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Effect of Harvesting Index on Browning Reaction and Changes of Tissue Structure in Santol Fruits

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Abstract: This investigation was carried out to justify the relationship between harvesting ages of santol fruit, browning reaction and other concerning data. Growers of santol plantations in Thailand have always accustomed to problems on inconsistency in qualities of santol fruits var. Pui Fai due to inappropriate harvesting index. Thus these encourage to decrease marketable qualities of fruits and short shelf-life particularly browning discoloration. In order to investigate adequate information for growers of the santol orchard plants, it is important to carry out experiments on Polyphenol Oxidase (PPO) activity and changes of tissue structure by separating fruit into three parts: peel, flesh and seed of santol fruit var. Pui Fai harvested at four stages: 100, 115, 130 and 145 Days After Full bloom (DAF). A factorial in randomized complete block design with five replications was used. In each replication ten fruits were used. This investigation was carried out during the period of October 2006 to March 2007 at The Department of Agricultural Technology, Mahasarakham University, Northeast Thailand. The results showed that PPO activity which related to browning reaction changed with harvesting stage. The highest PPO activities were obtained and highly significant increased in mature fruit at 130 and 145 DAF. When the fruits were ripening at 145 DAF, PPO activities from peel, flesh and seed had no significant differences and high levels by 160.76-184.44, 158.88-180.76 and 154.52-181.08 U mg^{-1} fresh weight, respectively. While cross-section study in peel and flesh of santol fruit var. Pui Fai at different ages indicated that immature fruits (100 and 115 DAF) showed small cells arranged one to two layers in epidermis. Trichome like-hair also appeared on this layer. When santol fruits grew through 145 DAF, epidermis converted to periderm and trichome disappeared. In addition, parenchyma cells in flesh accumulated several substances in form of druse with the fruit age increment. These high PPO activities and fruit tissue conversions of peel and flesh in mature fruit may led to be more sensitive to browning reaction.

Key words: Harvesting index, browning reaction, tissue structure, santol fruit, polyphenol oxidase activity

INTRODUCTION

Santol (*Sandoricum koetjape* (Burm.f.) Merr.) is one of the popular and highly demand of the markets in Thailand. Most of consumption characteristics are fresh fruit and processed for different consuming purposes including pickled santol fruits, santol in syrup, dressed salad and oven-dried crispy santol slices etc. By nature, santol trees are simplicity in growing. They could thrive on well in all types of soil even under drought conditions, particularly in most areas of tropics. In addition, santol fruit is delicious and composes of high nutrition to human (i.e., vitamin C, vitamin A, calcium and crude fiber). Sangkitikomol (2000) found that santol fruits contain the total antioxidants which protect several diseases (i.e., rickets, arthritis, heart disease, paralysis and cancer). From benefit and importance role above, the land areas used for cultivation santol trees in all the regions of Thailand were increasing and scattering continually reached a figure of 5471.04 ha in 2006 (Department of Agricultural Extension, 2005). Nevertheless, growers in all regions of the country have come across with many problems such as fruit browning, which affected the santol quality standard demand by customers. This causes to reduce quality and short distribution. Fruit browning, as a consequence of bruising and wounding to epidermal cells, caused by inappropriate harvesting or postharvest means. It also occurred during various processes of cutting, slicing, or peeling, with effects being observed very rapidly (Chutichudet, 2001). Due to the importance of visual appearance as tissue browning has long gained attention from horticultural researchers. Level of browning reaction

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appearance depended on plant species and different parts of plant. Chutichudet *et al.* (2007) indicated that browning reaction of santol fruit mostly occurred on peel, flesh and seed. This reaction caused by enzyme activity namely 1,2-benzene diol: oxygen oxidoreductase (Polyphenol oxidase, PPO) in plant tissues was released and mixed with substance of browning reaction. Oxygen is stimulator to activate this reaction. The results from reaction affected to many fresh fruit qualities especially high demand of tropical and subtropical fruits. Chen *et al.* (2000) and Zheng and Tian (2006) stated that this discoloration is not appealing to consumers and reduces the market value. In addition, Chutichudet (2001) reported that browning reaction might be due to nonenzymatic reaction from amino acid and sugar. Therefore, in order to produce an acceptable quality of santol fruit, it is an urgent need for researchers to obtain more information on such problems in order to attain the crop with high quality. One way to improve fruit quality is to search PPO activity and compare fruit cell structure modification by using optical microscopy.

Therefore, this investigation focused on appropriate harvesting index associated with the cell structure of santol fruit var. Pui Fai to enhance the better understanding of the processes leading to fruit skin browning. In addition, the attained information could possibly assist the growers of santol plants in Thailand to achieve the high quality fruits.

MATERIALS AND METHODS

Forty santol trees var. Pui Fai (i.e., the 8 year old trees) with a similar girth diameter of the trunk at approximately 0.15 m above ground and similar bushy structures were chosen for the experiment. Santol fruits were obtained from orchard's Chiang Rai grown on Hang Chat (Hc) soil series with the distances between rows and within rows of 8×8 m. The experiment was set up a factorial in randomized complete block design with two factors and five replications. The first factor is fruit age four levels : 100, 115, 130 and 145 DAF. The second factor is separation of fruit into three parts: peel, flesh and seed. In each replication ten fruits were used.

The following items were used for data collection:

- Polyphenol Oxidase (PPO) activity determination separated from three parts of santol fruit (peel, flesh and seed) were carried out according to the method reported by Jiang and Fu (1998). The attained enzyme extracts were measured by spectrophotometer model V-325-XS, China to record the quantified from absorbance at 420 nm every 30 sec for 5 min. One unit of PPO activity was defined as the amount of enzyme causing a change of 0.01 in absorbance (420 nm) per 30 sec. The unit of PPO activity is U mg^{-1} fresh weight. The obtained data were statistically analysed using SPSS Computer Programme, Base 9 (SPSS, 1999).
- Comparative santol fruit tissues from peel and flesh at four ages (100, 115, 130 and 145 DAF) The explants of santol fruits from peel gather with flesh were cut into size of 5×5 mm and 5-10 mm depth by using free hand section methods. The explants were dyed with sulfamin 1% for 15 min then dehydrated the tissues with alcohol series at concentrations of 15, 30, 50, 70, 95 and absolute alcohol, respectively. The following procedure were dipping in absolute alcohol mixing with xylene ratio 1:1 and pure xylene, respectively. The duration for steeping in each solution was 20 min. The slides were sealed by xylene, dibutyl phthalate (DePeX) and compared structure of tissues with the use of optical microscopy ZEISS model Axiostar Plus (serial 3108007777), China with the 10 and 40x lens. The photographs were taken by digital camera of Nikon CollPIX at 4500X.

RESULTS

PPO activities: PPO activities comparison as PPO extracts from peel, flesh and seed of santol fruits at 100, 115, 130 and 145 DAF by using 0.1 M catechol, revealed that PPO activities increased with time resulted from enzyme reaction with substrate. At 100 and 115 DAF (immature stage), PPO activities were similarly and nonsignificant differences with values ranged 84.88-186.96, 41.72-106.32 and 44.76-174.24 U mg^{-1} fresh weight from peel, flesh and seed, respectively (Table 1). When santol fruits were harvested at maturity stage (130 DAF), the results revealed that mean values of PPO activities were the higher contents than younger stage at 100 and 115 DAF. They also showed PPO activities with highly statistically significant than ages at 110 and 115 DAF with values of 160.76-184.44, 158.88-180.76 and 154.52-181.08 U mg^{-1} fresh weight from peel, flesh and seed, respectively. Whilst ripening fruits at 145 DAF indicated the highest concentration of PPO activities but nonsignificant differences from at 130 DAF with values of 160.76-184.44, 158.88-180.76 and 154.52-181.08 U mg^{-1} fresh weight from peel, flesh and seed, respectively. The results showed obviously all three parts of santol fruits at 145 DAF had the similar PPO activities.

Changes of tissue structure: Optical microscopy to compare the tissues in both peel and flesh of santol fruits at 100, 115, 130 and 145 DAF by free hand section, presented that peel of immature fruits at 100 DAF

Table 1: PPO activity from peel, flesh and seed of santol fruit at 100, 115, 130 and 145 DAF

	PPO activity (U mg^{-1} fresh weight)										
Treatments	0s	30s	60s	90s	120s	150s	180s	210s	240s	270s	300s
Peel 100 daf	103.84^{b}	129.20^{ab}	144.72^{abc}	157.60^{abc}	164.16^{ab}	170.36^{a}	175.16^{a}	179.40^{a}	182.52^{a}	185.16^{a}	186.96^{a}
Peel 115 daf	84.88^{b}	107.80^{b}	119.56^{bcd}	126.84^{bcd}	131.60^{abc}	135.64^{ab}	138.44^{ab}	139.64^{ab}	139.76^{ab}	137.52^{bc}	132.88^{cde}
Peel 130 daf	157.52^{a}	157.44^{a}	159.00^{ab}	159.72^{abc}	161.92^{ab}	164.00^{a}	165.12^{a}	167.60^{a}	169.40^{a}	167.08^{ab}	154.12^{abcd}
Peel 145 daf	160.76^{a}	164.72^{a}	166.64^{a}	170.44^{ab}	174.04^{ab}	176.96^{a}	179.28^{a}	181.84^{a}	182.96^{a}	184.12^{a}	184.44^{a}
Flesh 100 daf	41.72^{c}	63.96^{c}	78.60^{def}	87.80^{de}	93.80^{cd}	97.88^{bc}	100.72^{bc}	102.92^{bc}	104.56^{bc}	105.44^{cd}	106.32^{ef}
Flesh 115 daf	42.24^{c}	52.88^{c}	59.60^{f}	65.24^{e}	69.48^{d}	72.84^{c}	75.24^{c}	77.64^{c}	79.88^{c}	80.60^{d}	81.84^{f}
Flesh 130 daf	162.92^{a}	165.52^{a}	166.12^{a}	168.44^{ab}	169.68^{ab}	171.68^{a}	174.12^{a}	175.16^{a}	171.92^{a}	168.72^{ab}	162.88^{abc}
Flesh 145 daf	158.88^{a}	163.68^{a}	167.96^{a}	172.08^{a}	175.92^{a}	177.92^{a}	180.20^{a}	181.52^{a}	182.04^{a}	182.76^{a}	180.76^{ab}
Seed 100 daf	146.44^{a}	161.24^{a}	168.44^{a}	169.08^{ab}	164.28^{ab}	165.32^{a}	165.56^{a}	168.32^{a}	171.12^{a}	173.04^{ab}	174.24^{abc}
Seed 115 daf	44.76^{c}	61.16^{c}	75.28^{ef}	86.92^{de}	94.40^{cd}	100.00^{bc}	104.60^{bc}	108.60^{bc}	111.68^{bc}	112.60^{cd}	110.28^{def}
Seed 130 daf	98.00^{b}	105.80^{b}	111.68^{cde}	119.12^{cd}	129.72^{bc}	135.40^{ab}	140.84^{ab}	146.16^{ab}	144.76^{ab}	141.24^{abc}	138.52^{bcde}
Seed 145 daf	154.52^{a}	159.84^{a}	163.28^{a}	167.80^{ab}	170.80^{ab}	173.88^{a}	177.04^{a}	178.84^{a}	181.24^{a}	181.16^{ab}	181.08^{ab}
F-test	**	**	**	**	**	**	**	**	**	**	**
LSD	12.8294	14.6614	15.3678	15.5559	15.8123	16.0636	16.3263	16.0508	15.8569	15.6608	15.6658
CV (%)	25.3800	26.3500	26.0800	25.2800	24.9600	24.7500	24.6600	23.8300	23.3600	23.1000	23.4300

Letter(s) within columns and LSD indicate significant differences at **p = 0.01, daf: No. of days from full bloom to harvesting

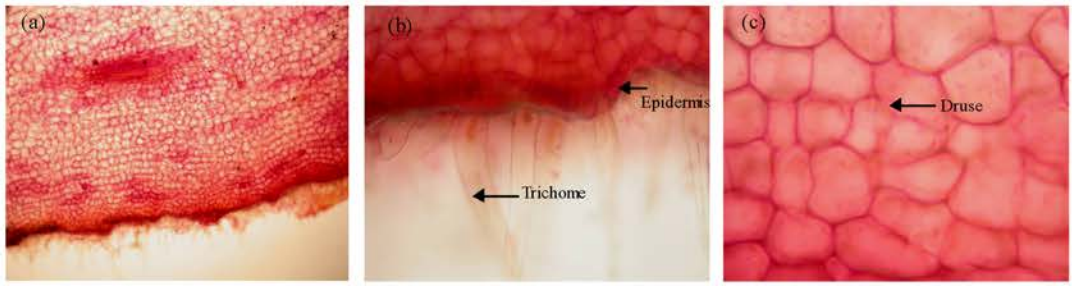

Fig. 1: Cross section from peel (a, b) and flesh (c) of santol fruits at 100 DAF

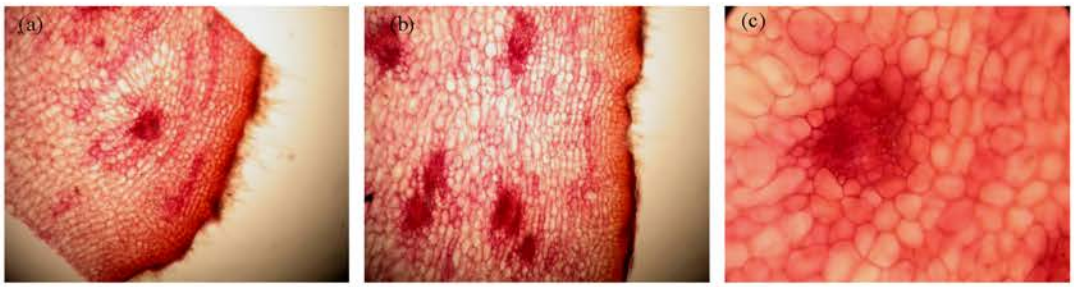

Fig. 2: Cross section from peel (a), flesh (b) and clusters of cell acted to store several substances (c) in santol fruits at 115 DAF

composed of epidermal cells which small, uncertain shape and rather angled. These cells laid in concentration on epidermis about one to two rows (Fig. 1a). From the microscopic images revealed that trichome like hair on epidermis as unicellular hair with long, acute and obese at the end. These trichomes also accumulated some substances (Fig. 1b). Whilst mesocarp or flesh layers showed parenchyma cells which had large cells with thin wall, uncertain shape and loosely arrangement. Some parenchyma cells composed of crystal-like star which accumulated the substances (Fig. 1c). Results also show that at 115 DAF, epidermis cells arranged more compaction (Fig. 2a). Similarly, parenchyma cells in pericarp also laid more tightly and distributed as clusters (Fig. 2b). We assumed that these clusters of cell acted to store the substances (Fig. 2c). As santol fruits aged at 130 and 145 DAF, trichomes like hair on external peel disappeared. At these fruit stages also showed the breakdown of membranes within cells of plant tissues in epidermis which converted into periderm with the thinner wall (Fig. 3a, 4a). The parenchyma cells in pericarp also changed to uncertain shape (Fig. 3b, 4b). However, it

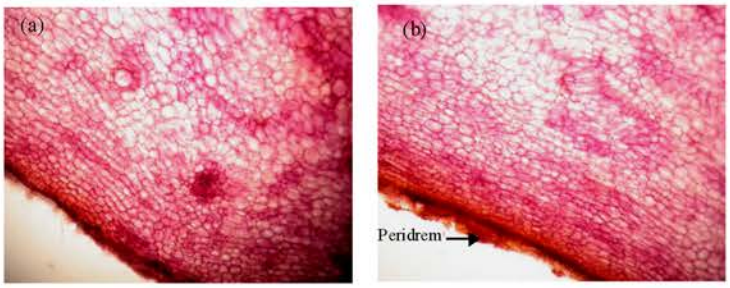

Fig. 3: Cross section from peel (a) and flesh (b) of santol fruits at 130 DAF

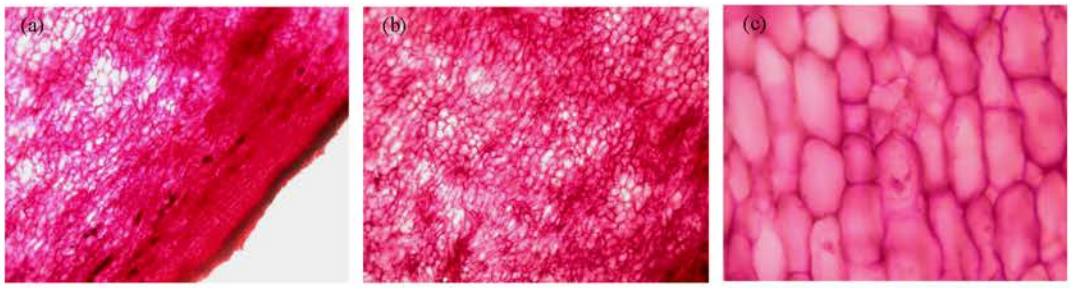

Fig. 4: Cross section from peel (a), flesh (b) and clusters of cell acted to store several substances (c) in santol fruits at 145 DAF

seems more likely that production of crystal like star was naturally occurring initiated in peel and flesh raised with fruit age particularly 145 DAF (Fig. 4c).

DISCUSSION

The results from the effect of harvesting index on browning reaction and changes of tissue structures of santol fruit in peel and flesh indicated that similar PPO activities found in all three sections (peel, flesh and seed) at immature stage (100 and 115 DAF). This could have been attributed to all three sections of santol fruit at young stage have low phenolic compounds (Lakshminarayana *et al*., 1970; Ueda *et al*., 2000). The higher PPO activities obtained from mature fruit at 130 and 145 DAF. The amount of PPO activities from both age were similar in all portions (peel, flesh and seed). Increment of PPO activities at these two stages affected to promote fruit discoloration. Based on the results it may be concluded that discoloration characteristics of santol fruit are thought to be result from maturity at the harvesting time. Due to maturity age increment, PPO activities tend to be concentrated in all of peel, flesh and seed which were major contributors to enzymatic browning. These caused mature fruits be more sensitive to browning reaction than young fruits. Altunkaya and Gökmen (2008) stated that PPO was importance enzyme related to enzymatic browning. In many plants, PPO located in plastids or chloroplasts of healthy cells (Mayer and Harel, 1979). Similarly, Yiping *et al*. (2001) showed that harvest maturity related directly to the occurrence of PPO activity due to the loss vigorous of membrane system. When fruits became ripening stage, enzyme PPO may be solubilized and proteolyzed (Murata *et al*., 1997) affected to the loss of sub-cellular compartmentalization. PPO would released to cytoplasm and reacted with phenolic compounds or substrates which leaked from vacuole (Tan and Li, 1984; Degl'Innocenti *et al*., 2005). Similarly, Marangoni *et al*. (1996) reported that senescence of plant tissues at ripening stage hastened the cell damage compartmentalization. Present results confirm the work reported by De Castro *et al*. (2008) that the loss of impermeable characteristics of membrane caused by sub-cellular compartmentalization or membranes degradation inside the cell of plant tissue (Toivonen, 2004). PPO was an oxygen transferring enzyme catalyzed two reactions: (1) hydroxylation of monophenols to diphenols and (2) oxidation of o-diphenols to quinones (Nicolas *et al*., 1994). As well as o-quinones was very unstable and reacted with amino acids or proteins which spontaneously polymerized to produce brown pigments responsible for tissue browning (Eidhin *et al*., 2005). However, level of enzymatic browning was complex reaction concerned with many factors such as endogenous polyphenol content, distribution and activity of PPO and oxygen concentration (Coseteng and Lee,

1987; Martinez-Cayuela *et al*.,1988; Zawistowsky *et al*., 1991). This pattern has also been observed in banana fruits, Min-Kyung (2007) stated that level of browning discoloration of banana fruit was associated to polyphenol oxidase activation and concentration of free phenolic substrate (Weaver and Charley, 1974). This confirms the study reported by Lee and Whitaker (1994) where they reported that during mature fruit, PPO would change from bound form to more soluble form. However, PPO activity in soluble form of immature fruits had lower than mature fruits. It may be attributed to the increase in PPO activity during fruit development. Ueda *et al*. (2000), Aydin and Kadioglu (2001) and Lin *et al*. (2005) showed that PPO, soluble protein, related to ripening and senescence of fruit. Furthermore, Ortega-García *et al*. (2008) suggested that alteration of PPO activity in olive fruit (*Olea europaea* L.) var. Picual showed the increased PPO activity associated with fruit maturity increment particularly ripening fruit. Fang *et al*. (2007) studied PPO activity in each fruit development stage of bayberry. The results revealed that fruits at ripening stage had the most PPO activity which was higher than immature fruit about three times. This implied that ripening bayberry fruits were more sensitive to enzymatic reactions than young fruits. Agreed with Ajila *et al*. (2007) found that peel and flesh of ripening mango fruits var. Irwin had higher PPO activity than raw fruits (Prabha and Patwardhan, 1986). These results suggest that santol fruits have sensitivity to browning reaction with fruit maturation. While the results from cross-section study showed that immature fruits at 100 and 115 DAF found a lot of trichome on peel and small cells with thick wall one to two layers laid on epidermis. However, trichome on peel disappeared and unnoticeable in mature fruits at 130 and 145. In addition, cells in epidermis at latter stage developed into periderm composed of cell with thin wall, several layers and accumulated several substances. Thus, tissue damages from browning reaction in each part of plant tissue depend upon type of plant tissue. Immunohistochemical study of Ortega-García *et al*. (2008) showed that mostly of PPO in leaf found in epidermis, parenchyma and companion vascular cells while in fruit, it found majority in epidermis. On the other hand, Cheng and Crisosto (1995) reported that Browning Potential (BP) in buffer extracts of peach (*Prunus persica* L. Batsch) and nectarine (*P. persica* var. nectarine (L.) Batsch) showed darkening coloration at fruit surface and damaged to epidermal layers (Cheng and Crisosto, 1994; Crisosto *et al*., 1992, 1993). Carlos *et al*. (1994) indicated that browning coloration found in core, carpels and flesh of Chinese pears fruits var. Ya Li and Seuri. The results showed that maturity stage influenced on browning discoloration. Immature fruits harvested at less than 180 DAF did not browning occurrence while browning disorder appeared only on the fruits harvested at color break stage from green to greenish yellow. In addition, luscious brown to dark brown tissue development found only in core and flesh of Chinese pear fruits but not shown on external surface of fruits. Gustavo *et al*. (2006) suggested that sensitive part and severe damage to browning reaction on slices of *Averrhoa carambola* L. fruit were central section. They implied that this portion had high PPO concentration. The results revealed that necessity to raise the research to control browning reaction. The results from this experiment indicated that santol fruits tend to be sensitive to browning reaction as fruit approached to the mature stage at 130 DAF and ripening at 145 DAF. Browning occurrence in fruit tissues led to storage limitation after harvesting (Jiang *et al*., 2003) because the qualities of organoleptic and nutrition values altered and related to fruit decay (Das *et al*., 1997).

From assay on the PPO activities of different ages of santol fruits and modification of cell's structures both peel and flesh may be useful in explaining the sensitivity of PPO activities that enable to promote browning occurrence. These confirmed that browning susceptibility of santol fruits is related to aging processes. From the loss of their membrane stabilities may explain why fruits at mature stage are sensitive to discoloration. Nevertheless, our results tend to agreed with Toivonen and Brummell (2008) accounted that unripe fruits had the strength of cell-to-cell adhesion. When these fruits riped, mostly of parenchyma cells became thinner and weaker because of primary cell walls continuous degraded by nature. This could have been attributed to decrease the strength and quality of cell wall and intercellular adhesion. While Toivonen and Brummell (2008) stated that alteration of cell size is one of factor affected to fruit texture. Under electron microscope showed that larger size of air spaces produced from degradation of middle lamellae during ripening (Crookes and Grierson, 1983). These factors associated with reduced intercellular adhesion. The destruction of fruit cellular compartmentation allows the phenolic substrates to be accessible to PPOs which catalyze the phenolic oxidation (Mayer and Harel, 1979). In addition, factors affecting PPO activity included changes in cell size, intercellular adhesion, starch/sugar conversion, water loss, cell wall composition and cell wall strength. During fruit ripening, cell wall polysaccharides are extensively modified by a variety of ripening-related enzymes secreted from the symplast into the cell wall space. These changes affected to the structure and strength of the wall and ultimately bring about fruit softening. The composition of cell wall

in both pectins and matrix glycans degraded into more soluble form. Meantime Zheng *et al.* (2007) suggested that senescence in high plant was the characteristics of cell wall components degradation and membrane disintegration with membrane permeability increment. These effected to loss of cellular decompartmentation thus enzymes and substrates came to mix together and promoted the enzymatic browning led to loss of tissue structure as reported by Paliyath and Droillard (1992), Jiang and Chen (1995), Lin *et al.* (1988) and Jiang *et al.* (2004). Thus rate limiting step in enzymatic browning reaction probably related to loss membrane integrity which is the importance factor for controlling browning reaction. However, production of crystal like star content was naturally occurring initiated in peel and flesh raised with fruit age particularly 145 DAF. This finding expected that most of them are insoluble salt of calcium oxalate (Liebman, 2002). In present, physiological role of oxalate in plant is relatively unclear. Whilst Rassam and Laing (2005) reported that *Actinidia chinensis* from six genotypes had the oxalate content by 18 and 45 mg/100 g of FW which widely occurred in skin, pericarp and seed. Therefore, it might be inferred that these substances accumulation may involve with sensitivity to browning reaction of santol fruit at maturity. It may be of interest to carry out more experiments on the browning reaction in order to obtain more intensive results for further evaluation.

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