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## Antioxidant, Polygalacturonase, Pectin Methyltransferase and Polyphenol Oxidase Activities of Fresh-Cut Wax Apple (*Syzygium samarangense*) Treated with Organic Acids

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### ABSTRACT

This study reports the effectiveness of various concentrations of ascorbic acid, citric acid and oxalic acid used as acidulants to regulate activities of antioxidant, polygalacturonase, pectin methyltransferase and polyphenol oxidase of fresh-cut wax apple (*Syzygium samarangense*). The samples of fresh-cut wax apple, cut in wedges (1/8), were dipped into ascorbic acid, citric acid and oxalic acid at four different concentrations (0, 0.5, 1.0, 1.5 and 2.0%) for 1 min. The changes in the parameters were monitored for nine days while they are in storage at 4°C. Fruit treated with oxalic acid contained the highest antioxidant activity, followed by those treated with citric acid and ascorbic acid. The activity of antioxidant increased with the increasing storage period. Similar effects of acid treatments were detected for total phenolic contents. There was no effect of types of organic acids on polygalacturonase activity but acid dipping reduced the activity of the enzyme. In contrast, types of acids and acid concentration did not alter the activity of pectin methyltransferase activity. Activity of pectin methyltransferase in fruit sampled on day 6 and 9 were higher than those of the earlier dates. Similar pattern of effects of acid treatment were observed for polyphenol oxidase activity.

**Key words:** Wax apple, minimally processed fruits, cell wall enzymes, antioxidant activity, organic acid treatments

### INTRODUCTION

Fresh-cut vegetables and fruits are more perishable than intact fruits (Gil *et al.*, 2006) and possessed a shorter shelf life following higher respiration, ethylene production and membrane degradation (Portela and Cantwell, 1998; Supapvanich *et al.*, 2011). For some fruits, their fresh-cut products are prone to enzymatic browning that could decrease the appearance value of the products. Enzymatic browning is the discoloration due to the action of polyphenol oxidase that occurs as a result of disruption of the cell integrity leading to the mixing of the plastid and vacuole content (Suttirak and Manurakchinakorn, 2010). They also claimed that the usage of anti-browning agents is effective and normally used in controlling the enzymatic browning in a number of fresh-cut

products. Weak organic acids including ascorbic acid, citric acid and oxalic acid are among the most popular anti-browning agents being used in reducing the enzymatic browning.

In delaying physiological decay in fruit tissues, it is vital to treat the surface of fresh-cut fruits that will help in stabilizing the surface consequently prevent degradative processes of the fresh-cut fruits. Dipping with the anti-browning solutions is one of the methods being used in preventing the enzymatic browning. Besides the dipping time, temperature and pH of the solution for dipping treatments also play some roles in influencing their effects of fresh-cut produces. After the dipping treatment, drying procedure has to be done properly in order to avoid microbial spoilage (Soliva-Fortuny and Martin-Belloso, 2003).

Ascorbic acid has been used in quite a number of studies in reducing enzymatic browning especially in fruits with the concentration range of 0.5-4.0% (Soliva-Fortuny and Martin-Belloso, 2003). Ascorbic acid reduces the o-quinones to colorless dihydroxyphenols and prevent oxygen diffusion into the product through reaction deactivation and inhibits browning temporarily depending on the concentration, pH, temperature, enzyme activity, oxygen, metals and substrate concentration (Zhu *et al.*, 2007). Citric acid is another organic acid that could be utilized for the purpose due to its ability to reduce the rate of enzymatic browning by lowering the pH of the tissue away from the optimum pH for polyphenol oxidase activity (pH 6.0-7.0). Citric acid inhibiting effect was reported to link with its phenolase Cu-chelating power (Pizzocaro *et al.*, 1993). Polyphenol oxidase activity could be reduced at the concentration of 2% and below. Oxalic acid is an acidulant that minimizes the activity of polyphenol oxidase by lowering the pH of the product and consequently retard the enzymatic browning. Besides, oxalic acid also can acts as chelating agents that chelates the polyphenol oxidase active site with copper that reduces the rate of discoloration. Oxalic acid treatment has been used to reduce the enzymatic browning in banana and apples (Yoruk *et al.*, 2002). Kayashima and Katayama (2002) stated in their study that oxalic acid is available as a natural antioxidant.

Polygalacturonase is an enzyme that catalyzes the cleavage of pectin chains with a low degree of methylation (Riov, 1974). Pectin methylesterase is another enzyme that involves in the softening of fresh produces that produces pectin with a lower degree of methylation which later being used as the substrate for polygalacturonase. Pectin methylesterase can be found abundantly in citrus fruits and vegetables. The research on polygalacturonase and pectin methylesterase in wax apple, whole or fresh-cut fruits had not been done extensively by other researchers previously, hence, through this study it is expected to contribute some knowledge regarding this matter.

Antioxidant activity of any food item is an important criteria of food that attract consumers as its involvement in reducing in the incidence of degenerative diseases (Alothman *et al.*, 2009). Anyasi *et al.* (2015) reported that pretreatment of the unripe banana pulp with ascorbic and lactic acids has resulted in the increase in the antioxidant activity of the flour extracted from the fruits. They also recorded that the increase in antioxidant was strongly positively correlated to the total polyphenol content. Such relationship could also exist in the fresh-cut fruits.

In this study, the effectiveness of various concentrations of ascorbic acid, citric acid and oxalic acid used as acidulants in view of their potential application as anti-browning agents and regulation of cell wall enzymes for quality retention of fresh-cut wax apple were evaluated. The impacts of organic acid treatments on antioxidant activity and total phenolic content were also determined.

## MATERIALS AND METHODS

**Fruit materials:** *Syzygium samarangense* (red skin wax apple) were obtained from a commercial farm (Poh Keong Realty Sdn. Bhd, Melaka, Malaysia) at maturity stage that is suitable for consumption (*ca.* 45 days after anthesis). Before further processing, the fruits were stored at 10°C for overnight. Fruits with similar characteristics (size and colour), free from disease and physical defects were selected. The fruits were cleaned with tap water for five min and then were rinsed with tap water before being air-dried at room temperature (24°C).

The fruits were cut into 1/8 (wedges), then dipped into ascorbic acid, citric acid and oxalic acid at four different concentration (0, 0.5, 1.0, 1.5 and 2.0%) for 1 min. The fruits were then blotted with tissue paper, packed into plastic containers (130-150 g per container) and stored in domestic refrigerator (4°C) until day 9. The evaluation of fruits quality was done on day 0, 3, 6 and 9.

**Determination of antioxidant activity:** Three gram of wax apple was grinded with 30 mL of 80% methanol before being shaken on orbital shaker for 2 h. The mixture was then filtered through filter paper (Whatman No. 2) and the filtrates obtained were used for the determination of antioxidant activity and total phenolic content. For antioxidant activity determination, 0.2 µM methanolic DPPH was prepared by dissolving 2.1 mg DPPH powder in 80% methanol. One milliliter of extract was introduced into 2 mL methanolic DPPH in an aluminum-wrapped test tube. After 30 min, the absorbance of the mixture was measured by using spectrophotometer at the wavelength of 517 nm. The antioxidant activity was then expressed as the percentage of inhibition as shown as in the formula (Lim *et al.*, 2006):

$$\text{Inhibition (\%)} = \frac{A_{517 \text{ nm of methanolic DPPH}} - A_{517 \text{ nm sample}}}{A_{517 \text{ nm of methanolic DPPH}}}$$

**Determination of total phenolic content:** Folin-Ciocalteu (FC) solution was prepared by diluting FC reagent with distilled water with the ratio of 1: 10. 0.3 mL of extract (the same as the one used for antioxidant determination) was introduced into aluminum-wrapped test tube containing 1.5 mL of the diluted FC reagent, followed by the addition of 7.5% sodium carbonate (Na<sub>2</sub>CO<sub>3</sub>) before being stored in darkness for 30 min (Lim *et al.*, 2006). The absorbance of the mixture was then taken at the wavelength of 765 nm and the total phenolic content was expressed in mg gallic acid/100 g.

**Enzyme extraction:** Five gram of fresh-cut wax apple was homogenized with buffer containing 0.1 M sodium citrate, 1 M NaCl, 13 nM EDTA, β-mercaptoethanol and 2% polyvinylpyrrolidone (PVP)-40 before being centrifuged at 29000×4 at 4°C. The supernatant was collected and kept at 4°C before enzyme extraction.

**Polygalacturonase assay:** The assay for polygalacturonase (PG) activity was done according to Gross (1982) by using cyanoacetamide method. About 0.1 mL of enzyme extract was added into test tube together with 0.5 mL 0.25% polygalacturonic acid in 40 mM sodium acetate buffer (pH 4.4). The mixture was incubated at 30°C for 2 h. The reaction was then terminated by the addition of 2 mL cold borate buffer (0.1 M, pH 9.0). About 0.02 mL 1% 2-cyanoacetamide was added into the mixture and immersed in boiling water for 10 min. The mixture was then cooled at room temperature before measuring the absorbance of the mixture at 276 nm by using spectrophotometer.

**Pectin methylesterase assay:** The same extract obtained for PG assay was used for pectin methylesterase activity (PME) assay. Twenty five milliliter aliquot of 1.0% pectin and 0.3 M NaCl was equilibrated at 30°C and the pH was adjusted to 7.3. Following the addition of 0.5 mL of sample extracts, the pH was re-adjusted to 7.3 using 0.01N NaOH. The activity of PME was calculated following method of Ali *et al.* (2004).

**Polyphenol oxidase assay:** The procedure as described by Kim and Jung (2011) was adopted for polyphenol oxidase (PPO) activity assay. Five g of fresh-cut wax apple was homogenized with 10 mL of sodium phosphate buffer (0.1 M, pH 7.0 and 0.5 g PVP) and centrifuged at 12,000 rpm for 20 min. The centrifuged supernatant was then used as an

enzyme extract. About 0.2 mL of the enzyme extract was mixed with 4-methylcatechol in sodium acetate buffer (0.1 M) before it's absorbance measured at 420 nm for 1 min with the interval of 10 sec using spectrophotometer.

**Statistical analysis:** The study involved a three factorial experiment and treatments were laid-out in a Completely Random Design (CRD) with three types of organic acids (ascorbic acid, citric acid and oxalic acid) for the dipping treatment at five different concentration (0, 0.5, 1.0, 1.5 and 2.0%) throughout nine days of storage, with four replications except for assay on polygalacturonase, pectin methylesterase and polyphenol oxidase activities which was done with 3 replications. Each replication consisted of three fruits. Data obtained was subjected to analysis of variance (ANOVA) and Least Significance Difference (LSD) at  $p \leq 0.05$  level was performed for mean comparisons. Data was analyzed using Statistical Analysis System (SAS version 9.4 Cary, NC, USA).

## RESULTS AND DISCUSSION

The effects of types of acids, their concentrations and storage period on all parameters measured in the study are summarized in Table 1. When the interaction effects among the main factors are significant, then the data obtained was presented in such a way so that it depicts how one factor behave under varying levels of the other factors.

Table 1: F-test of ANOVA for various parameters on the effects of different types of organic acids applied at different concentration during 9 days of storage period of fresh-cut wax apple

Treatments	Antioxidant activity (%)	Total phenolic content (mg/100 g)	Polygalacturonase activity (U/FW g)	Pectin methylesterase activity (equivalent carboxyl group released/FW g sec <sup>-1</sup> )	Polyphenol oxidase activity (U min <sup>-1</sup> mg <sup>-1</sup> protein)
<b>Types of acid (A)</b>					
Ascorbic acid	81.17	30.43	2.51	230.72	0.0297
Citric acid	86.16	33.64	2.60	242.35	0.0297
Oxalic acid	90.64	33.84	2.81	249.70	0.0310
LSD <sub>0.05</sub>	1.46	00.91	0.85	038.98	0.0020
<b>Concentration (%) (C)</b>					
0.0	87.69	32.67	3.39	238.72	0.0318
0.5	83.98	30.51	2.09	228.98	0.0295
1.0	84.45	31.41	2.64	244.74	0.0300
1.5	86.20	33.16	3.06	242.28	0.0298
2.0	87.63	35.44	2.01	249.89	0.0296
LSD <sub>0.05</sub>	1.886	1.18	1.10	050.32	0.0030
<b>Storage period (days) (S)</b>					
0	80.64	31.21	2.28	187.18 <sup>b</sup>	0.0260
3	83.39	32.11	2.66	196.79 <sup>b</sup>	0.0306
6	88.44	33.41	2.77	285.60 <sup>a</sup>	0.0316
9	91.49	33.81	2.85	294.11 <sup>a</sup>	0.0324
LSD <sub>0.05</sub>	1.69	01.05	0.99	045.01	0.0026
<b>F-test</b>					
A	***	***	ns	ns	ns
C	***	***	**	ns	ns
S	***	***	ns	***	***
A×C	***	***	ns	ns	ns
A×S	ns	ns	ns	ns	ns
C×S	ns	ns	ns	ns	ns
A×C×S	ns	ns	ns	ns	ns

ns: Non significant, \*\*, \*\*\*: Significant F-test at  $p < 0.01$  and  $p < 0.001$ , respectively

**Antioxidant activity:** The interaction between the types of organic acids used in the dipping treatment and the concentration of the acids was significantly affecting antioxidant activity ( $p \leq 0.001$ , Table 1). Among the three acids used, fruit treated with oxalic acid possessed the highest antioxidant activity (90.64%), followed by those treated with citric acid (86.15%) and ascorbic acid (81.17%). Compared to treatment with the other two acids, increasing concentration of ascorbic acid in the dipping solution reduced the antioxidant activity. Antioxidant activity in those treated with oxalic acid tended to increase slightly from 87.69-93.66% when the oxalic acid concentration has increased from 0-2.0% (Fig. 1).

Simirgiotis *et al.* (2007) reported that phytochemicals such as reynoutrin, hyperin, myricitrin, quercitrin, quercetin, guaijaverin, gallic acid and ellagic acid are the well known antioxidant compounds found in wax apple. However, there is a very little (maybe none on wax apple) studies have been done on the effect of organic acids on the antioxidant of fresh-cut fruits, so it is quite difficult to compare the data obtained in this study with the results of published earlier. Among limited literature available, Altunkaya and Gokmen (2008) reported on the effect of various inhibitor of enzymatic browning on antioxidant activity and total phenolic content and stated that citric acid and oxalic acid had insignificant effect on the antioxidant activities of lettuce.

Results of the study also show that the activity of antioxidant of fresh-cut wax apple has increased as the period of storage from 0-9 days by 13.45% ( $p \leq 0.001$ ). This observation is supported with the discovery of the increased of the total antioxidant activity in plum, asparagus and yellow pepper over storage period that is believed to be correlated with the total phenolic content (Kevers *et al.*, 2007).

**Total phenolic content:** Phenolic compounds are widely known to have a strong antioxidant activities and their ability to scavenge free radical (Chan *et al.*, 2009). There was a significant interaction between the types of acids and their concentrations in affecting the total phenolic content of fresh-cut wax apples ( $p \leq 0.001$ ) (Table 1) which is similar to the finding in antioxidant activity suggesting that total phenolic compound could be one of the contributing substances to the high antioxidant activity in organic acid dipped fresh-cut wax apples. Khandaker *et al.* (2012) revealed that the pulp of wax apples is rich with phenolics and also other substances that link to antioxidant activity of the fruit. Besides, Kahkonen *et al.* (2001) stated that the synergistic effect of ascorbic acid with total phenolic contents in fruits may also be one of the factors associated with the increase of antioxidant activity but this phenomenon was not recorded in this study.

Fresh-cut wax apples treated with oxalic acid and citric acid has resulted in a significantly higher total phenolic compound in comparison to those dipped in ascorbic acid (Fig. 2) with their respective means of 33.84, 33.64 and

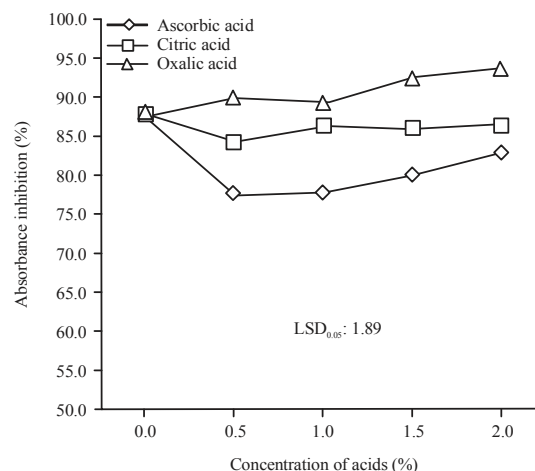


Fig. 1: Antioxidant activity for fresh-cut wax apple dipped into three types of organic acids

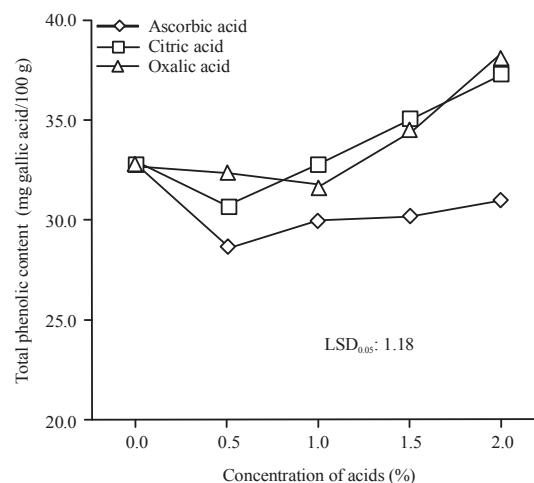


Fig. 2: Total phenolic content for fresh-cut wax apple dipped into three types of acids.

30.43 mg/100 g. The trends in the effect of organic acids on the total phenolic contents were somewhat similar at low concentration (0.5-1.0%) but total phenolic contents of citric acid and oxalic acid treated fruits were enhanced at higher acid concentration (1.5-2.0%). For example, the respective total phenolic contents for fruit treated with ascorbic acid, citric acid and oxalic acid at 1.0% concentration were 29.89, 32.74 and 31.59%. The corresponding values at 2.0% acid concentration were 30.90, 38.20 and 38.20%.

Additionally, the total phenolic content at the later period of the study (6 and 9 days) was found to be significantly higher than those on day 0 and day 3 ( $p \leq 0.001$ ). Similarly, Shiri *et al.* (2011) in their study using fresh-cut grapes treated with ascorbic acid discovered that ascorbic acid-treated fruits contained a higher total phenolic content after 14 days of storage.

**Polygalacturonase activity:** Polygalacturonase activity is one of the major enzymes that cause the change in the structure of the cell wall in fruits. It is well known that the decrease in fruit firmness is related to cell wall degrading enzymes (Brummell, 2006). In this study, the activity of PG was determined in organic acids treated fresh-cut wax apple. Results show that there was no effect of types of organic acids on PG activity but organic acid treatment reduce the activity of the enzyme (Table 1).

Throughout 9 days of storage, the PG activity was relatively constant and did not significantly affected by the treatments despite the significantly decrease in the firmness of the fresh-cut wax apples (data not shown). This is similar to the results of Barrelet and Gonzalez (1994) in cherry. The result obtained here suggests that polygalacturonase is present in the cell wall of fresh-cut wax apples but perhaps it is not the main enzyme that cause the reduction in the firmness of fresh-cut in wax apples. Beside PG, pectin methylesterase, glucosidase, cellulose, pectatelyase and rhamnogalacturonase A are the other major cell wall enzymes involve in fruit ripening (Brummell and Harpster, 2001).

**Pectin methylesterase activity:** Pectin methylesterase catalyzes the de-esterification of pectin with other pectinases that results in liberated carboxyl groups and methanol (Simsek and Yemencioğlu, 2007). In this study, the enzyme activity was assayed and the result obtained is summarized in Table 1. Results show that different types of acids and variation in their concentration did not affect the activity of PME. At day 0 and day 3, the activity did not differ among each other but the activity increased with the storage period whereby the PME activity in fruit sampled on day 6 and day 9 of storage were higher than those sampled on the earlier dates.

In contrary to PG, the increase in activity of PME over period of storage was observed here and this is inline with the reduction of firmness of the fresh-cut wax apple (data not shown). The role of PME in affecting fruit firmness has been highlighted in many studies earlier such as by Chuni *et al.* (2010) on dragon fruits and Ali *et al.* (2004) on selected tropical fruits including guava, banana and papaya. Ali *et al.* (2004) suggested that the pectin demethylesterification activity catalyzed by PME is not only meant for the PG activity but also affecting other cell wall hydrolysis that leads to the decrease of fruit firmness.

**Polyphenol oxidase activity:** Another enzyme investigated in this study was polyphenol oxidase (PPO). The PPO normally involves in the browning of fruits and would cause one of the main problems in fresh-cut industries. Fresh-cut wax apple are prone to browning and the white flesh of wax apple makes the fruit even more obvious especially near the cavity tissue in the middle of the fruits (Supapvanich *et al.*, 2011).

Results in Table 1 show that PPO activity of fresh-cut wax apples was not significantly affected by types of organic acids

and their respective concentrations. However, prolonging of the storage period produced a marked effect on PPO activity where its activity on day 3, 6 and 9 were higher than that recorded on day 0. The increase in PPO right activity after the processing of the fresh-cut fruits (day 0) suggesting that the accumulation of PPO that occurred in the tissue of fresh-cut wax apples over storage period, which was reported due to the mixing of the polyphenol substrates with PPO post cutting process (Toivonen and Brummell, 2008). Similar observation was also reported occurred in fresh-cut 'Jonagored' apple slices (Rocha and Morais, 2002).

In this study, the degree of browning was not measured but general observation made on the samples shows that browning occurred on the surface of the fresh-cut wax apples suggesting the increase in the PPO activity especially in the cavity tissue in the middle of the fresh-cut wax apples as reported by Supapvanich *et al.* (2011). However, the browning was not that severe that it could reduce the appearance of the sample.

Theoretically, acids used in this study would have different mechanism in reducing the PPO activity. In general, ascorbic acid acts as reducing agent would inhibit the browning reaction by reducing quinones to diphenol or reduces the  $\text{Cu}^{2+}$  to  $\text{Cu}^+$  at the active site of PPO (Altunkaya and Gokmen, 2008). On the other hand, citric acid and oxalic acid can either act as acidulants by reducing the pH and/or acting as chelating agents in delaying the enzymatic browning (Suttirak and Manurakchinakorn, 2010).

## CONCLUSION

In conclusion, within the limit of the study, fresh-cut wax apple treated with oxalic acid could be the best treatment as it has resulted in the highest antioxidant activity which is coupled with increase in total phenolic contents. Furthermore, organic acid treatment, regardless of types of acids and their concentration would reduce the polygalacturonase activity and this could contribute to the retention of fruit firmness.

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