DEVELOPMENT OF CHITOSAN COATING TO IMPROVE QUALITIES OF THAI TANGERINE (*CITRUS RETICULATE* BLANCO CV. KIEW WAN)

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ABSTRACT

The coating of tangerine is widely practiced in Thailand. At high temperature the coated tangerine developed off-flavor and decayed rapidly. The weight loss of coated tangerine was minimized at low temperature storage. The suitable coating for tangerine storage at high temperature is needed. The effect of storage temperature and time on total soluble solid, pH, titratable acidity, ascorbic acid, total polyphenol, phenolic acids and antioxidant activity of Thai tangerine (*Citrus reticulate* Blanco cv. Kiew Wan) coated with shellac (commercial coating) was investigated. The coated fruits were stored at 4, 12, 20 and 30°C for 5 weeks and samples were taken out weekly for analysis. The total soluble solid, pH and titratable acidity of coated tangerine were affected by coating treatment, storage temperatures and time. Ascorbic acid decreased during storage at all tested temperatures. The major phenolic acids found were ferulic acid followed by sinapic, caffeic, and *p*-coumaric acid. The 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2 azinobis (3-ethylbenzothiazoline-6-sulfonate) (ABTS) antioxidant activity increased during storage and were correlated with total polyphenol and phenolic acids. Sinapic, caffeic, and *p*-coumaric acid are significantly correlated with the DPPH while ferulic acid was correlated with the ABTS. All hydroxycinnamic acids increased during the early stage of storage at 4 and 12°C but decreased at the end. At high temperature (30°C) the hydroxycinnamic acids in coated were increased except ferulic acid increased at the end of storage of coated tangerine, but it decreased in uncoated tangerine. Chitosan is a biodegradable film or coating. It have some positive advantages of chitosan are antifungal activity against several species. For development of chitosan coating the Response surface method (RSM) with central composite design was used to optimize the coating formula from chitosan and oleic acid suitable for tangerines displaying at room temperature (30±2°C). The response effect of chitosan (1-2%) and oleic acid (1-4%) on weight loss, ethanol in juice, carbon dioxide, and oxygen in fruits were investigated. The optimum formulation for tangerine was found to be 1% of chitosan (v/v) and 2.5% of oleic acid (v/v), which was based on the composite desirability value of 0.80. Chitosan-oleic acid coating can delay fruit degreening and inhibit spoilage of tangerine. The comparison of the chitosan-oleic coating and the shellac was conducted. The shellac coated tangerine produced the higher light reflectance than the chitosan-oleic. Tangerine coated with shellac produced more volatile compounds than the chitosan-oleic acid coated one. Weight loss was higher with chitosan-oleic than shellac. Tangerine coated with chitosan-oleic retained ascorbic acid content more than with shellac. Both coating material showed no significant change of hydroxycinnamic acids. The antioxidant activity measured by DPPH and ABTS increased during storage regardless of coating types. The phenolic compound was related to the enhancement of antioxidant capacity of tangerine. The coating treatment affected on the sensory evaluation of tangerine juice. The shellac based coating showed lower sensory scores with taste, flavor and acceptability when compared to the chitosan-oleic coating. The tangerine coated with chitosan-oleic acid can be stored for 4 weeks, but the uncoated and shellac coated for 3 weeks at 30±1°C. From the results it can be suggested chitosan-oleic coating is suitable for tangerine, in addition it can reduce the use of fungicide was in tangerine packing house.

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CHAPTER 1

INTRODUCTION

Tangerine (*Citrus reticulate*, Blanco), locally called Som kiew wan, is the major citrus fruit grown in Thailand. It is mostly consumed fresh but some are used for juice. Citrus fruits are sources of important nutrients for human health including ascorbic acid, dietary fiber, phenolic compounds and phenolic acids. Rapisarda et al. (2008b) reported that bioactive compounds, total polyphenol, vitamin C and hydroxycinnamic acids in blood oranges increased during storage at low temperatures. However, the nutrition, chemistry and flavor of tangerine can change during postharvest storage and treatment. To maintain the quality of fruits after harvesting many practices have been applied. Storage condition is a prime factor influencing the quality changes particular with chemical composition such as the ascorbic acid. It has been accepted that ascorbic acid and other antioxidants in fruits including phenolic compound, lignin, flavonoid and phenolic acids play the significant role in human health. The changes of these compounds may reduce the quality of fruits.

Application of a coating to fruits is a method to maintain fruit quality and extend shelf life. Coatings of apples and oranges have long been practiced to extend shelf-life and improve glossiness. Presently in Thailand, coating of oranges has become common practice in many packing houses but coated fruits can result in quality detioration if storage conditions are unsuitable. Coating tends to restrict the exchange of O_2 and CO_2 if the O_2 in fruit is too low and CO_2 is high then anaerobic respiration can occur which leads to off-flavor development (Hagenmaier 2002; Baldwin et al., 1995). Many commercial coatings have been developed to coat citrus fruit such as shellac, carnauba wax, hydrocolloid and polyethylene based. Most of coating used in packing houses imported from foreign country and exact formulations of commercial coatings are trade secrets.

Using of suitable of coating materials and storage temperature are important to maintain quality of tangerine. In the tropical countries most of fruits are displaying and transportation at ambient temperature of around 28-30°C. At high temperature, changes are rapid softening of fruit and development of objectionable taste and off-flavor.

Chitosan is one of interesting polymers that gain more attention from food industry. It is a deacetylated form of chitin and a biodegradable polymer soluble in dilute organic acid solution.

It is used in formation of films and coatings. The advantages of chitosan are antifungal activity against several species and decreasing of transpiration losses in fruits. For coating, chitosan was used to prolong shelf life and control decay of many fruits including Chinese water chestnut, strawberry, longan, apple, mango, murcott tangor, fortune mandarins, and valencia oranges. In Thailand, chitosan can be produced from fishery industrial waste, therefore using chitosan coating base is beneficial to the country. Currently, no studies have been reported using chaitosan-oleic acid coating for tangerine during storage at room temperature. The objective of this study is to provide the suitable postharvest treatments to prolong shelf life of tangerine for storage at high temperature.

The objectives of this research are:

1. To study changes in chemical composition, phenolic acid and antioxidant activity of commercial coated tangerines at different storage temperatures.
2. To optimize the chitosan formulation for coat tangerines storage at high temperature.
3. To compare the quality of fruit coated with chitosan and the commercial based coating material.

CHAPTER 2

LITERATURE REVIEWS

2.1 Tangerine

Tangerine (*Citrus reticulate*, Blanco) is an orange to orange-yellow in color citrus fruit, flattened in shape, 2 to 4 inches in diameter, and a type of mandarin orange. They are smaller than the most oranges, and the skin of some varieties peels off easily. They taste more sour and sweet than other oranges. In general, tangerines are grown in the north of Thailand. It is the loose-skin citrus are citrus fruits that Thailand has been producing in large quantity, available all the year and the peak period is from October to February. Standard of tangerine mandarin included the juice content of not less than 35% juice weight divided by fruit weight (w/w), total soluble solids content (TSS) is 9.0 degrees Brix up for Sai Num Phueng or Shogun cultivars. For other loose-skin (easy peeler) a citrus fruit is 8.0 degrees Brix up and the ratio between degrees Brix and %acid is not less than 13:1(National Bureau of Agricultural Commodity and Food Standards, 2007).

2.2 Nutrition and health benefit of tangerine

Citrus fruits are sources of important nutrients for human health including ascorbic acid, dietary fiber, phenolic compounds and phenolic acids (Tripoli et al., 2007; Rapisarda et al., 1999; Wang et al., 2007). Many studies have been reported that ascorbic acid and phenolic compounds in citrus fruit play the important roles in antioxidant activity (Abeysinghe et al., 2007; Franke et al., 2004; Gorinstein et al., 2004). Ascorbic acid is the water soluble antioxidant while some other antioxidants may be soluble in both hydophillic and hydrophobics media including limonoids and flavonoids. Phenolic acids are aromatic secondary plant metabolites which are classified into hydroxycinnamic and hydroxybenzoic structures. Hydroxycinnamic acids in berries (Kahkonen et al., 2001) and oranges (Rapisarda et al., 2008a) showed a high correlation with antioxidant activity. Caffeic, *p*-coumaric, ferulic and sinapic acid are hydroxycinnamic acids found in nearly all food plants, and exist in fruits in both free and bound forms. Only small fractions exist as free acid. The major fractions are bound to structural component such as cellulose, lignin and protein.

Ferulic acid is the major phenolic acid in citrus fruits (Rapisarda et al., 1998). Tangerine and orange also have carotenoids, especially β-criptoxanthine (Rodriguez-Amaya. 1999).

Vitamin C, phenolic acid, and pectin provide various health benefits. Vitamin C, acting as an antioxidant, may reduce the risk of arteriosclerosis, cardiovascular diseases, and some forms of cancer (Diplock, 1994). Economos and Clay (1999) reported several classes of phytochemicals including monoterpenes, limonoids (triterpenes), flavanoids, carotenoids and hydroxycinnamic acid, have been isolated from citrus which being qualified them for prevention of many diseases, such as anemia, neural tube defects, cardiovascular diseases, stroke and cancer. Baker (1994) reported that both the edible and inedible parts of citrus fruit are rich source of pectin, which has multiple biological activities, including glycemic and cholesterol level control.

Hydroxycinnamic acid compounds, such as caffeic acid, ferulic acid, chlorogenic acid, and *p*-coumaric acid can be inhibit oxidation of low-density lipoprotein (Meyer, et al., 1998) which have anticancer (Kual and Khanduja, 1998) and antimicrobial activities (Kernan et al., 1998). Zhao and Moghadasian (2008) reported that the intake of ferulic acid might offer beneficial effects against cancer, cardiovascular disease, diabetes and Alzheimer's disease.

2.3 Storage of tangerines

Tangerines are specialty fruits and generally not suited to long-term storage when compared to others sweet oranges, grapefruits, limes, and lemons. These fruits are very susceptible to stem-end rots and blue-mold decay. Successful postharvest storage of citrus depends to a large extent on minimizing losses from fungal decay, weight loss and softening; the latter leads to fruit deformation that adversely affects its marketability (Grierson and Ben-Yehoshua, 1986).

Specific conditions are necessary for the storage of each cultivar, as fresh fruit is likely to develop off-flavor, lose fresh appearance, and marketability. Longer storage duration with more than 10–15% spoilage is not economical (Ladaniya, 2008). Storage of mandarin is not generally recommended, but if some storage is needed for orderly marketing it should be limited to 2–3 weeks or at the most 4 weeks at 4-6°C (Hardenburg et al., 1986). These fruits can be stored best at 2-6°C in general with slight variations in temperature range depending on the tolerance of the specific cultivar. The recommend storage condition for mandarin and tangerine was showed in Table 2.1.

Table 2.1 Recommended temperatures, relative humidity, and storage life of mandarin and tangerine

Citrus fruit	Temperature (°C)	Relative humidity (%)	Storage life (Weeks)
Ponkan	4–6	85–90	4–5
Satsuma	2–3	80–85	12–18
Clementine	3–4	85–90	4–6
Nagpur	6–7	85–90	6–8
Coorg (main crop)	5–6	85–90	6–8
Temple, Orlando, Dancy	4–6	90–95	2–5
Kinnow mandarin	3–4	85–90	8–12
Thai tangerine	2-5	90-95	2-4

Source: Adapted from Ladaniya. (2008) and National Bureau of Agricultural Commodity and Food Standards (2007)

2.4 Physical and chemical changes in citrus

Citrus fruits are non-climacteric, with persistently low in rate of respiration and ethylene production. They do not undergo any major softening or compositional changes after harvest, and can normally be stored for relatively long periods of 6–8 weeks at 10-12°C for lime and 2-4 weeks at 5-8°C for mandarin (Kader and Arpaia, 2002). Softening of fruit and development of insipid taste and off-flavor are the major deteriorative changes that take place during storage of citrus fruits. Changes are rapid if fruits are held under hot and dry ambient conditions; while under optimum refrigerated conditions with high relative humidity changes are gradual and at times seem insignificant. But at the end of long-term refrigerated storage, changes can be quite conspicuous. Postharvest treatments such as coatings, packaging, and various chemicals also influence these changes. Sometimes there are inconsistent and contradictory findings with respect to these changes; these can be attributed to experimental procedures and materials used (Ladaniya, 2008).

2.4.1 Physical changes

Weight loss

The loss of moisture as water vapor from fresh fruits and vegetables to the atmosphere is governed by Fick's law which states that the diffusion gases through a barrier varies inversely with the permeability of the pathway and directly with the magnitude of driving force (Purvis, 1994). Weight loss during storage is a major factor in the postharvest deterioration of citrus fruit. Weight loss is a combination of loss of water vapor from transpiration, and loss of carbon from the exchange of carbon dioxide for oxygen in respiration. Weight loss of fruit results in direct loss of sealable weight in addition, as 5-10% weight loss of orange can result in a shriveled appearance that can render them unsealable (Davies and Albrigo, 1998). The loss of weight involves mainly the peel, not the pulp of the fruit. Grierson and Ben-Yehoshua (1986) wrote the peel is a visible marketing feature, peel appearance is just as economically important than the flavor of the pulp. Washing operation in commercial packing houses was caused on the higher rate of weight loss due to the removal natural wax by the action of detergent and washing brushes (Hagenmaier and Baker, 1993). Coating treatments were affected on weight loss and reductions in transpiration rates of fruits by covering of the cuticle and blocking pores (Bank et al, 1993).

2.4.2. Chemical change in fruit composition during storage

2.4.2.1 Total soluble solids, sugars, and acidity

Changes in total soluble solids (TSS), sugars and titratable acidity (TA) contents of the juice depend on the conditions under which citrus fruits are stored (Ladaniya, 2008). Acidity of citrus juice is due primarily to citric and malic acid (Table 2.2). The titratable acidity of orange and grapefruit plays an important role in determining the maturity of these fruits. The sweetness of citrus fruits is due to the presence of glucose, fructose, and sucrose. The most important factor governing sugar content is maturity, especially with the varieties of citrus. Most soluble solid in orange, tangerine, and grapefruits, are sugars which occur in juice vesicle. Concentrations of reducing and non-reducing sugars are equal at maturity.

Higher ambient temperatures coupled with lower relative humidity result in rapid water loss and cause an increase in TSS and titratable acidity contents of mandarins (Dhillon et al., 1976; Chundawat et al., 1978). At high relative humidity acidity declines in various citrus fruits during ambient storage (Angadi and Shanthakrishnamurthy, 1992; Chattopadhyay et al., 1992). Somsrivichai et al. (1992) reported that TA was reduced and increase of TSS was not different in both unsealed and tangerine sealed with plastic film held at 13°C and room temperature (30°C). The higher level of TSS in unsealed fruit may be resulted from water loss during storage. Warming the fruits also reduced the ascorbic acid content but had little effect on sugar content. Reduction in acidity was mainly due to the result of a decrease in citric acid. Both citric and malic acid contents declined during cold storage.

Juice color, soluble solid content, pH and TA of Valencia orange kept in 60% CO_2 at 5°C for 5 to 14 days followed by holding in air at 5°C for 7 days were not significantly (Ke and Kader, 1990). Ladaniya and Sonkar, (1996) reported that under refrigerated storage conditions (about 4–6°C at 90% RH), juice content, TSS, and acidity contents declined gradually in Nagpur mandarins. Temple oranges (*C. reticulata* x *C. sinensis*) stored for 4 weeks at 14, 5, or 2°C, followed by 1 week at a shelf-life temperature of 17°C did not show any significant changes in the internal composition of the fruit. However, after 8 weeks, titratable acids and internal CO_2 were higher with lower O_2 content and unacceptable quality (Cohen et al., 1984). Loss in titratable acidity, sugars and vitamin C contents of Hayashi Satsuma were very slow when storage at 5°C than at 15°C up to 3 months (Izumi, et al. 1990). In Robinson tangerine increased in TTS (°Brix) value and significant decrease in citric acid during storage, however sucrose remained constant indicating that the in some citrus fruit cultivars is not always directly related to changes in the fruit's simple sugar content (Echeverria and Ismail, 1987).

Table 2.2 Organic acids in juice and peels of citrus fruits

Variety	juice (g/100 ml)		peel (meq/g dry wt)			
	Malic	Citric	Malic	Citric	Oxalic	Malonic
Orange						
Washington Navel I	0.06	0.56	0.02	0.01	0.11	0.02
Washington Navel II	0.20	0.93	0.02	0.01	0.10	0.03
Valencia	0.16	0.98	0.02	trac	0.13	0.03
Tangerine						
Dancy I	0.18	1.22	0.06	0.02	0.15	0.01
Dancy II	0.21	0.86	0.09	0.02	0.20	0.02

Source: Gorini and Testoni (1988)

2.4.2.2 Volatiles

Volatiles which give typical citrus odor emanate from fresh citrus fruit during storage. They include essence oil compounds from peel. The higher the storage temperature, the more rapid the loss. The volatile aroma of the stored Hamlin oranges was contributed by ethyl esters, particularly ethyl butyrate, while sesquiterpene hydrocarbons appeared to be responsible for the persistent odor from the cuticle of fresh oranges. Volatile compounds are definitely identified include ethyl acetate, ethanol, ethyl butyrate, limonene, ethyl caproate and ethyl caprylate. Production of ethanol and acetaldehyde increases with storage duration and temperature. The occurrence of ethanol is attributed to spoilage (Attaway and Oberbacher, 1968).

2.4.2.3 Enzymes

Activities of polyphenol oxidase and peroxidase (Chauhan et al., 1980) and polymethylesterase, polygalacturonase, and cellulase (Nagar, 1994) have been reported to be increased during ambient storage, indicating the senescence of citrus fruits. During CA storage (reduced O_2 and increased CO_2) of citrus fruits, activity of alcohol dehydrogenase increases, indicating anaerobic respiration (Davis et al., 1973). Superoxide dismutase activity is reported to decrease with an increase in malonaldehyde during storage of C. *reticulata* fruits at 5.4–14.6°C and 82–92% RH (Ladaniya, 2008).

Echeverria and Valich (1989) studied the relationship between the activities of gluconeogenic and glycolitic enzyme with postharvest changes in sugar and organic acids. Activities of the enzymes of acid metabolism (malic enzyme, isocitrate dehydrogenase/aconitase and alcohol dehydrogenase) either increased during the first 3 weeks (malic enzyme) or remained constant during storage of Valencia oranges at 15°C for 9 weeks. Activities of enzymes involved in sugar catabolism (hexokinase, sucrose synthase, UDPG pyrophosphorylase and PPi-dependent phosphofructokinase) increased during storage.

2.4.2.4 Ascorbic acid

Vitamin C is the most important vitamin in citrus fruits. The amount of this vitamin varies with variety, maturity, and other factors. As the fruit matures, the vitamin C content gradually decreases. During the harvesting season, the vitamin C content ranges from

0.3 to 0.6 mg/ml. Ascorbic acid is relatively stable in citrus products during process and storage. Ascorbic acid (vitamin C) content decreases during storage of citrus fruits under ambient and refrigerated conditions. The loss is more rapid at higher temperatures. There is a loss of 10–20% vitamin C in usual handling and marketing practices of fresh produce. The longer the storage duration was the greater the loss (Ladaniya, 2008).

L-Ascorbic acid (AA) is the main biologically active form of vitamin C and reversibly oxidized to form L-dehydroascorbic acid (DHA), which also exhibits biological activity. Further oxidation generates diketogulonic acid which has no biological function (Davey et al., 2000; Deutsch, 2000). DHA is easily converted to AA in human body, it is important to measure both AA and DHA in fruits and vegetables in order to monitor vitamin C of the activity (Lee and Kader, 2000).

2.4.2.5 Pectic substances

Pectic substances are polygalacturonides with non-uronide carbohydrate covalently bound to an unbranched chain of 1–4 linked α-galacturonic acid units. The carboxyl groups of polygalacturonic acids may be partly esterified by the methyl group and partly or completely neutralized by one or more bases. Important pectic substances include protopectin, pectinic acid, pectin, and pectic acid. These compounds have varying degrees of methyl ester content and neutralization. Pectic substances are deposited in the cell wall and middle lamella. The meristematic and parenchymatous tissues are rich in pectic substances. Pectic substances as a combination of hemicellulose and pectin gel are a part of cell structure. Pectin in the middle lamella can be called cement or adhesive between cells. Polysaccharides also form the important part of cell sap.

Fruit firmness decreases from the degradation of insoluble protopectin into the more soluble pectic acid and pectin. In citrus fruits these changes are relatively slow and less pronounced compared with climacteric fruits (Ladaniya, 2008). Echeverria and Valich (1989) reported that the increases in ^{O}Brix and solubilized sugars in the valencia orange juice as observed during postharvest storage is also due to cell wall hydrolysis by various enzymes such as pectinase, cellulase, hemicellulase, or pectinesterase.

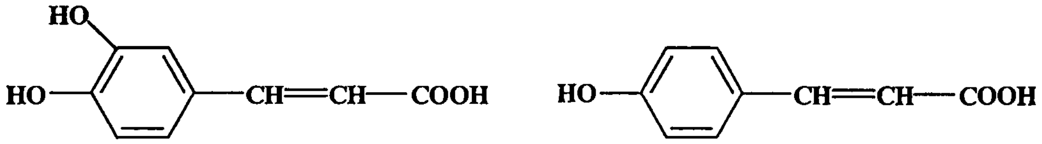
2.4.2.6 Phenolic compounds

Phenolic compounds are widely distributed in the plant kingdom and are considered to be secondary metabolites. Structurally they contain an aromatic ring bearing one or more hydroxyl groups, together with a number of other substituents. Plants provide nearly all the phenols found in higher animals, since higher animals are incapable of synthesizing compounds with benzonoid rings from aliphatic precursors. The polyphenolic composition of fruits varies in accordance with species, cultivar, degree of ripening and environmental conditions of growth and storage. Phenolics also contribute to colour, astringency, bitterness, and flavour in fruits. Phenolic compounds occurring in food materials are mostly of the flavonoid type. Of the naturally occurring flavonoid compounds, anthocyanidins, flavonols, and cinnamic acid derivatives occur most frequently in foods. Flavonols, together with flavones and flavanones are collectively termed anthoxanthin pigments which are light yellow in color.

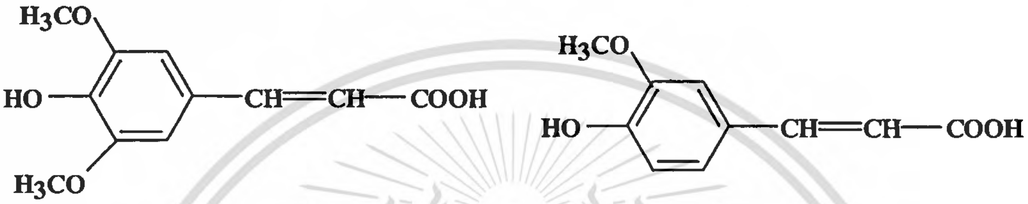
The hydroxycinnamic acids have their biosynthetic origin from phenylalanine. The conversion of phenylalanine to the various hydroxycinnamic acids and coumarins is referred to as general phenylpropanoid metabolism (Mann, 1987). The first step in phenylpropanoid metabolism is the elimination of ammonia from phenylalanine by the enzyme phenylalanine ammonia lyase (PAL) to produce cinnamic acid. Cinnamic acid is then hydroxylated to *p*-coumaric acid via the enzyme cinnamic acid 4-hyroxylase (Dewick, 1993). Other hydroxycinnamic acids are obtained by further hydroxylation and methylation reactions. Caffeic acid and ferulic acid are the most prominent phenolic acids occurring in foods of plant origin such as cereals, coffee, fruits and vegetables (Andreasen et al., 2000; Scalbert and Williamson, 2000; Robbins, 2003).

Major of phenolic compounds of oranges and juices are hydroxycinnamic acids (HCA) and flavonoids, among which flavanones are predominant. Hydroxycinnamic acids occur mainly as esters of ferulic, *p*-coumaric, sinapic and caffeic acids. Some references of hydroxycinnamic acids and flavanone content in commercial orange juices are available (Peleg et al., 1991; Robards et al., 1997; Tomás-Barberan and Clifford, 2000; Gil-Izquierdo et al., 2002; Belajová and Suhaj, 2004), however, they are differences margin because of differences in varieties of oranges, their ripeness and the technological processes used to obtained commercial juice. Hydroxycinnamic acids are more abundant in blood orange than in blond orange. Ferulic acid is the major phenolic acid of orange (Rapisarda et al., 1998).

The role of hydroxycinnamic acid compounds as antioxidants and free radical scavengers has been pointed out (Chen and Ho., 1997). The common natural hydroxycinnamic acids are caffeic, ferulic, *p*-coumaric and sinapic acid. These structures are shown in Figure 2.1.



Caffeic acid **p-coumaric acid**



Sinapic acid **Ferulic acid**

Figure 2.1 Structures of hydroxycinnamic acids

2.5 Coating materials

Many fruits such as tangerine, apples, bananas, mangoes, and grapes make their own natural waxy coating to help retain moisture. They develop a waxy coating on the epidermis, which is either felt or seen as a powdery bloom. Wax production by plants is known to be greatly influenced by environmental factors. Therefore it is not surprising that storage conditions can affect the postharvest changes in the amount and composition of wax.

Washing of the fruit is the first process in commercial packing line for remove dust, mold, and bacterial spores that can infect fruit and case decay. The dirt and spores have been removed, but also removed natural waxes of the cuticle. The removal of the waxes during washing has side effects that limit market life including the loss of water by transpiration and increase the respiration rate of the fruit. To prolong the market life, the industry used a coating to replace the natural wax. Some coatings are use for substitute natural wax in the fruit surface, to improve the barriers to water vapor. Thus reduces the rate at which water evaporates from the fruit surface and thereby slows loss of saleable weight. In many fruits, wax coating can also delay loss of juiciness and the onset of visible shrivels, protect produce from loss of value because of decline in quality. The advantages of coating fruit are as followed:

1. To reduce shrinkage due to water loss.
2. To provide a barrier to free gas exchange.
3. To improve appearance and marketability by application of shiny, sweat-resistant film.
4. To provide a carrier for decay control fungicides and growth regulators. (Kaplan, 1986)

Many material have been developed to suit the fruits, some of them are summarized as followed.

2.5.1 Wax type

Citrus fruits are commonly waxed because the natural wax has been removed at the washing stage in a packing house. Contemporary coatings include a collection of wax-type products which are generally accepted. This type of coating can provide good water vapor barriers and add gloss and shine to produce for attractive. Most wax coatings applied to citrus are microemulsions (Hagenmaier and Baker, 1993, 1994, 1996, and 1997).

Wax can be obtained from natural source or synthesis from petrochemical products.

2.5.1.1 Carnauba wax

Carnauba wax is derived from the palm tree *Copernicia prunifera*. Carnauba wax has a very high melting point and is used as an additive to other waxes to increase toughness and luster. In the 1950s, carnauba wax oil in water emulsions were developed for coating fresh fruits and vegetable (Kester and Fennema, 1986). Mannheim and Soffer (1996) shown the weight loss of mandarin coated with carnauba wax higher than fruits coated by carnauba mixed with shellac. Moreover carnauba waxes also affected on off-taste and off-flavor of mandarin.

2.5.1.2 Candelilla wax

Candelilla wax is obtained from the desert plant *Euphorbia antisiphylitica*. The wax is extracted from the plant with boiling water. Hagenmaier and Baker (1996) reported that the good gloss wax is made from candelilla and addition of gelatin or hydroxypropyl methylcellulose. However this wax type affects the internal atmosphere and anaerobic fermentation.

2.5.1.3 Polyethylene wax

Polyethylene wax is produced by the oxidation of polyethylene which is a petroleum by-product. Polyethylene wax is used primarily to make emulsion coatings. Polyethylene wax is permitted in the United States as a protective coating for fresh avocado, banana, limes, mango, and orange (Hagenmaier and Shaw, 1991). The polyethylene wax coating used in fruit often mixed with carnauba wax and other polymers. The disadvantage of polyethylene wax is less permeable coating, it has remarkable effects on fruit quality and increase the off-flavor in many citrus varieties.

2.5.1.4 Shellac wax

Shellac is a purified product of the hardened resinous secretion of the scale insect Kerria lacca (Kerr), a plant parasite found in India, Myanmar, and Thailand. The most commonly used coating applied to fruits in the U.S.A. citrus packing houses as their major ingredients a mixture of shellac and wood resin. The shellac coatings are designed to impart high-gloss (Hagenmaier, 2000) but this coating can affect low permeability gases; more affect the internal atmosphere and flavor change (Hagenmaier and Shaw, 1991, 1992). Hagenmaier and Shaw (2002) suggested that the high gloss shellac coating was not appropriated for tangerines due to high production of ethyl acetate, ethyl butyrate, isopentanol and 2-methyl-3-buten-2-ol, and were highly correlated with ethanol content. The optimum formulation for tangerine coating was 9.27% shellac and 1.3% carnauba wax showed the highest glossiness (Luangwilai et al., 2007).

2.5.2 Chitosan

Chitosan is a β-1,4-linked polymer of glucosamine (2-amino-2-deoxy-β-D-glucose) and lesser amounts of N-acetylglucosamine. It is formed by the deacetylation of chitin (poly-N-acetyl-glucosamine), in the presence of alkali (Figure 2.2). It is an abundant by product of the crab and shrimp processing industries. Chitosans are described in terms of the degree of deacetylation and average molecular weight and their importance resides in their antimicrobial properties in conjunction with their cationicity and their-forming properties (Muzzarelli, 1996).

The molecular weight of chitosan has an influence on the thermal, mechanical, and permeability properties of the films (Butler et al., 1996). The antimicrobial activity of chitosan was depend on several factors such as the kind of chitosan (DD and MW) used, pH of the medium, temperature, and food components (Devlighere et al., 2004). No et al, (2002) reported that chitosan showed higher antibacterial activities than chitosan oligomers.

Chitosan is a biodegradable film or coating which has been widely used in food and cosmetic industries. Some positive advantages of chitosan are antifungal activity (Devlighere et al., 2004, Galed et al., 2004), and decreasing of transpiration losses in fruits (Jiang and Li, 2001).

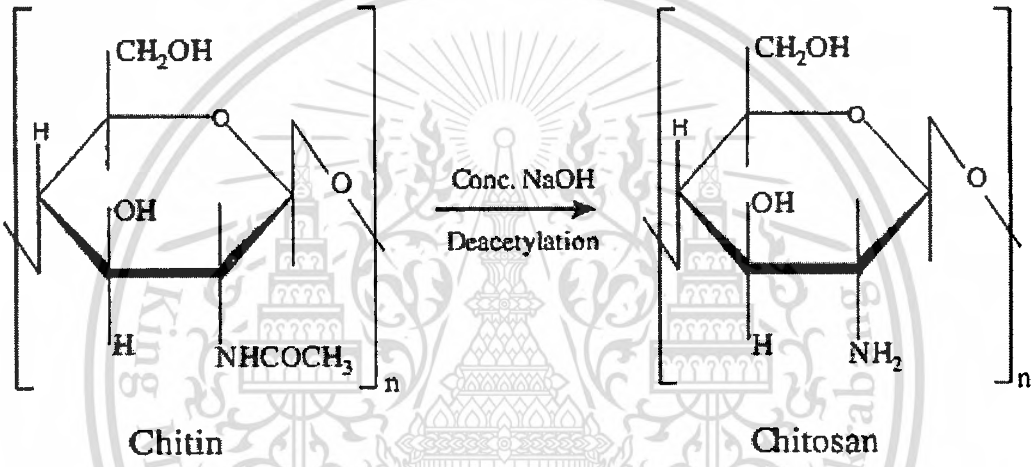


Figure 2.2 Structures of chitin and chitosan

Source: Dutta et al. (2009)

Chitosan as a coating material

The chitosan coating has been developed to prolong shelf life of egg, banana, Chinese water chestnut, strawberry, longan, apple, mango, murcott tangor, fortune mandarins, and valencia oranges (Choi, et al., 2002; Bhale, et al., 2003; Pen and Jiang, 2003; Galed, et al., 2004; Chien, et al., 2007a; Chien, et al., 2007b). The coating emulsion was prepared by dispersing chitosan (0.5-4%) in different concentration of acetic acid solutions (0.1-2%), the solutions were then adjusted to pH 5-6 with NaOH, and a surfactant (Tween 80) was added to improved wettability of chitosan coating.

Chien, et al. (2007b) suggests using 0.1% low molecular weight chitosan (LMWC) coating to help preserve the quality of minimally process fruits. In the other study, the formulation of chitosan coating for mandarin was in the range 1-2% (Nilprapruck, 2002).

The limitation of using chitosan in coating is due to its high water vapour permeability (Wong et al., 1992; Caner et al., 1998) which can result in high weight losses in fruits. To overcome this problem lipid materials and hydrocolloids may be added to improve the moisture barrier property of coating or film (Amarante and Banks 2001). Many lipid materials have been used to improve water vapour permeability of films including fatty acid, natural waxes, and resin (Vargas et al., 2009b). Lai and Padua, (1998) reported that many plasticizers can be used in coating film such as polyols, mono-, di-, or oligosaccharides, lipid and lipid derivatives. Oleic acid was used as the plasticizer in zein films. Morillon et al. (2002) reported fatty acids used as emulsifier and surface active agents in film and coating. Oleic acid is one of the fatty acids has been used in coating formulations for oranges and mandarin, it was used in range 0.5-3.8% (Chen and Nussinovitch, 2000a; Chen and Nussinovitch, 2000b; Hagenmaier, 2002). Vargas et al. (2006) found that addition oleic acid (1-4%) to chitosan formulation could increase a water vapour resistance of coated strawberries. Vargas et al. (2009a) reported that addition of oleic acid into the chitosan matrix decreased the water vapour permeability and increased the gloss of the film.

2.6 Effect of coating on fruit's physiology

Coating fruit with waxes restrict gas exchange through the peel surface, so that the internal atmosphere of the fruit is modified, with enhanced CO_2 and reduced O_2 levels (Hagenmaier and Baker, 1993; Petracek et al., 1999). Build up of anaerobic conditions in waxed fruits leads to an enhanced anaerobic respiration and increased production of off-flavor volatiles, such as ethanol and acetaldehyde (Cohen et al., 1990; Baldwin et al., 1995; Hagenmaier and Shaw, 2002). This risk can be minimized through selection of coatings with appropriate gas permeability characteristics and careful management of storage and ripening temperatures. Coatings can retard ripening, water loss, and reduce decay (McGuire and Hallman, 1995; Baldwin et al., 1997), but may also alter flavor (Cohen et al., 1990; Hagenmaier and Baker, 1993). Coating fruits and vegetables with chitosan helps a long-term storage (El Ghaouth et al., 1992) because a chitosan film provides a type of an active package. The chitosan can act as preservatives which released from the film deposited on the surface of the food and these inhibit fungal growth.

2.7 Application of response surface methodology (RSM)

Response surface methodology

The treatment structure known as RSM is essentially a designed regression analysis. The objective of an RSM experiment is to predict the value of a response variable (call the dependent variable) based on the controlled values of the experimental factors (call independent variables). All of the factors in RSM experiment must be quantitative. RSM treatment structures provide an economical way to predict the value of one or more responses over the range of values of the independent variables. A set of sample is prepared under the conditions specified by the selected RSM treatment structure.

To optimize the formulation, the response surface methodology (RSM) can be used. The RSM has been used to determine the optimization of process condition, ingredient levels (Gan et al., 2007) and coating formulation in areas of food industry. It is an effective tool to minimize the numbers of trials in the experiment design. Authors have used the RSM with central composite design to determine the effects of shellac and carnauba wax on coating's properties. Contour plot from quadratic model was generated to determine the optimization. They found that both shellac and carnauba wax affected binding, viscosity, gloss, and weight loss of Sai Nam Pueng orange. Increasing shellac resulted in increased binding, viscosity and gloss, but decreased weight loss of orange compares to control (Luangwilai et al., 2007). Tungsawas et al. (2007) proposed the optimal rice bran wax and shellac applying the central composite design (CCD) and found that optimum coating of production was that 2.00% rice bran wax and 11.00% shellac. They reported the optimal formula at 50% concentration could extend shelf life of orange from 12 to 24 days at temperature 25±2°C and RH 75±2%, weight loss and spoilage of orange were 17.00% and 6.45%, respectively.

Carvalho et al., (2009) studied on some physical properties of biodegradable films based on blends of gelatin and poly (vinyl alcohol) using a response surface methodology. The linear model was statistically significant and predictive for puncture force and deformation, elongation at break, solubility in water, the moisture content, the color difference and the opacity. The concentration of concentration of PVA was affected strongly the elongation at break of the film.

Srinivasa et al. (2007) reported the effect of temperature (4-50°C), relative humidity (RH, 40-80%) and storage period (39 days) on the mechanical properties such as tensile strength (TS), percent elongation (%E) and modulus of elasticity (ME) of biodegradable chitosan film was studied by applying response surface methodology and Box Behnken design of experiment.

The optimum levels of the independent variables used desirability function. It was found that at 20°C, 40% RH and storage period of 7 days, the optimum values for TS, %E and ME were 35.8 MPa, 29.9% and 896.7 MPa respectively.

For calculation of the optimal levels of ingredients for short dough biscuits the desirability function method was performed by Gallagher et al. (2003). Wang et al. (2009) reported the use of RSM for investigating the effect of pH and corn oil on the properties of gelatin biopolymer film and they used the desirability function to generate the optimum combinations of film.

2.8 Optimization by desirability function

The desirability function approach was introduced by Harrington (1965) and Derringer and Suich (1980) further developed it (Lazic, 2004). It is based on transforming the measured property of each response to a non-dimension desirability scale, *di*, defined as a partial desirability function whose value varies from zero, undesirable response, to one, desirable response. Once these individual *di* functions are defined for all responses, they are combined into one objective function *D*, representing the overall desirability which is calculated as the geometrical average of *n* partial desirability functions. The overall desirability function *D* also varies from 0 to 1.

$$D = \left[\prod_{i=1}^{n} d_i^{p_i}\right]^{1/n}$$

The standard estimates on desirability scale are show in Table 2.3.

Table 2.3 Standard estimates on desirability scale

Standard estimates	Desires	Quality of product
1.00	Excellent	The ultimate in satisfaction or quality, and improvement beyond this point have no appreciable value
1.00-0.80	Very good	Acceptable and excellent, represent unusual quality, or performance, well beyond anything commercially available
0.80-0.63	Good	Acceptable and good, represent an improvement over the best commercially quality, the latter having the value of 0.63
0.63-0.37	Satisfactory	Acceptable but poor quality is acceptable to the specification limits, but improvement is desired
0.37-0.20	Bad	Unacceptable. Materials of this quality would lead of failure of the project
0.20-0.00	Very bad	Completely unacceptable

Source: Lazic (2004)

CHAPTER 3

MATERIALS AND METHODS

3.1 Raw materials

3.1.1 Thai tangerine oranges (*Citrus reticulate*, Blanco cv. Kiew Wan) from orchard in Kamphaengphet province. Fruits (size 5, 5.6-6.0 cm diameter) were harvested at the commercial fully matured stage.

3.2 Chemical and Reagent

3.2.1 Sodium hydroxide (Ajax Finechem, Australia)

3.2.2 Caffeic acid (Sigma, USA.)

3.2.3 Coumaric acid (Sigma, USA.)

3.2.4 Sinapic acid (Sigma-Aldrich, USA.)

3.2.5 Ferulic acid (Sigma, USA.)

3.2.6 Acetronitrile (Merck, Germany)

3.2.7 Acetic acid (Merck, Germany)

3.2.8 ABTS (2,2 azinobis (3-ethylbenzothiazoline-6-sulfonate)) (Sigma-Aldrich, USA)

3.2.9 DPPH (2, 2-diphenyl-1-picrylhydrazyl) (Merck, Germany)

3.2.10 Phosphoric acid (Merck, Germany)

3.2.11 Ethyl acetate (Merck, Germany)

3.2.12 Methanol (Merck, Germany)

3.2.13 Folin-Cocialteu reagent (Merck, Germany)

3.2.14 Sodium carbonate (Ajax Finechem, Australia)

3.2.15 Ascorbic acid (Merck, Germany)

3.2.16 Metaphosphoric acid (Merck, Germany)

3.2.17 Gallic acid (Sigma-Aldrich, USA)

3.2.18 2, 6 dichloroindophenol (Merck, Germany)

3.2.19 Oleic acid (Merck, Germany)

3.2.20 Tween 80 (Merck, Germany)

3.2.21 Shrimp chitosan (MW, 248 kDa), with deacetylation degree of 96.5% was purchased from Muew biosafe CO., LTD., Phathumthani, Thailand

3.2.22 Commercial Shellac based (Natural Zivdar wax, Safepack Products Ltd., Israel)

3.3 Equipments

3.3.1 High performance Liquid Chromatography (HPLC) (LDC/Milton Roy USA.)

3.3.2 Gas Chromatography (GC) model GC-8A (Shimadzu Corporation, Kyoto, Japan)

3.3.3 pH meter model Suntex SP 701 (Suntex instrument, Taipei, Taiwan)

3.3.4 Hand refractometer model ATAGO N1 (ATAGO, Japan)

3.3.5 UV-VIS spectrophotometer model UV-1601 (Shimadzu, Japan)

3.3.6 Environment Scanning Electron Microscope model S-3400N (E-SEM, Hitachi Japan.).

3.3.7 Freeze dryer (Freezone 18®, Labconco, U.S.A.).

3.3.8 Incubator (Sanyo incubator, MIR 253, Sanyo electric Co. Ltd, Japan)

3.3.9 High performance Liquid Chromatography (HPLC) (Agilent 1100 series Agilent Technology, Wilmington DEL)

3.3.10 Centrifuge (FX 6100, Beckman Coulter ™, U.S.A.)

3.3.11 Spectrophotometer (Minolta CM-2600D, Konica Minolta Sensing, Inc.)

3.3.12 Gas Chromatography (GC) (6890N, Agilent Technologies, Wilmington DEL)

3.3.13 Mass spectrometer (5973 Network Mass Selective Detector, Agilent Technologies)

3.4 Methods

3.4.1 Changes of chemical composition, total polyphenol, phenolic acids and antioxidant activity of commercial coated tangerines during different storage conditions

3.4.1.1 Sample preparation

Tangerines were harvested at the fully matured stage and then coated with a commercial coating (18% shellac, Natural Zevdar wax) using a coating machine at the orchard. The ratio of coating material to fruit was 1:1 (liters per tonne). Fruit size was 5.6-6.0 cm in diameter. The coated and uncoated tangerines were divided in to four groups then packed in cardboard boxes and stored at 4±1, 12±1, 20±1 and 30±1°C for 5 weeks. Five fruits were randomly chosen, peeled, removed seed, and juice was squeezed out using a fruit juice separator. The juice was used for chemical analyses and antioxidant activity determination. It was kept in amber glass bottles and stored at -18°C for further analysis. Before analysis the juice was thawed for 30 min in running trap water at room temperature.

3.4.1.2 Analytical methods

1) Total soluble solid

The total soluble solid of orange juice was determined by using the refractometer (ATAGO N1, Japan). Result was reported as °Brix.

2) pH

The pH of samples was measured using Suntex SP 701 (Suntex instrument, Taipei, Taiwan). Triplicates of pH determinations were made for each sample.

3) Titratable acidity

Ten milliliters of clear juice was passed through filter paper (Whatman, No.1) and added with 100 ml of distilled water and drops of 1% phenolphthalein. The mixture was titrated with standardized 0.1 N NaOH, to pink color persisting for 30 s. Report as milliliter of NaOH. Titratable acidity was calculated in term of grams of citric acid anhydrous per 100 ml of the sample by using the formula below. (AOAC, 2000).

TA %=(ml of NaOH)x(Normality of NaOH)x(0.64) / (ml of sample)

4) Weight loss

Five fruits were taken from each treatment and weighed using a top load balance and weight loss was calculated as a percentage on weekly basis.

5) Ascorbic acid

Ascorbic acid will be determined by 2, 6 dichloroindophenol titrimetric method (AOAC, 2000).

6) Total polyphenol content

The total polyphenol content was measured following a modified method of Gorinstein et al. (2001). The juice samples were centrifuged at 10,976×g at $4^{o}C$ for 20 min. A volume of 0.2 mL juice samples and 1 mL of 5-fold diluted Folin-Ciocalteu reagent were transferred into a test tube, then 0.8 mL of 7.5% Na_2CO_3 solution was added and mixed. The solution was made up to a final volume of 10 mL with distilled water and left to stand for 30 min at room temperature. The absorbance was read at 765 nm by a UV-VIS spectrophotometer (UV 1601, Shimadzu, Japan), distilled water was used as a blank and gallic acid as the standard. Results were calculated as mg of gallic acid per 100 mL of juice.

7) Antioxidant activity

(a) DPPH Radical Scavenging Activity

The DPPH scavenging activity followed a modified method of Brand-Williams et al. (1995). The juice samples were centrifuged at 10,976×g at $4^{o}C$ for 20 min. The juice sample (0.1 mL) was added to 3.9 mL solution of 80 μM DPPH in 80% ethanol, the mixture was vigorously shaken and allowed to stand at room temperature in the dark for 30 min. The sample was substituted for 50% ethanol as a control. The absorbance was read at 517 nm and the antioxidant activity was calculated as a percentage of DPPH discoloration using the following equation.

Antioxidant activity% =100 x (1-absobance of sample/absorbance of control)

(b) ABTS Radical Scavenging Activity

Measurement of the ABTS radical scavenging activity followed a modified method of Landult et al. (2001). The juice sample (0.1 mL) was added to a mixture of 2.5 mM ABTS in phosphate buffer saline (PBS) (0.4 mL), 2.5 μM metmyoglobin solution (0.72 mL), 10 mM of PBS (3.10 mL), and 10 mM of H_2O_2 (0.48 mL) then the volume was adjusted to 4.32 mL by PBS. The sample was substituted for phosphate buffer as a control.

The absorbance was read at 734 nm after 10 min of reaction at room temperature in the dark place and calculated the antioxidant activity using the following equation.

Antioxidant activity% =100 x (1-absobance of sample/absorbance of control)

8) HPLC analysis for phenolic acids

(a) Extraction of phenolic acids

The juice samples were centrifuged at 10,976×g at 4°C for 20 min. Ten milliliters of clear orange juice was added to 10 mL of 2 N NaOH and stored in the dark for 4 hours. The juice was then acidified with concentrated phosphoric acid to pH 4.5 (Rouseff et al., 1992) and 20 mL of ethyl acetate was added to the mixture. The extraction was done twice; the extract was evaporated at 40°C under vacuum to dryness, and then dissolved in 1 mL of methanol. The methanol extract was used for HPLC analysis.

(b) HPLC determination

The clear methanol extract was passed through a 0.22 µm membrane filter prior to injection. A constaMetric 3000 pump (LDC/Milton Roy) was connected to an HPLC column (Hypersil ODS C-18, 5 µm 250x4.6 mm ID, Thermo Hypersil, UK.) with a C-18 guard column. The phenolic acids were detected at 300 nm with Spectra Monitor 3000 Detector (LDC/Milton Roy). Twenty microlitres of sample was introduced to the system by Rheodyne injector (20 µl Fixed loop). The isocratic elution was performed at 1 mL/min with a mobile phase consisting of 80% H_2O (with 2% acetic acid) and 20% acetronitrile. Chromatograms were recorded and processed by a Chromatopac C-R6A (Shimadzu Japan). Quantification of phenolic acids was done by the external standard method.

3.4.1.3 Statistical analysis

Data were analyzed by ANOVA using a split-split plot in a Randomized Complete Block Design (RCBD), with temperature as a main plot, storage time as a sub-plot and coating treatment as a sub-subplot. Means were compared by Duncan's multiple range tests at the significant difference $p \leq 0.05$.

3.4.2 Development of chitosan formulation for tangerine coating

3.4.2.1 Sample preparation

1) Preparation of coating solution

Chitosan-oleic acid coating was prepared according to method described by Vargas et al. (2006). Chitosan (1-2%) was dispersed in aqueous solution of glacial acetic acid (1%, v/v) at 40°C. Tween 80 at 0.1% (v/v) was added to improve wettability. After 8 h of stirring, oleic acid (1-4%) was added to the chitosan solution. The concentration of chitosan and oleic acid in the coating solution was varied according to the conditions defined in the experimental design (Table 3.1). The pH of each mixture was adjusted to 5 with 1 N NaOH (Chein et al. 2007b). All mixtures were emulsified using an Ultra Turrax T25 (IKA®, Germany) at 13,500 rpm for 4 min and the solution was filtered through a filter paper to remove undissolved material before use.

2) Coating application

The tangerine was coated by a self made coating apparatus shown in Appendix D, brush rollers speed was controlled at 160 rpm, the coating solution was sprayed at 2 bars and the ratio of coating to fruits was 50 mL/10 kg. Coated fruits were then dried with an electric fan at room temperature (30±2°C) for 30 min. Fifty coated fruits were packed in a cardboard box and stored in a laboratory room at 30±2°C (to simulate Thai market), 79±5% RH for 3 weeks.

3.4.2.2 Analytical methods

1) Weight loss (same as 3.4.1.2.4)

2) Internal O_2 and CO_2

Samples of ten fruits from each treatment were randomly withdrawn. A gas tight syringe was pierced into the core of the fruit submerging in water and then five milliliters of gas was taken out, the needle was removed and rubber stopper was used to seal the syringe. The gas sample was used for analysis of internal O_2 and CO_2. A gas tight syringe was pierced through the rubber stopper and then one milliliter of gas was taken out. The internal concentration of gases were analyzed by a gas chromatograph (GC-8A Shimadzu, Japan) equipped with a column; Molecular sieve (1 m, 4 mm o.d., GL science Inc, Japan) for O_2, and a thermal conductivity detector (at 90mA). Helium was the carrier gas at flow rate 50 ml/min, and column temperature was set at 60°C, injector temperature at 100°C. The internal concentration of gases was determined by a gas chromatograph (GC-8A Shimadzu, Japan) equipped with a column ; Porapak Q (2 m, 4 mm o.d., GL science Inc, Japan) for CO_2, and a thermal conductivity detector (at 90 mA). Operating condition was helium as carrier gas at 50 ml/min, column temperature at 60°C, injector temperature at 100°C.

3) Ethanol

Ten fruits were randomly chosen, peeled, removed seed, and juice was squeezed out using a fruit juice separator and then centrifuged at 10,976×g at 4°C for 20 min. The supernatant was filtered before use. The ethanol concentration was analyzed with a gas chromatograph (GC-8A Shimadzu, Japan) equipped with a BX-10 column (3 m, 4 mm o.d., GL science Inc, Japan) and a flame ionization detector. The operating condition was helium as carrier gas at 50 ml/min, column temperature at 85°C, injector temperature and detector temperature at 100°C.

3.4.2.3 Experimental design and statistical analysis

The response surface methodology (RSM) with central composite design (CCD) was applied to determine the experimental design and to optimize the combination of chitosan (1-2%) and oleic acid (1-4%) concentrations in the coating formulation (Table 3.1). The five coded levels of chitosan: -1.41 (1%), -1 (1.15%), 0 (1%), 1 (1.85%), 1.41 (2%) and oleic acid: -1.41 (1%), -1 (1.44%), 0 (2.5%), 1 (3.56%), 1.41 (4%) were incorporated into the design. The central point of design (0, 0) was repeated five times to calculate the reproducibility of the method. The effect of two independent variables on the fruit properties (weight loss, O_2, CO_2, ethanol) was modeled using the second-order to fit the response. The second-order model equation used to fit responses follows Lazic (2004).

$$Y=\beta_0+ \beta_1X_1+ \beta_2X_2+\beta_{11}X^2_1+ \beta_{22}X^2_2+\beta_{12}X_1X_2$$

Where Y is the predicted response value (weight loss; ethanol, CO_2 and O_2), X_1 is chitosan level and X_2 is oleic acid level, β_0 is a constant value, β_1 and β_2 are linear terms, β_{11}, β_{22} are quadratic terms, and $\beta12$ is an interaction term. Design-Expert 7.6.1 trial version (Stat-Ease, Inc., MN, 2007) was used to fit the model, draw contour plots and determine desirability.

Table 3.1 Central composite experimental design for the independent variables (coded and uncoded levels)

Run	Coded		Uncoded[a]	
	X_1	X_2	Chitosan (%)	Oleic acid (%)
1	-1	-1	1.15	1.44
2	-1	1	1.15	3.56
3	1	1	1.85	3.56
4	1	-1	1.85	1.44
5	0	0	1.50	2.50
6	0	0	1.50	2.50
7	0	0	1.50	2.50
8	0	0	1.50	2.50
9	0	0	1.50	2.50
10	1.41	0	2.00	2.50
11	-1.41	0	1.00	2.50
12	0	1.41	1.50	4.00
13	0	-1.41	1.50	1.00

[a] uncoded = concentration of chitosan level in coating formulation (w/v), concentration of oleic acid level in coating formulation (v/v)

For detail of codification of the levels see Appendix A4

3.4.3 Comparative study of qualities of tangerine coated with chitosan and commercial material

3.4.3.1 Sample preparation

The tangerines were coated by a chitosan-oleic acid formula as proposed by 3.4.2. The commercial coating was shellac based. The self made coating apparatus was used for coating. The brush rollers speed was controlled at 160 rpm, the coating solution was sprayed at 2 bars, the ratio of chitosan-oleic acid and shellac coating per fruits (50mL/10 kg.). Fifty coated fruits were packed in a cardboard box and stored in incubator (Sanyo incubator, MIR 253, Sanyo electric Co. Ltd, Japan) at 30±2°C (simulate the market condition) and 90±4% relative humidity for 5 weeks. Five fruits were randomly chosen, peeled, removed seed and juice was squeezed out using a fruit juice separator. The juice was used for chemical analyses and antioxidant activity determination. It was kept in amber glass bottles and stored at -18°C for further analysis. Before analysis the juice was thawed for 30 min in running trap water at room temperature. The juice was measured on the following:

3.4.3.2 Analytical methods

1) Viscosity and solid content of coating

The viscosity of coating solution was measured using Brookfield viscometer (Model DV III ultra Brookfield, U.S.A.) with a spindle No. 18. Coating solution was poured into a small sample adapter; the viscosity was read at 250 rpm with high toque and measured at room temperature.

The moisture content (MC) of the coating was determined by AOAC (2000) and the solid content was calculated by equation;

$$\text{Solid content} = 100\text{-}\%\text{MC}.$$

2) Measurement of glossiness

The glossiness of coated tangerine with different coating was scanned by the reflectance using a spectrophotometer Minolta CM-2600D.

3) Analysis of volatile components

The coated tangerine was cut in half and extracted with an electrical juice maker with rotating head, 6 g of sample was mixed together with 6 mL $CaCl_2$ (1g/mL) in flask and closed with cap and shaken for 30 sec, the flask was frozen in liquid nitrogen and stored at -80°C for further analysis. Before analysis the sample was thawed for 15 min in a water bath at 35°C (Haake SWB-25, Thermo Fischer Scientific, USA). Ten grams of juice samples were put into vials then sealed with TFE/silicone septa seals and incubated at 40°C for 1 h in a heat tray of a gas chromatography (6890N, Agilent Technologies, Wilmington DEL) to form a headspace. A solid phase micro extraction fiber (SPME), (50/30 μm DVB/Carboxen/PDMSS, Supelco) was inserted into the vial headspace and remained 30 min to absorbing the volatiles. The injection port was operated at 280°C in splitless mode and subjected to a pressure of 70 psi. Volatiles were separated on a 30 m 0.25 mm i.d. 0.25 m thickness capillary column (HP-5MS, Agilent

Technologies). The carrier gas was helium with a flow rate of 1.3 mL/min. The initial oven temperature was 35°C, followed by a ramp of 4°C per min up to 200°C, and then at 30°C per min reach the final temperature of 280°C, which was held for 5 min. A mass spectrum was obtained by electron ionization at 70 eV, and a spectrum range of 40–450 m/z was used. A detector worked at 230°C and in full scan with data acquisition and ion mass captured between 30-300 amu. Chromatograms and mass spectra were evaluated using the ChemStation software (Agilent Technologies). The peaks were identified using a mass spectrometer (5973 Network Mass Selective Detector, Agilent Technologies) coupled to the GC by comparison of experimental spectra with mass spectra reference from the National Institute for Standards and Technology library (NIST 98, search version 2.0).

4) Weight loss (same as 3.4.1.2.4)

5) Soluble solid (same as 3.4.1.2.1)

6) pH (same as 3.4.1.2.2)

7) Titratable acidity (same as 3.4.1.2.3)

8) Ascorbic acid (same as 3.4.1.2.5)

9) Total polyphenol content (same as 3.4.1.2.6)

10) Antioxidant activity (same as 3.4.1.2.7)

11) HPLC analysis for phenolic acids

Sample preparation for hydroxycinnamic acids analysis was same as 3.4.1.2.8. The equipment for HPLC analysis was Agilent 1100 series (Agilent Technology, Wilmington DEL) with diode array detector (DAD, Agilent Technology) and Agilent Chemstation software.The column was a C18 Synergi Hydro RP 80A (4 µm 250x4.6 mm ID, Phenomenex, Torrence, CA) with a C18 guard column. The isocratic elution was performed at 1 mL/min with a mobile phase consisting of 80% H_2O (with 2% acetic acid) and 20% acetronitrile. The phenolic acids were detected at 300 nm. Quantification of phenolic acids was done by the external standard method.

12) Sensory evaluation

Sensory evaluation on taste, color, flavor and acceptability of juice qualities was evaluated by 7 selected experienced panelists from laboratory at the Faculty of Agro-Industry, King Mongkut's Institute of Technology Ladkrabang. Juice was pooled from sampled fruits to ensure serving (Hagenmaier, 2002). Ten milliliters of juice samples were served at room temperature (30±2°C). The preference test a 5 point hedonic scale was used; 1=unacceptable, 2=a little acceptable, 3=moderate acceptable, 4=good acceptable, and 5=excellent acceptable. (Appendix C)

3.4.3.3 Statistical analysis

Data from 3.4.3.2.1-3.4.3.8 were analyzed by ANOVA using a split plot in a completely randomized design, with coating material as a main plot and storage time as a sub-plot. Means were compared by Duncan's multiple range tests at the significant difference $p \leq 0.05$.

CHAPTER 4

RESULTS AND DISCUSSIONS

4.1 Changes of chemical composition, total polyphenol, phenolic acids and antioxidant activity of commercial coated tangerines during different storage conditions

The effect of postharvest conditions i.e.; temperatures and storage period, on changes in coated tangerines was investigated. Fruits were coated with shellac based coating material and stored at 4, 12, 20 and 30^{o}C for 5 week. Each week, samples were taken out for measurement of total soluble solid, pH, titratable acidity, weight loss, ascorbic acid, total polyphenols, phenolic acids and antioxidant activity. However, during the storage of tangerines at 30^{o}C for 4 weeks some samples were spoiled due to mold, shrivel and flavor change; therefore, the analysis was done up to 3 weeks for this storage condition. The results are on the following.

4.1.1 Total soluble solid

Both coated and uncoated fruits showed gradual increased in total soluble solids (TSS). Storage temperature and coating treatment resulted in tangerines significantly different TSS (Appendix B, Table 1B). At high storage temperature the samples showed higher TSS than those stored at low temperature (Figure 4.1a-b). The higher level of TSS in the uncoated fruits may be resulted from the high rate of water loss during storage (Somsrivichai et al, 1992). The rise in TSS in valencia orange depended on storage temperature was reported by EL-Zeftawi (1976). Storage period showed no significant effect on TSS in tangerines with or with out coating. During storage period, total soluble solid in the coated and uncoated tangerine were in the range of 10.27 to 10.64 and 10.25 to 10.80 ^{O}Brix, respectively. Generally, sugar in fruit continues to increase for a time after harvest (Echeverria and Burn, 1989). This is true to all climacteric fruit where as starch was converted to sugar. Orange is a non climacteric fruit the increase of brix may not link with starch. Echeverria and Valich (1989) reported that the increase in sugars in Valencia orange during 9 weeks storage at 15^{O}C and 95% RH was due to the conversion of organic acid to glycolytic intermediates to sugars and other physiological can caused a release soluble sugar such as starch and cell wall hydrolysis could also contribute to increase in TSS. These results correspond with the experiments of Echeverria and Ismail (1987) who reported that the total soluble solid in Robinson tangerine significant increase during 4 weeks storage at 15°C and 95% RH.

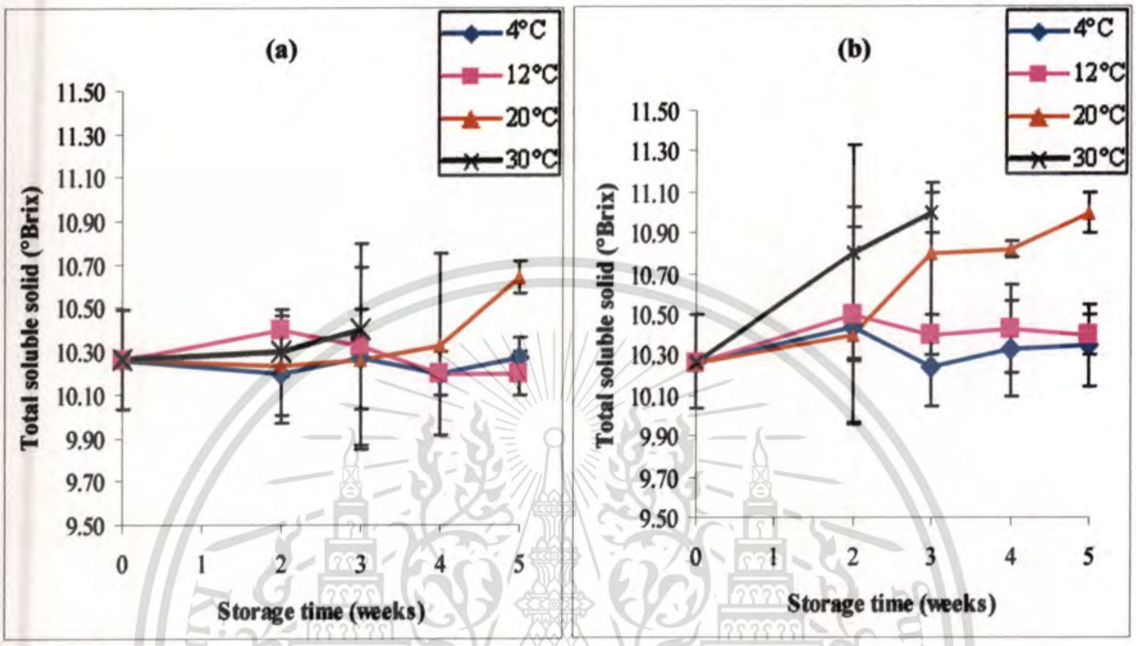


Figure 4.1 Change in total soluble solid of coated (a) and uncoated (b) Thai tangerines during storage at 4, 12, 20°C for 5 weeks and at 30°C for 3 weeks, 90±4% RH

4.1.2 pH value

The coating treatment and storage temperature of tangerines affected no significantly different on pH values (Appendix B, Table 2B). The storage time showed significant effect on the change of pH value during storage period, pH values increased in all storage temperatures (Figure 4.2a-b). The pH value of tangerine at initial was 4.34 increased to 4.39 for coated and 4.57 for uncoated samples at the end of storage. The increase of pH value of coated and uncoated tangerine juice may due to acidity decreased during storage. Etxeberria and Gonzalez (2005) reported that the acidity of murcott mandarin declined approximately 60% with a concomitant raise in pH from 2.7 to 3.5 during the same period. The increase of pH value reflects the H^+ concentration vacuolar which resulted from sucrose accumulation. Echeverria and Burn (1989) reported the similar results with murcott mandarin juice.

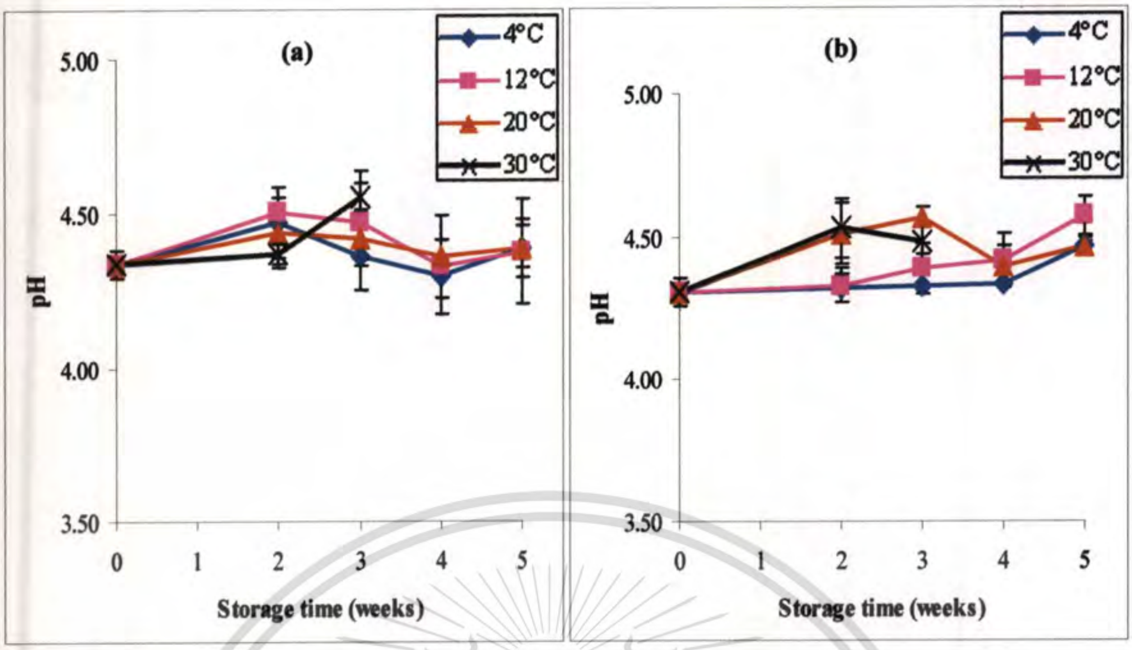


Figure 4.2 Change in pH of coated (a) and uncoated (b) Thai tangerines during storage at 4, 12, 20°C for 5 weeks and at 30°C for 3 weeks, 90±4% RH

4.1.3 Titratable acidity

The titratable acidity was decreased by storage temperature. At high temperature (30°C) titratable acidity in the tangerines decreased with greater extent than those stored at lower temperature. Both coated and uncoated tangerine showed gradually titratable acidity decrease during storage (Figure 4.3a-b). The titratable acidity of coated tangerine was decreased when stored at all temperatures during the first collection (2 weeks) and decreased throughout storage period when stored at 4°C. However at 12 and 20°C the titratable acidity of the coated tangerines slightly increased after 5 week storage. For uncoated samples showed the titratable acidity decreasing trend during stored at all temperatures. The initial of titratable acidity was 0.52% and decreased to the range from 0.38 to 0.44% at the end of storage. These results are similar to those of Rapisarda et al. (2001) who reported that total acidity of blood orange decreased during storage when stored at both 8 and 22°C. Citric acid has been reported to decrease in stored citrus fruits which declined in acid content due to the use of organic acid for energy production and alcoholic fermentation (Echeverria and Valich, 1989). Organic acids are known to be utilized as energy source for the synthesis of other organic compounds and it affected on the decline in acidity and corresponding increase in pH (Murata, 1977). In previous study, the tangerine coated with polyethylene wax stored for 4 weeks at 5°C plus 1 week at 17°C decreased in acid content form 1.2 to 0.9 (Cohen et al, 1990).

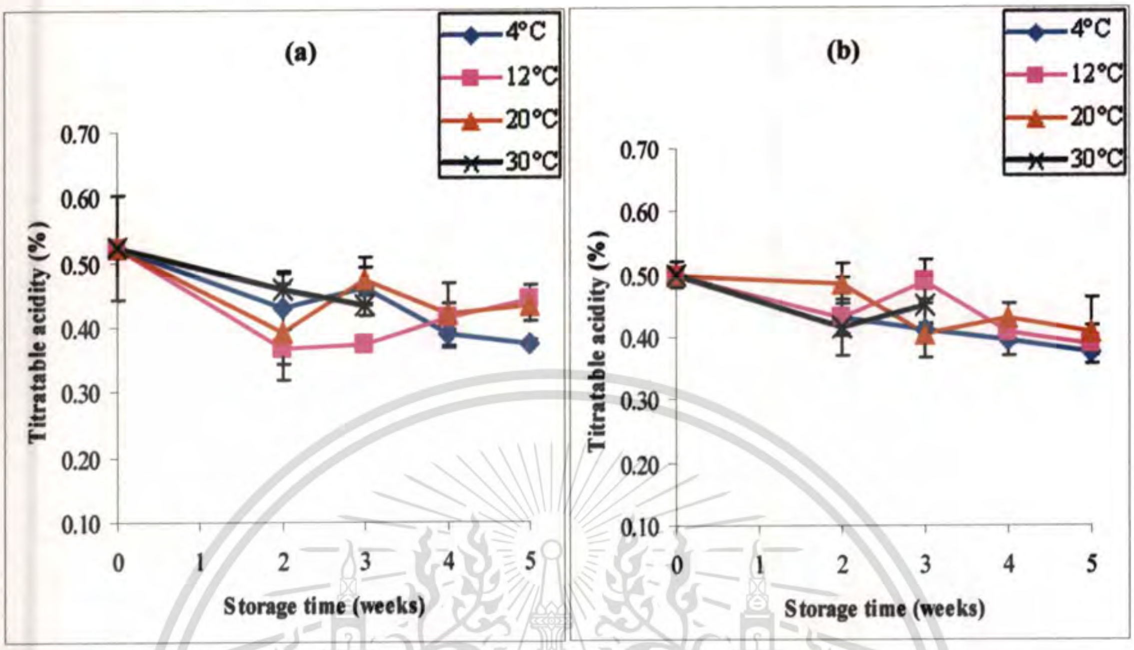


Figure 4.3 Change in titratable acidity of coated (a) and uncoated (b) Thai tangerines during storage at 4, 12, and 20°C for 5 weeks and at 30°C for 3 weeks, 90±4% RH

4.1.4 Weight loss

Storage temperature, time and coating treatment showed the significantly different effect on weight loss of tangerine (Appendix B, Table 4B). Coated samples, after 5 weeks at 4, 12 and 20°C, the weight losses were 3.69±0.24, 13.17±0.82 and 17.74±0.39%, respectively (Figure 4.4a). At high storage temperature, the weight loss of uncoated sample increased significantly (Figure 4.4b). Weight loss of coated tangerines at 4, 12 and 20°C in comparison to uncoated fruits were reduced by 36.82, 46.31, and 41.22%, respectively. Weight loss during storage is the major factor in the postharvest deterioration of citrus fruit which transpiration is the main cause leading to other aspects of physiological deterioration of citrus fruits (Grierson and Ben-Yehoshua, 1986). Many authors have reported that coating and low storage temperatures can reduce weight loss of murcott tangor and mandarin (Chien et al., 2007b; Mannheim and Soffer, 1996). It was reported that weight loss of fruits higher than 5-10% resulted in a quality loss that made them unsaleable (Davies and Albrigo, 1998). The advantage of coating on weight loss reduction for tangerine is obvious because fruit were purchased by weight. However others effect of coating on qualities need to be taken in to consideration.

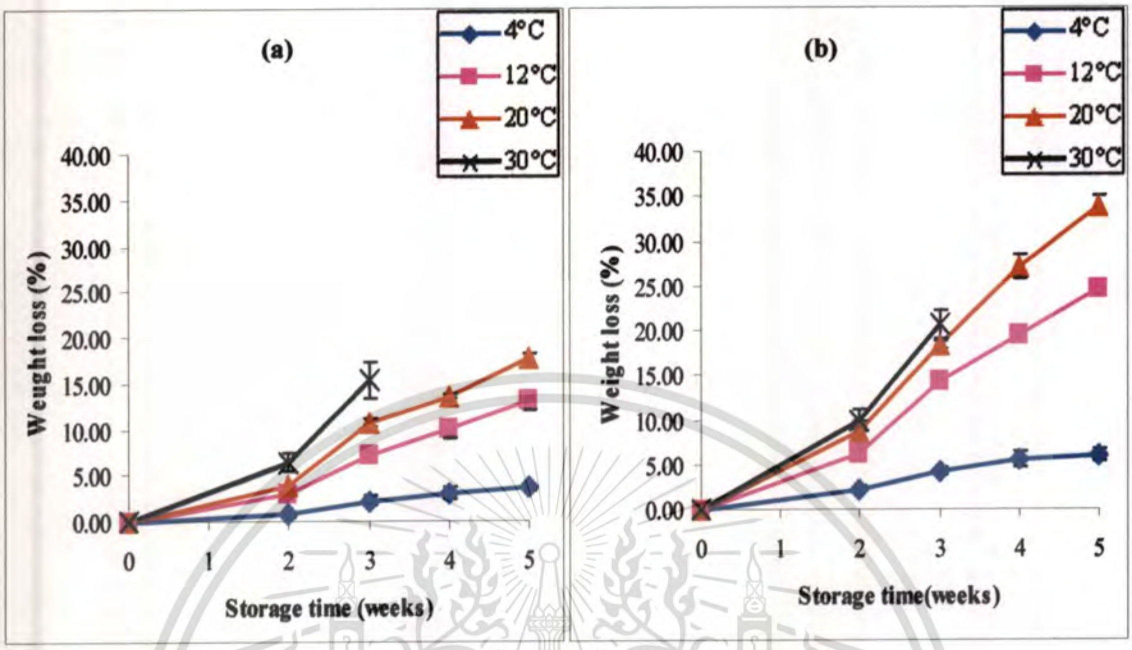


Figure 4.4 Change in weight loss of coated (a) and uncoated (b) tangerines during storage at 4, 12, 20°C for 5 weeks and at 30°C for 3 weeks, 90±4% RH

4.1.5 Ascorbic acid

Tangerine is rich in ascorbic acid that makes it a popular fruit to be consumed for promoting good health. The changes of ascorbic acid contribute to the fruit's quality. During storage, the tangerine at different temperature the storage periods showed significantly effect on the ascorbic acid content (Appendix B, Table 5B). However the storage temperature and coating treatment showed no significant effect on ascorbic acid content in tangerines. The ascorbic acid content in tangerine samples decreased during storage for 5 weeks at 4, 12 and 20°C which had previously been reported by Burdurlu et al. (2006). Prolong storage of coated and uncoated samples affected the ascorbic acid content regardless of temperatures (Figure 4.5a-b). The initial content in the coated tangerine juice was 20.83±0.84 mg/100mL which gradually decreased to 14.38±3.04, 14.77±0.49 and 15.14±1.48 mg/100mL at 4, 12 and 20°C respectively after five weeks storage. The ascorbic acid content in uncoated samples showed the same trend, however when storage at high temperature (30°C) the ascorbic content was lower than coated samples. Decreases of ascorbic acid in fruit may be associated with enzymes such as cytochrome oxidase, ascorbic acid oxidase and polyphenol oxidase (Nagy, 1980). Oxidation of ascorbic acid may lead to the formation of dehydroascorbic acid which can not be detected by the method used. The results indicated that the storage period has more significantly effect on the content of ascorbic acid than the temperatures. It has previously been reported that loss of ascorbic acid in citrus fruit was not affected by storage temperature (Rapisarda et al., 2001) and that there was no loss in ascorbic acid during storage of oranges at low temperature (Schirra and Chessa, 1988). In addition, the results showed that ascorbic acid loss in Thai tangerine was not affected by coating material, compared to the uncoated, which are similar to results reported by Badwin et al. (1995).

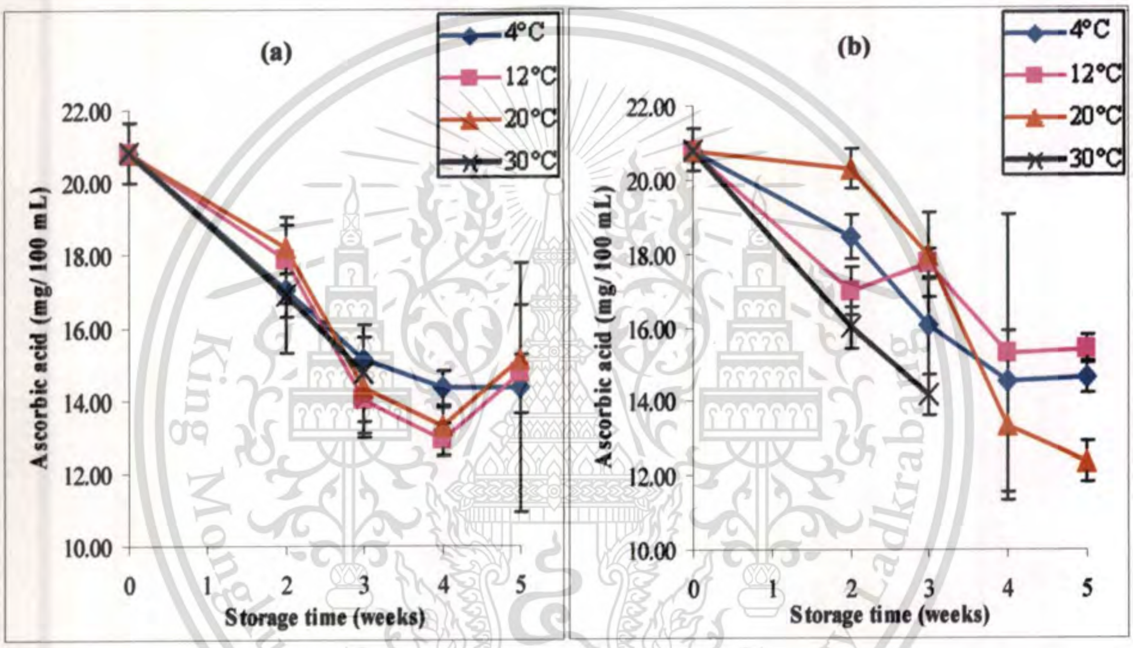


Figure 4.5 Change in ascorbic acid of coated (a) and uncoated (b) Thai tangerines during storage at 4, 12, and 20°C, for 5 weeks and at 30°C for 3 weeks, 90±4% RH

4.1.6 Total polyphenol

Total phenolic content was determined using the Folin-Ciocalteu reagent. The results showed that the coating treatment has a more significantly effect on the content of total polyphenol than the temperatures and storage time (Appendix B, Table 6B). The total polyphenol content of coated tangerine before storage was 26.30±3.42 mg gallic acid/100mL which increased to about 28.40±1.04, 29.99±0.05 and 28.65±0.58 mg gallic acid/100mL after 5 weeks storage at 4, 12 and 20°C (Figure 4.6a), respectively. Figure 4.6b showed that the total polyphenol content in uncoated tangerine decreased when storage was at high temperature (12 and 20°C). At 30°C total polyphenol was sharply decreased both uncoated and coated samples. This result may be due to the oxygen can be passing into the uncoated fruit more than the coated one and affected on the oxidation of polyphenols. Dietrich et al. (2004) reported polyphenols can be degraded during storage fruit and juice under inappropriate storage conditions such temperature, access to light and oxygen. The polyphenol content in fruit is associated with antioxidant capacity; normally measured by Folin-Ciocalteu reagent which can react with all types of antioxidants through electron transfer-based antioxidants including non-phenolic compounds such as vitamin C, dehydroascorbic acid, amino acid and carotenoid (Huang et al., 2005). During post harvesting, many physiological changes can take place in fruit that lead to senescence and decomposing of the cell structure thus the liberation of free phenolic acids and free amino acids can occur contributing to the increase of total polyphenol. Therefore the antioxidant activity may be assumed to be increased during storage. The results correspond with the experiments of Moussaid (2000) who reported that the total polyphenol content in coated mature oranges increased during 9 weeks storage at 20°C.

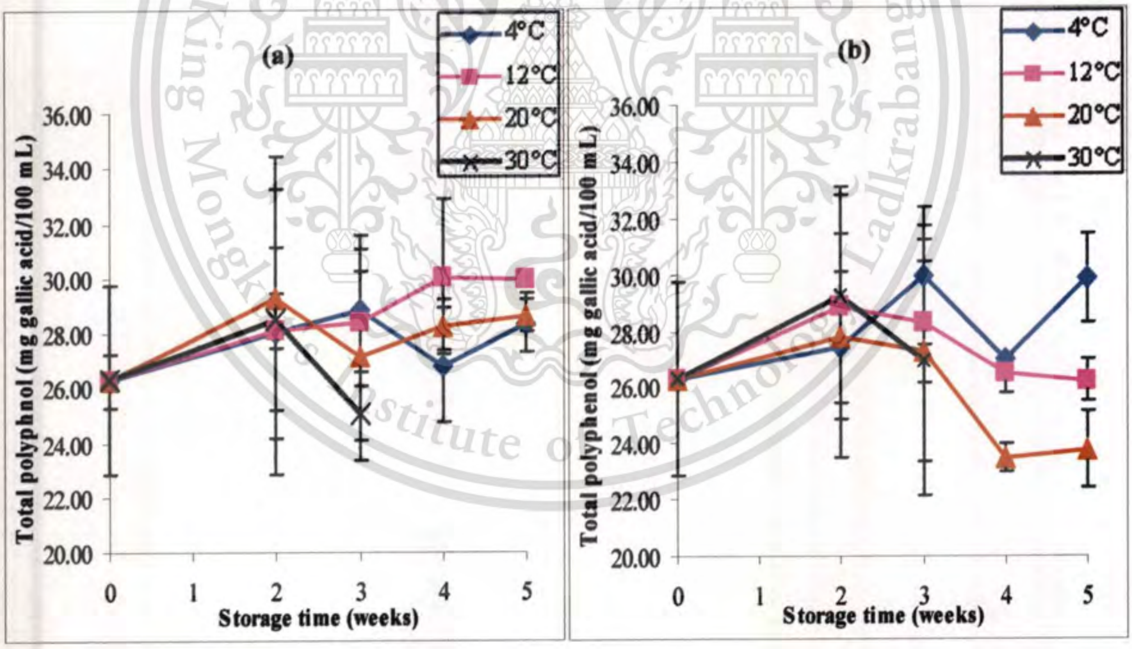


Figure 4.6 Change in total polyphenol of coated (a) and uncoated (b) Thai tangerines during storage at 4, 12, and 20°C for 5 weeks and at 30°C for 3 weeks, 90±4% RH

4.1.7 Hydroxycinnamic acids

The major hydroxycinnamic acids found in juice of coated tangerine were identified as ferulic acid followed by sinapic, caffeic, and *p*-coumaric acid. The hydroxycinnamic acids found in this experiment were reported to be similar to that found in blood orange (Rapisarda et al., 1998). The phenolic acids in tangerine samples were significantly different by storage period and coating treatment (Appendix B, Table 7B). Only ferulic acid in both coated and uncoated tangerine slightly decreased at 20°C but this was not statistically significance. The initial contents of caffeic, *p*-coumaric, sinapic, and ferulic acid were 2.54±0.13, 1.86±0.08, 4.79±0.23, and 37.49±2.41 mg/L, respectively. The phenolic acids increased during storage regardless of temperatures, but the increase was higher at the early stage of storage at 4 and 12°C and slightly declined at the end. The phenolic acids in uncoated tangerines have changed in the similar trend. With other fruits such as tomato the soluble phenolic acid slightly increased during 10 days of early storage at 7, 15 and 25°C (Toor and Savage, 2006). In Tacle and Clara hybrid mandarin oranges, the total hydroxycinnamic acids increased during early storage at 6°C when stored for 104 days (Rapisarda et al., 2008a).

Caffeic acid increased in the first 2 weeks and slightly declined till the end of storage at all tested temperatures (Figure 4.7a, e). However the content was significantly higher than the initial one. *p*-coumaric acid showed steadily increased during 5 weeks storage at 20°C meanwhile at 4 and 12°C slightly declined after 4 weeks (Figure 4.7b, f). Sinapic acid increased during storage at all temperatures particularly at 20°C where the content was higher at the end of storage (Figure 4.7c, g).

Ferulic acid was the most abundant phenolic acid found in the juice. It increased at all temperatures and declined at the end of storage particularly at 20°C (Figure 4.8d, h). The effect of coating treatment was showed by phenolic acids content in coated tangerine higher than in the uncoated one.

It may be concluded that phenolic acids in coated tangerines increased during storage at 4, 12 and 20°C for 4 weeks but when the storage was prolonged to 5 weeks the increase of caffeic, *p*-coumaric and sinapic acid at 20°C was noted but ferulic acid decreased. The increased of phenolic acids was higher at 4°C and 12°C than at 20°C. Generally phenolic acids were reported to be synthesized in plants when subjected to stress as a mechanism for defensive (Naczk and Shahidi, 2006). At low temperature stress the L-phenyalanine was converted to *tran*-cinnamic acid catalyzed by phenyalanine ammonia-lyase (PAL) and then hydroxylation and/or methylation of *p*-coumarate yields caffeic, ferulic and sinapic acids. Rapisarda et al. (2001) reported that *p*-coumaric, ferulic, caffeic and sinapic acid increased during storage of Tarocco orange for 85 days at 8°C. There are some reports of total hydroxycinnamic acids decreasing due to senescence during storage (Rapisarda et. al., 1998 and 2008b).

The detrimental off-flavors in blood orange during storage was reported to the *p*-vinylguaiacol and *p*-vinylphenol which are derived from ferulic and *p*-coumaric acid (Rapisarda et al., 2001). In this experiment the decreased of ferulic acid during storage at 20 and 30°C was obvious but with *p*-coumaric acid it occurred only at 4 and 12°C. This may suggest the optimum storage period for tangerines. In addition at 20 and 30°C the desiccation and senescence of tangerine may affect the analysis when storage was prolonged.

At 30°C, the hydroxycinnamic acids in coated tangerine were slightly increased except ferulic acid at the end of storage (3 weeks). The hydroxycinnamic acids in uncoated tangerines were increased at the early stage of storage at 30°C and sharply declined when stored for 3 weeks. This result showed that coating treatment may not suitable to tangerine stored at high temperature that it may affect on flavor change due to ferulic acid increase during storage, in addition to that a short shelf life was observed due to decay.

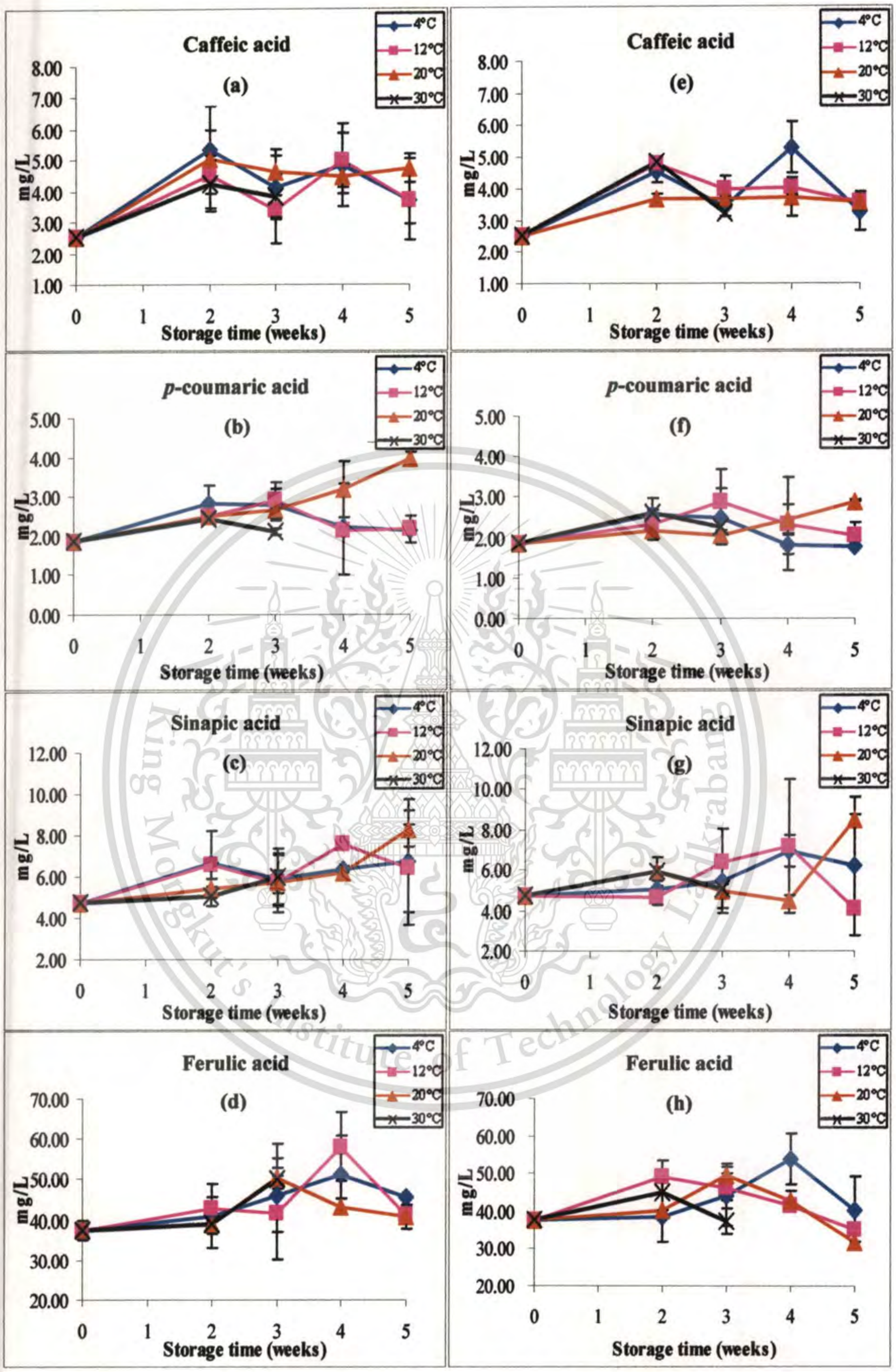


Figure 4.7 Changes in caffeic, *p*-coumaric, sinapic, ferulic acid of coated (a-d) and uncoated (e-h) Thai tangerines during storage at 4, 12 and 20°C for 5 weeks and at 30°C for 3 weeks, 90±4% RH

4.1.8 Antioxidant activity

The antioxidant activity in coated and uncoated tangerine during storage were measured by DPPH and ABTS methods. The DPPH increased significantly during storage regardless of temperatures and coating treatment, but the ABTS showed no significant difference by all of treatments (Appendix B, Table 8B). The DPPH and ABTS assay show the difference in correlating with various antioxidants. The DPPH antioxidant activity is correlated significantly with caffeic acid, *p*-coumaric acid, and sinapic acid not with ferulic acid (Table 4.1) but the ABTS assay is correlated with ferulic acid at a significant level but failed to be correlated with other phenolic acids. This might due to the properties of certain phenolic acids with ferulic acid being more hydrophilic (Kim et al., 2001; Chen and Ho, 1997). The number of hydroxyl groups of each hydroxycinnamic acid can affect the scavenging DPPH radical (Kim et al, 2001). The total polyphenol was more correlated with the ABTS assay than the DPPH assay which indicated antioxidants in tangerine were both hydrophilic and lipophilic (Cano et al., 2000; Arnao et al., 2001). It may be concluded that the antioxidant activity in tangerine was associated with more than a single compound. The synergistic effect of ascorbic acid and phenolic compounds on antioxidant activity has been reported by Kahkonen et al. (2001). In addition the reduced form of ascorbic acid may play the role of antioxidant in addition to those phenolic acids.

Table 4.1 Correlation coefficients between radical scavenging activity and antioxidant components in coated Thai tangerine during storage at 4, 12, and 20°C, 90±4% RH for 5 weeks

Antioxidant component	**Correlation coefficients**	
	DPPH	**ABTS**
Ascorbic acid	-0.361	-0.286
Total polyphenol	0.327*	0.641**
Caffeic acid	0.491**	0.184
p-coumaric acid	0.558**	0.135
Sinapic acid	0.625**	0.296
Ferulic acid	0.288	0.459*

Significant at ** $p \leq 0.01$ and * $p \leq 0.05$

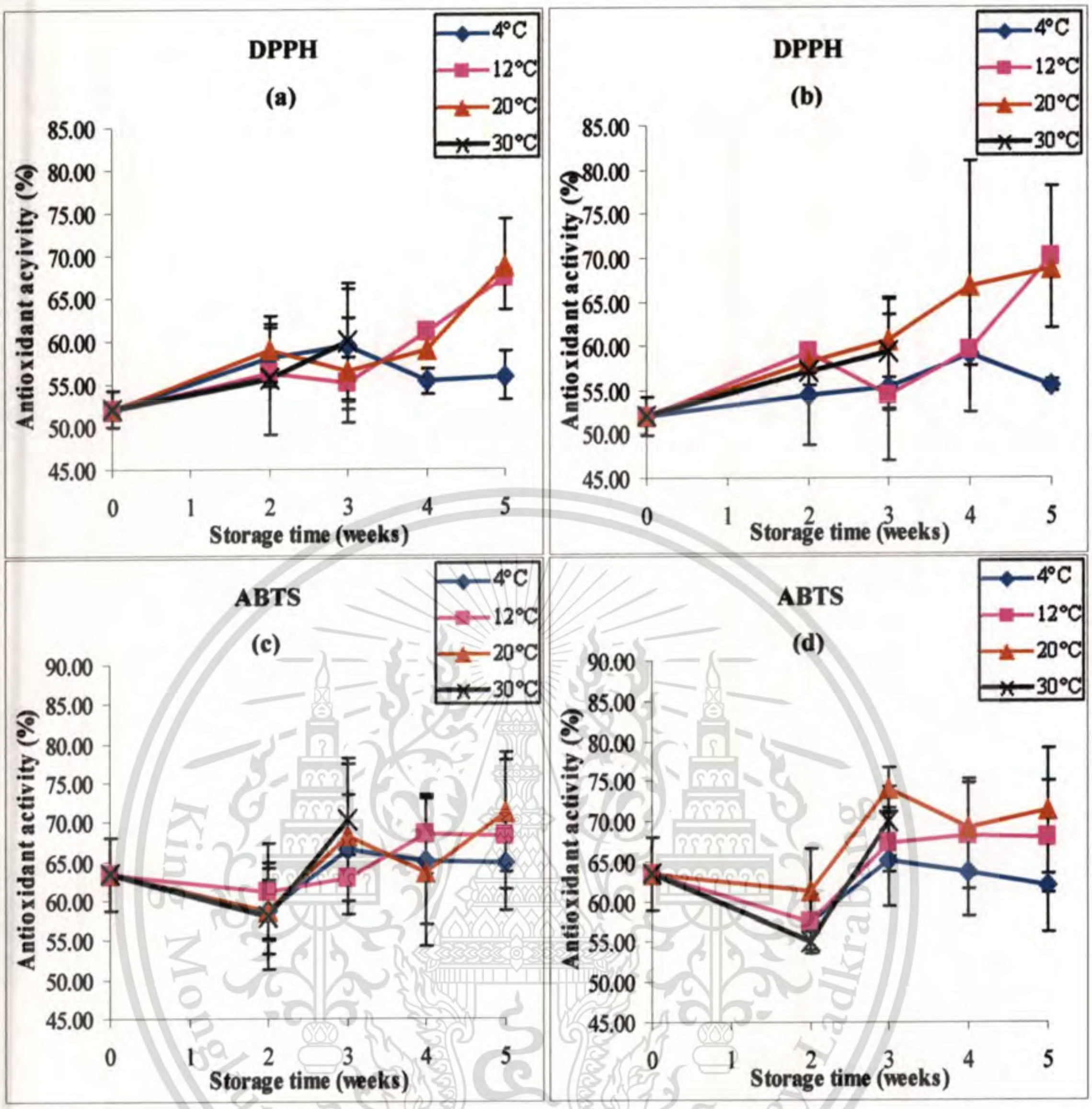


Figure 4.8 Change in antioxidant activity of coated Thai tangerines during storage at 4, 12, and 20°C for 5 weeks and at 30°C for 3 weeks, 90±4% RH, DPPH of coated and uncoated sample (a) and (b), ABTS of coated and uncoated sample (c) and (d)

It may be concluded from these experiments that storage temperatures affected the quality of coated tangerine. At 30°C the storage period was 3 weeks which at 4°C was 5 weeks. To store coated tangerine at 30°C, the commercial coating material may need improvement. The phenolic acid increased except the ferulic acids decreased upon prolong storage. The weight loss of coated tangerine was minimized at all temperature storage, which is the great benefit with respect to sale profit. The antioxidant activity of coated tangerine was correlated with phenolic acids more than ascorbic acid.

4.2 Development of chitosan formulation for tangerine coating

The response surface methodology with a central composite design was used to generate the combinations of chitosan and oleic acid. It was found out that a total of 13 experiments were needed for those two factors. The combination of chitosan and oleic acid in each formulation and the response on weight loss, ethanol, CO_2 and O_2 are presented in Table 4.2. To predict the optimum concentration of chitosan and oleic acid, the Design-Expert 7.6.1 software was applied and the results are shown in Table 4.3 and 4.4. The optimal level of response variables was determined by the desirability with the scale from 0–1.0. The desirability function was based on the transforming of the measured property of each response to a dimensionless desirability scale. The overall desirability is calculated as a geometrical average of partial desirability functions. The partial response is to be maximized, minimized, or assigned a target value (Derringer and Suich, 1980).

Table 4.4 shows the quadratic model of weight loss, oxygen, carbon dioxide, and ethanol. These models were found to be adequate as there was a no significant lack of fit in all the response variables (Table 4.3). Coefficient values (R^2) of all models ranged between 77-93% indicating that the model can be used to predict the effect of chitosan and oleic acid in the formulation on the parameters associated with the quality of coated tangerines.

4.2.1 Weight loss

As the concentrations of chitosan and oleic acid increased, the weight loss decreased (Figure 4.9a, Table 4.4). Only oleic acid not chitosan that showed a quadratic effect on the weight loss (Table 4.3, Figure 4.9a) as its concentration increased. It appeared to have a greater effect on weight loss than chitosan. Addition of oleic acid into chitosan film can enhance the film hydrophobicity (prevention on losing water) by increasing the surface solid density which consequently increased the water vapour resistance of the film (Vargas et al., 2006). Gontard et al. (1994) also reported that oleic acid has a certain degree of mobility due to its double bond, which resulted in reducing the moisture barrier properties of film. Vargas et al. (2009a) reported that water vapour permeability value of chitosan-oleic film was lower than pure chitosan film. It is confirmed by this experiment.

Table 4.2 Central composite design and response values

	Coded		Uncoded		Response			
Run	X_1	X_2	Chitosan (%)	Oleic acid (%)	Weight loss (%)	O_2 (%)	CO_2 (%)	Ethanol (ppm)
control	-	-	-	-	15.15	9.61	3.97	1011.74
1	-1	-1	1.15	1.44	13.53	9.48	4.35	1849.43
2	-1	1	1.15	3.56	12.91	7.54	4.67	2086.33
3	1	1	1.85	3.56	12.04	6.36	5.89	2222.61
4	1	-1	1.85	1.44	13.29	6.37	5.17	2038.80
5	0	0	1.50	2.50	12.64	6.92	4.26	2064.99
6	0	0	1.50	2.50	12.42	7.13	4.66	2162.18
7	0	0	1.50	2.50	12.53	7.73	5.62	2268.19
8	0	0	1.50	2.50	12.99	7.91	4.58	2044.10
9	0	0	1.50	2.50	12.88	7.81	4.64	2038.50
10	1.41	0	2.00	2.50	12.06	5.83	6.87	2245.19
11	-1.41	0	1.00	2.50	12.72	8.05	4.04	1885.80
12	0	1.41	1.50	4.00	12.57	6.60	5.93	2350.56
13	0	-1.41	1.50	1.00	13.94	8.30	4.43	1973.45

Table 4.3 Analysis of variance of independent variables on the response variables for chitosan-oleic acid coating

Source	**F-value**				
	df	Weight loss	O_2	CO_2	Ethanol
Model	5	17.71***	13.46***	4.89*	5.65*
X_1 (Chitosan)	1	14.03**	43.94***	17.26**	11.45**
X_2 (Oleic acid)	1	48.72***	15.09**	7.04*	14.99**
$X_1 X_2$	1	2.67	5.93*	0.15	0.09
X^2_1	1	2.74	2.13	2.02	1.55
X^2_2	1	18.15***	0.06	0.51	0.05
Residual	7				
Lack of Fit	3	0.20	0.52	1.03	0.48
Pure Error	4				
R^2	-	0.9267	0.9058	0.7774	0.8013

* Significant at $p \leq 0.05$.

** Significant at $p \leq 0.01$.

*** Significant at $p \leq 0.001$.

Table 4.4 Predictive models from the response surface methodology

Dependent values	**Models**	
Weight loss	$12.88+3.23*X_1-1.20*X_2-0.42*X_1X_2-0.97*X^2_1+0.28*X^2_2$	(1)
O_2	$13.80-0.58*X_1-2.60*X_2+1.29*X_1X_2-1.75*X^2_1+0.03*X^2_2$	(2)
CO_2	$7.38-5.18*X_1-0.65*X_2+0.27*X_1X_2+2.22*X^2_1+0.12*X^2_2$	(3)
Ethanol	$558.00+1371.23*X_1+133.99*X_2-35.39*X_1X_2-32930*X^2_1+6.30*X^2_2$	(4)

X_1=chitosan

X_2=oleic acid

4.2.2 Internal gases

The internal gases (O_2 and CO_2) of coated tangerines were affected by the combination of chitosan and oleic acid in the formulation (Table 4.3). The increase of chitosan and oleic acid concentration significantly decreased the internal oxygen and increased the carbon dioxide (Figure 4.9b and 4.9c), which shows that coating of tangerine can restrict the exchange of gases between fruits and the surrounding atmosphere. The results correspond with the report of Wu et al. (2002) who found that increase concentration of chitosan could lower the internal O_2 and elevate the CO_2 and the effect of chitosan on gas permeability was directly associated with its concentration. Therefore the inappropriate coating may result in off flavor as a consequence of too low oxygen and too high carbon dioxide levels.

From Table 4.3 it can be seen that the interaction effect of chitosan and oleic acid was found only on the internal O_2 but not on CO_2. It was found that to a certain limit the increase of concentration of oleic acid reduced the internal O_2 level (Figure 4.9b). This might be due to the effect of oleic acid on the structure formation of the film. The gas permeability of edible films or coatings may be depend on several factors including the integrity of the film, the ratio between crystalline and amorphous zones, the hydrophilic-hydrophobic ratio, the polymeric chain mobility; the interaction between the film-forming polymer and the presence of a plasticizer (Garcia et al., 2000). For the starch-based film the O_2 permeability is lower than the CO_2. It was explained to a higher solubility of CO_2 in the starch films. The permeability of the film is strongly dependent of the interaction between the polymer matrix, the permeating gas and the environmental conditions such as temperature and relative humidity.

By using the RSM, the combinations of chitosan and oleic acid were designed and the response variables were measured. The range of O_2 was 5.83-9.48% and CO_2 was 4.26-5.93%. In general the respiration of fruits can be shifted to anaerobic if the O_2 and CO_2 levels are less than 1-3% and greater than 10% respectively (Kader, 1986). Hagenmaier (2002) also proposed the extinction point of internal O_2 was 4% and CO_2 was 5% for a mandarin hybrid at 21 $^\circ$C. Thus the range of O_2 and CO_2 generated from this experiment by the use of RSM are possible to apply to create formula from chitosan with oleic acid. However the optimal combination must be pointed out.

4.2.3 Ethanol content

Naturally ethanol can occur in many citrus fruits along with the formation of off flavor at high concentration affecting consumer acceptability (Ahmed and Khan, 1987). Table 4.3 shows that ethanol concentrations are affected by the concentrations of chitosan and oleic acid in coating formula. With the uncoated tangerine the ethanol content was lower than the coated ones. The ethanol level increased with the increased amount of chitosan and oleic acid (Figure 4.9d).

Sinclair (1984) had reported that the ethanol concentration in juice of orange was affected by waxing. Tangerines kept at 21°C for 5 days was rated as less fresh when the ethanol content was greater than 1500 ppm (Hagenmaier, 2002). Alonso et al. (2005) found the ethanol concentration in stored mandarin ranged 1524.3 to 4009.1 ppm but these levels did not affect consumer acceptance. From this experiment the range of ethanol was 1849 to 2350 ppm which is corresponding to that reported by Alonso et al. (2005). This experiment confirmed the relationship between concentrations of ethanol and the coating formulations.

4.2.4 Optimization of chitosan-oleic acid coating formula

The advantage of using RSM is to optimize the formulation by taking into account of more reponse variables and minimize number of treatment. Optimization was based on the generation of results which minimized response for weight loss, CO_2, ethanol and maximized response for O_2. The desirability function value varies from zero, undesirable response, to one, desirable response. The optimum desirability range proposed by Lazic (2004) is 0.80-0.63. The desirability values for weight loss, O_2, CO_2 and ethanol ranged from 0.66 to 0.95 while the composite desiribility was 0.80 (Table 4.5). The optimum chitosan-oleic acid concentration for coating was found to be 1% chitosan and 2.50% oleic acid. This optimum formula gave the predicted values of weight loss 12.81%, internal oxygen 8.38%, carbon dioxide 4.24%, and ethanol 1885.71 mg/L (Table 4.5). While the uncoated tangerine showed the higher of weight loss (15.15%) but lower carbon dioxide (3.97%), and ethanol (1011.74 mg/L). The great advantage of chitosan oleic coating is the reduction of weight loss which can be beneficial to fruit grower and retains fresh look to consumers.

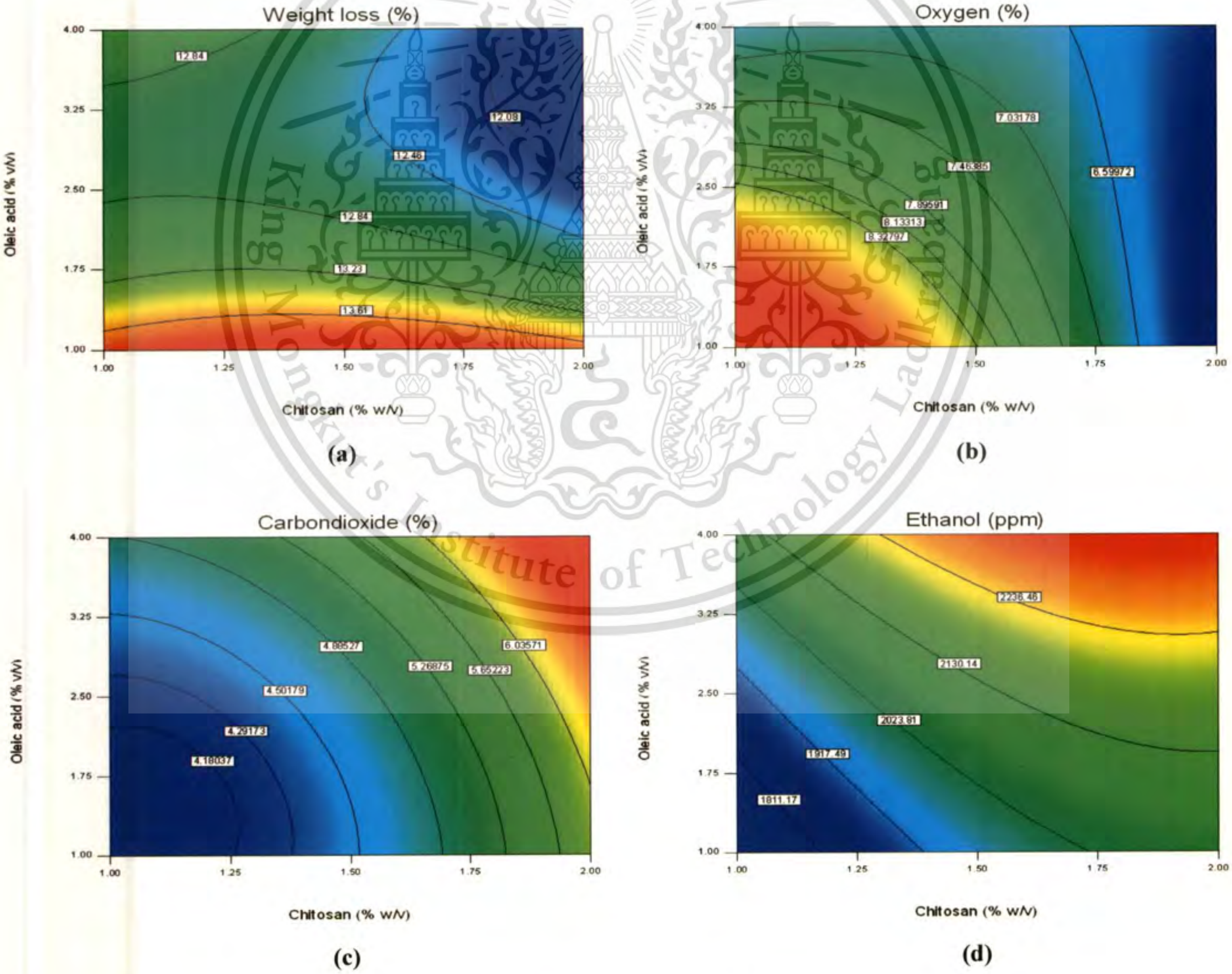
In conclusion the RSM was a useful tool to generate the optimal combination formula for a coating mixture to tangerine. It generates the model that can explain the effect of independent variables on the response variables. Both chitosan and oleic acid had a linear effect on weight loss, ethanol, carbon dioxide, and oxygen. Only the oleic acid showed a quadratic effect on the weight loss. The desirability values for weight loss, O_2, CO_2 and ethanol ranged from 0.66 to 0.95 while the composite desiribility was 0.80. The optimum formulation for coating tangerines was 1% (w/v) of chitosan plus 2.5% (v/v) of oleic acid which was suitable for tangerine storage at room temperature (30±2°C), 79±5% RH for 3 weeks.

Table 4.5 Optimization of chitosan coating in terms of composite properties

Response variable	**Optimization of chitosan-oleic coating**						
	Goal	**Lower**	**Target**	**Upper**	**Weight**	**Predicted responses**	**Desirability**
Weight loss (%)	Minimize	0	0	13	0.1	12.81	0.66
O_2 (%)	Maximize	4	20	20	0.1	8.38	0.88
CO_2 (%)	Minimize	0	0	15	0.1	4.24	0.95
Ethanol (ppm)	Minimize	0	0	2000	0.1	1885.71	0.75

Solution :Chitosan 1.0%, oleic acid 2.50%

Composite desirability = 0.80



(a)

(b)

(c)

(d)

Figure 4.9 Contour plots for the effect of chitosan (X_1), and oleic acid (X_2) on (a) weight loss, (b) oxygen, (c) carbon dioxide, and (d) ethanol of coated tangerine.

4.3 Comparative study of qualities of tangerine coated with chitosan-oleic acid and commercial based on chemical composition, total polyphenol, phenolic acids, antioxidant activity, and sensory evaluation

From the previous experiment, the chitosan-oleic acid formulation was proposed to be 1% chitosan (w/v) and 2.5% oleic acid (v/v). Therefore in this experiment the chitosan-oleic acid will be compared to the shellac based coating. Fruits were coated with chitosan-oleic and shellac (based material) using a self made machine. Some characteristics of coating were compared. The coated fruit were stored at 30°C for 4 weeks. Each week 15 fruits samples were taken out for measurement as described in topic 4.1. Moreover, this topic the effect of coating on external appearance, reflectance (glossiness) and change of flavor was investigated. The results are as follow.

4.3.1 Viscosity and solid content of coating

The chitosan-oleic acid and shellac based coatings were prepared and measured for viscosity and solid content and shown in Table 4.6. Viscosity of the chitosan-oleic acid and 18% shellac based coatings were not difference that eases the spraying by coating machines. However, the solid content of these two coating were different. This may be due to the difference in molecular weight of the chitosan (248 kDa) which is much higher than the shellac (1006 Da, Sharma et al, 1983) used in this experiment may affect the viscosity. Kwaambwa et al. (2007) reported that the viscosity of polymers related to size of molecule, molecular weight, and molecular shape. The binding property of the film is increased with the increasing of concentration. Luangwilai et al. (2007) reported that increasing concentrations of shellac resulted in increasing of binding and viscosity of the coating. Cisneros-Zevallos and Krochta (2003) reported that the film thickness of coating depends on the coating solution properties such as density, viscosity, surface tension and concentration. The difference of solid content of coatings using in this experiment may effect on the structure of the film (Figure 4.13) and affected on change of quality of tangerine during storage.

Table 4.6 Viscosity and solid content of chitosan-oleic acid and shellac coating solution

Type of coating	Viscosity (cp)	Solid content (%)
Chitosan-oleic acid	3.55±0.02	3.11±0.07
Shellac based (commercial)	3.50±0.03	19.13±0.13

4.3.2 The change of external appearance (peel and decay)

The appearance of fresh fruits is a primary criterion in making purchasing decisions (Kays, 1991). The appearances of uncoated and coated tangerine were changed during storage. The peel color of uncoated and shellac coated samples were changed more than those coated with chitosan-oleic acid during storage for 4 weeks (Figure 4.10). The chitosan-oleic acid coating delayed fruit degreening for 2 weeks but the shellac coating did not. This result corresponds to Foenes et al. (2005) who reported that chitosan delayed natural clementile mandarin peel pigmentation for several days. The mode of action is not known. They suggested that chitosan has a hormone-like effect (gibberellic acid), acting inside the cell. Gibberellic acid has been applied for delaying peel senescence in clementile. This hormone delays natural chlorophyll degradation and carotenoid synthesis in Satsuma and clementile mandarin. Chitosan may act on the regulation of some key enzymes such as chlorophyllase, which is responsible for chlorophyll degradation. Chitosan's affect was reported on the inactivation the activity of polyphenol oxidase and peroxidase in lichi (Zhang and Quantick, 1997) and longan (Jiang and Li, 2001).

The uncoated fruit and that coated with shellac were observed on spoilage when stored for 3 weeks, but not with the fruits coated with chitosan-oleic acid (Figure 4.11). In Table 4.7, the result showed that tangerine coated with chitosan-oleic acid could inhibit spoilage of tangerine. The spoilage of fruit that were uncoated and coated with shellac was 10.00 and 16.25%, respectively. During storage the fruits spoilage 10–15% is not economical (Ladaniya, 2008). The citrus fruit can easily become diseased with blue mold, green mold, scald, or watery rot, therefore the lost during postharvest storage must be minimized (Porat et al., 2000). Several studies have reported that chitosan was effective inhibiting fungal growth and maintaining the external appearance of fortune mandarin, valencia orange and murcott tangor for a longer time (Galed et al., 2004; Chien et al., 2007b).

Chitosan has a dual function working as a fungistaic and as a biostiulant. It directly interferes with fungal growth and with activation of several defense processes. These defense mechanisms include the accumulation of chitinases, synthesis of proteinase inhibitors and induction of callous synthesis (El Ghaouth, 1992). The results can indicate that uncoated and shellac coated tangerine can not store more than 3 weeks, but tangerine coated with chitosan-oleic acid can stored for 4 weeks.

Uncoated day 0 **Chitosan oleic acid day 0** **Shellac day 0**

Uncoated 2 weeks **Chitosan oleic acid 2 weeks** **Shellac 2 weeks**

Uncoated 3 weeks **Chitosan oleic acid 3 weeks** **Shellac 3 weeks**

Uncoated 4 weeks **Chitosan oleic acid 4 weeks** **Shellac 4 weeks**

Figure 4.10 Photographs of tangerine uncoated and coated with chitosan-oleic acid and shellac during storage at 30°C, 90±4% RH for 4 weeks

Uncoated 3 weeks	Shellac 3 weeks

Uncoated 4 weeks	Shellac 4 weeks

Figure 4.11 Photograph of spoiled tangerines during storage at 30°C, 90±4% RH for 4 weeks

Table 4.7 Percentage of spoiled tangerines with chitosan-oleic acid and shellac coating during storage at 30°C, 90±4% RH for 4 weeks

Treatment / **weeks**	**% of spoilage**				
	0	**1**	**2**	**3**	**4**
Uncoated	0.00	0.00	0.00	6.25	10.00
Chitosan-oleic acid	0.00	0.00	0.00	0.00	0.00
Shellac	0.00	0.00	0.00	6.25	16.25

4.3.3 Glossiness

The glossiness of citrus fruits peel is one of the important characteristics for customers demand. Surface appearance is associated with the distribution of light by the object. The four types of light distribution and their respective relationships to surface appearance are: diffuse reflection (shiny), specular reflection (glossy), mirror-like, diffuse transmission (cloudy or opaque) and specular transmission (translucent) (Hunter, 1975). The method for measurement glossiness of fruit and vegetable was used glossmeter and measurement of light refraction in a spectrophotometer. In this study, the light reflection was used to evaluate the glossiness of tangerine samples. Light reflectance of sample was read between 650 and 700 nm. If the curves absent the sample was loss light reflectance (Quezada Gallo et al., 2005). The reflectance of coated tangerines with chitosan-oleic acid and shellac based was showed in Figure 4.12. The shellac based coating produced more light reflectance (35-40%) than the chitosan-oleic acid (15-20%) and uncoated (10-15%). The result showed that coating materials affected on glossiness of coated fruits. Chen and Nussinovitch (2001) reported that shellac coating formulation with out hydrocolloid is responsible for creating uniform layers of large flakes covering the fruit. Chitosan based coating provide the slight sheen on products when used at 1-2% (Baldwin, 1994). Gloss properties of coating may due to the formation and structure of film covering the fruits (Figure 4.13). The surface of fruit coated with chitosan-oleic acid coating showed the structure of film similar to the uncoated one. Shellac coating showed that a film layer with more uniform structure will be produced. The gloss of edible coatings as affected by surfactants, lipids, relative humidity and storage time has been studied by Trezza and Krochta (2000). It was shows that type and level of surfactants, particle size of lipid dispersion were influenced gloss value of coating, however storage time did not affected coating gloss when storage at 23°C and 75% relative humidity for 7 month. This result indicated that the concentration of oleic-acid in chitosan coating formulation was improving glossiness of fruit.

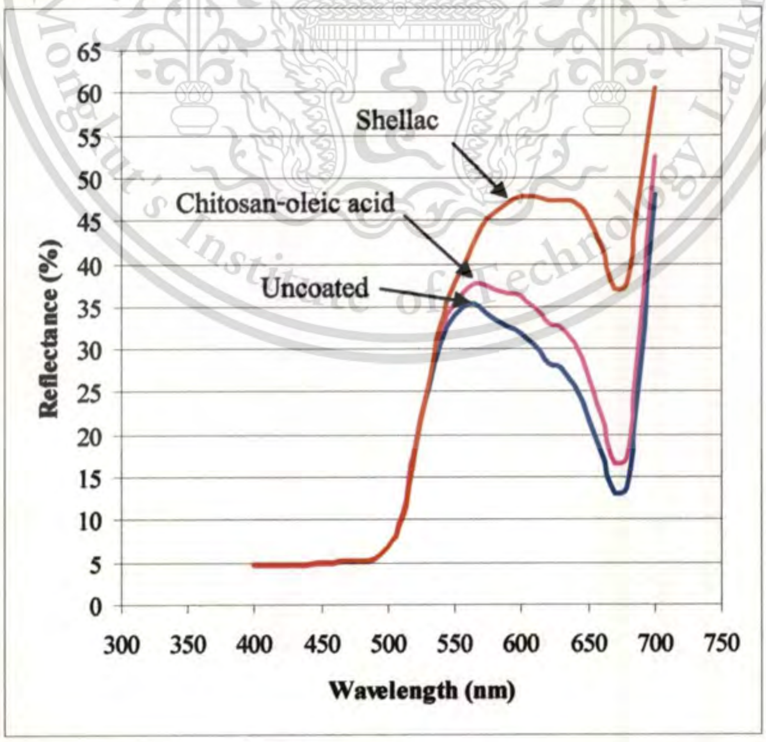


Figure 4.12 Effect of coating materials on light reflectance of tangerine coated with chitosan-oleic acid and shellac

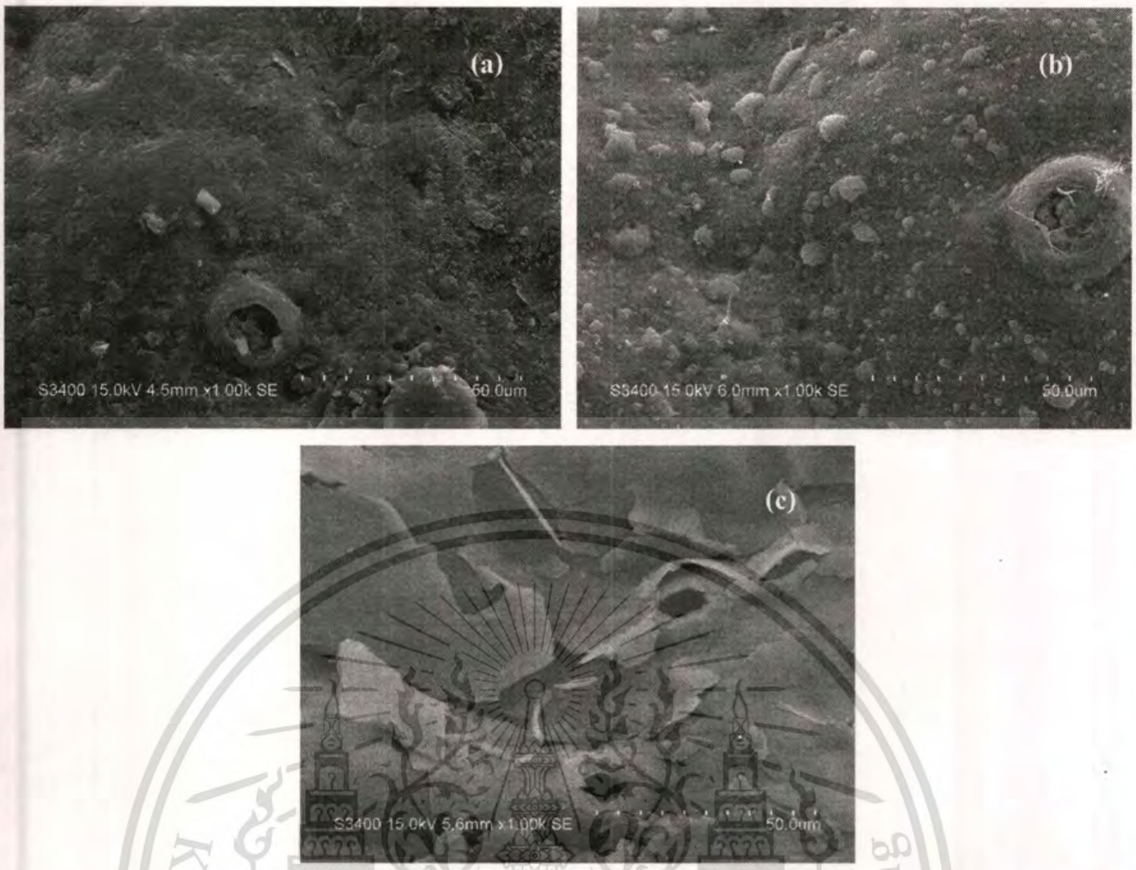


Figure 4.13 Structure of film coating on tangerine peel by scanning electron microscope (SEM) (a) uncoated (b) chitosan-oleic acid and (c) shellac coating

4.3.4 Volatile compounds analysis

The volatile profile of tangerine juices was determined by GC-MS coupled with solid phase micro extraction (SPME). Eighteen volatile compounds (i.e. ethanol, ethyl acetate, ethyl propanoate, ethyl isobutyrate, ethyl butanoate, butanoic acid, α-pinene, sabinene, β-pinene, β-myrcene, α-phellandrene, α-terpinene, ρ-cymene, *d*-limonene, ocimene, ξ-terpinene, terpinolene, 3-carene) were identified and quantified in juices from tangerine coated with chitosan-oleic acid and shellac coating during storage for 2 weeks. Fresh tangerine juice contained thirteen volatile compounds (ethanol, α-pinene, sabinene, β-pinene, β-myrcene, α-phellandrene, α-terpinene, ρ-cymene, *d*-limonene, ocimene, ξ-terpinene, terpinolene, 3-carene) which was less volatile compounds than the stored samples. The predominant compounds in fresh tangerine were *d*-limonene (95.11%), β-myrcene (2.94%), ethanol (0.86%), α-pinene (0.22%) and 3-carene (0.17%) (Table 4.8). Ethyl acetate, ethyl propanoate, ethyl butanoate, ethyl isobutyrate and butanoic acid were accumulated in coated tangerine during storage, where as the volatile compound in tangerine coated with shellac increased more than those with chitosan-oleic coating. The result indicates that the coating treatments affected on the change of volatile compounds in coated tangerine during storage. The tangerine coated with shellac coating showed high levels of ethyl acetate ethyl propanoate, ethyl butanoate, ethyl isobutyrate and butanoic acid that make this coating not appropriated for tangerines due to off-flavor. For this experiment, the ethanol was detected in fresh tangerine that is due to ethanol is naturally present in fruit and is thought to be enhancer of the flavor if it presents in low concentrations (Nisperos-Carriedo and Shaw, 1990). The increase in concentrations of ethyl acetate, isopentanol and ethyl butanoate in Orlando and temple orange during storage was reported by Hagenmaier and Shaw (2002). They suggest that pre-harvest or harvest temperatures are important factors to determine the postharvest qualities when fruit is coated with high-gloss shellac-resin based. Nisperos-Carriedo and Shaw, (1990) suggested the high level of ethyl acetate in unpasteurized orange juice was due to enzyme activity in the juice. Ethyl butanoate is considered as an indicator of quality in the aromatic fraction of a juice, consequently a reduction in the concentration of this component resulted in an improvement of the aromatic quality (Nisperos-Carriedo and Shaw, 1990).

d-limonene was affected by both coating treatments and storage time. In fresh tangerine the limonene content was higher than the coated ones. Reduction of limonene in chitosan-oleic acid was less than the shellac. It was reported that limonene degraded to other terpene during storage period (Perez-Lopez et al., 2006). These results can be explained that the flavor change of coated tangerines was affected by many volatile compounds not only ethanol. Obenland et al. (2008) suggested that both minimizing storage time and application of coating do not influence the volatile flavor profile in the navel orange.

Table 4.8 Volatile compound profile in juice of coated tangerine with different coating during storage at 30°C, 90±4% RH for 2 weeks

Volatile compound	Relative peak area (%)						
	1 week				2 weeks		
	fresh	uncoated	Chitosan-oleic acid	shellac	uncoated	Chitosan-oleic acid	shellac
ethanol	0.86	2.24	1.92	4.71	2.42	4.62	4.88
ethyl acetate	-	0.32	0.31	1.80	0.69	1.81	4.29
ethyl propanoate	-	-	-	0.45	0.08	0.23	0.50
ethyl isobutyrate	-	-	-	-	-	0.02	0.13
ethyl butanoate	-	-	-	-	0.05	0.11	0.35
butanoic acid	-	-	-	0.13	-	0.02	0.18
α-pinene	0.22	0.22	0.18	0.24	0.29	0.32	0.43
sabinene	0.04	0.05	0.09	0.02	0.08	0.10	0.06
β-pinene	0.19	0.33	0.22	0.23	0.34	0.45	0.25
β-myrcene	2.94	2.35	2.43	2.47	2.74	2.64	2.34
α-phellandrene	0.12	0.08	0.07	0.08	0.15	0.11	0.11
α-terpinene	0.05	0.07	0.05	0.05	0.04	0.06	0.06
ρ-cymene	0.07	0.09	0.08	0.07	0.06	0.05	0.08
d-limonene	95.11	93.88	94.30	89.55	93.37	89.96	85.99
ocimene	0.06	0.06	0.08	0.12	0.08	0.06	0.06
ξ-terpinene	0.06	0.08	0.06	0.06	0.05	0.06	0.06
terpinolene	0.10	0.09	0.08	0.08	0.06	0.07	0.08
3-carene	0.17	0.15	0.11	0.13	0.18	0.13	0.37

4.3.5 Weight loss

The tangerine coated with shellac showed lower weight loss than chitosan-oleic acid (Figure 4.14). Weight loss of uncoated tangerine and coated with chitosan-oleic acid and shellac coating losses were 12.17±0.88, 10.17±0.90 and 8.88±0.91, respectively after 4 weeks of storage. This result showed that chitosan-oleic coating can be retarded the water loss to some extent. The result is similar to the report of Baldwin et al. (1995). However, the shellac coating is more effective with respect to weight loss than chitosan-oleic acid coating. Because that chitosan films have relatively high water vapor permeability (Wong, et al., 1992; Butler, et al., 1996; Caner, et al., 1998). The addition of lipid materials to hydrophilic coatings can improve their moisture barrier properties (Amarante and Banks, 2001). Chein et al. (2007b) reported that murcott tangor orange coated with chitosan was slower water loss than the control. The weight loss of all treatment in section 4.1.4 were higher than in this section that may be due to the difference in washing operation before coating treatment. Hagenmaier and Baker (1993) reported that the higher rate of weight loss for the fruit from commercial packinghouses is caused by washing operation which remove natural wax by the action of detergent and washing brushes. In this experiment the fruit was washed in the lab with only soaking in trap water.

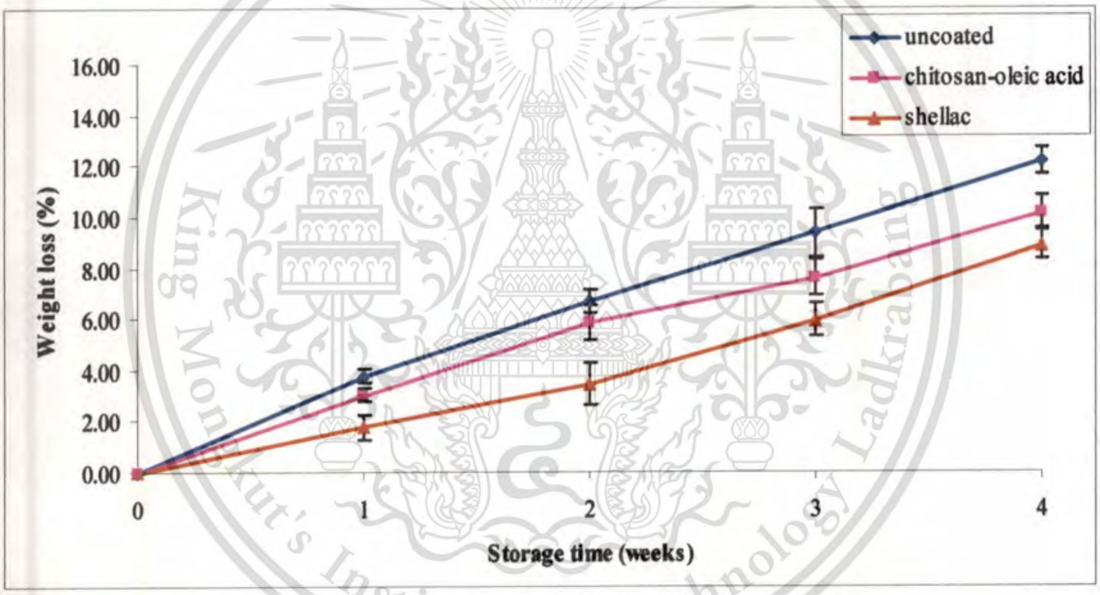


Figure 4.14 Change in weight loss of tangerine coated with different coating during storage at 30°C, 90±4% RH for 4 weeks

4.3.6 Total soluble solid

Tangerines showed gradual increase in total soluble solids (TSS) during storage. Storage period significantly effect TSS. The coating formulations were not significantly different in TSS (Appendix B, Table 9B). The TSS in the uncoated tangerine and coated with chitosan-oleic acid and shellac slightly increased at the end of storage for 4 weeks (Table 4.9). Dou et al.

(2004) found that TSS in LB8-9 mandarin coated with shellac and carnauba wax fruit changed after storage for 12 weeks. This result showed that total soluble solid was affected by weight loss.

4.3.7 pH

The pH value of tangerine with uncoated and coated with chitosan-oleic acid and shellac coating at initial was 4.13 then increased to 4.28, 4.39 and 4.40 respectively, at the end of storage (Table 4.9). Shellac and chitosan-oleic acid coating showed increasing trend of pH value. This experiment confirmed the results of section 4.12 and corresponds with Roongruangsri et al. (2009) who reported coating treatment had no significant change of pH values in tangerine juice.

4.3.8 Titratable acidity

Titrable acidity decreased in all coating treatments during storage period. There was no significant difference in the titratable acidity among the samples with uncoated and coated with chitosan-oleic acid and shellac (Appendix B, Table 11B). The titratable acidity of tangerine with uncoated and coated with chitosan-oleic acid and shellac were 0.36±0.01, 0.32±0.03 and 0.33±0.06, respectively (Table 4.9). The result indicated that coating treatment did not affect the titratable acidity of tangerine stored for 4 weeks. Roongruangsri et al. (2009) observed the similar results.

4.3.9 Ascorbic acid

During storage the tangerine coated with different coating showed the significant decrease of ascorbic acid content (Appendix B, Table 13B). The ascorbic acid content at 0 day was 25.27±0.55 mg/100mL and reduced to 18.41±1.06, 16.97±0.93 and 16.56±1.22 mg/100mL in uncoated, chitosan-oleic acid and shellac coating, respectively at the end of storage. In uncoated tangerine the ascorbic acid content was higher than the coated fruits. According to Mahrouz et al. (2002), vitamin C is sensitive to waxing and washing with cold water treatment. The tangerine coated with chitosan-oleic acid had more ascorbic acid content than the ones coated with shellac after storage for 4 weeks (Table 4.9). The result corresponded with Oms-Oliu et al. (2008) reported vitamin C retention in fresh cut melon was significantly affected by the coating composition and storage time, although vitamin C of both coated and uncoated samples decreased throughout storage.

4.3.10 Total polyphenol

The total polyphenol content in tangerine at 0 day was 21.05±1.76 mg gallic acid/100mL and increased to 26.34±0.91, 25.55±0.95 and 26.02±1.97 mg gallic acid/100mL in uncoated, chitosan-oleic acid and shellac coating, respectively, at the end of storage period (Table 4.9). This result similar to previous study that reported the total polyphenol content in coated tangerine increased during storage. The fruit with uncoated and coated with shellac was higher in the total polyphenol content than the chitosan-oleic acid coated fruit but not significantly different. The accumulation of phenolic compounds shown in both the coated and uncoated samples may be promoted by phenyl alanine ammonialyase (PAL) activity which increased in the synthesis of phenolic compounds. Puschmann et al. (2007) observed an increase in PAL activity during storage of both starch-chitosan coated baby carrots and uncoated ones.

Table 4.9 Total soluble solid, pH, titratable acidity, ascorbic acid and total polyphenol in juice of coated tangerine with different coating materials during storage at 30°C, 90±4% RH for 4 week

Coating types	Storage time (weeks)	Total soluble solid (°brix)	pH	Titratable acidity (%)	Ascorbic acid (mg/mL)	Total polyphenol (mg gallic acid/100mL)
uncoated	0	8.20±0.10	4.13±0.07	0.36±0.04	25.27±0.55	21.05±1.76
	1	8.67±0.07	4.38±0.11	0.33±0.05	25.53±0.55	23.00±1.95
	2	8.92±0.04	4.21±0.09	0.35±0.04	24.36±2.18	25.67±1.35
	3	8.67±0.30	4.30±0.03	0.29±0.01	21.72±0.30	24.55±0.59
	4	9.55±0.35	4.28±0.02	0.36±0.01	18.41±1.06	26.34±0.91
Chitosan-oleic acid	1	8.67±0.07	4.43±0.18	0.34±0.05	24.29±0.31	21.50±0.49
	2	8.92±0.04	4.25±0.11	0.37±0.05	23.64±0.66	26.90±0.54
	3	8.67±0.30	4.28±0.08	0.30±0.02	20.83±0.90	24.91±1.01
	4	9.20±0.35	4.39±0.07	0.32±0.03	16.97±0.93	25.55±0.95
Shellac	1	8.60±0.14	4.41±0.09	0.34±0.06	21.55±0.18	23.00±0.84
	2	8.83±0.20	4.29±0.05	0.32±0.03	22.33±1.46	29.21±0.13
	3	8.93±0.12	4.39±0.05	0.30±0.02	19.94±0.30	24.63±0.97
	4	9.20±0.01	4.40±0.20	0.33±0.06	16.56±1.22	26.02±1.97

4.3.11 Hydroxycinnamic acids

The hydroxycinnamic acids content in uncoated tangerine was lower than the tangerine coated with chitosan–oleic acid and shellac coating. The initial content of caffeic, *p*-coumaric, sinapic, and ferulic acid were 1.99±0.19, 1.07±0.24, 2.76±0.16, and 44.82±3.46 mg/l respectively (Table 4.10). However, the coating treatment showed no significant change of hydroxycinnamic acids (Appendix B, Table 15B). Caffeic, *p*-coumaric, and sinapic acid in uncoated and coated samples showed the increasing trend during storage. Ferulic acid in uncoated tangerine and those coated with chitosan-oleic acid showed a little increase, but decreased in tangerine coated with shellac when stored more than 3 weeks. This result may suggest that tangerine coated with shellac should store less than 3 weeks due to the senescence. Rapisarda et al. (1998 and 2008b) reported that decreasing of hydroxycinnamic acids was due to senescence during storage.

Table 4.10 Hydroxycinamic acid in juice of coated tangerine with different coating materials during storage at 30°C, 90±4% RH for 4 weeks

Coating types	Storage time (weeks)	Caffeic acid (mg/L)	*p*-coumaric acid (mg/L)	Sinapic acid (mg/L)	Ferulic acid (mg/L)
uncoated	0	1.99±0.19	1.07±0.24	2.76±0.16	44.82±3.46
	1	2.53±0.46	1.15±0.13	2.60±0.41	44.38±5.44
	2	2.30±0.38	2.02±0.36	2.63±0.29	44.95±5.36
	3	2.96±0.68	1.94±0.38	2.77±0.38	41.91±7.91
	4	2.26±0.15	2.15±0.10	3.05±0.40	45.37±1.27
Chitosan-oleic acid	1	2.10±0.51	1.08±0.10	2.42±0.30	39.01±3.97
	2	2.58±0.31	2.17±0.23	2.88±0.35	49.03±4.42
	3	2.48±0.28	2.29±0.32	3.69±0.83	51.39±8.47
	4	2.49±0.19	2.16±0.07	3.39±0.21	46.97±0.68
Shellac	1	2.32±0.12	1.47±0.56	2.81±0.18	45.77±2.07
	2	2.82±0.56	2.50±0.60	3.04±0.36	39.73±2.00
	3	3.20±0.18	1.90±0.11	3.04±0.40	52.17±9.73
	4	2.12±0.20	2.04±0.20	3.23±0.20	44.69±4.42

4.3.12 Antioxidant activity

The antioxidant activity measured by DPPH and ABTS increased significantly during storage regardless of coating types (Appendix B, Table 16B and Table 4.11). The antioxidant activity in tangerine juice showed the similar trend as those in section 4.1.8. In this result the increase in phenollic compounds was related to the enhancement of antioxidant capacity of tangerine. Some authors have also related the accumulation of phenolic compounds to an increase in antioxidant activity (Reyes and Cisneros-Zevallos, 2003). In the study of fresh-cut Pielde Sapoi melon found the contribution of phenolic compounds to antioxidant activity was grater than vitamin (Oms-Oliu et al., 2008). Therefore, the increases of antioxidant activity in tangerine coated with both coatings are mainly from the phenolic compound and phenolic acids. Eberhardt et al. (2000) suggested antioxidant capacity comes from other several different phytochemicals. The results obtained have support their suggestion.

Table 4.11 Antioxidant activity in juice of coated tangerine with different coating materials during storage at 30°C, 90±4% RH for 4 weeks

Coating types	Storage time (weeks)	DPPH (%)	ABTS (%)
uncoated	0	54.11±6.02	67.07±2.80
	1	54.43±4.15	70.02±0.60
	2	58.21±4.95	66.67±1.47
	3	59.28±9.11	66.44±3.04
	4	66.03±4.10	68.58±1.55
Chitosan-oleic acid	1	59.54±6.59	72.21±0.70
	2	61.15±4.79	70.21±2.86
	3	57.59±5.06	71.19±1.81
	4	65.95±6.23	68.91±0.81
shellac	1	58.98±10.34	71.70±3.80
	2	56.24±6.05	70.40±4.97
	3	58.50±2.29	69.59±2.18
	4	63.28±6.50	68.06±0.38

4.3.13 Sensory evaluations

Sensory evaluation of juice extracting from tangerines with uncoated and coated with chitosan-oleic acid and shellac after storage for 3 weeks at 30°C was presented in a spider web (Figure 4.15). The quality attributes were color, flavor, taste, and overall acceptability. Tangerine with uncoated and coated with chitosan-oleic acid had no difference sensorial score with all attributes but lower than fresh sample (day 0). Coating treatments did not affect color of juice samples. The shellac based coating showed different significantly and lower sensorial score with taste, flavor and acceptability than those with uncoated and with chitosan-oleic acid. The result indicated that shellac coating was not suitable for tangerine due to off-flavor. The flavor score confirmed the previous finding that tangerine coated with shellac increased ethanol, ethyl acetate, ethyl propanoate, ethyl butanoate, ethyl isobutyrate and butanoic acid more than the fruits coated with chitosan-oleic acid coating during storage. The off-flavor of coated tangerine was detected when internal CO_2 and ethanol increased (Cohen et al., 1990) or other flavor components were produced (Hagenmaier and Shaw 2002). The result corresponded with Hagenmaier and Shaw (2002) who suggested that high-gloss coatings (shellac and resin) are not suitable for tangerine varieties due to this coating often reduce internal O_2 to value below 4% at 21°C. The chitosan-oleic acid was more appropriated for coat tangerine and storage at high temperature than the shellac coating.

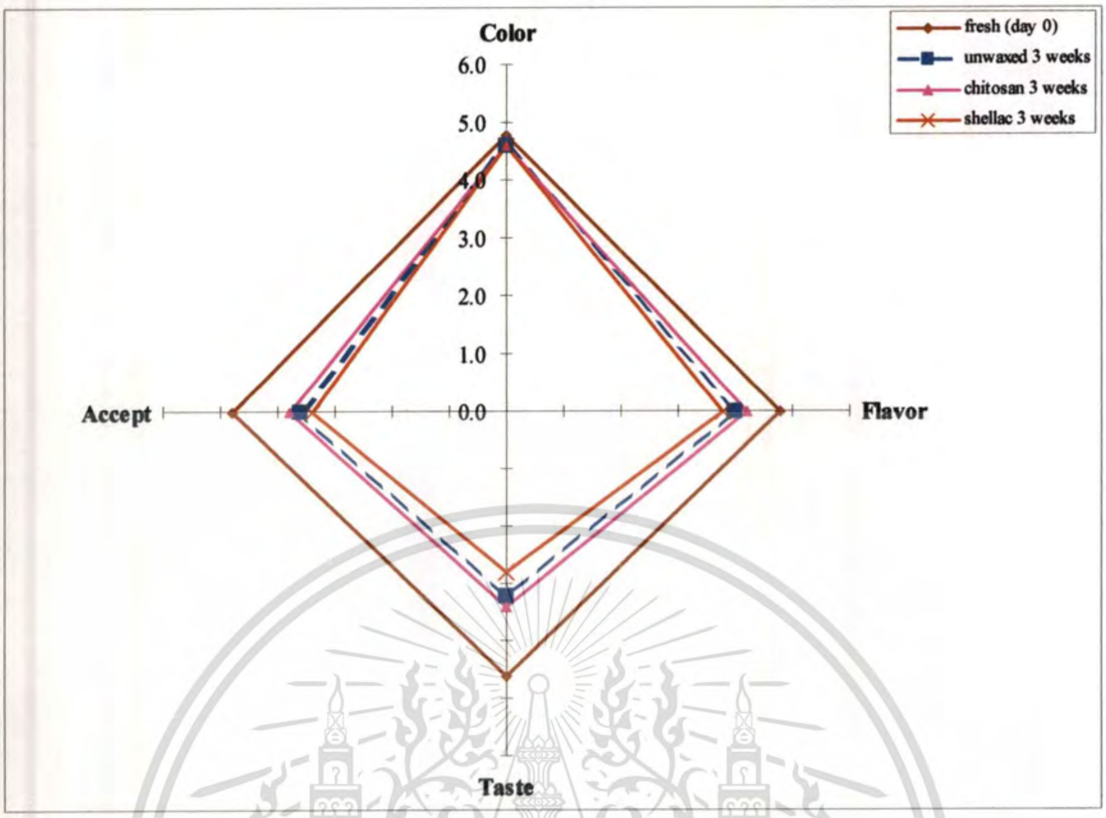


Figure 4.15 Sensory profile of coated tangerine juice during storage at 30°C, 90±4% RH for 3 weeks

In conclusion chitosan-oleic acid coating was appropriated for tangerine by improve external appearance and inhibit the fungi growth. At 30°C tangerine coated with shellac coating and uncoated fruit have the storage period of 3 weeks where as chitosan-oleic acid coating was 4 weeks.

Both coating did not affect changes of chemical compositions and antioxidant activity, however shellac coating affected the flavor and sensorial characteristic of tangerine.

CHAPTER 5

CONCLUSIONS

Changes of total soluble solid, pH and titratable acidity in coated tangerine were affected by storage temperatures. During storage period total soluble solid and pH increased and titratable acidity was decreased. Uncoated fruits showed slightly higher total soluble solid than those of the coated fruit. Changes of ascorbic acid, total polyphenol, hydroxycinnamic acids and antioxidant activity in coated tangerine were affected by storage period regardless of temperatures. Ascorbic acid content decreased during the storage period irrespective of storage temperature.

Hydroxycinnamic acids found in coated tangerines were caffeic, *p*-coumaric, sinapic and ferulic acid and all these hydroxycinnamic acids increased during the early stage of storage and slightly declined at the end. At high temperature the hydroxycinnamic acids in coated were slightly increased except ferulic acid which highly increased at the end of storage. The DPPH correlated well with caffeic, *p*-coumaric sinapic acid and ferulic acid. Total polyphenol increased during storage and it correlated with ABTS and DPPH antioxidant activity.

Storage of coated tangerine at low temperature did not affect the antioxidant components. The weight loss of uncoated and coated tangerines was minimized at low temperature storage.

The response surface methodology (RSM) was a useful tool to generate the optimal formula for a coating mixture to tangerine. It generates the model that can explain the effect of independent variables on the response variables. The concentrations of chitosan and oleic acid in a coating formulation affected the internal gases (CO_2 and O_2) and ethanol. Both chitosan and oleic acid had a linear effect on weight loss, ethanol, carbon dioxide, and oxygen. Only the oleic acid showed a quadratic effect on the weight loss.

The optimum formulation for coating Thai tangerines was 1% (v/v) of chitosan plus 2.5% of oleic acid (v/v) which was suitable for tangerine storage at room temperature ($30\pm2^{\circ}C$) for 3 weeks. The desirability values for weight loss, O_2, CO_2 and ethanol ranged from 0.66 to 0.95 while the composite desirability was 0.80. This optimum formula gave the predicted values of weight loss 12.81%, internal oxygen 8.38%, carbon dioxide 4.24%, and ethanol 1885.71 mg/L.

The types and formulation of coatings affected the solid content, but not affected on the viscosity of the chitosan-oleic acid and shellac based coatings. The external appearances of uncoated and coated tangerine were changed during storage. The color of the peel of uncoated and shellac coated samples were changed more than the samples coated with chitosan-oleic acid during storage for 3 weeks. Chitosan-oleic acid coating can be delayed fruit degreening and inhibit spoilage of tangerine. The spoilage of uncoated tangerine and the ones coated with shellac coating were 10.00 and 16.25% respectively, stored for 4 weeks. The tangerines coated with shellac produced more light reflectance than those coated with chitosan-oleic acid and uncoated.

The volatile profile of tangerine juices was affected by coating treatment and storage time. The predominant compounds in tangerine included *d*-limonene, β-myrcene, ethanol, α-pinene and 3-carene. The volatile compound in fresh tangerine juice was less than those in juices from tangerine coated with chitosan-oleic acid and shellac coating during storage for 2 weeks. Off-flavor in coated tangerine occurred when ethanol, ethyl acetate, ethyl propanoate, ethyl butanoate, ethyl isobutyrate and butanoic acid increased and limonene decreased. The coating and storage period affected directly on weight loss. Shellac coating is more effective on reducing weight loss than chitosan-oleic acid coating.

The comparison of shellac and chitosan-oleic acid based coating showed that the total soluble solids, pH and titratable acidity of the coated tangerines were not significantly difference. The uncoated tangerine and those coated with shellac have the total polyphenol more than the chitosan-oleic acid coated fruit. Both coating material showed no significant change of hydroxycinnamic acids. Caffeic, *p*-coumaric, and sinapic acid in coated samples increased during storage but ferulic acid in tangerine coated with chitosan-oleic acid showed a little change, and decreased with shallec coated one.

The antioxidant activity measured by DPPH and ABTS increased during storage regardless of coating materials. The coating treatment affected on sensory evaluation of tangerine juice. The sensorial score of chitosan-oleic acid coated tangerine and the uncoated were not different in all attributes, however the score was lower than the fresh one. The shellac based coating showed lower sensory scores with taste, flavor and acceptability when compared to the uncoated and chitosan-oleic acid coated. At room temperature storage the chitosan-oleic acid coating can prolong shelf life of tangerine more than the shellac coating.

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APPENDIX

Appendix A

Analytical Method

A1 Ascorbic acid

(AOAC, 2000)

Chemical

1. Acetic acid (CH_3COOH)
2. Ascorbic acid
3. 2,6 Dichloroindophenol (sodium salt)
4. Metaphosphoric acid (HPO_3)
5. Sodium carbonate ($NaHCO_3$)

Reagents

1. Metaphosphoric acid–acetic acid solution

Dissolve, with shaking, 15 g HPO_3 pellets or freshly pulverized stick HPO_3 in 40 mL CH_3COOH and 200 mL H_2O; dilute to ca 500 mL, and filter rap idly through fluted pa per into glass-stoppered bottle.

2. Metaphosphoric acid–acetic acid–sulfuric acid solution

Mix metaphosphoric acid with 0.15M H_2SO_4 in place of H_2O.

3. Ascorbic acid standard solution (1 mg/mL)

Accurately weigh 50 mg of ascorbic acid standard that has been stored in desiccator away from direct sun light. Transfer to 50 mL volumetric flask. Dilute to volume immediately before use with HPO_3–CH_3COOH solution.

4. Indophenol standard solution

Dissolve 50 mg 2,6-dichloroindophenol Na salt that has been stored in desiccator over soda lime, in 50 mL H_2O to which has been added 42 mg $NaHCO_3$; shake vigorously, and when dye dissolves, dilute to 200 mL with H_2O. Filter through fluted paper into amber glass-stoppered bottle. Keep stoppered, out of direct sun light, and store in refrigerator.

Transfer three 2.0 mL aliquots ascorbic acid standard solution to each of three 50 mL Erlenmeyers containing 5.0 mL HPO_3–CH_3COOH solution. Titrate rapidly with indophenol solution from 50 mL buret until light but distinct rose pink persists ≥5 s. (Each titration should require ca 15 mL indophenol solution, and titrations should check within 0.1 mL.) Similarly titrate 3 blanks com posed of 7.0 mL HPO_3–CH_3COOH solution plus volume H_2O ca equal to volume indophenol solution used indirect titrations. After subtracting average blanks (usually ca 0.1 mL) from standardization titrations, calculate and express concentration of indophenol solution as mg ascorbic acid equivalent to 1.0 mL reagent (F). Standardize indophenol solution daily with freshly pre pared ascorbic acid standard solution.

Sample preparation

The juice of citrus fruits was filter. Add aliquots of 100 mL prepared juice to equal volumes of HPO_3–CH_3COOH solution. Designate total volume as V mL. Mix, and filter through rapid folded paper.

Determination

Titrate 3 test solution aliquots each containing ca 2 mg ascorbic acid and make blank determinations for correction of titrations of indophenol standard solution using proper volumes of HPO_3–CH_3COOH solution and H_2O. If ca 2 mg ascorbic acid is contained in test solution aliquot <7 mL, add HPO3–CH3COOH solution to give 7 mL for titration.

Mg ascorbic acid/ml =(X-B)x(F/E)x(V/Y)

when

X = average mL for test solution titration

B = average mL for test blank titration

F = mg ascorbic acid equivalent to 1.0 mL indophenol standard solution

E = number of g, tablets, mL

V = volume initial test solution

Y = volume test solution titrated

A 2 Moisture content

1. The moisture aluminium can is dried for 1 hour at 105°C. and allowed to cool for 30 minutes in a desiccator then weighed on balance.

2. Approximately 2.0000 to 5.0000 grams of the sample are placed in the dish.

3. The samples are dried, in hot air oven at 105°C. for 6 hours

4. After drying, the samples are placed in a desiccator for about one hour, or until they reach room temperature, and each is weighed, without further delay.

5. The moisture content, W, as percentage by mass of the sample (grams per 100 grams), is equal to:

$$W = \frac{M_1 - M_0}{M_1 - M_2} \times 100$$

where

M_0 is the mass, in grams, of the aluminium can

M_1 is the mass, in grams, of the aluminium can and the test portion before drying

M_1 is the mass, in grams, of the aluminium can and the test portion after drying.

A 3. Structure of the film coating on peel by scanning electron microscope (SEM)

The coated peel was removed and cut into a suitable size. The samples were dried with a freeze dryer (Freezone 18®, Labconco, U.S.A.) for 8 hrs. The dried sample fixed on a stups. The dried peels were analyzed using an environmental scanning electron microscopy (E-SEM, model S-3400N, Hitachi, Japan). The structure of the film was viewed with E-SEM under high vacuum conditions at an accelerating voltage of 15 kV.

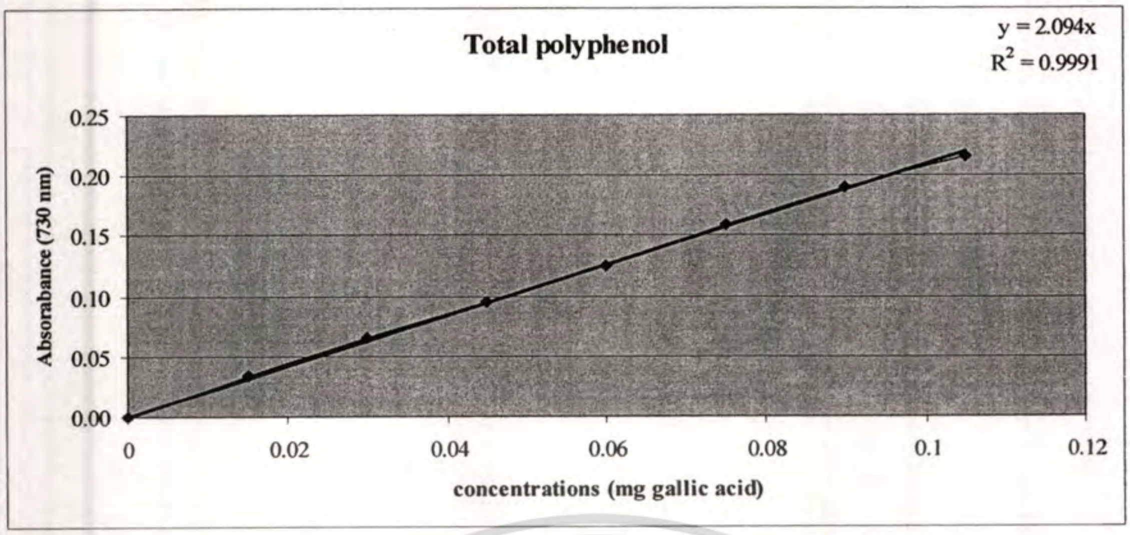


Figure A1 Standard curve of Gallic acid for total phenolics acid calculation.

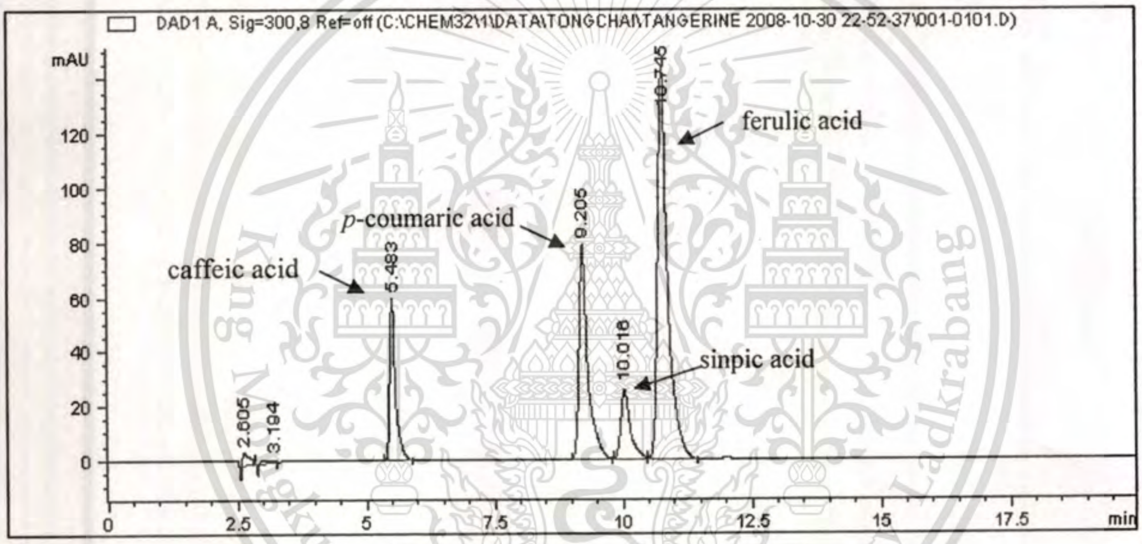


Figure A2 Chromatogram of standard hydroxycinnamic acids

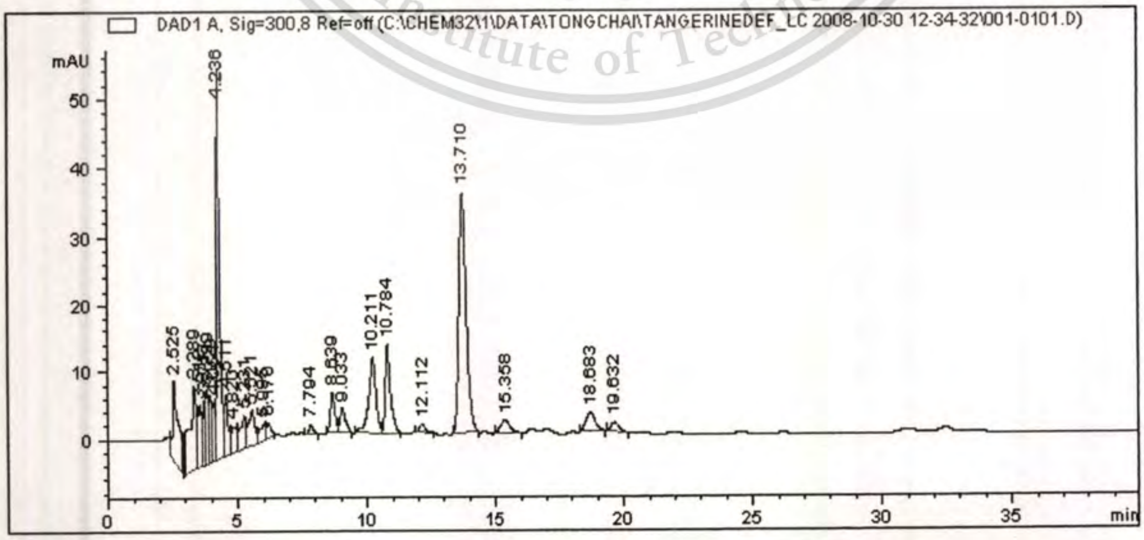


Figure A3 Chromatogram of hydroxycinnamic acids in stored coated tangerine with shellac

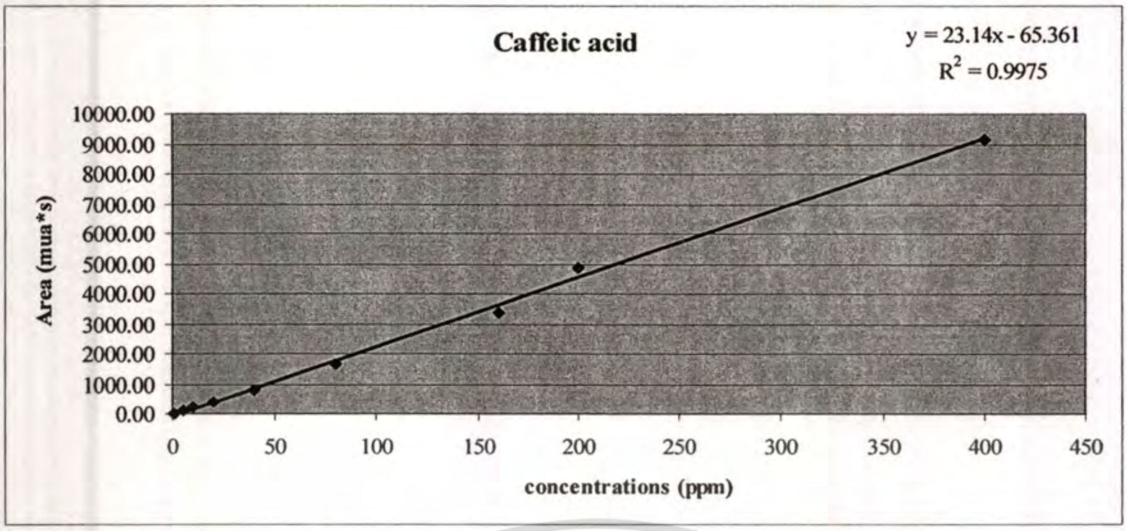


Figure A4 Chromatogram of standard caffic acids

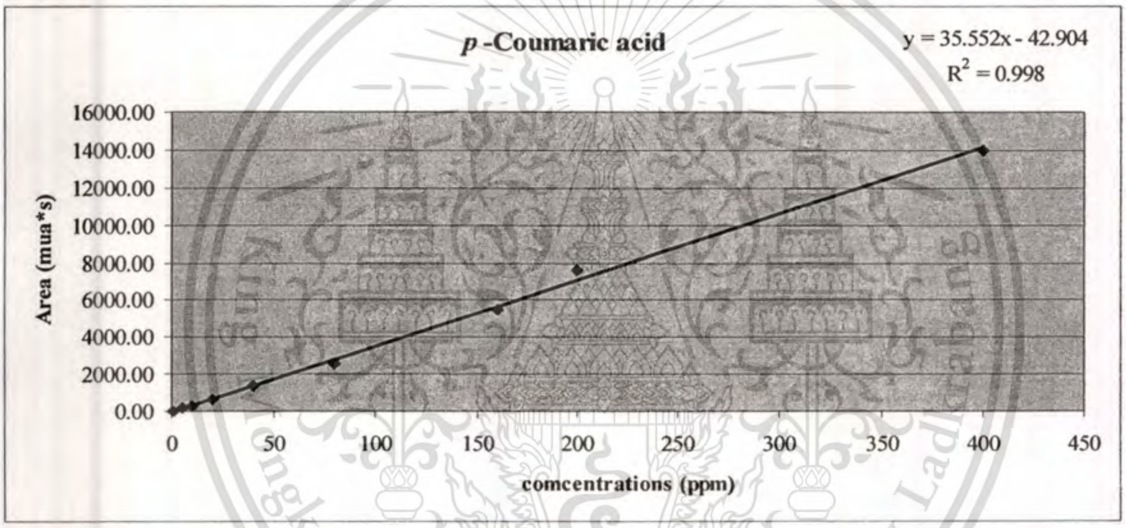


Figure A5 Chromatogram of standard *p*-coumaric acids

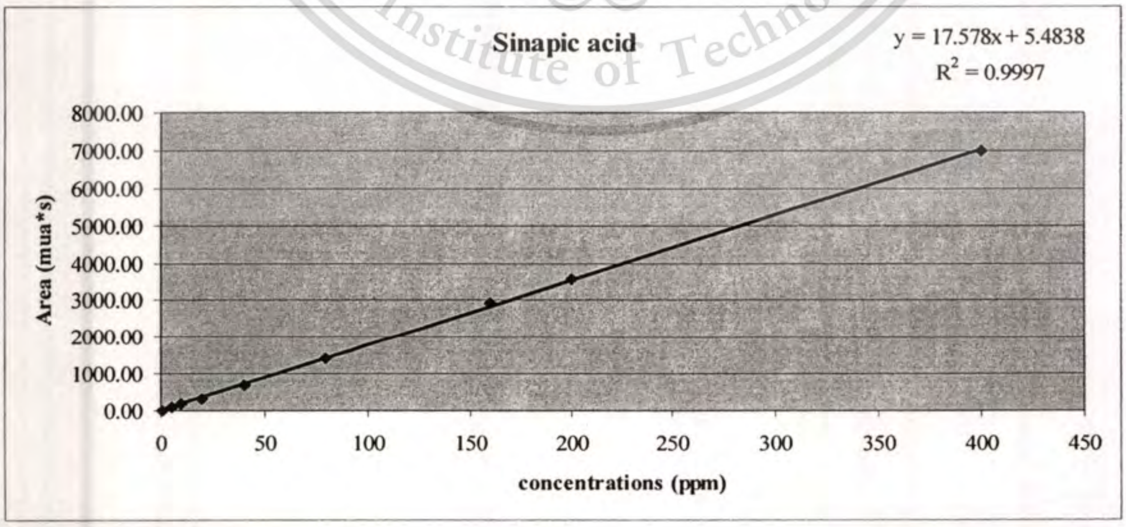


Figure A6 Chromatogram of standard sinapic acids

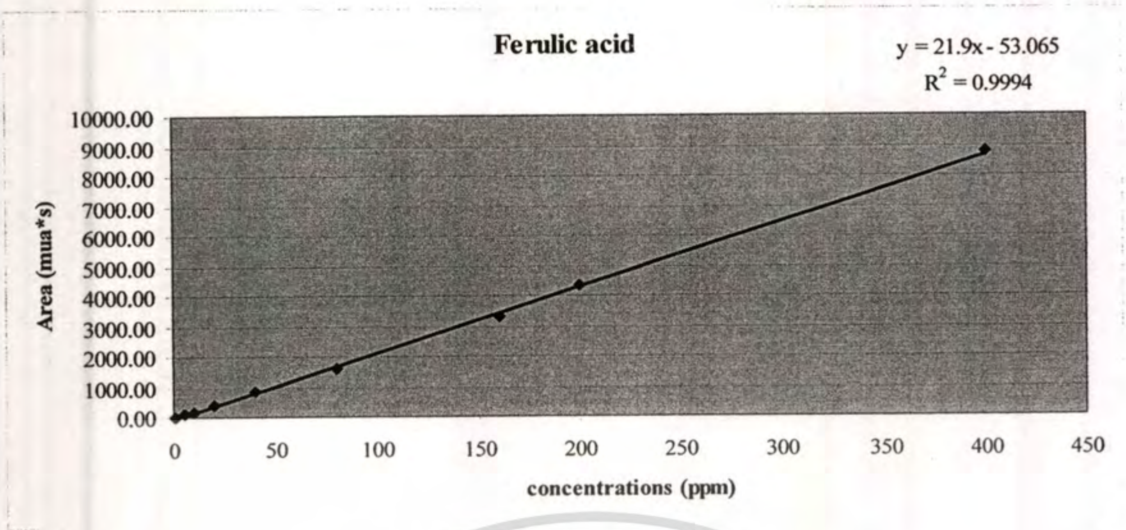


Figure A7 Chromatogram of standard ferulic acids

A 4. Codification of the levels of the variable

The initial ranges of the two variables were chosen as 1 to 2% (w/v) for chitosan and 1-4% (v/v) for oleic acid. The value for the variables, coded central point (0) was obtained from (max+min/2), and coded so that 1 and -1 are obtained from the followings.

Chitoan

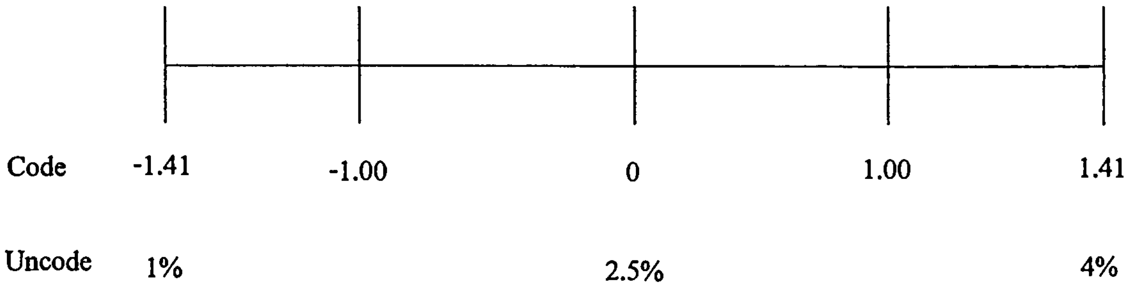
Code	-1.41	-1.00	0	1.00	1.41
Uncode	1%		1.5%		2%

Difference space from 0 to 1.41 is 1.41 unit		differences actual value is	0.5%
If the space is	1.00 unit	" ——————— "	(0.5/1.41)*1= 0.35%

At code 1 concentration is 1.5 + 0.35 = 1.85%

At code -1 concentration is 1.5 - 0.35 = 1.15%

Oleic acid



Difference space from 0 to 1.41 is 1.41 unit difference actual value is 1.5%

If the space is 1.00 unit ″——————″ (1.5/1.41)*1= 1.06%

At code 1 concentration is 2.5 +1.06 = 3.56%

At code -1 concentration is 2.5 - 1.06 = 1.44%

Appendix B

Statistical Analyses

Table 1B Analysis of variance of total soluble solids (TSS).

SOV	df	SS	MS	F	Sig
Replicate	1	.711	.355	12.771	.018
Storage temperature	2	.789	.395	14.180	.015
Error	2	.056	.028		
Storage time	4	.359	.090	1.479	.240
Temp x Time	8	2.584	.323	5.317	.001
Error	12	.0732	.061		
Coating treatment	1	.284	.284	5.067	.032
Temp x coated	2	.243	.122	2.168	.132
Time x coated	4	.140	.035	.625	.648
Temp x Time x coating	8	.778	.097	1.733	.131
Error	15	.840	.056		
Total	47				

Table 2B Analysis of variance of pH value.

SOV	df	SS	MS	F	Sig
Replicate	1	.024	.012	1.109	.414
Storage temperature	2	.074	.037	3.434	.135
Error	2	.022	.011		
Storage time	4	.164	.041	3.081	.035
Temp x Time	8	.568	.071	5.329	.001
Error	12	.156	.013		
Coating treatment	1	.025	.025	1.284	.266
Temp x coated	2	.027	.014	.695	.507
Time x coated	4	.232	.058	2.934	.037
Temp x Time x coating	8	.230	.029	1.460	.213
Error	15	.300	.020		
Total	47				

Table 3B Analysis of variance of titratable acidity.

SOV	df	SS	MS	F	Sig
Replicate	1	.001	.001	.582	.600
Storage temperature	2	.017	.009	7.754	.042
Error	2	.002	.001		
Storage time	4	.033	.008	4.422	.008
Temp x Time	8	.062	.008	4.125	.003
Error	12	.024	.002		
Coating treatment	1	.005	.005	3.100	.089
Temp x coated	2	.018	.009	5.404	.010
Time x coated	4	.111	.028	16.236	.000
Temp x Time x coating	8	.025	.003	1.859	.105
Error	15	.030	.002		
Total	47				

Table 4B Analysis of variance of weight loss.

SOV	df	SS	MS	F	Sig
Replicate	1	.630	.630	1.699	.322
Storage temperature	2	1176.66	588.331	1585.79	.001
Error	2	.742	.371		
Storage time	4	2148.30	537.07	1833.00	.000
Temp x Time	8	589.23	73.65	251.34	.000
Error	12	3.52	.293		
Coating treatment	1	448.65	448.649	1124.00	.000
Temp x coated	2	119.39	59.694	149.545	.000
Time x coated	4	197.64	49.409	123.778	.000
Temp x Time x coating	8	66.369	8.296	20.783	.000
Error	15	5.988	.399		
Total	47				

Table 5B Analysis of variance of ascorbic acid.

SOV	df	SS	MS	F	Sig
Replicate	1	6.926	6.926	15.438	.059
Storage temperature	2	1.690	.845	1.883	.347
Error	2	.897	.449		
Storage time	4	455.330	113.832	66.550	.000
Temp x Time	8	49.418	6.177	3.611	.023
Error	12	20.526	1.710		
Coating treatment	1	4.148	4.148	2.437	.139
Temp x coated	2	11.526	5.763	3.386	.061
Time x coated	4	30.950	7.738	4.546	.013
Temp x Time x coating	8	28.351	3.544	2.082	.105
Error	15	25.529	1.702		
Total	47				

Table 6B Analysis of variance of total polyphenol.

SOV	df	SS	MS	F	Sig
Replicate	1	145.664	145.664	59.904	.016
Storage temperature	2	15.271	7.635	3.140	.242
Error	2	4.863	2.432		
Storage time	4	37.143	9.286	1.170	.372
Temp x Time	8	24.616	3.077	.388	.907
Error	12	95.232	7.936		
Coating treatment	1	16.310	16.310	14.900	.002
Temp x coated	2	18.440	9.220	8.423	.004
Time x coated	4	23.857	5.964	5.449	.007
Temp x Time x coating	8	21.721	2.715	2.480	.062
Error	15	16.419	1.095		
Total	47				

Table 7B Analysis of variance of hydroxycinnamic acids.

SOV	df	SS	MS	F	Sig
Caffeic acid					
Replicate	1	.353	.353	.515	.547
Storage temperature	2	.231	.116	.169	.856
Error	2	1.371	.685		
Storage time	4	35.195	8.799	15.451	.000
Temp x Time	8	3.733	.467	.819	.601
Error	12	6.833	.569		
Coating treatment	1	2.439	2.439	6.831	.020
Temp x coated	2	1.593	.796	2.230	.142
Time x coated	4	.784	.196	.549	.702
Temp x Time x coating	8	2.814	.352	.985	.484
Error	15	5.356	.357		
Total	47				
***p*-coumaric acid**					
Replicate	1	.952	.952	2.253	.272
Storage temperature	2	.531	.266	.628	.614
Error	2	.845	.423		
Storage time	4	4.887	1.222	5.534	.009
Temp x Time	8	5.794	.724	3.281	.032
Error	12	2.649	.221		
Coating treatment	1	1.864	1.864	26.679	.000
Temp x coated	2	1.357	.679	9.712	.002
Time x coated	4	.808	.202	2.889	.059
Temp x Time x coating	8	1.201	.150	2.148	.096
Error	15	1.048	.070		
Total	47				

Table 7B (continue)

SOV	df	SS	MS	F	Sig
Sinapic acid					
Replicate	1	.006	.006	.001	.977
Storage temperature	2	.936	.468	.082	.924
Error	2	11.378	5.689		
Storage time	4	35.192	8.798	3.580	.038
Temp x Time	8	44.585	5.573	2.268	.097
Error	12	29.493	2.458		
Coating treatment	1	5.951	5.951	7.254	.017
Temp x coated	2	.407	.203	.248	.784
Time x coated	4	4.397	1.099	1.340	.301
Temp x Time x coating	8	8.073	1.009	1.230	.347
Error	15	12.306	.820		
Total	47				
Ferulic acid					
Replicate	1	6.634	6.634	.549	.536
Storage temperature	2	58.705	29.353	2.429	.292
Error	2	24.166	12.083		
Storage time	4	1046.914	261.728	4.450	.020
Temp x Time	8	418.995	52.374	.890	.552
Error	12	705.844	58.820		
Coating treatment	1	55.156	55.156	2.758	.118
Temp x coated	2	2.143	1.071	.054	.948
Time x coated	4	170.981	42.745	2.137	.126
Temp x Time x coating	8	279.846	34.981	1.749	.167
Error	15	300.005	20.000		
Total	47				

Table 8B Analysis of variance of antioxidant activity.

SOV	df	SS	MS	F	Sig
DPPH					
Replicate	1	10.367	10.367	.600	.520
Storage temperature	2	209.605	104.803	6.065	.142
Error	2	34.558	17.279		
Storage time	4	995.946	248.987	4.178	.024
Temp x Time	8	355.598	44.450	.746	.653
Error	12	715.089	59.591		
Coating treatment	1	6.131	6.131	1.080	.315
Temp x coated	2	23.318	11.659	2.055	.163
Time x coated	4	28.289	7.072	1.246	.334
Temp x Time x coating	8	81.919	10.240	1.804	.154
Error	15	85.125	5.675		
Total	47				
ABTS					
Replicate	1	.612	.612	.041	.858
Storage temperature	2	116.130	58.065	3.888	.205
Error	2	29.871			
Storage time	4	611.322	152.831	.877	.500
Temp x Time	8	133.711	16.714	.096	.999
Error	12	2090.459	174.205		
Coating treatment	1	3.582	3.582	.543	.473
Temp x coated	2	46.225	23.112	3.503	.056
Time x coated	4	32.689	8.172	1.239	.337
Temp x Time x coating	8	37.882	4.735	.718	.674
Error	15	98.957	6.597		
Total	47				

Table 9B Analysis of variance of total soluble solids (TSS).

SOV	df	SS	MS	F	Sig
Coating	2	.116	.058	1.572	.283
Error	6	.222	.037		
Storage time	4	5.661	1.415	24.376	.000
Coating x storage time	8	.416	.052	.899	.546
Error	24	1.393	.058		
Total	44				

Table 10B Analysis of variance of pH value.

SOV	df	SS	MS	F	Sig
Coating	2	.031	.015	2.581	.155
Error	6	.036	.006		
Storage time	4	.426	.107	9.795	.000
Coating x storage time	8	.032	.004	.365	.929
Error	24	.261	.011		
Total	44				

Table 11B Analysis of variance of titratable acidity.

SOV	df	SS	MS	F	Sig
Coating	2	.004	.002	2.605	.153
Error	6	.005	.001		
Storage time	4	.041	.010	6.408	.001
Coating x storage time	8	.011	.001	.866	.557
Error	24	.039	.002		
Total	44				

Table 12B Analysis of variance of weight loss.

SOV	df	SS	MS	F	Sig
Coating	2	42.527	21.264	282.418	.000
Error	6	.452	.075		
Storage time	4	589.929	147.482	3669.00	.000
Coating x storage time	8	14.001	1.750	43.542	.000
Error	24	.965	.040		
Total	44				

Table 13B Analysis of variance of ascorbic acid.

SOV	df	SS	MS	F	Sig
Coating	2	11.082	5.541	4.599	.062
Error	6	7.229	1.205		
Storage time	4	510.392	127.598	86.280	.000
Coating x storage time	8	13.468	1.683	1.138	.374
Error	24	35.493	1.479		
Total	44				

Table 14B Analysis of variance of total polyphenol.

SOV	df	SS	MS	F	Sig
Coating	2	12.669	6.334	.739	.517
Error	6	51.446	8.574		
Storage time	4	121.116	30.279	10.536	.000
Coating x storage time	8	24.463	3.058	1.064	.419
Error	24	68.970	2.874		
Total	44				

Table 15B Analysis of variance of hydroxycinnamic acids.

SOV	df	SS	MS	F	Sig
Caffeic acid					
Coating	2	.208	.104	1.047	.407
Error	6	.597	.099		
Storage time	4	3.999	1.000	7.754	.000
Coating x storage time	8	1.520	.190	1.474	.219
Error	24	3.095	.129^b		
Total	44				
***p*-coumaric acid**					
Coating	2	.124	.062	.308	.746
Error	6	1.208	.201		
Storage time	4	10.947	2.737	42.345	.000
Coating x storage time	8	.913	.114	1.765	.134
Error	24	1.551	.065		
Total	44				
Sinapic acid					
Coating	2	.580	.290	4.705	.060
Error	6	.370	.062		
Storage time	4	2.488	.622	4.295	.009
Coating x storage time	8	1.374	.172	1.186	.348
Error	24	3.475	.145		
Total	44				
Ferulic acid					
Coating	2	25.149	12.574	.509	.625
Error	6	148.349	24.725		
Storage time	4	247.777	61.944	2.726	.053
Coating x storage time	8	358.449	44.806	1.972	.095
Error	24	545.370	22.724		
Total	44				

Table 16B Analysis of variance of antioxidant activity (DPPH and ABTS).

SOV	df	SS	MS	F	Sig
DPPH					
Coating	2	19.417	9.709	.134	.877
Error	6	433.260	72.210		
Storage time	4	791.043	158.209	3.161	.021
Coating x storage time	8	240.073	24.007	.480	.890
Error	24	1501.362	50.045		
Total	44				
ABTS					
Coating	2	1.138	.569	.063	.939
Error	6	54.000	9.000		
Storage time	4	514.934	102.987	20.977	.000
Coating x storage time	8	90.613	9.061	1.846	.095
Error	24	147.289	4.910		
Total	44				

Appendix C

Questionnaire for sensory evaluation

Name..**Date**...

Sample Orange juice

Instruction: Please test the samples in the orders presented from left to right and wright your score for each sample below.

1=unacceptable
2=a little acceptable
3=moderate acceptable
4=good acceptable
5=excellent acceptable

CODE	___	___	___	___	___
Color					
Flavor					
Taste					
Acceptability					

Suggestion

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Table 1C Description of scoring used for sensory evaluation of tangerine juice

Score	Description			
	Color	**Flavor**	**Taste**	**Acceptability**
1	Off yellow	Strong fermented	Objectionable taste	Off color, strong fermented flavor, Objectionable taste (over ripe)
2	Pale yellow to yellow-orange	Moderate strong fermented	Slight objectionable taste	Pale color, Moderate strong fermented flavor, slightly objectionable taste
3	Moderate yellow to yellow-orange	Moderate fermented	Moderate taste	Moderate color, fermented flavor, taste
4	fairly bright yellow to yellow-orange	Slight fermented	Pleasant taste	fairly color, slightly fermented flavor, good taste
5	Bright yellow to yellow-orange	Non fermented	Excellent taste	Bright color, non fermented flavor, excellent taste (very fresh)

Appendix D

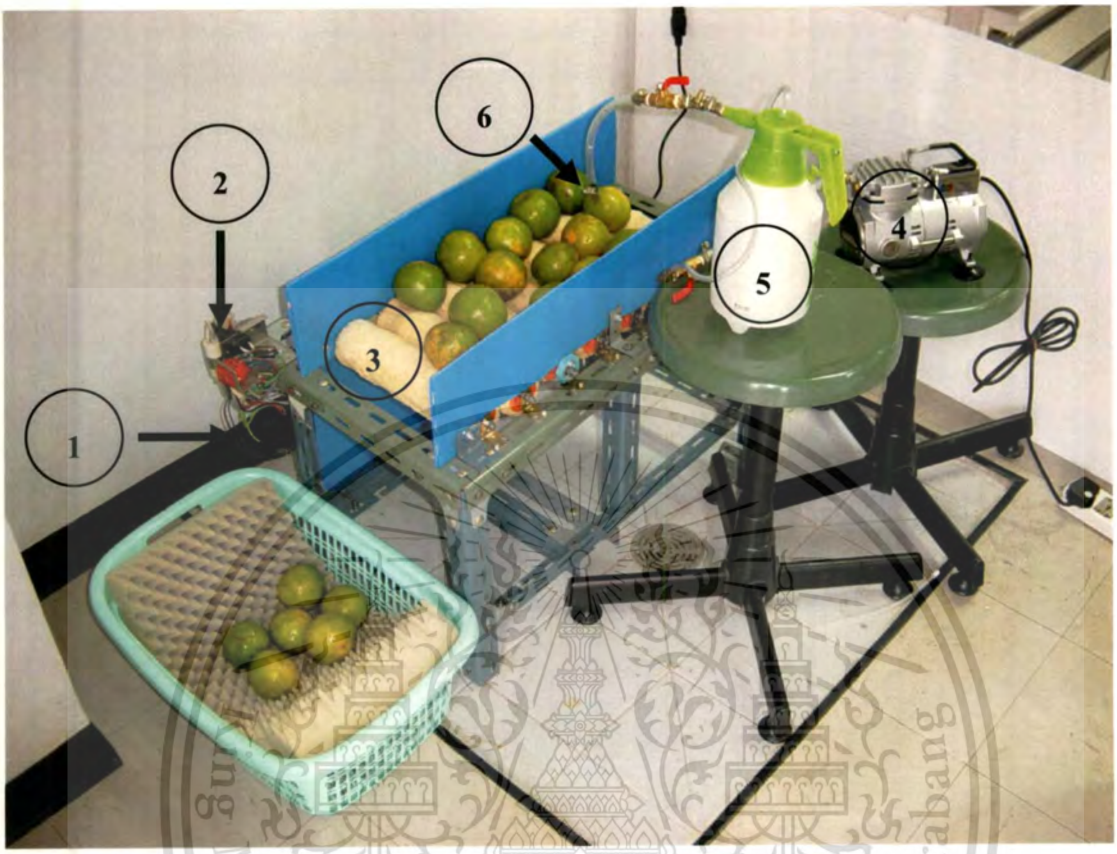

Figure 1D Coating apparatus used in the experiment

Components of the apparatus

1. Gear motor
2. Switch (on-off) and speed controller
3. Paint roller
4. Air pump
5. Coating tank
6. Spray nozzle

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