CHAPTER 2

LITERATURE REVIEW

Fresh longan fruit is one of the major produce exports of Thailand. Longan is a non-climacteric fruit and classified as Sapindaceae family as well as litchi and rambutan. Longan cv. Daw is mostly planted in the northern part of Thailand, especially Chiang Mai and Lumphun provinces and another area in the eastern of Thailand, for example Chanthaburi. Good Agricultural Practice (GAP) orchards of ~100,000 farmers are registered by Department of Agricultue (DOA) of the Ministry of Agriculture and Cooperative (MOAC) for controlling quality standard before export. Production and harvested area in year 2008 was 1,035,556 and 966,831 rai, respectively. The total production in 2008 was 476,930 tons. Thirty percent of the total produce is consumed within the country. The rest is exported as fresh longan fruit, frozen and dried longan, dried longan flesh and process were 4,000 million baht. Thailand ranks the 2nd world production by China and the 1st rank exported country in the world. The fresh and frozen exported longan fruit was about 149,919 tons (2,350.4 million baht) (Office of Agricultural and Economics, 2009). The major importing countries for fresh longan are China and the others such as: Hong Kong, Singapore, Indonesia, EU, Canada and the USA.

2.1 Postharvest longan fruit/ the longan fruits

Longan fruits are normally harvested during June to August of every year and is also presently produced thoughout the year in off-season by using potassium chlorate which can be soil drenched around the longan tree to induce flowering. Optimal harvesting after bloom is 149 days or 21 weeks or 5-6 months and chemical content indices are 18.55 %brix total soluble solid (TSS) and 149.70 mg/100 ml ascorbic acid. The mature pericarp (peel) has yellowish brown color and smooth skin (Wara-Aswapati, 1994; Jitpang, 1989). Surface characteristics show small cracks and

lenticels. The pericarp consists of 3 layers. The exocarp originates next to 2-3 cuticle layers and upper epidermis. The mesocarp, 70% thickness of the peels, has large intercellular spaces scattered with groups of xylem, phloem and stone cells. The endocarp characterizes wavy skin when observed under the Scanning Electron Microscope (SEM). Soluble solid content (SSC), titratable acidity (TA) and total sugars increased during optimum harvesting and then decreased gradually after harvest. The main sugars presented in longan are sucrose, fructose and glucose (Paul and Chen, 1987) (Table 2.1) and differences are found at different stages of maturity and cultivars. TA and SSC of the pulp have been shown to decrease slightly during cold storage due to its non-climacteric fruits (Jiang *et al*., 2002).

Table 2.1 Longan fruit compositions at harvest.

Constituent	Aproximately value
Aril (% dry weight)	16.5 ± 0.7
Pericarp (% dry weight)	35.6 ± 0.4
Soluble solids (%)	18.3 ± 0.2
Total sugars (mg/g)	154.0 ± 11.0
Sucrose (mg/g)	29.0 ± 3.0
Glucose (mg/g)	17.0 ± 1.0
Fructose (mg/g)	23.0 ± 1.0
Titratable acidity (meq/g)	2.1 ± 0.1
pH	6.4 ± 0.1
Citric acid (meq/g)	0.12 ± 0.01
Malic acid (meq/g)	0.35 ± 0.07
Succinic acid (meq/g)	1.15 ± 0.11
Ascorbic acid (meq/g)	1.40 ± 0.20
Total phenol (mg/g)	0.5 ± 0.1

(Source: Paul and Chen, 1987).

2.2 Postharvest losses

The major factors reducing the storage life and marketability of longan fruit are skin browning and microbial decay, both of which detract from the appearance and can impart off-flavours.

2.2.1 Pericarp browning

Browning of longan fruit results in a short life and a reduced commercial value. During storage, pericarp turns brown within 2 days at ambient temperature or in 15 days at 5°C because of desiccation (Tongdee, 1992; Sardsud *et al*., 1998). Longan tissue deterioration results from physical handling (Tongdee, 1992). This causation is a browning reaction between polyphenol oxidase (PPO) and phenolic substrates in O_2 conditions. Phenol is oxidized to quinone which is polymerized to brown-colored by-products (Prapaipong and Rakariyatham, 1990). In additon, pericarp browning is also related to chilling injury, heat stress, senescence, pathogens and insect pests.

2.2.1.1Factors affected

- Enzymatic browning: PPO

PPO is a copper associated enzyme with two binding sites for phenolic substrates. It was first found in mushroom to be a tetramer containing four atoms of copper per molecule. It is located in the chloroplast bound to thylakoid membranes (Figure 2.1). PPO is activated by releasing into the cytosol when plant tissues undergo physical damage such as bruising, cutting or blending. During storage of longan fruit, there is a gradual breakdown of the cellular ultrastructure resulting in loss of compartmentalization of PPO and its substrates, which initiates enzyme-catalyzed browning reaction in the presence of oxygen (Qu *et al*., 2001) and then forms the brown-coloured by-products. Polyphenol oxidase catalyses two basic reactions: hydroxylation to the *o*-position adjacent to an existing hydroxyl group of the phenolic substrate (monophenol oxidase activity) and oxidation of diphenol to *o*-benzoquinones (dipehnol oxidase activity). Both reactions utilize molecular oxygen as a co-substrate. Whether a single enzyme system exhibits both mono- and di-phenol oxidase activities is still unclear.

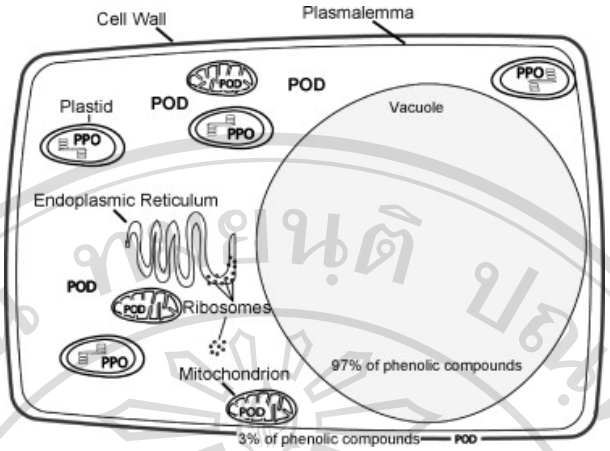


Figure 2.1 The internal and external localization of phenolic compounds and phenolic oxidizing enzymes (polyphenol oxidase and peroxidase) in a typical plant cell. This model was constructed from previously published work (Hrazdina and Wagner, 1985; Marangoni *et al*., 1996; Toivonen, 2004). POD: phenol peroxidase; PPO: polyphenol oxidase.

Polyphenol oxidase catalyses the oxidation of phenols to *o*-quinones, which are highly reactive compounds. *O*-quinones thus formed undergo spontaneous polymerization to produce high-molecular-weight compounds or brown pigments (melanins). These melanins may in turn react with amino acids and proteins leading to enhancement of the brown colour produced (Figure 2.2). The hydroxylation reaction is relatively slow and results in colorless products, while the oxidation reaction is relatively rapid and the resultant quinones are colored. Subsequent reactions of the quinones lead to melanin accumulation, which is the brown or black pigment associated with "browning" in plant tissues. The specific reaction sequence which results in brown or black-colored products depends on the specific structure of the polyphenolic substrate (Tovinen, 2008). There are two aspects of the temperature effect: heat effect and cold effect. Heat treatment is the most widely used method due to its capability of inactivating enzymes and destroying microorganisms (Mashall *et al.,* 2000). One of the most direct influences of enzyme activity is acidity. The pH can change the state of ionization of acidic or basic amino acids in the active site of enzymes. This leads to the alteration of enzyme as well as its substrate's tertiary structure.

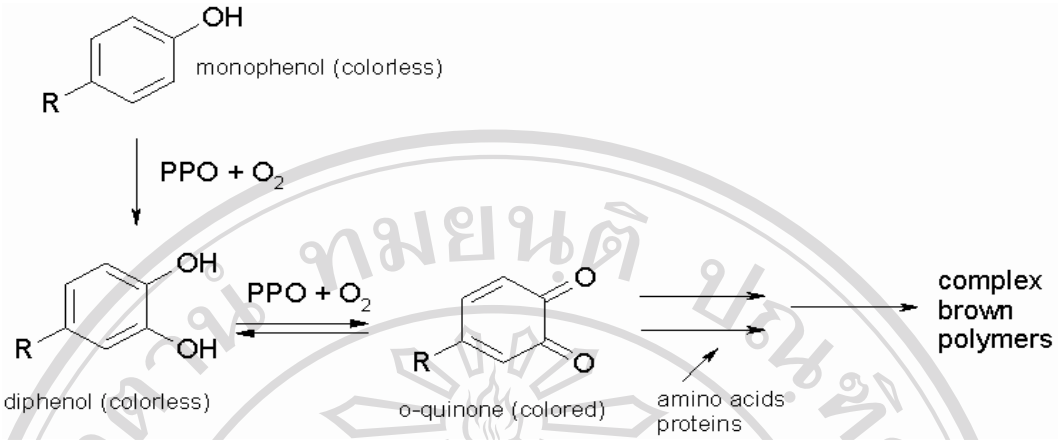


Figure 2.2 Schematic representation of oxidation reaction by PPO.
(Source: McEvily *et al*, 1992; Marshall *et al*., 2000).

Changes in pH not only affect enzyme solubility but also influence the charge distribution of the enzyme, phenolic substrates and products (Serradell *et al*., 2000). The enzyme, PPO, has been isolated and purified from "Shi Xia" fruit pericarp and has been shown to have the optimum pH and temperature of 6.5 and 35°C, respectively (Jiang, 1999). PPO activity in longan was relatively low at harvest and decreased initially during low temperature storage for 7 days. It increased again, reached a peak after storage for 30 days and finally decreased again (Wu *et al*., 1999).

The substrates for this reaction include simple phenols, such as catechol and gallic acid; cinnamic acid derivatives, such as dopamine; and flavonoids, such as catechin and epicatechin (Fennema, 1975). Longan fruit contains a large amount of phenolic compounds (Jiang *et al*., 2002). The major polyphenolic components in pericarp tissues of longan fruit have been identified as gallic acid, corilagin and ellagic acid (Rangkadilok *et al*., 2005). Shi *et al*. (2008) found that the direct substrate for the PPO from pericarp tissues of longan fruit was identified to be (-)-epicatechin. Furthermore, the contents of (-)-epicatechin of pericarp tissues of longan fruit of two major cultivars were determined by high performance liquid chromatography (HPLC). The HPLC analysis exhibited that the contents of (-)-epicatechin of fruit pericarp of 'Shixia' and 'Chuliang' were 0.26 and 0.56 mg/g on fresh weight (FW) basis at harvest and 0.15 and 0.09 mg/g FW after 3 days of storage. The more rapid decrease in the (-)-epicatechin content of 'Chuliang' was due to the

oxidization catalyzed by PPO, which was in agreement with the higher browning index.

The substrate specificity of polyphenol oxidase varies in accordance with the source of the enzyme. Phenolic compounds and polyphenol oxidase are, in general, directly responsible for enzymatic browning reactions in damaged fruits during postharvest handling and processing. The relationship of the rate of browning to phenolic content and polyphenol oxidase activity has been reported for various fruits such as apples (CoSeteng and Lee, 1987), grapes (Lee and Jaworski, 1988) and peaches (Lee *et al*. 1990). This hypothesis is supported by work with fresh-cut potatoes that clearly shows that browning is not rate limited by either the enzymes associate with browning or polyphenol substrate concentration (Cantos *et al*., 2002).

The most common assay for PPO activity is spectrophotometric measurement of quinones formation at 400–500nm, depending on the substrates (Serradell *et al*., 2000; Yoruk and Marshall, 2003). For examples, *o*-quinones formed from epicatechol, pyrocatechin, caffeic acid and L-DOPA substrates exhibit an absorption maximum at 420, 400, 400, and 475 nm, respectively (Casado-Vela *et al*., 2005). In the plot of absorbance versus reaction time, the absorbance increases linearly in the initial time and then decreases later. The reason of the decrease in absorbance is the formation of 18 insoluble brown polymers called melanins. During the reaction with o-diphenol, PPO is inactivated by reacting with intermediate free radicals that act on copper in the PPO active site (Miyawaki, 2006). Vamos-Vigyazo (1995) reviewed that the principles of browning prevention have not changed with the time and are essentially the same as those applying to the inhibition of any tissue enzyme, i.e.:

A/ inhibition/ inactivation of the enzyme

B/ elimination/ transformation of the substrate (s)

C/ combination of a/ and b/

Various techniques and mechanisms have been developed over the years for the control of these undesirable enzyme activities. These techniques attempt to eliminate one or more of the essential components (oxygen, enzyme, copper, or substrate) from the reaction. Prevention does not necessarily consist of postharvest treatment only. Much can be done to reduce browning occurring during storage or

processing by selecting cultivars off slight browning tendency (Amiot *et al*., 1992) and by appropriate agricultural techniques (Mondy and Munshi, 1993).

- Water loss

Meenaphan and Ketsa *et al*., (2003) found that browning development of baby corn correlated with weight loss and total phenolics but not with phenylalanine ammonia lyase and polyphenol oxidase activities. Lin *et al*. (2005) found that water loss of non-wrapped longan cv. Fuyan was more than wrapped fruit during storage at 10±1°C with 50% RH. Pericarp browning development was significantly related to water loss of the pericarp (except for the others causes: chilling injury, heat stress, senescence and pest or pathogen attack).

Desiccation is the main factor of induced-pericarp browning in longan. The relationship between water loss from pericarp and pericarp browning in longan cv. Fuyan fruits using open plastic punnets and sealed polyethylene bags at 10±1°C, 50% RH and the effect of pericarp water loss on active oxygen metabolism and phenolics metabolism were investigated. Water loss resulted in rapid pericarp browning. Development of pericarp browning was higher with a higher rate of water loss from the pericarp and longer storage time (from 0 to 6 days). Water loss from the pericarp was positively correlated with pericarp browning index significantly ($P<0.01$). Water loss from the pericarp resulted in an increase in activity of PPO, and obvious reductions in total phenolic and flavonoid contents, whereas there were not obvious changes in anthocyanin content. These results show that phenolics and flavonoids are the main substrates for PPO during desiccation-induced browning. Water loss from the pericarp caused a significant increase in activity of POD which also plays an important role in desiccation-induced browning in the pericarp of longan fruit. Water loss from the pericarp caused an increase in pericarp pH value, which resulted in changes in anthocyanin structure and color, the degradation of anthocyanin became easier. The results suggest that desiccation-induced browning of longan pericarp may be due to a decrease in activities of reactive-oxygen-scavenging enzymes and amounts of endogenous antioxidant substances, an accumulation of active oxygen, an increase in membrane lipid peroxidation, an injury of the integrity of cellular membrane structure, which, in turn, may cause cellular decompartmentalization,

resulting in PPO and POD, located in the plastid and other organelle, to come into contact with phenolic and flavonoid substrates, located in vacuoles, to form brown polymers.

2.2.1.2 Symptoms and mechanism

- Pericarp browning during ambient temperature

During storage, the pericarp turns brown in 2-3 days at ambient temperature. Pan (1994) observed that the mesocarp cells were the first to turn brown, followed by the endocarp. This browning spread over the entire pericarp surface, mainly in the epicarp and outer layers of the mesocarp. There was a gradual breakdown of cellular ultrastructure resulting in loss of compartmentalization of enzymes, such as PPO and their substrates (Qu *et al*., 2001).

- Chilling injury

Cold preservation and storage during distribution and retailing are necessary for the prevention of browning in fruit and vegetables are effective in lowering PPO activity. The rate of enzyme- catalyzed reactions is controlled to a great extent by temperature. For every 10^{o}C temperature increase (in biologically important ranges), there is a two-fold increase in the rate of an enzyme-catalysed reaction, referred to as the temperature coefficient (Q_{10}). On the other hand, for every 10 ^{o}C reduction in temperature a similar decrease in the rate of biological activity occurs. At low temperatures, reduced kinetic energy of the reactant molecules results in a decrease in both mobility and "effective collisions" necessary for the formation of enzyme-substrate complexes and their products. Chilling involves the temporary storage of foods at temperatures above freezing. Subtropical fruits are susceptible to chill injury (CI) and should therefore not be stored below their respective critical temperature (Marshall *et al*., 2000). The visual symptoms that develop after removal from chilling to non-chilling temperature are not unique to this stress. However, there are several symptoms that commonly result from chilling upto their structural plant damages and regions. CI symptoms of longan pericarp were water-soaking or drying and darkening of the pericarp during storage (Tongdee, 1992; Jiang *et al*., 2002) and this symptom was positively associated with water loss (Boonyakiat *et al*., 2002).

Pericarp (peel) browning is often subjectively often indicative of injury severity area such as discoloration of similar fruit pericarp of longan, litchi and longkong. The other symptoms of chilling injury were internal pitting (pine apple), core break down, surface pitting, internal discoloration, loss of capacity to ripen, wilt, decay was reported in apple (Katz and Reinhold, 1965; Morris, 1982; Wang, 1982, 1990). Zhou *et al*. (1997) also reported CI as irregular patches of browning on the longan pericarp cv. "Shi Xia" after exposure to less than 4^{o}C.

Longan fruit is a subtropical fruit and non-climacteric. Skined fruit is very prone to chilling damage therefore they can not be stored at low temperatures. Also low humidity storage can be used in some crops to reduce the incidence of disease during storage but in longan this causes dehydration of the skin causing it to go brown. After 14 day storage at 1˚C, 90% RH, longan fruit showed susceptibility to CI and the inner peel turned dark brown. Membrane permeability was broken down as assessed from electrolyte leakage and rapid reduction of fresh weight. These symptoms rarely happened in the fruit stored at 5°C and 10°C. Darkening of the inner peel and the leakage of electrolyte indicated strong CI index (Boonyakiat, 2002). Additionally, CI may be due to field practices and handling during transit or wholesale distribution (Kays, 1991). The critical threshold temperature below which chilling stress occurs varies widely between fruit species. For example, the low temperatures that induce CI of longan fruit vary among cultivars such as Kohala (7.5^{o}C), Homestead (6.5-7.5^{o}C), Kuhko (10^{o}C) and Chompoo (7-8^{o}C) (Drinnan, 2004). The chilling process is generally accomplished either with the use of moving air, water (hydrocooling), ice, or vacuum (vacuum cooling) (Marshall *et al*., 2000).

Jaitrong *et al*. (2004); (2006) studied CI development in longan when fresh longan fruit (cv. Daw) with 0.5 cm pedicels were packed in cardboard boxes and stored at 5±1°C, 90±1% RH. CI symptoms of water soaking and/or browning areas on the pericarp, appeared on the inner side of pericarp after 6 days storage and on the outer side of whole fruit within 14 days. Both sides of the peel became brown in color. During CI there was an increase in the PPO activity and electrolyte leakage and decreases in total phenolic compounds and protein content. High performance liquid chromatography – photodiode array (HPLC-PDA) analysis of the methanol

extracts showed a very large number of phenolic compounds that were similar in both cultivars. The main classes of phenolic compounds were tentatively identified as ellagic acid and flavone glycosides as well as a set of unknown compounds. The primary flavones in longan pericarp were quercetin and kaempferol. Microscopic anatomy of CI pericarp showed flaking of cuticle damage flaking of the cuticle, damage trichomes surface and parenchyma cell walls in the mesocarp. Separation and dissolution of the middle lamella and cell walls was evident resulting in a lack of cellular adhesion when assessed by light microscope (Jaitrong, 2006).

The events leading to CI can be separated into primary and secondary events. The primary cause of CI is membrane damage; the secondary cause may include ethylene production, increase respiration, interference with energy production, accumulation of toxic compounds (ethanol and acetaldehyde) and alteration of cellular structure. If the product is stored below the critical temperature for a short period, the plant can repair the damage. If the exposure is prolonged, irreversible damage occurs and visible symptoms often result (Wang, 1982). For many years, CI had been considered to be a consequence of phase transitions of membrane lipids occurring at a critical temperature that led to a complete loss of permeability control (Lyons, 1973). The current knowledge strongly suggests that membranes of chilling sensitive fruit undergo alterations in their biophysical properties related to their composition that can alter functionality. Modern theories focus on the plasma membrane as the site for chill-induced membrane damage (Marangoni *et al*., 1996). The primary event in CI is the induction of a phase change in the membrane lipids. This would be reversible until secondary events have caused a modification in the normal properties of the membrane lipid. Lateral phase seperations may be reversible up to the point in time where lipid degradation and accumulation of lipid degradation products induce irreversible membrane damage (Figure 2.3). Permanent and extensive CI sympotoms may be due to the irreversible phase of the reaction (Marangini *et al*., 1996). The increased respiration and ethylene production might be a rapid method which indicates the CI has been suggested by Gomolmanee (2001) who found that the respiration after 3 week storage were 18-27 mg CO_2/kg-hr and ethylene productions were 0.1-0.4 ppm/kg-hr. When the fruits were placed at 25˚C, the respiration rates were increased to 140-180 mg CO_2/kg-hr and ethylene

productions were increased due to mold growth. While the ion leakage of the peel increased at 2.5, 5 and 7.5˚C after 2, 3 and 4 storage weeks, respectively. Prevention of CI is possible in several ways. SO_2 fumigaion in longan completely inhibited CI. Storage at 0°C, non-treated fruit turned brown while the SO_2 fumigated fruit remained yellowish (La-Ongsri *et al*., 1993). Presently, SO_2 was also used to fumigate litchi fruit and grape and it inhibits CI and fruit rot.

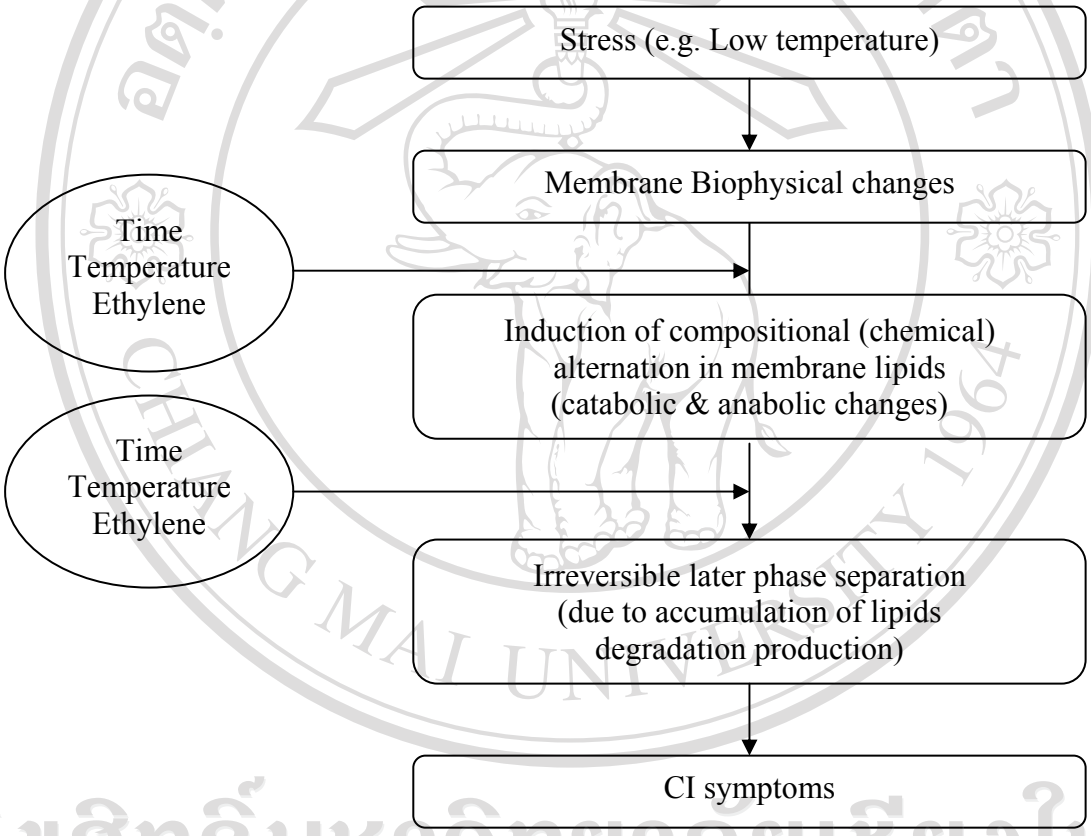


Figure 2.3 A model for the sequence of metabolic events that leads from the stress-induced alteration in the properties of membranes to the observation of macroscopic tissue damage. (Source: Wang, 1982; Marangini *et al*., 1996).

2.2.2 Fruit rot

2.2.2.1 Fungal disease species on longan fruit

High sugar and moisture content in longan fruit induce various decay organisms to rot the fruit rapidly. Fruit rot usually follows peel browning. Upon storage at 5°C and 10°C, the peel turns brown within 24-26 days and 10-18 days respectively. In fresh longan fruits, the surface has characteristically small cracks and lenticels. The pericarp consists of 3 layers such as exocarp, mesocarp and endocarp (Jaitrong *et al*., 2004; Suwanakood *et al*., 2007). Lin *et al.* (2001) reviewed that both the storability and disease resistance of longan fruits were related to the structure of skin and pulp. Some cultivars which were strongly resistant to diseases and more suitable for storage and transportation, had thick skin, thick corky layer of good continuity, many tumor shape projecting and setas, many stone-cell groups with closer arrangement, developed vascular, and a thick cuticle. Common fruit rots pathogens belong to 10 species, but only 2 species, such as *Lasiodiplodia theobromae* and *Pestalotiopsis* sp. cause rapid peel browning and they were reported to be entophytes of longan tree (Chaiwangsri, 1992; Nachaiwiang, 1994; Sardsud *et al*., 1992; Sardsud *et al*., 1998; Suwanakood *et al*., 2007).

Suwanakood (2007) revealed that examination of the surface appearance using a stereo microscope showed that the fruit skin was rough and uneven. Under a scanning electron microscope, the surface of longan fruit consisted of scale and epidermal hairs and in some areas the remnant of cuticles could be observed. Filamentous fungi were also observed. Many genera of fungi were isolated from the healthy skin of harvested longans by a tissue transplanting method. These were *Aspergillus*, *Cladosporium*, *Colletotrichum*, *Fusarium*, *Lasiodiplodia*, *Mucor*, *Penicillium Pestalotiopsis*, *Phomopsis*, *Rhizopus*, *Trichoderma*, *Verticillium* and 7 unidentified genera. Each of the fungal isolates was inoculated onto the pericarp of the fruit for pathogenicity determination. The pathogenic ability examination showed that *Lasiodiplodia* and *Pestalotiopsis*, which were common fungi found on the fruit skin, caused the most severe symptoms, e.g. the diseased fruit rotted rapidly. The

most virulent isolate was identified as *Lasiodiplodia theobromae* based on its morphological characteristics and by DNA sequencing.

2.2.2.2 *Lasiodiplodia theobromae* (Pat.) Griff.& Maubl.

Syn*:* *Botryodiplodia theobromae* Pat, *Diplodia natalensis* Pole (Evans) *Lasiodiplodia triflorae* (Higgin) Griff. & Maubl.

- Taxonomy category

Kingdom Eumycota

Phylum Deuteromycota

Class Deuteromycetes

Form-class Coelometes

Order Sphaeropsidales

Family Sphaerioidaceae

Botryodiplodia sp. belongs to the class of Ascomycetes and lives in a saprophytic way. It is a mould which normally needs injured tissue to parasitize the plant. The fast and constantly growing mycelium of the fungus is snow-white at first, changing its colour within three to four weeks to black. This fungus is characteristically the generation of pycnidia in which the spores of the fungus are formed. The mature colony stage of this fungus changed to darken black and imperfect stage by oval-shaped fruiting bodies (pycnidia). Pycnidia develop on artificial media only rarely and after a long time. Pynidia size of 172.6 µm located on stroma and may be single or cluster characteristics or not present. The inside of pycnidia shows spores (conidia) based on conidiophores branches. The spores are elliptical and relatively big compared to other spores. Immature or young conidia are colorless and unicellular with no septum whereas mature conidia were 20-37 x 10-17 µm size, have a brown pigment color with one septum and a thick wall. A double layer of thick wall of the mature conidia consisted of inside layer (clear color) and an outside layer as well as septate (brown pigment) (Zitter *et al*., 1996)

Zhang *et al*. (2005) found that a temperature of 28-30˚C and pH of 6-7 were optimum for the mycelial growth of *L. theobromae*. Sporodochium yield was highest at 30˚C and pH 5-8. Conidial germination increased with increasing relative humidity. The germination rate of conidia was 88.5% when the conidiospores were incubated in

a water drop and did not germinate when the relative humidity was below 86%. Sucrose and glucose promoted mycelial growth. A solution of sucrose, glucose, maltose and mannitol promoted conidial germination. Pycnidium production was induced more quickly under fluorescent light. The lethal temperature for the mycelium was 53˚C for 10 minutes, whereas the lethal temperature was 52˚C for 10 minutes. The relationship between pH medium and rate of growth including survival rate was found by Frazier and Westhoff (1998) and the effect on growth rate of microbes by Garbutt (1997). Conidia germination increased with relative humidity.

Disease symptoms on fruits were darken brown rot and water soaked stalk. In the mean time, water contaminant like exudate from rot as substance degradation caused by this fungus was found. Fruiting body (pycnidia) showing black spot on disease symptoms was sometimes observed. This fungus can attack and damage during all parts of fruit growth in the tree and during postharvest, transportation and storage. Disease epidemiology is the releasing of spores from the ostiole with rain, air and insect and increasing with high relative humidity (%RH) (Visathanon, 2000; Snowdon, 1990). There were many hosts of this disease such as mango, guava, rambutan, passion fruit, banana, and rose apple including soil, dry branches on and under tree (Jantaratep, 1989) and different virulence of fungus isolates. The mature spores can store for 1 month with high relative humidity and dormant until harvest.

This fungus lives as a saprophyte in dead organic matter or latent infection on longan tree from orchard in many parts: shoot, stalk, seed and pericarp and had endophytic fungus on longan tree (Sardsud *et al*., 1998). Jantaratep (1989) studied the infection process of *L. theobromae* on mango fruit. Results revealed that most hypha of the fungi invaded into plant tissue about 5 mm in depth after inoculation for 24 to 48 hours. The hypha can penetrate for 1 cm after 72 hours of inoculation. The mycelium proliferated and mango was ripened and stem-end rot was clearly observed. Saoha (1997) studied using SEM and found that the hypha directly invaded to grow near the artificial wound on the stem end fruit after inoculation. Conidia were germinated to be by germ tube and encountered the stem end fruits and perforations.

2.2.2.3 Plant defense response during disease attack

General structure of fungi, chemically, the fungal cell wall is 80 to 90 percent polysaccharides with most of the remainder consisting of protein and lipid. Sometimes substantial amounts of pigments (melanin), polyphosphate, inorganic ions, etc., may also be presented. Polysaccharides of fungal cell walls are built from a variety of sugars. At least 11 monosaccharides have been reported as occurring in fungal cell walls, but only three, i)-glucose, N-acetylglucosamine and D-mannose, are consistently found in most fungi. Pathogenisis-related protein is that protein induced in several plant species when they are infected by viruses, viroids, fungi or bacteria. The occurrence of these proteins is not pathogen-specific, but determined by the type of reaction of the host plant.

They form a protective barrier against pathogens by collecting at infection sites and act to decrease susceptibility of plants. They may have anti-fungal or anti-bacterial activity (Figure 2.4).

(1) The perception of a signal by the plant cell.

(2) The intracellular transduction of this recognition signal.

(3) The synthesis of defence molecules.

(4) Transport of defence molecules to strategic sites.

Besides the known PRs inducers of biotic origin (pathogens, insects, nematodes, herbivores). Chemicals, such as salicylic, polyacrylic and fatty acids, inorganic salts, as well as physical stimuli (wounding, UV-B radiation, osmotic shock, low temperature, water deficit and excess), are involved in PRs induction

Phytoalexins are antimicrobial compounds of low molecular weight that are both synthesized by and accumulated in plants after exposure to microorganisms (Paxton, 1981). Even when phytoalexins appear critical to the defense of the plant, they are almost certainly not the only response of the plant required for effective resistance. Phytoalexins are absent in healthy plants.

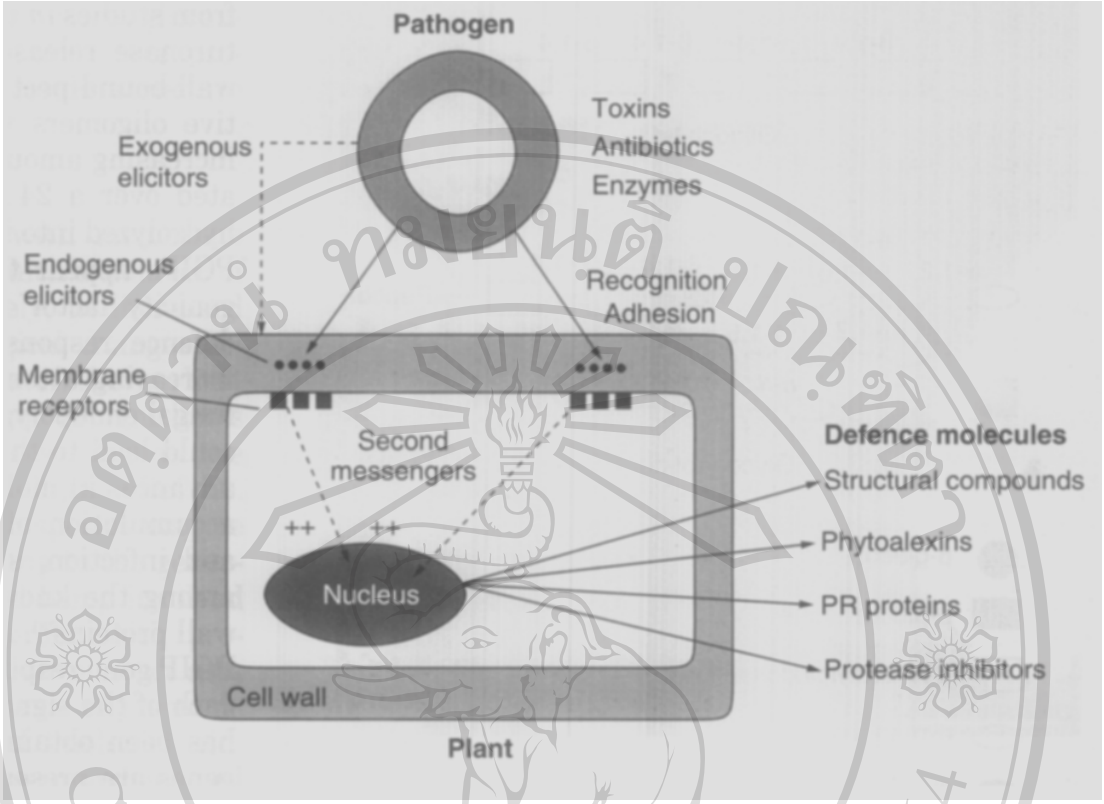


Figure 2.4. Signals and responses in plant-pathogen interactions. Upon recognition between both partners, the pathogen produces an array of metabolites, including endopolygalacturonases, that contribute to the release of signalling pectic oligomers (the endogenous elicitors). Binding of these elicitors to specific membrane receptors causes membrane depolarization, leading to the activation of second messengers that transduce the signal to the nucleus. Defence genes, encoding structural compounds, enzymes of secondary metabolism, pathogenesis-related (PR) proteins and protease inhibitors, are then triggered. Among the PR proteins, chitinases and β-l,3-glucanases may cause fungal cell wall hydrolysis, leading to the release of chitin and β-l,3-glucan oligomers (the exogenous elicitors) that bind to specific membrane receptors and trigger a cascade of events similar to that induced by the endogenous elicitors (Benhamou, 1996).

2.3 Present status of SO_2 fumigation

Sulfur dioxide (SO_2) is a colorless gas with a pungent odor. It is liquid under pressure. SO_2 dissolves in water very easily. It can not catch fire. Sulphites are widely used to inhibit enzymatic browning. SO_2 gas is one of the sulfiting agents: sodium metabisulfite, potassium metabisulfite, sodium bisulfite, sodium sulfite and it is generally recognized as safe (GRAS) approved by Food and Drug Administration (FDA) and used as food preservative. Prior to 1986, aqueous solutions of sulfite, particularly sodium metabisulfite, were used to wash mushrooms to remove unwanted particulate matter, and to enhance mushroom whiteness. In 1986, however, the U.S. FDA banned the use of sulfite compounds to fresh mushrooms, due to severe allergic reactions to sulfites among certain asthmatics. In Naveretheless, it was still not a concern to longan growers, because SO_2 fumigations were low cost, effective at removing unwanted particulates, and gave excellent initial quality.

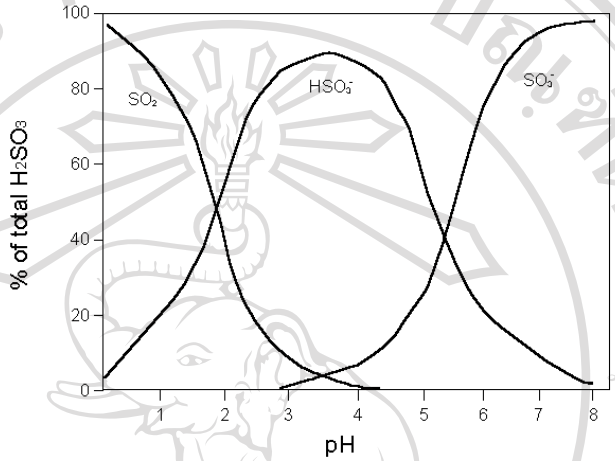
2.3.1 Mechanism to prolong shelf life

Sulfiting agents include SO_2 and several forms of inorganic sulphite that liberate SO_2 under the conditions of their use; SO_2: sulfur dioxide, SO_3^{-2}: sulfite. HSO_3^-: bisulphate, $S_2O_5^{-2}$: metabisulfite. SO_2 and sulfite salts form sulfurous acid (H_2SO_3) and exist as a mixture of the ionic species, bisulfite (HSO_3^-) and sulfite (SO_3^{2-}) anions in aqueous solution. The predominant ionic species varies in accordance with pH, ionic environment, water activity, presence of non-electrolytes and concentration of the medium in which they are dissolved. Maximum $\mathbf{HOS^-_3}$ concentrations exist at pH 4, while at pH 7; both SO_3^{-2} and HSO_3^- exist in approximately equivalent concentrations (Green, 1976). Increasing pH solution of sulfite less than 5 were observed to enhance the inhibition of polyphenol oxidase-catalysed browning (Sayavedra-Soto and Montgomery, 1986). The dibasic acid undergoes ionization according to the following reaction scheme (Figure 2.5) with pKa values of 1.89 and 7.18 (25 ^oC, zero ionic strength) for the first and second ionizations, respectively.

$H_2O + SO_2 \rightleftharpoons H_2SO_3 \rightleftharpoons H^+ + HSO_3^- \rightleftharpoons 2H^+ + SO_3^=$

Acid pH alkaline

(a)



(b)

Figure 2.5 The distribution of species of sulfurous acid at various pH values (a-b). (Source: Ough, 1984).

They possess antimicrobial activity and inhibit both enzymatic and non-enzymatic browning reactions. While the mode of action of SO_2 gas can react with H_2O ingredient within pericarp to be H_2SO_3 form at pH below 3 or HSO_3^- at pH below 5 to inhibit microbial effectively including pericarp browning as reducing agent and antioxidant (Marshall *et al*., 2000). Sulfites may control enzymatic browning in foods in several ways. First, there is an indication that sulfites react with PPO itself. It may irreversibly inhibit PPO by modification of the protein structure (Sayavedra-Soto and Montgomery, 1986). Secondly, it may interact with the intermediates in the reaction and thus, prevent the formation of the brown pigments (Taylor *et al*., 1986). There is evidence of the formation of quinine-sulfite complexes which indicates that sulfites may complex with diphenol or quinones, therefore removing them from the reaction (Sayavedra-Soto and Montgomery, 1986). Lastly, sulfites are best known as reducing

agents due to their ability to reduce the colored orthoquinones back to the colorless and less reactive diphenol (McEvily *et al*., 1992).

As a reducing agent, sulfur dioxide is able to protect longan fruit from turning brown as a result of PPO activity. It also plays a role in decay inhibition and acts as bleaching agent (Tongdee, 1994). SO_2 showed the best result in reducing PPO activity and total phenol loss which is in accordance with the results of Wu *et al*. (1999) and Whangchai *et al*. (2006). Wu *et al*. (1999) indicated that mechanism after SO_2 fumigation, the pH of the cytoplasm declined and in the pericarp, PPO activity was inhibited and the contents of free and total phenols and the content of reduced ascorbic acid increased. Primarily by affecting reaction conditions and inhibiting PPO, sulfur dioxide treatment controlled enzymatic browning of longan pericarp at 4˚C. Li *et al*. (1999) showed that SO_2 treatment significantly inhibited the respiration of the fruits and the PPO activity in the pericarp, as well as inhibiting pericarp browning. After removal from low temperature, the respiration and the PPO activity of the SO_2-treated fruits were also inhibited significantly and resulting in a longer shelf life.

Pan *et al*. (1999) reported the effects of the amount of sulfur and the treatment duration on pericarp browning and mass fraction of residual SO_2 were investigated. In longan fruits of 7 longan cultivars, i.e., Fuyan, Wulongling, Pumingan, Songfengben, Youtanben, Jiaoyan and Xingshan Wanshu, which were fumigated with SO_2. The results showed that the SO_2 absorbtion rates of the fruits were significantly different amongst the various cultivars. The proper fumigating conditions were determined as 100 g S for 20 minutes for Fuyan and 75 g for 20 minutes for Jiaoyan.

Han *et al.* (2001) reported on the effect of SO_2 treatment on the overall quality of longan fruit (cv. Shixia) during cold storage (4˚C). Results indicated that the content of anthocyanin in longan pericarp decreased and the fruit colour was improved after SO_2 treatment. No obvious effect on fruit total soluble solids and Vitamin C in the pulp was observed, while the titratable acid content was increased. Most of the SO_2 residue was located in the pericarp. Appropriate SO_2 treatment lowered the SO_2 residue level in the pulp to as low as 10 μg/g. The eating quality was maintained during the early stage of storage and the shelf life was extended compared with the control fruit. Fruit taste worsened and the shelf life was shortened with

prolonged storage. SO_2 treatment caused the pulp to partially redden during the later stage of storage.

2.3.2 Situation of SO_2

Although sulfites are very effective in controlling browning, they are subjected to regulatory restrictions owing to their potentially adverse effects on health. Many reports have described allergic reactions in humans, following the ingestion of sulfite-treated foods by hypersensitive asthmatics. Presently, fresh longan exported to China, Canada and EU, has to be fumigated for a longer shelf life. Sulfur powder is burned to SO_2 which is then blown to disperse in the closed room. After fumigation, the gas is blown out and neutralized with lime water. The factories which deal with SO_2 fumigation have to show a table of optimized dosages of sulfur powder usage and the room volume including the number of basket containers and others as Good Manufacturing practice (GMP) roles (Tongdee *et al*., 1998). High dosage of SO_2 is needed to inhibit pathogen on the peel. After fumigation, the residue on the peel will be reduced by half within 2 days. Too low dosage of fumigation cause SO_2 injury: brown line or brown circle scattered over the inner peel will be observed wthin 2 days after the fumigation (Tongdee, 1994). Fumigated longan with or without precooling should be stored at 2°C cold room or refrigerated container during transportation.

2.3.3 SO_2 residue problem and quality control

Spayd *et al*. (1984) reported that the influence of SO_2 generators on red raspberry quality during postharvest storage. Use of generators (SO_2 released pads) is not recommended owing to this bleaching and to SO_2 residues remaining in the fruit. The SO_2 gas is produced by burning sulfur. SO_2 monitoring of some exported fresh longan showed high residue contamination, their standard quality producing more problems (Sinchaipanit and Jaengsawang, 1997; Tongdee, 1998). Currently, SO_2 monitoring from 14 fumigation factories in the north of Thailand showed SO_2 residue of 1,500-3,000 ppm in the pericarp and 100-400 ppm in the flesh (Jaroenkit *et al*., 2005). More residues in fumigated fruit was detected during storage at 5±1°C and decreased slowly at 10±1°C (Rinapol, 2005). Recently, Chungyusuk *et al.* (2009) who found that factors affecting SO_2 residue in the longan fruits depended on orchard

locations, moisture content, fruit size, maturity index and storage condition. The joint WHO/FAO Expert Committee on Food Additives (1974) indicated that SO_2 residue of acceptable daily intake(ADI) at 0.70 mg/weight 1 kg/day e.g. 42 mg.SO_2 for a 60 kg man (Taylor *et al*., 1986). In the US, SO_2 is generally recognized as a safe substance in food processing, but it is not allowed in postharvested perishable produces except grape (FDA, 1996). Presently, a cooperative committee of Thailand and China indicated maximum SO_2 flesh residue not to exceed 50 ppm on SO_2 certificate. All packing houses, factories and companies with SO_2 fumigation activity and export the fumigated fresh longan to China and Hongkong, have to register with the Department of Agriculture (DOA) (Department of Agriculture, 2005).

2.3.4 Reduction of SO_2 residue

Ozone application reduced or eliminated SO_2 residue in longan fruit. SO_2 fumigated fruit were subjected for 3 hrs with ozone gas in a closed chamber. The result indicated that SO_2 residue in the pericarp and flesh were reduced to 57.63% and 77.87% respectively (Kankriagwong *et al*., 2002). In another experiment, fumigated fruits were dipped in water with ozone released for 2 hrs, SO_2 residue in the pericarp and flesh reduced to 34.65 and 49.70 % respectively and their shelf life were 40 days at 5°C (Whangchai *et al.*, 2004). Dipping fumigated fruit in tap water for 15 min decreased only peel residue to 34.50 % (Tongsri *et al*., 2005).

2.4 Alternatives to SO_2

SO_2 fumigation is facing increasing consumer and regulatory use. It has recently had strict guidelines placed over its use by the US-FDA, the Australian and New Zealand Food Authority (ANZFA) because of concerns over health risks to workers and consumers. So the longan industry is under threat of its removal in the future. Without the use of SO_2, major problems are expected from the lack of control of storage quality and disease in the fruit by a large part of the industry. The removal of SO_2 could have a significant detrimental effect on the longan industry's ability to maintain current domestic and export markets as well as take advantage of new export opportunities. This is largely due to the lack of knowledge on the postharvest aspects of longan storage. Current postharvest handling recommendations for longan are not

complete and need to be refined and handling practices to improve the effectiveness of the alternative studies are required.

2.4.1 Precoolings

Precooling is temperature management before either container or shipment storage (Tongdee, 1992). It is used on a commercial scale; hydrocooling and top-icing (Maneesin, 2004). Historically, hydrocooling was used to precool longan before SO_2 fumigation. Postharvested longan fruits in the plastic basket were immersed immediately in iced water at 0-2°C for 10-12 min before cold storage (Wara-Aswapati, 1989; Yaacop and Subhadrabandha, 1995). However, cold room insufficiency causing freshness losses and chilled water contamination were difficult to control. Thus, wet longan was susceptible to decay. Commercially, fumigated longan fruits will be precooled by forced-air cooling or room cooling except hydrocooling. Forced-air cooling and room cooling reduced SO_2 residue more rapidly than hydrocooling and less brown spot was observed on the pericarp (Tongdee, 1988; Suwanakul, 1997). Dipping longan fruit in chlorinated iced water maintained pericarp color, delayed pericarp browning and decays more slowly in a 3-week storage experiment (Apai *et al*., 2007). Addition of citric acid showed brighter pericarp (L*) (Apai *et al*., 2005). In nervertheless, hydrocooling was not suitable method to prolong storage life of longan due to wet fruit which accumulated pathogenic attack.

2.4.2 Physical treatments

Physical treatment importantly becomes phytosanitary guarantee of Thai longan exporter to overseas: USA, Australia and New Zealand. Biosecurity Australia has recommended that the importation of fresh longan and lychee fruit for human consumption into Australia from the People's Republic of China and Thailand be permitted subject to the application of risk management measures and phytosanitary procedures.

Dipping longan fruit in hot water at 40-50°C for a period of time and stored at 1°C did not reduce CI or improve fruit quality. During storage at 1°C the fruit lost ascorbic acid content (Hayeesaleh and Booyakiat, 2003) and hence, lessen the activity

of polyphenol oxidase; PPO in the peel (Sukvivat, 2004). The stepwise temperature to 5°C both start at high and low temperature up to 5°C storage showed different results. Those fruit started at high temperature showed higher electrolyte leakage and weight losses (Rattanapanone *et al*., 2003).

Follett and Sanxter (2002) reported that hot-water immersion and irradiation quarantine treatments are used to disinfest longan of fruit flies and other pests before export from Hawaii to the US mainland. One day after harvest, longan (cultivars Chompoo and Biew Kiew) fruits were subjected to hot-water immersion at 49°C for 20 minutes, irradiation treatment at a minimum absorbed dose of 400 Gy, or left untreated as the controls. Fruits were then stored at 10˚C in perforated plastic bags and quality attributes were evaluated after 7, 14 and 21 days. Chompoo and Biew Kiew fruits treated by hot-water immersion were darker and less intensely coloured than irradiated or untreated fruits after 14 days of post-treatment storage. For both cultivars, the external appearance of fruits treated by hot-water immersion was rated as unacceptable after 14 and 21 days of post-treatment storage, whereas irradiated and nontreated fruits were rated as acceptable on all days. *Penicillium* mold contributed to the unacceptable external appearance ratings after 21 days for fruits that were treated by hot-water immersion. With both cultivars, the taste of fruits treated with hot-water immersion was rated as unacceptable after 21 days of storage, whereas irradiated fruits remained acceptable. Overall, under these experimental conditions, irradiation was superior to hot-water immersion as a quarantine treatment based on the maintenance of fruit quality.

2.4.3 Postharvest treatments

In Thailand, dipping for 5 min in citric acid **(**CA) solution as well as sodium metabisulfite and formic acid could bleach longan peel. CA reduced fruit decay better than sodium metabisulfite (Sardsud *et al*., 2003). CA + sorbic acid and CA + potassium sorbate (PS) delayed fruit decay and extended shelf life for 30 days at 5°C (Kheuenmanee *et al*., 2005) similar results were observed in either sodium benzoate or PS dip (Chartupos and Kongbangkerd, 2002) and for 21 days in sodium metabisulfite dip without sulfite residue detected in the flesh (Boonyong, 2001). Dipping in ozonized-cold water extended longer shelf life than dipping in cold water

without ozone for 6-9 days (Ruangyuttikarn and Pankasemluk, 2002). The former treatment with vacuum bag could be kept as long as 40 days at 5°C (Hunthavee *et al*., 2005). Dipping in oxalic acid before fumigated with ozone gas showed the brightest (L*) peel and the shelf life was 30 days (Chuajedton *et al*., 2005). However, these treatments have not yet been applied in commercial scale. So, they need to investigate more intrinsically and together with each imported country committees.

Different region of longan growing may have affecting on fruit characteristic. Edible coating in longan fruit was popularly tested in lab scale. Jiang and Li (2001) reported that dipping longan in chitosan at concentration of 1-2% (w/v) at pH 5.5 showed the best fruit quality at least for 30 days at 2˚C: delayed fruit decay, browning, weight loss, PPO activity. Accordingly to Vangnai *et al*. (2006) found that chitosan coating could prolong shelf life for 15 days at 4˚C.

Drinnan (2004) recommended that HCl dip would be 5% solution (300 g/l HCl) for 20 min; surface dried and packed in medium humidity packaging could prolong shelf life for 40-60 days at 5°C. Sodchit *et al*. (2008) studied the effect of N-acetyl-L-cysteine and 4-hexylresorcinol on browning inhibition of 500 g bunches of longan fruits cv. Daw. The results showed that N-acetyl-L-cysteine prevented pericarp browning of the fruits better than 4-hexylresorcinol, which resulted in better color values, lower disease incidence and weight loss during storage at 15±2°C and 85 %RH for 6 days. In conclusion, consistency in the effectiveness of postharvest dip will initially be concerned on very importance prior to be appropriated in the commercial use more than primary concern with the low cost. Chemical dip might be strictly combined with the others to improve its efficacy to control both browning and decay.

2.4.4 Modified and controlled atmosphere

Different in cultivar and growning region may affect the storage life of longan. Longan with or without SO_2 had 50 and 30 days storage life, respectively (Ratanachinakorn *et al*., 2001). Tian *et al*., (2002ab) reported that longan fruits were stored in different controlled atmospheres. The results indicated that in comparison with modified atmosphere packaging (MAP), controlled atmosphere storage was associated with: a significantly reduction in fruit decay, inhibition of PPO activity and

prevention of skin browning, decreased ethylene productions and extension of storage life of longan fruits. Fruit kept in controlled atmospheres of 15% CO_2+4% O_2 retained good taste and quality, without any deterioration or off-flavour development after 60 days of storage.

Additionally, application of controlled atmospheres needs to be precooled before storage. This practice is costly at the present time for longan produce. Modified atmosphere packaging (MAP) might be applied in commercial practice. However, more research is needed.

2.5 Improvement of anti-browning efficacy by organic acid mixed in chitosan-based coating

2.5.1 Citric acid dip

Citric acid, a main component in lemon juice is one of the hydroxytricarboxylic acid, used as acidity/regulator in a category of preservatives for inhibiting browning from PPO of perishable products according to the 84th Ministry of Health's declare in 1984. Citric acid is one of the most widely used acidulants in the food industry. It is typically applied between 0.5 and 2 percent (w/v) for the prevention of browning in fruits and vegetables. In addition, it is often used in combination with other antibrowning agents such as ascorbic or erythorbic acids and their neutral salts, for the chelation of pro-oxidants and for the inactivation of polyphenol oxidase. Recommended usage levels for citric acid vary between 0.1 and 0.3 percent (w/v) with the appropriate antioxidant at levels ranging between 100 and 200 ppm (Dziezak, 1986). Citric acid exerts its inhibitory effect on polyphenol oxidase by lowering the pH as well as by chelating the copper at the active site of the enzyme. In longan fruits, Sardsud *et al.* (2003) reported that citric acid dip can extend shelf life at ambient temperature and at 5°C. As results, Kheuenmanee *et al.* (2005) showed that efficacy of citric acid dipping on decay inhibition was approved when mixing with potassium sorbate. The shelf life of longan can be extended for 30 days when stored at low temperature. In litchi fruits, Terdbaramee *et al.* (2002) reported that dipping in citric acid can maintain shelf life at 5°C for 42 days.

Table 2.2 Study on citric acid to control browning in longan.

Treatments	Browning delay	References
-Dip in 5%CA solution	28 days	Sardsud *et al.* (2003)
-Dip in 5%CA solution prior O_3 gas	21 days	Whangchai *et al.* (2003; 2006)
-5%CA + 0.3% PS	30 days	Kheuenmanee *et al*. (2005)

CA = citric acid, PS = potassium sorbate

2.5.1.1 Mechanism on browning control

Citric acid is one of the inhibitors or has synergist effects in browning reaction caused by polyphenol oxidase; PPO (EC 1.10.3.1) in fresh and fresh-cut perishables. The mechanisms of inhibitions, CA lower pericarp pH (Joas *et al*., 2005) which normally is at at pH 6.5 (Jiang, 1999) and binding Cu^{2+} in active site of PPO to form an inactive complex. Therefore activity of PPO can be inhibited (Marshall *et al*., 2000). Citric acid is identified as non-competitive browning inhibition to reduce catechol-quinone product. The other acids as browning inhibitor, such as oxalic acid, kojic acid and cysteine were also reported in fresh-cut apple (Son *et al*., 2001).

2.5.1.2 Dipping method problems of organic acids

Ascorbic acid or sodium erythorbate, in combination with citric acid, were not effective to prevent discoloration of fresh-cut fruits and vegetables (Sapers and Miller, 1995; Buta *et al*., 1999; Son *et al*., 2001). However, dipping longkong fruits in citric acid showed pericarp damage at high concentration, therefore the pericarp browning was rapidly found within a few days storage in comparison with untreated fruit (Lichanporn *et al.*, 2002; 2003). This causation hypothesis assumed that pH in pericarp and water loss after dip may be higher during storage cause cell damage and plasmolysis. As a concequence, increase contact between PPO from chloroplast and phenol from vacuoles, accelerates enzymatic action (Underhill and Critchley, 1994).

Table 2.3 Damaging of perishable produce after dip in citric acid or the others.

Damage characteristics	Plants	Treatments	References
1. Pericarp damage fruit	Longkong	Dip in 2, 4 or 6% CA for 5 min	Lichanporn *et al*. (2002); Lichanporn *et al*. (2003)
	Litchi	Dip in 5, 10 or 15% CA	Seangnil *et al.* (2007)
	Longan	Dip in 1, 3 or 5% CA for 5 min	Jarinthorn *et al.* (2008)
2. Tissue breakdown	Fresh-cut apple	Dip in 2, 4 or 6% CA	Ponting *et al*. (1971)
	Apple	Dip in CA+AA	Cocci *et al*. (2006)

CA = citric acid, AA = ascorbic acid

In addition to this caution, be rapid degradable of the chemical after dipping because pericarp may be due to different morphology, porosity and thickness. The active substances may be reacted with other substances or they were directly inhibited by the food ingredients. In addition, the structure of active substances were modified or evaporated from food surface or diffusion of active substances in food not to be thoroughly (Ouattara *et al*., 2000). Lichaporn *et al*. (2003) found the effects of 0.5 and 1.0% ascorbic acid and 2.0, 4.0 and 6.0 % citric acid on skin damage. The skin of longgong showed browning with weight loss and increasing in respiration rate and ethylene production. The fruits were also deteriorated more rapidly than those treated with water.

2.5.2 Chitosan coating

2.5.2.1 Property

Chitosan is a modified (Figure 2.6), natural carbohydrate polymer derived by deacetylation of chitin [poly-β-(1 → 4)-N-acetyl-Dglucosamine], a major component of the shells of crustacean such as crab, shrimp, and crawfish and the 2nd most

abundant natural biopolymer after cellulose. Its high molecular weight cationic polysaccharide had a group of β-1, 4 linkages with glucosamine (main) and N-acetyl glucosamine (minor). During the past several decades, chitosan has received increased attention for its commercial applications in the biomedical, food and chemical industries. Chitosan has been used for drug delivery because of its biocompatibility, biodegradability and bioadhesiveness.

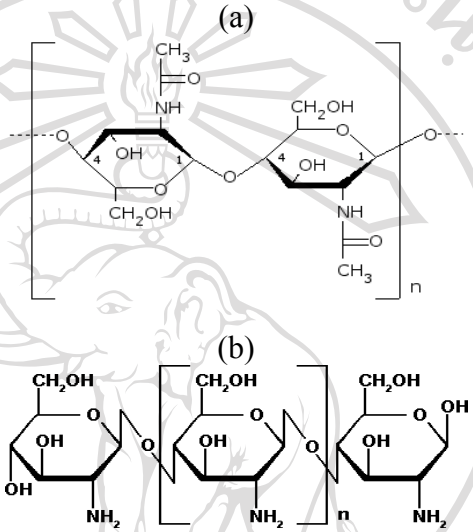


Figure 2.6 Chitin (a) and chitosan (b)

It has been more popular used as an edible-based coating on fruits produce and mixed with many substances: organic acids, preservatives, antioxidants, antimicrobials because of its good biocompatibility and carrier (Zhao, 2005). The chitosan, carrier, had matrix and ionotropic gel characterization. It maintains active substances at the surface and prevents the diffusion of active substances into bulk food. So, it may help to protect the quality and water loss from food in addition to the protection of surface contact with O_2 as a semi-permeable coating (Conca and Yang, 1993). Furthermore, it was used in controlling the diffusion of medicine and chemical by capsule production in pharmaceutical.

Chitosan is now widely produced commercially with different deacetylation grades and molecular weights (thus, viscosities of chitosan solutions) and hence different functional properties. Chitosan can be dissolved in organic acid solvents especially acetic acid. It had been reported in longan fruits (Jiang and Li, 2001), litchi

(Zhang and Quantick, 1997; Jiang *et al*., 2005), fresh-cut (peeled) litchi (Dong *et al*., 2004). However, this acid can burn the longan surfaces to damage (Drinnan, 2004). Chitosan might be soluble in the other organic acids such as citric acid and oxalic acid while the water temperature was 80°C. Solubility of chitosan depended on content of acetylglucosamine block and reaction of hydrocarbon chain of carboxylic acids group (Hamdine *et al*., 2005).

2.5.2.2 Mechanisms in browning control

Shelf life was dramatically improved. A research study conducted by Zhang and Quantick (1997) indicated that chitosan coating had potential inhibitory activity on PPO and peroxidase activity in lychee (*Litchi chinensis* Sonn.) fruit. Chitosan has been shown to improve the storability of fruits. Its effectiveness in this respect is therefore thought to be due to the formation of a protective barrier on the surface of fruit, which reduces the supply of oxygen (Figure 2.7) for the enzymatic oxidation of phenolics. Chitosan is non-toxic and is biologically safe. Thus, the application of a chitosan coating for the control of browning and quality improvement in fruits and vegetables might be accomplished in combination with other methods such as low temperature and suitable packaging.

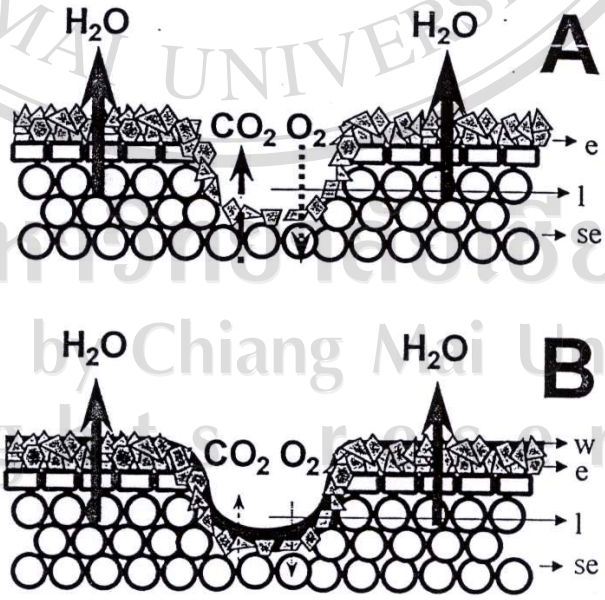


Figure 2.7 Hypothetical models for the difference in the skin permanence to O_2 (↓), CO_2 (↑) and water vapor (↑) in a non-coated (A) and coated (B)

pear with a lignified cell in the skins. (Cr = crack in the cuticle, se = sub epidermis, w = wax, l = lenticel) (Amarante and Banks, 2001).

2.5.2.3 Edible coating in conjunction with antibrowning agents

Edible coating are thin layers of edible material applied to the product surface in addition to or as a replacement for natural protective waxy coatings and provide a barrier to moisture, oxygen and solute movement for the food. They are applied directly on the food surface by dipping, spraying or brushing to create a modified atmosphere (Krochta and Mulder-Johnson, 1997). An idea coating is defined as one that can extend storage life of fresh fruit without causing anaerobiosis and reduces decay without affecting the quality of the fruit (El Ghaouth *et al*., 1992). Previously, edible coatings have been used to reduce water loss, but recent developments of formulated edible coating with a wider range of permeability characteristics has extened the potential for fresh produce application (Avena-Bustillos *et al*., 1994). The effect of coating on fruits and vegetables depends greatly on temperature, alkalinity, thickness and type of coating, and the variety of and condition of fruits. The functional characteristics required for the coating depend on the product matrix (low to high moisture content) and deterioration process to which the product is subjected (Guilbert *et al*., 1996).

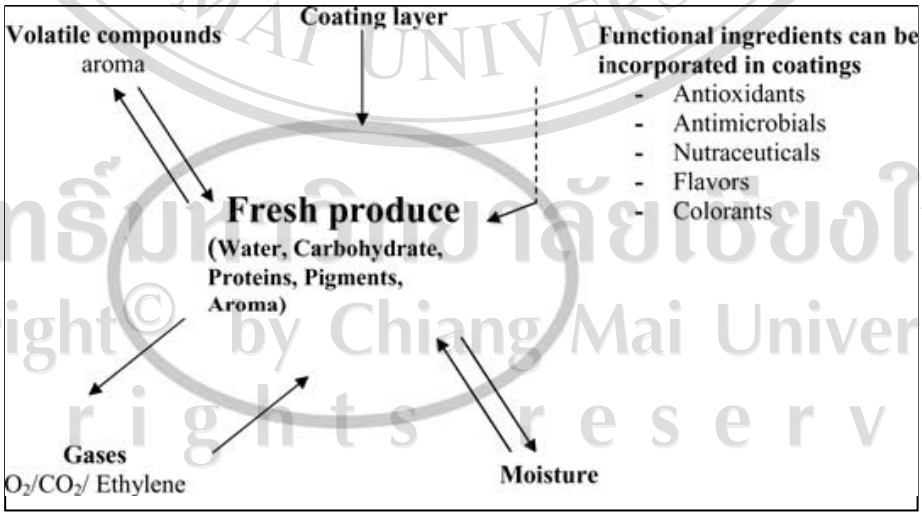


Figure 2.8 Functional ingredients can be incorporated in coatings.

(Souce: Lin and Zhao, 2007).

2.5.3 Chitosan coating with citric acid to retard pericarp browning

Currently, citric acid and tartaric acid mixed with chitosan as a pericarp browning inhibition, the organic acid mixing until pH of chitosan-based coating reduced to 0.8, 1.0 or 1.3 after storage at 10°C for 2 weeks. The results showed that the important mechanism to reduced pericarp browning depend on lower pH and water loss of pericarp after coated and during storage. Consequently, pericarp browning can be predicted by pericarp pH and weight loss of fruits (Joas *et al*., 2005). In addition, pericarp browning may be estimated from an acid impregnation index (AII), defined as the titratable acidity-to-postharvest weight loss (in %). Citric acid+chitosan showed higher potential on inhibition of browning more than tartaric acid when compared to effects on pericarp browning and consistency of dispersion of chitosan in surfaces (Caro and Joas, 2005). This treatment has been demonstrated to be more effective when pericarp pH is close to 3, i.e. the pericarp PPO and POD pKs and Pericarp pH of treated fruits is correlated to the pH of the soaking solution (citric acid+chitosan).

2.6 Application of potassium sorbate mixed in chitosan coating to control fruits decays: new emerging technology

2.6.1 Potassium sorbate as food preservative

Preservatives were mixed in edible-based coating such as sorbic acid, propionic acid, potassium sorbate, benzoic acid, sodium benzoate and citric acid (Quintavalla and Vicini, 2002). Zhao (2005) reported that chitosan might be a good carrier and mixing with preservatives, such as potassium sorbate or sodium benzoate, to inhibit microbial in perishable produce. Potassium sorbate ($C_6H_7O_2K$; molecular weight at 150.22), potassium salt of sorbic acid ($C_6H_7O_2COOH$) and a polyunsaturated fatty acid salt, are generally recognized as safe (GRAS) by the US Food and Drug Administration (FDA) (Pylypiw and Grether, 2000). Park *et al.*

(2005) reported that potassium sorbate mixed in chitosan-based coating in strawberry reduced aerobic count and coliforms including water loss. These treatments showed good growth inhibition in vitro study of fungi: *Cladosporium* sp. and *Rhizopus* sp., and maintained fruits quality through storage (Park *et al*., 2005). In addition, potassium sorbate had been reported as a mixture in chitosan-based edible films and inhibited many bacteria in meats by gradually slowly diffusion (Pranoto *et al*., 2005).

The effectiveness of chitosan-based coating to inhibit postharvest diseases of fruitss and vegetables also depended on factor in characteristic of surface skin and morphology of pericarp. In fresh longan fruits, surface characteristic shows small cracks and lenticels. Lin *et al.* (2001) reviewed that both the storability and disease resistance of longan fruits were related to the structure of skin and flesh, including cultivars. Some cultivars which were strongly resistant to diseases and more suitable for storage and transportation, had thick skin, thick corky layer of good continuity, many tumor shape projecting and setas, many stone-cell groups with closer arrangement, developed vascular and a thick cuticle.

2.6.1.1 Problems with potassium sorbate dip

Direct surface application like spraying or dipping of antimicrobial agent has some limitations because the active substances could be neutralized, evaporated or diffused inadequately into the bulk of food (Torres *et al*., 1985; Siragusa and Dickson, 1992). Subsequently, upon encountering the higher pH inside the cell, the molecule dissociates resulting in the release of charged anions and protons, which can not cross the plasma membrane (Brul and Coote, 1999). In longan fruits that was dipped in 1000 ppm potassium sorbate, had been a report of sorbic acid residue in pulp (Chartupos and Kongbangkerd, 2002). Therefore its efficacy is limited because of its ability to diffuse into foods, which results in reduced preservatives concentration on the surface and thus microbes can overcome.

2.6.1.2 Application of food additives incorporating edible coating to reduce migration loss

While testing the effectiveness of antimicrobial coatings on food products gives the most meaningful results, this approach can be the most difficult because of

the complexity of food systems. A coating method must be developed to coat the food product of interest evenly and effectively. The coating must withstand the product and storage conditions.

The antimicrobial agent like potassium sorbate can migrate to the surface of the chitosan film or chitosan-based coating and then to the food to inhibit microbial growth. The antimicrobial is effective against microorganisms on the food surface without migration of active agents to the bulk food. Limjaroen *et al*. (2005) reported that using sorbic acid–containing films, common spoilage organisms were also inhibited on meat product and cheese. After 28 days of contact with bologna and Cheddar cheese, these films retained 7% and 60% of their original sorbic acid content, respectively, with the control film retaining 85% of its original sorbic acid content. Given these findings, sorbic acid–containing films may be useful in enhancing the safety and shelf-life of ready-to-eat delicatessen products.

Edible coatings can also serve as carriers of additives. Coatings carrying antimicrobials can keep the preservatives on the cut fruit surface avoiding their diffusion into the fruit. Injuries allow the interaction of PPO and phenolic compounds that are normally separated by the cell compartments of intact fruits (Luo and Barbosa-Cinovas, 1996). Thus, techniques to concentrate the preservative on the surface would reduce the preservative dosage currently applied to fruit preserves. Edible coatings can help prevent water loss by acting as water loss barriers, causing high relative humidity in the surrounding atmosphere of the sliced fruits and thus reducing the gradient to the exterior. It seems it is unable to form good films under high relative humidity conditions. When working under high relative humidity, hydrophilic coatings may incorporate water into the film structure, increasing their permeability (McHugh and Krochta 1994). Polysaccharide-based coatings, such as pectin, cellulose, carrageenan, starch and chitosan coatings were also studied. Use of Nature Seal 1020, a cellulose-based edible coating, as carrier of antioxidants, acidulants and preservatives prolonged the storage life of cut apple cv. Red Delicious and potato cv. Russet. Ascorbic acid delayed browning more effectively when applied in an edible coating than in an aqueous solution. Similarly, the preservatives sodium benzoate and potassium sorbate were more effective in controlling certain microbial populations when applied in Nature Seal than in aqueous solutions, but less

effective for others. Adjustment of coating pH to 2.5 gave optimal control of browning and microbial populations (Baldwin *et al*., 1996).

2.6.2 Mode of reaction of coating components on disease control

2.6.2.1 Potassium sorbate

The high efficacy activity of potassium sorbate (PS) is in dissociated form of sorbic acid (pKa = 4.8) which inhibited microorganism in food at lower pH by disturbing cell membrane of yeasts, fungi and bacteria and structure of enzyme in cell wall (Branen *et al*., 1990). Other mechanisms were to inhibition of essential metabolic reaction as glycolysis, induction of energetically expensive stress response or accumulation of toxic anions (Kralj Cigic *et al*., 2001). In a part of these salt compound, K ion had a toxic directly to microbial cell, protein in cell was denature and and reduction of growth of microbial cell in solution can be inhibit by plasmolysis.

- Effect of potassium sorbate on *in vitro* fungal development

Potassium sorbate inhibited spore and radial growth of *Aspergillus flavus* in PDA, pH 3.5 (Lo´pez-Malo *et al.,* 2005). 150 ppm K-sorb can inhibit the growth of *Penicillium digitatum*, 700 ppm for *P. glabrum* in PDA, pH 3.5 (Matamoros-Leon *et al*., 1999) and 10 ppm of PS produced a total inhibition of fungal development and toxin biosynthesis of *Alternaria alternata* (Combina *et al*., 1999).

- Changes of fungal morphology due to the effect of potassium sorbate

Influence on cell walls and membrane, such as morphological alterations, genetic material changes and inhibition of enzymes or transport function, are found during growth inhibition. A visible shrinkage of the cytoplasm and slight cell wall damage in *Listeria monocytogenes* were found in the presence of 0.3% PS during chilled storage (4°C) for 4 weeks (Dykes, 1999), including cell wall damage, shrinkage of the cytoplasm and leakage of nucleic acid and protein from cell.

- Control of postharvest diseases

0.2 M of PS dip at 40.6°C for 120 s reduced 70-80% green mold (*Penicillium digitatum) in vivo* of inoculated lemon or orange after 7 days. The mixture between PS and sodium benzoate did not significantly enhance the effectiveness of the substances (Palou *et al*., 2002). PS suppressed postharvest decay of sweet cherry, but less effective to sodium bicarbonate (Karabulut *et al.*, 2001). However, in stone fruits (sweet cherry, apricot, peach and nectarine), 3.0% of sodium bicarbonate showed phytotoxic effects on fruits. While 1.5% PS showed significantly to reduce *Monilinia* infection in sweet cherry (61.6%), apricot (78%) and nectarines (31.8%) with respect to the control, without any visible damage on the skin (Mari *et al*., 2004). Besides, PS was found to be the more suitable preservatives to use in bakery product for prevent contaminants from *Eurotium, Aspergillus* and *Penicillium* (Marn *et al*., 2002).

In addition of preservatives like potassium sorbate may effectively inhibit the decay in fresh longan fruits though 30 days storage at 5°C (Chartupos and Kongbangkerd, 2002) and more effective near longan fruits fumigated with SO_2 gas when it was mixed in citric acid dip (Kheuenmenee *et al*., 2005). Citric acid is one of the organic acids that were severally adjusted pH in solution to 3-5 to reducing the microbial population (Juliot *et al*., 1989). In ready-to-use desalted cod, PS mixed in citric acid can inhibit mesophillic, psychrotrophic and mold and yeasts as more effectiveness (Fernandez-Segovia *et al*., 2003).

2.6.2.2 Chitosan

Chitosan had inherently antimicrobial property depends on the kind of chitosan (deacetylation degree percentage; %DD), molecular weight) used, the pH of medium, temperature, the presence of food components, etc. Mode of reaction has been well documented both in vitro and in vivo studies. The level of inhibition of fungi is highly correlated with chitosan concentration and polycationic nature of this compound to its antifungal properties, including to the length of the polymer chain (Hirano and Nagao, 1989). In addition, polycationic of amine group of chitosan can react with electronegative charges on cell wall of bacteria caused permeability loss of protein, metabolism and died. Recently, chitosan have shown not only effective to

inhibit fungal growth, but also induces marked morphological changes, structural alternations and molecular disorganization of the fungal cell (Benhamou, 1996; El Ghaouth *et al*., 1999; Ait Barka *et al*., 2004). In ready-to-use desalted cod, PS mixed in citric acid can inhibit mesophillic, psychrotrophic and mold and yeasts as more effectiveness (Fernandez-Segovia *et al*., 2003).

- Effect of chitosan on *in vitro* fungal development

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

Tanapaisankit *et al.* (2005) reported effects of chitosan to *Lasiodiplodia theobromae* in rambutan that 0.18% chitosan inhibited mycelium growth to 69% in PDA, 0.04% chitosan inhibited mycelium growth to 34.94% in PDA and 0.18% coated fruits showed to inhibit fruits rot as well as dipping in 1000 ppm benomyl. For example, as chitosan concentration increased (0.075-0.6%) the redial growth of *Alternaria alternate*, *Botrytis cineria*, *Colletotrichum gloeosporioides* and *Rhozopus stolonifer*, decreased (El Ghaouth *et al.,* 1992b). 0.075% chitosan upwards reduced spore viability and germ tube growth of both *B. cineria* and *R. stolonifer* (El Ghaouth *et al*., 1992a).

- Changes of fungal morphology due to the effect of chitosan

Microscopic observation of fungi treated with chitosan revealed that it can affect the morphology of the hyphae. Cell wall of the tip of the hyphae of *Trichoderma longibrachiatum* was thin in presence of chitinase and β-1,3-glucanase (Arlorio *et al*., 1992). Excessive mycelial branching, abnormal shapes, swelling and hyphae size reduction were observed in *Fusarium oxysporum* f.sp. *radicis-lycopersici*, *R. stolonifer* and *Sclerotium sclerotiorum* (Benhamou, 1992; El Ghaouth *et al*., 1992a,b; Cheah *et al*., 1997). Morphological changes such as large vesicles or empty

cells devoid of cytoplasm in the mycelium of *B. cineria* an *F. oxysporum* f.sp. *albedinis* (Ait Barka *et al*., 2004; El Hassni *et al.*, 2004). There are rarely reports of effects of chitosan on morphological parameters of spores of these fungi like length and form of conidia of each the fungi. Spore of *Aspergillus niger* was only one reported in the present time (Plascencia-Jatomea *et al*., 2003).

- Control of postharvest diseases

Chitosan can delay the onset of infection and slow down the infection process. Chucheep *et al*. (2002); Romanazzi *et al*. (2002) reported that 1 or 2% chitosan had a reduction of fruits decay and severity of *Botrytis cinerea* in tomato, grape and strawberry. Zhang and Quantick (1997) reported that chitosan-treated with litchi delayed the infection process during the 33 days storage period, but was not as effective as TBZ in controlling rots. Besides, chitosan was severally reported reduction of rot during storage in other plants like apple, kiwifruits, pears, longan and it was extremely recently reported to combine with the others methods for improving the antimicrobial properties to diseases (Bautissa-Banos *et al*., 2006)

- Chitosan inducing pathogenesis-related protein (PR-protein) in plant cell

Most cell wall of fungi has been consisted of 80% polysaccharide like chitin and glucan in cell wall. Pathogenesis-related protein (PR) gene in plant cell can be activated by infection, wounding, stress from chemicals and environment. Chitosan can induce host cell to synthesis enzyme in gene that accumulated protein content to inhibit infection or colonization by pathogens. The systematic nature and persistence of defense enzymes in plant tissue on elicitation by chitosan could be important in retarding the resumption of latent infections which typically become active when tissue resistance declines (Wilson *et al*., 1994).

Bautissa-Banos *et al*. (2006) reviewed that chitosan induces a series of defense reaction correlated with enzymatic activities. Chitosan has been shown to increase the production glucanohydrolases (Pathonogenesis-related protein; PR-protein). Zhang and Quantick (1998) showed that chitosan-based coating had significantly an efficacy on decay inhibition in strawberry and raspberries by increase

of chitinase and ß-1, 3-glucanase activity of the fruits as compared with the uncoated controls. El Ghouth *et al*. (1997) reported that chitosan also reduces production of polygalacturonases of *B. cineria*. In bell pepper tissue and markedly reduced the maceration of the host cell wall components, pectin and cellulose. Phenolic compounds and synthesis of specific phytoalexins with antifungal activity were reported in grape and tomato (Bautissa-Banos *et al*., 2006). In Thailand, Jongchotsirikul (2001) reported that PR-protein in tissue of grape were induced by chitosan during pre-harvest and acted synergistically having a role in defense of grape against antracnose (*Collectotrichum gleosporoides*). In addition on phenolic compounds like phytoalexin (stilbene) were also detected.

Chitosan induces structural barriers for example inducing the synthesis of lignin-like material. Cellular suberization and lignification among others were elicited during the infection process in some plant organs (Bautissa-Banos *et al*., 2006). For Bell pepper was observed by a TEM, structural defense responses were observed only in the first tissue layers beneath the ruptured cell such as thickening of the host cell, formation of hemispherical and spherical protuberances along the cell walls, and occlusion of intercellular spaces with fibrillar material (El Ghaouth *et al*., 1994; 1997).

2.7 Effects of coating components on chilling tolerance

Chitosan, edible-coatings form a protective barrier on the surface of fresh produce, reduce the supply of oxygen, water loss, retard senescence and consequently, delay browning of fruits and vegetables (Jiang and Li, 2001). Chitosan showed potential to induce PR-protein in plant tissue in strawberry fruits (Zhang and Quantick, 1997). In addition to treatment of tomato fruits with a naturally occurring substance like methyl jusmonate significantly also increased the same expression of some pathogenesis-related (PR) genes. The increased accumulation of these (PR) genes may be related to the increased resistance of fruits tissue to chilling injury and decay. These results suggest that induced the synthesis of some stress proteins, such as PR proteins (chitinase and ß-1, 3-glucanase activity), which leads to increase chilling tolerance and resistance to pathogens, thereby decreasing the incidence of decay (Ding *et al*., 2002).