CHAPTER 1

INTRODUCTION

1.1) STATEMENT AND SIGNIFICANCE OF THE PLOBLEM

The longan (*Euphoria longana* Lam.) is one of the most important commercial fruits of Thailand. Usually, the longan fruit is processed in the form of dried-longan pulp, as a high value-added product. However, the major problem in the dried-longan pulp products is a very short-shelf life due to the enzymatic browning and non-enzymatic browning reactions during processing and storage periods. The enzymatic browning reaction is an important problem in the dehydrated fruit and vegetable products (Shewfelt, 1987) because of undesirable colors, flavors and market values. This reaction is attributed to oxidation of phenolic compounds caused by polyphenol oxidase (PPO) and peroxidase (POD) producing brown pigments. Jiang and Li (2001) reported that PPO can catalyze the reaction in the presence of atmospheric oxygen. While POD can rapidly catalyze the enzymatic browning reaction in the presence of H_2O_2. Phenylalanine ammonia lyase (PAL) is involved to the biosynthesis pathway of phenolic compounds (Peng & Jiang, 2004) and also associated with enzymatic browning in fruit and vegetables. Currently, there are different agents to control the browning including PPO, POD and PAL inhibitors, such as acids, halides, phenolic acids and sulfites (that exclusion of oxygen inhibitors), ascorbic acid and cysteine (an chelating agent) (Pongsakul *et al.*, 2006). However, these chemical treatment especially

bisulphites are dangerous for human health because of acute allergic reaction (Saper, 1993). Thus, alternative treatments without toxic effects are needed.

One of the alternative treatments to inactivate enzymes is thermal treatment (Chutintrasri & Noomhorm, 2006; Peng & Jiang, 2004; Abreu *et al.,* 1999) especially the microwave pretreatment. Many reports (Rodriguez-López *et al.*, 1999; Ancos *et al.*, 1999; Brewer & Begum, 2003) referred that the microwave pretreatment could inactivate enzymes such as PPO, POD and catalase that, therefore, can improve product flavor, color, texture and nutritional values. Moreover, microwave energy can reduce drying time and save energy when compared with conventional heating (Pereira *et al.*, 2007). Besides, there are other factors affecting browning in dehydrated products during storage such as water activity and moisture content, packaging materials and storage conditions. However, there is no available information concerning the microwave pretreatment on PPO, POD and PAL inactivation and studies on subsequent storage of dried longan pulp. Thus, the application of microwave pretreatment is required to inactivate PPO, POD and PAL activities of dried-longan pulp results in improved color, texture and nutritional value and extended shelf- life leading to the high-quality and desirable quality of dried products.

1.2) BOTANICAL DESCRIPTION OF LONGAN FRUIT

1.2.1) Scientific classification (Rangkadilok *et al.,* 2005; Wichaipanich & Ramingwang, 2004)

Figure 1.1 Longan fruits

Euphoria langana Lam. (longan) is a member of Sapindaceae family that also includes litchi (*Litchi chinensis*), rambutan (*Nephilium lappaceum* L.), and horse chestnut (*Aesculus hippocastanum* L.). However, other scientific names given to longan include *Dimocarpus longan* Lour. and *Nephilium longana* (Lam.) Cam.

Longan is an evergreen tree, growing to 20 m and possesses a spreading or erect habit, brittle trunk, rough bark. The compound leaves are alternate and paeipinnate with 6-9 pairs of leaflets. The flowers of longan are made up of staminate, pistillate and hermaphroditic. Fruits are round, 1-2.5 cm. in diameter with light brown peel. The black seed is surrounded by translucent whitish pulp with juicy texture and sweet and aromatic flavor. It grows and crops in China, South East Asia including Thailand, Vietnam, Philippines, Florida and Hawaii in the USA and Australia.

Longan fruit is consumed in fresh, dried, frozen and canned forms especially the dried longan which is used to prepare a refreshing drink, liqueur and an ingredient of herbal medicine.

1.2.2) Reviews of pharmacological activities

The flesh of the longan has been used in Chinese medicine as a stomachic, febrifuge, vermifuge and an antidote for poison (Mortin, 1987). The dried longan is used as a tonic and for treatment of insomnia and neurasthemic neurosis and methanolic extract of longan that has been shown in anxiolytic-like effect.

The corilagin that is extracted from seed of longan fruit has been shown to the lowmblood pressure of spontaneously hypertensive rats through blocking nordrenaline release and (or) by direct vasorelaxation (Cheng *et al.,* 1995). Moreover, corilagin has been shown to have the antifungal activity especially the *Candida glabrata* strains (Latte et al., 2000), inhibit HIV-1 replication in HeLa $CD4^+$ cells and tumor necrosis factor-α (TNF-α) release (Okaba *et al*., 2001).

1.2.3) Cultivars of longan fruits

There are numerous longan cultivars in Southeast Asia, under the family Sapindaceae and genus *Euphoria* which has been reported to contain 7 species. In Thailand, the most popular longan cultivar is 'Daw' covering about 73% of total planting longan in the country. It is an early-maturing cultivar that is harvested in Thailand in late June to late August, whereas harvesting period in China is from late July to late September. Moreover, it has been reported that the cultivar Daw and Biewkiew contained the highest levels of gallic and ellagic acid (Rangadilok *et al.,* 2005). This cultivar has large fruits and it also has a big seeds. The aril is rather dense and not crispy. The fruits can be consumed in fresh or processed that is highly esteemed in dried food processing in Thailand. Daw is normally grown in the northern provinces of Thailand because the cool winter months induce flowering of longan trees.

'Chompoo' is a well known cultivar among Thai people because it is very sweet (total soluble solid of 21-22 percent) and has a pleasant aroma (Wong, 2000). The fruit is of medium size, oval shape and greenish light-brown peel. It has small seed, the aril is slightly pink and thus the name 'Chompoo' which means pink color in Thai. However, this cultivar is not used in dried food processing since it has brown darker than other cultivars. It is mid-maturing cultivar. Flowering period of Chompoo cultivar is during December to early January and then the fruits can be harvested from middle July to early August.

'Biewkiew' is another well known cultivar. It is late-maturing cultivar which flowers in late January and its fruits are harvested in late August to September. The fruit has round shape, large size and brownish green peel. The aril is crispy, with pleasant aroma and sweet. The peel is rather thick. This cultivar has irregular bearing and susceptible to witches' broom disorder. Generally, it is grown in the northern provinces of Thailand.

'Haew' is a late bearing cultivar which flowers in late of January to early February. The harvest season is in mid to late August. The botanical characteristic of this cultivar is large size, small seed and has an average recovery percentage. The aril is firm and good eating quality and, therefore, suitable for canning. The fruit peel is rather rough and thick, advantageous for longer shelf life.

'Dang' has reddish-brown peel cultivar and thus the name 'Dang' meaning red (in Thai). The mature fruit is large and also has large seed leading to rather poor recovery percentage. This is a mid-bearing cultivar which is harvested in mid July to early August. The trees are susceptible to waterlogging.

'Baidum' is classified as mid-maturing cultivar which is harvested in mid-July to early August. The leaves are small and dark green color. The fruit has medium size with rough peel, small seed and an average aril recovery percentage. The aril is of acceptable flavor, crispy and very sweet and bright white color.

'Talub Nak' is early-maturing cultivar which is harvested in mid to late July. This fruit has medium size, small seed and high aril recovery percentage. The aril is bright white-color but it is less sweet when compared to other cultivars.

'Phetsakon' cultivar is different from all the other cultivars. It is lowland cultivar which does not require a cool climate for induction of flowering. Phetsakon is an early maturing cultivar. It is grown in the central region of the country in Samutsakon and Ratchaburi provinces.

1.3) DREID LONGAN PRODUCTION

Thailand is the biggest longan exporter and longan processed product in the world. Countries which import the longan fruit from Thailand are China, Hong Kong, Indonesia, Singapore, Malaysia, Canada, United Kingdom and France. China is the major importer of dried longan from Thailand. Table 1.1 showed the amount and value of exported dried longan of Thailand from 2005 to 2007. There has been an increasing trend in the export of dried longan of Thailand in each year.

Table 1.1 Amount and value of dried longan exported of Thailand in 2005-2007

month	2005		2006		2007	
	Amount (Metric ton)	Value (Million Baht)	Amount (Metric ton)	Value (Million Baht)	Amount (Metric ton)	Value (Million Baht)
Jan	3,859	83.59	983	33.23	1,701	34.46
Feb	751	23.22	618	22.49	623	14.94
Mar	1,417	63.19	4,639	94.83	801	12.64
Apr	1,106	40.06	2,955	60.82	1,041	23.20
May	1,297	39.38	7,736	160.10	1,300	21.57
Jun	260	10.83	6,225	145.87	187	5.38
Jul	7,196	184.30	4,395	90.00	4,261	69.49
Aug	29,137	779.36	21,195	403.15	28,221	458.66
Sep	13,034	294.06	15,735	280.95	17,039	313.84
Oct	12,640	250.46	6,162	146.83	19,206	349.93
Nov	15,003	301.40	4,030	92.93	20,000	394.53
Dec	8,388	208.06	3,692	73.62	18,404	319.38
Total	94,088	2,277.91	78,365	1,604.82	112,784	2,018.02

Source: Official of Agricultural Economics, Thailand (http://www.oae.go.th)

The longan fruit has high sugar content causeing the short shelf life under ambient temperature as observed by skin color loss (browning) during storage and transportation (Prapaipong and Rakariyatham, 1990). Thus, their usable period is extended by processing them into various products. In Thailand, longan fruits have been processed into canned longan, frozen longan and dried longan products. The drying processing is the preferred preservation method. Varith *et al.* (2007) has described detailed processing of longan into dried fruits in Thailand. Hot air drying is common method for the industry to dry unpeeled and peeled longan. The processing of unpeeled longan can be done by drying at 75°C for 48-52 hour or longer. While the peeled longan requires

about 12-15 hour at 70°C, the moisture content of dried longan is decreased to 18-19 percent. The schematic diagram (Figure 1.2) showed the method of dried longan production.

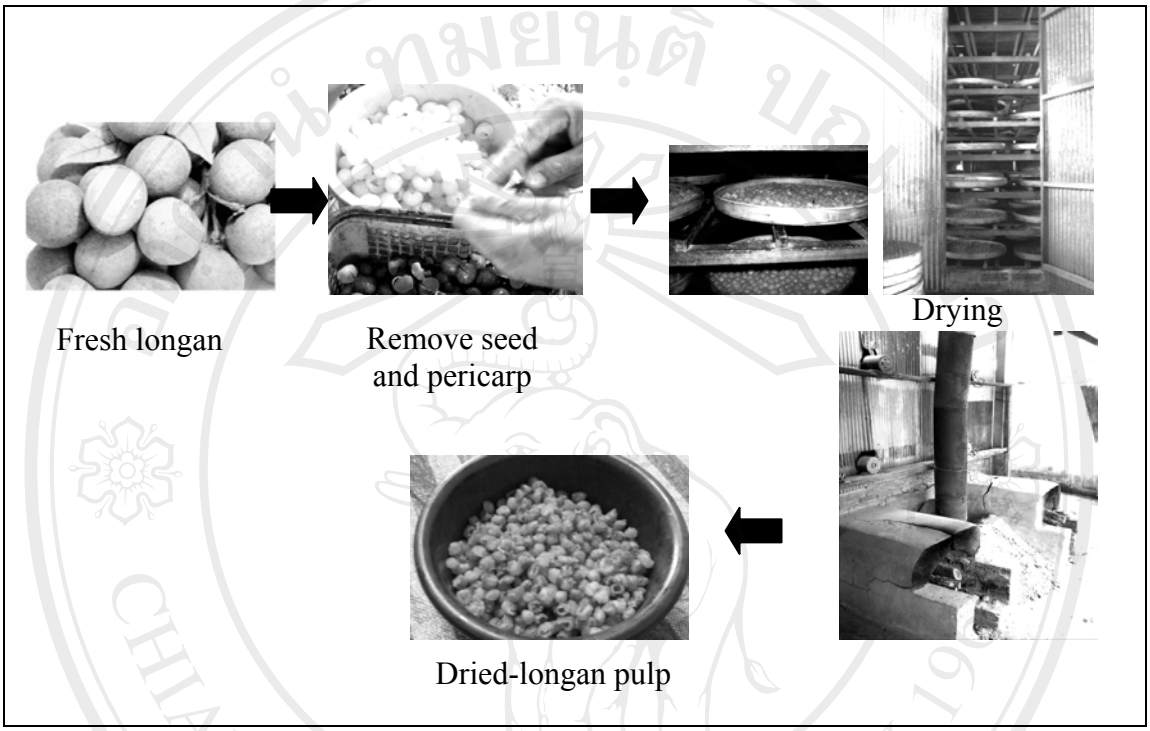

Figure 1.2 Schematic diagram of method dried-longan pulp production in Thailand.

However, browning of dried longan pulp is a serious problem for food industry. The dried longan turns to brown color in short time of storage. It causes from the enzymatic browning and non-enzymatic browning reaction.

1.4) BROWNING REACTION

Browning of food results from enzymatic and non-enzymatic browning reaction during processing and storage, especially during processing of meat, sea foods, fruit and vegetable products, leading to decreased sensory properties of those products associated with color, flavor and softening besides nutritional properties (Eskin, 1990; Martínez and Whitaker, 1995). In tropical and subtropical countries, enzymatic

browning results in over 50 percent losses in fruit and vegetable (Whitaker and Lee, 1995). Such lettuce, potatoes and other starchy staple such as sweet potato, bread fruit, yam, mushrooms, apples, avocados, bananas, grapes, peaches and a variety of other tropical and subtropical fruits and vegetables are susceptible to browning and therefore cause to economic losses for the agriculturist. Therefore, the knowledge in browning mechanism is necessary to understand in controlling the quality of dried food products.

1.4.1) ENZYMATIC BROWNING REACTION

Enzymatic browning is one of the most important problems of fruits such as apricot, apple, pears, peaches, bananas and grapes, vegetables such as potato, mushroom and lettuce, and sea food such as shrimp, lobsters and crabs. This discoloration decreases the shelf life of minimally processed and is also a problem in the production of dehydrated and frozen fruit and vegetables products (Shewfelt, 1987).

Enzymatic browning is attributed to oxidation of phenolics caused by polyphenol oxidase (PPO), peroxidase (POD), producing brown-colored by-products (Saltveit, 1997) and phenylalanine ammonia lyase (PAL) which is the first enzyme in the biosynthesis of phenolics that also associated with browning in fruits and vegetables.

(1) Polyphenol oxidase (PPO)

This enzyme causes the discoloration in fruits and vegetables that found in both plants and animals. The phenolic compounds in fruits and vegetables were oxidized in the presence of atmospheric oxygen and are catalyzed by PPO. The monophenols are hydroxylated to *o* – diphenols and then are oxidized to *o* – quinones.

The quinones condense to produce the complex brown polymers (Saper and Hicks, 1989; Saper, 1993) as shown in Figure 1.3.

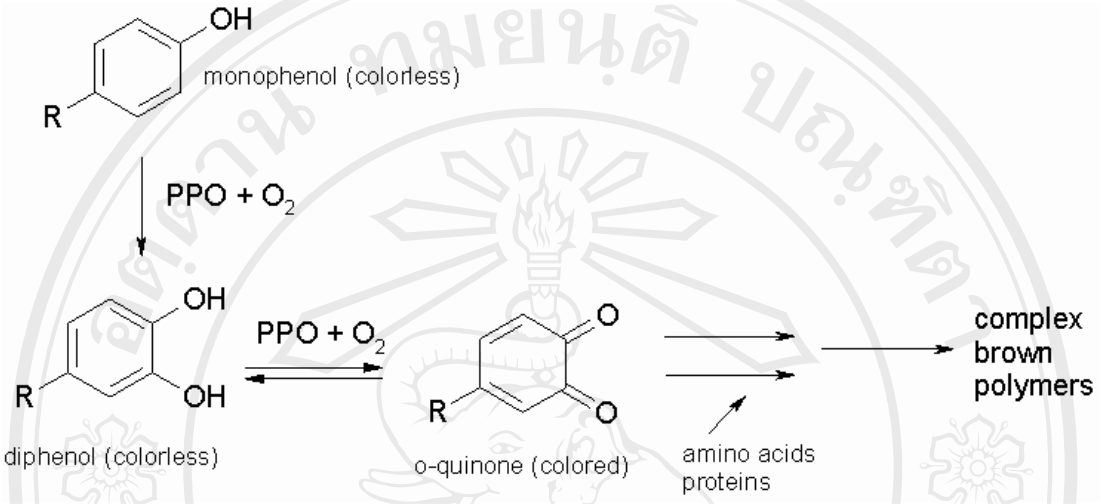

Figure 1.3 The mechanism of enzymatic browning catalyzed by polyphenol oxidase

Polyphenol oxidase is classified as an oxidoreductase that oxygen act as the hydrogen accepter. This enzyme is widely distributed in the plant, higher animal and human. The localization of polyphenol oxidase is found in the plastid in plants. However, it is also in free from the cytoplasm in degenerating or sensescent tissues such as ripe or ripening fruit. The polyphenol oxidase has the copper as prothestic group and can be divided into two entries in the International Union of Biochemistry (IUB) as EC 1.14.18.1, monophenol monooxygenase, and EC 1.10.3.1, catechol oxidase. Common names for monophenol monooxygenase are tyrosine, phenolase, monophenol oxidase and cresolase. Figure 1.4 showed the oxidation of tyrosine and catechol that are catalyzed by this enzyme.

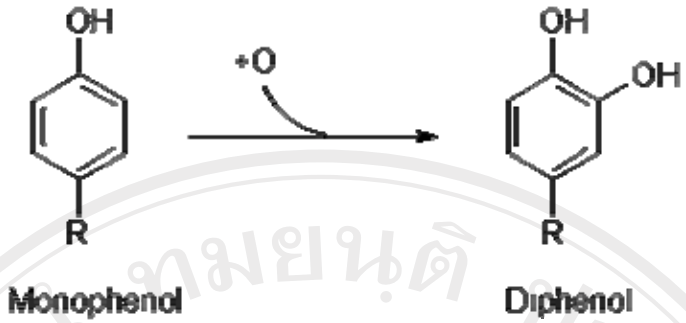

Figure 1.4 Monophenol oxidase pathway producing the diphenol

While the common names for catechol oxidase are diphenol oxidase, *o*-diphenolase, phenolase and polyphenol oxidase. The enzyme catalyzes the conversion of *o*-diphenols to *o*-quinones (Figure 1.5).

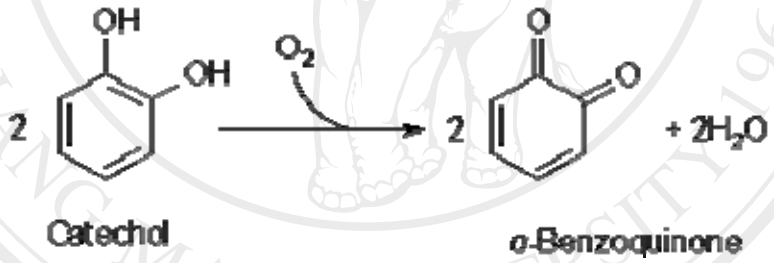

Figure 1.5 Diphenol oxidase pathway producing the *o*-quinones

The polyphenol oxidases catalyze the oxidation of monohydroxy phenol to *o*-dihydoxy phenols and then catechol oxidases catalyze the dehydrogenation of o-dihydroxy phenols to o-quinonees, which lead to brown pigment polymers, melanoidins (Figure 1.6).

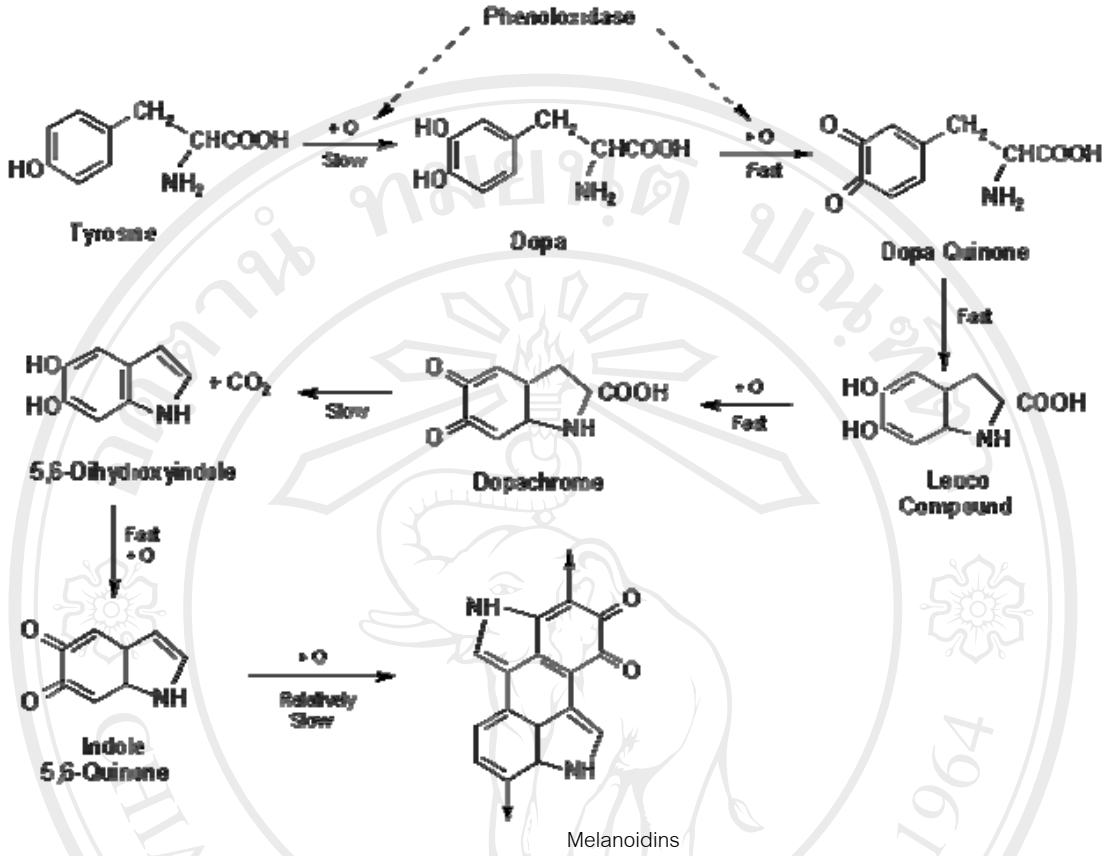

Figure 1.6 The mechanism of enzymatic browning reaction

The substrates of the polyphenol oxidase enzymes are phenolic compounds. The phenolic compounds are considered to be secondary matabolites that are found in plants and higher animals in the vacuole of cells. Structurally, they contain an aromatic ring bearing one or more hydroxyl group, together with a number of other substituents. The polyphenolic composition of fruits varies in accordance with species, cultivar, degrees of ripening and environmental conditions of growth and storage. However, there are few of phenolic compounds in fruits and vegetables served as substrate for polyphenol oxidase such as catechins, tyrosine, 3,4-dihydroxy phenylalanine (DOPA)

and cinnamic acid and chlorogenic acid (Figure 1.7). The substrate specificity of polyphenol oxidase varies in accordance with the source of the enzyme. Such the longan PPO has the greatest activity was detected towards pyrogallol followed by 4-methyl-catechol and catechol, respectively (Jiang, 1999).

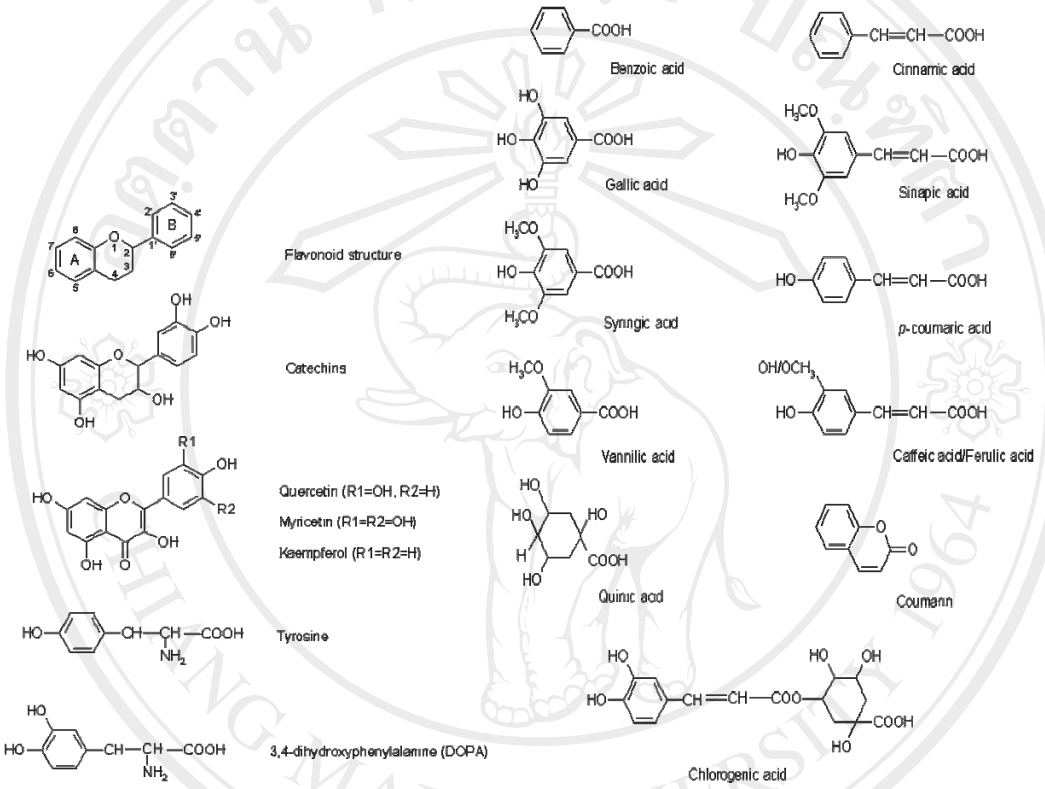

Figure 1.7 Structures of common phenolic compounds

Characterization of the enzyme thermostability of polyphenol oxidase (PPO) has been reported. In general, temperature at 70-90°C is mostly adequacy for destruction of the enzyme. Weemaes *et al.* (1998) has been determined the thermal inactivation kinetics of PPO from apples, avocados grapes plums and peachs in juices. It found that in the case of apple, pear, avocado and plum PPO inactivation at about 60-65°C, plum and peach PPO were inactivated about 75and 70°C, respectively.

Peng and jiang (2004) reported that the polyphenol oxidase (PPO), peroxidase (POD) and phenylalanine ammonia lyase (PAL) activities in slices of

Chinese water chestnut significantly decreased, when the products were immersed in boiling water for 30 s.

Moreover, it has been studied the effect of thermal treatment on the inactivation of polyphenol oxidase in pineapple puree. It was found that PPO activity was decreased rapidly at 75°C and then residual activity was only about 7% after 5 min at 85 °C and 1.2% after 5 min at 90°C.

(2) Peroxidase (POD)

Peroxidases (POD) are distributed ubiquitously in both the plant and animal kingdoms. The peroxidases can be classified into the 2 groups, iron-containing peroxidases (ferriproporphyrin peroxidase) and flavoprotein peroxidases (verdoperoxidases). The first group contains ferriprotoprophyrin ш (protohemin) as the prosthetic group which is found in common plant, and the enzyme is brown when highly purified. The second groups include the peroxidases of animal tissue and milk. The prosthetic group of this enzyme is an iron porphyrin. When highly purified, this enzyme is green color (Hammer, 1993). All of peroxidases act on hydrogen peroxide as an electron acceptor and oxidize a multitude of donor compounds, many times to brown colored end product as shown in Figure 1.8.

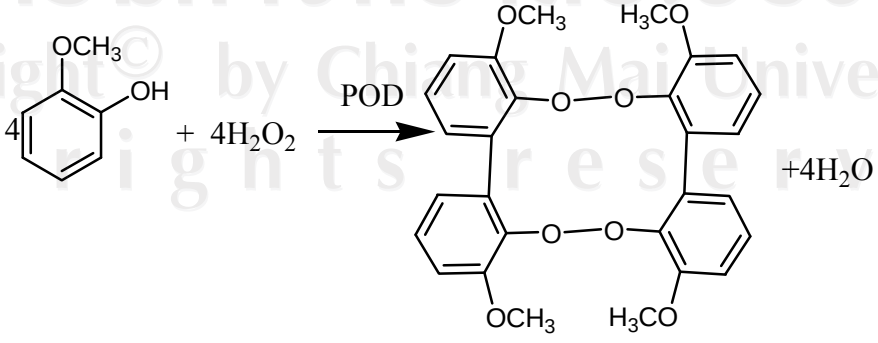

Figure1.8 The mechanism of enzymatic browning catalyzed by peroxidase

Peroxidases, one of the most heat stable enzymes that are often used as an indicator for adequacy of blanch (Begum and Brewer, 2000). However, it has been found that POD in food causes to the formation of undesirable end product such as off-flavor, aroma and color as well as loss of some nutrients. Therefore, the inactivation of peroxidase is required. Many methods to inactivate peroxidases in various food products have been studied. Heating, however, is still the most widely used to inactivate peroxidasese especially blanching. Previous paper reported that blanching of vegetables prior to freezing effectively inactivated the peroxidase because it maintained the texture, color, flavor and nutritional quality of product (Williams *et al.* , 1986).

(3) Phenylalanine ammonia lyase (PAL)

Phenylalanine ammonia lyase is the first enzyme involved in the phenylpropanoid pathway that is synthesis of phenolic compounds. PAL catalyzes the first committed step in the conversion of the amino acid L-phenylalanine to trans-cinnamic acid. The phenolic compounds, among which 5-caffeoylquinic acid (chlorogenic acid), 3,5-dicaffeoylquinic acid, caffeoyltartaric acid and dicaffeoyltartaric acid were produced in subsequent reactions that these compounds have been reported, associated with increased browning (Tomás-Barberán *et al*., 1997a,b). While the induction of PAL activity has been reported to respond to wounding (Seltveit, 2000), UV light (Droby *et al.,* 1993), gamma irradiation (Riov *et al.,* 1968), heat treatment (Martínez-Téllez and Lafuente, 1997) and ethylene (Riov et al., 1969). Previous study, reported that wounding (cutting, cracking or breaking) of lettuce generated a signal that migrated through the tissue and induced the synthesis of PAL in the metabolic pathway

which was responsible to increase the phenolic compounds and browning (Figure1.9) (Ke and Salveit, 1989; Lopez-Galvez *et al.,* 1996b; Peiser *et al.,* 1998).

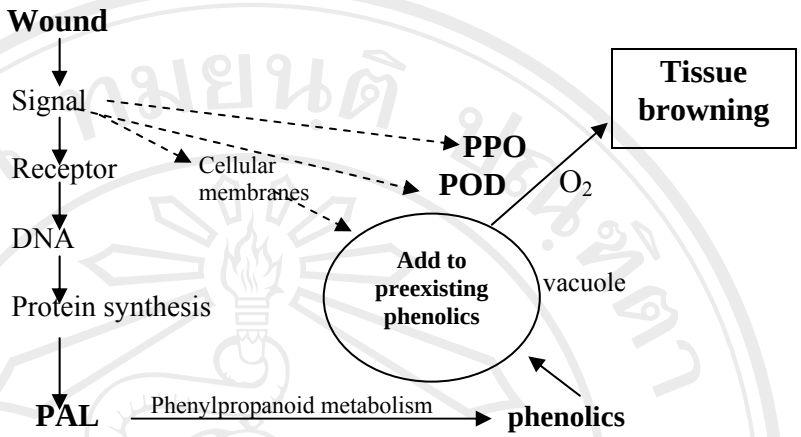

Figure 1.9 The relationship between wounding and PAL activity leads to tissue browning

1.4.2) NON-ENZYMATIC BROWNING REACTION

These browning reactions do not require enzymatic catalysis and are referred to as non-enzymatic occuring during processing as well as during storage of food products. There are evidences obtained 3 main reaction pathways: (1) Maillard reaction; (2) caramelization; (3) oxidation of ascorbic acid.

(1) Maillard reaction

The Maillard reaction can be defined as the reaction of an amino group of amino acids, peptides or proteins with a glycosidic hydroxyl group of sugars, aldehydes and ketones leading to the formation of brown nitrogenous polymers or melanoidins (Deman, 1990). The Maillard reaction occurs to be the main cause of browning developing during the heating or prolonged storage of foods. Moreover, Maillard reaction can occur in both acidic or alkaline media, although it is favored

under the more alkaline conditions and processed readily in aqueous solution. The mechanism of these reactions is shown in Figure 1.10. There are 5 steps involved in the process.

Step 1 the production of N-substituted glycosylamine from an aldose and ketose reacting with a primary amino group of an amino acid, peptide and protein.

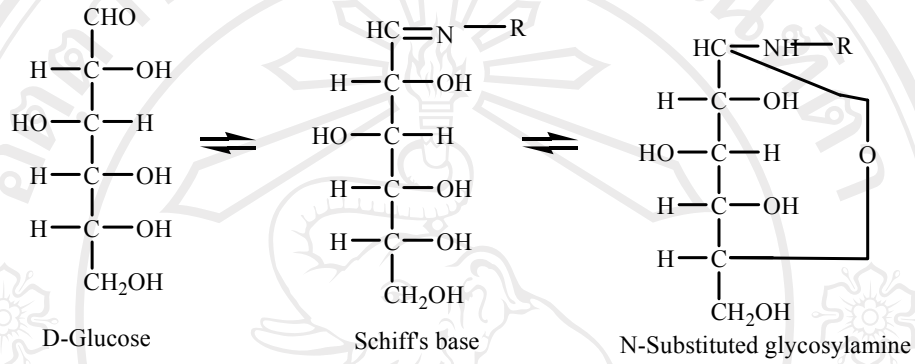

Figure 1.10 The production of N-substituted glycosylamine

Step 2 rearrangement of the glycosylamine by an Amadori rearrangement type of reaction to yield an aldoseamine or ketoseamine.

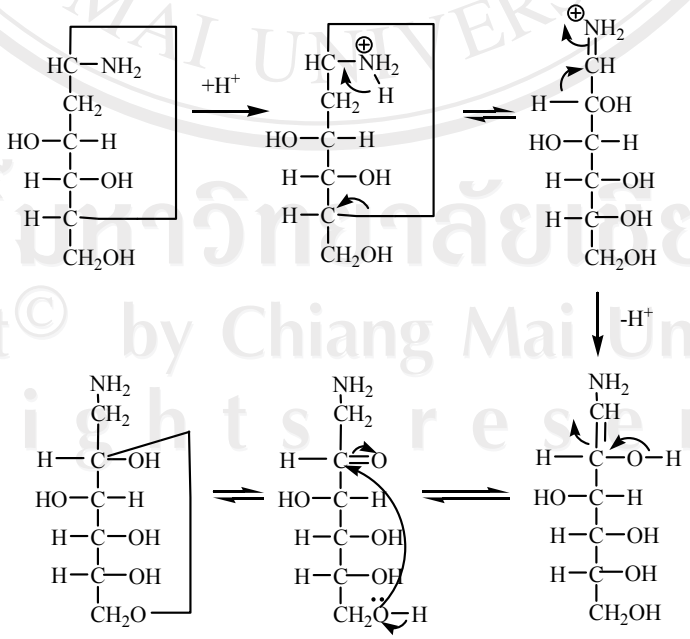

Figure 1.11 Amadori rearrangement

Step 3 a second rearrangement of the ketoseamine with a second mole of aldose to form a diketoseamine, or the reaction of an aldoseamine with a second mole of ascorbic acid to yield a diamino sugar.

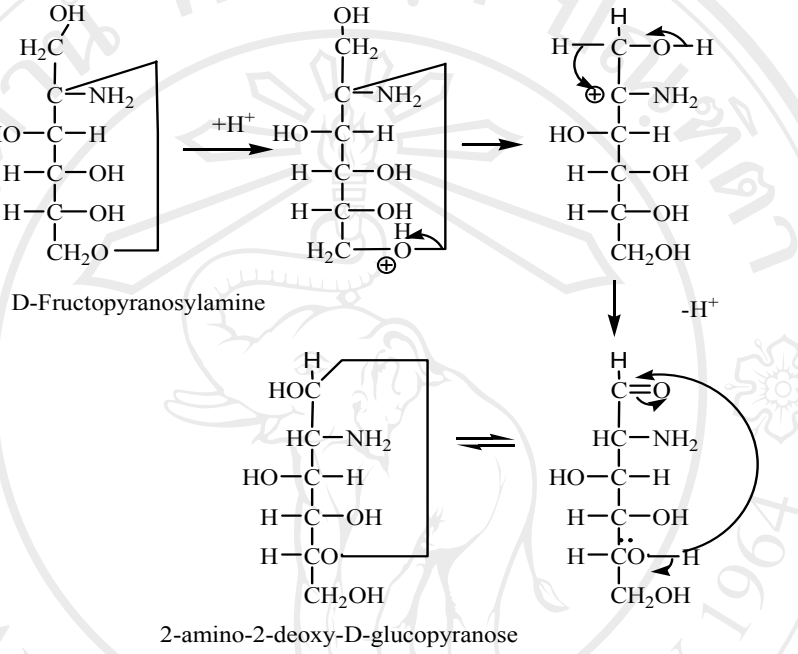

Figure 1.12 Second rearrangement

Step 4 the degradation of the amino sugars with loss of one or more molecules of water to give amino or nonamino compounds.

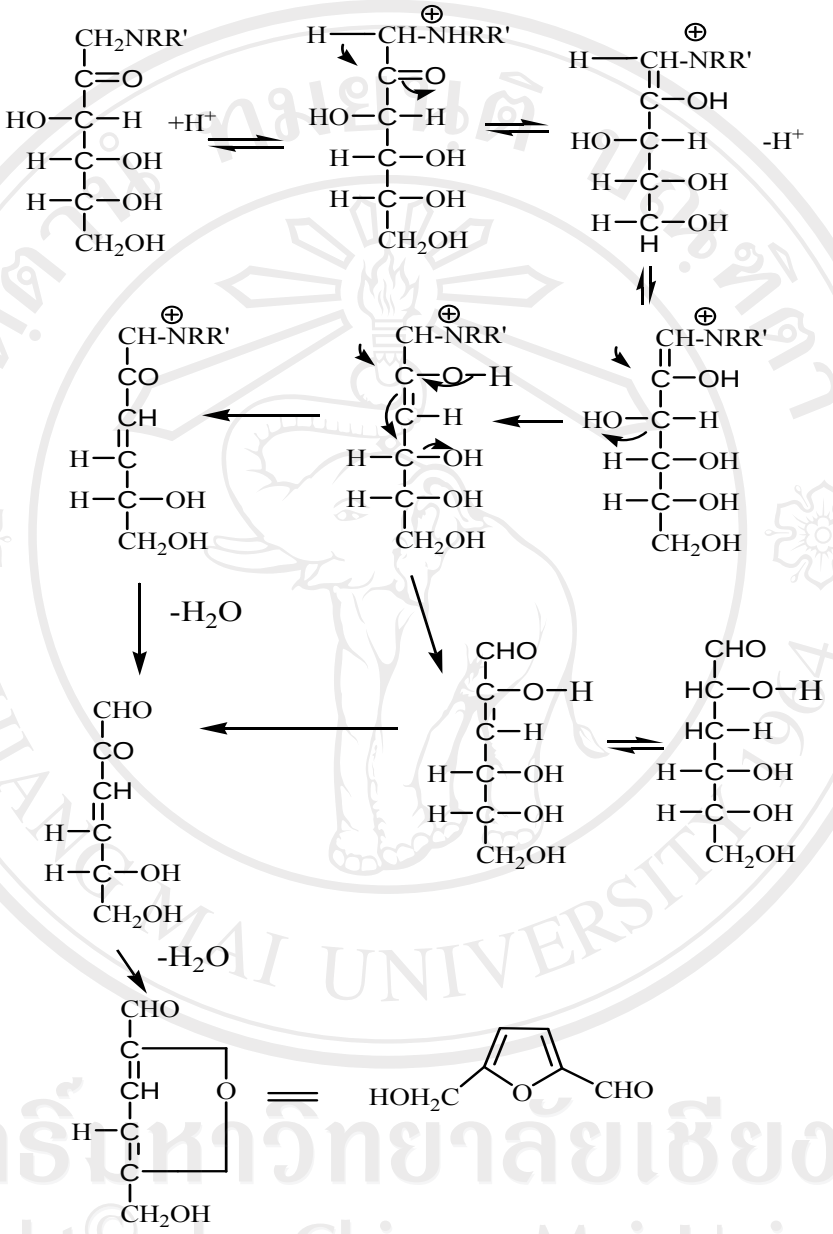

Figure1.13 1, 2-Enolization mechanism of browning reaction

Step 5 the condensation of the compounds formed in step 4 with each other or with amino compounds to form brown pigments and polymers (meaning melanoidins).

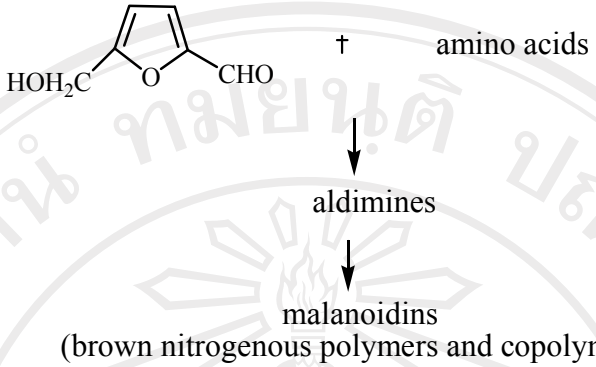

Figure 1.14 The formation of brown pigment of Maillard reaction

(2) Caramelization

This reaction is another non-enzymatic browning involving in the sugar degradation in the absence of amino acid or protein when sugars are heated above their melting points they darken to a brown coloration. This reaction proceeds under acidic or alkaline conditions. This process cause changes in product quality such as burned and bitter products and flavor. A common pathway is shown in Figure 1.15.

This pathway showed the dehydration of 1,2-enol to 5-(hydroxymethyl)-2-furaldehyde, if the initial sugar was a pentose then the final product would be a 2-furaldehyde. The formation of the furfurals leads to the production of the colored products that is a complicated series of polymerization reactions.

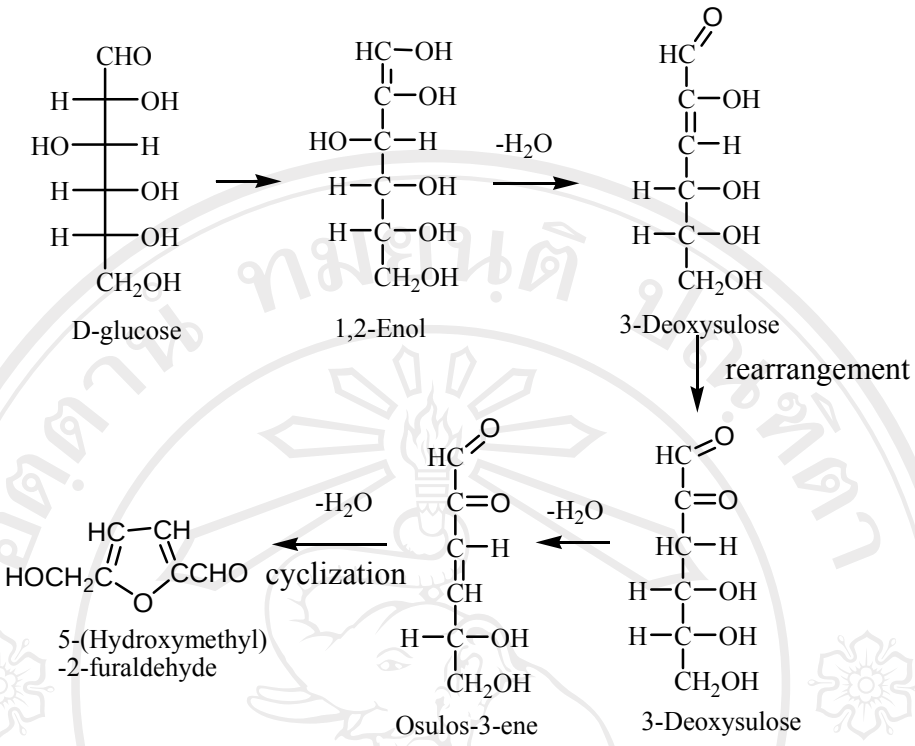

Figure 1.15 The pathway of caramelization

(3) Oxidation of ascorbic acid

The mechanism of ascorbic acid oxidation is complex; a possible pathway from decomposition of ascorbic acid to furfural accompanied by liberation of carbon dioxide is shown in Figure 1.16. The reactions of ascorbic acid are dependent on pH, concentration and temperature. The further mechanism of furfural leads to the discoloration of foods.

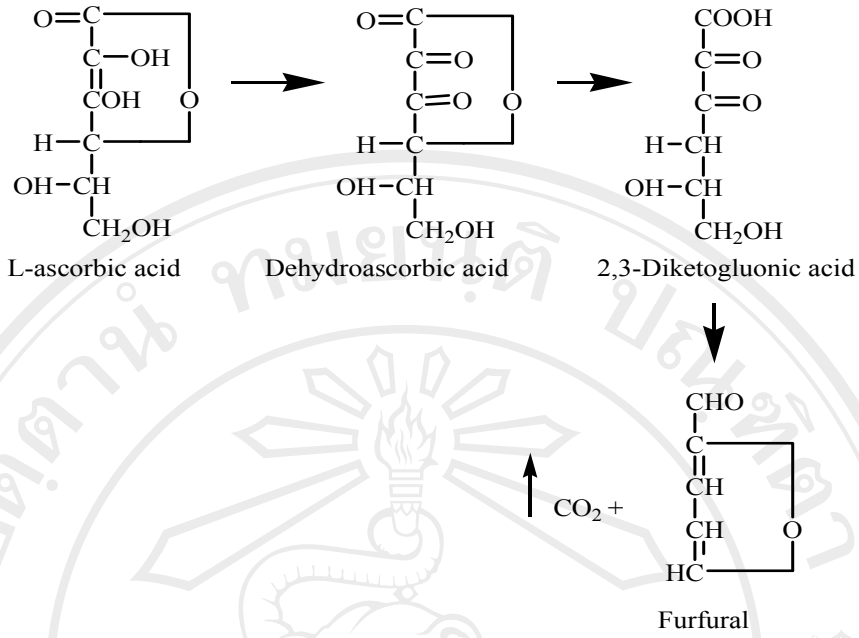

Figure 1.16 The pathway of oxidation of ascorbic acid

1.5) CONTROLLING OF BROWNING REACTION

Many techniques are applied to prevent enzymatic browning in fruits and vegetables as reviewed below;

1) Heating

Heat treatment is the most widely utilized method for foods industry because it will inactivate enzymes and destroy microorganism. Heat is applied commonly in process such as blanching and high temperature – short time (HTST) pasteurization, used in the pretreatment of vegetables and fruits for canning, freezing preservation and dehydration. Thermal inactivation for important enzymes such as polyphenol oxidase, peroxidase and lipoxygenase in fruit and vegetable processing shows deterioration during processing and storage time.

Steam blanching is a preferred method for the prevention of browning in foods. Temperatures applied in steam blanching treatment vary in accordance with

thermostablity of the enzyme to be inactivated as well as with the nature of the food product. Blanching techniques are often operated at temperature ranging between 70°C and 105°C or higher. In general, exposure of polyphenol oxidase to temperature of 70-90°C, results in the destruction of their catalytic polyphenol oxidase and peroxidase activities in fruit and vegetable (Vámos-VigyázÓ, 1981). Previous paper has been reported that blanching of green beans at 98°C for 30s almost completely inactivated peroxides (Katsaboxakis and Papanicolaou, 1984). Several problems may arise through the application of heat. The fruit and vegetable become cooked, and, in turn, leads to unfavorable texture, color and flavor changes. Thus, alternative thermal treatments are required to inactivate enzyme in food processing.

Microwave heating has been used to prevent browning and improve quality in some commodities. It has been applied successfully in the enzymatic inactivation in whole tomato fruits and soybeans (Porreta and Leonic, 1989; Klinger and Decker, 1989). Guenes and Bayin (1993) studied effect of water- and microwave-blanching method on activities of peroxidase and lipoxygenases in green beans, peas and carrots. They concluded that the application of microwave treatment could inactivate the enzymes resulting in improved product flavor, color, texture and nutritional value.

Cano *et al.* (1990) who studied the effect of steam and microwave blanching of bananas on polyphenol oxidase and peroxidase enzymes found that the optimal quality in frozen bananas was obtained with microwave pretreatment.

Brewer and Begum (2003) have been reported the inactivation of peroxidase in a variety of vegetables. It found that microwave treatment at powers of 70% and 100% for 1 min had the greatest effect on peroxidase inactivation.

Ancose *et al.* (1999) who applied the microwave to inactivate the peroxidase and polyphenol oxidase in kiwi, strawberry and papaya purees fruits products reported that microwave heating could be an effective treatment to inactivate PPO and POD in processed fruit products prior to storage.

Application of microwave energy in the enzyme inactivation of tropical fruit pulps such as guava, papaya and mango fruits were reported by Abd-El-Al MG *et al.* (1994). They found that the exposure times of enzyme inactivation in pulps varied from 20 to 80s depending on enzyme and pulp types.

The advantages of microwave heating are also reduced processing time, cost and energy (Anatasis, Katerina and Michael, 1999).

2) Refrigeration

The rate of enzyme-catalysed reactions is controlled to a great extent by temperature. For every 10 ^{o}C temperature increasing (in biological important ranges), there is a two fold increase in the rate of enzyme catalytic reaction. At low temperatures, reduced kinetic energy of the reactant molecules results in a decrease in both mobility and "effective collisions" which are necessary for the formation of enzyme-substrate complexes and their products. Cold preservation and storage during distribution and retailing are necessary for the prevention of browning in fruit, vegetables, and seafood, since refrigerated temperatures are effective in lowering polyphenol oxidase activity.

3) High pressure treatment

Pressure can influence biochemical reactions by reducing molecular spacing and increasing interchain reactions. High-pressure treatment is a potentially viable technique for preserving food quality through the inactivation of endogenous food enzymes. Pressures exceeding 5 kbar generally cause irreversible denaturation of enzymes due to the weakening of hydrophobic interactions and the breaking of intramolecular salt bridges (Cheftel 1992). High-pressure treatments can result in either reversible or irreversible changes in protein structures. Loss of catalytic activity under high-pressure conditions, however, varies in accordance with the enzymes, the nature of the substrates, the temperature and the duration of high pressure processing (Cheftel, 1992).

Polyphenol oxidase is highly pressure resistant. Pressures of 5 and 7 kbar are required for the inactivation of apple polyphenol oxidase at pH 4.5 and 5.4, respectively. Weemaes *et al*. (1998) showed that inactivation of polyphenol oxidase from apple, grape, avocado and pear at room temperature (25 ^{o}C) became noticeable at 600, 700, 800 and 900 Mpa, respectively.

4) Elimination of oxygen during storage

This technique attempts to eliminate the oxygen, the use of oxygen impermeable and degradable films may be useful in preventing the onset of browning (Martinez and Whitaker, 1995). Vacuum packaging of pre-peeled potatoes to exclude oxygen, was observed to extend their shelf life (Langdon, 1987). Modified atmosphere packaging reduces oxygen concentration in the atmosphere surrounding a product. Packaging headspace of nitrogen reduces browning rate in sulfured dried peaches (Bolin et al., 1976; Bolin and steele, 1987). Kleinhenz *et al*. (2000) studied the postharvest

deterioration of bamboo shoot during storage at ambient temperatures, which easily leads to browning and lignification. The studies showed that the shelf life of bamboo shoots limited to one day at ambient temperature (20-25°C), while the combination of low temperature and package with low-density polyethylene (LDPE) bags could be extended the shelf life to 28 days. Shen *et al.* (2006) also supported that the bamboo shoots were packed in LDPE with low oxygen concentration which could prevent the peroxidase and phenylalanine ammonia lyase activities and maintain the bamboo shoots unbrowned. Besides, previous studies have been reported that the dehydrated cauliflower was packed in laminated aluminium foil for 6 months at room temperature which could decrease the loss of ascorbic acid and browning better than other treatments (Kadam *et al*., 2006).

5) Inhibitors

The application of browning inhibitors in food processing is restricted to relevant consider action of toxicity, wholesomeness, and effect on taste, flavour, texture, and cost. There are 6 classified categories of polyphenol oxidase inhibitors applicable in the prevention of enzymatic browning. These include reducing agents, acidulants, chelating agents, complexing agents, enzyme inhibitors and enzyme treatments.

5.1) Reducing agents

Reducing agents play a role in the prevention of enzymatic browning either by reducing *o*-quinones to colorless diphenols, or by reacting irreversibly with *o*-quinones to form stable colorless products. Reducing compounds are very effective in

the control of browning. Sulphiting agents are the most widely applied reagents for the control of browning in the food industry. However, Sulphiting agents have severe effect to asthmatic patients bcause of acute allergic reaction (Sapers, 1993). The United States Food and Drug Administration (FDA) 1995 regulations prohibited the use of sulphites in salad bars. As a result, there has been a considerable focus on identifying appropriate sulphite substitutes for use in foods. The FDA has proposed maximum residual sulphur dioxide levels allowed in certain foods. In accordance with these proposed limits, residual sulphur dioxide levels for fruit juices, dehydrated potatoes, and dried fruit, are 300, 500, and 2000 ppm respectively (Martinez and Whitaker, 1995). Other reducing agents are inhibitors such as ascorbic acid and analogs, cysteine, glutathione, erythorbic acid and phenolic antioxidants.

5.2) Acidulants

Acidulants are generally applied in order to maintain the pH well below that required for optimum catalytic activity of an enzyme and also decreased the rate of enzymatic browning. Acidulants such as citric, malic, and phosphoric acids are capable of lowering the pH of a system, thus rendering polyphenol oxidase polyphenol oxidase inactive.

5.3) Chelating agents

Enzymes generally possess metal ions at their active sites. Removal of these ions by chelating agents can therefore render inactive enzyme. Chelators used in the food industry include sorbic acid, polycarboxylic acids (citric, malic, tartaric, oxalic,

and succinic acids), polyphosphates (ATP and pyrophosphates), macromolecules (porphyrins, proteins), and EDTA.

5.4) Complexing agents

It has been reported that cyclodextrin inhibited the enzymatic browning in apple products (Billaud *et al*. 1995). Chitosan is another complexing agent that had potential inhibitory activity on polyphenol oxidase of longan fruit (Jiang and Li, 2001).

5.5) Enzyme inhibitors

Inhibitors of enzymatic browning are effective such as 4-hexylresorcinols, halide salts, honey, aromatic carboxylic acid and aliphatic alcohol.

5.6) Enzyme treatments

Enzyme action can be exploited for the control of undesirable enzyme activities which inactivate other enzymes via direct proteolytic activities such as ring-cleaving oxygenases, catechol transferase and protease**.**

1.6) OBJECTIVE OF THE STUDY

In this study, the optimum condition of microwave pretreatment (treatment times and power levels) for polophenol oxidase (PPO), peroxidase (POD), phenylalanine ammonia lyase (PAL) inactivation of dried-longan pulp will be determined. The preliminary qualities of dried-longan pulp in terms of total phenolic content, color appearance, firmness, water activity, moisture content, titratable acidity and total soluble solid and proximate analysis including lipid content, protein content, moisture content, ash content, carbohydrate content, ascorbic acid and total plate count and yeast and molds will be also investigated. Then, the effect of microwave pretreatment and different packaging materials on storability of dried longan pulp was determined.