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Control of Skin Colour and Polyphenol Oxidase Activity in Santol Fruit by Dipping in Organic Acid Solution

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Abstract: This laboratory experiment was carried out at the Department of Agricultural Technology, Mahasarakham University, Northeast Thailand during July and August 2008. The experiment aimed to determine an effective natural organic acid that would delay the unattractive skin browning of santol fruit, while at the same time not damaging the quality of the fruit. The experiment included a study of the fruit's polyphenol oxidase (PPO) activity, phenolic content and quinone content, as they relate to colour and a study of total soluble solid content, pH, titratable acidity and vitamin C content as they relate to fruit quality. A Completely Randomized Design (CRD) with four replications was used. Each replication consisted of 10 fruits. Santol fruit was harvested at 145 days after full bloom and dipped for 30 min in aqueous solutions of two organic acids that were used as treatments, i.e., 0% for T_1 (control), 5% citric acid for T_2, 5% ascorbic acid for T_3, 10% citric acid for T_4 and 10% ascorbic acid for T_5 and stored at room temperature (28°C, 90% R.H.) to investigate the effect of the acid on fruit weight, skin colour, PPO activity and other internal parameters. The results showed that the most appropriate anti-browning agent for santol fruit was found with T_2. It gave the highest mean values, 57.37 and 55.95, of brightness (L*) at 4 and 10 Days After Storage (DAS), respectively. In addition, PPO activity of flesh tissue was lowest for T_2 with mean values of 0.0078 to 0.0092 by 0 and 300 S, respectively. The phenolic content in the flesh tissue significantly increased with an increase in numbers of DAS, whereas the reverse was found with the pH level in the fruits. They were lowest for T_2, with mean values of 6.00, by 10 DAS. There were no significant differences among the treatments in any of the measured Total Soluble Solids (TSS), Titratable Acidity (TA) and vitamin C content.

Key words: Skin colour, polyphenol oxidase activity, santol fruit, organic acid, enzymatic browning

INTRODUCTION

Santol [(*Sandoricum koetjape* (*Burm. f.*) *Merr.*] is a tropical fruit in the family of Meliaceae. It is one of the most economically important tree fruit crops cultivated throughout the East, central and Northern parts of Thailand. The soft tissue of flesh in santol fruit is juicy and delicious with an exquisite flavour. It is recognized as a good source of antioxidants. Sangkitikomol (2000) cited that santol fruit could provide antioxidants up to 6.5 millimhos 100^{-1} g of fresh weight. These substances play a major role; several are in human nutrition where the substance possess its medicinal properties against human illness, particularly healing of Coronary Heart Disease through anti-oxidative and anti-carcinogenic substances (Sangkitikomol, 2000; Stoclet *et al*., 2004; Samappito and Butkhup, 2008). It is well known that the golden-yellow skin of santol fruit is one of the most important aspects of the fruit's quality. However, harvested santol fruits rapidly lose their skin appearance due to their susceptibility to postharvest browning. The skin browning of santol fruit has largely been attributed to the enzymatic oxidation of phenolic compounds by Polyphenol oxidase (PPO) (Benjwan *et al*., 2008), producing brown-colored by-products (Ferrar and Walker, 1996). Such changes have a major negative impact upon the market value of santol fruit and contribute to its relatively short storage life. One way of extending the storage life of santol fruit is to control the PPO activity. With the consumers' increased awareness of safety, chemical compounds for application in fruit and vegetable products and chemicals without toxic effects for controlling santol skin colour are needed for commercial use. Very limited information is currently available on controlling PPO activity in santol fruit. Some natural antioxidant compounds are found in all higher plants and they include citric acid and ascorbic acid which can inhibit enzymatic browning of some agricultural products.

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Whitaker and Lee (1995) suggested that treatment with organic acid is a safe and promising method for controlling some fruit browning during postharvest storage. Thus, the main purpose of this study was to find the effective natural organic acid such as citric acid and ascorbic acid to reduce browning of santol fruit during storage at room temperature.

MATERIALS AND METHODS

This laboratory experiment was carried out at the Department of Agricultural Technology, Faculty of Technology, Mahasarakham University, Mahasarakham, in Northeast Thailand during the months of July and August 2008. The fruits of santol cv. Pui Fai were hand-harvested from an experimental orchard at the Department of Agricultural Technology, Faculty of Technology, Mahasarakham University, Mahasarakham Province, in the Northeast region of Thailand. The santol fruits were harvested from four year old plants with an average plant height of approximately five m above ground level. Measurements of the trunk diameter were made at approximately 30 cm above ground level. The grafted seedlings of Pui Fai cultivar were planted with distances between rows and within rows of 6×6 m, respectively. After harvesting, santol fruits were selected for uniform size and colour and any blemished or diseased fruits were discarded. The experiment was laid in a Completely Randomized Design (CRD) with four replications. Each replication consisted of ten fruits. The antibrowning organic acids being used as treatments were: 0% (T_1, control), 5% citric acid (T_2), 5% ascorbic acid (T_3), 10% citric acid (T_4) and 10% ascorbic acid (T_5). Each treatment was soaked in 5000 mL of each organic acid solution for 30 min and drained. Samples were kept on a laboratory bench at room temperature (28°C, 90% R.H.). The following determinations were made on every other day. They include (1) Average fresh weight of fruits of each replication. (2) Skin colour of fruits was determined using a Hunter Lab Model No. 45/0-L, Serial No. 7092, USA. The measurements made were: L* = brightness (black = -100 and white = +100), a* = green or red on skin surface of fruits where - = green and + = red, b* = yellowness (- = blue and + = yellow). (3) Polyphenol oxidase (PPO). Activity determination was carried out according to the method reported by Jiang and Fu (1998). The attained enzyme extracts were measured by spectrophotometer model V-325-XS, from China. 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. (4) Phenolic contents were performed as described by Ribeiro *et al.* (2008). They were expressed as absorption at 765 nm/100 g fresh weight of fruit. (5) Quinone content was extracted as described by Pirie and Mullins (1976). Quinone content was expressed as absorbance at 437 nm. (6) Total Soluble Solid (TSS) content (juices being squeezed from flesh tissue with the use of distilled water at a ratio between flesh and distilled water of 1:3) was measured by a digital refractometer (Atago-Palette PR 101, Atago Co., Ltd., Itabashi-ku, Tokyo, Japan). (7) The measurement of pH values was carried out with the use of juices described earlier in number 6 and a pH meter ID 100D, from Singapore was used. (8) Titratable Acidity (TA) evaluation was made by the use of juices described in number 6 with the method of AOAC (1984). (9) Vitamin C content was measured by the use of juices described in number 6 with the method of AOAC (1990). The collected data were statistically analyzed using the SPSS Computer Programme, Version 6 (SPSS, 1999).

RESULTS

Fruit weight: The results showed that organic acid treatment did not affect santol fruit weight. There was a further decrease in fruit weight during storage but no significant difference for the whole fruit weight was observed during storage (up to 12 days after storage or DAS). Fruit weight was substantial 262.74-311.78 g fruit^{-1} after the first 6 DAS and decreased to 242.06-309.42 g fruit^{-1} by day 12 (Table 1). These results confirmed the view that santol fruit weight decreased with duration of storage.

Fruit skin colour: With respect to fruit skin colour, the degree of browning was expressed by L* value. The results showed that lightness index L* of all treatment decreased with an increase of storage time. This suggested that changes in the skin colour of santol fruit proceeded quickly. The lightness (L*) of T_2, or 5% citric acid-treated fruit, was significantly higher when compared to all other treatments at 4 and 10 DAS (57.37 and 55.95, respectively) (Table 2). Thus, T_2 gave the best skin brightness and good visual appearance.

Table 1: Mean values of fruit weight fruit^{-1} of santol dipped at 0% (T_1, Control), 5% citric acid (T_2), 5% ascorbic acid (T_3), 10% citric acid (T_4) and 10% ascorbic acid (T_5)

	Fruit weight fruit^{-1} (g) after storage					
	2	4	6	8	10	12
Treatments	(days)					
T_1 (control)	305.23	294.21	271.08	265.76	254.65	246.25
T_2	281.89	258.22	262.74	248.14	231.11	242.06
T_3	337.27	333.92	311.78	302.35	291.61	255.85
T_4	338.07	324.54	299.74	282.96	292.85	256.40
T_5	319.51	309.67	303.03	308.77	258.38	309.42
F-test	ns	ns	ns	ns	ns	ns
CV (%)	22.55	19.81	19.65	21.78	25.03	20.84
LSD	19.07	22.56	21.45	21.88	23.07	15.91

ns: Non significant, CV : Percentage of covariance

Table 2: Mean values of fruit skin colour of L* of santol fruit as influenced by treatments, i.e. 0% (T_1, Control), 5% citric acid (T_2), 5% ascorbic acid (T_3), 10% citric acid (T_4) and 10% ascorbic acid (T_5)

	L* values (days after storage)						
Treatments	0	2	4	6	8	10	12
T_1 (control)	57.39	57.34	57.26ab	55.88	57.07	55.46ab	52.40
T_2	57.33	56.35	57.37a	56.07	55.58	55.95a	47.90
T_3	57.74	56.92	56.85abc	56.19	56.75	54.00abc	53.85
T_4	57.47	56.43	55.97bc	56.21	55.58	51.93c	49.11
T_5	57.32	55.63	55.55c	56.79	55.66	52.64bc	49.97
F-test	ns	ns	*	ns	ns	*	ns
CV (%)	3.78	3.37	0.46	0.55	0.53	1.04	1.68
LSD	0.58	0.51	2.84	3.09	2.65	4.73	6.64

Letter(s) within columns indicate Least Significant Differences (LSD) at probability, *p = 0.05, ns: Non significant, CV : Percentage of covariance

Table 3: Mean values of fruit skin colour of a* of santol fruit as influenced by treatments, i.e. 0% (T_1, Control), 5% citric acid (T_2), 5% ascorbic acid (T_3), 10% citric acid (T_4) and 10% ascorbic acid (T_5)

	a* values (days after storage)						
Treatments	0	2	4	6	8	10	12
T_1 (control)	11.98	12.36	13.31bc	13.73	14.49	15.14	13.12
T_2	13.42	12.89	14.70a	14.68	15.44	14.24	9.44
T_3	11.96	11.90	13.00bc	13.98	14.55	14.36	14.49
T_4	11.51	12.79	14.12ab	13.99	14.82	12.98	11.96
T_5	12.18	11.23	12.92c	14.25	14.57	13.52	10.60
F-test	ns	ns	*	ns	ns	ns	ns
CV (%)	20.67	13.38	0.41	0.31	0.28	0.78	1.47
LSD	0.67	0.44	10.36	7.01	5.28	13.55	24.63

Letter(s) within columns indicate Least Significant Differences (LSD) at probability, *p = 0.05, ns: Non significant, CV : Percentage of covariance

a* values of fruit skin colour did not change and the results showed that there was little or no effect on a* values during storage (Table 3). b* values (Table 4) of fruit skin colour were similar to a* values except that b* values showed a highly significant difference in colour change immediately after dipping on the first day and there was a decrease in b* values over longer storage periods. Table 4 also showed the marked increases of b* that occurred after dipping in the two organic acids. The b* values were significantly higher in the fruits treated with organic acid than in the untreated fruits ($p<0.01$). Afterwards, there was no significant difference in b* values between control and fruits-treated with the two organic acids.

PPO activity: For the PPO activity of the flesh tissue, the results showed that PPO activity did not show any significant difference during the first four days of storage (data not shown). The lowest activity of the PPO was on day 12 with a mean values of 0.0270 and 0.0422 units in the extract for T_2 by 0 and 300 S, respectively (Table 5). This implied that citric acid showed much more potent enzymatic browning than ascorbic acid. Thus, citric acid at lower concentration (5%) can be used effectively against enzymatic oxidation for santol fruit.

Table 4: Mean values of fruit skin colour of b* of santol fruit as influenced by treatments, i.e. 0% (T_1, Control), 5% citric acid (T_2), 5% ascorbic acid (T_3), 10% citric acid (T_4) and 10% ascorbic acid (T_5)

	b* value (days after storage)						
Treatments	0	2	4	6	8	10	12
T_1 (control)	41.02b	40.38	40.38	39.02	40.26	37.98	31.61
T_2	52.47a	41.74	41.04	39.29	38.27	33.25	24.29
T_3	53.47a	40.59	39.71	38.93	38.74	35.85	33.74
T_4	52.00a	41.13	40.00	39.98	40.07	33.17	29.52
T_5	53.43a	40.19	39.40	38.85	39.57	34.42	28.29
F-test	**	ns	ns	ns	ns	ns	ns
CV (%)	4.27	6.11	0.75	1.21	1.04	2.02	3.01
LSD	0.58	0.67	6.46	9.76	7.47	14.18	20.44

Letter(s) within columns indicate Least Significant Differences (LSD) at probability, **p = 0.01, ns: Non significant, CV : Percentage of covariance

Table 5: PPO activity found in flesh tissue of santol fruits as influenced by dipping organic acids (treatments) by 12 days after storage

	PPO activity at different time (sec)					
Treatments	0	60	120	180	240	300
T_1 (control)	0.0170ab	0.0172abc	0.0177abc	0.0182bc	0.0185bc	0.0188bc
T_2	0.0270a	0.0292a	0.0320a	0.0352a	0.0385a	0.0422a
T_3	0.0167ab	0.0162bc	0.0163bc	0.0163bc	0.0168bc	0.0173c
T_4	0.0078b	0.0078c	0.0083c	0.0087c	0.0090c	0.0092c
T_5	0.0182ab	0.0208ab	0.0243ab	0.0282ab	0.0325ab	0.0372ab
F-test	*	*	*	*	*	**
CV (%)	0.0039	0.0044	0.0050	0.0054	0.0060	0.0065
LSD	57.80	54.95	50.76	66.4	61.22	69.56

Letter(s) within columns indicate Least Significant Differences (LSD) at probability, **p = 0.01, *p = 0.05, ns: Non significant, CV : Percentage of covariance

Table 6: Phenolic contents found in flesh tissue of santol fruits as influenced by dipping organic acids (treatments)

	Phenolic (mg/100 g FW) contents					
	2	4	6	8	10	12
Treatments	(days)					
T_1 (control)	2519.73c	1039.65	5689.18ab	2627.11a	6303.29	5021.38c
T_2	5987.76a	3399.45	4084.21bc	1554.30b	7538.10	6446.45ab
T_3	5175.85ab	2134.12	6098.90a	1312.24b	7217.86	5606.29bc
T_4	4897.05b	1774.33	3104.64c	1579.73b	5692.95	7281.91a
T_5	4638.97b	1722.52	4284.83bc	1720.07b	6923.99	5828.58bc
F-test	**	ns	*	**	ns	*
CV (%)	16.48	9.49	6.20	16.61	4.62	4.71
LSD	312.52	115.50	32.67	23.13	16.83	19.14

Letter(s) within columns indicate Least Significant Differences (LSD) at probability, **p = 0.01, *p = 0.05, ns: Non significant, CV : Percentage of covariance

Phenolic content: For phenolic content, which was the main substrate for PPO activity, the results showed that the concentrations of phenolic compounds were quite variable, as they clearly decreased by 4 DAS, then increased rapidly to very high values at 10 DAS. Santol fruit-treated with two organic acids had less phenolic content (1312.24-1720.07 mg/100 g FW) than control (2627.11 mg/100 g FW) by 8 DAS (Table 6).

Quinone content: For quinone content of flesh tissue, the results showed that quinone content decreased meaningfully with storage time. The quinone content in the santol flesh of the different treatments were shown to

Table 7: Quinone contents found in flesh tissue of santol fruits as influenced by dipping organic acids (treatments)

	Quinone (g^{-1} fresh weight) contents					
	2	4	6	8	10	12
Treatments	(days)					
T_1 (control)	0.1197	0.1007a	0.0386c	0.0465b	0.0604a	0.0676
T_2	0.1383	0.0628b	0.0513bc	0.0491b	0.0351b	0.0528
T_3	0.0920	0.0542b	0.0526bc	0.0445b	0.0605a	0.0603
T_4	0.0881	0.0637b	0.0926a	0.0405b	0.0523a	0.0575
T_5	0.1169	0.0686b	0.0593b	0.0584a	0.0648a	0.0590
F-test	ns	**	**	**	**	ns
CV (%)	32.48	20.20	29.41	20.92	25.90	16.84
LSD	0.0150	0.0059	0.0069	0.0031	0.0055	0.0045

Letter(s) within columns indicate Least Significant Differences (LSD) at probability, **p = 0.01, ns: Non significant, CV : Percentage of covariance

Table 8: Total soluble solids (°Brix values) found in flesh tissue of santol fruits as influenced by dipping organic acids (treatments)

	TSS (°Brix) contents					
	2	4	6	8	10	12
Treatments	(days)					
T_1 (control)	2.67bc	2.40bc	3.00	3.40	3.33	3.63
T_2	2.47c	2.73ab	2.67	3.27	3.47	2.83
T_3	2.73abc	2.33bc	3.20	3.00	3.53	3.00
T_4	3.40a	2.13c	2.73	3.00	3.27	3.23
T_5	3.27ab	3.07a	2.50	2.87	3.27	3.10
F-test	*	*	ns	ns	ns	ns
CV (%)	12.82	10.79	15.99	8.47	9.05	9.60
LSD	0.2150	0.1578	0.2603	0.1520	0.1764	0.1751

Letter(s) within columns indicate Least Significant Differences (LSD) at probability, *p = 0.05, ns: Non significant, CV : Percentage of covariance

be significantly different. Fruit-treated with 5% citric acid (T_2) showed a lower quinone content with a mean value of 0.0351 g^{-1} fresh weight when compared with the others by 10 DAS. Afterwards, the contents of quinone were relatively constant by the end of the storage period (Table 7).

Total Soluble Solids (TSS): For total soluble solids (°Brix values) of flesh tissue, the results showed that TSS of santol flesh tended to increase with storage time. The TSS content was significantly different; only 2 and 4 DAS were observed. The highest Brix value was found with the flesh tissue of santol fruit with a mean value of 3.07° Brix for T_5 by 4 DAS. Afterwards, TSS content showed quite invariable by the end of storage (Table 8).

pH: With acidity levels (pH), the results showed that pH values of santol flesh highly increased with storage time. Table 9 presents the lowest pH value with a mean value of 6.20 and 6.00 for T_2 by 8 and 10 DAS, respectively. These suggest that T_2 markedly decreased the acidity levels of santol fruit.

Titratable Acidity (TA): Percentages of TA of the flesh tissue tended to decrease with storage time. On the sixth day of storage the TA content showed a significant difference between treatments. The results revealed that

Table 9: Acidity levels (pH) found in flesh tissue of santol fruits as influenced by dipping organic acids (treatments)

	Acidity levels (pH)					
	2	4	6	8	10	12
Treatments	(days)					
T_1 (control)	5.53	5.33bc	5.93a	6.43a	6.43a	6.20
T_2	5.57	5.30c	5.30b	6.20c	6.00b	6.27
T_3	5.60	5.60a	5.23b	6.50a	6.63a	6.17
T_4	5.60	5.43bc	5.20b	6.27bc	6.47a	6.43
T_5	5.57	5.47ab	5.33b	6.37ab	6.70a	6.17
F-test	ns	**	**	*	**	ns
CV (%)	0.03	3.19	3.80	1.41	2.94	3.68
LSD	0.80	0.05	0.12	0.05	0.11	0.13

Letter(s) within columns indicate Least Significant Differences (LSD) at probability, **p = 0.01, *p = 0.05, ns: Non significant, CV: Percentage of covariance

Table 10: Titratable acidity (TA) contents found in flesh tissue of santol fruits as influenced by dipping organic acids (treatments)

	TA (%) contents					
	2	4	6	8	10	12
Treatments	(days)					
T_1 (control)	0.5933	0.2880	0.3040ab	0.3147	0.2773	0.4800
T_2	0.5333	0.2560	0.3445a	0.2880	0.3093	0.4053
T_3	0.4640	0.3093	0.3040ab	0.3019	0.3093	0.4693
T_4	0.5440	0.2667	0.3040ab	0.3168	0.2827	0.3840
T_5	0.7680	0.2773	0.2635b	0.3093	0.3360	0.4480
F-test	ns	ns	*	ns	ns	ns
CV (%)	4.52	8.00	8.06	9.80	11.44	21.57
LSD	0.13	0.01	0.01	0.02	0.02	0.06

Letter(s) within columns indicate Least Significant Differences (LSD) at probability, *p = 0.05, ns: Non significant, CV: Percentage of covariance

Table 11: Vitamin C contents (mg/100 ml juice) found in flesh tissue of santol fruits as influenced by dipping organic acids (treatments)

	Vitamin C contents (mg/100 mL juice)					
	2	4	6	8	10	12
Treatments	(days)					
T_1 (control)	14.19	14.22	14.14c	14.18	14.16	14.17
T_2	14.14	14.18	14.19a	14.14	14.14	14.05
T_3	14.19	14.16	14.16bc	14.17	14.06	14.05
T_4	14.19	14.14	14.17abc	14.12	14.14	14.03
T_5	14.17	14.19	14.18ab	14.17	14.11	14.00
F-test	ns	ns	*	ns	ns	ns
CV (%)	0.25	0.24	0.01	0.01	0.03	0.04
LSD	0.02	0.02	0.35	0.77	0.38	0.47

Letter(s) within columns indicate Least Significant Differences (LSD) at probability, *p = 0.05, ns: Non significant, CV: Percentage of covariance

the highest TA value was found with a mean value of 0.3445% for T_2. Afterwards, no significant differences in any of the measured TA was observed (Table 10).

Vitamin C content: For vitamin C content, the results showed that it was quite invariable with storage time. The vitamin C content of flesh tissue from T_1 to T_5 with values ranged from 14.14 to 14.22 mg/100 mL of juice and 14.12-14.18 mg/100 mL of juice by 4 and 8 DAS, respectively (Table 11).

DISCUSSION

The results of dipping santol fruit in two organic acids at two concentrations (5% citric acid, 5% ascorbic

acid, 10% citric acid and 10% ascorbic acid) compared with 0% (Control) showed that organic acid treatment did not affect santol fruit weight. Fresh fruit weight still continued to decline with storage time and the differences in weight between the five treatments were not statistically significant. The reason for this may be derived from the fact that the santol fruits were kept at ambient temperature.

With the results on fruit colour, the results revealed that changes in the skin colour of santol fruit proceeded quickly. It was found that the lightness L* of 5% citric acid-treated fruit (T_2) was significantly higher compared to all other treatments by 4 and 10 DAS. Thus, T_2 gave the best skin brightness and good visual appearance. There was little or no effect on a* and b* values of santol fruit during storage. These implied that the effectiveness of organic acids for controlling browning in whole santol fruit could not withstand longer periods.

For PPO activity of santol flesh, the results showed that santol fruit treated with 5% citric acid had much lower PPO activity than those being treated by other substances. Thus, this treatment may be the appropriate treatment to be applied. The reason for this may be derived from the fact that citric acid has been reported extensively for its inhibitory activity on PPO by reducing the pH, so the activity of PPO may decrease slightly as a result of pH drop (Kavrayan and Aydemir, 2001; Aydemir, 2004). However, both organic acid applications in this experiment did not markedly inhibit PPO activity. It is possible that the effect of organic acids on enzymatic browning of santol fruit was partial. These substances may have lost their antibrowning properties during storage (Srilaong and Tatsumi, 2003; Degl'Innocenti *et al*., 2005). In addition, the preliminary results from Benjwan *et al*. (2008) indicated that activity of PPO could be found in different organs of santol fruit (peel, flesh and seed). This activity showed that santol fruit is very sensitive to enzymatic browning due to lengthen storage time. However, the mechanism of these organic acids to inhibit PPO activity is not clearly understood.

For the result on phenolic contents, it was found that the concentrations of phenolic compounds tended to increase markedly by the end of storage. Zawistowsky *et al*. (1991) stated that degree of browning depended on phenolic content and PPO activity. It was found, with santol fruits of this current study, that the increase of phenolic content during the storage time might result from the association between phenolic compounds and the sensitivity to enzymatic browning in santol fruit. This phenomenon indicated the phenolic compounds have shown complicated reactions in enzymatic browning. Certain phenolic acids inhibit the PPO activity by binding in the active site of the enzyme, whereas others can promote the browning reaction as reported by Janovitz-Klapp *et al*. (1990). For the quinone content of flesh of santol fruits, the results showed that quinone content was highly decreased with an increase in number of days after storage. The results suggested that quinone compounds were oxidized to form brown pigment as storage time increased (De Castro *et al*., 2008). The results showed that quinone content in santol fruit treated with 5% citric acid was much lower than those being treated with other substances. However, there was no clear association between phenolic content, quinone content and PPO activity in santol fruit.

The results on Total Soluble Solids (TSS) in flesh tissue of santol fruits showed that most of TSS contents were quite invariable during storage. The results indicated that the conversion of carbohydrates to sugar was the same when the santol fruits reached ripening stage after storage. Mwithiga *et al*. (2007) reported that the sugar content of fruit will initially increase with ripeness and then remain fairly constant as the fruit approaches senescence.

The result on acidity levels (pH) was highly decreased with an increase in storage age. The reverse was found with Titratable Acidity (TA) values. The advances in the ripening stage could possibly be the reason for this phenomenon. The results also indicated the citric acid was shown to be an anti-browning agent by decreasing the acidity level which affected lowering the enzymatic browning (Ibrahim *et al*., 2004). In addition, Zhang *et al*. (2005) also reported that organic acid may be lost in fruit during storage.

For vitamin C content, the results showed that most of vitamin C content in santol fruit was quite constant during storage. The reason for this may be derived from the fact that vitamin C content in some fruits from mature stage were relatively unchanged (Watada *et al*., 1976). Further, specific studies are needed to understand the mechanism of citric acid involvement with the enzymatic browning behavior of santol fruit.

The results of this study suggest that treatment with citric acid is a promising method for controlling santol browning during postharvest storage. Compared with the literature on PPO enzymes, there are very few publications relating to the role of organic acids to protect the enzymatic browning in santol fruit. Son *et al*. (2001) examined the antibrowning activities of ascorbic acid on the apple slices after treatment in a 1% dipping solution. Their observation on treated apple slices revealed that solutions of ascorbic acid appeared to be less effectively inhibit browning. Due to after the reducing power of ascorbic acid was depleted, within for a moment, the

brown colour developed rapidly thereafter. This result was similar to the previous studies of some researchers reported that ascorbic acid also has a reductant feature (Yamaguchi *et al*., 2003). While, Xiaolin and Tian (2006) cited that application of oxalic acid (2 and 4 mM) can effectively control the pericarp browning of litchi fruit cv. Huaizhi during postharvest storage at room temperature. The results showed that the pericarp browning indices of the fruit, treated with both oxalic acid concentrations, were significantly lower than that of the control, due to increase of membrane integrity, inhibition of pigment degradation, decline of oxidation and sustain the relatively low of some enzyme activity in the fruit during storage. Concellón *et al*. (2004) observed the enzymatic activity in Williams banana was characterized by a decrease of activity below pH. Moreover, Ibrahim *et al*. (2004) was also reported similar results that citric acid may have a dual inhibitory effect on PPO: it lowers the pH and chelates the copper at the active site of the enzyme (Martinez and Whitaker, 1995). Joas *et al*. (2005) cited that organic acids may also prove to be an interesting alternative with respect to colour stabilization on litchi fruit. This optimal performance may be explained by the formation of an acid favours a stable acidification of the epicarp during soaking and minimizes the fruit's buffering capacity. In addition, Zhang *et al*. (2001) presented that organic acid may strengthen the membrane stability. So the effect of organic acid on enzymatic browning of santol during storage was partly due to causing a relative decrease of PPO activity in the fruit.

Opposite results were found by Altunkaya and Gökmen (2008) studied the effects of four organic acids, namely ascorbic acid (10-20 μM), cysteine (5-10 μM), oxalic acid (0.1-0.14 mM) and citric acid (0.1-0.14 mM) on crude lettuce PPO activity. They concluded that the most effective inhibitor of lettuce PPO is l-cysteine. Because of nearly all ascorbic acid is converted to dehydroascorbic acid, the amount of *o*-quinones formed by action of PPO increases. The quinones then polymerise and or combine together with amino compounds to form high molecular weight brown pigments (Duangmal and Owusu Apenten, 1999). In their study, the decrease in antioxidant capacity was observed under different conditions. Furthermore, Limbo and Piergiovanni (2006) cited that among the anti-browning agents to inhibit enzymatic browning of peeled and sliced potatoes, ascorbic acid inhibits enzymatic browning very effectively, primarily because of its ability to reduce quinones to phenolic compounds before they undergo further reaction to form pigments.

In conclusion, it was found that the most appropriate controlling enzymatic browning for santol fruits was soaking the fruits in 5% citric acid for 30 min. At this treatment, brightness of fruit skin (L* value) was much greater than the other treatments. The lowest PPO activity or acidity level (pH) was also attained with the dipping by 5% citric acid. There was no significant difference in the internal qualities of santol fruit in terms of TSS, TA and vitamin C content during storage.

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