Changes of phenolic acids, total soluble solid, acidity of coated Tangerine oranges at low temperature storage

(코팅된 감귤의 저온저장 중 품질변화)

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Phenolic compounds are prominent in fruits playing a great role in determining flavour, and color. In citrus fruits the glycosides of trans hydroxycinnamic acid are found in a greater amount than other phenolic acids (Simon et al.,1992). Bound and free caffeic, *p*-coumaric, ferulic and sinapic acid were determined in Shanti orange (Peleg et al.,1991). The amount of phenolic acids in fruit very widely from variety to variety and season to season. Raspirada et al. (1998) found the amount of hydroxycinnamic acids in blood and blond orange were significantly different and ferulic acid was the most common acid found in both varieties. Now a day the health beneficial of phenolic acids as antioxidant and radical scavengers has been reported (Chen and Ho., 1997)

It has been increasingly used of coating for tangerine oranges in Thailand. The main purpose of such practice is toprovide glossiness to the fruits or prevent shrinkage. Coating of oranges has been practiced since ancient time by dipping fruits into molten wax. Coating restricts the exchange of CO2 and O2 of the fruits. Restriction of gases (O2) can affect the physiology of fruit. At low oxygen level the respiration will slow down, and anaerobic respiration can occur. Ben Yahoshua et al. (1985) found that stomata of citrus fruit were closed due to coating. The advantage of coating was pointed out to be associated with prevention of weight loss, alleviating chilling injury and prolong shelf life. However the effectiveness of coating are mainly due to formulation (coating material) and application methods. Selection of suitable material is therefore necessary. Off-flavour can be easily detected in coated fruit due to the formation of fermentation products. Type and thickness of coating material thus play the great role for fruit flavour and appearance.

It is the aim of our work to investigate the change of four prominent phenolic acids; caffeic, *p*-coumaric, sinapic and ferulic acid, in coated tangerine oranges stored at different temperatures.

Materials : Tangerine oranges were harvested and coated with Zivdar wax (Trade name) at the pack house where is about300 km from Bangkok. The fruits were transported by air conditioned van to our laboratory at KMITL Bangkok. The fruits were divided into 4 groups and stored at 4, 12, 20C in cardboard boxes.

Methods : Five fruits were peeled and juices separated by a fruit juice separator. Juices were bottled in amber glass bottles and stored at -18C for further analysis.

Extraction of phenolic acids : Samples were centrifuged at 10000 rpm for 20 min The upper liquid was used for extraction. Ten milliliters of clear juice was added to 10 ml of 2 N NaOH and stored in a dark place for 4 hours. The juice was then acidified with concentrated phosphoric acid to pH 4.5 (Rouseff et al., 1992). Twenty milliliters of ethyl acetate was added to extract phenolic acid. Extraction was done twice. Solution was evaporated at 40C under vacuum to dryness and 1 ml of methanol was added.

HPLC determination : The clear extract was passed through 0.22 m membrane filter prior to injection to HPLC. A constaMetric 3000 pump (LDC/Milton Roy) was connected to a column ODS-18 Lithosphere 5 m (250 mm, 4.6 mm ID) with C-18 guard column. Phenolic acids were detected at 300 nm with Spectra Monitor 3000 Detector (LDC/Milton Roy). Twenty microlitters of sample were introduced to the system by a Rheodyne injector (20 l Fixed loop). Isocratic elution was performed at 1 ml/min with a mobile phase consisted of 80% H2O (+ 2% acetic acid) and 20% Acetronitrile. The chromatograms were recorded and processed by Chromatopac C-R6A (shimadzu).

Standards : The standards used were analytical grade purchased from Sigma.

Total soluble solid : By hand refractometer (ATAGO N1 Japan).

Titratable acidity : By titration method.

Results and discussion :

By using chromatographic procedure, HPLC, we can analyze hydroxycinnamic acids in coated tangerine oranges. Phenolic acids in orange juice are present in both free and bound forms. Free caffeic and *p*-coumaric acid were not detected in our samples. This might due to too small amount of those acid presented in the fruit. Sinapic acid and ferulic acid were detected in small amount as free form, see Fig 1. By enriching the orange juice with standard caffeic and *p*-coumaric acid, peaks of these acids were detected as shown in Fig 2 and Fig 3. The caffeic acid appeared at 5.99 min and *p*-coumaric acid at 9.41, sinapic acid at 9.99 min and ferulic acid at 10.54 min. The discrepancy of the retention time was affected by the concentration of acids. The larger amount of ferulic acid made it difficult to detect sinapic acid (Rouseff et al.,1992)

Improvement of the resolution of sinapic acid ferulic acid can be achieved by adjusting their concentration to be not so much different. Saponification of acids with NaOH and acidified with phosphoric acid could liberate the acids from the bound form. Four hydroxycinnamic acids were found more in bound forms. Bound phenolic acids mostly exist as glycosides.

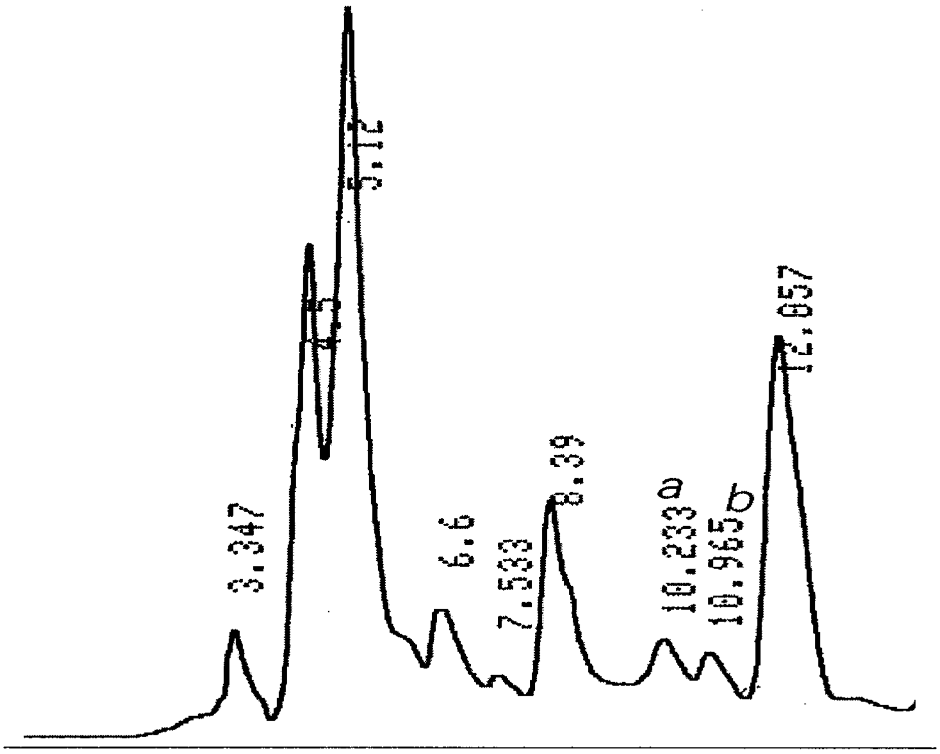

Fig. 1. Chromatograms of free phenolic acid, (a) sinapic acid, (b) ferrulic acid in coated tangerine orange

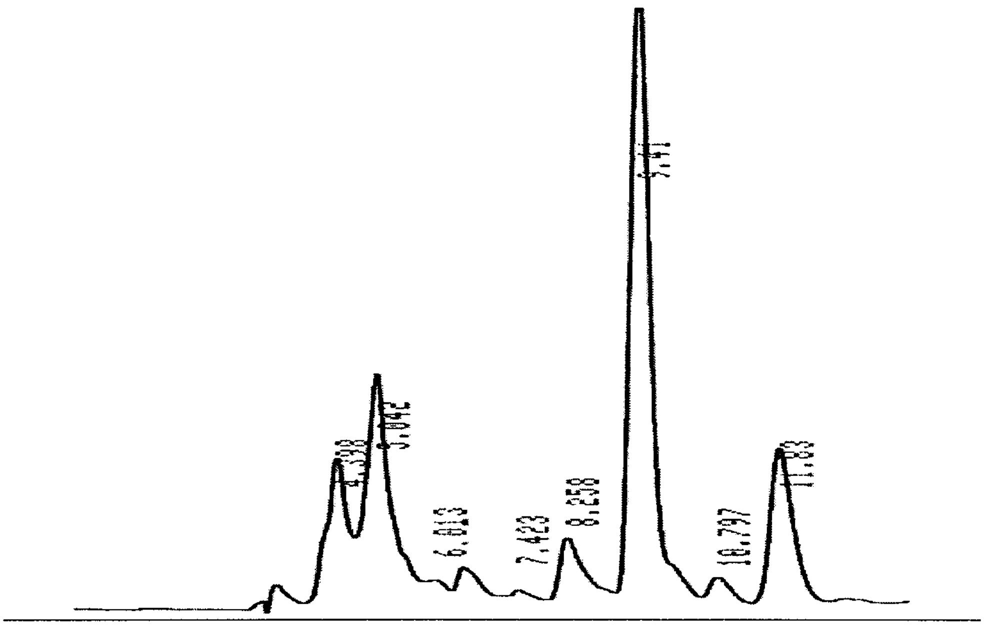

Fig. 2. Chromatograms of free phenolic acid in coated tangerine orange enriched with caffeic acid

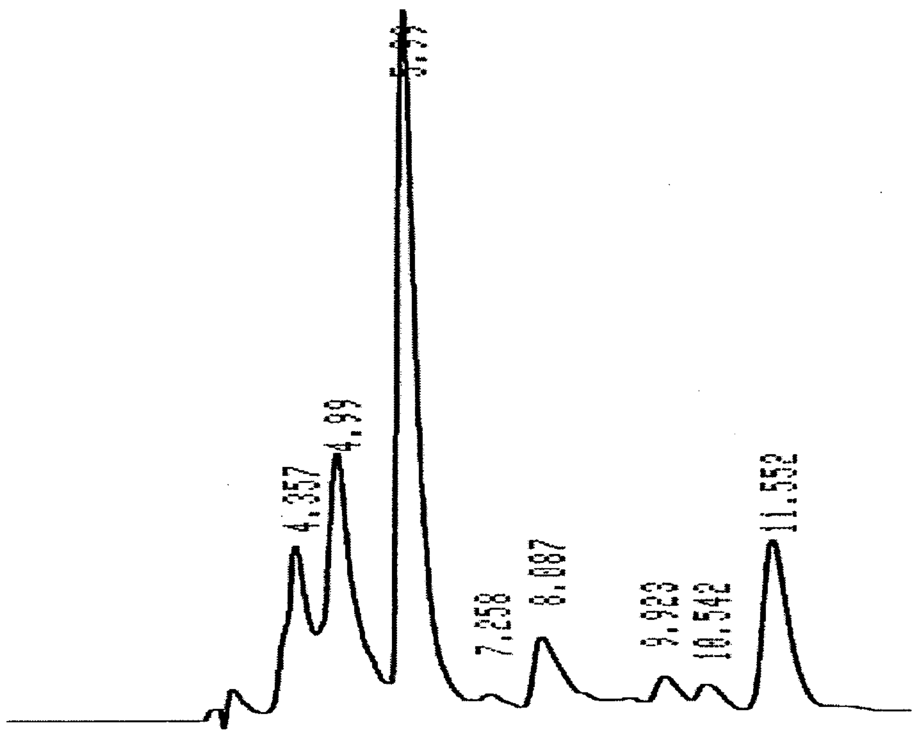

Fig. 3. Chromatograms of free phenolic acid in coated tangerine orange enriched with *p*-coumaric acid

The hydroxycinnamic acids content of coated oranges are present in Table I. The amount of each acids was from bound acids. The amount of phenolic acids found in the increasing order from *p*-coumaric, caffeic acid, sinapic acid and ferulic acid. By comparison to the uncoated oranges, the phenolic acids content were higher in the coated ones. This could be explained by the restriction of gas exchange in the fruit tissue due to the coating thus prevented the oxidation of phenolic acids.

Table I. Caffeic, *p*-coumaric, sinapic and ferulic acid in coated oranges stored at 4, 12, 20C for 5 weeks

Phenolic acids	Orange	Storage temperatures (5 weeks), ppm		
		4℃	12℃	20℃
Caffeic acid	Coated (2.99)*	3.86	5.80	6.21
	Control (3.04)*	3.02	2.56	3.92
Coumaric acid	Coated (2.21)*	2.18	2.64	3.28
	Control (2.28)*	1.86	1.98	3.75
Sinapic acid	Coated (11.28)*	6.58	7.49	10.44
	Control (10.12)*	8.17	6.58	17.04**
Ferulic acid	Coated (68.83)*	63.27	71.52	87.37
	Control (66.74)*	62.35	56.06	79.21

* Unstored fruit, **not well resolved

From our results it was found that at 4C the amount of hydroxycinnamic acid was lower than those found at higher temperatures. Its amount was close to the unstored oranges. This might indicated that the metabolism of phenolic acids at low temperature was suppressed.

Acidity of coated oranges was slightly higher than that of the control. Coating could slow down the metabolism of acid in oranges. At higher storage temperatures (12, 20C) the acidity was retained better than that at a lower one (4C). Total soluble solid did not change during storage which was true for non climacteric fruits. Results shown in Table II. The off flavour of coated oranges were not obviously detected at low temperature but at higher temperature (28C) off flavour was detected after 3 weeks.

Table II. Acidity and total soluble solid of coated tangerine oranges stored at 4, 12, 20C for 5 weeks

	Orange	Storage temperature			
		4℃	12℃	20℃	Remark
Titratable Acidity (%)	coated (0.52)* Control (0.42)*	0.39 0.38	0.44 0.36	0.53 0.41	At 4℃ orange peel's colour remained green
Total soluble solid (Brix)	coated (10.28)* Control (10.40)*	10.00 10.20	10.20 10.00	10.64 11.00	

* Unstored fruit

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