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Changes in Ascorbic Acid, Total Polyphenol, Phenolic Acids and Antioxidant Activity in Juice Extracted from Coated Kiew Wan Tangerine During Storage at 4, 12 and 20°C

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ABSTRACT

The aim of this research was to investigate changes in ascorbic acid, total polyphenol, phenolic acids and antioxidant activity in juice extracted from Kiew Wan tangerines (*Citrus reticulate* Blanco cv. Kiew Wan) coated with shellac. The coated fruits were stored for five weeks at 4, 12 and 20°C and samples were taken weekly for analysis. Ascorbic acid decreased during storage at all tested temperatures. The major phenolic acids found were ferulic acid followed by sinapic, caffeic and *p*-coumaric acid. The 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2 azinobis (3-ethylbenzothiazoline-6-sulfonate (ABTS) antioxidant activities increased during storage and were correlated with total polyphenol and phenolic acid contents. Sinapic, caffeic and *p*-coumaric acid levels were significantly correlated with the DPPH while ferulic acid was correlated with the ABTS. All phenolic acids increased during the early stage of storage at 4 and 12°C but decreased at the end. Weight loss of coated fruit during storage was in the range of 4–17%.

Key words: tangerine, weight loss, ascorbic acid, total polyphenols, phenolic acids, antioxidant activity

INTRODUCTION

Tangerine (*Citrus reticulate* Blanco), locally called Som Kiew Wan, is the major citrus fruit grown in Thailand. It is mostly consumed fresh but is also used for juice. Citrus fruits are sources of important nutrients for human health, including ascorbic acid, dietary fiber, phenolic compounds and phenolic acids (Rapisarda *et al.,* 1999; Tripoli *et al.,* 2007; Wang *et al.,* 2007). Many studies have reported that ascorbic acid and phenolic compounds in citrus fruit play important roles in antioxidant activity (Franke *et al.,* 2004; Gorinstein *et al.,* 2004; Abeysinghe *et al.,* 2007). Ascorbic acid is a water-soluble antioxidant, while some other antioxidants may be hydrophobic, including limonoids and flavonoids. Phenolic acids are aromatic secondary plant metabolites, which are classified into hydroxycinnamic and hydroxybenzoic structures. Hydroxycinnamic acids in berries (Kahkonen *et al.,* 2001) and oranges (Rapisarda *et al.,* 2008a) showed a high correlation with antioxidant activity. Caffeic, *p*-coumaric, ferulic and sinapic acid are hydroxycinnamic acids found in nearly all food plants and exist in fruits in both free and bound forms. Only small fractions exist as free acid. The major fractions are bound to structural components such as cellulose, lignin and protein. Ferulic acid is the major phenolic acid in citrus fruits

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(Rapisarda *et al.,* 1998). Zhao and Moghadasian (2008) reported that the intake of ferulic acid might offer beneficial effects against cancer, cardiovascular disease, diabetes and Alzheimer's disease.

Application of a coating to fruits, including apples and oranges has long been practiced to extend shelf life and improve glossiness. Shellac-based coatings are commonly used commercially due to their highly glossy properties. Presently in Thailand, coating of oranges has become common practice in many packing houses, but coated fruits can result in quality deterioration if storage conditions are unsuitable. Coating tends to restrict the exchange of O_2 and CO_2 between the fruit and the surrounding atmospheres. If the O_2 in fruit is too low and CO_2 is high then anaerobic respiration can occur which leads to off-flavor development (Baldwin *et al.,* 1995; Hagenmaier, 2002; Hagenmaier and Shaw, 2002). It is common to store coated fruits at low temperature to extend their shelf life (Beaudry *et al.,* 1992; Rapisardan *et al.,* 2001). The consequence of coating and storage temperature may lead to other chemical changes in fruits (Xu *et al.,* 2008). Rapisarda *et al*. (2008a) reported that bioactive compounds, total polyphenol, vitamin C and hydroxycinnamic acids in blood oranges increased during storage at low temperatures. Tangerine oranges can be stored best between 4 and 6°C (Ladaniya, 2008) and shipping temperature recommendations for citrus fruit range from 10 to 15.5°C (Wardowski, 1981).

There has been no report published on coating of Thai tangerines with respect to changes in bioactive compounds and antioxidant activity. The aim of this study was therefore to determine the effect of temperature and storage time on the changes in ascorbic acid, phenolic acids, antioxidant activity and weight loss of coated Thai tangerines in order to provide suitable postharvest practices.

MATERIALS AND METHODS

Raw materials

Kiew wan tangerines were purchased from an orchard in Kamphaeng Phet province, Thailand. They were harvested at the fully matured stage and then coated with a commercial coating (shellac-based) using a coating machine at the orchard. The ratio of coating material to fruit was 1:1 (liters per tonne). Fruit size was 5.6-6.0 cm in diameter. The coated tangerines were divided into three groups then packed in cardboard boxes and stored at 4±1, 12±1 or 20±1°C for five weeks. Five fruits were randomly chosen, peeled and the juice was squeezed out using a fruit juice separator. The juice was used for chemical analyses and antioxidant activity determination. It was kept in amber glass bottles and stored at -18°C for further analysis.

Standard phenolic acids, caffeic acid, coumaric acid, sinapic acid, ferulic acid and gallic acid, were purchased from Sigma-Aldrich, USA. ABTS (2,2 azinobis (3-ethylbenzothiazoline-6-sulfonate)) was also purchased from Sigma-Aldrich, USA. DPPH (2,2-diphenyl-1-picrylhydrazyl), Folin-Cocialteu reagent, ascorbic acid and 2, 6 dichloroindophenol were purchased from Merck, Germany.

Determination of ascorbic acid

Ascorbic acid was determined by the 2, 6 dichloroindophenol titrimetric method (AOAC, 2000)

Determination of total polyphenol

The total polyphenol content was measured following a modified method of Gorinstein *et al*. (2001). The juice samples were centrifuged at 10,000 rpm for 15 min. A volume of 0.2 mL juice samples and 1 mL of diluted 5-fold Folin-Ciocalteu reagent were transferred into a test tube, then 0.8 mL of 7.5% Na_2CO_3 solution was added and mixed. The solution was made up

to a final volume of 10 mL with distilled water and left to stand for 30 min at room temperature. The absorbance was read at 765 nm by a UV-VIS spectrophotometer (UV 1601, Shimadzu Japan), distilled water was used as a blank and gallic acid as the standard. Results were calculated as mg of gallic acid per 100 mL of juice.

Determination of antioxidant activity

The antioxidant activity of the juice samples was determined by the DPPH radical scavenging activity method and the ABTS radical scavenging activity assay. The DPPH scavenging activity followed a modified method of Brand-Williams *et al*. (1995). The juice sample (0.1 mL) was added to 3.9 mL solution of 80 μM DPPH in 80% ethanol, the mixture was vigorously shaken and allowed to stand at room temperature in the dark for 30 min. The absorbance was read at 517 nm and the antioxidant activity was calculated as a percentage of DPPH discoloration using Equation 1:

Antioxidant activity % = 100 × (1-absobance of sample/absorbance of control) (1)

Measurement of the ABTS radical scavenging activity followed a modified method of Landult *et al*. (2001). The juice sample (0.1 mL) was added to a mixture of 2.5 mM ABTS in phosphate buffer saline (PBS) (0.4 mL), metmyoglobin solution (0.72 mL), 10 mM of PBS (3.10 mL) and 10 mM of H_2O_2 (0.48 mL), then the volume was adjusted to 4.32 mL by PBS. The sample was substituted for phosphate buffer as a control. The absorbance was read at 734 nm after 10 min of reaction in a dark place at room temperature and the antioxidant activity calculated using Equation 1.

HPLC analysis of phenolic acids

Extraction of phenolic acids

Ten milliliters of clear orange juice was added to 10 mL of 2 N NaOH and stored in the dark for 4 h. The juice was then acidified with concentrated phosphoric acid to pH 4.5 (Rouseff *et al*., 1992) and 20 mL of ethyl acetate was added to the mixture. Extraction was carried out twice; the extract was evaporated at 40°C under vacuum until dry and then dissolved in 1 mL of methanol. The methanol extract was used for HPLC analysis.

HPLC determination

The clear methanol extract was passed through a 0.22 μm membrane filter prior to injection. A constaMetric 3000 pump (LDC/Milton Roy) was connected to an HPLC column (Hypersil ODS C-18, 5 μm 250×4.6 mm ID, Thermo Hypersil UK.) with a C-18 guard column. The phenolic acids were detected at 300 nm with a Spectra Monitor 3000 Detector (LDC/Milton Roy). Twenty microlitres of sample was introduced to the system by Rheodyne injector (20 μl Fixed loop). The isocratic elution was performed at 1 mL/min with a mobile phase consisting of 80% H_2O (with 2% acetic acid) and 20% acetronitrile. Chromatograms were recorded and processed by a Chromatopac C-R6A (Shimadzu Japan). Quantification of phenolic acids was conducted by the external standard method.

Determination of weight loss

Five fruits were taken from each treatment and weighed using a top load balance and weight loss was calculated as a percentage on weekly basis.

Statistical analysis

Data were analyzed by ANOVA using a split plot in a completely randomized design, with temperature as the main plot and storage time as a sub-plot. Means were compared by Duncan's multiple range test, with values considered significantly different at $p \leq 0.05$.

RESULTS AND DISCUSSION

During five weeks of storage, significant

changes were found in phenolic acids, ascorbic acid, total polyphenol content and antioxidant activity at every temperature. The tested temperatures showed significant effects on ascorbic acid content but were not statistically significant with phenolic acids. Only ferulic acid levels decreased slightly at 20°C but this was not statistically significant. An increase in antioxidant activity at 20°C was observed.

Ascorbic acid

Tangerine is rich in ascorbic acid, which makes it a popular fruit eaten to promote good health. The changes in ascorbic acid contribute to the fruit's quality. Ascorbic acid content decreased during storage for five weeks at 4, 12 and 20°C, as had previously been reported by Burdurlu *et al*. (2006). Prolonged storage affected the ascorbic acid content regardless of temperature (Figure 1a). The initial content in the juice was 20.83 mg/100mL, which gradually decreased to 14.38, 14.77 and 15.14 mg/100mL at 4, 12 and 20°C, respectively, after five weeks storage. However, desiccation at 12 and 20°C may have contributed to the decrease in the level of ascorbic acid. Decreases in the level of ascorbic acid in fruit may be associated with enzymes, such as cytochrome oxidase, ascorbic acid oxidase and polyphenol oxidase (Nagy, 1980). Oxidation of ascorbic acid may lead to the formation of dehydroascorbic acid, which cannot be detected by the method used. The results indicated that the storage period had a more significant effect on the content of ascorbic acid than the temperatures did. It has previously been reported that a loss of ascorbic acid in citrus fruit is not caused by storage temperature (Rapisarda *et al*., 2001) and that there was no loss of ascorbic acid during storage of oranges at low temperature (Schirra and Chessa, 1988). In addition, the current research results showed that ascorbic acid loss in Thai tangerines was not affected by the coating material, compared to the uncoated, which were similar to results reported by Baldwin *et al*. (1995).

Total polyphenol

The total polyphenol content before storage was 26.30 mg gallic acid/100mL, which increased to about 28.40, 29.99 and 28.65 mg gallic acid/100mL after five weeks storage at 4, 12 and 20°C (Figure 1b). The polyphenol content in fruit is associated with antioxidant capacity and is normally measured by Folin-Ciocalteu reagent, which can react with all types of antioxidants through electron transfer-based antioxidants including non-phenolic compounds such as vitamin C, dehydroascorbic acid, amino acid and

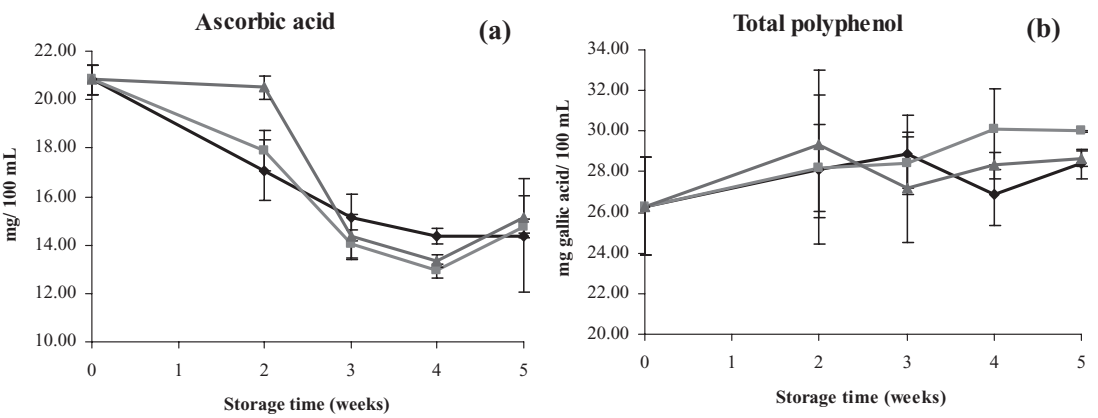

Figure 1 Change in (a) ascorbic acid; and (b) total polyphenol of coated Thai tangerines during storage at 4, 12 and 20°C for five weeks (—◆— 4°C, —■— 12°C, —▲— 20°C).

carotenoid. During post harvesting, many physiological changes can take place in fruit that lead to senescence and decomposition of the cell structure, thus the liberation of free phenolic acids and free amino acids can occur, which contributes to an increase in the total polyphenols. The current results corresponded with the experiments of Moussaid (2000), who reported that the total polyphenol content in coated mature oranges increased during nine weeks storage at 20°C.

Phenolic acids

The major phenolic acid found in juice of coated tangerine was identified as ferulic acid followed by sinapic, caffeic and *p*-coumaric acid. The hydroxycinnamic acids identified were similar to those found in blood orange (Rapisarda *et al*., 1998). The initial content of caffeic, *p*-coumaric, sinapic and ferulic acid was 2.54, 1.86, 4.79 and 37.49 mg/l, respectively. The phenolic acids increased during storage regardless of temperature, but the increase was higher during the early stage of storage at 4 and 12°C and declined slightly at the end. With other fruits, such as tomato, the soluble phenolic acid content increased slightly during 10 days storage at 7, 15 and 25°C (Toor and Savage, 2006). In the Tacle and Clara mandarin orange hybrids, the total hydroxycinnamic acid content increased during early storage at 6°C when stored for 104 days (Rapisarda *et al.,* 2008b). Caffeic acid increased in the first two weeks and declined slightly until the end of storage at all tested temperatures (Figure 2a). However, the content was significantly higher than initially. The *p*-coumaric acid content steadily increased during five weeks storage at 20°C, but at 4 and 12°C it declined slightly after four weeks (Figure 2b). The level of sinapic acid increased during storage at all temperatures, particularly 20° C, where the content was higher at the end of storage (Figure 2c). Ferulic acid was the most abundant phenolic acid found in the juice. It increased at all temperatures and declined at the end of storage, particularly at 20°C (Figure 2d).

It can be concluded that the phenolic acid contents in coated tangerines increased during storage at 4, 12 and 20°C over four weeks, however when the storage was prolonged to five weeks, an increase in caffeic, *p*-coumaric and sinapic acid at 20°C was noted, but the ferulic acid level decreased. The increase in phenolic acids was higher at 4°C and 12°C than at 20°C. Generally, phenolic acids were reported to be synthesized in plants as a defensive response mechanism to stress (Naczk and Shahidi, 2006). At low temperature stress levels, the L-phenyalanine is converted to *tran*-cinnamic acid catalyzed by phenyalanine ammonia-lyase (PAL) and then hydroxylation and/or methylation of *p*-coumarate yields caffeic, ferulic and sinapic acids. Rapisarda *et al*. (2001) reported that *p*-coumaric, ferulic, caffeic and sinapic acid levels increased during storage of Tarocco orange for 85 days at 8°C. There are some reports of total hydroxycinnamic acid levels decreasing due to senescence during storage (Rapisarda *et. al*., 1998 and 2008a). The detrimental off-flavor in blood orange during storage was reported to be caused by *p*-vinylguaiacol and *p*-vinylphenol, which are derived from ferulic and *p*-coumaric acid (Rapisarda *et al*., 2001). In the current finding, the decrease in ferulic acid during storage at 20°C was obvious, but with *p*-coumaric acid, it occurred only at 4 and 12°C. This may suggest the optimum storage period for coated tangerines. In addition, at 20°C, the desiccation and senescence of tangerine may have affected the analysis when storage was prolonged.

Antioxidant activity

The antioxidant activity measured by DPPH and ABTS increased significantly during storage regardless of temperature (Figure 3). The DPPH and ABTS assays showed a difference in their correlations with various antioxidants. The DPPH antioxidant activity correlated significantly

with caffeic acid, *p*-coumaric acid and sinapic acid, but not with ferulic acid (Table 1). The ABTS assay correlated with ferulic acid at a significant level, but failed to be correlated with other phenolic acids. This might have been due to the different properties of certain phenolic acids, with ferulic acid being more hydrophilic (Chen and Ho, 1997, Kim *et al.*, 2001). The number of hydroxyl groups in each hydroxycinnamic acid can affect the scavenging DPPH radical (Kim *et al.*, 2001). Total polyphenol was more correlated with the ABTS assay than the DPPH assay, which indicated

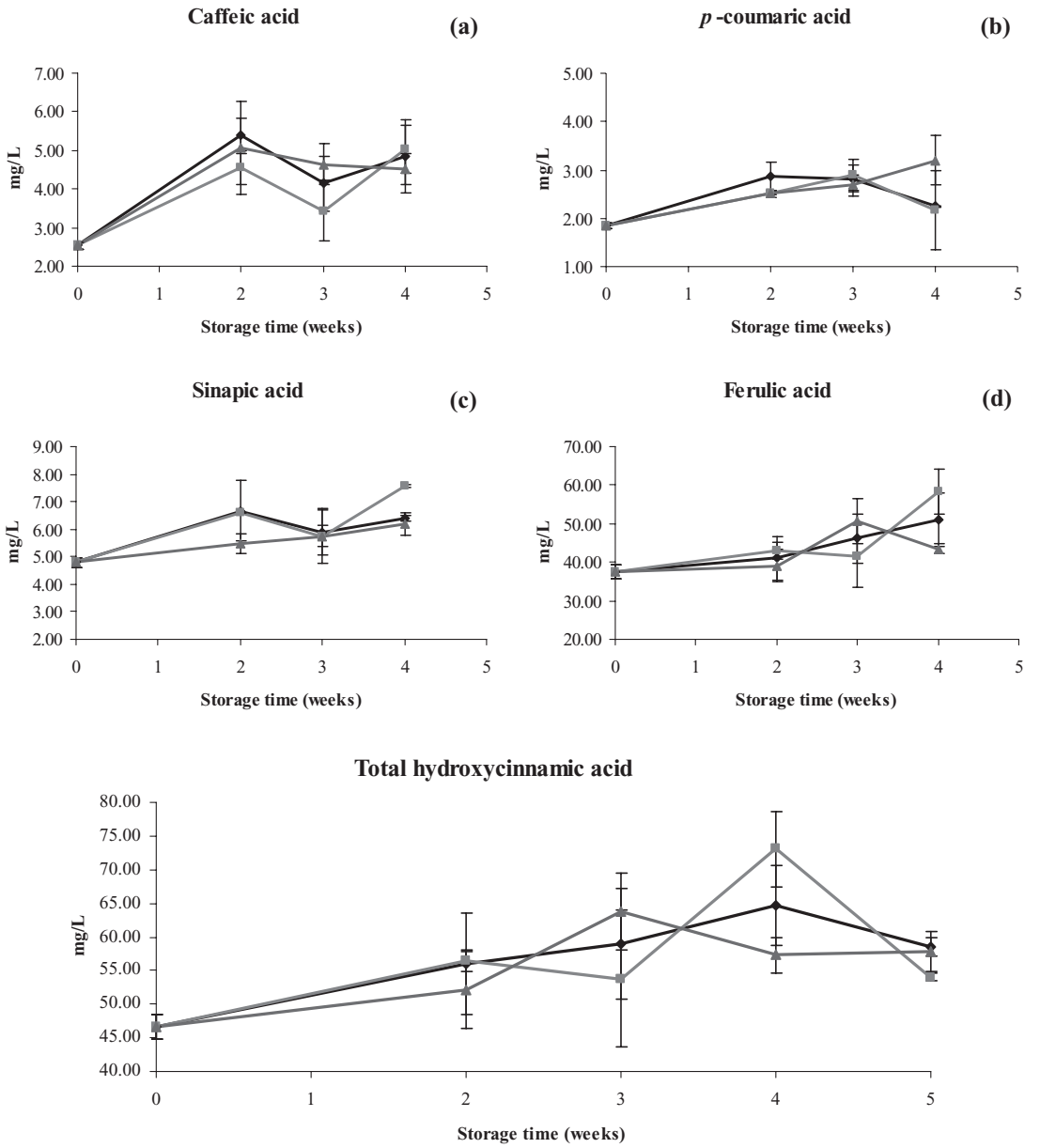

Figure 2 Changes in (a) cafeic, (b) *p*-coumaric, (c) sinapic, (d) ferulic acid and (e) total hydroxycinnamic acid of coated Thai tangerines during storage at 4, 12 and 20° for five weeks (—◆— 4°C, —■— 12°C, —▲— 20°C).

antioxidants in tangerine were both hydrophilic and lipophilic. It may be concluded that the antioxidant activity in tangerine was associated with more than a single compound. The negative correlation of antioxidant activity with ascorbic acid confirmed that phenolic acids contributed to antioxidant activity in coated tangerines. However, the synergistic effect of ascorbic acid and phenolic compounds has been reported by Kahkonen *et al*. (2001). In addition, the reduced form of ascorbic acid may play the role of an antioxidant in addition to the phenolic acids.

Weight loss

Coating of tangerines is common practice in Thai markets to maintain freshness. At high temperature, weight loss increased significantly (Figure 4) and after five weeks at 4, 12 and 20°C, the losses were 3.69±0.24, 13.17±0.82 and 17.74±0.39%, respectively. Weight loss of coated tangerines at all storage temperatures in comparison to uncoated fruits was reduced by 36.82, 46.31 and 41.22%, respectively. Many authors have reported that coating and reduced storage temperatures can reduce weight loss (Chaim and Soffer, 1996, Chien *et al*., 2007). It was reported that weight loss of fruits higher than 5-10% resulted in a quality loss that made them unsaleable (Davies and Albrigo, 1998).

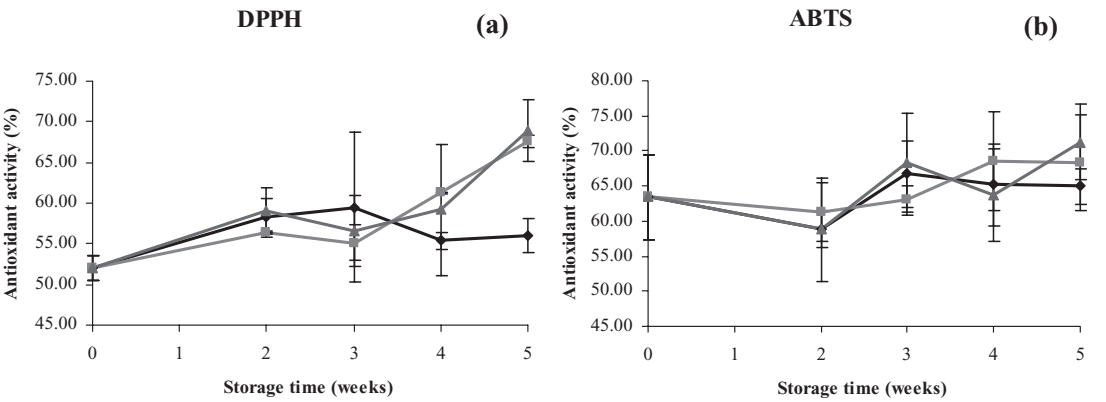

Figure 3 Change in antioxidant activity of coated Thai tangerines during storage at 4, 12 and 20°C for five weeks (—◆— 4°C, —■— 12°C, —▲— 20°C).

Table 1 Correlation coefficients between radical scavenging activity and antioxidant components in coated Thai tangerine during storage at 4, 12 and 20°C for five weeks.

Antioxidant component	Correlation coefficients	
	DPPH	ABTS
Ascorbic acid	-0.361	-0.286
Total polyphenol	0.327*	0.641**
Caffeic acid	0.491**	0.184
p-coumaric acid	0.558**	0.135
Sinapic acid	0.625**	0.296
Ferulic acid	0.288	0.459*
Total hydroxycinnamic acids	0.457*	0.437*

Significant at **. $p \leq 0.01$ and *. $p \leq 0.05$

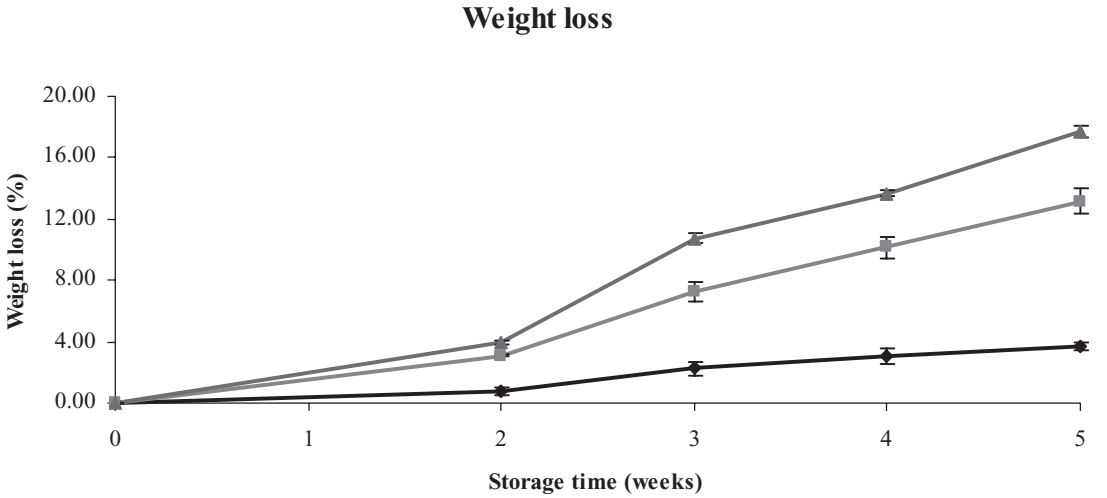

Figure 4 Change in weight loss of coated Thai tangerines during storage at 4, 12 and 20°C for five weeks (—◆— 4°C, —■— 12°C, —▲— 20°C).

CONCLUSION

The results of this study indicated that changes in the levels of ascorbic acid, total polyphenol, phenolic acids and antioxidant activity in coated tangerines were affected by storage period, regardless of temperatures. The ascorbic acid content decreased during the storage period, irrespective of temperature. The phenolic acids found were caffeic, *p*-coumaric, sinapic and ferulic acid and the level of each one increased during the early stage of storage and declined slightly at the end. The DPPH correlated well with caffeic, *p*-coumaric and sinapic acid, but not with ferulic acid. The ABTS assay correlated well only with ferulic acid. The total polyphenol level increased during storage and correlated with ABTS and DPPH antioxidant activity. Storage of coated tangerine at 4, 12 and 20°C did not affect the antioxidant components. The weight loss at 4, 12 and 20°C was between 4-17% for five weeks storage.

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