Changes in Quality and Enzymes of Longkong (*Aglaia dookkoo* Griff.) Fruit During Storage as Affected by Maturation, Package and Methyl Jasmonate Treatment

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ABSTRACT

The optimum harvesting period of longkong from 13th to 16th weeks of maturation were used to analyse the physiochemical quality changes. Fruit pericarp lightness (L*), yellowness (b*) decreased and redness (a*) increased throughout the maturation. Hue angle (H°) and chroma value (C*) was also decreased. The fruit weight ($P<0.05$) and diameter was increased throughout the maturation ($P\geq0.05$). Fruit total soluble solids (TSS), pH, total sugar (TS), reducing sugar (RS) and ascorbic acid (AsA) content increased throughout the maturation. Titratable acidity (TA) were decreased at the end of maturation period ($P<0.05$). Fruit DPPH radical scavenging activity, FRAP activity and total phenolic content (TPC) was increased throughout the maturation. Superoxide dismutase (SOD), catalase (CAT) activity was slightly decreased at the end of maturation (16th week) ($P<0.05$). Peroxidase (POD) activity was increased at the end of maturation (16th week) ($P<0.05$). Polygalacturonase (PG) and pectin methylesterase (PME) activities were increased throughout the maturation ($P<0.05$). Pericarp total phenolic content (pericarp TPC) increased during maturation. The pericarp polyphenol oxidase (PPO) activity increased throughout the maturation ($P<0.05$). Pericarp peroxidase (POD) and phenylalanine ammonia lyase (PAL) activities were decreased at the end of maturation (16th week). Peel epidermal trichomes losses on the surface and parenchyma cell changes in the cross section were found during the increased stage of maturation.

Longkong fruits stored in different OTR packages (PE1, PE2 and PE3) and temperatures (18°C and 25°C) were used to analyses the quality changes. Fruit pericarp L*, b*, H° and C* was continuously decreased, and a* value increased

throughout the storage. Fruit respiration gas and fruit weight loss were increased throughout the storage. TA, TSS, TS and RS were decreased during storage ($P<0.05$). Fruits stored in PE2 and PE3 at 25°C had retained the high values of DPPH scavenging activity, FRAP activity and TPC. Fruits stored at 25°C in PE2 and PE3 had retained the higher SOD and POD activities than PE1 ($P<0.05$). Fruit pericarp TPC decreased throughout the storage, but fruits stored at 18°C had retained higher phenolics than at 25°C. Pericarp PAL and POD activities increased throughout storage. Conversely, PPO activity increased at the beginning of the storage and then, it decreased ($P<0.05$). Pericarp surface epidermal hair loss and parenchyma cell damage was found more in fruit stored in PE2 and PE3 at 25°C than PE1 and 18°C storage.

Longkong fruit was stored at 18ºC and 25ºC under active modified atmospheric (5% O_2 and 5% CO_2 and 90% N_2) packaging to extend the quality and shelf life. Fruit stored at 18ºC increased the shelf life for up to 24 days whereas in fruit stored under 25ºC the shelf life was terminated within 12 days due to visible mold growth. Pericarp L*, b*, H° and C* was slightly decreased and a* value was increased during storage. An accumulation of CO_2 and depletion of O_2 was observed inside the fruit package during storage. The fruit weight loss was increased throughout the storage. The loss of fruit pH, TA, TS and RS were slightly reduced at 18ºC ($P<0.05$). The fruit total phenolics were increased at the initial days of storage and then it gradually decreased. Conversely, pericarp total phenolics decreased in the initial days of storage and afterwards, it increased ($P<0.05$). Fruit DPPH radical scavenging activity FRAP activity was maintained throughout the storage ($P<0.05$). Fruit SOD and CAT activities were gradually increased and POD activity was decreased throughout the storage ($P<0.05$). Pericarp PAL activity increased throughout storage. Pericarp PPO and POD activities were increased at the end of storage. Pericarp ultrastructural changes were controlled at 18ºC storage.

The quality changes of longkong fruit with (10, 20 and 30 µmol/l) and without methyl jasmonate (control) treatment in low temperature storage (13°C and 85% relative humidity) were determined. The higher concentration of methyl jasmonate (MeJA) treatment effectively decreased the longkong fruits pericarp colour (L*, a* b*, H° and C*) changes, browning index and weight loss than the lower

concentration and the control fruits ($P<0.05$). MeJA treatment was increased the fruit AsA content ($P\geq0.05$). MeJA treated fruits maintained the fruit pH, TA and TSS ($P<0.05$). Fruit DPPH radical scavenging ability, FRAP activity and TPC was contained high level in the 20 and 30 µmol/l MeJA treated fruits ($P<0.05$). Fruit SOD, CAT and POD activities were retained the higher level in MeJA treated fruits ($P<0.05$). The 20 and 30 µmol/l MeJA treated fruits were controlled the flesh PG and PME activities ($P<0.05$). MeJA treated fruits were decreased in pericarp PAL activity and consequently, low level of pericarp TPC was observed ($P<0.05$). The 20 and 30 µmol/l MeJA treated fruits were decreased in pericarp PPO, POD activities ($P<0.05$) and they controlled the pericarp ultrastructural changes.

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CHAPTER 1

INTRODUCTION AND REVIEW OF LITERATURE

1.1 Introduction

Longkong is a well-known commercial fruit of Thailand. It belongs to meliaceae family and classified as *Aglaia dookkoo* Griff. (Ketsa and Paull, 2008). The demand of longkong is increasing tremendously compared to other meliaceae family because the fruit is juicy and has a pleasant taste (Sapii *et al*., 2000). It is globular in shape and has a slightly thicker skin with less non-sticky sap. The matured longkong fruit skin has bright yellow in colour (Paull *et al.*, 1987). Longkong fruit grow in clusters of between 15 and 25 fruits per bunch and consist of five separate segments of white translucent flesh covered on one to five seeds each (Sangkasanya and Meenune, 2010). The fruit carries a variety of nutrients including high amount of antioxidants, carbohydrates, vitamins and minerals but low amount of protein and fat (Lim *et al.*, 2007; Ketsa and Paull, 2008). The sweet and sour taste of longkong joints with its faintly aromatic and nutritious qualities make this fruit a potentially precious export (Lichanporn *et al.*, 2009).

Harvested fruits are still living organs and suffer detrimental changes after harvest (Chakraborty and Mitra, 2006). These changes include the utilization of energy reserves through respiration, changes in biochemical composition, changes in texture associated with both water loss and biochemical change and the increased ethylene production associated with the fruit ripening (Burdon, 1997). Fresh fruits are more susceptible to disease organisms because of an increase in their respiration during postharvest. Therefore, the shelf life under ambient temperature is very limited (Sandhya, 2010). Especially, the shelf life of longkong fruit under room temperature is limited to about 4-7 days due to postharvest deterioration, such as pericarp browning, weight loss, soft texture and unpleasant flavour (Lichanporn *et al.,* 2009; Sangkasanya and Meenune, 2010).

The browning of harvested fruits and vegetables are mainly attributed to oxidation of phenolics by polyphenol oxidase (PPO), peroxidase (POD) and phenylalanine ammonia lyase (PAL) enzymes (Lichanporn *et al*., 2009). Phenylalanine ammonia lyase (PAL) enzyme converts phenylalanine to phenolics via

the phenylpropanoid pathway (André *et al.*, 2009). Polyphenol oxidase (PPO) and peroxidase (POD) can catalyze the oxidation of phenols to produce brown pigments (Francisco and Juan Carlos, 2001; Nokthai *et al.*, 2010).

The postharvest deterioration of fruits can be controlled by many postharvest techniques particularly, modified atmospheric package, low temperature and chemical treatments (Kader, 1986; Tano *et al.*, 2007; Sangkasanya and Meenune, 2010; Sandhya, 2010). Modified atmospheric packaging (MAP) is a technique used to decrease O_2 and/or elevates CO_2 levels (Kader, 1986; Kader, 1995; Sandhya, 2010). Changes in the concentrations of the respiratory gas may prolong the shelf life of fresh or minimally processed foods (Pirovani *et al.*, 1998; Allende *et al.*, 2004). MAP technique helps to maintain a high-humidity environment for fruit inside the sealed plastic film. However, most polymeric films used in MAP have lower water vapour transmission rates relative to transpiration rates of fresh produce (Tano *et al.*, 2007). Therefore, the excessive high relative humidity (RH) may occur, causing moisture condensation, microbial growth and decay of the produce (Ben-Yehoshua, 1987; Cameron *et al.*, 1995). MAP is also preventing cross-contamination during transportation and storage (Kader, 1995).

Longkong fruit with raceme, which was kept under MAP, has longer shelf life than that at atmospheric condition (Sangkasanya and Meenune, 2010). In addition, under MAP condition (5% CO_2 and 5% O_2); the longkong raceme that was treated with 1.5% citric acid for five min prior to storage under MAP at 18°C had its shelf life extended for 30 days (Meenune and Janthachum, 2004). One of the major problems encountered in using MAP is the accumulation of anaerobic metabolites inside the packages (Sangkasanya and Meenune, 2010). Under the condition where O_2 is low or not available, the build-up of an anaerobic condition in fruit leads to enhanced anaerobic metabolism and an increase in production of off-flavour volatiles, such as ethanol, which directly influence the consumer acceptability of fresh fruit (Shi *et al.*, 2005).

Storage under low temperature has been widely used to extend the shelf life of fruit and vegetable. Under this condition, it can also minimize the nutritional loss. However, the low temperature storage is susceptible to produce chilling injury in many tropical and subtropical plant produce (Wills *et al.*, 1998).

Longkong fruit is susceptible to chilling injury at temperature less than 15ºC and it causes skin browning, fermented flavour, softer fruit (Ketsa and Paull, 2008). Recently, methyl jasmonate (MeJA) and jasmonic acid (JA) are effective in controlling the chilling injury and extend the shelf life of fruits and vegetables (Kratsch and Wise, 2000; Nilpraprucк *et al.*, 2008; Cai *et al.*, 2011; Zhang *et al.*, 2012). MeJA, as a natural plant regulator compounds, plays important roles in plant growth and development, fruit ripening and responses to environmental stress (Fung *et al.*, 2004; Cao *et al.*, 2010; Sayyari *et al.*, 2011; Li *et al*., 2012). MeJA is reduced the postharvest diseases and an improved chilling tolerance and reduced incidence of chilling injury in several horticultural crops (Ding *et al.*, 2002; Feng *et al.*, 2003; González-Aguilar *et al.*, 2003; Cao *et al.*, 2010; Sayyari *et al.*, 2011; Cao *et al*., 2012). Conspicuously, the postharvest techniques are unique to control the fruit deterioration and sustain the quality of longkong. However, it also causes the adverse effect such as off-flavour and chilling injury on longkong fruit in a certain level. The outcome of present study will be a great resource of information to control the postharvest deterioration and minimize the adverse effect caused by postharvest techniques in longkong fruit. Therefore, this research will be extremely benefit to the longkong fruit cultivating farmers, shopkeepers, exporters and finally, to the consumers.

1.2 Review of literature

1.2.1 Longkong fruit

Longkong fruit is a non-climacteric and tropical fruit in nature (Paull, 2004). It is a member of meliaceae family and that includes langsat, duku-langsat and duku (Sangkasanya and Meenune, 2010). Longkong is classified as *Aglaia dookoo* Griff. and most likely derived from a natural hybrid of langsat (Salakpetch, 2000; Lim and Yong, 1996). A common name of longkong includes langsat, duku, lanzones, lansons, duku trengganu and duku jahore (Paull *et al*., 1987). The major cultivation of longkong found in peninsular of Thailand to Borneo. Longkong fruit is cultivated in several countries such as Philippines, Vietnam, Burma, India, Sri Lanka, Australia, Surinam and Puerto Rico (Paull, 2004). Longkong fruit is originally grown in Narathiwat province, located in the southern Thailand which gave the best quality of fruit since the pulp has mild pleasant aroma and almost seedless. So far, it has been introduced to the eastern and northern Thailand (Ketsa and Paull, 2008; Lim and Yong, 1996).

The globular shape of fruit develops on catkin inflorescences and are borne in bunches with 15 to 25 fruits in each (Paull *et al.*, 1987; Lichanporn *et al.*, 2009; Sangkasanya and Meenune, 2010). Longkong fruit has a slightly thicker skin and less sap that is not sticky. Skin of young fruit is green and it turns yellow when it ripens and frequently with the brown blemishes (Paull *et al*., 1987). The ripened longkong is contain five segments of hard white translucent flesh and covered on 1 to 5 green seeds (Paull *et al.*, 1987; Paull, 2004; Ketsa and Paull, 2008; Lichanporn *et al.*, 2009). Longkong holds a variety of nutrients, including proteins, carbohydrates, antioxidants, vitamins and minerals (Sabah, 2004; Lim *et al.*, 2007; Ketsa and Paull, 2008). The sweet and sour taste of longkong fruit combined with its faintly aromatic and nutritious qualities make this fruit a potentially precious export (Lichanporn *et al.*, 2009).

1.2.2 Harvest maturity of longkong

Longkong fruit is a non-climacteric fruit, which should be picked when fully ripe to ensure good quality (Paull, 2004). The season of longkong fruit production, mainly is between August and September (Wanichkul and Buasap, 1998). Nevertheless, the harvesting time of longkong fruit is vary with the areas. For example, the east of Thailand, longkong is harvesting at middle of November to the middle of February and then followed by the beginning of July to the middle of July. In the south (lower and west coast) of Thailand, at the beginning of April to middle of June and then followed by August to the end of October, longkong fruit is harvested (Wanichkul and Buasap, 1998). Longkong fruit trees that are 10 years old can produce 40-50 kg/tree increasing to 80-150 kg after 30 years (Ketsa and Paull, 2008).

During development, longkong fruit physical and chemical characteristic such as fruit weight, fruit size, skin colour, total soluble solid, total acidity and sugar content are changed (Sapii *et al*., 2000). Pantuvanid (1985) suggested that the development stage of longkong fruit divided into three stages based on fruit colour and weight and the stages as following. First stage is between the 1st and 7th week after anthesis. During the 1st to 4th week after anthesis, longkong fruit development is slow. However, in the 5th week after anthesis, the fruit weight is increased rapidly. After that, the cell division and cell enlargements are increased. Fruit skin develops to yield bright yellow skin, but some fruits are still green. Longkong fruit flesh at this stage is white, opaque and contains sourness. Second stage is between the 7th and 13th week after anthesis. Fruit weight is changed rapidly. The increase of longkong fruit flesh is stablished at the 13th week onwards after anthesis. At this stage, the flesh and skin weight is approximately 74% and 24%, respectively. Third stage is start between the 13th and 16th week after anthesis and this stage is the suitable time for optimal harvesting.

The pericarp colour is a best indicator for ripening, at matured stage the pale green pericarp tissue turns to bright yellow (Paull *et al.*, 1987; Ketsa and Paull, 2008; Lichanporn *et al.*, 2009; Sangkasanya and Meenune, 2010). At this fully ripened stage, fruit flesh turns to white translucent and the astringency decreases and sugar increases up to six folds in flesh (Paull *et al.*, 1987). At this stage, longkong has typical aromas with sweet and slight sour taste. A ripened longkong fruit could be

attained the full yellow skin and 15°Brix of total soluble solid (National Bureau of Agricultural Commodity and Food Standards, 2006). Several researchers reported that the early optimal harvesting period of longkong varies from 12^{th} to 14^{th} weeks after anthesis (Pantuvanid, 1985; Norlia, 1997; Sapii *et al*., 2000). Paull (2004) suggested that longkong fruit could be harvested since the ripen stage approach to 70-80 % to avoid excessive fruit drop. Longkong fruit needed to be dry, when it exported and if it wet can cause the mold growth on the surface. In addition, Sapii *et al*. (2000) recommended that longkong fruit could be harvested much earlier, about 4-5 days after the fruit turns to yellow if the fruit is to be transported to distant markets. At this stage, the whole bunch of fruit has turned yellow with 6-8 fruits per raceme, while the rest still having a greenish. The fruit harvested at this stage is able to turn to full yellow colour.

1.2.3 Longkong fruit types and grade classification

Longkong fruit is divided into three types: dried longkong, wet longkong and longkong kalamae and it generally graded by based on the size and colour (Yaacob and Bamroongrugsa, 1992; Paull, 2004; Ketsa and Paull, 2008; Lichanporn *et al.*, 2009). Dried longkong is very sweet and has a pleasant aroma. The fruit has five separate segments, with one to five seeds each. Ripe fruit has a dark yellow skin and dry and translucent flesh. The ripened wet longkong fruit has a light yellow skin and is softer than dried longkong fruit. The peel is thin and tough and is not easily peeled. The flesh is juicy, though not as sweet as dried longkong fruit. The ripe fruit of longkong-kalamae has a white-yellow, smooth, thin, soft skin with no latex. The flesh is dry, translucent and soft. The taste is a blend of sweet, sour and the fruit has fewer and smaller seeds.

1.2.4 Fruit Composition

The quality and characteristic of fruit is depending on its chemical composition and nutritional value. Moreover, the chemical composition and nutritional value could affect the considering of consumer to accept or reject the fruit. Table 1 shows the nutritional composition of longkong fruit (Phantumas, 1998). Generally, fruits principal contents are water, carbohydrates, amino acids and

proteins, fatty acids and lipids, organic acids, volatiles, vitamins and minerals. The details of each of fruit composition are as following.

Water is an essential for fruit quality and all life process. Most fruits contain more than 80%. Overall, the fresh fruit is mostly composed by water. Actual water content depends on the availability of water to the tissue at the time of harvest (Mercado *et al.*, 2011). For most produce, it is desirable to harvest when the maximum possible water content is present, as this result in a crisp texture (Chakraverty *et al.,* 2003). Chareoansiri and Kongkachuichai (2009) reported that longkong fruit has 80.6g moisture content/100g of fresh produce.

Carbohydrates are generally the most abundant constituent after water and widely distributed in food component derived from plants (Dai and Mumper, 2010). Fresh fruits vary greatly in their carbohydrate contents, generally the ranges between 10 and 25%. The structural framework, texture, taste and food value of a fresh fruit are related to its carbohydrate content (Kader and Barrett, 1996). Sucrose, glucose and fructose are the primary sugars found in fruits and their relative importance varies among commodities (Sudheer and Indira, 2007). Glucose and fructose occur in all produce and are often present in a similar level. Sucrose is present only two-thirds of produce. Produce with the highest sugar level is mainly tropical and subtropical fruits. Such variations influences taste since fructose is sweeter than sucrose and sucrose is sweeter than glucose (Wills *et al.,* 1998). In longkong fruit, primary sugar contents are fructose (7.94%), glucose (3.58%) and sucrose (2.56%) (Chareoansiri and Kongkachuichai, 2009; Sangkasanya and Meenune, 2010). Starch occurs as small granules within the cells of immature fruits. Starch is converted to sugar as the fruits mature and ripens (Vandeputte and Delcour, 2004). Other polysaccharides present in fruits include cellulose, hemicelluloses and pectin that are found mainly in cell wall. These large molecules are broken down into simpler and more soluble compounds resulting in fruit softening. Conversion of insoluble pectins to soluble pectins is controlled, for most part by pectinesterase and polygalacturonase (Nascimento *et al.,* 2006).

Fruits contain less than 1% protein. Fresh fruits are not an important source of protein in the diet (Knee, 2002). However, changes the level and activity of proteins resulting from permeability changes in cell membranes that involved with

chilling injury. There is an increasing evidence that chilling causes elevated levels of active oxygen species (AOS), which contribute significantly to chilling damage (Wang *et al.,* 2008; Promyou *et al*., 2012; Xu *et al*., 2012). The lipoxygenase, polyphenol oxidase, phenylalanine ammonia lyase, peroxidase enzymes are activated when the chilling injury occurs and produce chilling injury symptoms such as cell membrane damage and browning (Ding *et al.,* 2006). Antioxidant enzymes such as superoxide dismutase, catalase can react, neutralize the AOS activity, and decrease the chilling injury (Cao *et al.,* 2009). Enzymes, which catalyze metabolic processes in fruits, are proteins that are important and involved in the reactions of fruit ripening and senescence.

Lipids constitute only 0.1-0.2% of most fresh fruits, except avocados, olives and nuts. Lipids are very important because they make up the surface wax, contributes to fruit appearance, cuticle and therefore, resulting in reducing the water loss and pathogens. Lipids are also important constituents of cell membranes (Shah, 2005). Generally, low lipid content is seen as a positive factor in combating the rise of heart disease in the community and increases the consumptions of fresh fruits (Knee, 2002).

Most of the fruits are acidic and the organic acid level is excess for the operation of the TCA cycle and other metabolic pathways. The excess of organic acid is generally stored in the vacuole away from other cellular components (Tosun *et al.,* 2008). Acid contents usually decrease during ripening due to the utilization of organic acids during respiration or their conversion to sugars. Malic and citric acid are the most abundant in fruits except grapes and kiwi fruits (Knee, 2002). Chairgulprasert *et al.* (2006) reported that longkong fruit dried pulp contains four organic acids such as glycolic (0.14%), maleic (1.23%), malic (0.15%) and citric acid (0.22%).

Pigments, which are the chemicals responsible for skin and flesh colours, undergo many change during the maturation and ripening of fruits. These plant pigments include chlorophyll (green colour), carotenoids (yellow and orange colours) and anthocyanins (red, blue and purple colours) (Palapol *et al.,* 2009).

Total phenolics contents are higher in immature fruits than mature fruits and the ranges between 0.1 and 2.0%. Fruit phenolics are included chlorogenic acid, catechin, epicatechin, leucoanthocyanidins, flavonols, cinnamic acid derivatives

and simple phenols (Sidhu *et al.,* 2006). Chlorogenic acid (ester of caffeic acid) occurs widely in fruits. It is the main substrate involved in enzymatic browning of cut or damaged fruits, when fruits exposed to O_2 (Chung *et al.,* 2005).

Volatile compounds are responsible for the characteristic aroma of fruits (Hui, 2006). They are present extremely in small quantities (less than 100 µg fresh weight). The total amount of carbon involved in the synthesis of volatiles is less than 1% of that expelled as CO_2.Volatile compounds are largely esters, alcohols, acids, aldehydes and ketones and these contribute to typical fruit aromas (Ergönül and Nergiz, 2010). Chairgulprasert *et al.* (2006) found that longkong fruit sour flavour mainly attributed by maleic acid. Meenune *et al*. (2009) found that fully ripened longkong fruit contains 69 different volatile flavour compounds and among them, the most abundant groups are sesquiterpenes (36.23%), esters (31.88%), terpenes (17.39%), hydrocarbons (4.34%), alcohols (2.89%), aldehydes (2.89%), ketones (1.44%) and furans (1.44%).

Vitamins and minerals are importance in human nutrients and fresh fruits are essential source for vitamins and minerals. Vitamin C (Ascorbic acid) is only a minor constituent of fruits but it is an importance in human nutrition for the disease scurvy. Vitamin C is one of the most important antioxidant supplied by fruits and vegetables (Serrano *et al.,* 2007). Virtually all-human dietary vitamin C (approximately 90 %) is obtained from fruits. Major mineral in fruit is potassium and available abundant level in most fruit produce (Knee, 2002).

1.2.5 Physiological and biochemical changes during maturation

Maturation generally considered being the stage of development where the plant is capable of shifting from vegetative to reproductive growth that relative to human needs (Thuzar *et al.,* 2010). Growth and maturation are often referred as the development phase. Maturity at harvest is the most important factor that determines storage life and final fruit quality (Kader, 1999; Kader, 2002). Optimum maturity is determined by specific physiological and biochemical characteristics of the plants (Sinha, 2004). Changes in physiological and biochemical properties of longkong fruit during maturation are described as following.

1.2.5.1 Colour

Colour is the most obvious change that occurs in many fruits and is offer the major criterion used by consumers to determine whether the fruit ripe or unripe (Wills *et al.,* 1998; Lancaster *et al.,* 1997). The most common change is the loss of green colour. This change is due to the loss or degradation of chlorophyll, which is a magnesium-organic complex (Landahl *et al.*, 2003). The loss of green colour is due to degradation of the chlorophyll structure by changes of chlorophyllase, pH and oxidative systems (Hortensteiner, 2006). Combination of chlorophyll breakdown and the synthesis and degradation of carotenoids and phenolics pigments such as anthocyanins can be the chance of changing fruit colour (Palapol *et al.,* 2009). Longkong fruit pericarp colour is a primary maturity index to find its maturity. The immature longkong fruit has green colour pericarp and it turns to bright yellow when fruit attained the maturity (Paull *et al.*, 1987; Lichanporn *et al.*, 2008a; Lichanporn *et al.*, 2009; Sangkasanya and Meenune, 2010; Sangchote *et al.*, 2011; Taesakul *et al.*, 2012).

1.2.5.2 Texture and Firmness

The terms such as crispness, juiciness, hardness and mealiness are the broad spectrum of attributes that define the feel of fruit in the mouth (Peace and Norelll, 2009). They are experienced during mastication, which causes the breakdown of the tissues. In many fruits, the tissue is made up of parenchyma cells. The perception of texture is mainly contributed by the structural integrity of the cell well (Robert and Fischer, 2002). Normally, fruits soften gradually during ripening. It is believed that, softening is largely due to the breakdown of starch and other non-pectic polysaccharides in the pulp and thereby reducing cellular rigidity (Lizada *et al.*, 1990). Flesh firmness is a prime indicator of fruit quality and it is used as a ripening index in several fruits. A large amount postharvest research effort is dedicated to find ways of maintaining fruit firmness during postharvest storage and shelf life extension (Munoz *et al.,* 2008). Sapii *et al*. (2000) reported that longkong fruit has a decrease in firmness during the increased stage of ripening and easily yields to pressure when it was pressed with finger.

Table 1. Proximate analysis of longkong per 100 g edible portion of fruit

Constituent	Longkong fruit (Unit)
Edible portion proximate	94 g
Water	80.6 %
Energy	157 kcal
Protein	0.9 g
Fat	0.2 g
Carbohydrate	12.2 g
Fibre	0.8 g
Ash	NA
Calcium	19 mg
Phosphorous	25 mg
Potassium	28 mg
Iron	1.1 mg
Sodium	NA
Vitamin A	-
Thiamine	NA
Niacin	0.07 mg
Riboflavin	0.04 mg
Vitamin C	3 mg

Source: Phantumas (1998); Chareoansiri and Kongkachuichai (2009)

Note: NA: not available

1.2.5.3 Respiration

A major metabolic process-taking place in harvested produce or in any living plant product is respiration. Respiration is a serious of oxidation-reduction reactions where a variety of substrate from cell such as starch, sugar and organic acids are oxidised to yield CO_2 and produce water and vital heat **(**Sinha, 2004; Wills *et al*., 1998). Respiration can occur in the presence of oxygen (aerobic respiration) or in the absence of oxygen (anaerobic respiration), which is sometime called fermentation (Vignais and Billoud, 2007). Every plant tissue requires the energy to remain alive and to support development changes and therefore, it generated by respiration

(Chakraverty *et al.,* 2003). Respiration can be considered a series of enzymatic reactions, involving three major pathways namely, glycolysis (glucose to pyruvate), tricarboxylic acid cycle (pyruvate to CO_2), and oxidative phosphorylation (produce ATP) (Sinha, 2004; Fernie *et al.,* 2004). Respiration rate per unit weight is high for the immature fruit and then steadily declines with maturation. Non-climacteric fruits are having lower rate of respiration as compared to climacteric fruits (Saltveit, 2004). Previous studies have reported that longkong fruit has the higher respiration rate during ripening and after fruit harvested, reparation rates declines gradually (Srivastana and Mathur, 1955; Pantastico *et al*., 1968).

1.2.5.4 Ethylene production

Ethylene is a plant hormone and it mainly involves in the fruit ripening. Climacteric and non-climacteric fruits are differentiated by their ethylene production (Tian *et al.*, 2000). Climacteric fruits produce larger amounts of ethylene during ripening than non-climacteric fruits (Pech *et al.,* 2008). The internal ethylene concentration of climacteric widely varies; but non-climacteric fruit has little changes in ethylene during development and ripening, even though non-climacteric fruit have the capability to produce ethylene just like climacteric fruit but only lack the ability to produce ripening-associated ethylene (Yamane *et al.,* 2007). Fruit ripening and respiration is based on the involvement of gas ethylene. Ethylene produced from methionine via pathway that includes S-adenosyl-methionine (SAM) and 1-aminocyclopropane-1-carboxylic acid (ACC) by the enzyme ACC synthase. In higher plants, ACC is removed by conjugation to form malonyl or glutamyl ACC. In fruits, during un-ripened stage, the ethylene production is low; in this case, ACC oxidase enzyme needs to convert ACC to ethylene (Pathak *et al.,* 2003). ACC is a liable enzyme that is sensitive to oxygen. The ethylene synthesis is based on the presence of oxygen (Nazir and Beaudry, 2002). Longkong fruit during maturation has produced very minute amount of ethylene with internal concentration of 2 to 6 µl/kg (Paull *et al.*, 1987; Paull, 2004). In addition, during the storage period, longkong fruit had the gradual increase in ethylene production (Lichanporn *et al.*, 2009; Sangkasanya and Meenune, 2010).

1.2.5.5 Carbohydrate

The largest quantitative change associated with ripening is usually the breakdown of carbohydrate polymers, especially the conversion of starch to sugars. This alters both the taste and texture of the produce (Risenga, 2008). Starch can be converted back to glucose by the least three different enzymes namely, α-amylase, β-amylase and starch phosphorylase (Dieguez *et al.,* 2009). In non-climacteric fruits, the accumulation of sugar is associated with the development of optimum eating quality, although the sugar may be derived from sap imported into the fruit rather than from the fruit breakdown of starch reserves (Nascimento *et al.,* 2006). Starch is broken down to sucrose by the action of sucrose phosphate synthetase and non-reducing sugars from sucrose by acid hydrolysis. The onset of the starch-sugar conversion has been shown to be influenced by harvest maturity, with more mature fruits responding earlier (Madamba *et al*., 1997; Montenegro, 1988). The major sugars were identified as glucose, fructose and sucrose, with the last being in the largest proportion (Cocchi *et al*., 2006). During the early part of ripening process, sucrose is the predominant sugar, but in the later stage, glucose and fructose predominate (Thompson, 2008). The matured longkong fruit had an increase level in sugar (Sapii *et al*., 2000; Chareoansiri and Kongkachuichai, 2009). Paull (2000) reported that mature longkong fruit increased in sugar level up to six fold as compared to immature fruit. The predominant contribution of sugar in longkong was identified as fructose (7.8 g/100 g), glucose (6.9 g/100 g) and sucrose (0.5 g/100 g) (Chareoansiri and Kongkachuichai, 2009; Sangkasanya and Meenune, 2010).

1.2.5.6 Organic acid

Although the development of sweetness is important in fruit but organic acids also influence the overall fruit flavour (Sudheer and Indira, 2007a). The desirable sugar-acid balance is necessary for producing the pleasant taste in fruits. The acidity of fruits generally decreases during ripening (Muller, 2005). The astringency of longkong fruit tends to decreasing during maturation (Paull *et al*., 1987). Type and concentration of organic acids present in fruits are different with malic and citric acid being the most common (Thompson, 2004). The change in the concentration of acids during the development of fruit differs with the type of fruit

(Tosun *et al.,* 2008). Acids can be considered as a reserve source of energy to the fruit and would therefore be expected to decline during the greater metabolic activity that occurs with ripening (Wills *et al.,* 1998). In longkong, maleic acid is the predominant quantity present and malic acid, citric acid and glycolic acids are present in considerable quantities (Chairgulprasert *et al.,* 2006).

1.2.5.7 Flavour and aroma

Flavour is a subtle and complex perception combining taste, smell and texture or mouth feel (Kader, 2008). Ripening usually brings about an increase in simple sugars to give sweetness, a decrease in organic acids and phenolics to minimize astringency and an increase in volatiles to produce the characteristic flavour. The characteristic aroma of ripe fruit is due to the production of a complex mixture of individual volatile components (Ergönül and Nergiz, 2010). Total soluble solids, titratable acidity and aroma volatile composition are all associated with flavour and commonly measured as part of fruit quality (Ian and Linda, 2002). Most fruits contain in excess of 100 different volatile compounds. The compounds are mainly esters, alcohols, acids and carbonyl compounds (Kalua *et al.*, 2007). Non-climacteric fruits produce less volatile than climacteric fruits as they reach optimum eating quality. Nevertheless, the volatile produced are still appreciated by consumers (Carasek and Pawliszyn, 2006). The unique characteristics of longkong is majorly attributed by its pleasant aroma which makes longkong to be more valuable to export (Ketsa and Paull, 2008; Lichanporn *et al.*, 2009; Sangkasanya *et al.*, 2010). Chairgulprasert *et al.* (2006) reported, maleic acid is the major responsible for longkong fruit sour flavour. Meenune *et al*. (2009) reported that the matured longkong fruit contains 69 volatile flavour compounds and the predominant group of compounds are sesquiterpenes (36.23%), esters (31.88%), hydrocarbons (4.34%), alcohols (3.89%), aldehydes (2.98%), ketones (1.44%) and furans (1.44%).

1.2.5.8 Nitrogen compounds

Proteins and free amino acids are minor constituents of fruit and, as far as is known, have no role in determining eating quality. However, proteins are extremely important components of living cells in that they regulate metabolism, act as structural molecules and in some products represent storage forms of carbon and

nitrogen (Belitz *et al.,* 2009). Proteins synthesis and degradation are the two primary means of modulating the level of a specific protein (Wills *et al.,* 1998). The coded sequence is transcribed by the formation of a special type of RNA, the messenger RNAs (mRNA), thus transferring the required amino acid sequence to a molecule that can move from the nucleus into the cytoplasm where the actual synthesis of the protein molecule occur. The peptide bond in protein chain is degraded by peptidase or protease in cytoplasm or vacuole. Changes in nitrogenous constituents indicate variations in metabolic activity during different growth phases. During senescence, the level of free amino acids increases, reflecting a breakdown of enzymes and decreased metabolic activity (Barker and Bryson, 2007). Ketsa and Paull (2008) reported that the matured longkong fruit contains 0.9 g/ 100 g of protein.

1.2.5.9 Phenolic compounds

Phenolic compounds or polyphenols are one of the secondary metabolites occurring in all plant material and active as plant defence mechanisms against various factors of stresses, caused from pathogens, adverse environmental conditions or wounding (Bennett and Wallsgrove, 1994; Dixon and Paiva, 1995). Their biosynthesis depends on numerous enzymes involved in various metabolic pathways and their metabolism is completely combined with morphological and biochemical regulatory patterns of plants (Niemetz and Gross, 2005; Thitilertdecha and Rakariyatham, 2011). Fruits contain high levels of phenolic compounds, especially in the peel. Phenolics are common in many fruits and are responsible for the oxidative browning reaction when the pericarp and/or pulp of fruit are cut especially immature fruit. The polyphenol oxidase enzyme is responsible for this reaction. Recently, the interest in fruit phenolic compounds increased due to that function as free radical scavengers (Thitilertdecha and Rakariyatham, 2011; Awah *et al*., 2012; Nithyanantham *et al*., 2012). Matured longkong pericarp and flesh had a predominant level of phenolics (Lichanporn *et al.*, 2009). Phenolics such as tannins are polymerized to insoluble compounds, resulting in a reduction of astringent in the ripe banana fruit (Lizada *et al*., 1990). Tannins are perhaps the most important phenolic from the point of view of fruit utilization because they can give fruit astringent taste. As fruit ripens, their astringency becomes lower, which seems to be

associated with the reduction in tannin levels. Paull *et al*. (1987) have also found that the increased maturation had a decreased astringency in longkong fruit.

1.2.5.10 Vitamins

Vitamins are organic, low molecular weight component of the diet, which act essentially only as catalyst and required widely by the organism for the normal development (Friedrich, 1988; Fujii *et al.*, 1996). Vitamins are classified by their biological and chemical activity and not their structure. Vitamins have diverse biochemical functions. Some have hormone-like functions as regulators of mineral metabolism (e.g., vitamin D), or regulators of cell and tissue growth and differentiation (e.g., some forms of vitamin A), others function as antioxidants (e.g., vitamin E and vitamin C) (Paiva and Russell, 1999). Vitamins are essential for the normal growth and development of a multicellular organism. Major sources of vitamin A, B, C and folate are obtained from fruits and vegetables. Fruits are rich source of vitamins, particularly vitamin C (L-ascorbic acid) (Akinyele and Keshinro, 1980). Moreover, vitamin C can be a major metabolite (greater than 2 g/kg fresh weight) in fruit such as acerola, rosehip, quandong, kiwifruit, citrus, blackcurrants and guavas, and has strong antioxidant properties. This may account for a notable absence of browning in many fruits (in conjunction with relatively low levels of polyphenols and polyphenol oxidase in those tissues) (Atwell, 1999). Vitamin C levels increase in the fruit during early growth, and tend to be stable through to maturity (Beaulieu and Lea, 2007). The matured longkong pericarp, fruit and seed have a variety of vitamins especially, vitamin B1, B2 and C (Ketsa and Paull, 2008). Vitamin C in longkong fruit is the predominant level as compared to other vitamins (Sabah, 2004; Sapii *et al*., 2000; Chairgulprasert *et al.*, 2006; Ketsa and Paull, 2008; Lichanporn *et al.*, 2009).

1.2.5.11 Enzyme activity changes

1.2.5.11.1 Texture enzymes

Texture comes in many guises such as crispness, hardness, mealiness, flouriness and grittiness (Harker *et al.*, 1997). The complex carbohydrates such as, pectic substances, cellulose, hemicelluloses, starch and lignin are the primarily attributors to the fruits and vegetables texture (Kopjar *et al.*, 2008). During fruit

ripening, enzymatically mediated degradative changes in the cell walls found in most fruits (Paull *et al.*, 1999). One or more enzymes act on each of the complex carbohydrates that are important in food texture. The enzymes may be either synthesized and/or activated or a combination of both, at or near the onset of the ripening process (Prasanna *et al.*, 2007). Enzymatic mediated changes in the soluble pectin fraction and resulting in solubilisation or hydrolysis of pectin molecule. Pectolytic enzymes are widespread in plants, fungi and bacteria. The hydrolysis of pectin molecules involves the action of two types of enzymes namely, pectinmethylesterase (PME) and polygalacturonase (PG) (Rugkong, 2009) and their mode of action is given in Figure 1. Sapii *et al*. (2000) have reported that longkong fruit firmness during the different stage of ripening after fruit obtained the pericarp yellowing had a gradual decreased. However, there is no scientific data on PG and PME enzyme activity in longkong fruit during maturation. The general mechanism of PG and PME enzymes are as following.

PG is a texture related fruit enzyme that catalyzes the hydrolysis of the linear α-1, 4, D-galacturonan backbone of pectic polysaccharides (Poovaiah and Nukuya, 1979). There are two types of PG, namely exo- and endo-PG. These enzymes remove single galacturonic acid unit from the non-reducing end of polygalacturonic acid, whereas the endo-PG cleavages polymers randomly (Rugkong, 2009). The substrate for PG is mainly demethylated homogalacturonans. Because homogalacturonans are secreted to the cell wall in a highly methyl-esterified form, they must be de-esterified before becoming available as a substrate for PG (Wolf *et al.,* 2009; Redgwell and Fischer, 2002)

Pectinmethylesterase (PME) catalyses the de-esterification of pectin to expose its carboxyl groups and liberate methanol. The degree of methyl esterification influences the physicochemical properties of pectic polymers, relating to charge density of the molecule and gelation properties. The de-esterification of linear pectic polymers allows more calcium mediated junction zones and this contributes to the rigidity of the cell wall and fruit tissue integrity (Fraeye *et al.,* 2007). PME isoform are the variation with the same function and the different kinetic rates, different regulatory properties and or be expressed in a tissue-specific manner. The major PME isoform II as fruit specific and has a peak of activity at the mature breaker stage.

Reduction in PME isoform II levels resulted in higher esterification of pectin at all stage of fruit develop (Wolf *et al.,* 2009). Esterified pectin would be less susceptible to endo PG mediated hydrolysis; reduced pectin depolymerisation had a negative effect on shelf life. It was suggested that the loss of bound calcium in the walls of the transgenic fruit (with more esterified pectin) negated the potential benefits on wall integrity that decreased depolymerisation of the pectic polysaccharides (Lionetti *et al.,* 2009).

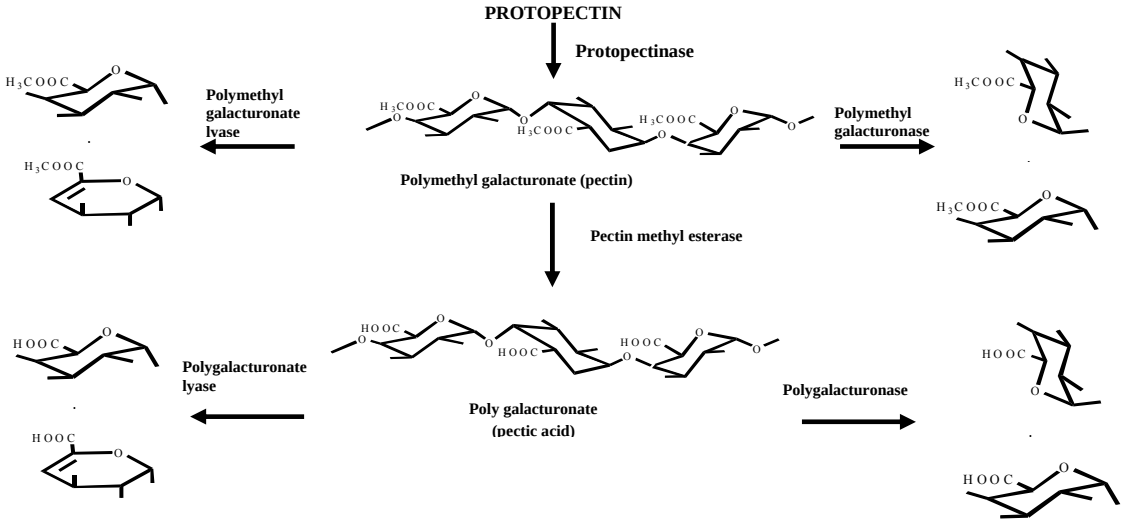


Figure 1. Mode of action of pectin degrading enzymes

Source: Prasanna *et al.* (2007)

1.2.5.11.2 Antioxidant enzymes

The evolution of aerobic metabolic processes such as respiration and photosynthesis unavoidably led to the production of reactive oxygen species (ROS) in mitochondria, chloroplast and peroxisomes. Abbasi *et al*. (2010) reported that the increased maturation of apple fruit was increased in ROS production. A common feature among the different ROS types is their capacity to cause oxidative damage to proteins, DNA and lipids. The extent of oxidative stress in a cell is determined by the amounts of superoxide, H_2O_2 and hydroxyl radicals. These ROS scavenged in plants by antioxidant enzymes such as superoxide dismutase (SOD), glutathione peroxidase (GPX) and catalase (CAT) (Klaus and Heribert, 2004). Scavenging mechanism of these antioxidant enzymes are given in Figure 2. Antioxidants in fruits are important

because it can protect fruit tissues from potentially toxic ROS and thereby contributing to the stress tolerance of crops (Mittler *et al*., 2004).

SODs act as the first line of defence against ROS; SOD converts hydrogen peroxide (Maleki and Gholami, 2010). CAT converts hydrogen peroxide into water. Hydrogen peroxide is also converted into water by the ascorbate glutathione cycle. The GPX cycle converts hydrogen peroxide into water using reducing equivalent from GSH. GPX requires several secondary enzymes (GR and G-6-PDH) and cofactors (GSH, NADPH and glucose 6-phosphate) to function. Generally, GR and G-6-PDH are considered as secondary antioxidant enzymes, because they do not act on ROS directly but enable GPX to function (Li *et al.*, 2000; Klaus and Heribert, 2004). ROS such as $O_2^{\cdot-}$, singlet oxygen, H_2O_2 and hydroxyl radical may contribute to the development of chilling injury and that the antioxidant enzymes, SOD, CAT and GPX play important roles in detoxifying ROS and alleviating chilling injury in fruits (Imahori *et al.,* 2008; Cao *et al.,* 2009). Lim *et al*. (2007) reported that the matured longkong fruit have higher level of antioxidant activity. However, there is no supportive study previously published on matured longkong fruit antioxidant enzymes activity.

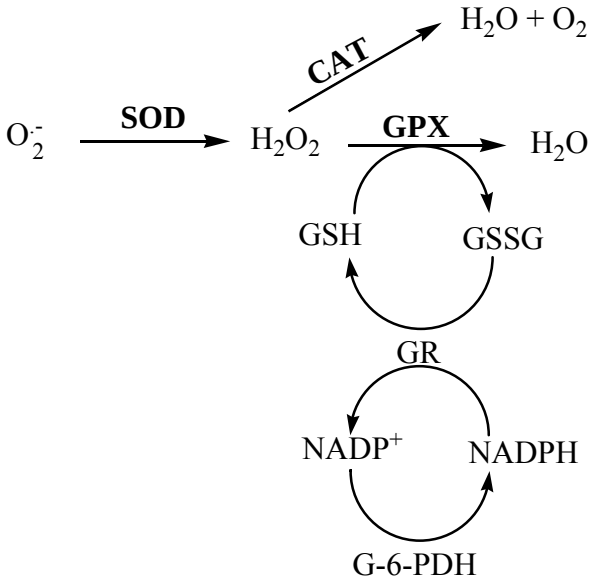


Figure 2. Mode of action of antioxidant enzymes on scavenging radicals
Source: Gill and Tuteja (2010)

1.2.5.11.3 Browning related enzymes

Enzymatic browning reaction is one of the most devastating reactions for many exotic fruits and vegetables, in particularly tropical and subtropical varieties (Quevedo *et al.,* 2009). It is estimated that over 50 percent losses in fruit occur because of enzymatic browning reaction (Whitaker and Lee, 1995). It is catalyzed by the enzyme polyphenol oxidase (EC 1.10.3.1) and peroxidase (EC 1.11.1.7) (Marshall *et al.,* 2000; Zocca *et al.,* 2008). Enzymatic browning reaction does not occur in intact plant cells since phenolic compounds in cell vacuoles are separated from the polyphenol oxidase, which is present in the cytoplasm (Figure 3). Phenolic compound synthesis is associated with the endoplasmic reticulum (Hrazdina and Wagner, 1985). The proteins involved with their synthesis either are incorporated into the endoplasmic reticulum membrane or are loosely associated with it. Once formed, these compounds are glycosylated and then transport vesicles formed from the endoplasmic reticulum membrane (Toivonen and Brummell, 2008). These vesicles are the vehicle by which the phenolic compounds transported to the vacuole or into the apoplast/cell wall compartment (Quevedo *et al.*, 2009).

There are smaller quantities of phenolic compounds that may be found in chromoplasts, cytoplasm and the mitochondria, but these are normally minute amounts and are associated with specialized metabolic functions (Hrazdina and Wagner, 1985). Once tissue is damaged by slicing, cutting or pulping, the formation of brown pigments occurs. Relatively few of the phenolic compounds in fruits and vegetables serve as substrates for polyphenol oxidase (Vámos-Vigyázó and Haard, 1981). Catechins, cinnamic acid esters, 3, 4-dihydroxy phenylalanine (DOPA) and tyrosine are the most important natural substrates of polyphenol oxidase in fruits and vegetables (Ayaz *et al.,* 2008). Polyphenol oxidase catalyses the initial step in the polymerization of phenolics to produce quinones, which undergo further polymerization to yield dark, insoluble polymers such as melanins (Figure 4) (Li *et al.*, 2008; Quevedo *et al.*, 2009)

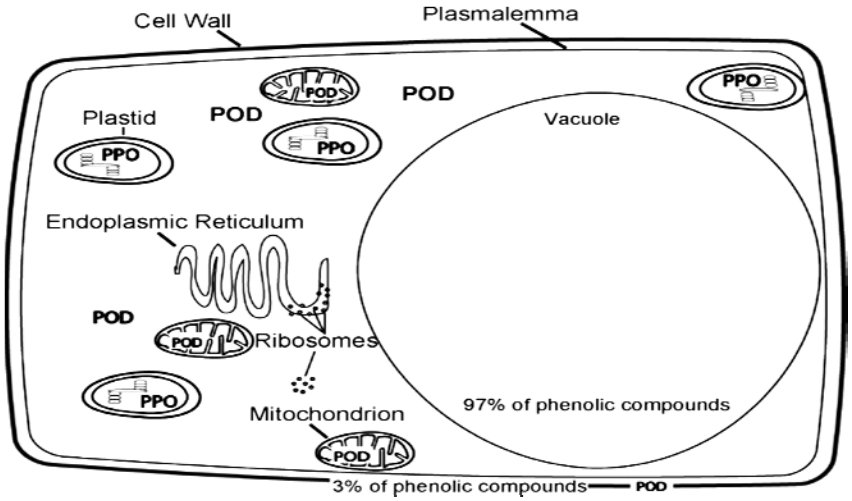


Figure 3. Localisation of phenolic compounds and phenolic oxidizing enzymes (polyphenol oxidase and peroxidase) in a typical plant cell

Source: Toivonen and Brummell (2008)

Peroxidase (POD) and phenylalanine ammonia lyase (PAL) also increased their activities when plants under stress or wounding. POD enzyme can rapidly oxidize 4-methylcatechol in the presence of H_2O_2 and form brown polymeric pigments (Jiang *et al.,* 2004). PAL is the first key enzyme involved in the biosynthesis of phenols in fruits and vegetables and can be induced by various stress conditions (Dixon and Paira, 1995). PAL might accelerate the browning of longkong fruit pericarp due to increased activity during the development of the brown colour. Moreover, the increase of PAL activity was observed to be concomitant with the accumulation of the total phenolic content. Many reports have found that the browning of fruits and vegetables was related to increasing PAL activity and increased levels of phenolic compounds (Sun *et al.,* 2009; Fujita *et al.,* 2006). Lichanporn *et al*. (2009) also reported that the PPO and PAL enzymes might be attribute to longkong pericarp browning.

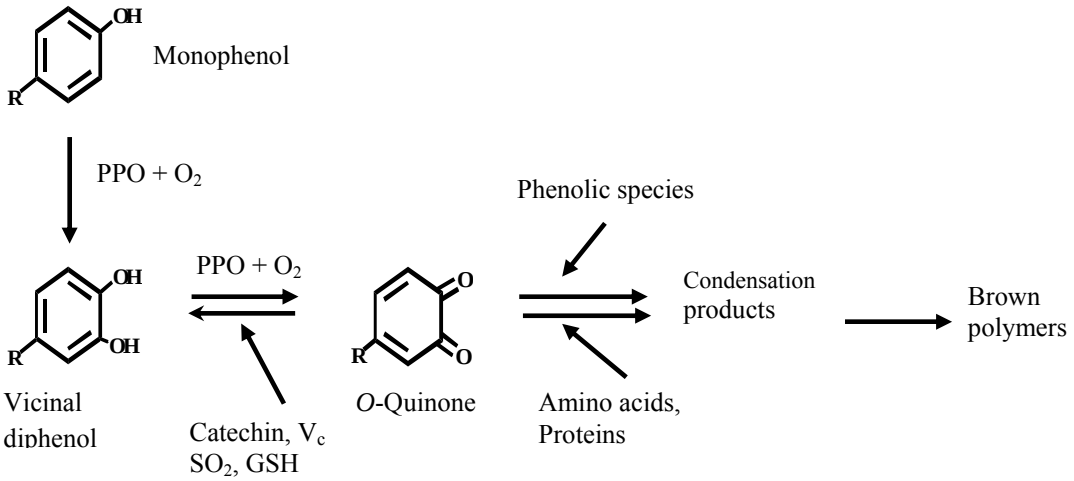


Figure 4. Enzymatic browning process

Source: Li *et al*. (2008)

1.2.6 Postharvest technology and sustaining fruit quality

The production of fruits and vegetables has been an important sector in the total world agricultural output. Asia is the largest producer of fruits and vegetables (Coyle, 2006). Human evolution was mainly linked to the consumption of naturally available fruits and vegetables (Eaton *et al.*, 2002). Fruits and vegetables are major sources of several essential nutrients that include vitamin A and C and folic acid. In addition, fruits and vegetables are rich in antioxidants such as carotenoids, polyphenols and anthocyanins that help combat free radicals produced within the body and excess production of health diseases (Paliyath and Murr, 2008). By virtue of their physiological properties, most fruits, vegetables are highly perishable commodities (Mogaji and Fapetu, 2011). Postharvest losses can occur at any point in the production and marketing chain, in where storage facilities are limited (Hodges *et al.*, 2010). Postharvest losses of commercial fruits and vegetables vary from 20 % to 50 %, before they reach consumers (Parfitt *et al.*, 2010).

Fruits and vegetables are containing high moisture, ranging from 70 to 95 %. Under normal atmospheric condition, they dry rapidly, which causes wilting and shriveling because of loss of rigidity and shrinkage of cells (Jennifer *et al.*, 2004). Fresh produce is more susceptible to disease organisms because of an increase in the respiration rate after harvesting (Sandhya, 2010). Therefore, the shelf life under

ambient conditions is very limited. The shelf life of longkong fruit is limited to about 4-7 days under room temperature and its due to the changes in physiochemical properties such as pericarp browning, texture, appearance and off-flavour after harvesting (Paull, 2004; Ketsa and Paull, 2008; Sangkasanya and Meenune, 2010; Sangkasanya *et al.*, 2010). Product respiration plays a major role in the postharvest life of fresh fruits (Saltveit, 2004). For controlling respiration and increasing the shelf life of plant commodities by several invented postharvest techniques such as modified atmospheric packaging, low temperature storage and chemical treatments. These techniques are extremely useful for the fruits and vegetables and widely used in worldwide (Sandhya, 2010; Morales *et al.,* 2010; Reichel *et al.,* 2010).

1.2.6.1 Modified atmospheric packaging

Modified atmosphere packaging (MAP) is a technique used for prolonging the shelf life of fresh or minimally processed foods (Park and Lee, 2008; Sandhya, 2010). MAP is created when fruit is sealed in plastic bags with a relatively low permeability to gases. Consequently, as the fruits respire, the O_2 level decreases, and the CO_2 level increases inside the bags (Kader, 1995). Under these atmospheric conditions, the respiration rate of the fruit is decreased, and as a direct effect, the consumption of respiration substrates such as organic acids and sugars is retarded (Tano *et al.,* 2007). O_2 and CO_2 concentrations in MAP change as a function of produce respiration rate, temperature, and the area, thickness, and O_2 and CO_2 permeability coefficients of the packaging material (Al-Ati and Hotchkiss, 2003).

Packaging in polymeric films can result in a commodity generated modified atmospheres such as reduced O_2 level and elevated CO_2 levels, ethylene, other volatiles (Kader, 1986; Kader, 1995). The concentration of both CO_2 and O_2 influence product quality. High barrier films can lead to anaerobic conditions and excessive CO_2 build up. On the other hand, low barrier films can result in less than optimal CO_2 concentrations and this condition may not provide maximum shelf life extension, especially for rapidly respiring produce, such as minimally processed fruits and vegetables (Gunes *et al.*, 2001). MAP is used with various types of products and the atmospheric modification within polymeric film packages depending upon film permeability, respiration rate, gas diffusion characteristic of the commodity, initial

free volume and atmospheric composition with in package and external environmental factors such as temperature, relative humidity and air velocity.

One of the major benefits of MAP is the prevention or retardation of fruit ripening and senescence and it associated biochemical and physiological changes (Ahmed *et al.*, 2011). MAP technology is largely used for minimally processed fruits and vegetables including fresh, ‘‘ready-to-use’’ vegetables (Beaulieu and Gorny, 2001). Modified atmospheres can be created either passively by the commodity or intentionally via active packaging (Kader, 1986; Kader *et al.*, 1989).

1.2.6.1.1 Passive MAP

Modified atmospheres can passively evolve within a hermetically sealed package because of a commodities respiration, i.e. O_2 consumption and CO_2 evolution (Guevara and Yahia, 2003; Sandhya, 2010). If a commodities respiration characteristics are properly matched to film permeability values, then a beneficial modified atmosphere can be passively created within a package (Fonseca *et al.,* 2002; Charles *et al.*, 2003; Valle *et al.,* 2009). If a film of correct intermediary permeability is chosen, then a desirable equilibrium modified atmosphere is established when the rates of O_2 and CO_2 transmission through the package equal a product respiration rate (Brecht *et al.*, 2003). Escalona *et al*. (2005) reported that fresh cut fennel stored under passive MAP was maintained the quality and extended the shelf life to 14 days. In another study, lettuce stored under passive MAP was efficiently controlled the indigenous microflora (Horev *et al*., 2012). Polyethylene is most commonly used to provide a hermetic seal and also as a medium of control for characteristics like anti-fogging, peeling and sealing abilities (Sandhya, 2010). Jacxsens *et al*. (2003) found that, strawberries and rasberries stored under high OTR packages were retained the fruit quality and shelflife. However, the use of passive MAP for fresh products is restricted mainly by the unavailability of appropriate films that provide O_2 and CO_2 gases fluxes, selectivities and temperature compensation to function effectively (Exama *et al*., 1993).

1.2.6.1.2 Active MAP

Active MAP consists essentially of gas flushing or gas-scavenging (O_2, CO_2 and N_2) to quickly establish equilibrium condition within the package and to avoid high content of unsuitable gases as compared to passive MAP (Kader and Watkins, 2000; Lange, 2000; Charles *et al.*, 2003). Another active packaging technique is the use of O_2, CO_2 or ethylene scavengers/emitters. Such scavengers/emitters are capable of establishing a rapid equilibrium atmosphere within hermetically sealed produce packages (Charles *et al.*, 2003). Meenune and Janthachum (2004) reported that the longkong fruit raceme, which was kept under MAP, has longer shelf life than that at atmospheric condition. In addition, under MAP condition (5% CO_2 and 5% O_2); the longkong fruit raceme that was treated with 1.5% citric acid for 5 min prior to storage under MAP at 18°C had its shelf life extension for 30 days (Meenune and Janthachum, 2004). Longkong fruit was stored in different MA conditions such as 5% CO_2: 5% O_2, 5% CO_2: 10% O_2 and 10% CO_2: 5% O_2 at 18ºC prolonging shelf life up to 24 days. In addition, fruits in 5% CO_2: 5% O_2 storage condition was maintained the better pericarp colours and reduced off-flavour formation than other storage conditions (Sangkasanya and Meenune, 2010).

1.2.6.2 Low temperature storage

Temperature is the single most important factor governing the maintenance of postharvest quality in fruits and vegetables. Temperature abuse during transportation, storage and marketing of fresh products is a primary concern in the fresh produce industry, because poor temperature control can lead to the deterioration of a packaged product due to an increase in product metabolism and growth of food spoilage organisms (Chakraverty *et al.,* 2003). Low temperature has been used to extend the shelf life of fruits and vegetables and low temperature can minimize the loss of nutritional in fruit and vegetable (Dangcham *et al.,* 2008; Sandhya, 2010). Low temperature is beneficial because the rates of respiration and of general metabolism are reduced and its beneficial effects for delaying senescence and maintain quality (Concellon *et al.,* 2007). Fruit deterioration such as skin desiccation, colour changes and/or losses, firmness and disease development can be delayed by low temperature storage (Meng *et al.,* 2009; Ali *et al.,* 2004). Lichanporn *et al.*

(2008b) reported, longkong fruit stored at low temperature such as 13ºC could maintain a better quality.

However, adverse low temperature effect is susceptible to produce chilling injury (Wills *et al.,* 1998). Chilling injury is a physiological damage that occurs when plants or plant parts are exposed to low, but not freezing temperature (Lyons, 1973; Raison and Lyons, 1986). In general, development of chilling injury is based on primary and secondary events (Wang, 1990). The primary event occurs when the tissue is exposed to temperature below the critical or threshold temperatures, which correlates with the onset of chilling injury. The secondary event, which follows the primary event include the metabolic and ionic imbalances, the loss of cellular integrity, and similar events that lead to visible symptoms of injury. Secondary event is related to both time and temperature dependants. If the chilling stress is short time exposure they are reversible. However if the stress is maintained, the imbalance of cellular integrity become excessive and the process becomes irreversible (Rugkong, 2009). The symptoms of chilling injury generally develop after removal from the chilling temperature to non-chilling temperature and they vary with the different plant commodities and the severity of injury (Lyons, 1973).

The commonly occurring symptoms include cellular change, altered metabolism, surface lesions, water soaking appearance, internal discoloration, pitting, scalding, accelerate senescence, increase susceptibility to decay and failure to normal ripen (Lisa, 1998; Rugkong, 2009). However, under low temperature longkong fruit is susceptible to chilling injury. Chilling injury symptoms of longkong fruit is pitting and scalding of the skin. Skin browning occurs rapidly if fruits are stored at temperatures less than 13ºC and the maximum storage life is about 2 weeks at 15ºC with relative humidity 85-90%. Chilling injury occurs after storage at 0°C for 5 days and the symptoms include skin browning, fermented flavour and softer fruit with a water soaked appearance (Wang, 1990; Ketsa and Paull, 2008).

1.2.6.3 Effect of some chemical treatments

Postharvest life of horticultural commodities are very short due to several problems persisted during storage such as pest attack and microbial growth (Del *et al.*, 2007; Najafi and Khodaparast, 2009; Sharma *et al.*, 2009), browning

(Yingsanga *et al.*, 2008; Segovia-Bravo *et al.*, 2009), off flavour formation (Sangkasanya and Meenune, 2010) and chilling injury (Florissen *et al.*, 1996; Wang and Qi, 1997; González-Aguilar *et al.*, 2000; Cao *et al.*, 2009). Initially, postharvest chemical treatments are mainly applied to fruits and vegetables to address pest and disease problems that they arise during the transport and storage of commodity (Barkai-Golan, 2001). Recently, chemical treatments are also used on improvement of quality and shelf life in plant commodities during storage, especially in fruits (Thompson, 2004). There are several chemicals widely used on fruit treatments, and those chemicals approved by food safety organization among several continents. The control of postharvest fruit deterioration by chemicals is explained in the following section.

1.2.6.3.1 Antibrowning agents

Many tropical, sub tropical and temperate fruits are susceptible to browning and therefore cause the economic losses to the agriculturist (Kasai and Arakawa, 2010; Sandhya, 2010). Many techniques such as, the use of killer enzymes, naturally occurring enzyme inhibitors and ionizing radiation have been explored and exploited as alternatives to heat treatment and the risks associated with certain chemical treatments to prevent enzymatic browning (Marshall *et al.,* 2000). Six categories of polyphenol oxidase inhibitors are applicable in the prevention of enzymatic browning. The reducing agents are 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. Enzymes generally possess metal ions at their active sites (He *et al.,* 2008). Removal of these ions by chelating agents can therefore render enzymes inactive. Complexing agents are forming a semi permeable membrane on the surface of the product to limiting the availability of oxygen to control the enzymatic browning reaction.

The optimum pH for PPO has been reported to be from acid to neutral in most fruits and vegetables. The optimum activity is observed at pH 6 to 6.5, and minimum activity is detected below pH 4.5 (Ayala-Zavala and Gonzalez-Aguilar, 2011). This is the reason behind the use of chemicals that lower the products pH or acidulants to control enzymatic browning (Rocculi *et al.,* 2007; Altunkaya and Gokmen, 2009). Acidulants, such as citric, malic and phosphoric acids are capable of

lowering the pH of a system, thus reducing the polyphenol oxidase activity (Rojas-Graü *et al.*, 2007.). Citric acid is widely used as an acidulant and is typically applied at levels ranging between 0.5% and 2% (w/v) for the prevention of browning in fruits and vegetables. Citric acid can be used in combination with other antibrowning agents, such as ascorbic or erythorbic acids and their neutral salts, for the chelation of prooxidants and for the inactivation of PPO. In addition to lowering the pH, citric acid acts by chelating the copper at the active site of the enzyme (Marshall *et al*., 2000). Table 2 shows the representative inhibitors of enzymatic browning with the examples.

1.2.6.3.2 Fungicide treatment

Fungicides are most effective when the treated fruit possesses some intrinsic resistance to infection; environmental conditions are least favorable to pathogen activity; and pathogen populations are low (Nallathambi *et al.,* 2009). A few chemicals such as chlorine, sulphur dioxide (SO_2), benzimidazole fungicides are true fungicides. Chlorine is commonly added to wash water to kill bacteria and fungi. Sulphur dioxide is lethal to botrytis on grapes. Benzimidazole fungicides, including benomyl, thiabendazole, thiophanate-methyl, and carbendazim are extensively studied and applied to a broad range of fruit crops (Yoshioka *et al.,* 2010; Moral *et al.,* 2009). The benzimidazole is highly effective in controlling decays caused by Penicillium spp. in multiple fruit crops, as well as botrytis cinerea, monilinia spp., and several less important postharvest pathogens, but are not effective against Alternaria spp., Mucor spp., or Rhizopus stolonifer. These fungicides, especially benomyl, are capable of controlling longkong from pathogens (Lichanporn *et al.,* 2009).

1.2.6.3.3 Methyl Jasmonate

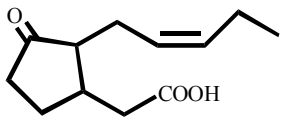
Methyl jasmonate (MeJA) and its free acid jasmonic acid collectively referred to jasmonates. These are important cellular regulators involved in diverse developmental processes, such as seed germination, root growth, fertility, fruit ripening and senescence (Meng *et al.,* 2009; Ziosi *et al.,* 2009). Jasmonates stimulate the plant defence mechanism to response the insect driven wounding, various pathogens and environmental stresses such as drought, low temperature and salinity.

Table 2. Representative inhibitors of enzymatic browning

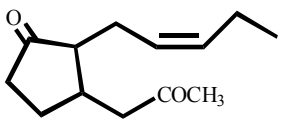
Representative inhibitors	Particulars
Reducing agent	Sulphiting agents
	Ascorbic acid and analogs
	Cysteine
	Glutathione
Chelating agents	Phosphates
	Ethylenediaminetetraacetic acid
	Organic acid
Acidulants	Citric acid
	Phosphoric acid
Enzyme inhibitors	Aromatic carboxylic acids
	Aliphatic alcohol
	Anions
	Peptides
Enzyme treatments	Oxygenase
	Catechol-*o*-methyl transferase
	proteases
Complexing agents	Cyclodextrins, Chitosan

Source: Marshall *et al.*(2000)

MeJA is a fragrant volatile compound initially identified from flowers of jasminum grandiflorum and has proven to be distributed ubiquitously in the plant kingdom. MeJA synthesized in plant via octadeconoid pathway. (Jong and Yang, 2003; Jong and Yang, 2007). Jasmonic acid (JA) and its methyl ester have been found to occur naturally in a wide range of higher plants (Rahim and Hadian, 2007). Since MeJA is more volatile, it can act as a messenger to neighbouring undamaged plants, telling them that an attack is under way and prompting them to produce defensive chemicals before they are attacked than JA, so that the efficacy of the MeJA more than JA (Ziosi *et al.*, 2009; Jong and Yang, 2007). MeJA are prime example of aroma compound that are also highly potent phytoeffectors (Dietmar *et al,* 1997). Figure 5 shows JA and MeJA structures.



Jasmonic acid (JA)



Methyl Jasmonate (MeJA)

Figure 5. Jasmonic acid (JA) and methyl jasmonate (MeJA) structures
Source: Jong and Yang (2003)

MeJA has been implicated in the signalling pathways mediating induced defense responses in chilling stressed plants and other onset of the tolerance has often correlated with the accumulation of defense related enzymes and compounds (Creelman and Mullet, 1997; Liu *et al.,* 2010). MeJA was shown to be involved in interplant communication of stresses (George and Moline, 1998). MeJA activity on chilling stress was studied by many researchers in various fruits and vegetables (Siripatrawan and Assatarakul, 2009; Wang *et al.,* 2009; Cao *et al*., 2010; Sayyari *et al*., 2011; Li *et al*., 2012; Zhang *et al*., 2012). When exogenously applied, it can be shown that postharvest diseases reduced and an improved chilling tolerance and reduced incidence of chilling injury in tomato, loquat, peach, okra and bamboo (Cao *et al*., 2012; Cai *et al*., 2011; Zhang *et al*., 2012). Protection of plant cells against environmental stress by MeJA may also associate with involving special gene expression. Exogenous treatment of MeJA was able to reduce chilling injury by enhancing the activity of antioxidant enzymes such as SOD, CAT, ascorbate peroxidase (APX) to maintain the cell membrane from chilling stress (Li *et al*., 2012). Therefore, it limits the interaction between the browning enzymes such as PPO, POD and PAL and substrate in many fruits and vegetables (Cao *et al.,* 2009; Jin *et al.,* 2009; Cao *et al.,* 2010). Recently, Cao *et al*. (2012) found that MeJA enhanced the accumulation of proline and γ-aminobutyric acid (GABA), which is helped to improve the stability against chilling stress. MeJA increased the proline and GABA by increased the delta-1-pyrroline-5-carboxylate synthetase (P5C5) and ornithine aminotransferase (OAT). Zhang *et al*. (2012) found that MeJA increased the polyamines to alleviate the chilling injury in cherry tomato fruit.

1.3 Objectives

1. To investigate the changes in physiochemical quality of longkong fruit during four different weeks of on-tree maturation
2. To study the physiological and biochemical changes of longkong fruit under passive and active MAP storage.
3. To study the effect of using methyl jasmonate on the quality changes of longkong fruit during storage under low temperature.

CHAPTER 2

PHYSIOCHEMICAL QUALITY CHANGES OF LONGKONG FRUIT DURING FOUR DIFFERENT WEEKS OF ON-TREE MATURATION

2.1 Abstract

The optimum harvesting period of longkong from 13 to 16 weeks of maturation were used to analyse the physiochemical quality changes. Lightness (L*), yellowness (b*), hue angle (H°) and chroma value (C*) was decreased and conversely redness (a*) increased. The fruit weight was significantly increased from 21.21 to 24.93 g and the diameter was increased ($P<0.05$). Total soluble solids, pH, total sugar and reducing sugar increased while titratable acidity decreased at the end of maturation period ($P<0.05$). The 1,1-diphenyl-2-picrylhydrazyl radical scavenging activity, ferric reducing antioxidant power and total phenolic content increased throughout the maturation period ($P<0.05$). The superoxide dismutase, catalase activities were increased during the maturation period and at the end, activities were decreased. Conversely, peroxidase enzyme activity was decreased during maturation and at the end it slightly increased. Polygalacturonase and pectin methylesterase activities were increased during maturation. Pericarp phenolics were found to be increased in throughout the maturation period. The polyphenol oxidase activity significantly increased throughout the maturation. Peroxidase and phenylalanine ammonia lyase activities were increased at the beginning but after that, they decreased at the end of the maturation period. Peel epidermal trichomes losses on the surface and parenchyma cell changes in the cross section were found during these stages.

2.2 Introduction

Longkong (*Aglaia dookkoo* Griff.) is a non-climacteric and tropical fruit, and belongs to the meliaceae family that also consists of langsat, lanson, duku, lanzones and duku trengganu and duku jahore (Ketsa and Paull, 2008; Sangkasanya and Meenune, 2010). The well-known and economic fruit longkong is much cultivated in peninsular Thailand and especially in the southern Thailand (Onthong *et al*., 2006; Paull, 2004). It is also cultivated in the Philippines, Vietnam, Myanmar, India, Sri Lanka, Australia, Surinam and Puerto Rico (Ketsa and Paull, 2008). The longkong fruit is globular in shape with an average size of 1.2-2.4 inches in diameter. It has a brittle and rough skin. Longkong develops between 15 and 25 fruits per bunch with little non-sticky sap on the skin. It is almost seedless with five segments of white translucent flesh (Paull, 2004; Salakpetch, 2000). The optimum harvesting condition of longkong lasts from 13 to 16 weeks after anthesis. The maturity index for a horticultural commodity is a set of measurements that can be used to identify whether a particular commodity is mature. The parameters often used as indicators of maturity are fruit size, titratable acidity, soluble solids content, peel colour and sugar content (Fadda and Mulas, 2010).

Generally, the unripe pale green colour of the longkong skin becomes bright yellow when it ripens, the astringency in the flesh tends to decline, and the sugar increases up to 6-fold (Paull *et al*., 1987). The sweet and sour taste of longkong, jointly with its faintly aromatic smell and nutritious qualities, make this fruit a potentially valuable export (Lichanporn *et al*., 2009). Longkong fruit contains a variety of nutrients, including proteins, carbohydrates, and has a low fat content and a high amount of minerals (Ketsa and Paull, 2008). Lim *et al*. (2007) have reported that langsat fruit had a high level of secondary antioxidant potential activity. Antioxidants are categorized into two groups such as enzymatic and non enzymatic antioxidants. Enzymatic antioxidants such as superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase is endogenously produced in human systems and they are in under normal conditions, defence against the free radicals and ROS (Wootton-Beard *et al*., 2011). Non-enzymatic antioxidants such as polyphenols, vitamins and minerals are substances that can avoid or delay oxidative damage of lipids, proteins and nucleic acids by reactive oxygen species (Du Toit *et al.*, 2001; Isabelle *et al.*, 2010; Lim *et al.*,

2007; Nurliyana *et al*., 2010). These can cause degenerative diseases, such as cancer, arthritis, arteriosclerosis, heart disease, inflammation, brain dysfunction and acceleration of the ageing process (Rodrigo *et al*., 2011).

On the other hand, longkong fruit deteriorates quickly after harvest due to pericarp browning, which is a major factor in affecting fruit quality, storage life and marketable value. Texture, appearance loss and off flavour occur after harvesting. The shelf life of most fruits are strongly limited by their loss of texture (Dray and Cutsem, 2008). Plant tissue softening is due to cell wall degradation by hydrolytic enzymes, including polygalacturonase and pectin methylesterase (Brummell and Harpster, 2001; Qiuping and Wenshui, 2007; Hangermann and Austin, 1986). Postharvest longkong pericarp browning has been attributed to the oxidation of phenolics caused by the polyphenol oxidase (PPO) and/or phenylalanine ammonia lyase (PAL) (Lichanporn *et al*., 2009). Phenylalanine ammonia lyase (PAL) converts phenyl alanine to phenolics via the phenylpropanoid pathway (André *et al*., 2009). Polyphenol oxidase (PPO) and peroxidase (POD) can catalyse the oxidation of phenols to produce brown pigments (Francisco and Juan, 2001; Nokthai *et al*., 2010). However, there is no published data available for antioxidant and browning related enzyme activity changes in longkong during maturation. This present study aimed to identify the physiochemical, antioxidant and browning enzyme quality changes during four different weeks of on-tree maturation.

2.3 Materials and methods

2.3.1 Plant material

Longkong fruits, at 13, 14, 15 and 16th weeks after anthesis, were obtained from tagged trees in a garden in Natawee, Songkhla province, Thailand. The fruits were harvested in the morning and transported to the laboratory on the same day within 2 h at an ambient temperature. After that, the fruits were immediately screened to obtain fruit that was free from any apparent skin damage and of uniform size. The fruits obtained were immediately used for the following experiments described below.

2.3.2 Physical quality

Measurement of fruit pericarp colour

The surface colour on four sides of an individual fruit was measured by using a Hunter Lab colourimeter in term of CIE lightness (L*), redness (a*) and yellowness (b*) values (Sapii *et al*., 2000). Hue angle and chroma value was measured in accordance to the method of McGuire (1992). Hue angle (H°) and chroma values (C*) was calculated as arctangent (b*/a*) (0° = red-purple, 90° = yellow, 180° = bluish-green, 270° = blue) and chroma (colour saturation) value was calculated as $C^* = (a^{*2} + b^{*2})^{1/2}$.

Measurement of fruit weight loss

Longkong fruit weight loss was measured by using digital weighing balance. Weight loss was expressed as the percentage of weight loss with respect to the initial weight.

Measurement of fruit diameter

Longkong fruit diameter was measured by using a vernier scale and the values were expressed in cm.

2.3.3 Chemical quality

Peeled and deseeded longkong flesh homogenate was prepared by blending, then filtered using cheesecloth and then subjected to chemical analysis.

Measurement of fruit pH

The pH was measured by using a Sartorius PB-20 (Germany) digital pH meter at ambient temperature after calibrated with pH 4.0 and 7.0.

Measurement of total soluble solids

The TSS was determined by using an Atago 1E (Japan) hand refractometer at 25°C. The results were expressed as °Brix.

Measurement of titratable acidity

The titratable acidity (TA) was determined in accordance to Sangkasanya and Meenune (2010). The results were expressed as percentages of citric acid content.

Measurement of total and reducing sugar

The total sugar (TS) and reducing sugar (RS) contents were quantified in according with the Ranganna (1986) volumetric method. The results were expressed as percentage of D-glucose content.

Measurement of ascorbic acid

Ascorbic acid (AsA) was analysed by an indophenol titration method in accord with Nielsen (2010). The results were expressed as ascorbic acid content (mg per g of fresh weight)

Measurement of total phenolic content

Total phenolic content of fruit flesh was determined by using the Folin-Ciocalteu reagent (Lim *et al*., 2007). Edible portion (5 g) was homogenized with 20 ml of 50% ethanol in a mortar and pestle method at 4°C. The homogenate was filtered with cheesecloth and then, the filterate was centrifuged at 10,000*g* at 4°C for 10 min. The supernatant collected and used for total phenolic measurement. A 0.3 ml of fruit extract was placed in test tubes followed by 1.5 ml of Folin-Ciocalteu reagent (1:10 dilution with distilled water) and 1.2 ml of sodium carbonate (7.5% w/v). The tubes were vortexed, covered with parafilm and allowed to stand for 30 min at room temperature. Absorbance was measured at 765 nm. Total phenol contents were expressed as gallic acid equivalents mg per g fresh fruit.

Total phenolic content of fruit pericarp tissue was analysed by the method of Singleton and Rossi (1965). Pericarp tissues 2 g were homogenized with 20 ml of 80% ethanol (1:1 w/v) in a mortar and pestle method. The homogenized sample was centrifuged at 12,000*g* for 20 min. A 0.4 ml of supernatant was mixed with 0.4 ml of Folin-Ciocalteu reagent and 1 ml of 7% sodium carbonate solution. The volume was increased to 10 ml in distilled water and vortexed the mixture, then incubated for

1 hr at room temperature. Absorbance was measured at 750 nm using a spectrophotometer. Total phenol contents were expressed as gallic acid equivalents mg per g fresh fruit.

2.3.4 Determination of radical scavenging ability

Edible portions of longkong, 25 g, were deseeded and homogenised using a mortar and pestle at 4°C. Then the homogenised sample was transferred into a 100 ml volumetric flask and the volume made up with 50% ethanol. The mixture was shaken with a vibrator for 10 min, and then filtered under suction if the filtrate appeared to be very cloudy. The filtrate was centrifuged at 10,000*g* at 4°C for 10 min to obtain a clear supernatant solution (Lim *et al*., 2007; Lichanporn *et al*., 2009). The supernatant was immediately used for radical scavenging ability analysis, such as 1, 1-diphenyl-2-picrylhydrazyl (DPPH) scavenging ability and ferric reducing power ability (FRAP).

Measurement of DPPH radical scavenging activity

DPPH assays were measured according to the method of Binsan *et al*. (2008). A 1.5 ml of the sample was added to the test tube and followed with 1.5 ml of 0.15 mM DPPH in 95% ethanol. The mixture was mixed vigorously and kept in a dark place for 30 min at room temperature and then a sample was measured at 517 nm. Distilled water was used instead of the sample for the blank. A standard curve was prepared by using ascorbic acid. The activity was expressed as ascorbic acid equivalents mg per g fresh weight.

Measurement of ferric reducing power

The ferric reducing power of the fruit extract was determined in accordance with the method of Benzie and Strain (1996). Stock solution was made, of 300 mM acetate buffer (pH 3.6), 10 mM 2,4,6-tripyridyl-s-triazine (TPTZ) solution in 40 mM hydrochloric acid, and 20 mM ferric chloride hexahydrate ($FeCl_3.6H_2O$). The FRAP solution was freshly prepared by adding 25 ml of acetate buffer, 2.5 ml of TPTZ solution and 2.5 ml of $FeCl_3.6H_2O$ solution. The mixed solution was incubated at 37°C for 30 min. A sample of 150 µl was mixed with 2850 µl of FRAP solution and kept for 30 min in dark. The absorbance was measured at 593 nm. A standard

curve was prepared by using ascorbic acid. The activity was expressed as ascorbic acid equivalents mg per g fresh weight.

2.3.5 Enzyme activity analysis

Longkong fruit flesh was used for antioxidant (SOD, CAT and POD), textural enzymes (PG and PME) analysis. On the other hand, the browning related enzyme (PAL, PPO and POD) activity was analyzed in longkong fruit pericarp.

Extraction and determination of superoxide dismutase activity

Longkong deseeded flesh (10 g) was homogenized with 25 ml of buffer solution (100 mM sodium phosphate buffer (pH 6.4)) and 0.5 g polyvinyl pyrrolidone in a mortar and pestle at 4°C. The homogenate was filtered through one layer of cheesecloth and then the filtrate was centrifuged at 19,000*g* for 30 min at 4°C. After that, the supernatants of crude enzyme extract were collected and used for superoxide dismutase (SOD) analysis. Determination of SOD was performed as described in a method of Constantine and Stanley (1977). The reaction mixture (3 ml) contained 50 mM sodium phosphate buffer (pH 7.8), 65 mM methionine, 150 µM nitroblue tetrazolium (NBT), 0.5 mM EDTA, 20 µM riboflavin and 0.1 ml of the enzyme extract. The mixtures were illuminated by fluorescent light (60 µmol/m^2/s) for 10 min and the absorbance was then determined at 560 nm. Identical solution held in the dark served as blank. One unit of SOD was defined as the amount of enzyme that caused a 50% decrease of the SOD-inhibitable NBT reduction. The specific activity was expressed as unit per g of fresh weight.

Extraction and determination of catalase activity

Longkong deseeded flesh (10 g) was homogenized with 25 ml of buffer solution (50 mM sodium phosphate buffer (pH 7.0)) and 0.5 g polyvinyl pyrrolidone in a mortar and pestle at 4°C. The homogenate was filtered through one layer of cheesecloth and then the filtrate was centrifuged at 19,000*g* for 30 min at 4°C. After that, the supernatants of crude enzyme extracts were collected and used for catalase (CAT) analysis. CAT activity was measured according to the method described by Beers and Sizer (1952). The reaction mixture contained, 2 ml of sodium phosphate buffer (50 mM, pH 7.0), 0.5 ml H_2O_2 (40 mM) and 0.5 ml crude enzyme

extract. The decomposition of H_2O_2 was measured by the decline in absorbance at 240 nm. One unit of CAT was defined as the amount of enzyme that decomposing 1 mM of H_2O_2 per minute at pH 7.0 and 25°C. The specific activity was expressed as unit per g of fresh weight.

Extraction and determination of peroxidase activity

Longkong deseeded flesh (10 g) was homogenized with 25 ml of buffer solution (100 mM sodium phosphate buffer (pH 6.4)) and 0.5 g polyvinyl pyrrolidone in a mortar and pestle at 4°C. The homogenate was filtered through one layer of cheesecloth and then the filtrate was centrifuged at 19,000*g* for 30 min at 4°C. After that, the supernatants of crude enzyme extracts were collected and used for peroxidase (POD) analysis. POD activity was assayed in accord with the method of Jiang *et al*. (2002). The 0.1 ml of enzyme was incubated in 2 ml buffered substrate (100 mM sodium phosphate, pH 6.4 and 8 mM guaiacol) at 30°C for 5 min and then, the increasing absorbance measured at 460 nm for 120 s after adding 0.9 ml of H_2O_2 (24 mM). One unit of POD activity was defined as the amount that caused of 0.01 in the absorbance per min. The specific activity was expressed as unit per g of fresh weight.

Extraction and determination of polygalacturonase (PG)

PG was extracted and assayed according to the method of Qiuping and Wenshui (2007). Longkong deseeded flesh (2 g) was homogenized with 10 ml of 0.2 mol/l acetic acid buffer (pH 6.0) in a mortar and pestle at 4°C. The homogenate was filtered through one layer of cheesecloth and then the filtrate was centrifuged at 11,000*g* for 20 min at 4°C. The supernatants of crude enzyme extract was collected and used for PG assay. The determination of PG activity was based on the hydrolytic release of galacturonic acid from polygalacturonic acid. The reaction mixture contained 0.3 ml of 1.0% (w/v) polygalacturonic acid in 40 mmol/l Na–acetate buffer (pH 4.6), 0.1 ml of crude enzyme extract and then followed by 1.9 ml of deionized water. The reaction mixture was incubated at 37 °C for 1 hr and the reaction was terminated by the addition of 1.5 ml of dinitrosalicylate reagent and immersion in a boiling water bath for 5 min. The final volume of the sample was adjusted to 25 ml with deionized water. Samples then cooled to room temperature and the absorbance

was measured at 540 nm. The released amount of galacturonic acid from polygalacturonic acid was obtained from the galacturonic acid standard curve. One unit of PG activity was defined as that amount of enzyme, which liberates 1 mg galacturonic acid under the given assay, conditions (60 min, 37°C). The specific activity was expressed as unit per g of fresh weight.

Extraction and determination of pectin methylesterase (PME)

Pectin methylesterase (PME) activity was measured according to the method of Hangermann and Austin (1986) with slight modification. Longkong deseeded flesh (5 g) was ground with 20 ml 8.8% (w/v) of NaCl and 0.5 g of polyvinylpyrrolidone (insoluble) at 4°C. The homogenate was filtered through one layer of cheesecloth and then the filtrate was centrifuged at 10,000*g* for 30 min at 4°C. The collected supernatant of crude extract was adjusted the pH to 7.5 and then assayed for PME activity. The activity was assayed in a reaction mixture containing 2.0 ml of 0.5% (w/v) pectin, 0.15 ml of 0.01% bromothymol blue, 0.75 ml of water, and 0.1 ml of enzyme extract. All solutions (pectin, indicator dye and water) were adjusted to pH 7.5 with 2 M NaOH just before the experiment starts. After adding the enzyme extract, the decrease in the absorbance at 620 nm was measured spectrophotometrically. Calculation of the activity was carried out against the standard curve as described by Hangermann and Austin (1986). One unit of PME activity was expressed as μmoles of H^+ produced per min. The specific activity was expressed as unit per g of fresh weight.

Extraction and determination of phenylalanine ammonia lyase

Pericarp tissues (2 g) from 20 fruits were homogenized in 20 ml of a 0.1 M sodium borate buffer (pH 8.0) solution contained 0.2 g of insoluble PVP, 5 mM mercaptoethanol, and 2 mM ethylene diamine tetra acetic acid (EDTA) at 4ºC. The homogenate was centrifuged for 20 min at 19,000*g* and 4ºC, and then the supernatant of crude extract was collected for the phenylalanine ammonia lyase (PAL) enzyme assay. PAL activity was determined in accord with the method of Jiang and Joyce (2003). A mixture of 0.1 ml enzyme extract and 2.9 ml of 0.1 M sodium borate buffer (pH 8.0) solution containing 3 mM l-phenylanine was incubated for 1 hr at 37ºC. An increase in the PAL activity at 290 nm, due to the formation of trans-cinnamate, was

measured spectrophotometrically. One unit of enzyme activity was defined as the amount that caused an increase of 0.01 in the absorbance per hr. The specific activity was expressed as unit per g of fresh weight.

Extraction and determination of polyphenol oxidase

Pericarp tissues (5 g) from 20 individual fruits were ground with 40 ml of 0.2 M sodium phosphate buffer (pH 6.4) and homogenised using a mortar and pestle at 4ºC. After that, the homogenised sample filtered through cheesecloth and then the filtrate was centrifuged at 12,000*g* for 30 min. The supernatant of crude extract was collected for measuring polyphenol oxidase (PPO) activity (Tian *et al*., 2002). The reaction mixture consisted of 3 ml of 0.5 M 4-methylcatechol in 0.2 M sodium phosphate buffer (pH 6.4) and 0.1 ml of the crude enzyme sample. The absorbance was measured at 398 nm at 25ºC for 1 min. One unit of enzyme activity was defined as an increased in one absorbance unit per min. The specific activity was expressed as unit per g of fresh weight.

Extraction and determination of peroxidase

Pericarp tissues (2 g) from 20 individual fruits were homogenized in 20 ml of 0.05 M phosphate buffer (pH 7) solution and 0.2 g of insoluble polyvinylpyrrolidone (PVP) using a mortar and pestle at 4ºC. The homogenate was filtered through cheesecloth and the filtrate was centrifuged for 20 min at 19,000*g* at 4ºC (Lichanporn *et al*., 2009). The supernatant of crude extract was collected for analysing peroxidase (POD) activity. POD activity, using guaiacol as a substrate, was analysed by the method of Zhang *et al*. (2005). A 3 ml reaction mixture contained 25 µl of crude enzyme extract, 2.78 ml of 0.05 M phosphate buffer (pH 7.0), 0.1 ml of 20 mM hydrogen peroxide (H_2O_2) and 0.1 ml of 20 mM guaiacol. An increased in POD activity was recorded at 470 nm for 2 min. One unit of enzyme activity was defined as the amount that caused a change of 0.01 in the absorbance per min. The specific activity was expressed as unit per g of fresh weight.

Ultrastructural analysis

Ultrastructural analysis of the pericarp tissue of both surface and cross-sections were measured (Lichanporn *et al*., 2009). The fruit pericarp tissues sampled on the zero day and 16th day of storage. Samples were fixed in 2.5% glutaraldehyde in a 0.1 M phosphate buffer with a pH of 7.2 for 2 hr. The specimens were rinsed twice in phosphate buffer for 20 min, and then once in distilled water for 20 min. The pericarp tissues dehydrated in a graded alcohol series, sputtered with platinum/palladium, and then dried by a critical point dryer. The dried peels were mounted on the stubs and twice gold-coated. Then, the samples were observed under the scanning electron microscope (Quanta 400, FEI, Praha, Czech Republic).

2.3.6 Statistical analysis

The experiment was laid out in a completely randomized design (CRD). Each physical and chemical analysis was done in three replications. The data represent the means ± standard deviation, and they were analysed by one-way analysis of variance (ANOVA) with the Statistical Package for Social Science (SPSS for windows, SPSS Inc., Chicago, IL, USA). The significance of the difference among maturation weeks were measured using Duncan's new multiple range test (DMRT), with a level of significance of 0.05.

2.4 Result and Discussion

2.4.1 Physical quality changes

Lightness (L*), redness (a*) and yellowness (b*), H° and C* in longkong pericarp was monitored at different weeks of on-tree maturation (Table 3). The L* value was non-significantly decreased, meanwhile b* value was significantly decreased and conversely a significant increase in a* value was observed during the maturation stages. On the other hand, H° and C* also decreased throughout the maturation. Longkong fruit on-tree maturation featured an increase in browning as evidenced by an increase in a* and decreased in L*, b*, H° and C* (Sapii *et al*., 2000). The increase in longkong browning was might be the oxidation of phenolics by PPO enzyme, which can be activated by air induced fruit surface damage (Lichanporn *et al*., 2009). Meanwhile, the longkong fruit weight and diameter were increased

gradually throughout the maturation stages (Table 4). The fruit weight was significantly increased, but the diameter of the fruit was not significantly increased.

Table 3. Physical quality changes of longkong fruit during four different weeks of on- tree maturation

Maturation Period	Colour			Hue angle (H°)	Chroma value (C*)
	Lightness (L*)	Yellowness (b*)	Redness (a*)		
13	68.97±1.8^{b}	39.73±1.09^{b}	5.84±0.80^{a}	80.70±0.90^{c}	41.28±1.19^{c}
14	67.22±1.6^{ab}	38.45±0.57^{b}	6.90±0.53^{a}	79.21±0.64^{b}	39.72±0.66^{b}
15	66.21±0.9^{ab}	36.52±1.06^{a}	9.19±1.02^{b}	74.59±1.13^{a}	39.00±1.27^{b}
16	65.54±1.3^{a}	35.36±0.57^{a}	9.28±1.11^{b}	73.87±1.47^{a}	37.37±0.83^{a}

Note: Different superscripts in the same column indicate the significant differences ($P<0.05$) (n=3).

2.4.2 Chemical quality changes

The longkong fruit chemical qualities, such as, pH, TA, TSS, TS and RS are represented in Table 5. The pH was increased significantly during on-tree maturation and in the meantime, TA was significantly decreased. Meanwhile, TSS increased significantly throughout maturation. The increase in TSS was probably due to the solubilisation of neutral sugars from carbohydrate polymer residues (Beirao-da-Costa *et al*., 2006). An increase in TSS and a decrease in TA resulted in an increase in the TSS/TA ratio and led to the sweetness of longkong (Sapii *et al*., 2000). The TSS/TA ratio of the initial value of longkong was 20.65, and it increased to 32 at the end of maturation. Longkong with a TSS/TA ratio of approximately 20 was the recommended level in terms of taste, with the fruit becoming sweeter as the ratio increased (Ahmad *et al*., 1998). The total sugar and reducing sugar content of longkong fruit was significantly increased and the increase in sugar was correlated with the increased level of TSS.

Table 4. Longkong fruit weight and diameter changes during four different weeks of on- tree maturation

Maturation Period	Fruit weight (g)	Fruit diameter (cm)
13	21.21±1.46[a]	3.41±0.10[NS]
14	22.12±0.95[a]	3.50±0.11[NS]
15	23.50±0.86[ab]	3.58±0.07[NS]
16	24.93±1.59[b]	3.61±0.19[NS]

Note: Different superscripts in the same column indicate the significant differences (P<0.05) (n=3). NS = Non-significant differences

2.4.3 Fruit radical scavenging ability

The most abundant antioxidants in longkong fruits are polyphenols and vitamin C (Ketsa and Paull, 2008; Lichanporn *et al*., 2009; Lim *et al*., 2007). TPC was significantly increased with the increased weeks of maturation and at the end, it was 0.59 mg (Table 6). Lim *et al*. (2007) have reported that longkong fruit had the higher level of polyphenols. The AsA content of longkong was not significantly increased at the initial stage of ripening, but after that, slight changes were noticed throughout the maturation (Table 6). An increase in AsA as the fruit matures was due to the breakdown of starch to glucose, which is used in the biosynthesis of ascorbic acid (Lim *et al*., 2006). Meanwhile the primary antioxidant activity, such as DPPH scavenging ability and FRAP was also found to be significantly increased during the on-tree maturation (Table 6). This result is in agreement with the maturation period of tropical and non-climacteric fruits, such as pomegranate, rambutan and cactus fruits (Kulkarni and Aradhya, 2005; Thitilertdecha and Rakariyatham, 2011; Cayupán *et al*., 2011). However, both antioxidant activities expressed in AsA equivalent were higher than the AsA content in longkong. It indicates that the increased DPPH scavenging ability and FRAP might be due to other compounds, such as polyphenols.

Table 5. Chemical quality changes of longkong fruit during four different weeks of on-tree maturation

Maturation period	pH	TSS (º Brix)	Titratable acidity (% as citric acid)	TSS/Titratable acidity ratio	Total sugar (% as D-glucose)	Reducing sugar (% as D-glucose)
13	3.99±0.01[a]	15.00±0.00[a]	0.73±0.00[d]	20.65±0.18[a]	12.61±0.06[a]	2.79±0.00[a]
14	4.29±0.03[b]	16.00±0.00[b]	0.65±0.01[c]	24.75±0.28[b]	12.80±0.11[b]	2.88±0.02[b]
15	4.41±0.02[c]	16.80±0.00[c]	0.60±0.01[b]	28.23±0.35[c]	14.15±0.01[c]	3.08±0.02[c]
16	4.51±0.01[d]	17.00±0.00[d]	0.53±0.01[a]	32.01±0.44[d]	15.00±0.04[d]	4.21±0.05[d]

Note: Different superscripts in the same column indicate the significant differences ($P<0.05$) (n=3).

Table 6. Antioxidant activity of longkong fruit during four different weeks of on-tree maturation

Maturation Period	Total phenolic content (Gallic acid equivalence mg/g of fresh weight)	Ascorbic acid (mg/g of fresh weight)	DPPH scavenging ability (Ascorbic acid equivalence mg/g of fresh weight)	FRAP activity (Ascorbic acid equivalence mg/g of fresh weight)
13	0.43±0.01[a]	0.025±0.00[a]	0.049±0.00[a]	0.050±0.00[a]
14	0.54±0.02[b]	0.035±0.00[b]	0.061±0.00[b]	0.053±0.00[b]
15	0.54±0.01[b]	0.037±0.00[b]	0.064±0.00[c]	0.058±0.00[c]
16	0.59±0.02[c]	0.034±0.00[b]	0.093±0.00[d]	0.070±0.00[d]

Note: Different superscripts in the same column indicate the significant differences ($P<0.05$) (n=3).

2.4.4 Fruit enzyme activity

Fruit antioxidant enzyme activity

Fruit ripening has been expressed as an oxidative phenomenon, which produced the reactive oxygen species (ROS), such as H_2O_2 and superoxide anion. The increased ROS production might be induced the fruit senescence and deterioration process. ROS detoxifying enzymes such as SOD, CAT and POD are implying the major role on the protection of fruits from oxidative damage (Sala, 1998). Longkong fruit antioxidant enzymes such as SOD, CAT and POD activity changes during different weeks of on-maturation are represented in Table 7. SOD and CAT enzymes increased in activity during the maturation period and at the end of maturation they were slightly decreased to 21.90 and 15.17 unit/g of fresh weight, respectively ($P<0.05$). On the other hand, POD activity at initially found in higher activity (0.051 unit/g of fresh weight), then it found to be decreased throughout the maturation and at the end it was 0.032 unit/g of fresh weight ($P<0.05$). Previous report on the non-climacteric fruits such as orange and blueberry maturation study were also had the relative correlation with our findings (Huang *et al*., 2007; Wang and Zheng, 2001). SOD enzyme catalyzes the breakdown of $O_2^{\cdot -}$ and H_2O_2, removes singlet oxygen as well as $O_2^{\cdot -}$ to prevents formation of $OH^{\cdot}$ and it has been implicated as an essential

defence against the potential toxicity of oxygen (Huang *et al*., 2007). CAT and POD is one of the most important enzymes scavenging the active oxygen species in plant cells and they are responsible for scavenging H_2O_2.

Table 7. Changes in antioxidant enzymes of longkong fruit during four different weeks of on-tree maturation

Maturation Period	SOD activity (units/g of fresh weight)	CAT activity (units/g of fresh weight)	POD activity (units/g of fresh weight)
13	15.72±1.32[a]	13.72±0.88[a]	0.051±0.00[c]
14	17.62±1.00[b]	13.80±0.05[b]	0.032±0.00[b]
15	23.49±1.47[d]	16.04±0.03[d]	0.024±0.00[a]
16	21.90±1.65[c]	15.17±0.06[c]	0.032±0.00[b]

Note: Different superscripts in the same column indicate the significant differences (P<0.05) (n=3).

Texture enzymes (PG and PME activity)

The textural enzymes such as PG and PME activity changes of longkong during four different weeks of on tree maturation are described in Table 8. PG and PME activity changes were significantly found in longkong fruit during different maturation weeks (P<0.05). The PG and PME activity was sharply increased for up to 15 weeks of maturation and then it tend to decreased at the end maturation period. The increased activity of textural enzymes was indicated the textural and firmness changes in longkong fruit during the increased maturation weeks. Draye and Van Cutsem (2008) have reported that strawberry fruit PME activity increased during early stage of maturation and lateral stage it decreased. Liu *et al*. (2008) have reported, PME played an important role in producing PG. PG plays an effect on maturation after PME worked actively. PG is a primary enzyme that plays an important role in the solubilisation of pectin during maturation in fruits (Prasanna *et al*., 2007). During fruit maturation, pectinases such as PG and PME activity increases in the fruit pulp and therefore, many pectins are dissolved by this enzymes and brings the softer fruit texture (Liu *et al*., 2008).

Table 8. Changes in textural enzymes (PG and PME) of longkong fruit during four different weeks of on tree maturation

Maturation period	PG activity (units/g of fresh weight)	PME activity (units/g of fresh weight)
13	0.14±0.00[b]	1.98±0.29[a]
14	0.12±0.00[a]	2.44±0.02[b]
15	0.16±0.00[d]	2.85±0.20[d]
16	0.15±0.00[c]	3.04±0.28[c]

Note: Different superscripts in the same column indicate the significant differences (P<0.05) (n=3).

Pericarp total phenolics and browning related enzymes

Longkong pericarp total phenolics and browning related enzyme activities are shown in Table 9. The pericarp phenolics were decreased significantly throughout the maturation period (P<0.05). Whereas, the PAL activity of longkong was significantly increased at the beginning of maturation, afterwards at the late stages of maturation it decreased slightly (Table 9). The decreases in pericarp phenolics were correlated with the decreased PAL activity. PAL is the first enzyme in the phenylpropanoid pathway and plays an important role in the synthesis of phenolic compounds in plants (André *et al*., 2009; Dixon and Paira, 1995; Pina and Errea, 2008). Phenolic compounds are a group of chemical substances, which can be responsible for colour. They are present in most fruits and vegetables and act as a key substrate for enzymatic browning (Alasalvar *et al*., 2001; Balasundram *et al*., 2006; Nokthai *et al*., 2010). The enzymatic browning of fruit and vegetables is mainly attributed to the oxidation of phenolics by PPO and/or POD (Jiang *et al*., 2004; Walker, 1995). Lichanporn *et al*. (2009) reported that the PAL and PPO activities were involved in longkong pericarp browning.

The PPO and POD activity of longkong increased significantly during the maturation of the fruit (Table 9). The increased activity of PPO and POD is highly linked with the decreased phenolics content in longkong pericarp. It was found that PPO increased during the ripening, but the increased level was less than the previously reported litchi (Sun *et al*., 2009), olives (Goupy *et al*., 1991), loquat fruit

(Vela *et al*., 2002) and apple (Murata *et al*., 1995). In the meantime, POD increased up to the 15th week of maturation and then at the 16th week, it decreased slightly. The increase in the POD activity could be associated with the enhanced lignifications of the pericarp tissue during fruit maturation and ripening (Cai *et al*., 2006). The increased browning related enzyme activity was lower than the litchi fruit during ripening (Sun *et al*., 2009).

Table 9. Changes in pericarp phenolics and browning related enzymes on longkong during four different weeks of on-tree maturation

Maturation period	Pericarp phenolics (gallic acid equivalence mg/g of fresh weight)	PAL (units/g of fresh weight)	PPO (units/g of fresh weight)	POD (units/g of fresh weight)
13	0.89 ± 0.02^{c}	0.097 ± 0.00^{c}	8.69 ± 0.54^{a}	0.33 ± 0.02^{a}
14	0.97 ± 0.01^{d}	0.099 ± 0.00^{d}	10.29 ± 0.61^{b}	0.38 ± 0.02^{b}
15	0.81 ± 0.00^{b}	0.092 ± 0.00^{a}	12.87 ± 0.50^{c}	0.50 ± 0.04^{c}
16	0.78 ± 0.00^{a}	0.093 ± 0.00^{b}	14.75 ± 0.21^{d}	0.41 ± 0.03^{b}

Note: Different superscripts in the same column indicate the significant differences ($P<0.05$) (n=3).

2.4.5 Pericarp ultrastructural changes

Ultrastructural changes in the longkong pericarp surface sections and cross sections were observed during the maturation stages (Fig. 6). Initially, at the 13th week of maturation, no changes were found in the epidermal trichomes on the surface and hyperdermal changes in the cross section. However, in the 14, 15 and 16th weeks of on-tree maturation, the epidermal trichomes losses were found on the surface section. Longkong pericarp epidermal trichomes contained the PPO enzyme, the changes in trichomes might be accelerated PPO enzyme activity and consequently, pericarp browning is increased. The changes in epidermal trichomes could be induced by prolonged exposure in the atmosphere. In addition, an enlarged size of parenchyma cells on the hyperdermal tissue was found.

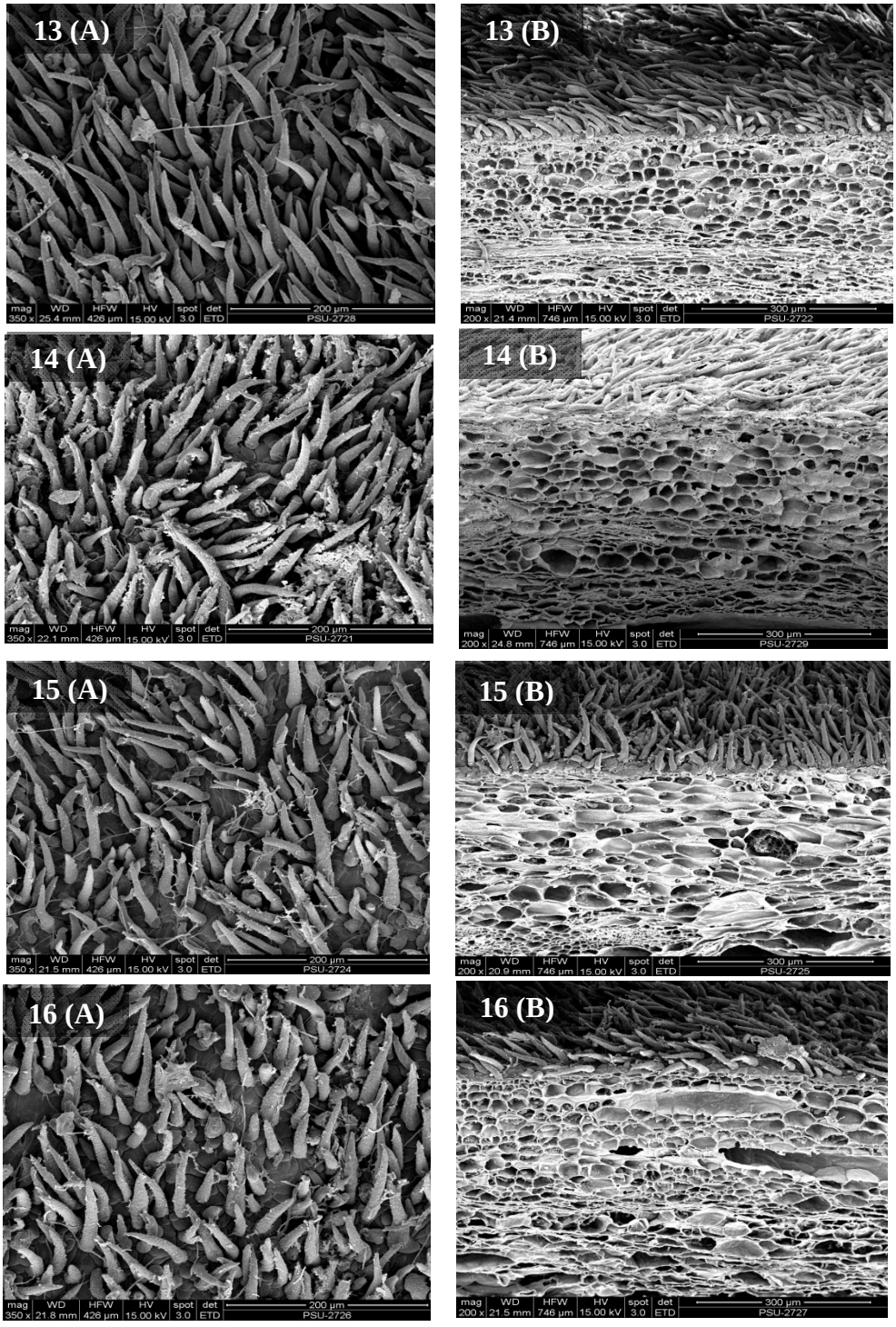


Figure 6. Ultrastructural changes of longkong peel on the 13, 14, 15 and 16th weeks of on-tree maturation

Note: 13 (A), 14 (A), 15 (A) and 16 (A) are represented the surface section of peel, while 13 (B), 14 (B), 15 (B) and 16 (B) are represented the peel cross section

2.5 Conclusion

Increased PPO, POD and PAL activities and ultrastructural changes were likely to be associated with the longkong pericarp colour changes. The fruit weight, the nutritional quality as indicated by TSS, TS, RS and the antioxidant scavenging ability and antioxidant enzyme activity were also increased during maturation. The fully matured 15th and 16th weeks of longkong gained more weight and nutritional qualities but lost in pericarp colour and ultrastructure. The increased textural enzyme were increased the textural losses in longkong during the increased weeks of maturation. This might be resulted in a reduction in the post-harvest shelf life. However, at the 13th and 14th weeks of on-tree maturation were caused the fewer changes in fruit colour, browning and textural related enzyme activities. Additionally, the optimum eating qualities such as sugar and sourness were achieved in the early stage (13th and 14th) of maturation. Therefore, the early stage of harvesting might be helpful for the post-harvest shelf life and consumer acceptability.

CHAPTER 3

PHYSIOLOGICAL AND BIOCHEMICAL QUALITY CHANGES OF LONGKONG FRUIT UNDER PASSIVE MAP STORAGE

3.1 Abstract

Physiological and biochemical quality changes of longkong fruits stored in different oxygen transmission rate (OTR) packages (PE1, PE2 and PE3) and temperatures (18°C and 25°C) were studied. Fruit pericarp lightness (L*), yellowness (b*), hue angle (H°) and chroma value (C*) was continuously decreased and redness (a*) increased throughout the storage. Fruit respiration gas and fruit weight loss were increased throughout the storage. Titratable acidity, total soluble solids, total sugar and reducing sugar was decreased during storage. Fruits stored in PE2 and PE3 at 25°C had retained the high values of DPPH scavenging activity, FRAP activity and TPC. Fruit superoxide dismutase (SOD), catalase (CAT) and peroxidase (POD) activities were increased during storage but CAT activity was slightly decreased at the end of storage. Fruits stored at 25°C in PE2 and PE3 had retained higher SOD and POD activities than PE1. Fruit pericarp phenolic contents decreased throughout the storage, but fruits stored at 18°C had retained higher phenolics than at 25°C. Pericarp phenylalanine ammonia lyase (PAL), peroxidase (POD) activities increased throughout storage. Conversely, PPO activity increased at the beginning of the storage and then the activity was decreased throughout the storage. Ultrastructural changes such as surface epidermal hair loss and parenchyma cell damage was found more in fruit pericarp storage in PE2 and PE3 at 25°C than PE1 and 18°C storage.

3.2 Introduction

Longkong (*Aglaia dookkoo* Griff.) is a well-known economical fruit in Thailand and mostly cultivated in southern part. It also cultivated in Borneo, India, Sri Lanka, Philippines, Australia and Puerto Rico. It is a non-climacteric and tropical fruit in nature and belongs to meliaceae family that also includes langsat, duku-langsat and duku (Paull, 2004; Ketsa and Paull, 2008; Sangkasanya and Meenune, 2010). Longkong is a globular in shape and the fruit size between 1.2 to 2.4 inches in diameter. It develops in clusters, among 15 to 25 fruits in per raceme (Salakpetch, 2000). The Immature longkong peel has green colour and it turns to yellow when it ripens and frequently with brown blemishes (Lichanporn *et al.*, 2009). The matured longkong fruit contains five separate segments of white translucent flesh covered on green seeds (Paull *et al.*, 1987). The green seeds are very bitter. Longkong contains variety of nutrients including, carbohydrates, proteins, vitamins, antioxidants, low fat and high percentage of minerals (Ketsa and Paull, 2008; Sabah, 2004). The sweet and sour taste of longkong flesh, along with pleasant aromatic smell makes the fruit more valuable export (Lichanporn *et al*., 2009).

The antioxidant substances are effectively delay and/or prevent the reactive oxygen species caused degenerative diseases such as cancer, arthritis, arteriosclerosis, heart disease, inflammation, brain dysfunction and acceleration of the aging process (Rodrigo *et al.*, 2011). Lim *et al*. (2007) reported that langsat fruit contains the higher level of antioxidant activity. However, the shelf life of longkong is very limited and it last around 4 to 7 days at an ambient temperature. This is due to its respiration accelerated deterioration by increasing pericarp browning, weight loss and off-flavour (Sangkasanya and Meenune, 2010; Lichanporn *et al*., 2009). Pericarp browning decreases the commercial value and it is considered as a main postharvest problem (Akamine, 1960). Whitaker and Lee (1995) have reported that enzymatic browning was predominantly responsible for the browning of fruits and vegetables. The postharvest browning of most fruits and vegetables are majorly attributed by oxidation of phenolics by polyphenol oxidase (PPO), peroxidase (POD) and phenylalanine ammonia lyase (PAL) enzymes (Lichanporn *et al*., 2009).

Modified atmosphere packaging (MAP) is a technique used to prolonging the shelf life of fresh and/or minimally processed fruits and vegetables.

Normally, MAP technique helps to reduce browning, controls postharvest diseases and maintains a high-humidity environment for fruit inside the sealed plastic film. It is also preventing cross-contamination during transportation and storage (Kader, 1995). MAP technique consists of the enclosure of respiring produce in polymeric films in which the gaseous environment is passively altered to slow down the respiration process (Sandhya, 2010; Guevara and Yahia, 2003). However, there is no information available on antioxidant and browning enzyme activity changes during passive MAP. The present research was observed the physiological and biochemical changes of longkong fruit under passive MAP storage.

3.3 Materials and methods

3.3.1 Plant material

Longkong fruits at commercially matured stage (roughly 80% or 13th weeks after anthesis) were purchased from a contact garden at Natawee, Songkhla province, in southern Thailand. The fruits were cut off from raceme and selected uniform in size. Defected fruits such as pericarp and flesh-damaged fruits were discarded. After that, fruits were washed in tap water and dipped in mixed solution of 500 ppm benomyl and 1.5% citric acid solution for 15 min and then dried at ambient temperature until the wet disappear on the fruit pericarp.

3.3.2 Passive modified atmospheric package and storage conditions

For each replication, a 15 individual fruit was placed on a polypropylene tray (4x10 inches) and passively MAP with different oxygen transmission rate packages such as PE1 (17,000-18,000 cc/m^2.day), PE2 (18,000-19,000 cc/m^2.day) and PE3 (19,000-20,000 cc/m^2.day) these packages were 8x15 inches in size and 25 μM thickness. Packages were obtained from Thantawan industry public company limited, Thailand. The sealed fruit PE packages were separated into two batches, the first batch was stored at 18°C, and the second batch was stored at 25°C. The storage was terminated when any fruits displayed a visible mold growth on fruit skin. Every 3 days, fruits were measured of following determinations.

3.3.3 Physical quality

Measurement of fruit pericarp colour

Four sides of an individual longkong fruit surface colour was measured by using a Hunter Lab colourimeter in term of CIE lightness (L*), redness (a*) and yellowness (b*) values (Sapii *et al*., 2000). Hue angle (H°) and chroma value (C*) was measured in accordance to the method of McGuire (1992). Hue angle was calculated as arctangent (b*/a*) (0° = red-purple, 90° = yellow, 180° = bluish-green, 270° = blue) and chroma (colour saturation) value was calculated as $C^* = (a^{*2} + b^{*2})^{1/2}$.

Measurement of fruit weight loss

Longkong fruit weight loss was determined by using digital weighing balance. Weight loss was expressed as the percentage of weight loss with respect to the initial weight.

Measurement of fruit package atmosphere

Determination of headspace CO_2 and O_2 gas concentration in the fruit package was measured by gas chromatography (Perkin Elmer (Auto system XL), USA) in accord with Sangkasanya and Meenune (2010). A gas sample (1 ml) from the headspace inside the bag was injected directly to Pora pak N column with a helium carrier flow of 50 ml/min and a thermal conductivity detector. GC oven and detector temperature was maintained at 60 and 150°C, respectively. The internal package atmosphere was identified and quantified by using external standard gas (CO_2 and O_2).

3.3.4 Chemical quality

Peeled and deseeded longkong flesh homogenate was prepared by blending and filtered using a cheesecloth and then subjected to chemical analysis.

Measurement of fruit pH

The pH was measured by using a Sartorius PB-20 (Germany) digital pH meter at ambient temperature after calibrated with pH 4.0 and 7.0.

Measurement of total soluble solids

The TSS was determined by using an Atago 1E (Japan) hand refractometer at 25°C. The results were expressed as °Brix.

Measurement of titratable acidity

The titratable acidity (TA) was determined in accordance to Sangkasanya and Meenune (2010). The results were expressed as percentages of citric acid content.

Measurement of total and reducing sugar

The total sugar (TS) and reducing sugar (RS) contents were quantified in according with the Ranganna (1986) volumetric method. The results were expressed as percentage of D-glucose content.

Measurement of total phenolic content

Total phenolic content of fruit flesh was determined by using the Folin-Ciocalteu reagent (Lim *et al*., 2007). Edible portion (5 g) was homogenized with 20 ml of 50% ethanol in a mortar and pestle method at 4°C. The homogenate was filtered with cheesecloth and then, the filterate was centrifuged at 10,000*g* at 4°C for 10 min. The supernatant collected and used for total phenolic measurement. A 0.3 ml of fruit extract was placed in test tubes followed by 1.5 ml of Folin-Ciocalteu reagent (1:10 dilution with distilled water) and 1.2 ml of sodium carbonate (7.5% w/v). The tubes were vortexed, covered with parafilm and allowed to stand for 30 min at room temperature. Absorbance was measured at 765 nm. Total phenol contents were expressed as gallic acid equivalents mg per g fresh fruit.

Total phenolic content of fruit pericarp tissue was analysed by the method of Singleton and Rossi (1965). Pericarp tissues 2 g were homogenized with 20 ml of 80% ethanol (1:1 w/v) in a mortar and pestle method. The homogenized sample was centrifuged at 12,000*g* for 20 min. A 0.4 ml of supernatant was mixed with 0.4 ml of Folin-Ciocalteu reagent and 1 ml of 7% sodium carbonate solution. The volume was increased to 10 ml in distilled water and vortexed the mixture, then incubated for 1 hr at room temperature. Absorbance was measured at 750 nm using a

spectrophotometer. Total phenol contents were expressed as gallic acid equivalents mg per g fresh fruit.

3.3.5 Determination of radical scavenging ability

Edible portions of longkong, 25 g, were deseeded and homogenised using a mortar and pestle at 4°C. Then the homogenised sample was transferred into a 100 ml volumetric flask and the volume made up with 50% ethanol. The mixture was shaken with a vibrator for 10 min, and then filtered under suction if the filtrate appeared to be very cloudy. The filtrate was centrifuged at 10,000*g* at 4°C for 10 min to obtain a clear supernatant solution (Lichanporn *et al*., 2009; Lim *et al*., 2007). The supernatant was immediately used for radical scavenging ability analysis, such as 1,1-diphenyl-2-picrylhydrazyl (DPPH) scavenging ability and ferric reducing power ability (FRAP).

Measurement of DPPH radical scavenging activity

DPPH assays were measured according to the method of Binsan *et al*. (2008). A 1.5 ml of the sample was added to the test tube and followed with 1.5 ml of 0.15 mM DPPH in 95% ethanol. The mixture was mixed vigorously and kept in a dark place for 30 min at room temperature and then a sample was measured at 517 nm. Distilled water was used instead of the sample for the blank. A standard curve was prepared by using ascorbic acid. The activity was expressed as ascorbic acid equivalents mg per g fresh weight.

Measurement of ferric reducing power

The ferric reducing power of the fruit extract was determined in accordance with the method of Benzie and Strain (1996). Stock solution was made, of 300 mM acetate buffer (pH 3.6), 10 mM 2,4,6-tripyridyl-s-triazine (TPTZ) solution in 40 mM hydrochloric acid, and 20 mM ferric chloride hexahydrate ($FeCl_3.6H_2O$). The FRAP solution was freshly prepared by adding 25 ml of acetate buffer, 2.5 ml of TPTZ solution and 2.5 ml of $FeCl_3.6H_2O$ solution. The mixed solution was incubated at 37°C for 30 min. A sample of 150 µl was mixed with 2850 µl of FRAP solution and kept for 30 min in dark. The absorbance was measured at 593 nm. A standard

curve was prepared by using ascorbic acid. The activity was expressed as ascorbic acid equivalents mg per g fresh weight.

3.3.6 Enzyme activity analysis

Longkong deseeded flesh was used for antioxidant enzyme (SOD, CAT and POD) activities and whereas, longkong pericarp used for browning related enzyme (PAL, PPO and POD) activities.

Extraction and determination of superoxide dismutase activity

Longkong deseeded flesh (10 g) was homogenized with 25 ml of buffer solution (100 mM sodium phosphate buffer (pH 6.4)) and 0.5 g polyvinyl pyrrolidone in a mortar and pestle at 4°C. The homogenate was filtered through one layer of cheesecloth and then the filtrate was centrifuged at 19,000*g* for 30 min at 4°C. After that, the supernatants of crude enzyme extract were collected and used for superoxide dismutase (SOD) analysis. Determination of SOD was performed as described in a method of Constantine and Stanley (1977). The reaction mixture (3 ml) contained 50 mM sodium phosphate buffer (pH 7.8), 65 mM methionine, 150 μM nitroblue tetrazolium (NBT), 0.5 mM EDTA, 20 μM riboflavin and 0.1 ml of the enzyme extract. The mixtures were illuminated by fluorescent light (60 $\mu mol/m^2/s$) for 10 min and the absorbance was then determined at 560 nm. Identical solution held in the dark served as blank. One unit of SOD was defined as the amount of enzyme that caused a 50% decrease of the SOD-inhibitable NBT reduction. The specific activity was expressed as unit per g of fresh weight.

Extraction and determination of catalase activity

Longkong deseeded flesh (10 g) was homogenized with 25 ml of buffer solution (50 mM sodium phosphate buffer (pH 7.0)) and 0.5 g polyvinyl pyrrolidone in a mortar and pestle at 4°C. The homogenate was filtered through one layer of cheesecloth and then the filtrate was centrifuged at 19,000*g* for 30 min at 4°C. After that, the supernatants of crude enzyme extracts were collected and used for catalase (CAT) analysis. CAT activity was measured according to the method described by Beers and Sizer (1952). The reaction mixture contained, 2 ml of sodium phosphate buffer (50 mM, pH 7.0), 0.5 ml H_2O_2 (40 mM) and 0.5 ml crude enzyme

extract. The decomposition of H_2O_2 was measured by the decline in absorbance at 240 nm. One unit of CAT was defined as the amount of enzyme that decomposing 1 mM of H_2O_2 per minute at pH 7.0 and 25°C. The specific activity was expressed as unit per g of fresh weight.

Extraction and determination of peroxidase activity

Longkong deseeded flesh (10 g) was homogenized with 25 ml of buffer solution (100 mM sodium phosphate buffer (pH 6.4)) and 0.5 g polyvinyl pyrrolidone in a mortar and pestle at 4°C. The homogenate was filtered through one layer of cheesecloth and then the filtrate was centrifuged at 19,000*g* for 30 min at 4°C. After that, the supernatants of crude enzyme extracts were collected and used for peroxidase (POD) analysis. POD activity was assayed in accord with the method of Jiang *et al*. (2002). The 0.1 ml of enzyme was incubated in 2 ml buffered substrate (100 mM sodium phosphate, pH 6.4 and 8 mM guaiacol) at 30°C for 5 min and then, the increasing absorbance measured at 460 nm for 120 s after adding 0.9 ml of H_2O_2 (24 mM). One unit of POD activity was defined as the amount that caused of 0.01 in the absorbance per min. The specific activity was expressed as unit per g of fresh weight.

Extraction and determination of phenylalanine ammonia lyase

Pericarp tissues (2 g) from 20 fruits were homogenized in 20 ml of a 0.1 M sodium borate buffer (pH 8.0) solution contained 0.2 g of insoluble PVP, 5 mM mercaptoethanol, and 2 mM ethylene diamine tetra acetic acid (EDTA) at 4ºC. The homogenate was centrifuged for 20 min at 19,000*g* and 4ºC, and then the supernatant of crude extract was collected for the phenylalanine ammonia lyase (PAL) enzyme assay. PAL activity was determined in accord with the method of Jiang and Joyce (2003). A mixture of 0.1 ml enzyme extract and 2.9 ml of 0.1 M sodium borate buffer (pH 8.0) solution containing 3 mM l-phenylanine was incubated for 1 hr at 37ºC. An increase in the PAL activity at 290 nm, due to the formation of trans-cinnamate, was measured spectrophotometrically. One unit of enzyme activity was defined as the amount that caused an increase of 0.01 in the absorbance per hr. The specific activity was expressed as unit per g of fresh weight.

Extraction and determination of polyphenol oxidase

Pericarp tissues (5 g) from 20 individual fruits were ground with 40 ml of 0.2 M sodium phosphate buffer (pH 6.4) and homogenised using a mortar and pestle at 4ºC. After that, the homogenised sample filtered through cheesecloth and then the filtrate was centrifuged at 12,000*g* for 30 min. The supernatant of crude extract was collected for measuring polyphenol oxidase (PPO) activity (Tian *et al*., 2002). The reaction mixture consisted of 3 ml of 0.5 M 4-methylcatechol in 0.2 M sodium phosphate buffer (pH 6.4) and 0.1 ml of the crude enzyme sample. The absorbance was measured at 398 nm at 25ºC for 1 min. One unit of enzyme activity was defined as an increased in one absorbance unit per min. The specific activity was expressed as unit per g of fresh weight.

Extraction and determination of peroxidase

Pericarp tissues (2 g) from 20 individual fruits were homogenized in 20 ml of 0.05 M phosphate buffer (pH 7) solution and 0.2 g of insoluble polyvinylpyrrolidone (PVP) using a mortar and pestle at 4ºC. The homogenate was filtered through cheesecloth and the filtrate was centrifuged for 20 min at 19,000*g* at 4ºC (Lichanporn *et al*., 2009). The supernatant of crude extract was collected for analysing peroxidase (POD) activity. POD activity, using guaiacol as a substrate, was analysed by the method of Zhang *et al*. (2005). A 3 ml reaction mixture contained 25 µl of crude enzyme extract, 2.78 ml of 0.05 M phosphate buffer (pH 7.0), 0.1 ml of 20 mM hydrogen peroxide (H_2O_2) and 0.1 ml of 20 mM guaiacol. An increased in POD activity was recorded at 470 nm for 2 min. One unit of enzyme activity was defined as the amount that caused a change of 0.01 in the absorbance per min. . The specific activity was expressed as unit per g of fresh weight.

3.3.7 Ultrastructural analysis

Ultrastructural analysis of the pericarp tissue of both surface and cross sections were measured (Lichanporn *et al*., 2009) on the 0 day and the end of storage day. The fruit pericarp tissues were fixed in 2.5% glutaraldehyde in 0.1 M phosphate buffer with a pH of 7.2 for 2 h. The specimens were rinsed twice in phosphate buffer for 20 min, and then once in distilled water for 20 min. The pericarp tissues were dehydrated in a graded alcohol series and sputtered with platinum/palladium and then

dried by a critical point dryer. The dried peels were mounted on the stubs and twice gold-coated. The pericarp was then observed under SEM (JEOL, JSM 5800 LV, England).

3.3.8 Statistical analysis

All the experiments were analysed in factorial (5X3X2) and laid out in a completely randomized design (CRD). Each experiment and the determinations were done in triplicates. The data represent the means ± standard deviation, and they were analysed by one-way analysis of variance (ANOVA) with the Statistical Package for Social Science (SPSS for windows, SPSS Inc., Chicago, IL, USA). The significance of the difference among treatments was measured using Duncan's new multiple range test (DMRT), with a level of significance of 0.05.

3.4 Results and Discussion

3.4.1 Physical quality changes

Longkong fruit pericarp colour (L*, b* and a*) changes were observed throughout the storage (Table 10). The decreased pericarp L* values were found in longkong throughout the storage period. The higher OTR packages and storage period was significantly affected the L* values ($P<0.05$). Fruits stored in PE1 and PE2 packages were certainly had high L* values as compared to the fruits in PE3 package. It indicated that, higher OTR package was influenced the pericarp L* values. However, different storage temperature had not significantly affected the L* values ($P\geq0.05$). Lichanporn *et al*. (2008) have reported that L* values itself can be used as an indicator for longkong pericarp browning. In addition, the b* and a* values were also provided much more knowledge support the changes in longkong pericarp colour. The b* values decreased continuously with L* values during storage. The results specified that, decreased b* values in PE1, PE2 and PE3 had not much differentiated. Although, the decreased b* values in PE3 slightly higher than PE1 and PE2. The interaction between different OTR packages, temperature and storage were significantly affected the b* values ($P<0.05$). In contrast with L* and b* values, the a* values were increased in longkong pericarp throughout the storage. The constant increased of a* values were found in all the PE packages throughout the storage. The

different OTR packages and storage period were significantly affected the a* values. However, different temperature had not significantly affected (P≥0.05). The decreased L*, b* values and increased a* values indicated that pericarp colour was gradually changed to brown. An increased in pericarp browning might be breakdown of membrane and thus allow the enzyme and substrate to react and cause the browning (Weller *et al*., 1997; Sodchit *et al*., 2008).

Table 10. Changes in pericarp lightness, yellowness and redness of longkong fruits in different OTR packages and temperatures during storage

Storage Period	Package	Temp	Lightness (L*)	Yellowness (b*)	Redness (a*)
0	PE1	18°C	69.91±1.48^{g}	38.12±1.5^{f}	7.10±1.15^{a}
		25°C	69.91±1.48^{g}	38.12±1.5^{f}	7.10±1.15^{a}
	PE2	18°C	69.91±1.48^{g}	38.12±1.5^{f}	7.10±1.15^{a}
		25°C	69.91±1.48^{g}	38.12±1.5^{f}	7.10±1.15^{a}
	PE3	18°C	69.91±1.48^{g}	38.12±1.5^{f}	7.10±1.15^{a}
		25°C	69.91±1.48^{g}	38.12±1.5^{f}	7.10±1.15^{a}
3	PE1	18°C	64.08±1.36^{def}	36.88±1.64^{defg}	8.80±1.22^{bc}
		25°C	64.26±3.16^{ef}	36.99±1.96^{efg}	8.93±1.01^{bcd}
	PE2	18°C	64.15±4.27^{ef}	37.04±1.98^{fg}	9.62±1.1^{cdefg}
		25°C	62.97±4.78^{bcdef}	35.99±2.12^{cedf}	9.2±1.36^{bcdef}
	PE3	18°C	64.26±3.16^{ef}	36.00±2.09^{cdef}	8.68±1.05^{b}
		25°C	64.50±3.75^{f}	38.04±1.99^{f}	8.54±1.28^{b}
6	PE1	18°C	63.91±1.12^{def}	36.65±1.28^{cdef}	9.03±0.73^{bcd}
		25°C	63.84±2.31^{cdef}	36.81±1.78^{defg}	9.00±0.79^{bcd}
	PE2	18°C	63.36±2.21^{bcdef}	36.08±2.52^{defg}	9.69±0.1^{cdefg}
		25°C	62.83±3.46^{bcdef}	36.74±2.48^{defg}	9.65±1.04^{cdefg}
	PE3	18°C	61.65±3.02^{bcd}	35.88±2.09^{bcdef}	9.06±0.86^{bcd}
		25°C	61.46±2.7^{bc}	35.72±3.43^{bcdef}	9.75±1.15^{defg}
9	PE1	18°C	62.77±1.63^{bcdef}	36.70±3.27^{efg}	9.14±1.26^{bcde}
		25°C	62.52±3.88^{bcdef}	35.43±1.86^{bcdef}	9.67±1.27^{cdefg}
	PE2	18°C	62.79±3.24^{bcdef}	35.94±2.34^{bcdef}	9.73±1.73^{cdefg}
		25°C	61.43±3.39^{bc}	35.85±1.94^{bcdef}	9.66±0.74^{cdefg}
	PE3	18°C	61.94±4.33^{bcde}	34.05±2.15^{ab}	10.05±0.98^{efg}
		25°C	61.98±2.42^{bcde}	35.40±2.51^{bcdef}	9.86±0.69^{defg}
12	PE1	18°C	61.94±1.76^{bcde}	35.87±3.32^{bcdef}	9.28±1.46^{bcdef}
		25°C	61.10±1.79^{b}	34.91±1.84^{bcd}	10.30±1.26^{g}
	PE2	18°C	61.99±3.54^{bcde}	35.69±2.2^{bcdef}	9.86±0.68^{defg}
		25°C	61.65±3.4^{bcd}	35.02±3.3^{bcde}	10.10±1.15^{fg}
	PE3	18°C	61.15±3.16^{b}	34.7±2.98^{abc}	10.01±1.05^{efg}
		25°C	58.67±2.26^{a}	33.21±1.67^{a}	10.05±1.07^{efg}

Note: Different superscripts in the same column indicate the significant differences (P<0.05) (n=3).

The H° and C* values of longkong pericarp were decreased throughout the storage period (Figure 7). The interaction between different storage period and OTR packages were significantly affected the fruit pericarp colour. Fruit stored at 18°C and the PE1 packages were reasonably controlled the decrease of longkong pericarp hue values ($P<0.05$). A decrease of the H° in fruit pericarp indicates a colour changes from yellow to red-purple (McGuire, 1992). On the other hand, pericarp C* was also decreased ($P<0.05$). A high chroma value represents a highly saturated and intense colour while a low value stands for dull colours (Kruger *et al*., 2011). Fruit stored at 25°C was retained more level of chroma as compared to 18°C. This could be due to the high temperature induced water vapour transmission that maintained the fruit pericarp freshness.

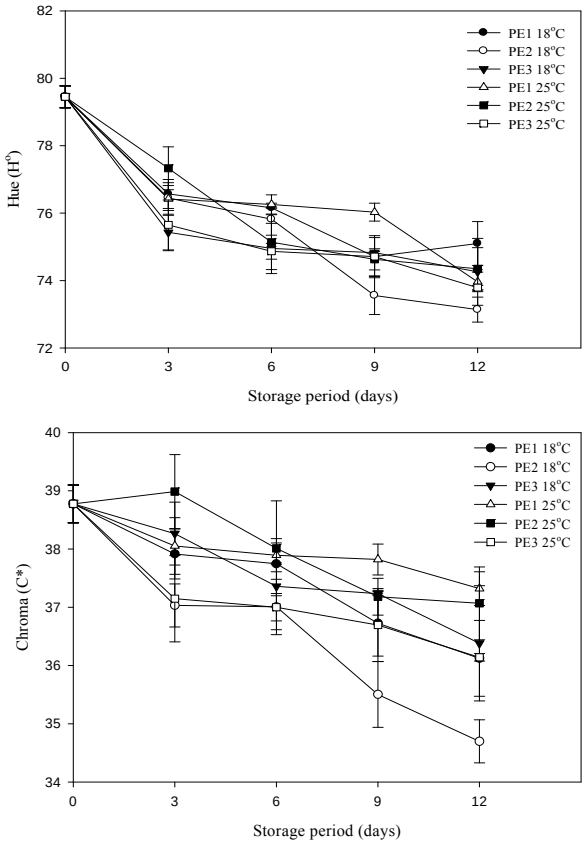


Figure 7. Changes in H° and C* of longkong fruit pericarp in different OTR packages and temperatures during storage

Note: The vertical bar indicates standard error (n=3).

Longkong fruits were respired constantly during the storage and thus resulted the increased accumulation of CO_2 in all permeable packages were stored at 18°C and 25°C (Table 11). On the other hand, decreased level of O_2 was found inside the packages throughout the storage. The decreased level of O_2 was highly correlated with the increased accumulation of CO_2. The interaction between storage, different temperatures and packages were significantly influenced the longkong fruit respiration gas ($P<0.05$). Various factors including film permeability, film thickness, product respiration and temperature plays a major role in increasing the respiratory gas concentration in the package headspace (Yehoshua *et al*., 2005).

Table 11. Changes in headspace respiration gas of longkong fruits in different OTR packages and temperatures during storage

Storage Period	Package	Temp	Carbon dioxide (%)	Oxygen (%)
0	PE1	18°C	1.50±0.04^{a}	6.70±0.01^{J}
		25°C	1.50±0.04^{a}	6.70±0.01^{j}
	PE2	18°C	1.74±0.2^{a}	5.82±0.13^{i}
		25°C	1.74±0.2^{a}	5.82±0.13^{i}
	PE3	18°C	1.73±0.06^{a}	5.38±0.02^{i}
		25°C	1.73±0.06^{a}	5.38±0.02^{i}
3	PE1	18°C	26.10±1.38^{cd}	3.88±0.07^{gh}
		25°C	24.64±3.46^{bcd}	2.67±0.37^{de}
	PE2	18°C	23.54±1.47^{b}	4.25±0.17^{h}
		25°C	23.14±1.26^{d}	3.90±0.35^{gh}
	PE3	18°C	21.14±4.30^{bcd}	3.88±0.38^{gh}
		25°C	27.85±0.32^{bc}	3.93±0.21^{gh}
6	PE1	18°C	55.64±3.87^{fg}	2.54±0.13^{cd}
		25°C	59.54±1.89^{gh}	2.68±0.18^{de}
	PE2	18°C	48.30±1.66^{e}	3.20±0.14^{f}
		25°C	48.99±2.29^{e}	3.49±0.44^{fg}
	PE3	18°C	54.62±3.59^{f}	3.09±0.04^{ef}
		25°C	52.09±0.29^{ef}	3.30±0.20^{f}
9	PE1	18°C	62.46±0.30^{hi}	2.12±0.01^{bc}
		25°C	64.47±3.00^{ij}	2.37±0.04^{bcd}
	PE2	18°C	62.46±4.12^{ij}	2.73±0.44^{de}
		25°C	66.07±4.54^{ij}	3.21±0.08^{f}
	PE3	18°C	65.96±3.13^{hi}	2.27±0.47^{bcd}
		25°C	65.09±3.19^{ij}	3.09±0.04^{ef}
12	PE1	18°C	56.35±2.15^{fg}	1.91±0.49^{b}
		25°C	65.30±4.28^{ij}	1.41±0.12^{a}
	PE2	18°C	55.69±2.00^{fg}	2.01±0.16^{b}
		25°C	66.18±0.79^{j}	2.48±0.16^{cd}
	PE3	18°C	56.12±3.06^{fg}	2.10±0.28^{bc}
		25°C	67.16±0.47^{ij}	2.18±0.39^{bc}

Note: Different superscripts in the same column indicate the significant differences ($P<0.05$) (n=3).

The continuous increased of fruit weight loss was found throughout the storage (Figure 8). Fruits stored in PE1 and PE2 in both temperatures had the minor changes in fruit weight than PE3. It indicates that the different permeable package had an effect on longkong fruit weight loss ($P<0.05$). The interaction between storage period, different OTR packages and temperatures were significantly increased the longkong fruit weight loss ($P<0.05$). However, the increased weight loss was less in fruits storage at 18ºC as compared to 25ºC. Longkong fruit weight loss was increased due to the respiration and transpiration process. This is in an agreement with previously published reports (DeEll *et al*., 2003; Jacxsens *et al*., 2003; Sammi and Masud, 2007; Yehoshua *et al*., 2005). Moreover, the higher OTR permeable thinner packages played a major role in inducing the fruit respiration and water vapour transmission rate (WVTR) and thus increased the fruit weight loss (Van der Steen *et al*., 2002).

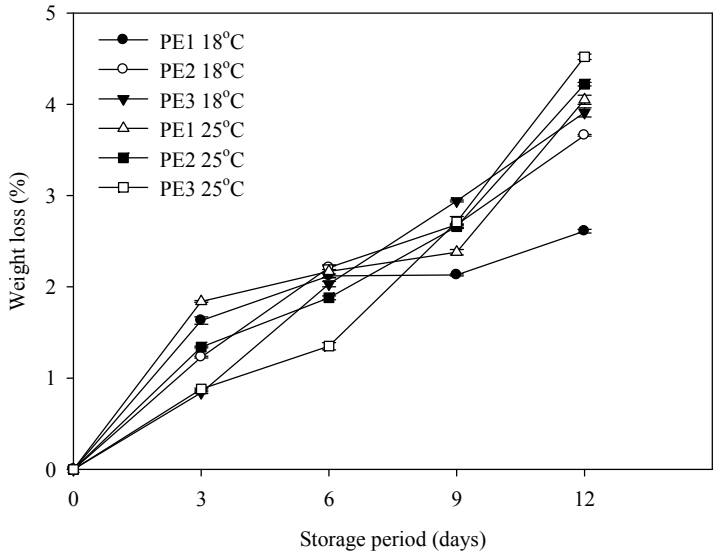


Figure 8. Changes in longkong fruit weight within different OTR packages and temperatures during storage

Note: The vertical bar indicates standard error (n=3).

3.4.2 Chemical quality changes

The longkong fruit pH was decreased continuously throughout the storage (Table 12). The different temperature and storage period significantly decreased the fruit pH ($P<0.05$). The different OTR packages were not significantly affected the pH ($P\geq0.05$). The end of storage, fruit stored in PE1 package had more reduction in pH as compared to PE2 and PE3. The increased CO_2 and ethanolic accumulation could be decreased the fruit pH. Titratable acidity (TA) of fruit was initially increased and at the 6^{th} day of storage it decreased and afterwards the continuous increased in TA level was found (Table 12). The interaction between storage, different OTR packages and temperatures were significantly influenced the TA level ($P<0.05$). Fruits in PE1 package at the end of storage had the higher TA level than PE2 and PE3 and it was correlated with the decreased pH values. The high accumulation of CO_2 and decreased pH could reduce the oxidative enzyme activity that involved with the Krebs cycle process and therefore the consumption of TA was reduced during storage (Davies, 1980; Kader, 1995).

Longkong fruit TSS at the 3^{rd} day of storage was slightly increased and then throughout the storage it significantly decreased ($P<0.05$). (Figure 9). Fruit stored at 18ºC had a better retention in TSS than 25ºC. The increased water loss might be concentrated the soluble solids in longkong fruit. It was greatly correlated with the fruit increased weight loss. Fruit TS and RS slightly increased at the 3^{rd} and 6^{th} day of storage and then it significantly decreased throughout the storage ($P<0.05$) (Figure 9). The interaction between storage, different temperature and packages were significantly affected the fruit TSS, TS and RS level ($P<0.05$). The decreased of TA, TSS, TS and RS could be related to the progression of ripening and senescence process as they used as a substrate for the respiration process.

Table 12. Changes in pH and titratable acidity of longkong fruits in different OTR packages and temperatures during storage

Storage Period	Package	Temp	pH	Titratable acidity (% as citric acid)
0	PE1	18°C	4.43±0.11^{e}	0.73±0.00^{i}
		25°C	4.43±0.11^{e}	0.73±0.00^{i}
	PE2	18°C	4.43±0.11^{e}	0.73±0.00^{i}
		25°C	4.43±0.11^{e}	0.73±0.00^{i}
	PE3	18°C	4.43±0.11^{e}	0.73±0.00^{i}
		25°C	4.43±0.11^{e}	0.73±0.00^{i}
3	PE1	18°C	4.21±0.13^{abcd}	0.70±0.02^{gh}
		25°C	4.31±0.12^{cde}	0.71±0.06^{gh}
	PE2	18°C	4.24±0.02^{bcd}	0.66±0.01^{ef}
		25°C	4.27±0.13^{cde}	0.72±0.00^{hi}
	PE3	18°C	4.26±0.01^{cde}	0.68±0.01^{efg}
		25°C	4.36±0.12^{de}	0.66±0.01^{ef}
6	PE1	18°C	4.19±0.15^{abcd}	0.62±0.01^{bcd}
		25°C	4.30±0.04^{cde}	0.55±0.04^{a}
	PE2	18°C	4.22±0.03^{abcd}	0.65±0.01^{cde}
		25°C	4.25±0.08^{bcd}	0.56±0.01^{a}
	PE3	18°C	4.26±0.12^{cde}	0.64±0.01^{cde}
		25°C	4.31±0.09^{cde}	0.57±0.01^{a}
9	PE1	18°C	4.07±0.07^{ab}	0.68±0.01^{fg}
		25°C	4.28±0.11^{cde}	0.61±0.01^{b}
	PE2	18°C	4.21±0.01^{abcd}	0.67±0.01^{efg}
		25°C	4.21±0.03^{abcd}	0.62±0.01^{a}
	PE3	18°C	4.16±0.13^{abc}	0.65±0.00^{ef}
		25°C	4.22±0.33^{abcd}	0.55±0.01^{a}
12	PE1	18°C	4.05±0.06^{a}	0.66±0.02^{ef}
		25°C	4.27±0.08^{cde}	0.62±0.01^{bcd}
	PE2	18°C	4.16±0.04^{abc}	0.64±0.02^{de}
		25°C	4.06±0.04^{a}	0.61±0.01^{b}
	PE3	18°C	4.15±0.05^{abc}	0.65±0.02^{def}
		25°C	4.16±0.11^{abc}	0.57±0.01^{a}

Note: Different superscripts in the same column indicate the significant differences ($P<0.05$) (n=3).

Longkong total phenolic contents were significantly increased throughout the storage period (Figure 9) ($P<0.05$). Fruits stored in 25ºC at the initial days of storage had maintained the higher level of phenolics as compared to 18ºC. However, at the end of storage fruit phenolics were retained more in 18ºC. The storage period and temperatures were significantly increased the phenolic contents in longkong fruit ($P<0.05$). Previous observation has found that longkong fruit pericarp and flesh contains high level of phenolics (Lichanporn *et al*., 2009; Lim *et al*., 2007).

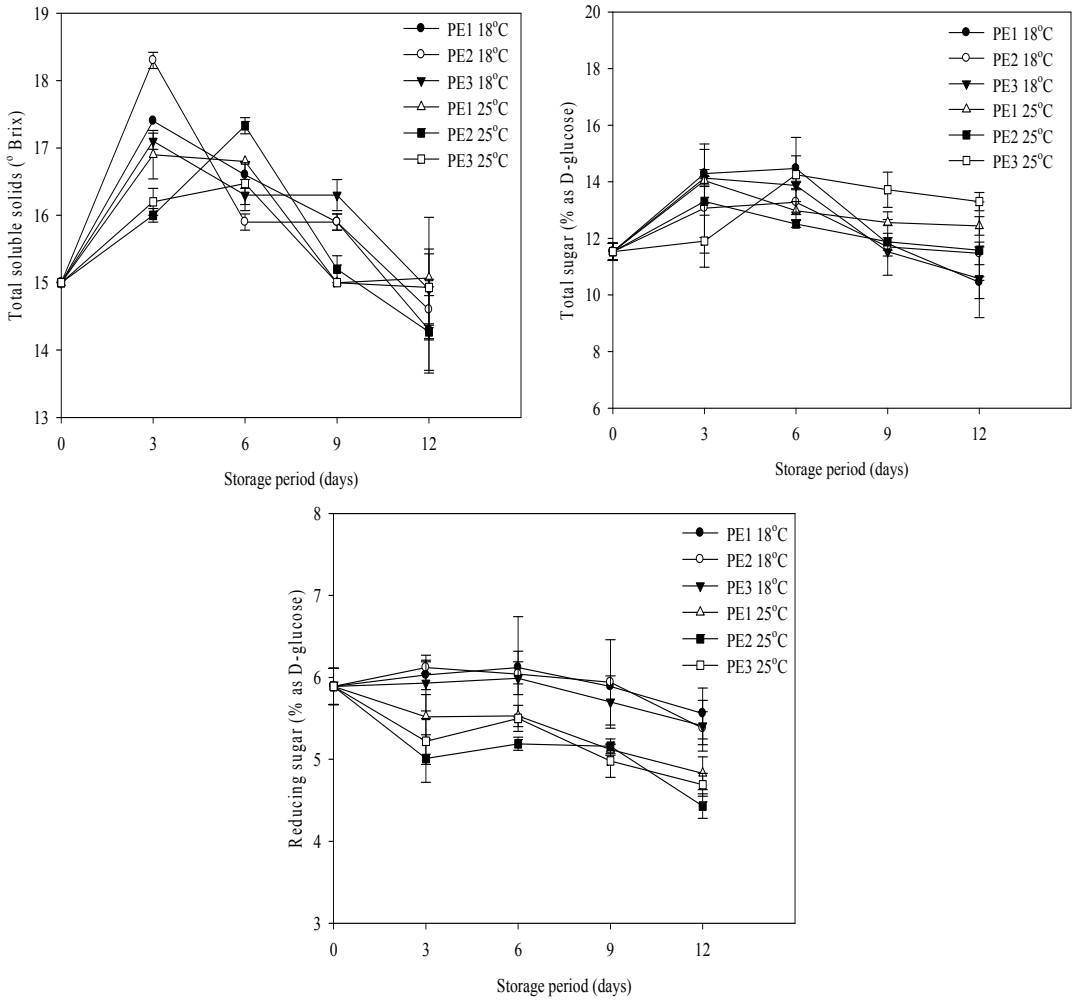


Figure 9. Changes in total soluble solids, total sugar and reducing sugar of stored longkong fruit in different OTR packages and temperatures

Note: The vertical bar indicates standard error (n=3).

Generally, fruit contains high levels of polyphenols and they are natural antioxidants and possess antioxygenic property with the advantage of low toxicity (Balasundram *et al*., 2006; Sun *et al*., 2010). Although, polyphenols are act as a donor to increase the activity of antioxidant enzyme such as guaiacol peroxidase (Arnnok *et al*., 2010; Chanjirakul *et al*., 2006). On the other hand, the fruit pericarp phenolics were significantly decreased throughout the storage period ($P<0.05$) (Figure 10). The decreased phenolics were found more in higher OTR packages (PE2 and PE3) at 25°C as compared to PE1 and 18°C. Fruit stored at 18°C was maintained the

pericarp phenolics than fruit stored at 25°C. The interaction between storage period, different temperatures and packages significantly influenced the longkong fruit pericarp phenolics ($P<0.05$). The decreased phenolics in pericarp might be utilized as substrates by the oxidoreductase enzymes and as results, pericarp browning occurred. This incidence was controlled at 18°C as compared to 25°C in this study.

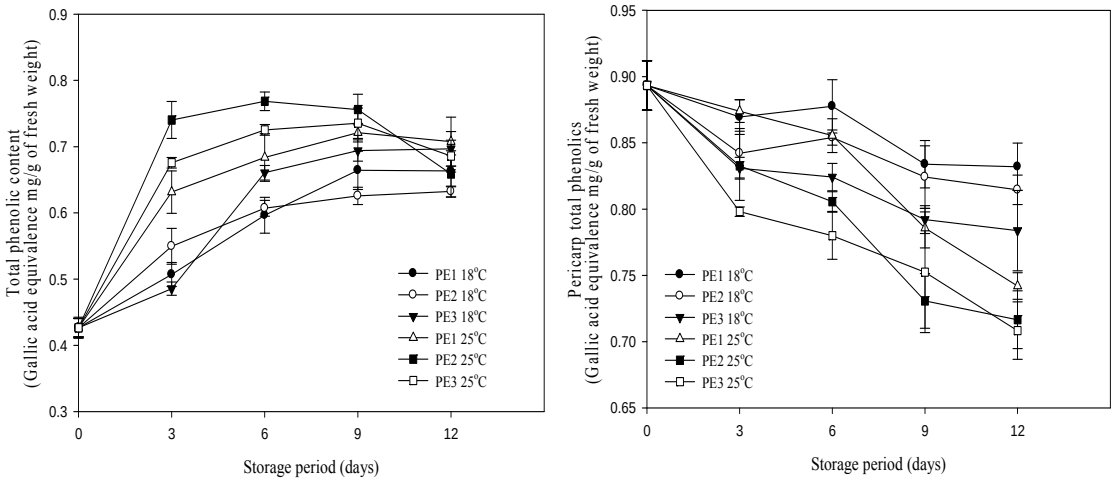


Figure 10. Changes in longkong fruit and pericarp total phenolics stored in different OTR packages and temperatures

Note: The vertical bar indicates standard error (n=3).

3.4.3 Fruit radical scavenging ability

Fruit radical scavenging ability in longkong fruit is shown in Figure 11. Longkong fruit DPPH scavenging activity was an increased in the 3rd day of storage and then significantly it decreased throughout the storage period ($P<0.05$). However, at the end of storage, fruits were stored at 18ºC had retained high DPPH scavenging activity as compared to 25ºC. The interaction between storage period, different temperature and packages were significantly decreased the DPPH scavenging activity ($P<0.05$). The higher OTR packages such PE2 and PE3 had the higher level of DPPH scavenging ability than PE1. The FRAP activity was decreased throughout the storage. The higher level of FRAP activity was observed in PE2 and PE3 than PE1 at both temperatures. The storage period and different packages were significantly influenced the longkong fruit FRAP activity ($P<0.05$). The increased

level of longkong fruit antioxidant activity might be contributed by the fruit phenolics and ascorbic acid. This finding was in an agreement with the previous study reported by Cordenunsi *et al*. (2005) and Lim *et al*. (2007). The decreased level of DPPH scavenging activity and FRAP activity in fruit was correlated with the decreased level of sugar content as can be seen in Figure 8. Sugar is the primary substrate for the ascorbic acid biosynthesis pathway. This is in an accord with the previous reports (Jemai *et al*., 2009; Petacci *et al*., 2010).

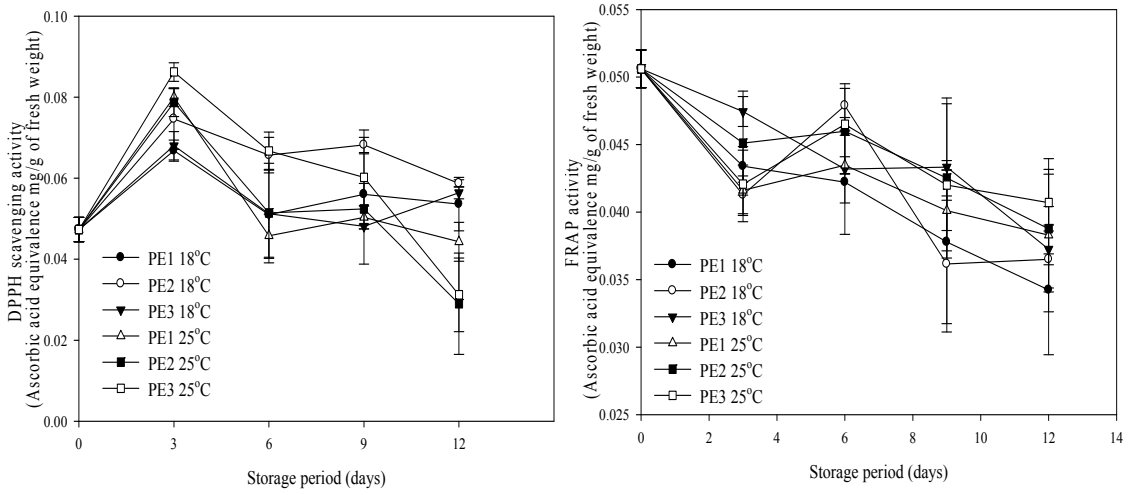


Figure 11. Fruit radical scavenging ability of longkong fruit in different OTR packages and temperatures during storage

Note: The vertical bar indicates standard error (n=3).

3.4.4 Antioxidant enzyme activity

Antioxidant enzymes have the ability to lesser the free radical burden and neutralize excess of free radicals created by stress conditions (Wang and Ballington, 2007). Longkong fruit antioxidant enzyme activity such as SOD, CAT and POD are shown in Figure 12. The activity of SOD was increased significantly throughout the storage ($P<0.05$). In the 3rd day of storage, SOD activity in both temperatures and OTR packages were observed at the constant level and then it increased continuously as the storage period increased. Fruits stored in PE3 package and at 25ºC had a higher level of SOD activity. The interaction between storage period, different temperatures and packages were significantly increased the SOD activity ($P<0.05$). SOD is a class of metal-containing proteins that catalyze the

dismutation reaction of superoxide radical anions into H_2O_2 and molecular oxygen (Chanjirakul *et al*., 2006; Gill and Tuteja, 2010). The high SOD activity might be the factor that plant retained the better tolerance to unfavourable environmental conditions and decrease the progress of senescence (Li and Zhang, 2010).

The significant increase of longkong fruit CAT activity was found during storage at 18ºC and at the end of storage it slightly decreased ($P<0.05$). Fruits stored at 25ºC had a lower level of CAT activity as compared to 18ºC. The interaction between storage period and temperatures were significantly affected the fruit CAT activity ($P<0.05$). The decreased CAT activity at 25°C possibly due to the elevated production of H_2O_2 by SOD enzyme at 25°C. CAT is a tetrameric heme containing enzymes with the potential to directly dismutate H_2O_2 into H_2O and O_2 and is essential for ROS detoxification during stressed conditions (Garg and Manchanda, 2009). The declined activity of CAT during the storage period might be the capacity for enzyme catalysed H_2O_2 scavenging became increasingly degraded (Hodges and Fomey, 2000).

Peroxidase (POD) activity in longkong fruit had significantly increased throughout the storage period. Fruits were stored at 25ºC in PE2 and PE3 packages had certainly increased the POD activity as compared to PE1 at 18ºC ($P<0.05$). The interaction between storage period, different temperature and packages were significantly affected the POD activity ($P<0.05$). The increased POD activity in longkong fruit at 25°C might be the higher temperature influenced the production and/or due to the decreased CAT activity. POD is an oxidoreductase enzyme and it found in animal, plant and microorganism tissues and it can catalyze oxidoreduction between hydrogen peroxide (H_2O_2) and various reductants (Chanjirakul *et al*., 2006). POD is an important active free radical scavenging enzyme and it is also participated in the growth, development and senescence processes of plants (Li and Zhang, 2010).

3.4.5 Pericarp browning enzyme activity

PAL enzyme activity was slightly decreased in the 3rd day of storage and then it continuously increased throughout the storage period (Figure 13). Fruit stored at 18ºC had a high value of the PAL enzyme activity as compared to 25ºC. The interaction between storage period and different temperatures were significantly affected the longkong pericarp PAL enzyme activity ($P<0.05$). However, the different OTR packages did not have any significant effect on PAL activity ($P\geq0.05$). However, the different OTR packages did not have any significant effect on PAL activity. PAL is a key enzyme for the generation of phenolics in plants via phenylpropanoid pathway (Lichanporn *et al*., 2009). Low temperature storage and accumulation of CO_2 might be induced the stress and thus increased the PAL enzyme activity (Dixon and Paiva, 1995; Toma´s-Barbera´n and Carlos Espı´n, 2001)

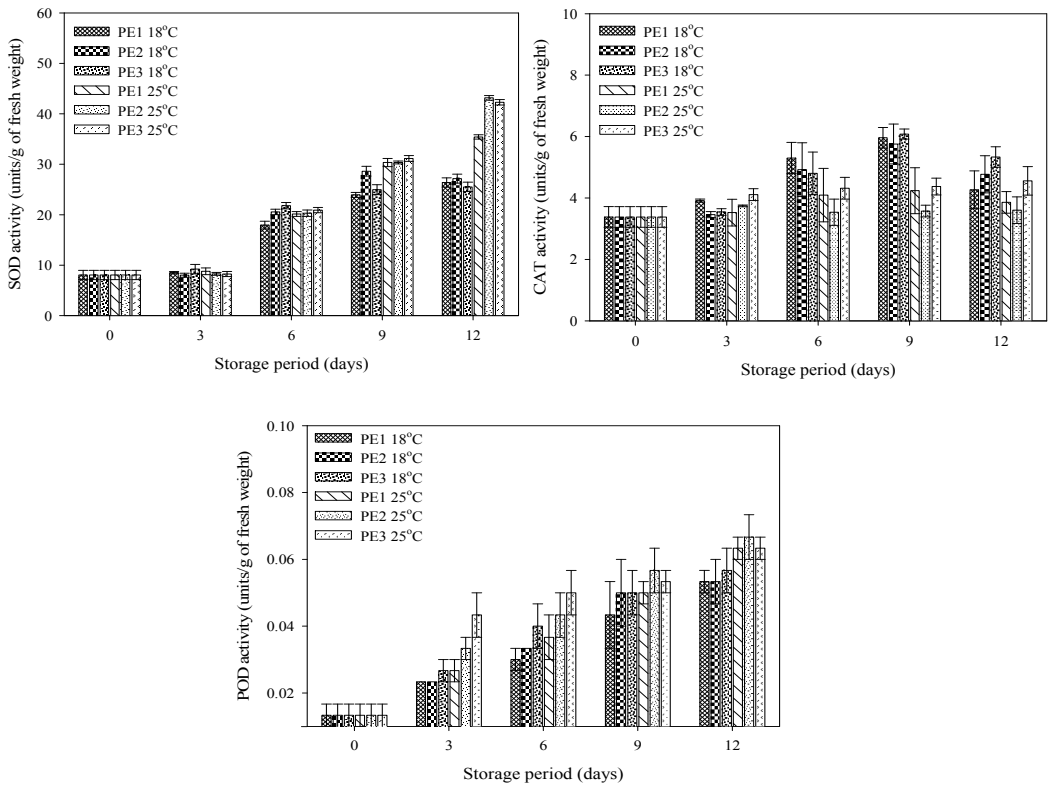


Figure 12. Antioxidant enzymes activity of longkong fruit in different OTR packages and temperatures during storage

Note: The vertical bar indicates standard error (n=3).

PPO activity was significantly increased at the early stage of storage and then it continuously decreased (Figure 13). At the end of storage, fruits stored at 18ºC had a slightly higher level of PPO activity than 25ºC ($P<0.05$). This could be due to the low temperature storage controlled the PPO enzyme activity degradation that induced by continuous oxidation process. The interaction between storage period, different temperatures and packages were significantly reduced the PPO activity in longkong fruit ($P<0.05$). An enzymatic catalyst such as PPO and POD oxidized the plant phenolic compounds to quinones and then condenses the tannins to brown polymers (Duan *et al*., 2007). The decreased level of PPO activity was due to the citric acid treatment and the increased accumulation of CO_2 (Collin *et al*., 2008; Zagory and Kader, 1989).

On the other hand, the significance increased of pericarp POD activity was found throughout the storage ($P<0.05$) (Figure 13). The higher level of fruit pericarp POD activity was found in PE2 and PE3 packages stored at 25°C than PE1 and 18°C ($P<0.05$). The interaction between storage period and different temperatures, storage period and different packages were significantly influenced the longkong fruit pericarp activity ($P<0.05$). The increased accumulation of internal CO_2 content in package induced the anaerobic respiration and thus produced free radicals and membrane degradation (Maguire and MacKay, 2003). As a result, an increased accumulation of CO_2 might be induced the POD activity and possibly accelerates the oxidation of polyphenols.

3.4.6 Pericarp ultrastructural changes

Ultrastructural changes in fruit pericarp surface and cross sections stored in different OTR packages at 18°C and 25°C were observed in the zero day and at the end of storage day. Pericarp surface observation was stood for the epidermal hair changes and the pericarp cross section was stood for the parenchyma cell changes of longkong. The initial day of longkong pericarp had not showed any apparent changes in epidermal hair and parenchyma cells. While, at the end of storage (12 days) fruit stored at 25°C had much more changes in epidermal hairs and parenchyma cells as compared to 18°C. Fruits were stored at 18°C in PE1 package did not found much changes on epidermal hairs and parenchyma cells (Figure 14). Whereas, fruits

in PE2 and PE3 was observed the loss of epidermal hairs on the surface and parenchyma cell wall destruction on the cross section (Figure 14).

On the other hand, fruits stored at 25°C in PE1, PE2 and PE3 packages were had a much difference in their ultrastructure as compared to zero day. Fruits stored in PE1 package, the surface section was found in epidermal losses and it increased as the gas permeability rate increased in PE2 and PE3. Meanwhile, the cross section was also had the detrimentally changes in parenchyma cells. It indicates that, low temperature storage might be reduced the respiration rate and therefore, the cell degradation due to respiration as controlled in low temperature.

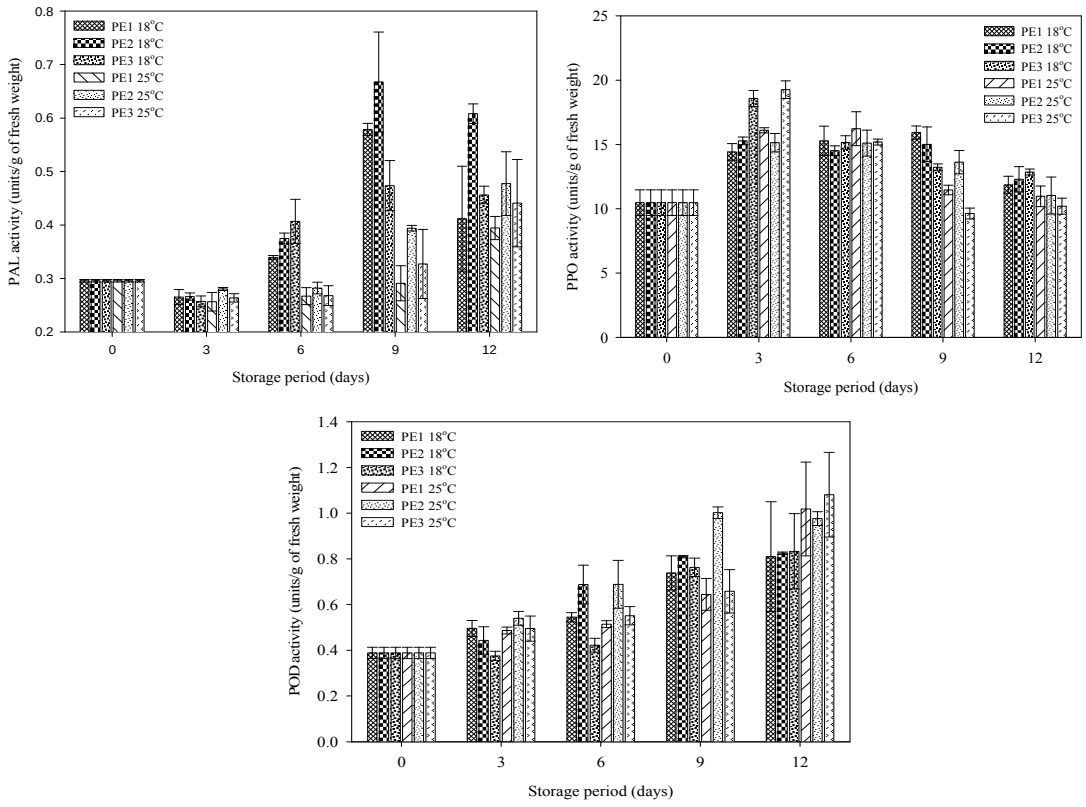


Figure 13. Changes in browning related enzymes of longkong fruit in different OTR packages and temperature during storage

Note: The vertical bar indicates standard error (n=3).

Whereas, the high temperature storage and higher gas permeability might be induced the fruit respiration and consequently, the cell wall degradation occurs. Therefore, the phenolic substrate and oxidoreductase enzyme react together to produce browning on the fruit pericarp. The observation of ultrastructural pericarp

changes are an important tool to provide more evidence on the cell wall degradation induced longkong browning. This finding is an associated with the previous report in longkong (Lichanporn *et al*., 2009).

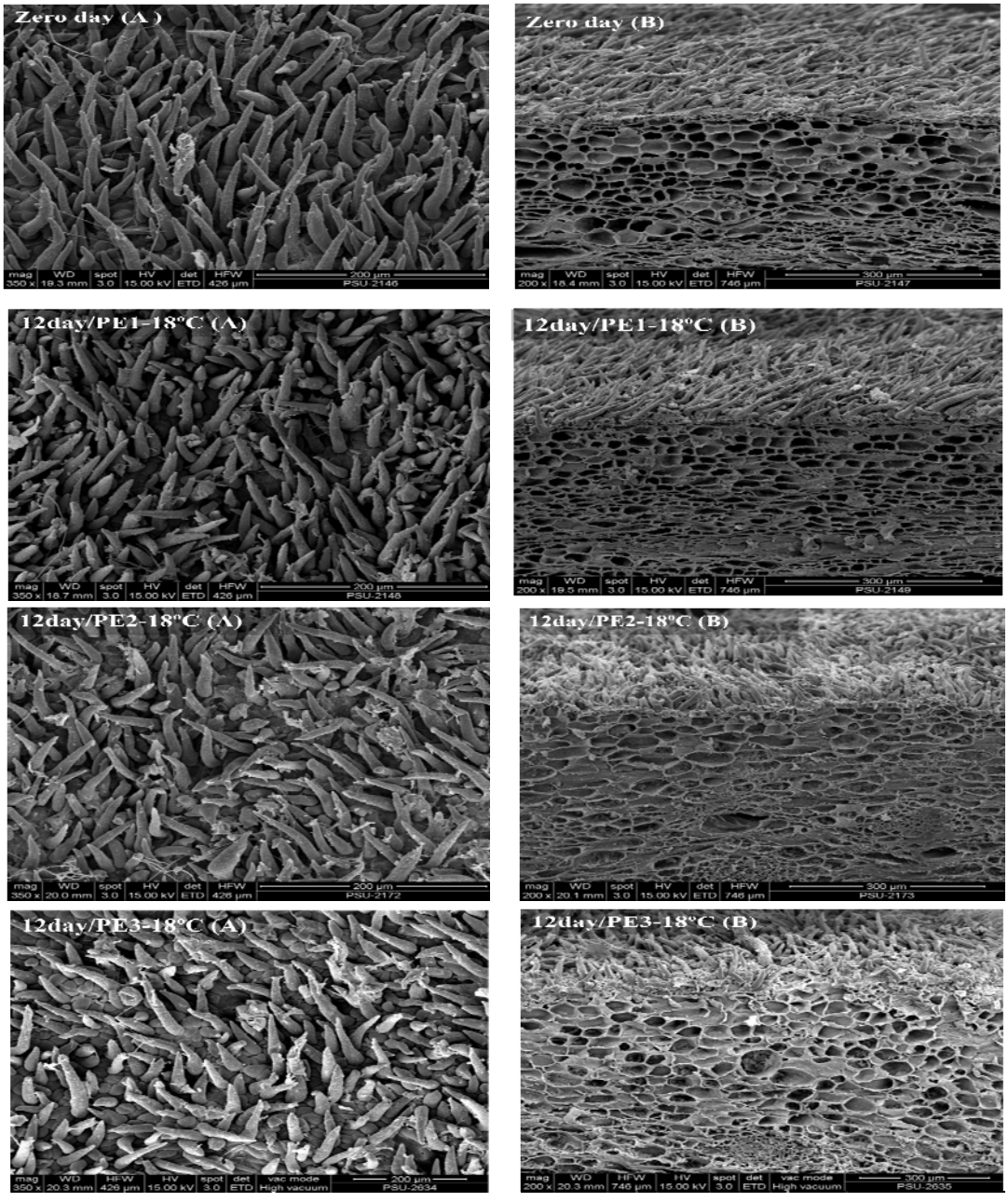


Figure 14. Ultrastructural changes of longkong peel on the zero day and the 12th day of storage in different OTR packages at 18°C

Note: A represented the surface and B represented the cross section of pericarp (n=3).

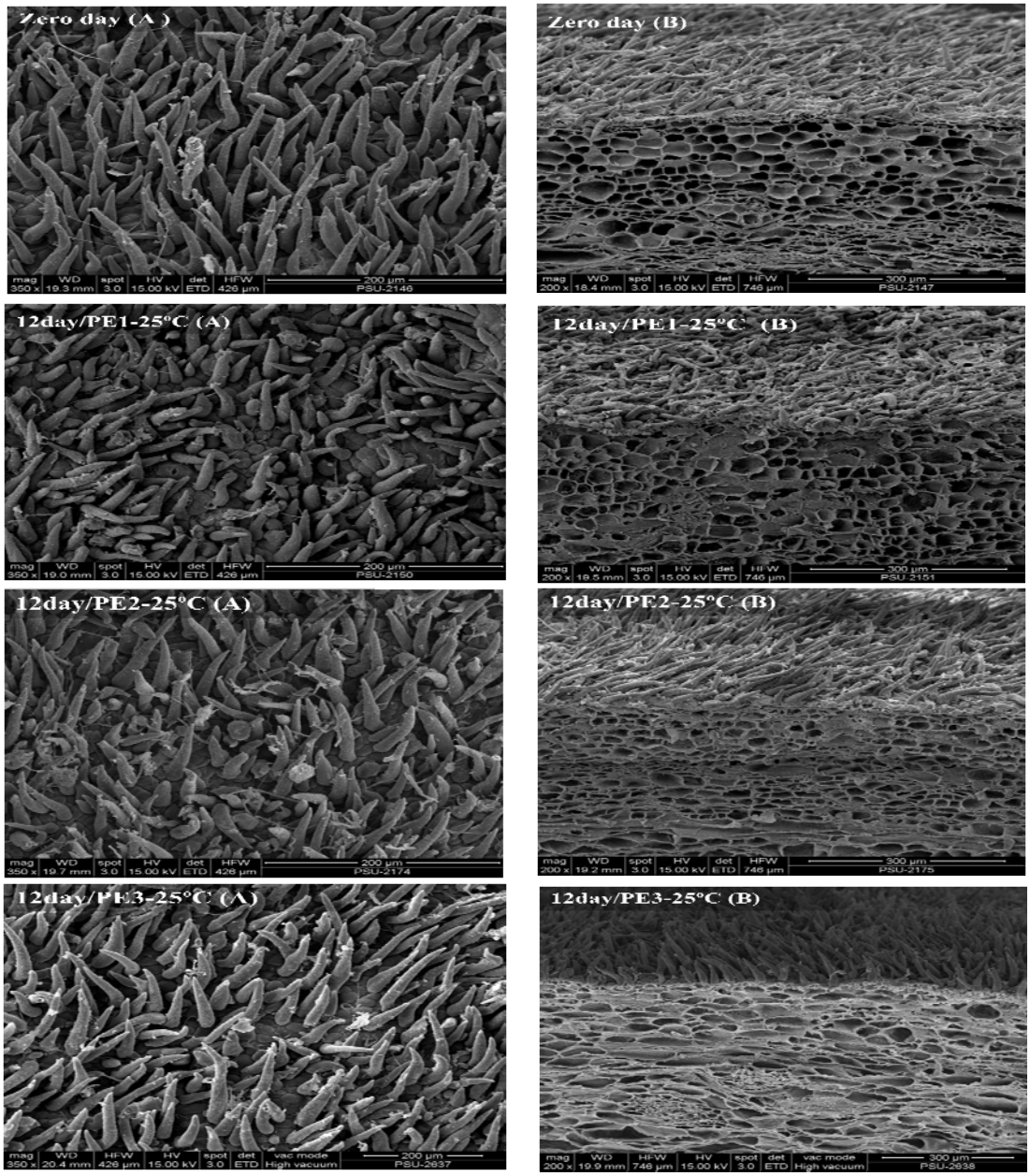


Figure 15. Ultrastructural changes of longkong peel on the zero day and the 12th day of storage in different OTR packages at 25°C

Note: A is represented the surface and B is represented the cross section of pericarp (n=3).

3.5 Conclusion

The interaction between different OTR packages, temperatures and storage days had a positive effect on the overall longkong fruit quality. The loss of fruit weight, titratable acidity and sugar were reasonably controlled in fruits that stored at 18°C as compared to 25°C. Radical scavenging ability and antioxidant enzyme activities were increased in fruits that stored at 25°C in higher OTR packages (PE2 and PE3). On the other hand, the browning related enzyme activities and ultrastructural changes were rationally controlled in fruits pericarp that stored in PE1 package at 18°C. The OTR packages and storage temperatures were influenced and controlled the overall enzyme activities in this study. However, the storage period was tending to minimized to 12 days due to the insufficient activity of benomyl upon mold growth.

CHAPTER 4

PHYSIOLOGICAL AND BIOCHEMICAL QUALITY CHANGES OF LONGKONG FRUIT UNDER ACTIVE MAP STORAGE AT DIFFERENT TEMPERATURES

4.1 Abstract

Longkong fruit stored at 18ºC and 25ºC under active modified atmospheric packaging was used to monitor quality changes during storage. Fruit stored at 18ºC increased in shelf life up to 24 days whereas in fruit stored under 25ºC the shelf life was terminated within 12 days due to visible mold growth. Pericarp lightness (L*), yellowness (b*), hue angle (H°) and chroma value (C*) was slightly decreased and redness was increased during storage at different temperatures. An accumulation of headspace CO_2 and depletion of O_2 was observed inside the fruit package during storage. The fruit weight loss at the end of storage was 6.06% at 18ºC (24 days) and 3.67% at 25ºC (12 days). Fruit pH, titratable acidity, total sugar and reducing sugar decreased throughout storage, but these were retained better at 18ºC ($P<0.05$). The fruits total phenolics were increased at the initial days of storage and then they decreased gradually throughout the storage. Conversely, pericarp total phenolics decreased in the initial days of storage and afterwards it increased throughout storage ($P<0.05$). 1, 1 diphenyl-2-picrylhydrazyl scavenging activity and ferric reducing antioxidant power were maintained in longkong fruit throughout the storage at different temperatures ($P<0.05$). Superoxide dismutase and catalase activities were gradually increased and peroxidase was decreased throughout the storage ($P<0.05$). Phenylalanine ammonia lyase (PAL) activity was increased throughout storage and polyphenol oxidase (PPO) and peroxidase (POD) activities were slightly decreased up to 16 days of storage and then increased. PAL, PPO and POD activity was found more in fruit stored at 25ºC than 18ºC ($P<0.05$). Pericarp ultrastructural changes were also better maintained at 18ºC.

4.2 Introduction

Modified atmosphere packaging (MAP) combined with low temperature storage has been successfully used to increase the shelf life of horticultural produce (Floros *et al.*, 2000). MAP is defined as a technique in which an atmosphere inside the package is created by altering the normal air composition, in order to provide a suitable atmosphere for the product to reduce its deterioration rate and extend its shelf life (Farber *et al.*, 2003; Rodriguez-Aguilera *et al.*, 2011). The MAP of fresh produce is generally packed in polymeric film bags and the atmospherics inside the bags are modified by either respiration of the produce (passive MAP) and/or dispersion of gases through the package films (active MAP) (Sandhya, 2010; Rodriguez-Aguilera *et al.*, 2011). MAP increases the CO_2 and reduces the O_2 inside the package and consequently reduces the respiration rate, ethylene production and sensitivity, decay and physiological changes and increases the shelf life of fresh produce (Ares *et al.*, 2007). The O_2 consumption and CO_2 elevation rates of a fresh produce are more sensitive to change in temperature than the O_2 and CO_2 mass transfer coefficients through the packaging materials (Jacxsens *et al.*, 2000). Increasing the temperature in MAP storage elevates the level of CO_2, which has a negative effect on packed fresh produce and reducing its shelf life and consumer acceptability follows. Therefore, low temperature and/or optimum temperature storage can extend the quality and shelf life of fresh produce (Bunsiri *et al.*, 2000).

Longkong fruit is an important economic fruit of Thailand and is widely produced in many Asian countries and is exported to many European and other Asian countries (Paull *et al.*, 1987; Salakpetch, 2000). The value of longkong fruit is its pleasant taste and aroma and its several nutritional benefits. However, the shelf life of longkong fruit is very limited (3-7 days) at an ambient temperature, due to pericarp browning, weight loss, nutritional loss and off flavour formation (Lichanporn *et al.*, 2008; Lichanporn *et al.*, 2009). Several postharvest techniques have been studied for longkong fruit. Of all the techniques, MAP is an inexpensive technique, which is successfully applied to longkong fruit and extends the shelf life (Bunsiri *et al.*, 2002; Sangkasanya and Meenune, 2010; Sangkasanya *et al.*, 2010). Active MAP along with optimum temperature storage has extensively increased the shelf life of longkong fruit (Meenune and Janthachum, 2004). Low gas permeable packaging with

high concentrations of CO_2 and O_2 has induced the longkong fruits pericarp browning, ethanolic accumulation and off flavour formation and thus lowered consumer acceptance. Previous available information has been obtained and they derived about the physical and chemical qualities of longkong. There are no reports on browning related and antioxidant enzymes during active MAP storage. Therefore, the present objective was to evaluate mainly the quality in terms of the physiological and biochemical changes of longkong during active MAP storage in moderate gas permeable packaging at two different storage temperatures.

4.3 Materials and methods

4.3.1 Plant material

Longkong fruits at a commercially mature stage (13^{th} weeks after anthesis) were purchased from a contact garden in Songkhla province, Thailand. The fruit was cut off from the raceme and selected for its uniform size. Defective fruit was discarded. The longkong fruit was then washed in a tap water and dipped in a mixed solution of 500 ppm benomyl and 1.5% citric acid for 15 min and then dried at ambient temperature until the moisture disappear on the fruit pericarp.

4.3.2 Active MAP preparation and storage conditions

For each replication, 15 individual fruits were placed on a polypropylene tray (4x10 inches) and active MAP was undertaken by filling with 5% O_2 and 5% CO_2 (N_2 balance) in an oxygen transmission rate (O_2TR) polyethylene (PE) package with 5,000-6,000 cc/m^2.day. The PE packages were 8x15 inches in size, 25 μM thickness and they were obtained from Thantawan Industry Public Company Limited, Thailand. The sealed fruit PE packages were separated into two batches, the first batch was stored at 18°C, and the second batch was stored at 25°C. The storage was terminated when any fruit displayed a visible mold growth on the fruits skin. Every 4 days the fruit were measured in the following ways.

4.3.3 Physical quality

Measurement of fruit pericarp colour

Four sides of an individual longkong fruit surface colour were measured by using a Hunter Lab colourimeter in terms of CIE lightness (L*), redness (a*) and yellowness (b*) values (Sapii *et al*., 2000). Hue angle (H°) and chroma value (C*) was measured in accordance to the method of McGuire (1992). Hue angle was calculated as arctangent (b*/a*) (0° = red-purple, 90° = yellow, 180° = bluish-green, 270° = blue) and chroma (colour saturation) value was calculated as $C^* = (a^{*2} + b^{*2})^{1/2}$.

Measurement of fruit weight loss

Longkong fruit weight loss was determined by using a digital weighing balance. Weight loss was expressed as the percentage of weight loss with respect to the initial weight.

4.3.4 Fruit package atmosphere

The determination of headspace CO_2 and O_2 gas concentration in the fruit package was measured by a gas chromatography (Perkin Elmer (Auto system XL), USA) in accord with Sangkasanya and Meenune (2010). A gas sample (1 ml) from the headspace inside the bag was injected directly to a Pora pak N column with a helium carrier flow of 50 ml/min and a thermal conductivity detector. The GC oven and detector temperature was maintained at 60 and 150°C, respectively. The internal package atmosphere was identified and quantified by using external standard gases (CO_2 and O_2).

4.3.5 Chemical quality

Peeled and deseeded longkong flesh homogenate was prepared by blending and was filtered using a cheesecloth and then subjected to chemical analysis.

Measurement of fruit pH

The pH was measured by using a Sartorius PB-20 (Germany) digital pH meter at ambient temperature after calibrated with pH 4.0 and 7.0.

Measurement of total soluble solids

The TSS was determined by using an Atago 1E (Japan) hand refractometer at 25°C. The results were expressed as °Brix.

Measurement of titratable acidity

The titratable acidity (TA) was determined in accord with Sangkasanya and Meenune (2010). The results were expressed as percentages of citric acid content.

Measurement of total and reducing sugar

The total sugar (TS) and reducing sugar (RS) contents were quantified in accord with the Ranganna (1986) volumetric method. The results were expressed as percentage of D-glucose content.

Measurement of total phenolic content

Total phenolic content of fruit flesh was determined by using the Folin-Ciocalteu reagent (Lim *et al*., 2007). Edible portion (5 g) was homogenized with 20 ml of 50% ethanol in a mortar and pestle method at 4°C. The homogenate was filtered with cheesecloth and then, the filterate was centrifuged at 10,000*g* at 4°C for 10 min. The supernatant collected and used for total phenolic measurement. A 0.3 ml of fruit extract was placed in test tubes followed by 1.5 ml of Folin-Ciocalteu reagent (1:10 dilution with distilled water) and 1.2 ml of sodium carbonate (7.5% w/v). The tubes were vortexed, covered with parafilm and allowed to stand for 30 min at room temperature. Absorbance was measured at 765 nm. Total phenol contents were expressed as gallic acid equivalents mg per g fresh fruit.

Total phenolic content of fruit pericarp tissue was analysed by the method of Singleton and Rossi (1965). Pericarp tissues 2 g were homogenized with 20 ml of 80% ethanol (1:1 w/v) in a mortar and pestle method. The homogenized sample was centrifuged at 12,000*g* for 20 min. A 0.4 ml of supernatant was mixed with 0.4 ml of Folin-Ciocalteu reagent and 1 ml of 7% sodium carbonate solution. The volume was increased to 10 ml in distilled water and vortexed the mixture, then incubated for 1 hr at room temperature. Absorbance was measured at 750 nm using a

spectrophotometer. Total phenol contents were expressed as gallic acid equivalents mg per g fresh fruit.

4.3.6 Determination of radical scavenging ability

Edible portions of longkong of 25 g, were deseeded and homogenised using a mortar and pestle at 4°C. Then the homogenised sample was transferred into a 100 ml volumetric flask and the volume made up with 50% ethanol. The mixture was shaken with a vibrator for 10 min, and then filtered under suction if the filtrate appeared to be very cloudy. The filtrate was centrifuged at 10,000*g* at 4°C for 10 min to obtain a clear supernatant solution (Lichanporn *et al*., 2009; Lim *et al*., 2007). The supernatant was immediately used for radical scavenging ability analysis, such as 1,1-diphenyl-2-picrylhydrazyl (DPPH) scavenging ability and ferric reducing power ability (FRAP).

Measurement of DPPH radical scavenging activity

DPPH assays were measured according to the method of Binsan *et al*. (2008). A 1.5 ml of the sample was added to the test tube and followed with 1.5 ml of 0.15 mM DPPH in 95% ethanol. The mixture was mixed vigorously and kept in a dark place for 30 min at room temperature and then a sample was measured at 517 nm. Distilled water was used instead of the sample for the blank. A standard curve was prepared by using ascorbic acid. The activity was expressed as ascorbic acid equivalents mg per g fresh weight.

Measurement of ferric reducing power

The ferric reducing power of the fruit extract was determined in accordance with the method of Benzie and Strain (1996). Stock solution was made, of 300 mM acetate buffer (pH 3.6), 10 mM 2,4,6-tripyridyl-s-triazine (TPTZ) solution in 40 mM hydrochloric acid, and 20 mM ferric chloride hexahydrate ($FeCl_3.6H_2O$). The FRAP solution was freshly prepared by adding 25 ml of acetate buffer, 2.5 ml of TPTZ solution and 2.5 ml of $FeCl_3.6H_2O$ solution. The mixed solution was incubated at 37°C for 30 min. A sample of 150 µl was mixed with 2850 µl of FRAP solution and kept for 30 min in dark. The absorbance was measured at 593 nm. A standard

curve was prepared by using ascorbic acid. The activity was expressed as ascorbic acid equivalents mg per g fresh weight.

4.3.7 Enzyme activity analysis

Longkong deseeded flesh was used for the antioxidant enzyme (SOD, CAT and POD) activities whereas, longkong pericarp was used for browning related enzyme (PAL, PPO and POD) activities.

Extraction and determination of superoxide dismutase activity

Longkong deseeded flesh (10 g) was homogenized with 25 ml of buffer solution (100 mM sodium phosphate buffer (pH 6.4)) and 0.5 g polyvinyl pyrrolidone in a mortar and pestle at 4°C. The homogenate was filtered through one layer of cheesecloth and then the filtrate was centrifuged at 19,000*g* for 30 min at 4°C. After that, the supernatants of crude enzyme extract were collected and used for superoxide dismutase (SOD) analysis. Determination of SOD was performed as described in a method of Constantine and Stanley (1977). The reaction mixture (3 ml) contained 50 mM sodium phosphate buffer (pH 7.8), 65 mM methionine, 150 μM nitroblue tetrazolium (NBT), 0.5 mM EDTA, 20 μM riboflavin and 0.1 ml of the enzyme extract. The mixtures were illuminated by fluorescent light (60 $\mu mol/m^2/s$) for 10 min and the absorbance was then determined at 560 nm. Identical solution held in the dark served as blank. One unit of SOD was defined as the amount of enzyme that caused a 50% decrease of the SOD-inhibitable NBT reduction. The specific activity was expressed as unit per g of fresh weight.

Extraction and determination of catalase activity

Longkong deseeded flesh (10 g) was homogenized with 25 ml of buffer solution (50 mM sodium phosphate buffer (pH 7.0)) and 0.5 g polyvinyl pyrrolidone in a mortar and pestle at 4°C. The homogenate was filtered through one layer of cheesecloth and then the filtrate was centrifuged at 19,000*g* for 30 min at 4°C. After that, the supernatants of crude enzyme extracts were collected and used for catalase (CAT) analysis. CAT activity was measured according to the method described by Beers and Sizer (1952). The reaction mixture contained, 2 ml of sodium phosphate buffer (50 mM, pH 7.0), 0.5 ml H_2O_2 (40 mM) and 0.5 ml crude enzyme

extract. The decomposition of H_2O_2 was measured by the decline in absorbance at 240 nm. One unit of CAT was defined as the amount of enzyme that decomposing 1 mM of H_2O_2 per minute at pH 7.0 and 25°C. The specific activity was expressed as unit per g of fresh weight.

Extraction and determination of peroxidase activity

Longkong deseeded flesh (10 g) was homogenized with 25 ml of buffer solution (100 mM sodium phosphate buffer (pH 6.4)) and 0.5 g polyvinyl pyrrolidone in a mortar and pestle at 4°C. The homogenate was filtered through one layer of cheesecloth and then the filtrate was centrifuged at 19,000*g* for 30 min at 4°C. After that, the supernatants of crude enzyme extracts were collected and used for peroxidase (POD) analysis. POD activity was assayed in accord with the method of Jiang *et al*. (2002). The 0.1 ml of enzyme was incubated in 2 ml buffered substrate (100 mM sodium phosphate, pH 6.4 and 8 mM guaiacol) at 30°C for 5 min and then, the increasing absorbance measured at 460 nm for 120 s after adding 0.9 ml of H_2O_2 (24 mM). One unit of POD activity was defined as the amount that caused of 0.01 in the absorbance per min. The specific activity was expressed as unit per g of fresh weight.

Extraction and determination of phenylalanine ammonia lyase

Pericarp tissues (2 g) from 20 fruits were homogenized in 20 ml of a 0.1 M sodium borate buffer (pH 8.0) solution contained 0.2 g of insoluble PVP, 5 mM mercaptoethanol, and 2 mM ethylene diamine tetra acetic acid (EDTA) at 4ºC. The homogenate was centrifuged for 20 min at 19,000*g* and 4ºC, and then the supernatant of crude extract was collected for the phenylalanine ammonia lyase (PAL) enzyme assay. PAL activity was determined in accord with the method of Jiang and Joyce (2003). A mixture of 0.1 ml enzyme extract and 2.9 ml of 0.1 M sodium borate buffer (pH 8.0) solution containing 3 mM l-phenylanine was incubated for 1 hr at 37ºC. An increase in the PAL activity at 290 nm, due to the formation of trans-cinnamate, was measured spectrophotometrically. One unit of enzyme activity was defined as the amount that caused an increase of 0.01 in the absorbance per hr. The specific activity was expressed as unit per g of fresh weight.

Extraction and determination of polyphenol oxidase

Pericarp tissues (5 g) from 20 individual fruits were ground with 40 ml of 0.2 M sodium phosphate buffer (pH 6.4) and homogenised using a mortar and pestle at 4ºC. After that, the homogenised sample filtered through cheesecloth and then the filtrate was centrifuged at 12,000*g* for 30 min. The supernatant of crude extract was collected for measuring polyphenol oxidase (PPO) activity (Tian *et al*., 2002). The reaction mixture consisted of 3 ml of 0.5 M 4-methylcatechol in 0.2 M sodium phosphate buffer (pH 6.4) and 0.1 ml of the crude enzyme sample. The absorbance was measured at 398 nm at 25ºC for 1 min. One unit of enzyme activity was defined as an increased in one absorbance unit per min. The specific activity was expressed as unit per g of fresh weight.

Extraction and determination of peroxidase

Pericarp tissues (2 g) from 20 individual fruits were homogenized in 20 ml of 0.05 M phosphate buffer (pH 7) solution and 0.2 g of insoluble polyvinylpyrrolidone (PVP) using a mortar and pestle at 4ºC. The homogenate was filtered through cheesecloth and the filtrate was centrifuged for 20 min at 19,000*g* at 4ºC (Lichanporn *et al*., 2009). The supernatant of crude extract was collected for analysing peroxidase (POD) activity. POD activity, using guaiacol as a substrate, was analysed by the method of Zhang *et al*. (2005). A 3 ml reaction mixture contained 25 µl of crude enzyme extract, 2.78 ml of 0.05 M phosphate buffer (pH 7.0), 0.1 ml of 20 mM hydrogen peroxide (H_2O_2) and 0.1 ml of 20 mM guaiacol. An increased in POD activity was recorded at 470 nm for 2 min. One unit of enzyme activity was defined as the amount that caused a change of 0.01 in the absorbance per min. The specific activity was expressed as unit per g of fresh weight.

4.3.8 Ultrastructural analysis

Ultrastructural analysis of the pericarp tissue of both surface and cross sections were measured (Lichanporn *et al*., 2009) on the 0 day and the end of storage day. The fruit pericarp tissues were fixed in 2.5% glutaraldehyde in 0.1 M phosphate buffer with a pH of 7.2 for 2 h. The specimens were rinsed twice in phosphate buffer for 20 min, and then once in distilled water for 20 min. The pericarp tissues were dehydrated in a graded alcohol series and sputtered with platinum/palladium and then

dried by a critical point dryer. The dried peels were mounted on the stubs and twice gold-coated. The pericarp was then observed under SEM (JEOL, JSM 5800 LV, England).

4.3.9 Statistical analysis

All the experiments were carried out in a completely randomized design (CRD). The significant difference between temperatures was measured in a t-test. The fruit was sampled three times for each experiment and after that, each experiment was done in triplicates. The data shows the mean ± standard deviations, and they were analysed by one-way analysis of variance (ANOVA) with the Statistical Package for Social Science (SPSS for windows, SPSS Inc., Chicago, IL, USA). The significance of the differences among treatments was measured using Duncan's new multiple range test (DMRT), with a level of significance of 0.05.

4.4 Results and Discussion

4.4.1 Physical quality changes

The longkong fruits pericarp lightness (L*), yellowness (b*) decreased and conversely, the redness was increased incessantly during storage (Figure 16). The different temperatures significantly influenced the pericarp colour changes ($P<0.05$). Longkong fruit stored at 18°C was significantly affected in terms of pericarp colour loss as evidenced by a retarding in the decrease of L*, b* values and an increase of a* values ($P<0.05$). Longkong fruit which was stored at 25°C showed rapid and severe changes in L*, a* and b* values. The sustained storage period significantly reduced the L* and b* values of longkong fruit pericarp and increased a* values in both temperatures at 18°C or 25°C ($P<0.05$). However, the increased pericarp colour changes in longkong fruit were better controlled at 18°C as compared to 25°C during storage. Longkong fruit pericarp browning is mainly attributed to the oxidoreductase (PPO and POD) enzymes (Lichanporn *et al.*, 2008). However, the combination of using low temperature and active MAP can limit the oxidoreductase activity in fruit pericarp and thus reduce the pericarp browning (Tian *et al.*, 2005; De Reuck *et al.*, 2010; Ahmad *et al.*, 2011).

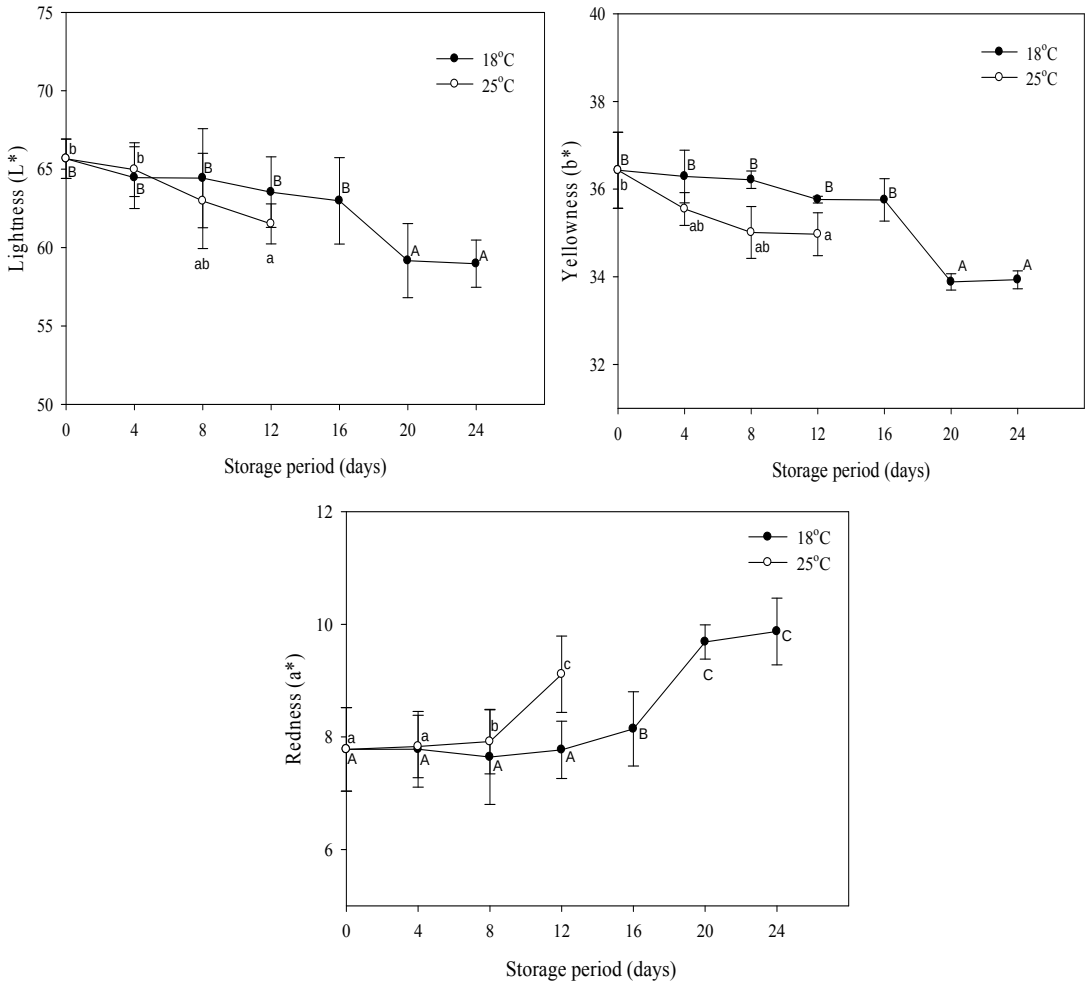


Figure 16. Pericarp colour changes in longkong fruit during storage under active MAP at different temperature (18°C and 25°C)

Note: The vertical bar indicates standard error.

Different capital or small alphabets within the same storage temperature indicate the significant differences ($P<0.05$) (n=3).

Additionally, fruits pericarp hue and chroma values were decreased continuously throughout the storage (Figure 17). The storage period and different temperatures were significantly influenced the H° and C* fruit pericarp ($P<0.05$). The decreased in H° was more specified to the increased browning on the fruit pericarp. On the other hand, changes in C* were also correlated with the pericarp browning. Fruit package stored at 18°C was controlled the decrease of H° and C* better than at 25°C.

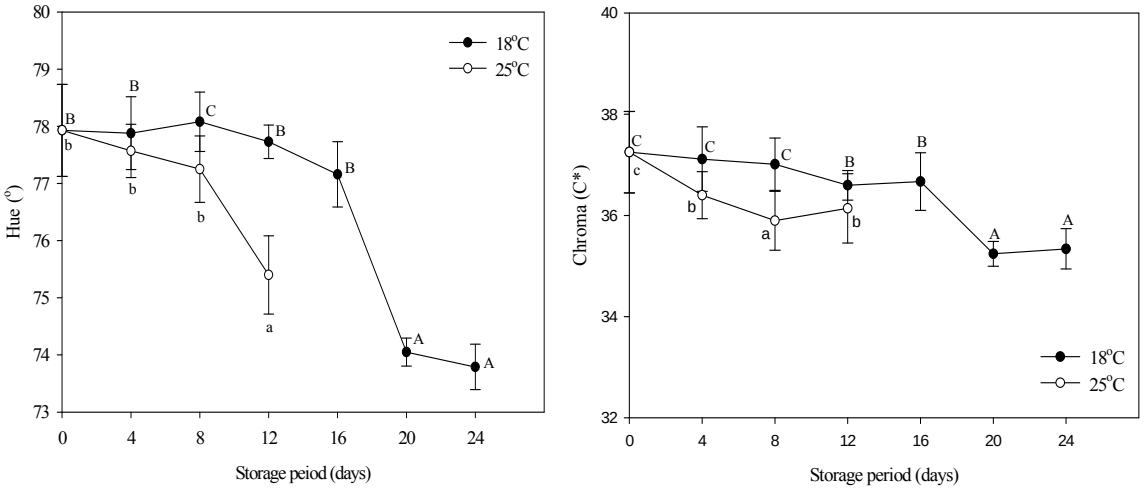


Figure 17. Changes in H° and C* of longkong pericarp during storage under active MAP at different temperature (18°C and 25°C)

Note: The vertical bar indicates standard error.

Different capital or small alphabets within the same storage temperature indicate the significant differences ($P<0.05$) (n=3).

A sustained increase of fruit weight loss was noticed under active MAP storage at different temperatures (Figure 18). The different temperatures significantly influenced the fruit weight loss. Fruit stored at 18°C had kept a higher level of fruit weight than at 25°C ($P<0.05$). Conversely, the storage period significantly increased the fruit weight loss ($P<0.05$). Packaging played a crucial role in minimizing the fruit weight loss (Somboonkaew and Terry, 2011; Sivakumar *et al*., 2008). DeEll *et al*. (2003) reported that packaging reduced the availability of inner atmospheric O_2 and consequently reduced the weight loss induced by the respiration rate. In addition, low temperature storage reduced the transpiration process that induced fruit weight loss. Wasna *et al*. (2007) reported that longkong fruit stored under an ambient temperature could maintain the fruit quality and extend the storage life by reducing the fruit weight loss. Piyasaengthong *et al*. (1997) reported that longkong fruit stored at 18°C had a low percentage of weight loss and extension of the shelf life.

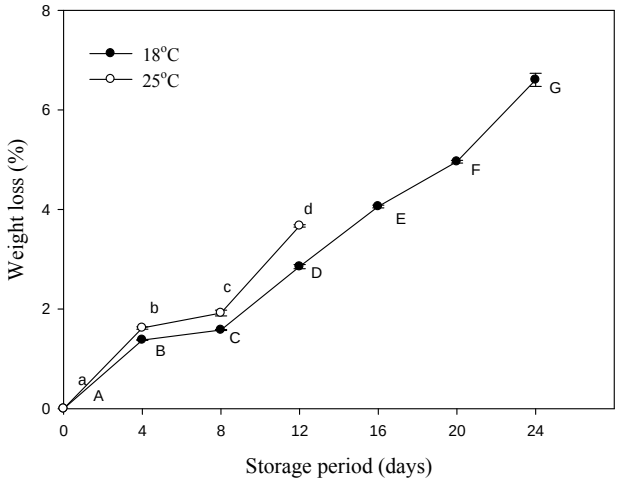


Figure 18. Percentage of weight loss in longkong fruit during storage under active MAP at different temperature (18°C and 25°C)

Note: The vertical bar indicates standard error.

Different capital or small alphabets within the same storage temperature indicate the significant differences ($P<0.05$) (n=3).

An increased accumulation of CO_2 and an increased depletion of O_2 were observed in the inner headspace of the longkong fruit stored packages during storage (Figure 19). The different temperatures significantly affected the inner headspace atmospheric gas changes ($P<0.05$). A steady increase of CO_2 accumulation and the consumption of O_2 was observed in longkong fruit packages stored at 25°C. The increased storage temperature substantially increased the products respiration rate (Exama *et al.*, 1993; Beaudry, 2000; Tano *et al.*, 2007). Fruit stored at 18°C had less changes in the inner atmospheric gas content as compared to that at 25°C. Low temperature storage lowers the rate of respiration and therefore fruit can tolerate the low O_2 and elevated CO_2 inside the package. However, the increased storage period caused significant changes in the inner atmosphere gas ($P<0.05$).

Longkong fruit stored at 18°C had partly maintained the O_2 level inside the package for 8 to 16 days of storage and after that, it mainly decreased. DeEll *et al*. (2003) reported that any fluctuation in storage temperature could increase the demand of O_2 intake of the fruit inside the package and consequently, the O_2 level may go

down below the extinction point for that fruit. Therefore, the anaerobic pathway is engaged and produces ethanolic accumulation. Optimal creation and maintenance of the atmosphere inside the modified atmospheric package depends on the plant producing a good respiration rate and the gas permeability of the film; both are controlled by temperature (Tano *et al.*, 2007). Sangkasanya and Meenune (2010) observed that longkong fruit stored in a low gas permeable package at 18°C induced more than 60% CO_2 accumulation and ethanolic formation. Fruit ethanolic accumulation is a catabolism process of changing pyruvate to ethanol (DeEll *et al*., 2003). On the other hand, our previous study (in chapter 3) suggested that passive MAP with the higher gas permeable package stimulated the mold growth on longkong fruit surface. However, in the present study, the active MAP storage and gas permeable package effectively maintained the respiratory gas and the overall accumulation of CO_2 was observed to be less than 35 %.

4.4.2 Chemical quality changes

The fruits pH and titratable acidity changed during storage under active MAP at different temperatures (Figure 20). An increase in the fruits pH was observed during storage at 18°C and 25°C up to 8 days and after that a continuous reduction in the fruits pH was found throughout the storage. The different temperatures significantly influenced the fruits pH ($P<0.05$). In addition, the increased storage period also significantly influenced the fruits pH ($P<0.05$). The fruits titratable acidity decreased continuously throughout the storage at 25°C. On the other hand, fruit stored at 18°C had fluctuations in the titratable acidity level. On the 4^{th} day it slightly decreased and then it increased up to the 12^{th} day of storage and afterwards it decreased gradually throughout the storage. Similar observations were found in previously published reports on longkong fruit (Lichanporn *et al*., 2009; Sangkasanya and Meenune, 2010). The storage temperatures and times were significantly affected the fruits titratable acidity level ($P<0.05$). The decline in the titratable acidity level might be because the fruit used the organic acid as a substrate for the respiration process.

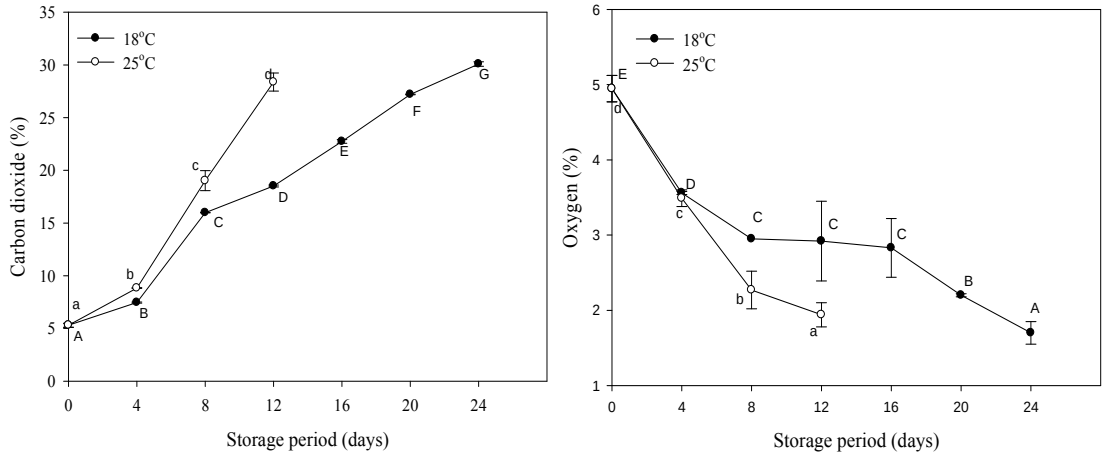


Figure 19. Respiration gas changes of longkong fruit during storage under active MAP at different temperature (18°C and 25°C)

Note: The vertical bar indicates standard error.

Different capital or small alphabets within the same storage temperature indicate the significant differences ($P<0.05$) (n=3).

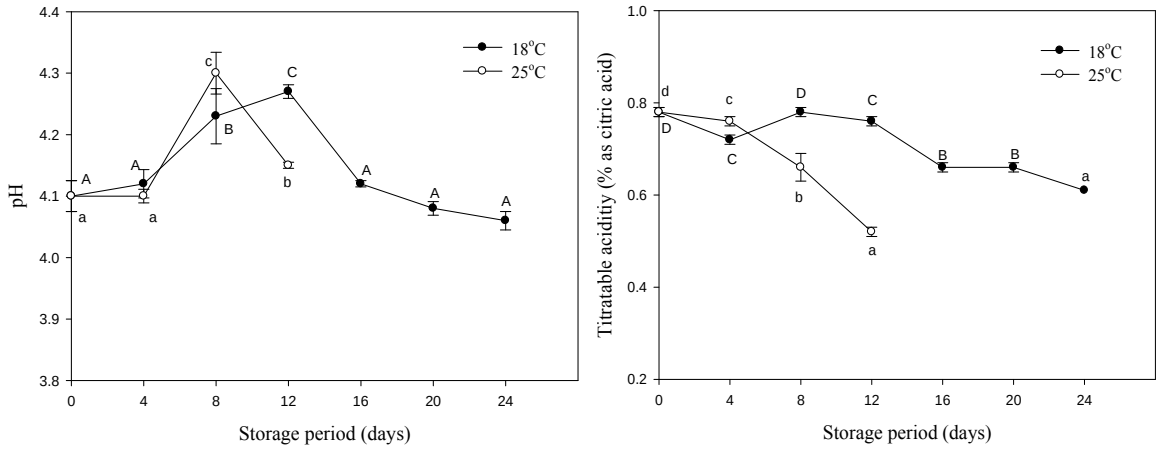


Figure 20. pH and titratable acidity changes in longkong fruit during storage under active MAP at different temperature (18°C and 25°C)

Note: The vertical bar indicates standard error.

Different capital or small alphabets within the same storage temperature indicate the significant differences ($P<0.05$) (n=3).

The total soluble solids (TSS), total sugar (TS) and reducing sugar (RS) content in longkong fruit showed changes in their levels during storage under active MAP at different temperatures (Figure 21). The different temperatures and sustained storage period significantly decreased the TSS level ($P<0.05$). Fruit stored at 18°C retained a higher level of TSS than at 25°C. On the other hand, the TS and RS levels in fruit stored at 18°C were maintained during storage. At the end of storage the TS and RS were slightly decreased at 18°C. The fruit stored at 25°C steadily decreased in TS levels and at the same time a steady increase in the RS level was observed. The decreased level of TS at 25°C might be a conversion to RS by invertase enzymes and it correlated with an increased level of RS at 25°C. This finding is in an agreement with a previous report which dealt with broccoli, permission and strawberry (Coupe *et al*., 2003; Del *et al*., 2009; Basson *et al*., 2010). Temperatures and storage period significantly influenced the TS and RS levels ($P<0.05$). Respiration is the oxidative breakdown of complex substrate molecules normally present in plant cells, such as sugars and organic acids, to simpler molecules such as CO_2 and H_2O (Saltveit, 2004). The increased percentage of CO_2 in the inner package atmosphere of fruit and the decreased level of titratable acidity might reduce the consumption of RS as the substrate for the respiration process. Therefore, higher levels of RS were observed in longkong fruit during storage.

Changes in the total phenolic content of longkong flesh and pericarp during active MAP storage at different temperatures are shown in Figure 22. Longkong fruits phenolic content increased up to 12 days of the storage period and then it gradually decreased throughout the storage. Fruit stored at 18°C retained the phenolic content a higher level than that stored at 25°C. On the other hand, longkong pericarp phenolic contents decreased up to 8 days of storage, and it then suddenly increased and afterwards a slight fluctuation was observed in the pericarp phenolics throughout storage. The different temperatures and storage period did not significantly affect the fruits flesh and pericarp phenolic content ($P\geq0.05$). The active MAP storage condition increased the novo biosynthesis of phenolics in plants (Simões *et al.*, 2011). The PAL enzyme might have contributed to the increased phenolic content in longkong (Lichanporn *et al*., 2009). The decrease in the phenolic

content during storage might be its utilization as a substrate for oxidoreductase enzymes and the cause of the increased concentration of CO_2 and N_2 inside the package atmosphere (Ahmad *et al.*, 2011; Bibi *et al.*, 2007; Cheng *et al.*, 2009).

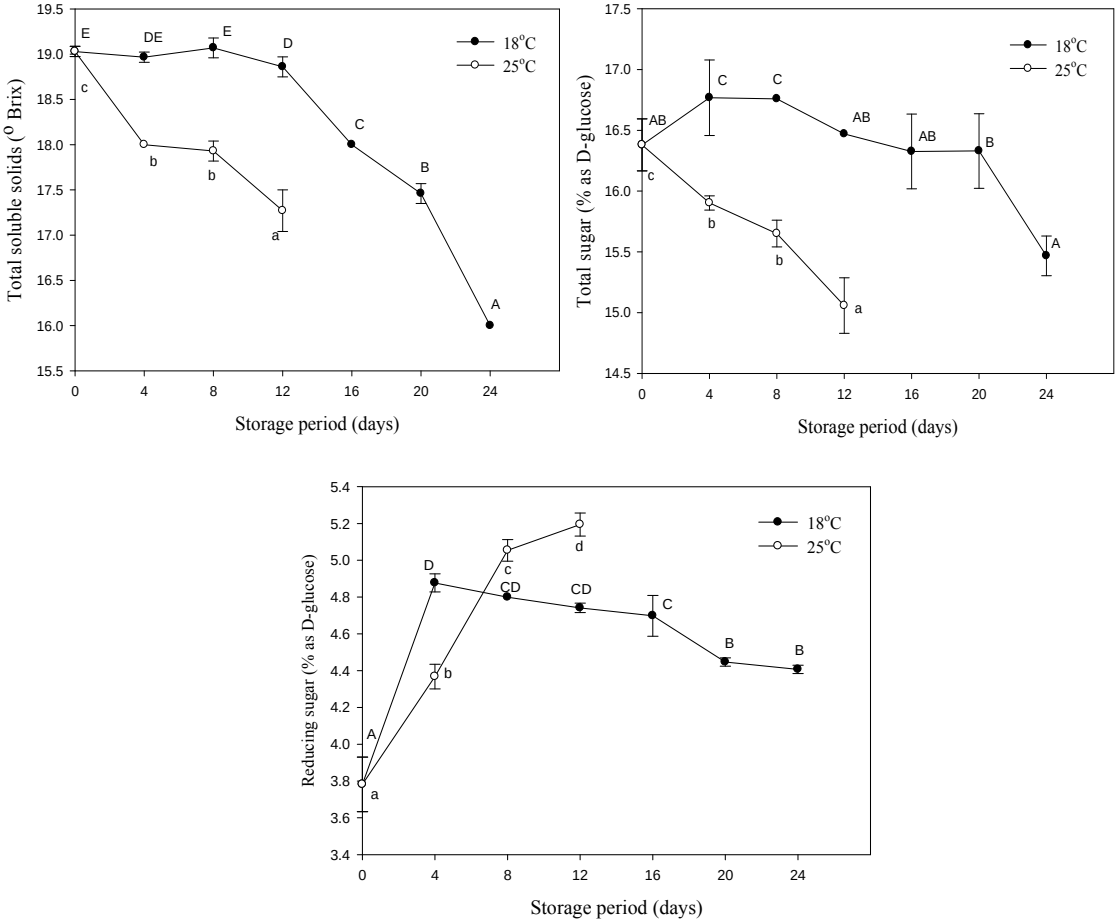


Figure 21. Changes in total soluble solids, total sugar and reducing sugar in longkong fruit during storage under active MAP at different temperature (18°C and 25°C)

Note: The vertical bar indicates standard error.

Different capital or small alphabets within the same storage temperature indicate the significant differences ($P<0.05$) (n=3).

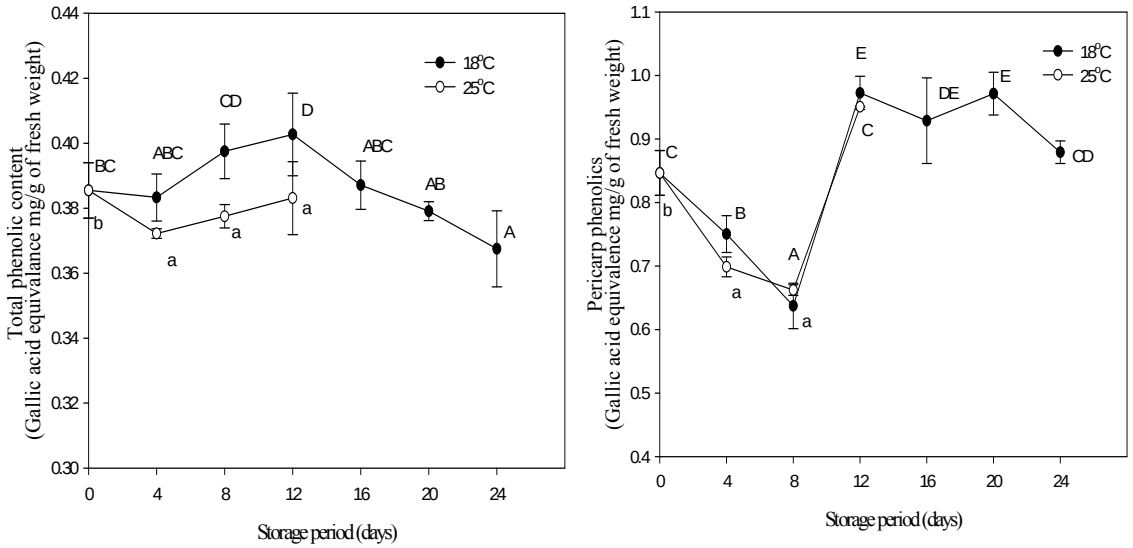


Figure 22. Changes in longkong fruit and pericarp total phenolic contents during storage under active MAP at different temperature (18°C and 25°C)

Note: The vertical bar indicates standard error.

Different capital or small alphabets within the same storage temperature indicate the significant differences ($P<0.05$) (n=3).

4.4.3 Fruit radical scavenging ability

Changes in fruit radical scavenging activity, such as DPPH scavenging ability, and FRAP activity during active MAP storage at different temperature are shown in Figure 23. The gradual increase of the fruits DPPH scavenging ability was found throughout the storage. Fruit stored at 18°C and 25°C had a slight fluctuation in DPPH scavenging activity at the end of storage. The temperatures and storage period significantly affected the fruit DPPH scavenging activity ($P<0.05$). On the other hand, the fruits FRAP activity steadily increased at the initial days of storage and at the end it slightly decreased. The continuous storage period significantly influenced the fruits FRAP activity ($P<0.05$). The reduction in FRAP activity at the end of the storage was mainly due to its strong relationship to total phenolic content. However, the different temperatures had not significantly affected the FRAP activity ($P\geq0.05$). Fruit stored at 25°C had a slightly higher radical scavenging activity than at 18°C. The higher

temperature storage could increase the fruit antioxidant activity (Moretti *et al*., 2010; Wang and Zheng, 2001). Normally, a fruits antioxidant activities depend on a wide range of compounds such as polyphenols, vitamins and pigments. (Liu and Wang, 2012). Longkong fruits antioxidant activity is contributed to by polyphenols and ascorbic acid (Lim *et al*., 2007). The slight decrease in antioxidant activity might be due to the prolonged storage or the process of senescence (Duan *et al*., 2011).

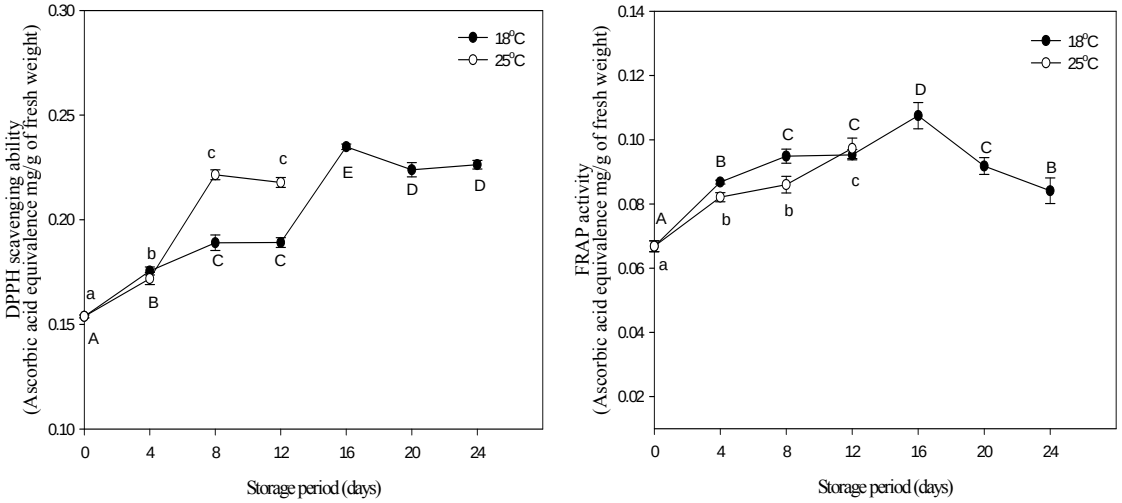


Figure 23. Changes in longkong fruit radical scavenging ability during storage under active MAP at different temperature (18°C and 25°C)

Note: The vertical bar indicates standard error.

Different capital or small alphabets within the same storage temperature indicate the significant differences ($P<0.05$) (n=3).

4.4.4 Fruit enzyme activity

Fruit antioxidant enzyme activity

Changes in longkong fruits antioxidant enzyme activities during under active MAP storage at different temperatures are given in Figure 24. The different storage temperatures and times had a significant effect on longkong fruits SOD activity ($P<0.05$). The SOD activity increased throughout the storage at different temperatures. A higher level of SOD activity was observed in fruit stored at 25°C than at 18°C. SOD is considered as the primary antioxidant enzyme to eliminate

superoxide radicals (O_2^-) caused by oxygen radicals in plants when responding to stress factors (Jaleel *et al.*, 2009). SOD activity changes during storage are induced by the substrate level-superoxide radical, which increases during stress conditions (Li and Zhang, 2010). Ding *et al*. (2006) reported that in loquat fruit, stored under active MAP with 5% O_2 and 5% CO_2, was controlled the production of oxidative stress, which can produced the super oxide radicals (O_2^-) and therefore, higher SOD activity was retained.

CAT belongs to an important antioxidant enzyme, which scavenges the active oxygen species in plant cells (Li and Zhang, 2010). Higher CAT activity was observed in fruit stored at 25°C than at 18°C in the initial storage period. Fruit stored at 25°C had a higher level of CAT activity on the 4th day during storage and it then gradually decreased. Fruit stored at 18°C slightly decreased in CAT activity for up to 12 days of storage and then it gradually increased throughout the storage. The temperatures and times significantly affected the CAT activity ($P<0.05$). POD enzyme is mainly involved in plant growth, and the development and senescence process, and it is an important free radical scavenging enzyme (Lee and Lee, 2000). The POD activity in longkong fruit flesh was observed to be at an unsteady level throughout the storage at both temperatures. Fruit stored at 25°C had a higher level of POD activity as compared to that at 18°C. The temperatures and times during storage were significantly influence the POD activity in longkong fruit flesh ($P<0.05$). CAT and POD are important enzymes that eliminate the H_2O_2, which is produced by SOD when scavenging the superoxide radicals (Bloknina *et al.*, 2003; Duan *et al.*, 2011).

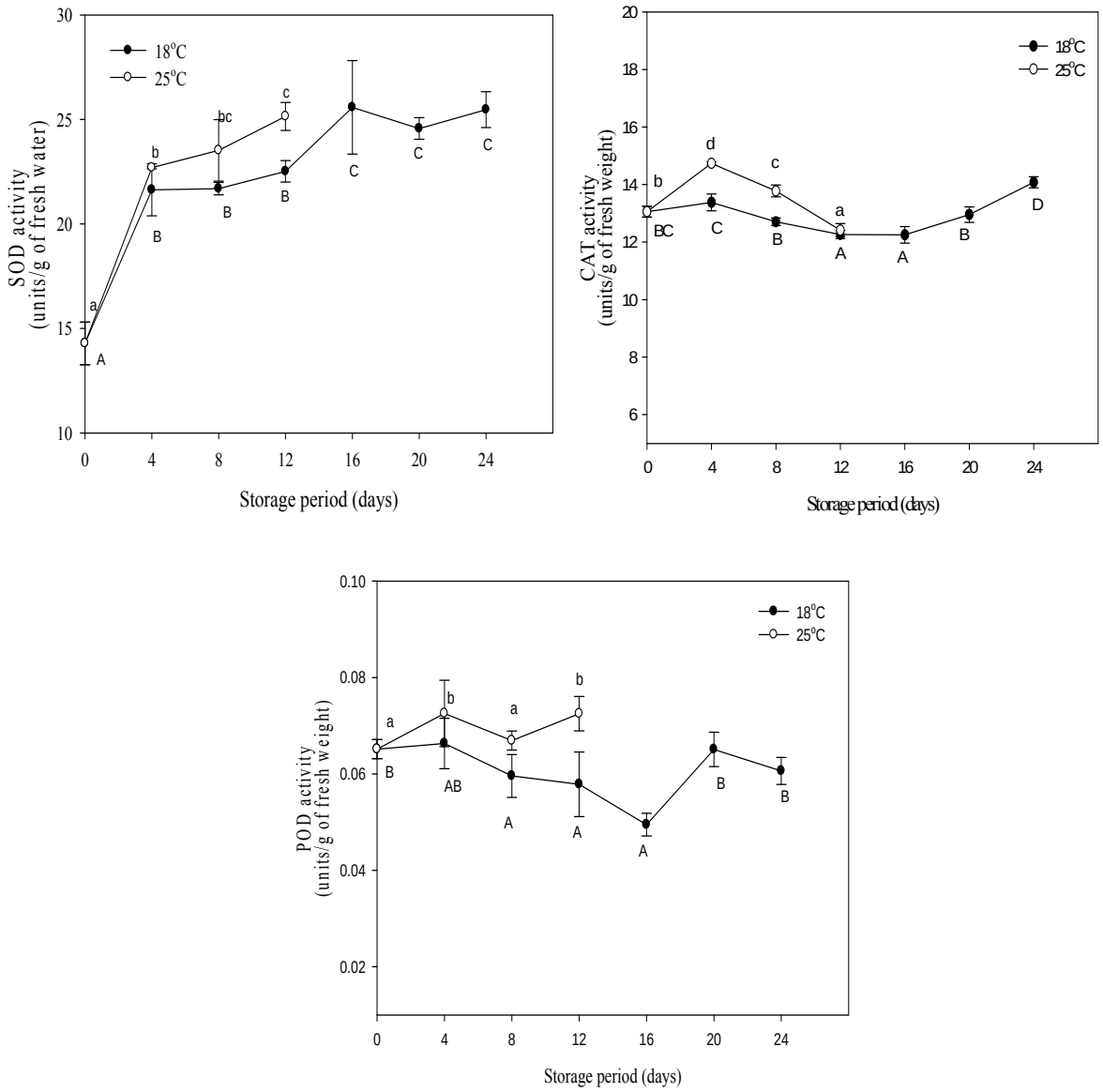


Figure 24. Antioxidant enzyme activity in longkong fruit during storage under active MAP at different temperature (18°C and 25°C)

Note: The vertical bar indicates standard error.

Different capital or small alphabets within the same storage temperature indicate the significant differences ($P<0.05$) (n=3).

Fruit pericarp browning enzyme activity

Fruit pericarp browning enzymes such as PAL, PPO and POD activities were changed during storage under active MAP at different temperatures (Figure 25). The PAL activity increased immensely in fruit stored at 25°C throughout the storage. Fruit stored at 18°C saw a gradual increased in PAL activity during the storage and at the end of storage PAL activity was slightly decreased. However, the PAL activity was at a lower level in fruit stored at 18°C than at 25°C. The temperature and times of storage significantly influenced the PAL activity ($P<0.05$). The increased PAL activity could be due to the stress that was induced by the storage temperature. The PAL enzyme synthesizes the free phenolics by deamination of phenylalanine and thereby produces trans-cinnamate and monophenols. Furthermore, PPO can convert these mono phenols to di-phenols that consequently oxidises and produces browning (Choehom *et al.*, 2004).

On the other hand, longkong fruit pericarp browning was mainly attributed to the PPO and POD enzymes (Lichanporn *et al.*, 2009). PPO activity in fruit pericarp on the 4^{th} day of storage at different temperatures attained the maximum level and afterwards, it gradually decreased. Fruit stored at 25°C had a higher level of PPO activity than that at 18°C. However, at the end of storage, fruit stored at 18°C showed a slight increase in PPO activity. Mishra *et al*. (2012) reported that increased browning in fruit stored at an ambient temperature might be due to the increased senescence process that induced the activities of oxidoreductase enzymes. The retarded PPO activity in longkong fruit pericarp was brought about by soaking longkong in 1.5% citric acid solution for 15 min. This was a consequence of the reduced availability of O_2 inside the package (Ducamp-Collin *et al.*, 2008). At the same time, fruit pericarp stored at 25°C had a higher level of POD activity throughout the storage. POD activity in fruit pericarp stored at 18°C was maintained at a constant level for up to 16 days and then it steadily increased until the end of storage. The different temperatures and storage periods significantly influenced the PPO and POD activity in longkong pericarp ($P<0.05$).

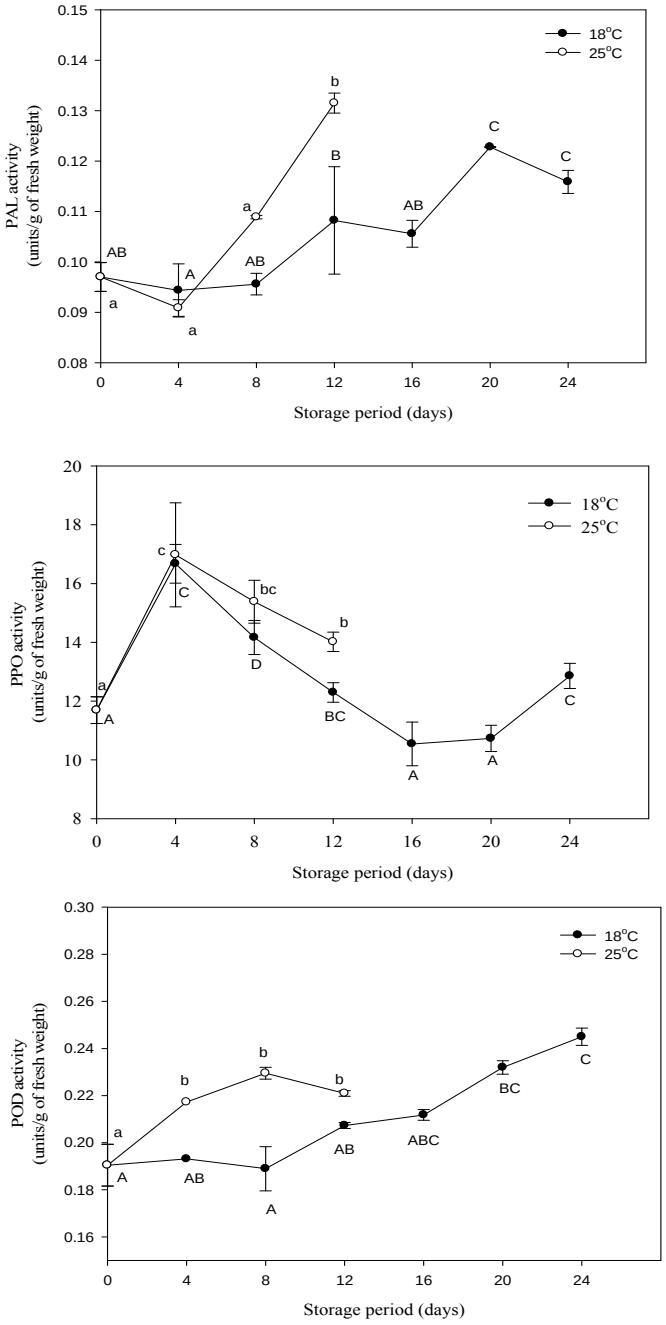


Figure 25. Longkong fruit pericarp browning related enzyme activity during storage under active MAP at different temperature (18°C and 25°C)

Note: The vertical bar indicates standard error.

Different capital or small alphabets within the same storage temperature indicate the significant differences ($P<0.05$) (n=3).

4.4.5 Pericarp ultrastructural changes

The pericarp browning of longkong fruit is mainly attributed to the oxidoreductase enzyme, which is present in the cytoplasm and mitochondria and phenols located in the vacuole of plant cells (Lichanporn *et al*., 2009). The oxidoreductase enzyme and substrate interact with each other and produce browning when the cell membrane system is damaged (Wang, 1990). The study of the fruits ultrastructural cell is predominantly useful for observing the browning reaction that is caused by cell damage. Longkong fruit ultrastructural changes, such as surface epidermal trichomes and the pericarp cross section hyperdermal tissues were observed on the 0 day and at the end of storage day (Figure 26). The fruits pericarp surface was fully covered by epidermal trichomes because it was fresh (on the 0 day). The continuous storage period significantly affected the fruit pericarp trichomes. At the end of storage, fruit stored at 25°C had showed the higher losses in surface trichomes and shrinkage on the trichomes as compared to that at 18°C. Mayer (2006) and Hare and Walling (2006) reported that damage or loss in glandular trichomes could induce the browning on fruit pericarp. This was due to a PPO enzyme that is present in the glandular trichomes.

On the other hand, the fruits parenchyma cells in the hyperdermal tissues had not had any changes in their shapes on the 0 day, as it is fresh. When the storage life increased, changes in the parenchyma cells began to appear. Fruit stored at 18°C had minor changes in their parenchyma cell structure. The oval shape in the parenchyma cells were extended slightly. Fruit stored at 25°C had a remarkable change in their hyperdermal tissues, which resulted in many of the parenchyma cells being damaged. The changes in the fruits hyperdermal cells at 25°C could be respiration-induced desiccation in the cell membranes. Fruit stored at 18°C at the end of storage had a slight change in their hyperdermal parenchyma cells. This change was a bit higher compared to the fruit stored at 18°C by day 12, but the damage was less than at 25°C.

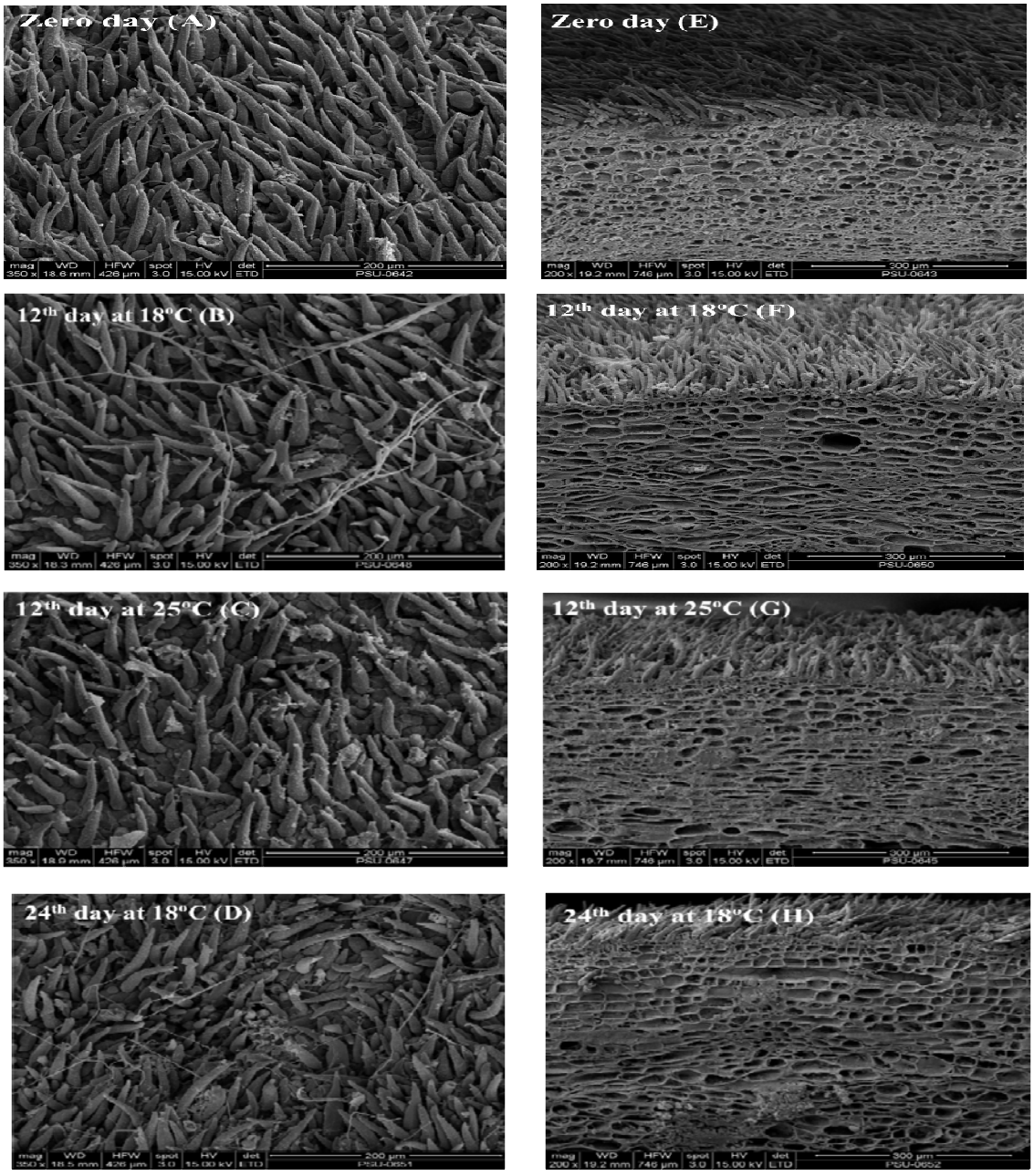


Figure 26. Ultrastructural changes on the 0 day, 12th day and 24th day of longkong fruit pericarp surface (A-D) and cross section (E-H) during storage under active MAP at different temperatures (18°C and 25°C)

4.5 Conclusion

Longkong fruit stored under active MAP at 18°C effectively maintained its quality and the shelf life were extended for up to 24 days. Fruit stored at 25°C had an extensive loss in quality and, consequently, the shelf life was limited to 12 days. Active MAP storage at 18°C maintains fruit quality by slowing down the respiration process, weight loss and browning related enzyme activities. Ultrastructural changes were also shown a low level of hyperdermal cell damage at 18°C. The fruits titratable acidity, sugar level and antioxidants were significantly maintained at 18°C.

CHAPTER 5

EFFECT OF METHYL JASMONATE ACTIVITY ON THE QUALITY CHANGES OF LONGKONG FRUIT DURING STORAGE UNDER LOW TEMPERATURE

5.1 Abstract

The quality changes of longkong fruit with (10, 20 and 30 μmol/l) and without methyl jasmonate (control) treatment in low temperature storage (13°C and 85% relative humidity) were determined. The higher concentration of methyl jasmonate (MeJA) treatment effectively decreased the changes in longkong fruits pericarp colour (L*, a*, b*, H° and C*), browning index and weight loss than the lower concentration and the control fruits ($P<0.05$). MeJA treatment was non-significantly increased the fruit ascorbic acid content than the control ($P\geq0.05$). MeJA treatments were also maintained the fruit pH, titratable acidity and total soluble solids ($P<0.05$). Fruit flesh total phenolics, DPPH scavenging ability and FRAP activity was contained high level in the higher concentration of MeJA treated fruits ($P<0.05$). Fruit superoxide dismutase, catalase and peroxidase activities were retained the higher level in MeJA treated fruits than the control fruits ($P<0.05$). The higher concentration of MeJA treated fruits maintained the texture by suppressed the activities of polygalacturonase and pectin methyl esterase ($P<0.05$). MeJA treated fruits were inhibited the increase of phenylalanine ammonia lyase enzyme and consequently, MeJA treated fruits contained the lower level of pericarp phenolics ($P<0.05$). Longkong fruits treated with the higher concentration of MeJA decreased the polyphenol oxidase and peroxidase activities ($P<0.05$). Longkong fruits treated with the higher concentration of MeJA maintained the pericarp ultrastructural epidermal hair and hyperdermal cell changes as compared to the lower concentration and the control fruits.

5.2 Introduction

Longkong fruit is an economically important plant produce in Thailand. It is originated in southern Thailand and then it widely spread to the rest of Thailand (Ketsa and Paull, 2008; Sangchote *et al.*, 2011). It is also cultivated in Borneo, Malaysia, Sri Lanka, India, Australia, Burma and Vietnam (Paull *et al.*, 1987). Longkong fruit is a non-climacteric and tropical fruit, it belongs to meliaceae family and classified as *Aglaia dookoo* Griff (Salakpetch, 2000; Paull, 2004; Lichanporn *et al.*, 2009). It contains high level of antioxidant activities, nutritional and health benefits (Lim *et al.*, 2007; Ketsa and Paull, 2008). However, the longkong fruit is highly perishable at ambient temperature due to pericarp browning and off flavour symptoms and thus, limits the fruit storage life to 4-7 days (Sangkasanya and Meenune, 2010). Low temperature storage extensively used to prolong the shelf life and maintain the quality of fruits and vegetables by delayed their senescence and deteriorations process (Lichanporn *et al.*, 2008). Longkong fruit stored below the ambient temperature has increased the shelf life to more than a week. However, too low temperature during storage can causes the severe deterioration such as chilling injury especially in tropical and subtropical fruits and vegetables (Paull, 2004; Ketsa and Paull, 2008).

Chilling injury is a physiological disorder persuaded by low but not freezing temperature. The indication of chilling symptoms differ with the crop, storage temperature, physiological period and chilling exposure length (Cao *et al.*, 2009a; Luo *et al.*, 2011; Sayyari *et al.*, 2011). Previous reports suggested that longkong fruit stored under low temperature between 12°C and 15°C had a chilling injury disorder (Piyasaengthong *et al.*, 1997; Paull, 2004; Ketsa and Paull, 2008). Chilling injury might be the consequence of oxidative stress caused by reactive oxygen species (ROS) via the over production of photosynthetic electrons from chloroplasts in the presence of light reaction and the production from mitochondria in the presence of dark reaction (Hasselt van, 1990; Paull, 1990; Purvis *et al.*, 1995; El-hilali *et al.*, 2003; Fung *et al.*, 2004). Plant resistant to chilling injury is linked with the increase of antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT) and peroxidase (POX) (Fung *et al.*, 2004; Zheng *et al.*, 2008). Chilling injury symptom appears at low temperature storage in fruits and vegetables and it is

frequently enhanced or more visible when transferred to non-chilling temperatures (El-hilali *et al.*, 2003). Longkong fruit chilling injury symptoms are skin browning, pitting and softer fruit with a water soaked appearance (Ketsa and Paull, 2008). Polygalacturonase (PG) and pectin methyl esterase (PME) are the pectin-degrading enzymes could attribute to fruit textural changes (Hadfield and Bennett, 1998; Willats *et al.*, 2001). The enzymes such as phenylalanine ammonia lyase (PAL), polyphenol oxidase (PPO) and peroxidase (POD) mainly attribute longkong fruit skin browning (Lichanporn *et al*., 2009).

Methyl jasmonate (MeJA) is a cyclopentanone compound and it considered as endogenous plant regulator that play a vital role on the stress response, plant growth and development (Cao *et al.*, 2009a; Cao *et al.*, 2010; Zhang *et al.*, 2012). Recently, MeJA has been used widely as a potential postharvest application for alleviation of chilling injury in many horticultural crops and maintain their quality (Ding *et al.*, 2002; Meng *et al.*, 2009; Zhang *et al.*, 2012). MeJA is an inexpensive treatment and easy to apply on various fruit produce (González-Aguilar *et al.*, 2003). However, the mechanism of MeJA on alleviation of chilling injury is still unclear. Several reports have been elucidated that, MeJA is easing the chilling injury by increasing the expression of a set of defence genes and enhance the antioxidant capacity in crops (Cao *et al.*, 2009a; Cao *et al.*, 2009b; Jin *et al.*, 2009; Zhang *et al*., 2012; Li *et al*., 2012; Cao *et al*., 2012). However, longkong fruit chilling injury information is very limited and it has not been studied with any compound to controlling their chilling injury symptoms. Therefore, the present objective was to determine the effect of MeJA on controlling the longkong quality loss and chilling injury during low temperature storage.

5.3 Materials and methods

5.3.1 Plant material

Longkong fruits at commercially matured stage (13^{th} weeks after anthesis) were purchased from a contact garden in songkhla province, Thailand. The harvested fruits were taken in to the laboratory within 2 hr at ambient temperature. Received fruits carefully removed from raceme without any apparent damage and

then washed in distilled water and kept at ambient temperature until fruit pericarp wet dried (approximately 30 min). Then, longkong fruits were used for the methyl jasmonate treatment and storage.

5.3.2 Methyl jasmonate treatment and storage

Longkong fruits separated randomly into four groups. The first group (MeJA not treated) served as a control. The second, the third and the fourth groups treated with 10, 20 and 30 µmol/l MeJA, respectively. All the groups treated in an air-sealed container for 24 hr at room temperature and then ventilated for at least 2 hr. After treatment, 15 individual longkong fruit placed on a polypropylene tray per replication. Then, the fruits were stored in an incubator at 13°C and 85% RH for 16 days. Fruits were measured the following quality determinations at four day intervals.

5.3.3 Physical quality

Measurement of fruit pericarp colour

The surface colour on four sides of an individual fruit was measured by using a Hunter Lab colourimeter in term of CIE lightness (L*), redness (a*) and yellowness (b*) values (Sapii *et al*., 2000). Hue angle (H°) and chroma value (C*) was measured in accordance to the method of McGuire (1992). Hue angle was calculated as arctangent (b*/a*) (0° = red-purple, 90° = yellow, 180° = bluish-green, 270° = blue) and chroma (colour saturation) value was calculated as $C^* = (a^{*2} + b^{*2})^{1/2}$.

Measurement of browning index

The severity of chilling induced browning was evaluated after longkong fruits transferred from the cold temperature to room temperature for 2 hr. The degree of browning index was measured by the extent of surface browning on the fruit pericarp. Five-trained panellists were used for the browning index measurement. They rated on a scale from 1 to 5, based on the intensity of surface browning; Score 1 = no chilling injury symptoms; Score 2 = browning symptoms covered 1-25% of the surface area; Score 3 = browning symptoms covered 26-50% of the surface area; Score 4 = browning symptom covered 51-75% surface area; Score 5 = browning

symptoms covered 76-100% of the surface area (Appendix A) (Nilprapruck *et al*., 2008).

Measurement of fruit weight loss

Longkong fruit weight loss was measured by using digital weighing balance. Weight loss was expressed as the percentage of weight loss with respect to the initial weight.

5.3.4 Chemical quality

Peeled and deseeded longkong flesh homogenate was prepared by blending, then filtered using cheesecloth and then subjected to chemical analysis.

Measurement of fruit pH

The pH was measured by using a Sartorius PB-20 (Germany) digital pH meter at ambient temperature after calibrated with pH 4.0 and 7.0.

Measurement of total soluble solids

The TSS was determined by using an Atago 1E (Japan) hand refractometer at 25°C. The results were expressed as °Brix.

Measurement of titratable acidity

The titratable acidity (TA) was determined in accordance to Sangkasanya and Meenune (2010). The results were expressed as percentages of citric acid content.

Measurement of ascorbic acid

Ascorbic acid was analysed by an indophenol titration method in accord with Nielsen (2010). The results were expressed as ascorbic acid content (mg/g of fresh weight).

Measurement of total phenolic content

Total phenolic content of fruit flesh was determined by using the Folin-Ciocalteu reagent (Lim *et al*., 2007). Edible portion (5 g) was homogenized with 20 ml of 50% ethanol in a mortar and pestle method at 4°C. The homogenate was filtered

with cheesecloth and then, the filterate was centrifuged at 10,000*g* at 4°C for 10 min. The supernatant collected and used for total phenolic measurement. A 0.3 ml of fruit extract was placed in test tubes followed by 1.5 ml of Folin-Ciocalteu reagent (1:10 dilution with distilled water) and 1.2 ml of sodium carbonate (7.5% w/v). The tubes were vortexed, covered with parafilm and allowed to stand for 30 min at room temperature. Absorbance was measured at 765 nm. Total phenol contents were expressed as gallic acid equivalents mg per g fresh fruit.

Total phenolic content of fruit pericarp tissue was analysed by the method of Singleton and Rossi (1965). Pericarp tissues 2 g were homogenized with 20 ml of 80% ethanol (1:1 w/v) in a mortar and pestle method. The homogenized sample was centrifuged at 12,000*g* for 20 min. A 0.4 ml of supernatant was mixed with 0.4 ml of Folin-Ciocalteu reagent and 1 ml of 7% sodium carbonate solution. The volume was increased to 10 ml in distilled water and vortexed the mixture, then incubated for 1 hr at room temperature. Absorbance was measured at 750 nm using a spectrophotometer. Total phenol contents were expressed as gallic acid equivalents mg per g fresh fruit.

5.3.5 Determination of radical scavenging ability

Edible portions of longkong, 25 g, were deseeded and homogenised using a mortar and pestle at 4°C. Then the homogenised sample was transferred into a 100 ml volumetric flask and the volume made up with 50% ethanol. The mixture was shaken with a vibrator for 10 min, and then filtered under suction if the filtrate appeared to be very cloudy. The filtrate was centrifuged at 10,000*g* at 4°C for 10 min to obtain a clear supernatant solution (Lim *et al*., 2007; Lichanporn *et al*., 2009). The supernatant was immediately used for radical scavenging ability analysis, such as 1, 1-diphenyl-2-picrylhydrazyl (DPPH) scavenging ability and ferric reducing power ability (FRAP).

Measurement of DPPH radical scavenging activity

DPPH assays were measured according to the method of Binsan *et al*. (2008). A 1.5 ml of the sample was added to the test tube and followed with 1.5 ml of 0.15 mM DPPH in 95% ethanol. The mixture was mixed vigorously and kept in a dark place for 30 min at room temperature and then a sample was measured at 517

nm. Distilled water was used instead of the sample for the blank. A standard curve was prepared by using ascorbic acid. The activity was expressed as ascorbic acid equivalents mg per g fresh weight.

Measurement of ferric reducing power

The ferric reducing power of the fruit extract was determined in accordance with the method of Benzie and Strain (1996). Stock solution was made, of 300 mM acetate buffer (pH 3.6), 10 mM 2,4,6-tripyridyl-s-triazine (TPTZ) solution in 40 mM hydrochloric acid, and 20 mM ferric chloride hexahydrate ($FeCl_3.6H_2O$). The FRAP solution was freshly prepared by adding 25 ml of acetate buffer, 2.5 ml of TPTZ solution and 2.5 ml of $FeCl_3.6H_2O$ solution. The mixed solution was incubated at 37°C for 30 min. A sample of 150 µl was mixed with 2850 µl of FRAP solution and kept for 30 min in dark. The absorbance was measured at 593 nm. A standard curve was prepared by using ascorbic acid. The activity was expressed as ascorbic acid equivalents mg per g fresh weight.

5.3.6 Enzyme activity analysis

Longkong fruit flesh was used for antioxidant (SOD, CAT and POD) and textural enzymes (PG and PME) analysis. On the other hand, the browning related enzyme (PAL, PPO and POD) activity was analyzed in longkong fruit pericarp.

Extraction and determination of superoxide dismutase activity

Longkong deseeded flesh (10 g) was homogenized with 25 ml of buffer solution (100 mM sodium phosphate buffer (pH 6.4)) and 0.5 g polyvinyl pyrrolidone in a mortar and pestle at 4°C. The homogenate was filtered through one layer of cheesecloth and then the filtrate was centrifuged at 19,000*g* for 30 min at 4°C. After that, the supernatants of crude enzyme extract were collected and used for superoxide dismutase (SOD) analysis. Determination of SOD was performed as described in a method of Constantine and Stanley (1977). The reaction mixture (3 ml) contained 50 mM sodium phosphate buffer (pH 7.8), 65 mM methionine, 150 µM nitroblue tetrazolium (NBT), 0.5 mM EDTA, 20 µM riboflavin and 0.1 ml of the enzyme extract. The mixtures were illuminated by fluorescent light (60 µmol/m^2/s)

for 10 min and the absorbance was then determined at 560 nm. Identical solution held in the dark served as blank. One unit of SOD was defined as the amount of enzyme that caused a 50% decrease of the SOD-inhibitable NBT reduction. The specific activity was expressed as unit per g of fresh weight.

Extraction and determination of catalase activity

Longkong deseeded flesh (10 g) was homogenized with 25 ml of buffer solution (50 mM sodium phosphate buffer (pH 7.0)) and 0.5 g polyvinyl pyrrolidone in a mortar and pestle at 4°C. The homogenate was filtered through one layer of cheesecloth and then the filtrate was centrifuged at 19,000*g* for 30 min at 4°C. After that, the supernatants of crude enzyme extracts were collected and used for catalase (CAT) analysis. CAT activity was measured according to the method described by Beers and Sizer (1952). The reaction mixture contained 2 ml of sodium phosphate buffer (50 mM, pH 7.0), 0.5 ml H_2O_2 (40 mM) and 0.5 ml crude enzyme extract. The decomposition of H_2O_2 was measured by the decline in absorbance at 240 nm. One unit of CAT was defined as the amount of enzyme that decomposing 1 mM of H_2O_2 per minute at pH 7.0 and 25°C. The specific activity was expressed as unit per g of fresh weight.

Extraction and determination of peroxidase activity

Longkong deseeded flesh (10 g) was homogenized with 25 ml of buffer solution (100 mM sodium phosphate buffer (pH 6.4)) and 0.5 g polyvinyl pyrrolidone in a mortar and pestle at 4°C. The homogenate was filtered through one layer of cheesecloth and then the filtrate was centrifuged at 19,000*g* for 30 min at 4°C. After that, the supernatants of crude enzyme extracts were collected and used for peroxidase (POD) analysis. POD activity was assayed in accord with the method of Jiang *et al*. (2002). The 0.1 ml of enzyme was incubated in 2 ml buffered substrate (100 mM sodium phosphate, pH 6.4 and 8 mM guaiacol) at 30°C for 5 min and then, the increasing absorbance measured at 460 nm for 120 s after adding 0.9 ml of H_2O_2 (24 mM). One unit of POD activity was defined as the amount that caused of 0.01 in the absorbance per min. The specific activity was expressed as unit per g of fresh weight.

Extraction and determination of polygalacturonase (PG)

PG was extracted and assayed according to the method of Qiuping and Wenshui (2007). Longkong deseeded flesh (2 g) was homogenized with 10 ml of 0.2 mol/l acetic acid buffer (pH 6.0) in a mortar and pestle at 4°C. The homogenate was filtered through one layer of cheesecloth and then the filtrate was centrifuged at 11,000*g* for 20 min at 4°C. The supernatants of crude enzyme extract was collected and used for PG assay. The determination of PG activity was based on the hydrolytic release of galacturonic acid from polygalacturonic acid. The reaction mixture contained 0.3 ml of 1.0% (w/v) polygalacturonic acid in 40 mmol/l Na–acetate buffer (pH 4.6), 0.1 ml of crude enzyme extract and then followed by 1.9 ml of deionized water. The reaction mixture was incubated at 37 °C for 1 hr and the reaction was terminated by the addition of 1.5 ml of dinitrosalicylate reagent and immersion in a boiling water bath for 5 min. The final volume of the sample was adjusted to 25 ml with deionized water. Samples then cooled to room temperature and the absorbance was measured at 540 nm. The released amount of galacturonic acid from polygalacturonic acid was obtained from the galacturonic acid standard curve. One unit of PG activity was defined as that amount of enzyme, which liberates 1 mg galacturonic acid under the given assay, conditions (60 min, 37°C). The specific activity was expressed as unit per g of fresh weight.

Extraction and determination of pectin methylesterase (PME)

Pectin methylesterase (PME) activity was measured according to the method of Hangermann and Austin (1986) with slight modification. Longkong deseeded flesh (5 g) was ground with 20 ml 8.8% (w/v) of NaCl and 0.5 g of polyvinylpyrrolidone (insoluble) at 4°C. The homogenate was filtered through one layer of cheesecloth and then the filtrate was centrifuged at 10,000*g* for 30 min at 4°C. The collected supernatant of crude extract was adjusted the pH to 7.5 and then assayed for PME activity. The activity was assayed in a reaction mixture containing 2.0 ml of 0.5% (w/v) pectin, 0.15 ml of 0.01% bromothymol blue, 0.75 ml of water, and 0.1 ml of enzyme extract. All solutions (pectin, indicator dye and water) were adjusted to pH 7.5 with 2 M NaOH just before the experiment starts. After adding the enzyme extract, the decrease in the absorbance at 620 nm was measured

spectrophotometrically. Calculation of the activity was carried out against the standard curve as described by Hangermann and Austin (1986). One unit of PME activity was expressed as μmoles of H^+ produced per min. The specific activity was expressed as unit per g of fresh weight.

Extraction and determination of phenylalanine ammonia lyase

Pericarp tissues (2 g) from 20 fruits were homogenized in 20 ml of a 0.1 M sodium borate buffer (pH 8.0) solution contained 0.2 g of insoluble PVP, 5 mM mercaptoethanol, and 2 mM ethylene diamine tetra acetic acid (EDTA) at 4ºC. The homogenate was centrifuged for 20 min at 19,000*g* and 4ºC, and then the supernatant of crude extract was collected for the phenylalanine ammonia lyase (PAL) enzyme assay. PAL activity was determined in accord with the method of Jiang and Joyce (2003). A mixture of 0.1 ml enzyme extract and 2.9 ml of 0.1 M sodium borate buffer (pH 8.0) solution containing 3 mM l-phenylanine was incubated for 1 hr at 37ºC. An increase in the PAL activity at 290 nm, due to the formation of trans-cinnamate, was measured spectrophotometrically. One unit of enzyme activity was defined as the amount that caused an increase of 0.01 in the absorbance per hr. The specific activity was expressed as unit per g of fresh weight.

Extraction and determination of polyphenol oxidase

Pericarp tissues (5 g) from 20 individual fruits were ground with 40 ml of 0.2 M sodium phosphate buffer (pH 6.4) and homogenised using a mortar and pestle at 4ºC. After that, the homogenised sample filtered through cheesecloth and then the filtrate was centrifuged at 12,000*g* for 30 min. The supernatant of crude extract was collected for measuring polyphenol oxidase (PPO) activity (Tian *et al*., 2002). The reaction mixture consisted of 3 ml of 0.5 M 4-methylcatechol in 0.2 M sodium phosphate buffer (pH 6.4) and 0.1 ml of the crude enzyme sample. The absorbance was measured at 398 nm at 25ºC for 1 min. One unit of enzyme activity was defined as an increased in one absorbance unit per min. The specific activity was expressed as unit per g of fresh weight.

Extraction and determination of peroxidase

Pericarp tissues (2 g) from 20 individual fruits were homogenized in 20 ml of 0.05 M phosphate buffer (pH 7) solution and 0.2 g of insoluble polyvinylpyrrolidone (PVP) using a mortar and pestle at 4ºC. The homogenate was filtered through cheesecloth and the filtrate was centrifuged for 20 min at 19,000*g* at 4ºC (Lichanporn *et al*., 2009). The supernatant of crude extract was collected for analysing peroxidase (POD) activity. POD activity, using guaiacol as a substrate, was analysed by the method of Zhang *et al*. (2005). A 3 ml reaction mixture contained 25 µl of crude enzyme extract, 2.78 ml of 0.05 M phosphate buffer (pH 7.0), 0.1 ml of 20 mM hydrogen peroxide (H_2O_2) and 0.1 ml of 20 mM guaiacol. An increased in POD activity was recorded at 470 nm for 2 min. One unit of enzyme activity was defined as the amount that caused a change of 0.01 in the absorbance per min. . The specific activity was expressed as unit per g of fresh weight.

Ultrastructural analysis

Ultrastructural analysis of the pericarp tissue of both surface and cross-sections were measured (Lichanporn *et al*., 2009). The fruit pericarp tissues sampled on the zero day and 16th day of storage. Samples were fixed in 2.5% glutaraldehyde in a 0.1 M phosphate buffer with a pH of 7.2 for 2 hr. The specimens were rinsed twice in phosphate buffer for 20 min, and then once in distilled water for 20 min. The pericarp tissues dehydrated in a graded alcohol series, sputtered with platinum/palladium, and then dried by a critical point dryer. The dried peels were mounted on the stubs and twice gold-coated. Then, the samples were observed under the scanning electron microscope (Quanta 400, FEI, Praha, Czech Republic).

5.3.7 Statistical analysis

All the experiments were analysed in factorial (5X4) and laid out in a completely randomized design (CRD). Each experiment and the determinations were done in triplicates. The data represent the means ± standard deviation, and they were analysed by one-way analysis of variance (ANOVA) with the Statistical Package for Social Science (SPSS for windows, SPSS Inc., Chicago, IL, USA). The significance of the difference among treatments was measured using Duncan's new multiple range test (DMRT), with a level of significance of 0.05.

5.4 Results and Discussion

5.4.1 Physical quality changes

Longkong fruit pericarp colour such as lightness (L*), yellowness (b*) and redness (a*) changes are shown in Table 13. Fruit pericarp L* and b* values were continuously decreased in the control and MeJA treated fruits throughout the storage. In addition, H° and C* in fruit pericarp was also decreased (Figure 27). Conversely, the redness (a*) values on fruit pericarp were increased throughout the storage (Table 13). The control fruits pericarp were severely decreased in L*, b*, H° and C*, whereas the severe increased in a* values were observed during storage. The different concentration of longkong MeJA treatment had a significant control on their pericarp colour changes ($P<0.05$). However, pericarp H° and C* of fruit was non-significantly controlled by the different MeJA concentration. The interaction between different MeJA treatments and storage period was also affected the pericarp colour changes ($P<0.05$). The higher MeJA concentrations (20 and 30 μmol/l) were retained the better pericarp colour as compared to the lower concentration and the control fruits. The increased a* values and decreased L*, b*, H° and C* in the control fruits was pointed out the increased of browning on pericarp. The different values on treated longkong fruit pericarp on zero day could be influenced by the effect of MeJA. This finding is in an agreement with the previous reports (Meng *et al*, 2009; Cao *et al*., 2012). At the end of storage, the pericarp colour changes were sharply increased in all the treatments. This could be due to the loss of freshness and increased dryness of longkong fruit pericarp that induced by the sustained low temperatures storage. The increased browning on pericarp under low temperature is the primary symptom of chilling injury in longkong fruit (Wang, 1990; Paull, 2004; Ketsa and Paull, 2008). The suppressed increase of browning in MeJA treated fruits might be the inhibitory action of MeJA on the oxidoreductase enzymes (Nilprapruck *et al.*, 2008; Meng *et al.*, 2009).

Table 13. Effect of methyl jasmonate on colour changes in longkong fruit pericarp during storage at 13°C and 85% relative humidity

Storage Period (days)	Treatment (µmol/l MeJA)	Lightness (L*)	Yellowness (b*)	Redness (a*)
0	Control	63.73±1.97^{k}	37.10±0.85^{jk}	8.88±0.19^{abc}
	10	61.39±1.41^{jk}	35.77±0.51^{ijk}	8.69±0.27^{ab}
	20	60.73±1.03^{ijk}	35.77±1.40^{jk}	8.99±0.13^{abcd}
	30	60.39±0.65^{hij}	37.11±0.85^{k}	8.55±0.77^{a}
4	Control	58.95±3.27^{ghij}	35.18±1.96^{ijk}	9.16±0.75^{bcdef}
	10	57.68±2.98^{fghi}	34.46±2.16^{hij}	9.22±0.74^{bcdef}
	20	59.07±2.89^{ghij}	34.46±2.12^{hij}	8.54±0.69^{a}
	30	57.49±3.28^{fgh}	33.56±1.89^{fghi}	9.10±0.89^{abcde}
8	Control	53.22±1.15^{e}	32.21±2.84^{efg}	9.58±0.96^{defg}
	10	56.99±3.02^{fg}	34.28±1.87^{ghij}	9.25±0.72^{bcdefg}
	20	55.79±1.34^{efg}	33.41±2.77^{fghi}	9.47±0.84^{cdefg}
	30	55.94±1.10^{efg}	33.03±2.51^{fgh}	9.75±0.82^{fg}
12	Control	49.72±2.48^{d}	26.11±3.32^{d}	9.84±0.82^{g}
	10	55.09±3.88^{ef}	31.97±3.36^{ef}	9.62±0.77^{efg}
	20	55.24±1.43^{ef}	32.27±2.19e^{efg}	9.51±0.46^{defg}
	30	52.93±2.58^{e}	30.83±3.15^{e}	9.77±0.61^{fg}
16	Control	34.52±2.11^{a}	10.36±2.47^{a}	10.92±0.76^{h}
	10	41.22±3.73^{b}	15.15±3.36^{b}	9.79±0.70^{g}
	20	44.86±2.19^{c}	17.51±2.58^{c}	9.50±0.43^{defg}
	30	41.52±3.52^{b}	16.07±2.38^{bc}	9.84±0.44^{g}

Note: Different superscripts in the same column indicate the significant differences ($P<0.05$) (n=3)

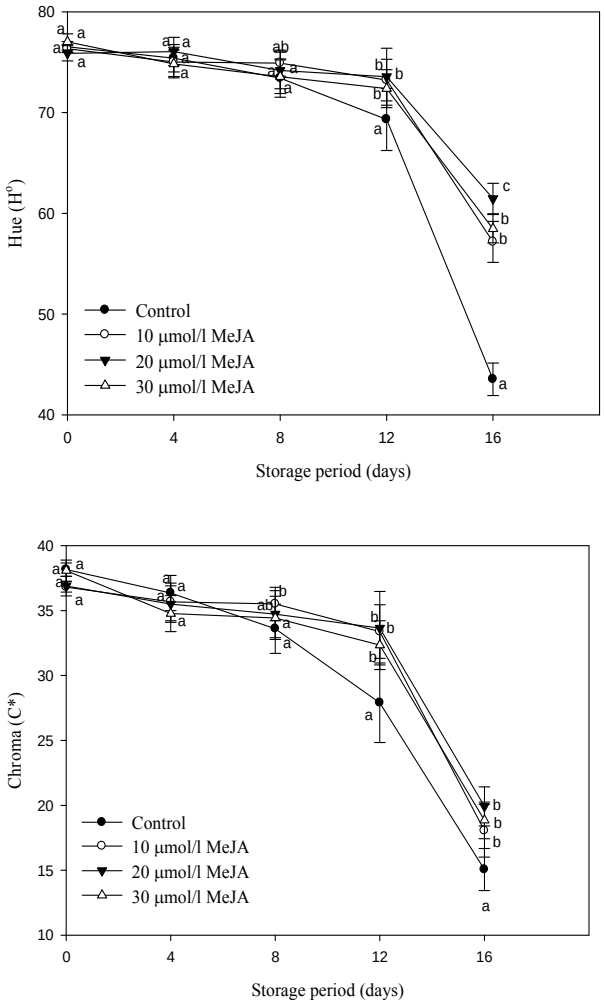


Figure 27. Effect of methyl jasmonate on H° and C* of longkong pericarp changes during storage at 13°C and 85% relative humidity

Note: The vertical bar indicates the standard error.

The different alphabets within the same storage period indicate the significant differences ($P<0.05$) (n=3).

Longkong fruit pericarp browning index (BI) is shown in Figure 28. The fruit pericarp browning rapidly increased as the storage period increased. The increased pericarp BI was directly correlated with the pericarp colour changes in Table 12. The control fruits had the higher BI score than the MeJA treated fruits. The lower BI score was noticed on MeJA treated fruits throughout the storage period. The different concentration of MeJA significantly controlled the fruit pericarp BI ($P<0.05$). At the end of storage, the higher concentration of MeJA treated fruits were attained the lower BI score as compared to the lower concentration and the control fruits. The interaction between MeJA treatments and storage period was significantly influenced the BI score ($P<0.05$). MeJA controlled the increase of fruit browning by the increase of defence mechanism and diminishing the complex chlorogenic acid (CA) and increased the free CA in fruits and vegetables (Tsao and Zhou, 2000; Alvarez-Parilla *et al*., 2007).

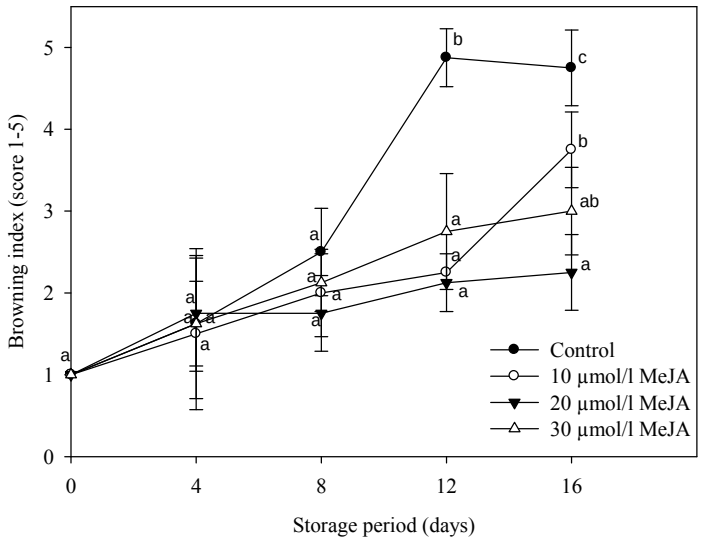


Figure 28. Effect of methyl jasmonate on the control of pericarp browning in longkong fruit during storage at 13°C and 85% relative humidity

Note: The vertical bar indicates the standard error.

The different alphabets within the same storage period indicate the significant differences ($P<0.05$) (n=3).

The evaluation of weight loss is an important parameter. This result can used to determine the quality of fruits (Nilprapruck *et al.*, 2008). The increased percentage of weight loss was found in the control and MeJA treated longkong fruits during the storage (Figure 29). However, the MeJA treated fruits were limited the increase of weight loss as compared to the control. The different concentration of MeJA was non-significantly controlled the fruit weight loss during the storage ($P<0.05$). However, the higher concentration of MeJA treated fruits were controlled the fruit weight loss better than the lower concentration and the control fruits. The interaction between MeJA treatments and storage period was also significantly affected the fruit weight loss ($P<0.05$). This result is an agreement with the previous study, which was reported by González-Aguilar *et al.* (2003). Nilprapruck *et al*. (2008) reported that the increased of weight loss might be stress induced by low temperature and consequently, fruit respiration increased. However, the MeJA treated fruits had the lower increase of weight loss. It could be due to the fruit stomatal closure by MeJA treatment and therefore reduced the water loss that induced by respiration process (González-Aguilar *et al.*, 2001; Cao *et al.*, 2007).

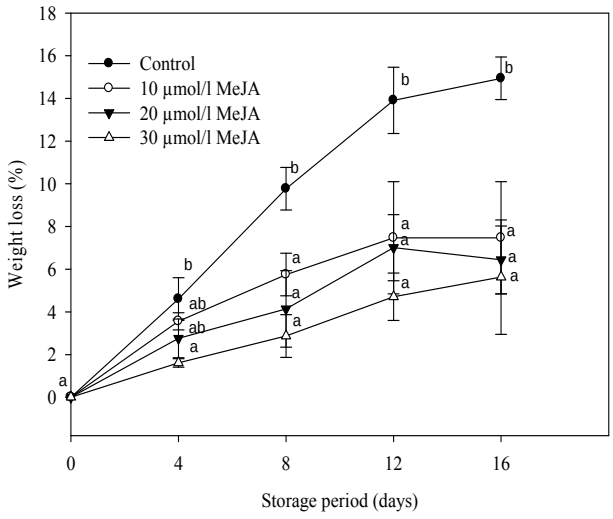


Figure 29. Effect of methyl jasmonate on the control of weight loss in longkong fruit during storage at 13°C and 85% relative humidity

Note: The vertical bar indicates the standard error.

The different alphabets within the same storage period indicate the significant differences ($P<0.05$) (n=3).

5.4.2 Chemical quality changes

Total soluble solids (TSS) in longkong fruit was maintained throughout the storage in all the treatments (Figure 30). The control fruits were having the higher level of TSS in longkong during 8^{th} and 12^{th} day of storage and at the end, it decreased. Whereas, the TSS in MeJA treated fruits gradually increased throughout the storage period. The different concentration of MeJA significantly increased the TSS level in longkong fruits throughout the storage ($P<0.05$). The higher concentration of MeJA treated fruits were maintained the TSS level throughout the storage as compared to the lower concentration of MeJA treated fruits. The interaction between MeJA treatments and the storage period was significantly maintained the TSS level in longkong ($P<0.05$). The increased level of TSS in the control fruit could be due to the increased fruit ripening and senescence process. Ayala-Zavala *et al.* (2004) and Nilpraprucк *et al.* (2008) reported that MeJA treated fruits were maintained the TSS level. The reduction in TSS could be used as a substrate for the fruit respiration process (Nilpraprucк *et al.*, 2008).

The ascorbic acid content in longkong fruits increased in all the treatments during the storage (Figure 30). The MeJA treated fruits contained high level of ascorbic acid than the control fruits. It is in an agreement with the previous studies, which were reported by Chanjirakul *et al.* (2006) and Jin *et al.* (2006). The different concentrations of MeJA treatment significantly increased the ascorbic acid content in longkong fruit ($P<0.05$). The higher concentration of MeJA treated fruits were retained the high level of ascorbic acid content than the lower concentration of MeJA treated and the control fruits. The interaction between MeJA treatments and storage period was not significantly influenced the ascorbic acid content in longkong fruit ($P\geq0.05$). Sugar is an important substrate in the ascorbic acid biosynthesis (Wolucka and Van Motagu, 2003). For that reason, the increased TSS could be induced the ascorbic acid content in this study. However, the higher level of ascorbic acid in MeJA treated fruits was due to an increased the transcription genes concerned in the de novo biosynthesis and regeneration of ascorbic acid by MeJA in fruits (Wolucka *et al*., 2005).

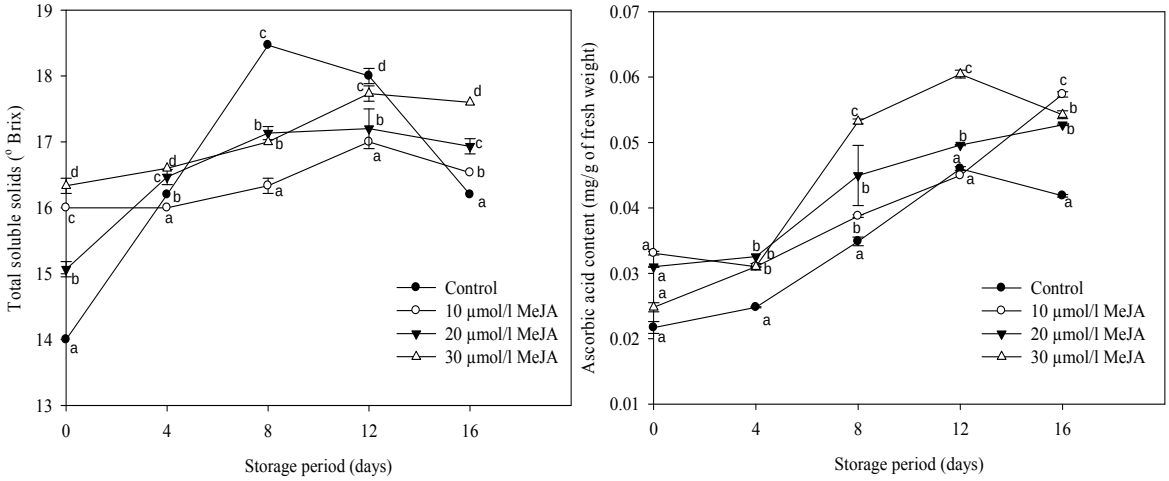


Figure 30. Effect of methyl jasmonate on longkong fruit TSS and ascorbic acid content during storage at 13°C and 85% relative humidity

Note: The vertical bar indicates the standard error.

The different alphabets within the same storage period indicate the significant differences ($P<0.05$) (n=3).

The decreased fruit pH was observed throughout the storage in all treatments (Figure 31). The different concentration of MeJA treated fruits had a low pH level than the control fruits during the storage ($P<0.05$). However, the sustained storage period, 30 µmol/l MeJA treated fruits had retained high fruit pH than 10 and 20 µmol/l MeJA. Wang *et al*. (2009) also found that the higher MeJA concentrations were increased the bayberry fruit pH during prolonged storage. The interaction between the different concentration and storage period was significantly influenced the fruit pH level ($P<0.05$). Ayala-Zavala *et al.* (2004) reported that MeJA treatment could not maintain the pH level in strawberry fruit. Longkong fruit had a variation in their titratable acidity level during storage in all the treatments (Figure 31). However, the TA level in control fruit was much lower than the MeJA treated fruits and the decreased TA level in all the treatment could be consumed as a substrate for the respiration process. The MeJA treatments and storage period was significantly affected the TA level in longkong fruit ($P<0.05$). The different concentration of MeJA were significantly influenced the fruit TA level at the initial storage period and at the

end it was not significantly maintained the TA level (P≥0.05). Fruit pH and TA had shown the correlated results, the treatment with high content of TA level had low level of fruit pH. These results are in an agreement with the previous reports on raspberry and bayberry fruits by Ghasemnezhad and Javaherdashti (2008) and Wang *et al.* (2009).

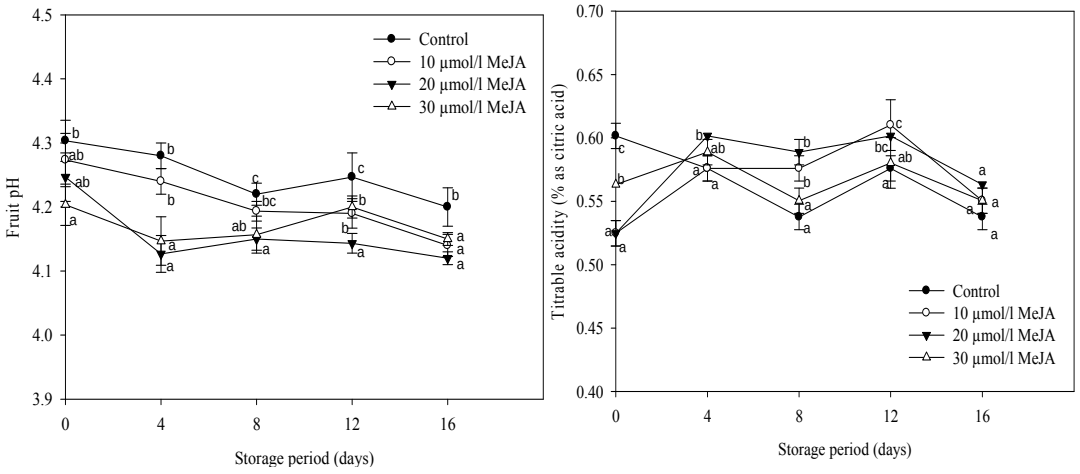


Figure 31. Effect of methyl jasmonate on longkong fruit pH and TA content during storage at 13°C and 85% relative humidity

Note: The vertical bar indicates the standard error.

The different alphabets within the same storage period indicate the significant differences (P<0.05) (n=3).

The total phenolic content increased in MeJA treated longkong fruits throughout the storage period (Figure 32). Conversely, total phenolic content in the control fruits decreased throughout the storage. The interaction between MeJA treatments and storage period was significantly affected the fruit total phenolic contents (P<0.05). Although, the different concentration of MeJA treatments were significantly retained the higher level of phenolic content in longkong fruit during the storage (P<0.05). Longkong fruit treated with 30 µmol/l MeJA maintained high level of phenolics as compared to other concentrations and the control fruit during storage. Similar study was found in Cao *et al.* (2009)b, Wang *et al.* (2009) and Sayyari *et al.* (2011) who reported that MeJA treated fruits contained higher level of total phenolics

as compared to the control. Phenylalanine ammonia lyase is an important enzyme involved in the biosynthesis of phenolic in longkong fruit (Lichanporn *et al.*, 2009). MeJA is an endogenous phytohormone, plays a major role in regulation a great range of physiological and biochemical process in plants including stimulating the biosynthesis of secondary metabolites such as phenolics (Wang *et al.*, 2009).

Conversely, pericarp phenolic content in both control and MeJA treated longkong fruits were decreased throughout the storage (Figure 32). However, the higher level of pericarp total phenolics was observed in control fruits as compared to the MeJA treated fruits ($P<0.05$). The different concentration of MeJA treated fruits were supressed the accumulation of pericarp phenolics as compared to the control fruits ($P\geq0.05$). The decreased level of phenolics could be consumed as a substrate for PPO and POD oxidation (Figure 36). Nevertheless, high concentration of MeJA treated fruits had a non-significant difference in controlling the fruit pericarp phenolics as compared to the low concentration. The interaction between MeJA treatments and storage period was significantly affected the longkong fruit pericarp phenolic content ($P<0.05$). Normally, PAL enzyme induced by external stress in plants such as chilling injury, air and physical wounds from rubbing and brushing. MeJA treatment could be decreased the stress which is mainly caused by chilling injury and therefore, the PAL activity decreased (Figure 36). This result is in agreement with the previous studies (Meng *et al.*, 2009; Yang *et al.*, 2011).

5.4.3 Fruit radical scavenging ability

The DPPH scavenging ability of longkong fruit was sustainably increased during storage and at the end; it slightly decreased (Figure 33). The MeJA treated fruits had retained more scavenging ability than the control fruits. At the end of storage, DPPH scavenging activity in the control fruits predominantly decreased as compared to MeJA treated fruits. The different concentration of MeJA was significantly increased DPPH scavenging activity throughout the storage period in longkong fruit ($P<0.05$). The interaction between MeJA treatments and storage period had significantly influenced the DPPH scavenging activity ($P<0.05$). Longkong fruit had a fluctuation on FRAP activity (Figure 33). At the end of storage, FRAP activity was slightly decreased. The control fruits had decreased more FRAP activity than

MeJA treated fruits. The storage period was significantly affected the fruit FRAP activity ($P<0.05$). The interaction between MeJA treatments and the storage period had significantly influenced the FRAP activity ($P<0.05$). Higher concentration of MeJA treated longkong fruits had retained better radical scavenging ability as compared to the lower concentration and the control fruits. Higher DPPH and FRAP activity in MeJA treated fruits might be stimulated by ascorbic acid (Figure 30) and high phenolics (Figure 32) in longkong fruit during storage. It is in an agreement with the previous reports (Wang and Zheng, 2005; Cao *et al.*, 2009a; Wang *et al.*, 2009; Sayyari *et al.*, 2011).

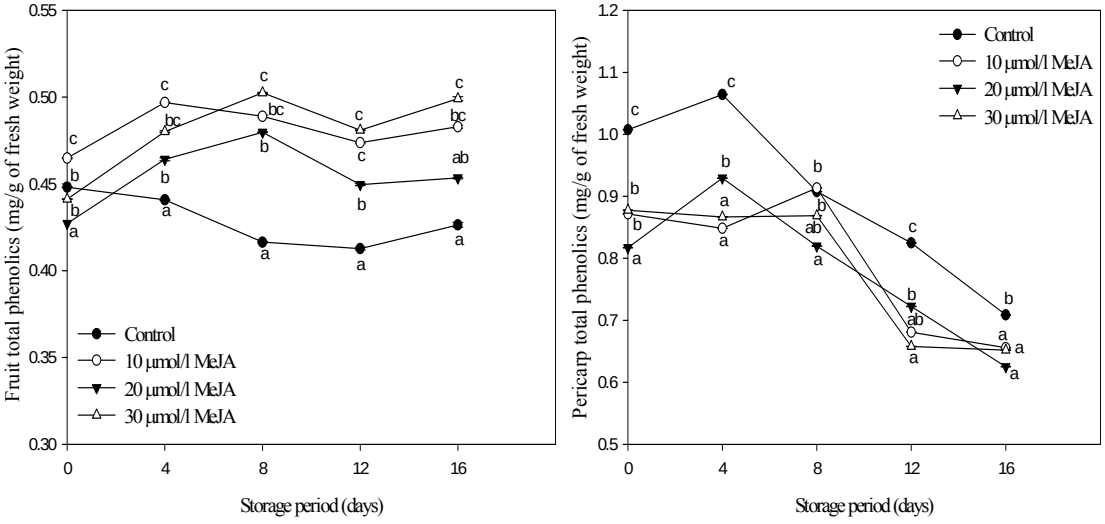


Figure 32. Effect of methyl jasmonate on total phenolics in longkong fruit and pericarp during storage at 13°C and 85% relative humidity

Note: The vertical bar indicates the standard error.

The different alphabets within the same storage period indicate the significant differences ($P<0.05$) (n=3).

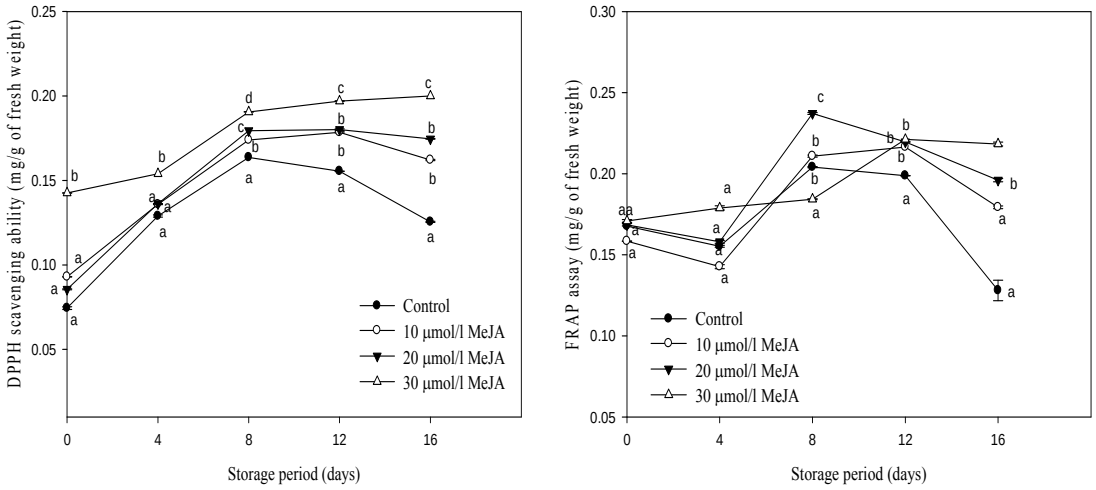


Figure 33. Effect of methyl jasmonate on longkong fruit radical scavenging ability during storage at 13°C and 85% relative humidity

Note: The vertical bar indicates the standard error.

The different alphabets within the same storage period indicate the significant differences ($P<0.05$) (n=3).

5.4.4 Fruit enzyme activity

Fruit antioxidant enzyme activity

SOD activity in the control and MeJA treated longkong fruits increased with the increased storage period (Figure 34). The MeJA treated fruits had retained more SOD activity than the control fruits ($P<0.05$). CAT and POD activities increased at the initial period of storage and then throughout the storage it slightly decreased (Figure 34) ($P<0.05$). The different concentration of MeJA had significantly retained the antioxidant enzyme activities in longkong fruit throughout the storage period ($P<0.05$). Conversely, the control fruits had low level of antioxidant enzyme activities. The higher concentration of MeJA treated fruits had the higher activity of SOD and CAT as compared to the lower concentration and the control fruits throughout the storage. Whereas, the POD activity was initially found high level in longkong fruit treated with MeJA higher concentration and after that, at the end of storage, the activity was observed slightly high level in MeJA treated fruits in low

concentration. The interaction between MeJA treatments and storage period was significantly affected the SOD, CAT and POD activities (P<0.05). Several findings reported that the increased ROS by oxidative stress during low temperature storage might be contributed to chilling injury due to damage the cell membrane (Fung *et al.*, 2004; Santos Soares *et al.*, 2010; Wang, 2011). Fruits under chilling stress, the MeJA effectively involved and increased the concurrent action of SOD, CAT and POD activity and subsequently, counteract the chilling injury onset (Cao *et al.*, 2009a; Sayyari *et al.*, 2011). MeJA is interacted in the signalling pathway mediating induced defence responses in chilling-stressed plants and the onset of the tolerance has frequently been correlated with the accumulation of antioxidant enzymes and compounds (Creelman and Mullet, 1997).

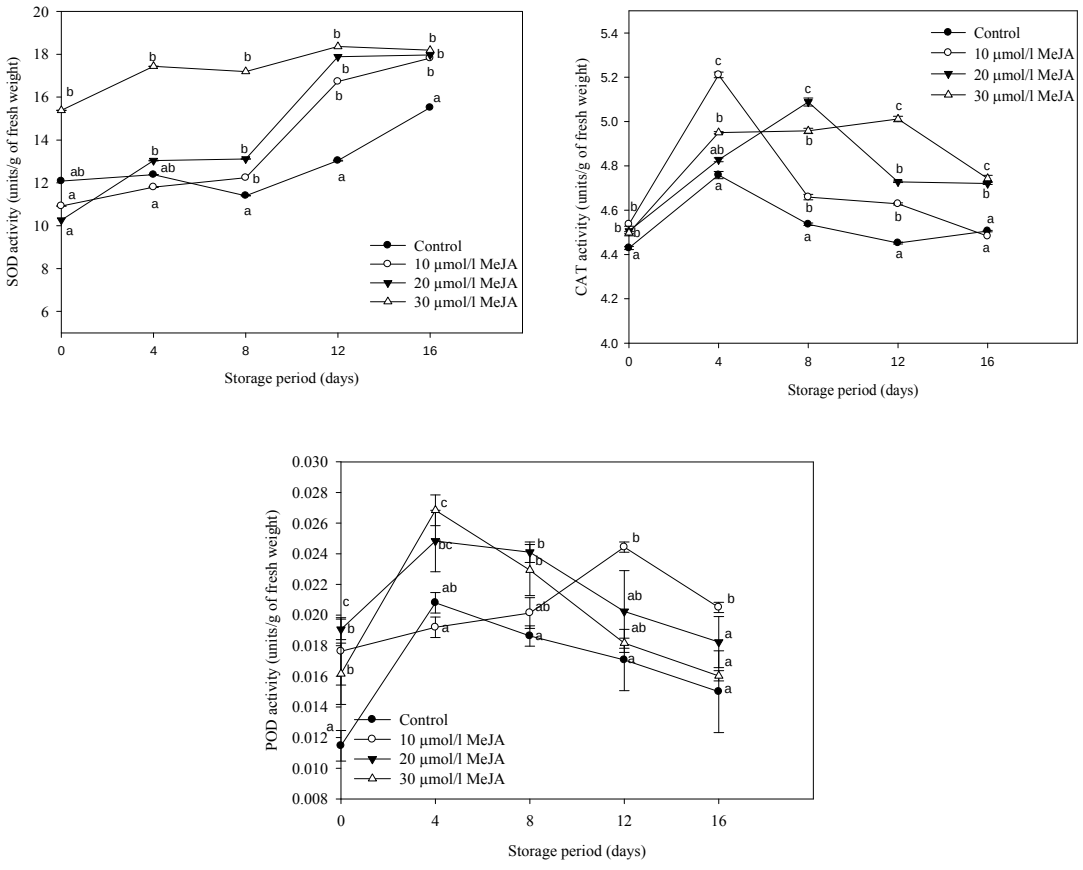


Figure 34. Effect of methyl jasmonate on longkong fruit antioxidant enzyme activity during storage at 13°C and 85% relative humidity

Note: The vertical bar indicates the standard error.

The different alphabets within the same storage period indicate the significant differences (P<0.05) (n=3).

Fruit textural enzyme activity

Longkong fruits PG and PME activities were increased continuously in all treatments during storage (Figure 35). The control fruits had high level of PG and PME activities during storage as compared to MeJA treated fruits. The MeJA treated fruits were suppressed the intensity of the increase of PG and PME enzyme activities during storage. Throughout the storage period, the different concentration of MeJA treatments significantly maintained the PG and PME activities in longkong fruits as compared to the control ($P<0.05$). In this study, the MeJA treated fruit with higher concentration was effectively suppressed the increased activity of PG and PME. The interaction between MeJA treatments and storage period was significantly affected the textural enzyme of longkong fruits ($P<0.05$). The fruits firmness reduced gradually during long-standing cold storage were in parallel with pectin harmonious modification resulted from enzymatic catalysis of metabolism and non-enzymatic cation change (Meng *et al.*, 2009). PG and PME is a key enzymes related to reducing the fruits firmness during ripening (Islas-Osuna *et al.*, 2010). Ziosi *et al*. (2008) reported that MeJA treated peach fruits were decreased in PG activity. Meng *et al*. (2009) reported that MeJA treatment reduced the PME activity by inhibiting the de-esterification of protein (Meng *et al.*, 2009).

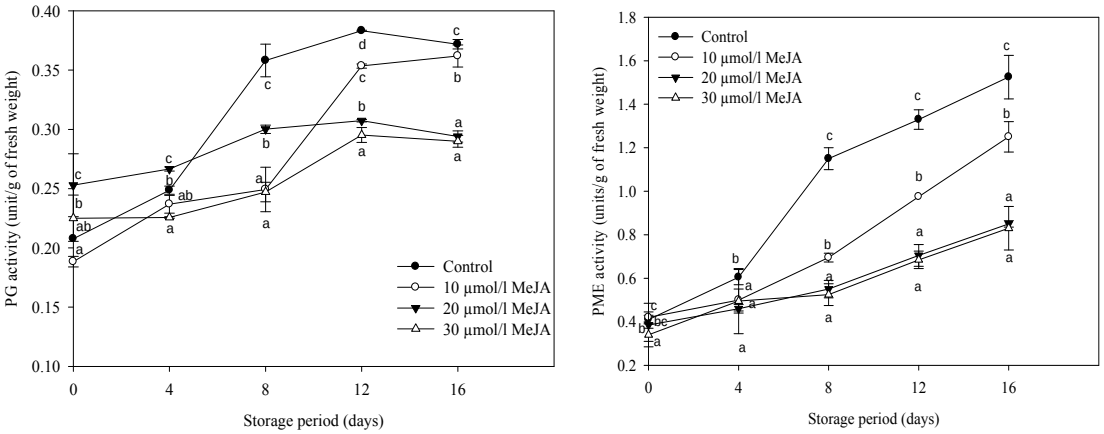


Figure 35. Effect of methyl jasmonate on longkong fruit textural enzymes (PG and PME) during storage at 13°C and 85% relative humidity

Note: The vertical bar indicates the standard error.

The different alphabets within the same storage period indicate the significant differences ($P<0.05$) (n=3).

Pericarp browning enzyme activity

The higher level of longkong fruits pericarp PAL activity was observed in the control than the MeJA treated fruits during the storage period ($P<0.05$) (Figure 36). The increased PAL activity was correlated with the increased level of fruit pericarp phenolics as shown in Figure 32. PAL is a key enzyme in the first step of the phenylpropanoid pathway and directly involved in the synthesis of phenols (Ryals *et al.*, 1996). In the meantime, the PPO and POD activities also increased in fruit pericarp during the storage period ($P<0.05$). However, the increased PPO and POD activities were observed in the higher level in control than MeJA treated fruits (Figure 36). PPO and POD has shown to be responsible for produce browning by oxidised the phenolics in longkong fruit pericarp (Lichanporn *et al.*, 2009). The different concentration of MeJA had the significant controlled in longkong fruit pericarp PAL, PPO and POD activities throughout the storage period ($P<0.05$).

The PAL, PPO and POD enzyme activities were effectively controlled by the higher concentration of MeJA treated fruits as compared to the low concentration and control fruits. The interaction between MeJA treatments and storage period was significantly influenced the longkong fruit pericarp browning enzyme activity ($P<0.05$). The inhibitory action of MeJA treatment on PAL, PPO and POD activity has been demonstrated in many fruits (Nilprapruck *et al.*, 2008; Meng *et al.*, 2009; Cao *et al.*, 2010). The increased activity of PPO and POD during storage in control fruits might be involved in the protecting effect against on fungal infections during low temperature and higher RH storage (Dixon and Paiva, 1995; Huckelhoven *et al.*, 1999). Longkong fruit stored under low temperature and at the high relative humidity conditions could be increased the visible mold growth on the fruit surface (Paull, 2004; Ketsa and Paull, 2008). Yao and Tian (2005) and Cao *et al*. (2008) reported that MeJA itself act as an antimicrobial activity. This finding is could be a reason that, MeJA controls the increase of fruit pericarp PPO and POD activities as compared to the control. MeJA effect on the browning enzyme activity is varied with the plant species.

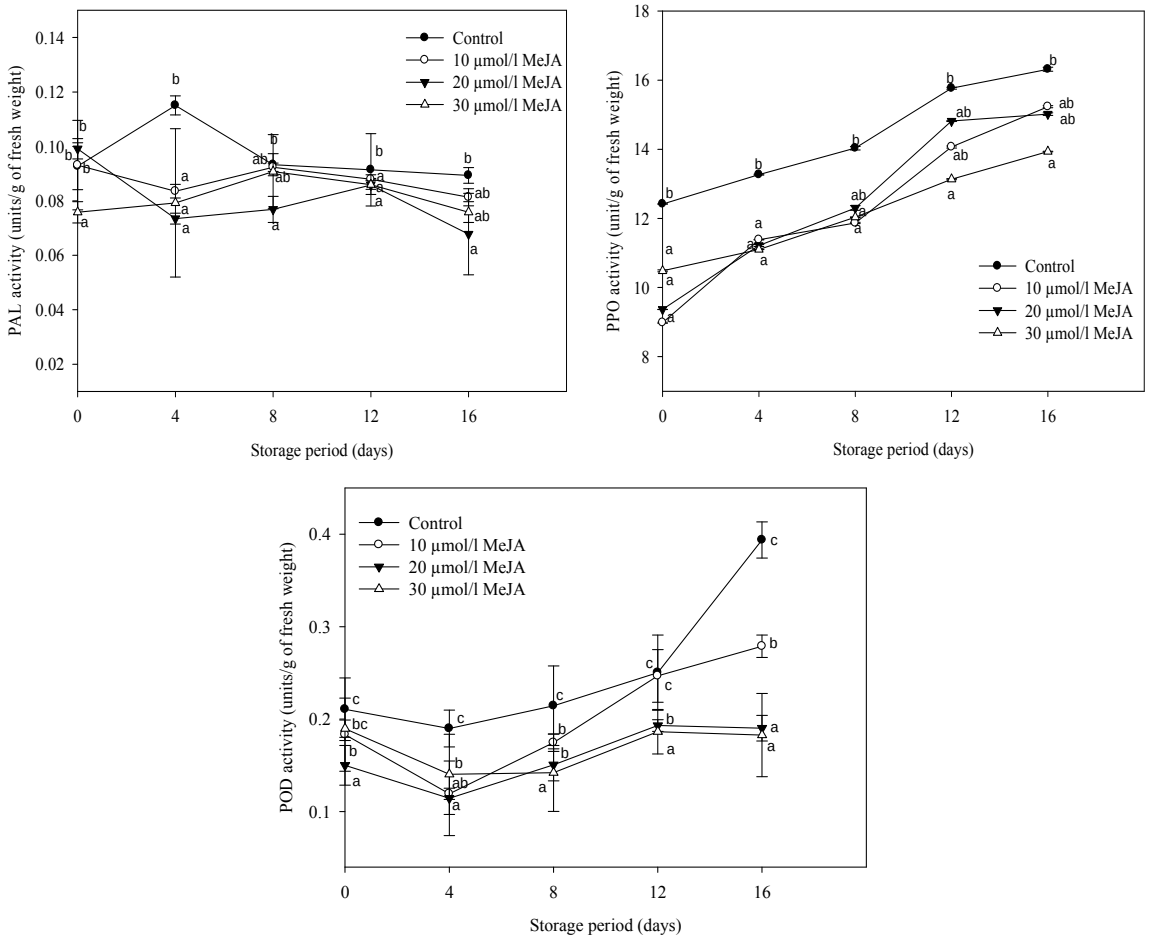


Figure 36. Effect of methyl jasmonate on longkong browning related enzymes (PAL, PPO and POD) during storage at 13°C and 85% relative humidity

Note: The vertical bar indicates the standard error.

The different alphabets within the same storage period indicate the significant differences ($P<0.05$) (n=3).

5.4.5 Pericarp ultrastructural changes

Longkong fruit pericarp ultrastructural changes in surface section on zero day and 16th day of the storage in control and MeJA treated fruits was shown in Figure 37. The surface section is represented the epidermal trichomes (hair) of the longkong fruit. During the zero day storage, the epidermal hair surface of longkong pericarp had not found any difference between the control and MeJA treated fruits.

Nevertheless, the observation on the 16th day of the storage was found the severe loss of epidermal trichomes in the control fruits. This could be due to the chilling air penetrate into the pericarp during low temperature storage and thus impaired the physiological function of pericarp and increasing its water permeability. Consequently, the pericarp tissue got damaged and increased in browning (Jaitrong *et al*., 2005). Whereas, in MeJA treated longkong fruit had not found any severe loss on their surface epidermal hair. The 10 µmol/l MeJA treatment had slightly lost in epidermal hair but, when it compared to control fruits, loss of trichomes was less observed. Meanwhile, the increased concentration of MeJA had maintained the epidermal surface section of longkong. Lichanporn *et al.* (2009) reported that longkong pericarp epidermal hair loss can be used as a tool to identify the stress that induced damage. The present results, epidermal hair loss in the control fruits confirmed that, chilling stress could induce the longkong fruit pericarp browning. On the other hand, chilling stress was extremely controlled in MeJA treated fruits. In addition, fruit pericarp cross section represented the parenchyma cell on the hyperdermal tissue (Figure 38). Zheng *et al.* (2000) have found that chilling stress induced cold stored fruit is attributed to the abnormal changes in the cell wall metabolism. The hyperdermal changes were not found during 0 day of storage and at the end of storage (16th day) fruits had the slight changes in their hyperdermal tissues. The severe destruction of parenchyma cells in the hyperdermal tissue were found in the control fruits. Whereas, in MeJA treated fruits had a slight enlargement in the hyperdermal parenchyma cells and it could be due to the penetration of MeJA vapour treatment and/or respiration effect. Hyperdermal tissue changes in longkong pericarp might be an indicator of the cell membrane damage (Lichanporn *et al*., 2009). Paull (1994) reported that chilling injured fruits had the abnormal changes in their cell membrane. MeJA treated fruits had maintained the cell membrane integrity and reduce the chilling injury incidence by decreasing the fruit browning (Nilprapruck and Yodmingkhwan, 2009; Cao *et al.*, 2010).

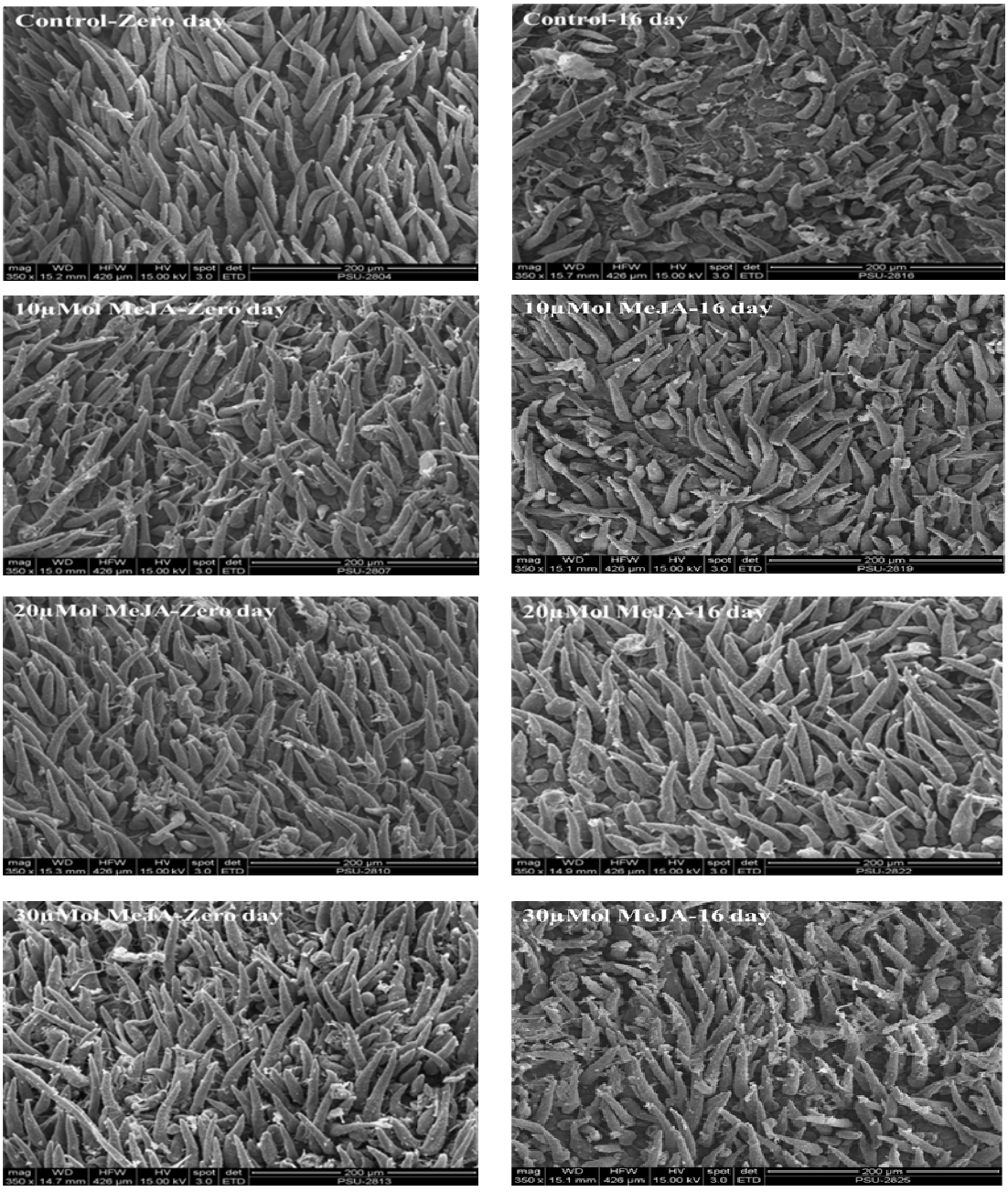


Figure 37. Effect of methyl jasmonate on ultrastructural changes of longkong pericarp surface section on the zero day and 16th day of storage at 13°C and 85% relative humidity

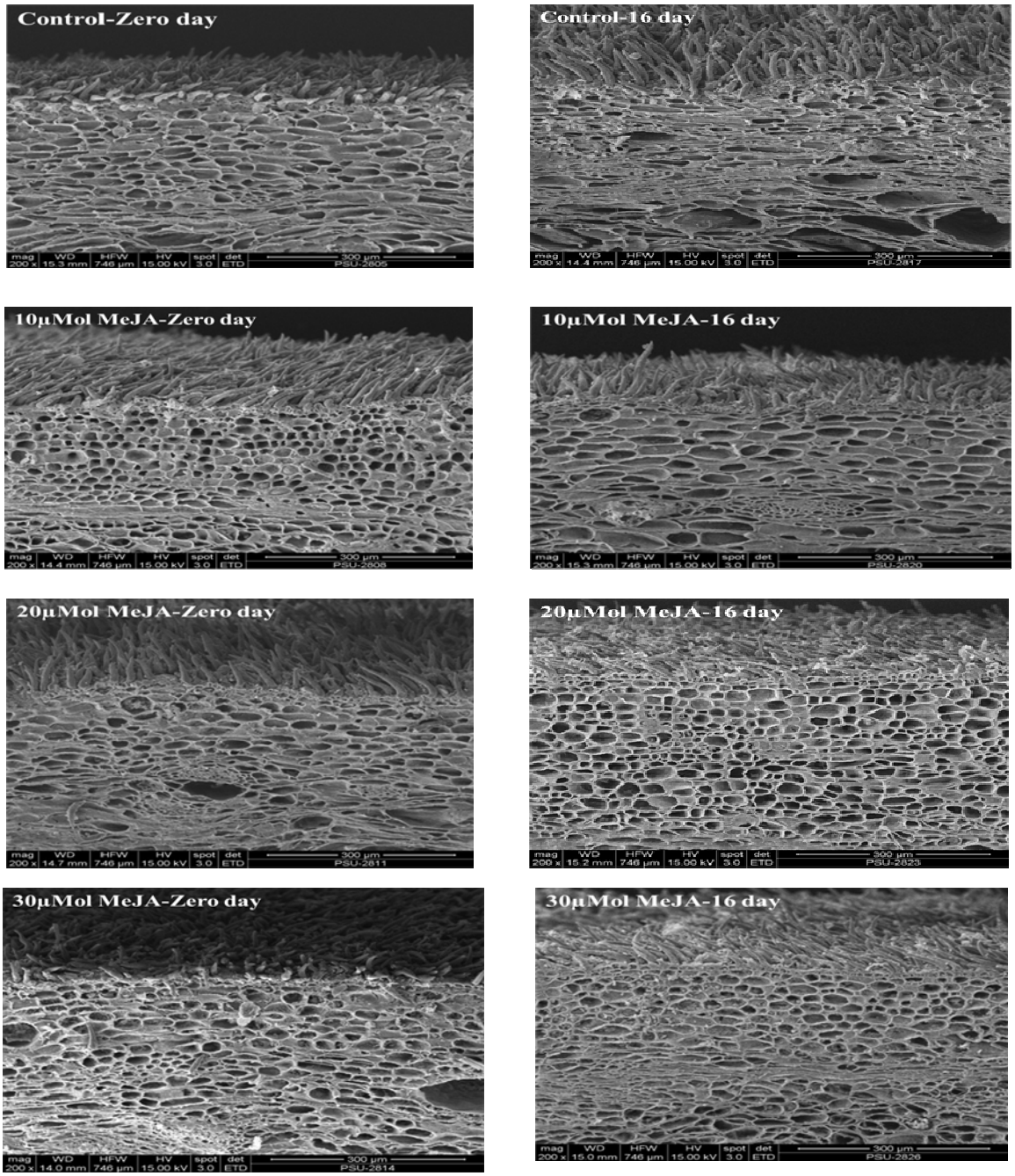


Figure 38. Effect of methyl jasmonate on ultrastructural changes of longkong pericarp cross section on the zero day and 16th day of storage at 13°C and 85% relative humidity

5.5 Conclusion

MeJA treatment effectively controlled and maintained the longkong fruit quality throughout the storage period from the chilling stress. The different concentration of MeJA treatment had a significant effect on sustaining the fruit quality. However, the 30 μmol/l MeJA concentration retained the higher level of fruit quality as compared to other treatment in this study. The controlled action of MeJA on chilling injury of longkong fruit has revealed by the suppressed action on an increased of browning enzyme and textural enzymes activities. The ultrastructural changes were highly controlled in MeJA treated longkong fruit. This is also an evident to support for the alleviation of chilling injury in longkong fruit by MeJA treatment.

CHAPTER 6

SUMMARY AND FUTURE WORK

6.1 Summary

1. The increased maturation period were increased the longkong fruit quality and nutritional level. The fully matured 15th and 16th weeks of longkong fruit gained the more fruit weight and nutritional qualities but lost in fruit physical qualities such as pericarp colour, texture and ultrastructure. Browning related enzymes and textural enzymes increased with the increased maturity of longkong. This might be resulted the reduction in fruit postharvest shelf life. Meanwhile, fruits at 13th and 14th weeks had less changes in colour and texture and fruit was attained the optimum eating and nutritional quality. Therefore, the early stage of harvesting might be helpful for the longkong fruit post-harvest practise.

2. The interaction between passive MAP with different OTR packages, temperatures (18°C and 25°C) and storage days had a positive effect on the overall longkong fruit quality. Fruit stored at 18°C was effectively controlled the loss of fruit weight and maintained the TA and sugar level as compared to 25°C. PE2 and PE3 packages were retained the more level of antioxidant and antioxidant enzyme activities in both temperatures as compared to PE1. Fruit stored in PE1 package and at 18°C storage was lowered the pericarp browning as compared to 25°C and PE2, PE3 packages and it was evidenced by lower changes in pericarp ultrastructure and browning related enzyme activities.

3. Longkong fruit stored under active MAP at 18°C effectively maintained its quality and the shelf life were extended for up to 24 days. Fruit stored at 25°C had an extensive loss in quality and, consequently, the shelf life was limited to 12 days. Active MAP storage at 18°C maintains fruit quality by slowing down the respiration process, weight loss and browning related enzyme activities. Ultrastructural changes were also shown a low level of hyperdermal cell damage at 18°C. The fruits titratable acidity, sugar level and antioxidants were significantly maintained at 18°C.

4. MeJA treatment was successfully controlled the longkong fruit chilling injury and maintained the quality throughout the storage period. The different concentrations of MeJA treatment were significantly maintained the fruit quality from chilling injury symptoms. MeJA treatment suppressed the browning enzymes and textural enzyme activities in fruits. MeJA treated fruits were reasonably increased in antioxidant activities. Fruit ultrastructural changes were significantly controlled in MeJA treated fruits.

5. The combination of methyl jasmonate treatment, fruit packing in a polyethylene package (8x15 inches in size and 25 μM thickness) with the oxygen transmission rate of 5,000-6,000 cc/m^2.day and at 18°C with 85% relative humidity storage might be the best condition for the longkong fruit postharvest quality and shelf life prolongation.

6.2 Future works

1. Analyse the different fungicides effect on controlling microbial growth on longkong fruit during under low temperature storage.

2. Detection and quantification of longkong pericarp and fruits phenolic compounds and their radical scavenging ability during different stages of fruit ripening.

3. Partial purification and characterization of polyphenol oxidase, peroxidase and phenylalanine ammonia lyase from longkong fruit.

4. Extraction and pharmacological properties of bioactive compounds from longkong fruit.

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APPENDIX

Appendix A

Table 14. Browning index evaluation form

Panellist name ……..	**Date**	**Time**	**Storage day**

Score	**Treatment**			
	Sample code No……	**Sample code No**……..	**Sample code No**…….	**Sample code No…….**
Score 1 No chilling injury symptoms				
Score 2 1-25% Browning symptoms occurred				
Score 3 26-50% Browning symptoms occurred				
Score 4 51-75% Browning symptoms occurred				
Score 5 76-100% Browning symptoms occurred				

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List of Publication and Proceedings

Publication

1. Venkatachalam, K. and Meenune, M. 2012. Physical and chemical quality changes of longkong (*Aglaia dookkoo* Griff.) during passive modified atmospheric storage. International Food Research Journal. 19(3):795-800.

2. Venkatachalam, K. and Meenune, M. 2012. Changes in physiochemical quality and browning related enzyme activity of longkong fruit during four different weeks of on-tree maturation. Food Chemistry. 131:1437-1442.

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1. Venkatachalam, K. and Meenune, M. 2012. Effect of methyl jasmonate on alleviation of chilling injury in longkong fruit during storage The 10th National Postharvest Technology conference, 23-24 August 2012. Centara convention center hotel, Khon Kean, Thailand. (Poster)

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