CHAPTER 2

LITERATURE REVIEW

2.1 Introduction

Longan is an evergreen subtropical fruit tree in the Sapindaceae family (Tindall, 1994). Blossoming starts between late December and late January, corresponding to the cool and dry winter months of the year (Tongdee, 1997). The fruit borne on panicles are harvested over 4-6 weeks in mid-summer (Menzel, 1989). Total soluble solids range from 15 to 25 ºB, depending on the cultivar and the stage of fruit maturity at harvest. Wara-Aswapati *et al*. (1994) recommended using total soluble solids as a maturity index for longan and established 15.5-16 ºB as minimum maturity standard. Mature longan fruit pericarp consists of three layers. The outer most epicarp has a discontinuous cuticle, a uniseriate epidermis and subepidermal sclerenchyma. The middle mesocarp is parenchymatous tissue. The inner endocarp is made up of small, thin-walled, unsuberized epidermal cells (Qu *et al*., 2001). Jaitrong *et al*., (2005) reported that the anatomy of pericarp thickness of longan fruit cv. "Daw" was 630 - 700 µm and consisted of three layers. The exocarp had discontinuous cuticle with many natural openings and some epidermal hairs. The subepidermals clerenchyma layer was thick. The mesocarp had some parenchyma cells with large intercellular spaces as the main body of the pericarp. The endocarp was made up of a single thin layer of epidermal cells.

Longan as a tropical fruit has very short shelf-life deteriorating easily and quickly within a few days at room temperature (25ºC) (Reed, 1986; Paul and Chen,

1987). The major factors reducing the storage life and marketability of longan fruit are microbial decay and skin browning, both of which detract from the appearance and can impart off-flavors to the flesh. In general, fruit can be stored at 1 - 5ºC for about 30 days depending upon the cultivar. In many crops if the fruit is stored at low temperature, losses of fruit can be reduced. However longan skin is very susceptible to chilling damage so that too low temperatures can injure the skin. Furthermore low humidity storage could dehydrate from pericarp causing brown skin (James, 2004)

There are many techniques to control postharvest decay and prolong storage life of longan fruit such as fungicide dips, applications of plant growth substances, waxes and chitosan coating, use of microbial antagonists such as *Bacillus subtilis*, irradiation, heat treatment and sulphur fumigation. Controlled atmosphere (CA) storage reduced postharvest decay, prevented peel browning and extended storage life (Tian *et al*., 2001). There have been several studies on the effects of elevated O_2 on fruit parameters such as respiration rate, ethylene production and colour formation (Su *et al*., 2005). Applications of chitosan coating of longan fruit reduced respiration rate and weight loss, delay the increase in PPO activity and the change in colour, and eating quality, and partially inhibited decay of fruit during storage (Jiang and Li, 2001). Currently, the widely applications were only fungicide dips and sulphur fumigation have been used commercially (Jiang *et al*., 2002). SO_2 fumigation has been reported to be the most effective postharvest treatment for controlling pericarp browning and saprophytic surface fungi and is used extensively in commercial situation at present (Underhill *et al*., 1992). But there are a few reports on the postharvest changes of Thai longan.

2.2 The botany of longan fruit

2.2.1 Botany

The longan was easier classified in a family Sapindaceae, genus *Dimocarpus longan* Lour. The basic morphology of the longan is similar to other sapindaceous tree such as lychee, rambutan, pulasan but most particularly related to lychee (Watson, 1984). Longan was formerly reported to be indigenous the lowlands of Ceylon, Southern India, Burma and China, but the area of distribution is now through to include the medium elevations (up to 1000 m) of the mountain chain from Burma to Southern China. Principal areas of cultivation are in Thailand, China and Taiwan. Common names are longan (dragon eye) and longyen (China-Taiwan), lamyai (Thailand) and lengkeng (Malaysia-Indonesia).

The tree is medium to large size, 10 to 20 m high. Leaves are petioled, alternate and propionate with 6-9 pairs of leaflets arranged opposite and alternate on the rachis. Leaflets are glossy dark green on the upper surface and paler on the lower, commonly up to 120 mm long. Inflorescences are large 300 mm long with widely branched erect and leafless. Flower are small and yellowish brown, with five, sometime six, petals. Filaments are pubescent and anthers glabrous.

The longan cultivated in various countries are:

China	Shin Hsia, Wu Yuan, She'P'I, Kuan Yen. Hua Kou
Taiwan	Aliou, Carambola leaf, Duan-Yu, Chien liou, Fu Yen, Tiang Suan Jou
Hong Kong	Shek Yip, Fa Haak and Fa Hok Chai
Thailand	Daw, Biew Kiew, Chompoo, Haew, Dang, Baidam, Luang and Talub nak
Florida	Kohala, Chompoo, K. Sweeney, Blackball, Ponyai, Kona, Homestead No.1, Homestead No.2 and Dagelmen
Hawaii	Fukho No.2, Kohala, Ilao,Wai and Carambo

Longan fruits are harvested during June to August of every year. The optimum maturity index of longan is 6-7 months after flowering. Fruits take from 5 to 7 months to mature according to cultivar and climatic conditions. Fruit surface have small rough, 2 cm fruit size and pericarp thickness about 500-1000 μm was shown. The aril is white to off white and may be up to 70% the total fruit weigh and sweet taste about 18-20 °B. The seed is small round, blackish brown and comes substantially free from the aril.

2.2.2 Anatomy

Qu *et al*. (2001) reported that the mature longan fruit pericarp consists of three layers. The outermost epicarp has a continuous cuticle, a uniseriate epidermis and subepidermis and subepidermal sclerenchyma. The middle mesocarp is

parenchymatous tissue, consists of an undeveloped cork layer, some stone cells and parenchyma cells with large intercellular spaces. The inner endocarp is made up of small, thin walled, un-suberized epidermal cells.

2.2.3 Harvesting maturity

Harvesting time is 149 days after blooming. Maturity is gauged by fruit shape, fruit weight, skin color become yellow-brown, flesh sugar concentration as 15.5-16% as a minimum maturity standard and flesh acid concentration. In Asia, the panicles are cut or broken from the tree. However fruit are not allowed to fall to the ground.

2.3 Sulphur Dioxide

Sulphur dioxide (SO_2) is one of the most widely used as food preservatives. The fumes of burning sulphur were used by the ancient Egyptians and Romans to sanitise wine vessels, an application which continues to this day in a more control manner (Tongdee, 1994). In the U.S., sulphur dioxide has been used in dried vegetable and fruits processing. Recently it has been used in fish and meat products. Sulphiting agents have been used in food such as SO_2, sodium metabisulphite ($Na_2S_2O_5$), sodium bisulphite ($NaHSO_3$), sodium sulphite (Na_2SO_3), potassium metabisulphite ($K_2S_2O_5$) and potassium bisulphite ($KHSO_3$). A variety of food technologies from sulphiting of food involve with one or more of the substances (Charles *et al*., 2000). Sulphites are unique compounds because they can perform many functions in food. The sulphites inhibit effectively enzymatic browning in foods and beverage, because these agents deactivate the mixed function enzyme, polyphenol

oxidase (PPO), which is present in fruits, vegetables and meat (McEvily *et al*., 1992), including inhibition of non-enzymatic browning (the formation of mellanoidin pigments), as an antioxidant, and as a reducing agent by inhibition of various enzymatic catalyse reaction, and inhibition and control of growth of microorganisms in foods; and acts as bleaching agents. Control of browning and antimicrobial effects maintain the quality and nutritional values of food.

2.3.1 Forms and Functions of Sulphur

SO_2 is a bifunctional acid. The distribution between the different forms is dependent upon the pH of the medium. SO_2 for food preserved is from burning sulphur or SO_2 in liquid. When sulphite salt is soluted, they are in forms of sulfurous acid (H_2SO_3), bisulphite ion (HSO_3^-) and sulphite ion (SO_3^-) (Poomipat, 2002).

$$SO_2 + H_2O \leftrightarrow H_2SO_3 \leftrightarrow H^+ + HSO_3^- \leftrightarrow 2H^+ + SO_3^{2-}$$

Molecular Sulphur dioxide — undissociated sulfurous acid — bisulphite ion — sulphite ion

1 <---------- pH ----------> 7

Figure 2.1: Illustration of sulphur dioxide reaction

SO_2, effective at low pH, is a gas that is used for fumigation. Sodium and potassium salts of metabisulphite and bisulphite, and sodium sulfite are approved to be used in aqueous solutions that are used as dips or sprays for foods. Bisulphite (HSO_3^-) is the predominant form of free SO_2. It causes an inactivation of polyphenol

oxidases (PPO), enzymes and the binding and/or reduction of brown quinine (Rotter, 2004). Bisulphite does bleach colour and slow anthocyanin (the predominant colouring matter in red fruit) polymerisation reaction with other phenols.

2.3.2 Regulatory Status of Sulphur Dioxide

The joint WHO/FAO Export Committee on Food Additives indicated SO_2 residues of acceptable daily intake (ADI) at 0.70 mg/weight 1 kg./day e.g.42 mg. SO_2 for a 60 kg (Taylor *et al*., 1987). The U.S. Food and Drug Administration (FDA) in 1986 required sulphites added to food must be declared. The only exception is made when sulphites are added indirectly (through a sulphites ingredient such as sulphited raisins in fruit cake) and the sulfite levels in the food product (such as the fruit cake) are below 10 ppm. SO_2 used as fumigation for table grapes is allowed and is required by the U.S. Environmental Protection Agency (EPA) to be less than detectable levels (10 ppm.). Although, sulphites is not used in postharvest perishable product (FDA, 1996), Europe, Australia and Japan have set a maximum residue limit of 10 ppm. Presently, longan fruit exported to China indicated maximum SO_2 in pulp residue not to exceed 50 ppm.

Allowable SO_2 levels of selected food items in selected countries are different and depend upon the laws (Table 2.1).

Table 2.1 Allowable SO_2 levels (ppm) in food

Country	Food	Allowable SO_2 level (ppm)
Canada	Dried fruit and vegetables	2500
	Fresh fruit	0
Hong Kong		350
Malaysia	Dried fruit	2000
	Wine	450
	Fresh fruit	0
Singapore	Dried fruit	2000-3000
	Yoghurt fruit	60
	Fresh fruit	0
Japan	Standard of usage for foods in general	30
The Netherland		100 (not exceeding 300 at exporting countries)
France	Fresh lychee	30
UK	Fresh fruit (pulp)	0
USA	Fresh grape	10

(Source: Tongdee, 1994)

2.3.3 The Properties of Sulphur Dioxide (SO_2)

Anti-enzymatic Function

SO_2 inhibits oxidation enzymes (enzymatic catalysts of oxidation such as tyrosinase and lactase and destroys them with time. Although, the most reaction occurred in fruit and vegetables, SO_2 inhibits polyphenol oxidase which catalyse oxidation reactions in juice. Oxidative protection at a must is sustained by this mechanism before fermentation begins. The use of SO_2 can help to avoid oxidasic case from rotten fruit (Rotter, 2004).

Browning can be associated with dehydration, heat stress, senescence, chilling injury or disease (Pan, 1994). Browning has been attributed to enzymatic oxidation of phenolics by polyphenol oxidase (PPO) (Jiang, 1999; Liu, 1999; Tian *et al*., 2002) the activity of PPO, oxygen and concentrations of antioxidants (Nicolas *et al*., 1994; Kader, 2002). PPO is activated by moisture, loss firmness the fruit, and/or chilling injury. SO_2 is an effect inhibitor of PPO, and SO_2 treatments effectively reduce fruit browning (Tongdee, 1994; Ji *et al*., 1999; Zhang *et al*., 1999). SO_2 reduce browning by obstructing polyphenol oxidase (PPO) enzyme. It seems that the bisulphite (HSO_3^-) form is responsible for these, and that it occurs by irreversible structural modification rather than binding inhibition (Sayavedra-soto and Mongomery, 1986). Fruit fumigated with SO_2 for 20 min could be stored at 4^{o}C for about 45 days without exhibiting peel browning (Han *et al*., 1999; Li *et al*., 1999).

2.3.4 Mode of action of sulphur dioxide

Sulphites may control enzymatic browning in food in several ways. Firstly, sulphites react with PPO itself that sulphites may irreversibly inhibit PPO by modification of the protein structure (Sayavedra-soto and Mongomery, 1986). Secondary, sulphites may interact with the intermediates in the reaction and thus, prevent the formation of the brown pigments (Taylor *et al*., 1987). There is an evidence of the formation of quinine-sulphite complex which indicates that sulphites may make a complex with diphenols or quinines, therefore removing them from the reaction. Finally, sulphites are best known as a reducing agent due to their ability to reduce the coloured orthoquinone back to the colourless and less reactive diphenol (McEvily *et al*., 1992). In some cases high concentrations of sulphites are used to bleach coloured pigment that have already formed. Since sulphites, for the most part, do not irreversibly inhibit enzymatic browning, they are consumed in the reaction and thus required concentrations are dependent on the length of time that the reaction must be inhibited (Taylor *et al*., 1987).

Anti non- enzymatic browning

Non-enzymatic browning is the overall term used to describe operation of any one of the following four processes: (a) Maillard browning as a result of a reaction between a reducing sugar and an amino compound; (b) breakdown of ascorbic acid under aerobic or anaerobic conditions; (c) caramelization or pyrolysis of sugars; (d) lipid browning, a result of oxidative deterioration. Control of non-enzymatic browning is often accomplished by adding sulphite. Non-enzymatic browning

processes involve formation of chemical intermediates that contain carbonyl groups, and sulphites prevent formation of brown-coloured compounds by forming alpha-hydroxysulphonates with these intermediates. The effectiveness of sulphite in preventing non-enzymatic browning depends, among other factors, on the number of carbonyl groups in the intermediates that are formed, and on the proportion of free carbonyl compound present in equilibrium with the hydroxysulphonate (Rose and Pilkington, 1989).

Antioxidant and Reducing Agent Function

As a reducing agent, SO_2 is able to protect 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). Moreover, SO_2 protects both must and wine from excessive oxidation (Rotter, 2004).

Reducing agents play a role in the prevention of enzymatic browning either by reducing o-quinones to colourless diphenols, or by reacting irreversibly with o-quinones to form stable colourless products. Reducing compounds are very effective in the control of browning. Mechanisms involved in the control of enzymatic browning are shown in below:

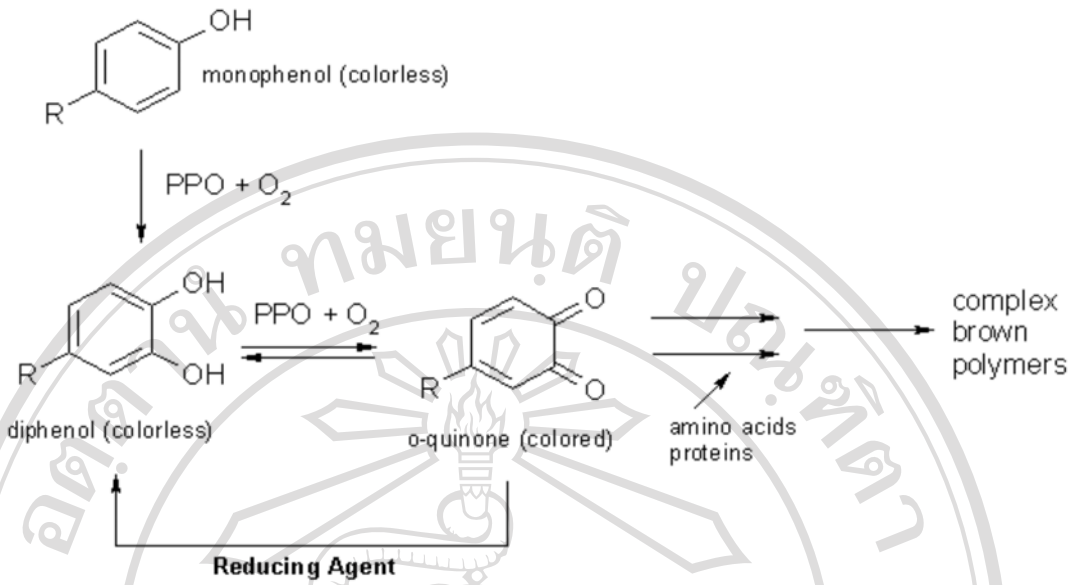


Figure 2.2: The role of reducing agent in the inhibition of enzymatic browning (Source: Walker, 1977)

2.3.5 Sulphur Dioxide Treatment on Fruits

Fresh longan exported to important countries such as China, Canada and EU, have to be fumigated for quality control and prolong shelf life. Fumigation is achieved by burning sulphur powder to SO_2 which is then blown to disperse in the enclosure room at ambient temperature for 20-30 min. Dipping in sodium metabisulphite is also effective against pericarp browning if followed by acid dip (Zhang *et al*., 1999). However, sodium metabisulphite is comparatively less effective and more variable than SO_2 treatment. After fumigation fruit sulphur residues were maximal at 150-300 ppm immediately (Ji *el al*., 1999) and were higher in the pericarp than in the aril, and decreased rapidly during the first few days (Pan *et al*., 1999). The effect of SO_2 treatment on the overall quality of longan fruit (Shixia) during cold storage (4ºC) indicated that content of anthocyanin on longan pericarp decreased and the fruit color

was improved after SO_2 treatment, and the SO_2 residue level in the pulp as low as 10 μg/g. The eating quality was maintained during the early stage of storage while the shelf life was extended as compared with the control fruit. Fruit taste worsened and the shelf life shortened as the storage prolonged. SO_2 treatment caused the pulp to partially redden during the later stage of storage (Han *et al*., 2005).

2.4 Phenolic compounds in fruit

Phenolic compounds are major constituents of fruits and play an important role in the nutritional, organoleptic and commercial properties of the fruits and their derived. Phenolic compounds contain aromatic ring(s) bearing hydroxyl group(s) and can range from simple molecules to very large oligomers (Seeram *et al*., 2006). The polyphenolic composition of fruit 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.

In addition to the primary metabolites common to all plants analysis has led to distinguishing numerous secondary metabolites which belong to a variety of chemical groups. Im particular, a number of compounds, for example, cinnamic acid, elenolic acid, shikimic acid and quinic acid are treated as phenolics because of metabolic considerations although they lack a phenolic group or even an aromatic ring. The major classes of phenol in fruits are listed in Table 2.2.

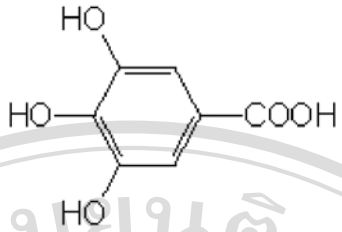
Table 2.2: The major classes of phenolics in longan fruits

Number of carbon atom	Basic skeleton	Class	Example	Fruit (example)
7	C_6-C_1	Hydroxybenzoic acid	*p*- Hydroxybenzoic	Strawberry
9	C6-C3	Hydroxycinnamic acid	Caffeic, ferilic acid	Apple
		Coumarins	Scopolin, aesculetin, umbelliferone	Citrus
10	C_6-C_4	Naphthoquinones	Juglone	Walnut
13	C_6-C_1-$_{C6}$	Xanthones	Mangiferin,mangostin	Mango
14	C_6-C_2-C_6	Stilbenes	Resveratrol	Grape
15	C_6-C_3-C_6	Flavonoids	Quercetin, cyaniding kaempferol	Cherry Apple
		Isoflavonoids	Daidzein	French bean
18	$(C_6$-$C_3)_2$	Lignins	Pinoresosinal	Stone fruits

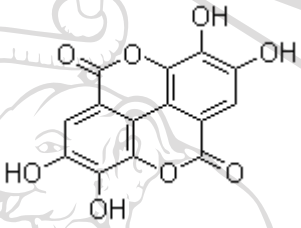
(Source: Macheix *et al.,* 1990; Robards *et al*., 1999; Antolovich *et al*., 2000)

Polyphenols have been demonstrated to act as antioxidants and are assumed to contribute to the beneficial health effects of fruits and vegetables (Tomas-Barberan and Robins, 1997). It is now well established that a diet high in fruit and vegetable is associated with a reduced risk of oxidative stress mediated diseases such as cancer, cardiovascular and neurodegenerative diseases (Halliwell, 1994).

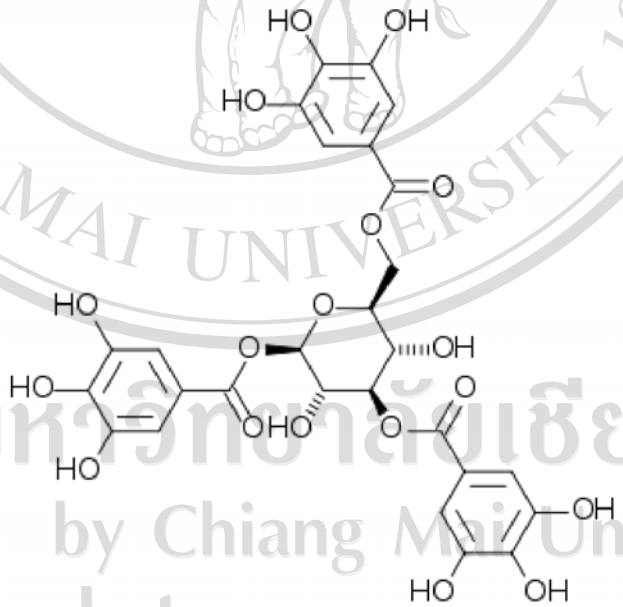
Phenolic compounds and polyphenol oxidase are, in general, directly responsible for enzymatic browning reaction in damage fruits during postharvest handling and processing. The relationship of the rate of browning to phenolic content and polyphenol oxidase activity has been reported for various fruits (Marshall *et al*., 2005). The browning has been attributed to polyphenol oxidase (PPO) which acts on the phenols. Phenolic compounds, such as chlorogenic acid, caffeoyl tartaric acid, catechol, gallic acid and catechin are good substrate for polyphenol oxidase. The phenolic content expressed as gallic acid in fresh tissue of cut jamaca increased after a week at 20 ^{0}C (Aquino-Bolanos and Mercado-Silva, 2004). Rangkadilok *et al*. (2005) reported that the major components were identified as gallic acid, corilagin (an ellagitannin) and ellagic acid. The content of these three compounds in different parts of the longan fruit and among different cultivars. The good substrates for polyphenol oxidase in longan cultivar 'Shixia' were pyrogallol, 4-methylcathecol and catechol (Jiang, 1999). Jaitrong *et al*. (2006) found that phenolic compouns in longan fruit peel cultivars 'Daw' and 'Beaw Kiew' were ellagic acid, quercetin and keamtferon. The quantitative of phenolic reduced during storage at low temperature. Zhang *et al*. (2000) reported that substrates of enzymatic oxidation, causing browning under storage were mainly flavan-3-ol-monomers, dimmers and cyaniding-3-glucoside in litchi peel. Longan pricarp contains higher amounts of phenolic compounds than the aril. Hsu and Chyn (1991) found that substrate for PPO in longan cultivar "Shixia" include pyrogallol, 4-methyl catechol and catachol. Phenolic compounds from fresh and dried longan pericarp, extract with 70% methanol, with compounds identified using HPLC-UV/VIS, were made up of gallic acid, corolagin (an ellagitannin) and ellagic acid (Figure 2.3).



Gallic acid ($C_{14}H_6O_8$, Molecular weight 302)



Ellagic acid ($C_{15}H_{10}O_7$, Molecular weight 302)



Corilagin ($C_{15}H_{10}O_6$, Molecular weight 286)

Figure 2.3: The structure of gallic acid, ellagic acid and corilagin

(Source: Rangkadilok *et al*., 2005)

Oxidative stress is believed to be an important contributing factor to the pathology of atherosclerosis, cancer and tissue damage in rheumatoid arthritis, as well as neurodegenerative diseases and the aging processes (Kelly, 1998). Phenolics have been reported to scavenge oxygen-derived free radical as well as to inhibit lipid hydroperoxide formation.

Fruit seeds are known to contain many phenolic compounds capable of protecting them from oxidative damage and defending them against yeast, fungi, virus and bacteria that might inhibit their germination. Soong and Barlow (2005) identified the phenolics of longan (*Dimocarpus longan* Lour.) seed by HPLC-ESI-MS). Gallic acid, ellagic acid, monogalloyl-glucose, monogalloyl-diglucose, digalloyl-diglucose, penta-to heptagalloyl-glucose, ellagic acid-pentose conjugate, galloyl-HHDP (Hexahydroxydiphenoyl)-glucopyranose, pentagalloy – HHDP - glucopyranose, procyanidin and quercetin-3-*o*-rhamnoside were found in longan seed.