C and the shelf life extended up to 6 weeks with less symptoms of chilling injury compared to the control (Abdullah *et al.*, 2008).

2.1.6.2 Controlled atmosphere and modified atmosphere storage

As with other non-climacteric fruit, controlled atmosphere via decrease oxygen levels carbon dioxide have shown only minimal effectiveness in extending pineapple shelf life. According to Paull and Rohrbach (1982), some beneficial effect might be gained from controlled atmosphere (4% oxygen) treatment in reducing chilling injury symptom development. Low oxygen (2-12%) has been shown to enhance water loss from the crown leaves. There is no published work on lower oxygen levels with or without various controlled carbon dioxide concentrations.

Cellophane and polyethylene bags have been tested on numerous occasions to extend postharvest life (Paull and Rohrbach, 1985). Both types of bags delay shell degreening when compared with unbagged fruit. Polyethylene bagging is objectionable because of the condensation problem, which leads to yeast and mould development, while cellophane bags allow moisture vapor exchange and the crown especially tends to dry out. Additional problems with all bags are the development of off-flavors and the difficulty of avoiding puncturing the bag with crown leaves. An

overwrap on the carton overcomes the puncturing problem but lead to problems in market inspection, and the wrap would need to allow some gas exchange to prevent excessive CO2 build-up, which apparently leads to off-flavors (Bartholomew *et al.*, 2002).

2.1.6.3 Chemical treatment

A number of chemicals used in preharvest or postharvest have been shown to be effective in fruit quality, postharvest spoilage, softening, ethylene production, and senescence rate such as calcium salts (Antoniolli *et al.*, 2003; C.-C. Chen, 1999; Gomez-Gadindo *et al.*, 2004; Herath *et al.*, 2000, 2001; Hewajulige *et al.*, 2006), 1-MCP (Dantas Junior *et al.*, 2009; Machado *et al.*, 2009; Selvarajah *et al.*, 2001), and fungicide (Biswal *et al.*, 2007; Bolkan *et al.*, 1978; Diep, 2008; Julca-Otiniano and Bello-Amez, 2005; Oliveira and Nascimento, 2009). Calcium treatment is potentially effective method applied as a postharvest treatment by dipping in solution of calcium salts. The dipping time ranges from 5 to 120 minutes and the calcium concentration from 0.5 to 6%; the most used source of calcium being calcium chloride followed by calcium nitrate, calcium lactate, calcium propionate, and calcium gluconate (Hernández-Muñoz *et al.*, 2006). Dipping pineapple cv. Perola with sodium hypochlorite (NaOCl) solutions 200 mg/l for 2 minutes combined with sanitization of pineapple slices with 20 mg chlorine solution/l significantly reduced microbial populations (Antoniolli *et al.*, 2005).

The efficacy of different fungicides on postharvest fruits was reported. Accoding to Biswal et al. (2007), combination of carbendazim and mancozeb at 0.1% could inhibit the mycelial growth of *Cladosporium herbarum* and *Aspergillus flavus* to maximum extent whereas carbendazim at 0.1% was found to be the best against *Fusarium solani* and *Fusarium oxysporum*. Dipping Queen pineapples in carbendazim solution 150 ppm for 30 minutes and storing at 12°C reduced the internal browning symptoms after 21 days storage. A similar result was seen for Smooth Cayenne treated with carbendazim solution 250 ppm for 30 minutes and stored at 14°C (Diep, 2008). In addition, triadimefon, imazalil and triadimenol were used to control Thielaviopsis black rot caused by *Ceratocystis paradoxa* on pineapple cultivars Red Spanish and Smooth Cayenne in the Canary Islands. Triadimenol gave the best

control but none of the fungicides gave complete disease control. Red Spanish was more prone to black rot than Smooth Cayenne (Hernandez-Hernandez and Sala-Mayato, 1990).

2.2 Coating Material for Fruit

2.2.1 Chitosan

Chitosan is derived from chitin, which is the second most abundant and naturally available biopolymer after cellulose, is a co-polymer of β (1-4)-linked D-glucosamine and N-acetyl- D-glucosamine units (Figure 2.6, 2.7). It is a high molecular weight polymer, which is about 100,000 - 1,200,000 Dalton. Chitosan is produced commercially by deacetylation of chitin, which is the structural element in the exoskeleton of crustaceans (crabs, shrimp, etc.), cell walls of fungi and other biological materials. Chitosan exhibits antimicrobial properties, in addition to its strong cationicity and film forming properties. Positive charges of chitosan (natural cationic polysaccharides) promote cell adhesion, since free amino groups interfere with the negative charges on bacterial cell membranes, causing leakage of intracellular constituents (Goldberg *et al.*, 1990). Furthermore, it provides a film with a good mechanical and oxygen barrier (Caner *et al.*, 1998).

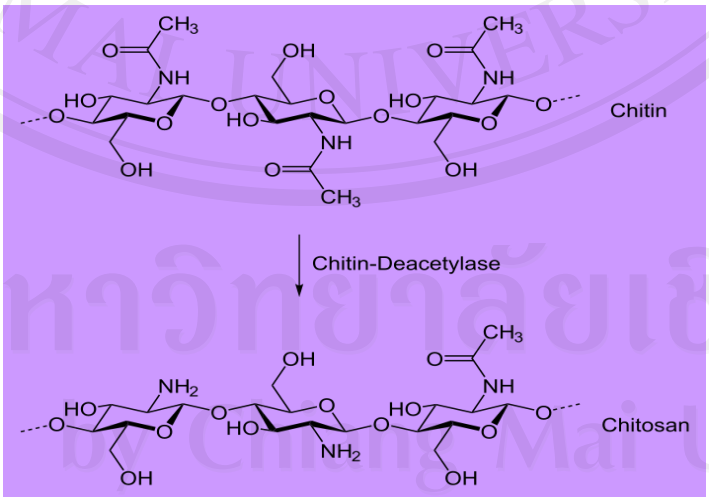


Figure 2.6 Structure of chitin and chitosan

(https://commons.wikimedia.org/wiki/File:Chitosan_Synthese.svg)

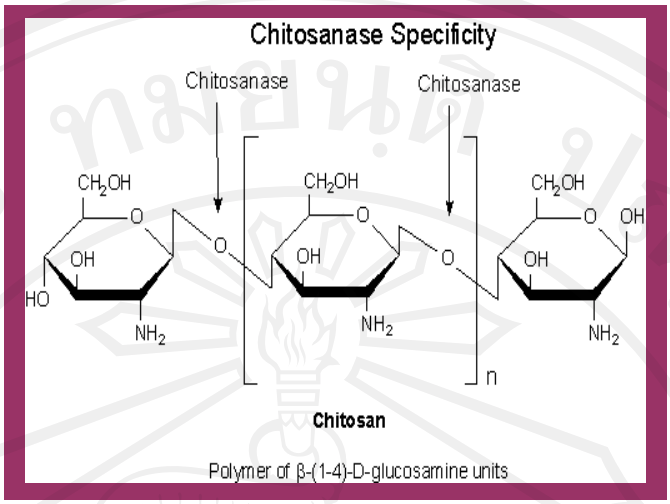


Figure 2.7 Structure of chitosan

(http://edu.go.vn/e-tap-chi/tin/9/90/6446/chitosan)

Chitosan is not soluble in pure water or organic solvents but it is soluble in slightly acidic solutions. Caner *et al.* (1998) studied the effect of different acids; acetic, formic, lactic and propionic acids, on their ability to dissolve chitosan. They discovered that acetic acid is the best solvent to use to produce a film with high water barrier and good mechanical properties. Kim *et al.* (2006) also show that the WVP of chitosan film was low for acetic acid and propionic acids and unaffected by pH (in a range of 3- 5). The tensile strength of chitosan film dissolved in aqueous acetic acid was the highest.

Properties of chitosan film depend on chitosan itself (molecular weight, degree of deacetylation), solvent used, drying condition, storage time, and temperature. Park *et al.* (2002) report that increasing molecular weight of the chitosan molecules resulted in improving strength of the films but did not significantly affects water vapor permeability (WVP). Cervera *et al.* (2004) found that high molecular weight chitosan absorbs more water than low molecular weight chitosan. Kim *et al.* (2006) studied the effect of the degree of deacetylation on properties of chitosan film. They found that low degree deacetylated chitosan films had lower WVP and high tensile strength compared with highly deacetylated chitosan films. The elongation values were not affected by degree of deacetylation. As pH increased, WVP of chitosan film

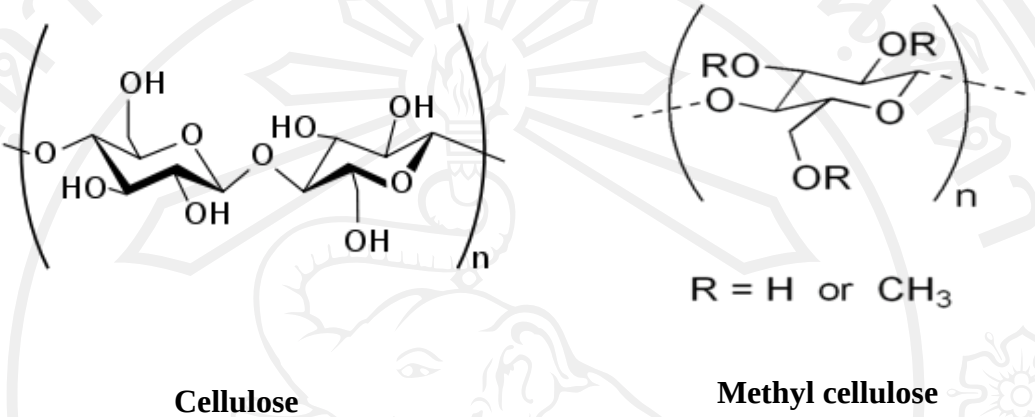
tended to increase while tensile strength decreased significantly. Highly deacetylated chitosan film was very sensitive to pH change in comparison to low degree deacetylated chitosan film. With increasing pH value, the degree of dissociation of chitosan decreased. High deacetylated chitosan has more amino groups that can be dissociated in an acid solvent than low deacetylated chitosan.

The ability of film to modify gas transport is important for tailoring such film to specific applications such as fresh fruits and vegetables (Guilbert *et al.*, 1996). The gas permeability of film from biopolymer depends on the nature of the gas, the structure of the material, temperature and moisture conditions. Generally, carbon dioxide permeates through plastic polymer more rapidly than oxygen. Film with high ratio values will allow carbon dioxide to escape from the package relatively easily, resulting in a low carbon dioxide concentration atmosphere. Since fruits and vegetables vary in their tolerance to carbon dioxide and in their ability to benefit from high percentages of this gas, the selectivity ratio value (ratio of carbon dioxide permeability to oxygen permeability) of a film is very importing for predicting the relative amounts of oxygen and carbondioxide that will accummulate in a package headspace. At very high relative humidity (RH) close to 100%, solubility of oxygen or carbondioxide molecules in the free water of the film becomes the main parameter for the transport of these molecules through the film. Microperforated synthetic films are marketed for their ability to achieve high permeability by allowing gases to move across the film via mass flow, which is much faster than the usual permeation process. However, mass flow does not provide the differential permeabilities to oxygen and carbondioxide that nonperforated films offer with resulting CO_2/O_2 permeability ratio values close to 1 (Gontard *et al.*, 1996).

2.2.2 Methyl cellulose

Methyl cellulose is a chemical compound derived from cellulose. It is a hydrophilic white powder in pure form and dissolves in cold (but not in water hot), forming a clear viscous solution or gel. It is used as a thickener and emulsifier in various food and cosmetic products, and also as a treatment of constipation. Like cellulose, it is not digestible, not toxic, and not allergenic. Methyl cellulose does not

occur naturally and is synthetically produced by heating cellulose with a caustic solution (e.g. a solution of sodium hydroxide) and treating it with methyl chloride. In the substitution reaction that follows, the hydroxyl residues (-OH functional groups) are replaced by methoxide (-OCH_3 groups) (Figure 2.8).



Cellulose **Methyl cellulose**

Figure 2.8 Structure of cellulose and methylcellulose (http://en.wikipedia.org/wiki/File:Methyl_cellulose.png)

Different kinds of methyl cellulose can be prepared depending on the number of hydroxyl groups substituted. Cellulose is a polymer consisting of numerous linked glucose molecules, each of which exposes three hydroxyl groups. The Degree of Substitution (DS) of a given form of methyl cellulose is defined as the average number of substituted hydroxyl groups per glucose. The theoretical maximum is thus a DS of 3.0, however more typical values are 1.3–2.6. Different methyl cellulose preparations can also differ in the average length of their polymer backbones.

Methyl cellulose (MC) has a low~~er~~ critical solution temperature (LCST) between 40°C and 50°C. At temperatures below the LCST, it is readily soluble in water; above the LCST, it is not soluble, which has a paradoxical effect that heating a saturated solution of MC will turn it solid, because methyl cellulose will precipitate out. The temperature at which this occurs depends on DS-value, with higher DS-values giving lower solubility and lower precipitation temperatures because the polar hydroxyl groups are masked.

MC has an excellent film-making property, high solubility, efficient oxygen and lipid barrier properties (Donhowe and Fennema, 1993; H. J. Park *et al.*, 1993). They are also selectively permeable to CO_2 and O_2, and hence, retard the respiration and ripening of many fruits and vegetables by limiting the availability of O_2. Hydrophilic films and coatings, such as polysaccharides, provide a good barrier to CO_2 and O_2 under certain conditions, but a poor barrier to water (Park and Chinnan, 1995). The poor water vapor barrier property allows the movement of water vapor across the film, thus, preventing water condensation that can be a potential source of microbial spoilage in horticultural commodities (Park *et al.*, 1994).

MC is nonionic cellulose and provides a strong and flexible water-soluble film. It offers superior toughness, flexibility, transparency and oil resistance. MC film has an excellent barrier to oxygen and aroma compounds. However, it is hydrophilic because of containing hydroxyl groups. Although MC is the least hydrophilic cellulose , MC film is still a poor moisture barrier (Biquet and Labuza, 1988). Turhan and Sahbaz (2004) pointed out that water vapor permeability (WVP) of MC films were 25-500 times greater than those of synthetic films.

2.2.3 Composite films

Composite films are generally designed to take advantages of the properties of the individual components. Generally, films composed of one primary substance either act as good barriers or have good mechanical properties, but not both. Polysaccharide and protein films have good barrier to oxygen but are hydrophilic. Lipid films provide good moisture barrier, but are weak. The mechanical and barrier properties of composite biopolymer films strongly depend on the constituting polymer characteristics and their compatibility (Butler *et al.*, 1996; Donhowe and Fennema, 1993).

Chitosan is the second most abundant polysaccharide on earth and is inherently antimicrobial (Goldberg *et al.*, 1990). Furthermore, it provides films with good mechanical and oxygen barrier properties (Caner *et al.*, 1998). However, chitosan has poor tensile strength when wet. It is rigid (high elastic modulus and small elongation) and has poor elongation properties. Blending methylcellulose with chitosan can be expected to correct these weaknesses. Higher elongation and lower

elastic modulus of composite films indicated the importance of hydrocolloid interactions. Film flexibility has been shown to increase with increasing methylcellulose content (García *et al.*, 2004; Pinotti *et al.*, 2007).

2.3 Antimicrobial Activity of Coating Solutions

Antifungal activity of films and solutions based on chitosan against typical seed fungi were also reported by Ziani *et al.* (2009). The antifungal activity was conducted against three fungi, *Aspergillus niger, Alternaria alternata and Rhizopus oryzae*. Results indicated important and significant differences of the antifungal activity between chitosan based solutions and chitosan based films. Furthermore, the antifungal activity of the different treatment depended on the type of fungus treated. Thus, chitosan film treatments were significantly more effective on *A. niger* than solution treatments. On the other hand, solution treatments resulted in higher radial inhibition when applied against *A. alternata* or *R. oryzae*. The highest radial inhibition was observed against *A. alternata* (97%) using a chitosan solution. The influence of the other parameters (concentration, molecular weight and surfactant type) on treatment efficiency was not as important and their significance depended on treatment type and fungus nature.

2.3.1 Mechanism of antimicrobial property of chitosan

Chitosan interfered with negatively charged residues of macromolecules exposed on the fungal cell surface. This interaction leads to the leakage of inter cellular-electrolytes and proteinaceous constitutes (Leuba and Stossel, 1986). Chitosan interferes with the interaction of diffused hydrolysis products with microbial DNA, which leads to the inhibition of mRNA and protein synthesis (Hadwiger *et al.*, 1986) and the chelating to metals, spore elements and essential nutrients (Cuero *et al.*, 1991).

Tanapaisankit *et al.* (2005) reported effects of chitosan on *Lasiodiplodia theobromae* in rambutan where 0.18% chitosan inhibited mycelium growth to 69% in PDA; 0.04% chitosan inhibited mycelium growth to 34.94% in PDA; 0.18% coated fruits was shown to inhibit fruit rot; dipping in 1000 ppm benom 0.18% coated fruits

inhibited fruit rot, as did dipping in 1000 ppm benomyl. For example, as chitosan increased from 0.075 to 0.6%, the redial growth of *Alternaria alternate*, *Botrytis cineria, Colletotrichum gloeosporioides* and *Rhozopus stolonifer*, decreased (El-Ghaouth *et al.*, 1992a). 0.075% chitosan upwards reduced spore viability and germ tube growth of both *Botrytis cineria* and *Rhozopus stolonife*r (El-Ghaouth *et al.*, 1992b).

The effect of chitosan and its components (citric acid and potassium sorbate) on the growth and morphology of *Lasiodiplodia theobromae*, one of the major postharvest pathogens of fresh longan fruit was investigated (Apai, 2009). The results indicated that chitosan and citric acid, potassium sorbate treatment, or combined, significantly reduced postharvest fungal rot of fruit compared with control challenged with *L. theobroma.* 1.2% chitosan + 0.3% potassium sorbate + 3% citric acid combination showed high efficacy to delay the lowest disease development compared to those treated with 0.3% potassium sorbate + 3% citric acid or only 0.3% potassium sorbate.

2.3.2 Antimicrobial agents

The incorporation of antimicrobial agents into chitosan film can create an environment inside the package that may delay or prevent the growth of microorganisms on the product's surface and lead to an extension of its shelf-life (Cha and Chinnan, 2004).

Vanillin (4-hydroxyl-3-methoxybenzaldehyde) is an organic compound with the molecular formula $C_8H_8O_3$. Its functional groups include aldehyde, ether, and phenol. Vanillin is the primary component of the extract of the vanilla beans and is a principle flavor compound used in numerous foods. It is also found in roasted coffee and the Chinese red pine. Synthetic vanillin, instead of natural vanilla extract, is sometimes used as a flavoring agent in foods, beverages, and pharmaceuticals. Vanillin has been reported to act as an antioxidant and recent reports have shown that vanillin can be effective in inhibiting bacteria, yeast and fungi.

The antimicrobial activity of vanillin depended on the time of exposure, concentration and the target organism. Recent reports have shown that vanillin can be effective in inhibiting bacteria, yeasts and molds (Cerrutti and Alzamora, 1996;

Fitzgerald *et al.*, 2004; Jay and Rivers, 1984). Cerrutti *et al.* (1997) treated strawberry puree with a mild heat treatment combined with 3000 mg/L vanillin and 500 mg/L ascorbic acid. They found the inhibition of native and inoculated flora growth for at least 60 days storage at room temperature. Penney *et al.* (2004) found that vanillin at 2000 mg/L suppressed fungal and total microbial growth in yoghurt significantly over a 3 week period.

The effects of vanillin and plasticizer on properties (mechanical, barrier, optical and thermal properties) of chitosan-methylcellulose based film was also studied (Sangsuwan *et al.*, 2008a). In this study, authors concluded that when the vanillin concentration was increased at the given PEG level, film flexibility decreased while tensile strength increased slightly. Vanillin also increased the barrier to oxygen but not water vapor. Increasing vanillin content resulted in less transparency and more yellowish tint. The bulky nature of vanillin reduced film crystallization. When PEG concentration was increased at a given vanillin level, it resulted in greater film flexibility but reduce film strength. Water vapor permeability (WVP) and oxygen permeability (OP) increased with increase in PEG content. Additionally, PEG contributed less to the opacity, yellowness and crystallization of the film.

2.3.3 Carbendazim

Carbendazim (methyl-2-benzimidazol carbamate) is a widely used broad-spectrum benzimidazole fungicide that plays a very important role in plant disease control. It is also used in postharvest food storage, in seed pre-planting treatment, and as a fungicide in paint, paper and wood. The molecular formula of carbendazim is $C_9H_9N_3O_2$ (Figure 2.9).

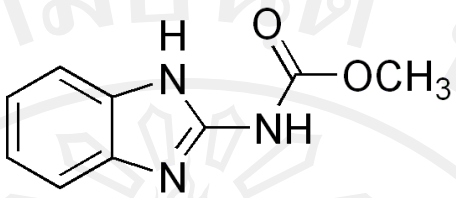


Figure 2.9 Structure of carbendazim
(www.alanwood.net/pesticides/carbendazim.html)

2.3.3.1 Carbendazim properties, antimicrobial mechanism and application

The main compounds of the benzimidazolic family are benomyl, carbendazim, thiabendazole and thiophanate-methyl. Benomyl is a systemic fungicide, applied to the soil to control a variety of fruit diseases. Benomyl rapidly degrades to carbendazim its main degradation product, and this is also a systemic fungicide, used to combat a wide range of diseases. It is believed that the fungicidal activity of benomyl is due to the presence of carbendazim (Chao-Hong Liu, 1990; Clemons and Sisler, 1971; Guan *et al.*, 1994). Carbendazim inhibits the polymerization of free tubulin molecules by binding an arginin residue of the β-tubulin subunit and acts by disrupting cell division through linking to the nuclear spindle, which inhibits fungal growth. This fungicide has been used in the control of fungal infections in vineyards and is indicated against *Botrytis cinerea*, *Uncinula necator*, *Plasmopara viticola* and other fungi. It can be used either alone or coupled with other fungicides that exhibit different mechanisms of action (IPCS-Inchem, 2010)

The inhibitory effect of carbendazim on *Arbuscular mycorrhiza* fungi is well documented (Kling and Jakobsen, 1997; Kough *et al.*, 1987) (Kough *et al.*, 1987; Thingstrup *et al.*, 1994, Kling *et al.*, 1997). But reports concerning plants are very limited. Dumpe and Wokoma (2003) observed that carbendazim at a lower concentration possesses germination stimulation of plantain hybrid seeds. Also, it is reported that carbendazim could improve the performance of seeds under PEG-6000 induced water stress conditions (Dumpe and Wokoma, 2003). Moreover, benomyl, carbendazim, thiophanate methyl and fluazinam were tested in vitro and in vivo for

their effects on *Rosellinia necatrix*. Benomyl, carbendazim and thiophanate methyl at $\geqslant$0.5 μg ml^{-1} totally inhibited *R. necatrix* mycelial growth on PDA medium. At 0.1 μg ml^{-1}, carbendazim and fluazinam inhibited growth by 97% and 84%, respectively, in comparison with fungicide-free medium. Benomyl and thiophanate methyl had less effect (53% and 22%, respectively) at this dose. Mycelia treated with fungicide were tested for their capacity to grow when transferred to fungicide-free medium one month after treatment, demonstrating the fungi static effect of fluazinam in comparison with the other systemic fungicides assayed.

Medina *et al.* (2007) evaluate carbendazim effectiveness against growth of *Aspergillus carbonarius* isolated from grapes and OTA production. A medium based on red grape juice was used in this study. Three factors assayed and the interactions a_w–carbendazim, concentration and a_w–temperature had significant effects on lag phase duration. The highest lag-times were observed at 0.94 a_w, 20 °C, and with 450 ng carbendazim/ml. The three factors also had significant effects of the growth rate and there was an interaction between a_w and temperature. The growth rate of *Aspergillus carbonarius* in these cultures is favored by high water availability and relatively high temperatures. However, addition of carbendazim at the assayed levels did not significantly influenced fungal growth rate. Accumulation of OTA was studied as a function of four factors (the three previously considered, and time). All factors had significant effects on the accumulation of OTA. There were also two significant interactions (a_w–temperature and temperature–time). On the basis of the results obtained, carbendazim does not increase the lag phase of *Aspergillus carbonarius* except at relatively low a_w and temperatures. It does not substantially decrease fungal growth rate once growth is apparent but it appears to cause an increase in OTA accumulation in the medium at the doses assayed. Carbendazim, which is widely used against fungal infections in grape, can positively influence OTA production by *A. carbonarius* in field, which can increase OTA content in grape juices and wines.

2.3.3.2 Carbendazim dose, tolerances and residue

Carbendazim is the ISO approved common name for methyl 2-benzimidazole carbamate, a systemically active benzimidazole fungicide that inhibits the synthesis of

-tubulin. It is absorbed through the roots and green tissues, with translocation acropetally and acts by inhibiting development of the fungal germ tubes, the formation of appressoria and the growth of mycelia. Carbendazim products are used for the control of a wide range of fungal diseases such as mould, spot, mildew, scorch, rot and blight in a variety of crops. The target crops include cereals, fruits (pome, stone, citrus, currant, strawberry, banana, pineapple, mango, avocado, etc.), vines, hops, vegetables, ornamentals, cotton, pasture, turf and mushrooms. Depending upon the crop, it is applied as either a spray (low and high volume) or a post-harvest dip, with multiple applications depending upon the crop and disease. Listed application rates for products are in the range 25-100 ml/100 L (all products registered for use on crops contains 500 g/L or 500 g/kg carbendazim). Products containing carbendazim are also registered for use as fungicides for the control of sapstain and mould on freshly sawn timber. Application is by spraying or dipping (APVMA, 2007).

Table 2.3 The maximum residue level (MRL) set for carbendazim in fruits and vegetable (IPCS-Inchem, 2010)

Country	Fruits	Maximum Residue Level (ppm)
Australia	Apples and pears	2.0
	Cucumbers	0.5
	Peanut kernels	0.1
Federal Republic of Germany	Citrus fruit	10.0
	Grapes	3.0
	Vegetables	1.0
	Citrus fruits (without peel)	1.0
	Stone fruit	1.0
	Cucumbers, cereals	0.5
	Bananas (without peel)	0.2
	Other plant produce	0.1
Netherlands	Fruit and vegetables	2.0
	Raw cereals (provisional)	0.5
The EU	Fruit and vegetables	0.2

Carbendazim was previously evaluated the accepted daily intake (ADI) by the Joint Meeting in 1973, 1977, 1983, 1985, and 1995. In 1995, an ADI of 0–0.03 mg/kg body weight (bw) was established based on the NOAEL of 2.5 mg/kg bw per day in a 2-year study in dogs and a safety factor of 100. Carbendazim has low acute toxicity: the oral LD50 is > 10,000 mg/kg bw in rats (FAO, 2005). According to (FAO, 2005), an acute reference dose (ARfD) was established, at 0.1 mg/kg bw for women of childbearing age and 0.5 mg/kg bw for the general population, including children. For tolerances of carbendazim in fruits and vegetables, it was variously established depending on the different countries. According to country, the Maximum Residue Level (MRL) set for carbendazim in fruits and vegetable have been established as Table 2.3 (IPCS-Inchem, 2010).

2.4 Application of Antimicrobial Coating Solutions for Fruits

With chitosan, this has numerous applications in preservation of fresh food, especially for smooth-skin fruit. Martín-Diana *et al.* (2009) reported that increases in chitosan concentration extended the quality of the orange juice significantly ($p < 0.05$), reducing enzymatic and non-enzymatic browning and controlling the spoilage during the storage time; however, concentrations > 1 g/l produced a significant ($p > 0.05$) reduction in the concentrations of ascorbic acid and carotenoids associated with the positive charge of chitosan and its ability to flocculate and coagulate negatively charged substances. Also, concentrations > 1 g/l were scored as unacceptable for the sensory panel due to an increase in bitterness. The study recommends the use of chitosan at concentrations up to 1 g/l to extend quality and preserve ascorbic acid and carotenoids during storage time of fresh orange juice**.**

Vargas *et al.* (2006) studied on quality of cold-stored strawberries as affected by chitosan–oleic acid edible coatings. Coatings had no significant effects on acidity, pH and soluble solids contents of strawberries throughout storage. In contrast, coatings slowed down changes in the mechanical properties and slightly modified respiration rates of samples. Addition of oleic acid not only enhanced chitosan antimicrobial activity but also improved water vapor resistance of chitosan-coated samples. Sensory analysis showed that coating application led to a significant

decrease in strawberry aroma and flavor, especially when the ratio oleic acid: chitosan was high in the film.

Devlieghere *et al.* (2004) reported that chitosan coating of strawberries was applicable while on mix lettuce the chitosan coating was not applicable due to the development of a bitter taste. Strawberries coated with chitosan tasted bitter on day 0, this abnormality disappeared after 3 days of storage at 7^{o}C. The microbiological load on the chitosan-dipped samples was lower for both products. The antimicrobial effect of chitosan on lettuce disappeared after 4 days of storage, while it maintained on the strawberries during 12 days. Chitosan treated strawberries had a better texture than untreated strawberries. Savage (1994) found that apples coated with chitosan had reduced incidence of mold growth over 12 weeks. Cheah and Page (1997) found that Sclerotinia rot of carrots coated with 2 or 4% chitosan was significantly reduced from 88 to 28%.

Srinivasa *et al.* (2002) studied mango storage packed using biodegradable chitosan film. Mature, green mangoes were desapped and later washed thoroughly with running tap water, then dipped in carbendazim (500 ppm) solution for 15 min to prevent fungal attack. Four mangoes were placed in each carton box (160 x 220 x 75 mm) (wax coated inside), whose top surface was covered with either chitosan film or LDPE film. All the boxes were kept at room temperature (27± 1 °C, at 65% RH). Results showed that fruits stored in chitosan-covered boxes showed an extension of shelf-life of up to 18 days and without any microbial growth and off-flavor. Moreover, chitosan films exhibit good antimicrobial activity which can help extend the storage life of food products (Dutta *et al.*, 2009; M. Vargas *et al.*, 2009).

Treated mandarin fruit with carboxymethyl cellulose from sugar beet pulp cellulose and stored in storage chamber at 25°C and 75% relative humidity delayed the soluble solids content, titratable acidity and ascorbic acid loses in comparison to the uncoated mandarins. Moreover, coating mandarin surface with emulsions containing carboxymethyl cellulose from sugar beet pulp was possible to extend the storage period with lower weight loss until 27 days by cellulose as a hydrophilic polymer (Togrul and Arslan, 2005).

The effect of potassium sorbate incorporated with chitosan on longan fruit decay at 10°C for 15 days storage was reported (Apai, 2009). Application of 0.3-1%

(w/v) potassium sorbate in Cts coating produced better fruit decay control in the fruit by reducing in mold population including disease incidence percentage in comparison with control fruit and no additive of potassium sorbate in the coating. Addition of 0.3% potassium sorbate in coating showed the optimal concentration to control fruit decay. Moreover, sorbic acid residue of this treatment was not detected on the aril whereas sorbic acid degradation in pericarp was increased as application of potassium sorbate concentration in coating was increased. However, addition of potassium sorbate at higher concentrations could disturb some coating properties by reducing viscosity and increasing the pH of solution.

With researching vanillin film used to preserve fruits**,** Sangsuwan *et al.* (2008b) researched the effect of chitosan/methyl cellulose films on microbial and quality characteristics of fresh-cut cantaloupe and pineapple. Chitosan/methyl cellulose film and vanillin film provided an inhibitory effect against *Escherichia coli* on fresh-cut cantaloupe. The chitosan/methyl cellulose film rapidly reduced the number of *Saccharomyces cerevisiae* yeast inoculated on cantaloupe and pineapple. Vanillin film was more efficient than chitosan/methyl cellulose in reducing the number of yeast, which decreased by 4 logs in fresh-cut pineapple on day 6. Vanillin film increased the intensity of yellow color of pineapple. However, vanillin film reduced the ascorbic acid content in pineapples. At the end of storage, ascorbic acid in pineapples wrapped with vanillin film was only 10% of its original concentration.

In Vietnam, chitosan film is a popular material that has been used recently. Dragon fruits were harvested at 28 to 30 days after blooming. Then the fruits were coated with chitosan film 1.5% (w/v). Preservation was maintained at low temperatures 6-8°C and relative humidity at 50-60%. Before being coated, the fruits had been treated with chlorine and benomyl solution at 100ppm and 500ppm, respectively. The results showed that the shelf-life of dragon fruits extend up to 6 weeks without changing quality (Phương, 2006). Chitosan films also apply for mangoes preservation. Dipping mangoes and oranges in chitosan solution and preserving them in low temperature, the shelf-life of the fruit were extended 4-6 weeks, and up to 8 weeks respectively, and pomelo coated with chitosan maintained quality to 3 months at normal temperature (VFVA, 2008).