

## Polymethoxylated Flavones, Flavanone Glycosides, Carotenoids, and Antioxidants in Different Cultivation Types of Tangerines (*Citrus reticulata* Blanco cv. Sainampung) from Northern Thailand

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Polymethoxylated flavones (PMFs) and flavanone glycosides (FGs) were analyzed in hand-pressed juice and the peeled fruit of 'Sainampung' tangerines (*Citrus reticulata* Blanco cv. Sainampung) grown in northern Thailand. The tangerines were collected from a citrus cluster of small orchard farmers and were cultivated as either agrochemical-based (AB), agrochemical-safe (AS), or organic grown fruits. Juice samples were also measured on contents of carotenoids, ascorbic acid, and tocopherols. The peel-deriving PMFs tangeretin, nobiletin, and sinensetin were found with high concentrations in juice as a result of simple squeezing, whereas amounts of those PMFs were negligibly low in peeled tangerine fruit. In contrast, the mean concentrations of the FGs narirutin, hesperidin, and didymin were several fold higher in peeled fruit than in tangerine juice and significantly higher in organic than AS and AB tangerines. Narirutin and hesperidin in juice from organic produces as well as narirutin in juice from AS produces were significantly higher than respective mean concentrations in juice from AB produces.  $\beta$ -Cryptoxanthin was the predominant carotenoid beside zeaxanthin, lutein, lycopene, and  $\beta$ -carotene in tangerine juice. Ascorbic acid concentrations were not predicted by the type of cultivation, whereas  $\alpha$ -tocopherol was significantly higher in juice from organic than AS produces. In summary, hand-pressed juice of *C. reticulata* Blanco cv. Sainampung serves as a rich source of PMFs, FGs, carotenoids, and antioxidants: 4–5 tangerine fruits (~80 g of each fruit) giving one glass of 200 mL hand-pressed juice would provide more than 5 mg of nobiletin and tangeretin and 36 mg of hesperidin, narirutin, and didymin, as well as 30 mg of ascorbic acid, >1 mg of provitamin A active  $\beta$ -cryptoxanthin, and 200  $\mu$ g of  $\alpha$ -tocopherol.

**KEYWORDS:** Tangerine; *Citrus reticulata* Blanco cv. Sainampung; polymethoxylated flavones; flavanone glycosides; carotenoids; antioxidants

### INTRODUCTION

Citrus produces have been known to be good sources of antioxidant species. For many years, the nutritional relevance of these foods resided almost exclusively in the fact that they were acknowledged as a good source of ascorbic acid and carotenoids. Recently, a growing interest in them has arisen regarding their content of flavonoids. Flavonoids are a large group of chemically related polyphenols of plant origin possessing bioactive potential. They have been assigned as antioxidants and compounds having anti-inflammatory, anti-allergic, and anti-carcinogenic, as well as blood-lipid- and cholesterol-lowering activity (1, 2).

Citrus-fruit-derived flavonoids include flavanone glycosides (FGs), flavone glycosides, and polymethoxylated flavones (PMFs). The most prevalent FGs in the fruit tissue and peels of citrus are hesperidin and naringin from oranges and grapefruit, respectively. PMFs, such as tangeretin and nobiletin, exist exclusively in the *Citrus* genus and are common especially in the peels of tangerine sweet orange and bitter orange (3, 4).

Recent studies indicated that citrus flavonoids could reduce the occurrence of cardiovascular disease (CVD) from atherosclerosis through their ability to reduce the hepatic production of cholesterol-containing lipoproteins and, hence, reduce total blood cholesterol (5–7). The citrus flavones tangeretin and nobiletin had been shown to have an even stronger potential lowering hepatic lipoproteins [very low-density lipoprotein (VLDL) and low-density lipoprotein (LDL)] and blood cholesterol than that observed for hesperidin and naringin. In addition, nobiletin intake has demonstrated improvement in memory

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impairment and demonstrated rescue from cholinergic neurodegeneration in mice (8).

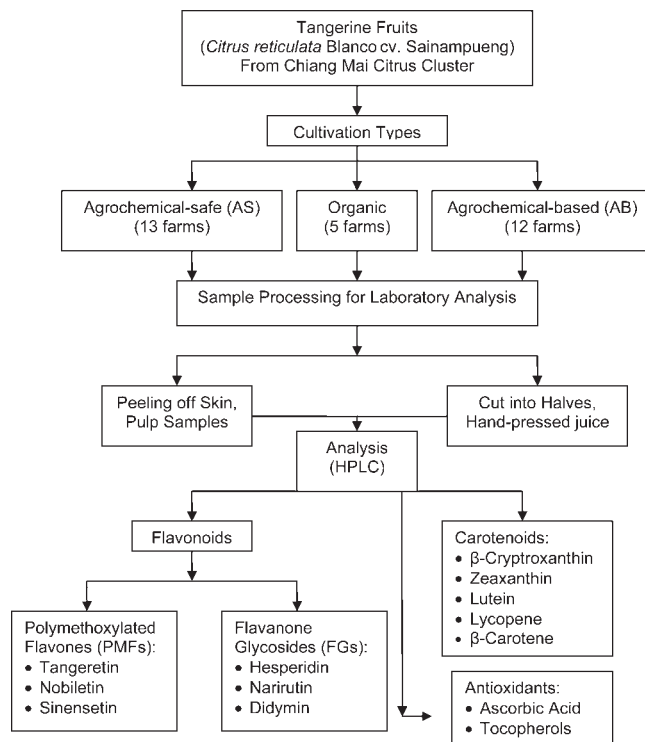
Carotenoids are yellow, orange, and red pigments present in many commonly eaten fruits and vegetables (9). Nutrition research has focused on those that account for most of the dietary intake having biological activity:  $\beta$ -carotene, lycopene, lutein, zeaxanthin, and  $\beta$ -cryptoxanthin. Tangerines, the smallest species in the economically important family of citrus fruits, contain high amounts of  $\beta$ -cryptoxanthin esters (10). Additionally tangerines have been reported as a source for  $\beta$ -carotene, lutein, and zeaxanthin (11). Subjects that frequently consumed tropical fruits high in  $\beta$ -cryptoxanthin, such as papaya or tangerine, had 2-fold the plasma  $\beta$ -cryptoxanthin concentrations of those with low intakes (12).  $\beta$ -Carotene and  $\beta$ -cryptoxanthin have provitamin A activity as well as biological actions, such as the ability to reduce lipid peroxidation and to scavenge free radicals that may be important in maintaining health and preventing serious diseases, such as cancer, pulmonary disorders, and cataracts (13, 14). Epidemiological studies have shown that a high intake of carotenoids is associated with a reduced risk of CVD and prostate cancer (15–17). Lycopene and  $\beta$ -cryptoxanthin can modulate gene expression to inhibit carcinogenesis via multiple pathways (18).

However, the validity of all food intake data in terms of nutrient intake is based on the availability and accuracy of food composition data. Detailed information on flavonoid, carotenoid, and antioxidant contents in tangerines from Thailand is limited. In the present study, tangerine produces from conventional agrochemical-based (AB), agrochemical-safe (AS), and organic farms were collected from a citrus cluster of small orchard farmers in the Fang district, Chiang Mai province, northern Thailand. The study objectives were to determine the frequency and quantity of major FGs (hesperidin, narirutin, and didymin) and PMFs (tangeretin, nobiletin, and sinensetin) in juice and peeled fruit as well as carotenoids (lutein, zeaxanthin,  $\beta$ -cryptoxanthin, lycopene, and  $\beta$ -carotene) and antioxidants (ascorbic acid and tocopherols) in juice using high-performance liquid chromatography (HPLC). Differences in quantity of PMFs and FGs between hand-pressed juice and peeled fruit as well as the impact of the type of agricultural cultivation on concentrations of PMFs, FGs, carotenoids, and antioxidants in juice were assessed.

## MATERIALS AND METHODS

**Study Design and Sampling.** From mid January to the end of February 2008, tangerines (*Citrus reticulata* Blanco cv. Sainampung) were collected from 30 different small farms, including 12 conventional AB, 13 AS, and 5 organic farms of a citrus cluster of small orchard farmers located in the Fang district, Chiang Mai province, northern Thailand (Figure 1). Up to 5 kg of fully ripe, 10–11 months old tangerines (in-season produced) were available from each farm. Conventional AB grown tangerines were used as a control because these are cultivated without a guideline on the usage of fertilizers and pesticides. The AS tangerines are grown according to good agricultural practice (GAP) to ensure no pesticide residues; agrochemicals are strictly controlled, and fruits are harvested after the safe period of a pesticide and are nonwaxed. Organic tangerines are cultivated under the Thailand organic guideline on the usage of fertilizers, nutritive supplements, and natural product insecticides, such as neem, lemon grass, and natural hormone. Tangerine fruits samples (5 kg) were collected from at least five fruit baskets of each cultivation type and performed by two cluster representatives. The fruit baskets contained approximately 22–25 kg tangerine fruits. Fruit samples were then transported to Toxicological Lab, Center for Pollution and Environmental Health Research, Research Institute for Health Sciences (RIHES), Chiang Mai University (CMU), for sample preparation.

**Preparation of Samples.** Tangerines (1 kg) of individual farms were cut into halves and extracted by a simple hand-pressed extractor (Winner Aluminum fruit press). After homogenization, aliquots of 15 mL from



**Figure 1.** Cultivation types of tangerine fruits (*C. reticulata* Blanco cv. Sainampung) and their bioactive constituent analyses.

each individual juice sample were separated for the analysis of polyphenols and carotenoids. For the determination of ascorbic acid, aliquots of 0.5 mL were stabilized with 0.5 mL of 10% (w/w) *meta*-phosphoric acid. For the analysis on polyphenolic compounds in peeled fruits, tangerines were peeled and whole cuts (pieces) were randomly combined from different fruits of each individual sampling. All samples were consequently frozen and stored at  $-80^{\circ}\text{C}$  before being shipped (in early March 2008) under dry ice to Stuttgart-Hohenheim, Germany, for HPLC analysis. All measurements were consequently performed from March to April 2008.

**Reagents and Standards.** Standards of flavonoids (hesperidin, narirutin, didymin, tangeretin, sinensetin, and nobiletin) were purchased from Roth (Karlsruhe, Germany) and Extrasynthese (Genay, France). Standards of lutein, zeaxanthin, and lycopene were provided by Hoffman-LaRoche (Basel, Switzerland),  $\beta$ -Cryptoxanthin and  $\beta$ -carotene were from Roth (Karlsruhe, Germany), while tocopherols were purchased from Fluka (Buchs, Switzerland). All reagents and solvents (acetonitrile, dioxin, methanol, ethanol, dimethylsulfoxide, sodium dihydrogen-phosphate, and formic acid) were of analytical and HPLC grade.

**Analytical Determination of FGs and PMFs in Tangerine Juice and Peeled Fruit.** Organic grown tangerines were mostly smaller than AB and AS grown tangerines, with the mean of 64.4 g ( $n = 43$ ) for organic, 84.1 g ( $n = 14$ ) for AB, and 99.2 g ( $n = 60$ ) for AS grown tangerines, respectively. Although the skin color was similar in golden yellow and smooth skin, the organic tangerines had slightly thicker skin than the other two tangerines. Samples of peeled fruits (edible cuts) were lyophilized by freeze-drying for homogenization. The mean dry weight as a percentage of its original fresh weight was 13.3% (11.9–14.1%) for AB, 14.3% (12.2–15.4%) for AS, and 17.8% (14.8–25.3%) for organic grown fruits. Dried samples were ground with a mortar, and aliquots were individually reconstituted with distilled water to original wet weight before extraction (i.e., 430  $\mu\text{L}$  was added to 70 mg of the freeze-dried sample).

For extraction, 0.5 mL of juice or reconstituted peeled fruit samples was mixed (vortexed) with 0.5 mL of ethanol/dimethylsulfoxide (1:1, v/v) in Eppendorf tubes. After sonication (15 min at  $50^{\circ}\text{C}$ ), samples were centrifuged (15 min at 13 500 rpm) and supernatants were transferred onto vials to be analyzed on a Varian-HPLC (Pro Star 210) coupled with a UV-vis detector (Waters 2487). Extraction and analysis of each sample were performed in duplicate. The separation of FGs and PMFs was achieved using a 5  $\mu\text{m}$  C<sub>18</sub> analytical column (Grom-Sil, 120 ODS-4 HE,

**Table 1.** FGs and PMFs in Hand-Pressed Juice and Peeled Fruit of Tangerines<sup>a</sup>

flavonoids	hand-pressed juice (mg/L)			peeled fruit (mg/kg)		
	AB <sup>b</sup> (n = 12)	AS <sup>c</sup> (n = 13)	organic (n = 5)	AB (n = 12)	AS (n = 13)	organic (n = 5)
narirutin	21.98 (17.7–27.3)	29.20 <sup>d</sup> (20.1–42.5)	34.03 <sup>d</sup> (26.7–43.4)	220.0 (181–268)	273.3 (196–382)	455.4 <sup>e,f</sup> (345–600)
hesperidin	137.2 (123.5–152.4)	155.0 (123.3–194.9)	175.3 <sup>d</sup> (148.6–206.7)	955.6 (841–1085)	1080.6 (904–1292)	1564.6 <sup>e,f</sup> (1290–1898)
didymin	5.29 (4.44–6.31)	6.60 (4.59–9.50)	6.38 (4.89–8.34)	53.4 (45.0–63.3)	66.46 (49.7–88.9)	91.3 <sup>e,g</sup> (74.6–112)
sinensetin	0.77 (0.30–2.00)	0.91 (0.42–1.94)	0.58 (0.41–0.81)	0.063 (n = 10) (0.041–0.096)	0.065 (n = 11) (0.047–0.088)	0.041 (0.025–0.069)
nobiletin	13.32 (6.40–27.7)	14.73 (7.72–28.2)	8.45 (5.49–13.0)	0.49 (0.27–0.87)	0.59 (0.31–1.13)	0.73 (0.44–1.21)
tangeretin	13.96 (6.78–28.8)	15.81 (7.86–31.8)	9.55 (5.99–15.2)	0.36 (0.21–0.62)	0.45 (0.22–0.95)	0.51 (0.35–0.73)

<sup>a</sup> Values are geometric mean (+1SD, –1SD) in mg/L juice or mg/kg fresh weight of edible whole fruit (pulp). <sup>b</sup> AB = agrochemical-based tangerines. <sup>c</sup> AS = agrochemical-safe tangerines. <sup>d</sup> Differences to mean values of juice and whole fruit of conventional AB grown fruits ( $p < 0.05$ ). <sup>e</sup> Differences to mean values of juice and whole fruit of conventional AB grown fruits ( $p < 0.001$ ). <sup>f</sup> Significant differences between organic and AS grown whole fruits ( $p < 0.01$ ). <sup>g</sup> Significant differences between organic and AS grown whole fruits ( $p < 0.05$ ).

125 × 4 mm, Grom, Germany) kept at 40 °C, with solvent A of 5% aqueous formic acid and solvent B containing 85% acetonitrile, 10% water, and 5% formic acid, with the following gradient at a flow rate of 0.8 mL/min: at the start, 15% solvent B was linearly increased in a first step to 25% in 4.5 min and then to 100% in 5 min (4.5–9.0 min), kept constant for 4 min (isocratic 100% B), ramped to the original composition of 15% B in 1.5 min (linear return), and finally kept isocratic for 5 min for equilibration, giving a total run time of 20 min. The injection volume was 20 µL, and compounds were detected at a wavelength of 330 nm for both PMFs and FGs. Data were acquired and evaluated with the Galaxy software (Varian). The limits of detection (LODs) of the method (10× noise) for FGs and PMFs were ≤0.05 and ≤0.002 mg/L, respectively. Concentrations of narirutin ≥0.1 mg/L, hesperidin and didymin ≥0.05 mg/L, and PMFs ≥0.01 mg/L were used as detected concentrations for statistical analysis. Pooled fruit juice (n = 8) analyzed along six batches gave interbatch coefficients of variation (CVs) of 6.8% for narirutin, 1.0% for hesperidin, 3.0% for didymin, 4.8% for sinensetin, 1.6% for nobiletin, and 2.7% for tangeretin. A pool of whole fruit (n = 8) analyzed along six batches gave interbatch CVs between 4.3% for sinensetin and 6.4% for narirutin.

**Ascorbic Acid in Tangerine Juice.** Thawed samples were centrifuged at 13 000 rpm for 3 min. Whole supernatants were transferred to autosampler vials, and 20 µL was analyzed on a Merck Hitachi HPLC (LaChrom) equipped with an autosampler (L-7250), a column oven (L-7360; set at 40 °C), a solvent degasser (L-7612), and a UV–vis detector (L-4250; set at 245 nm). Ascorbic acid was separated by a Reprosil-Pur 120 C18 AQ analytical column (5 µm, 250 × 4.6 mm, Trentec, Gerlingen). Sodium dihydrogen–phosphate buffer (0.1 M NaH<sub>2</sub>PO<sub>4</sub>·H<sub>2</sub>O, set at pH 2.5) was used as the mobile phase with a flow rate of 1 mL/min. Data were acquired, processed, and evaluated with the Clarity chromatographic station, DA-C50 (DataApex Ltd., Prague, Czech Republic). For internal quality control, aliquots of pooled fruit juice (n = 8) were analyzed along six batches of samples, giving an interbatch CV of <1.1%.

**Carotenoids and Tocopherols in Tangerine Juice.** Extraction and HPLC analysis of carotenoids and tocopherols were performed as follows: samples of 0.5 mL were mixed with 1 mL of ethanol containing pyrogallol (2.5%, w/v) and β-apo-8'-carotenal-methyloxime (internal standard) and 0.5 mL of 50% KOH solution for saponification at 38 °C under constant stirring. After 2 h, NaCl solution (15%, w/w) was added and vitamins were extracted twice with 1 mL of hexane. Combined hexane layers were washed with NaCl solution and transferred to tubes for evaporation under nitrogen. Residues were redissolved in ethanol/acetonitrile (1:4) to be analyzed on a Varian-HPLC (Pro Star 210) using a Spherisorb ODS-2 analytical column (3 µm, 250 × 4.6 mm, Trentec, Gerlingen) kept at 40 °C and a mobile phase consisting of acetonitrile, dioxine, and methanol (82:15:3) in recirculation mode with a flow rate of 1.5 mL/min. Carotenoids were detected by UV–vis at 450 nm (Waters 2487), while tocopherols were measured by fluorescence (Waters 472, excitation/emission set at 298/328 nm). Data were acquired and evaluated with the

Galaxy software (Varian). For calibration, a standard mixture of carotenoids, tocopherols, and internal standard β-apo-8'-carotenal-methyloxime was run on each day of analysis. Aliquots of pooled juice (n = 5) were analyzed within three batches of samples, giving interbatch CVs for carotenoids between 6.3% for zeaxanthin and 14.3% for β-carotene and <9 and <20% for α- and γ-tocopherol, respectively.

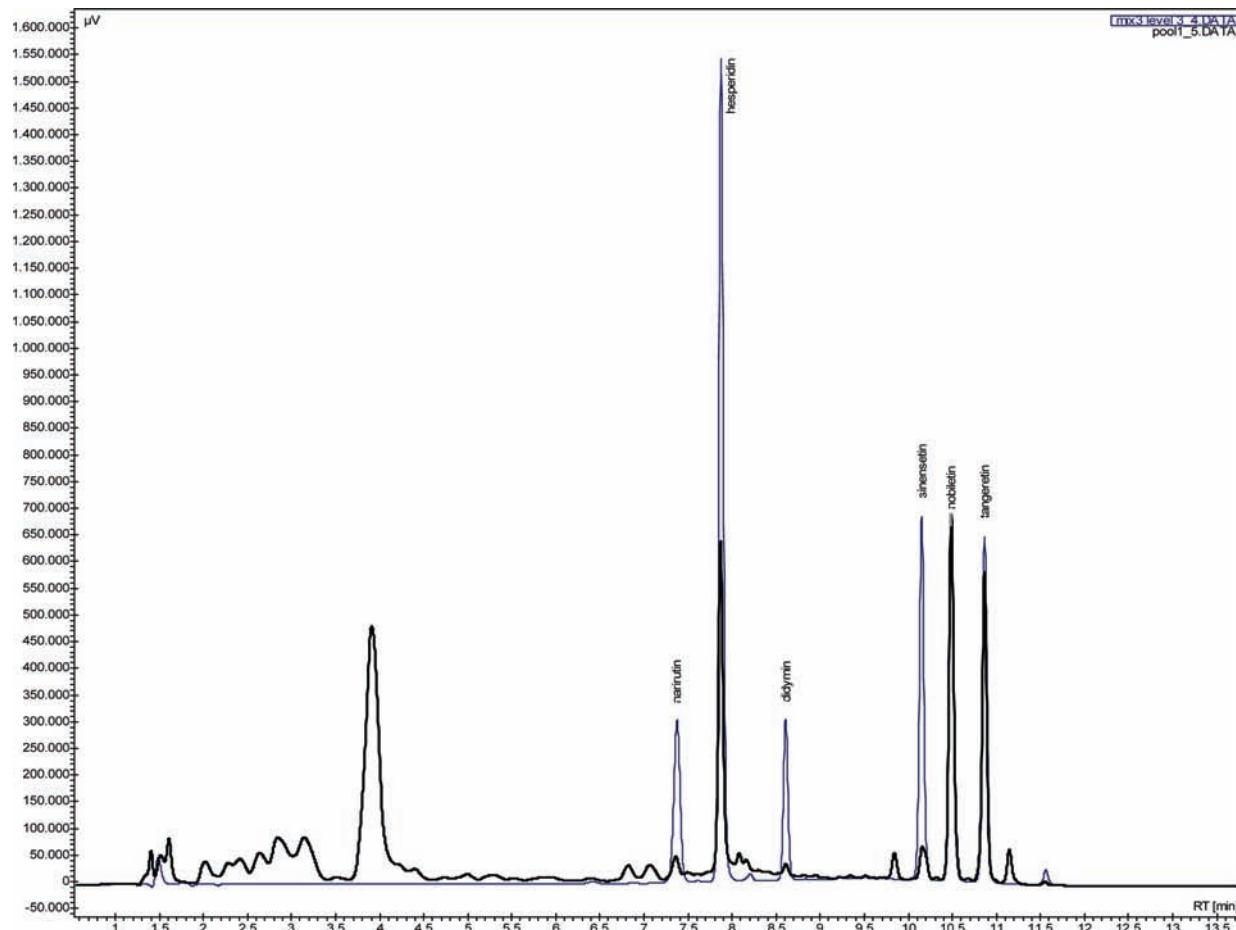
**Statistical Analysis.** Determinations of FGs, PMFs, and ascorbic acid were performed in duplicate, and mean values were used for final analysis. FGs and PMFs in juice and peeled fruit were log-transformed to achieve normal distribution. Concentrations of FGs and PMFs are presented as the geometric mean (+1SD, –1SD), while ascorbic acid, carotenoids, and tocopherols are shown as mean [standard deviation (SD)]. Comparisons of mean concentrations between the conventional AB, AS, and organic grown fruits were performed by analysis of variation (ANOVA) followed by a two-sided Dunnett test using AB fruits as a control. Independent-sample *t* tests were applied for the comparison between organic and AS grown fruits. *p* values <0.05 were regarded as being significant.

## RESULTS AND DISCUSSION

**FGs and PMFs in Juice and Whole Fruit.** Concentrations of FGs and PMFs in juice and peeled fruit of tangerines are given in **Table 1**. A typical chromatogram showing FGs and PMFs in tangerine juice and as standards is shown in **Figure 2**. Hesperidin was the predominant flavanone concentration, being much higher than those of narirutin and didymin. Nobiletin and tangeretin were the predominant PMFs, reaching concentrations that were >10-fold higher than of sinensetin. Mean narirutin, hesperidin, and didymin concentrations in peeled fruit were 5–10-fold higher than the concentrations found in hand-pressed juice samples; in contrast, mean nobiletin, tangeretin, and sinensetin in hand-pressed juice were >13-, 33-, and 22-fold, respectively, higher than the concentrations in peeled fruit samples. All FGs in peeled fruit were significantly higher in organic than AB or AS grown fruits. Juices of organic grown fruits showed higher concentrations of hesperidin and narirutin and juices of AS showed significantly higher concentrations of narirutin than those of AB grown fruits. PMFs tended to be higher in juices of AB and AS than organic grown fruits.

The present results show that simple hand-pressed juice of tangerines from northern Thailand contained considerably high amounts of the PMFs nobiletin, tangeretin, and sinensetin, being several fold higher (10–30 times) than respective concentrations measured in peeled fruits. In contrast, the FGs narirutin, hesperidin, and didymin had much higher concentrations (about 5–10 times) in peeled fruit than in the juice. The explanation is that





**Figure 2.** FGs (hesperidin, narinutin, and didymin) and PMFs (tangeretin, nobiletin, and sinensetin) in a typical juice sample and respective standard mixture.

PMFs occur mainly in the oil glands of the flavedo part (colored peel) of the skin, whereas FGs, such as hesperidin, are present in fruit sacs and tissues (19). Therefore, extracting juice with a hand-pressed extractor results in a fortification of PMFs in juice derived from the oil glands of the flavedo. This effect is confirmed by the observation of Leuzzi et al. (20), who found in particular much higher concentrations of PMFs in the second-pressure extracts than concentrated orange juice. Hand-pressed extraction of ponkan fruits (*C. reticulata*) from Japan resulted in highest concentrations of nobiletin, tangeretin, and sinensetin (3.6, 4.1, and 0.13 mg/100 mL, respectively) compared to much lower concentrations of those PMFs (0.6, 0.7, and a trace amount mg/100 mL, respectively) processed by an in-line extractor (21). In this study, the analysis in fruit tissues revealed that nobiletin, tangeretin, and sinensetin are exclusively in the flavedo and albedo (white inner layer of the peel). The flavedo contained more than 80% and the albedo contained more than 16% of the total amount of nobiletin, tangeretin, and sinensetin from ponkan tissues. Therefore, the extraction efficiency and consistency of the flavedo will significantly affect the concentrations of PMFs in juice. Comparable concentrations in flavonoids with slightly higher FGs but lower concentrations in PMFs than in the present study were measured in hand-pressed ortanique (*C. reticulata* var. *ortanique*, Israel) citrus juice (22).

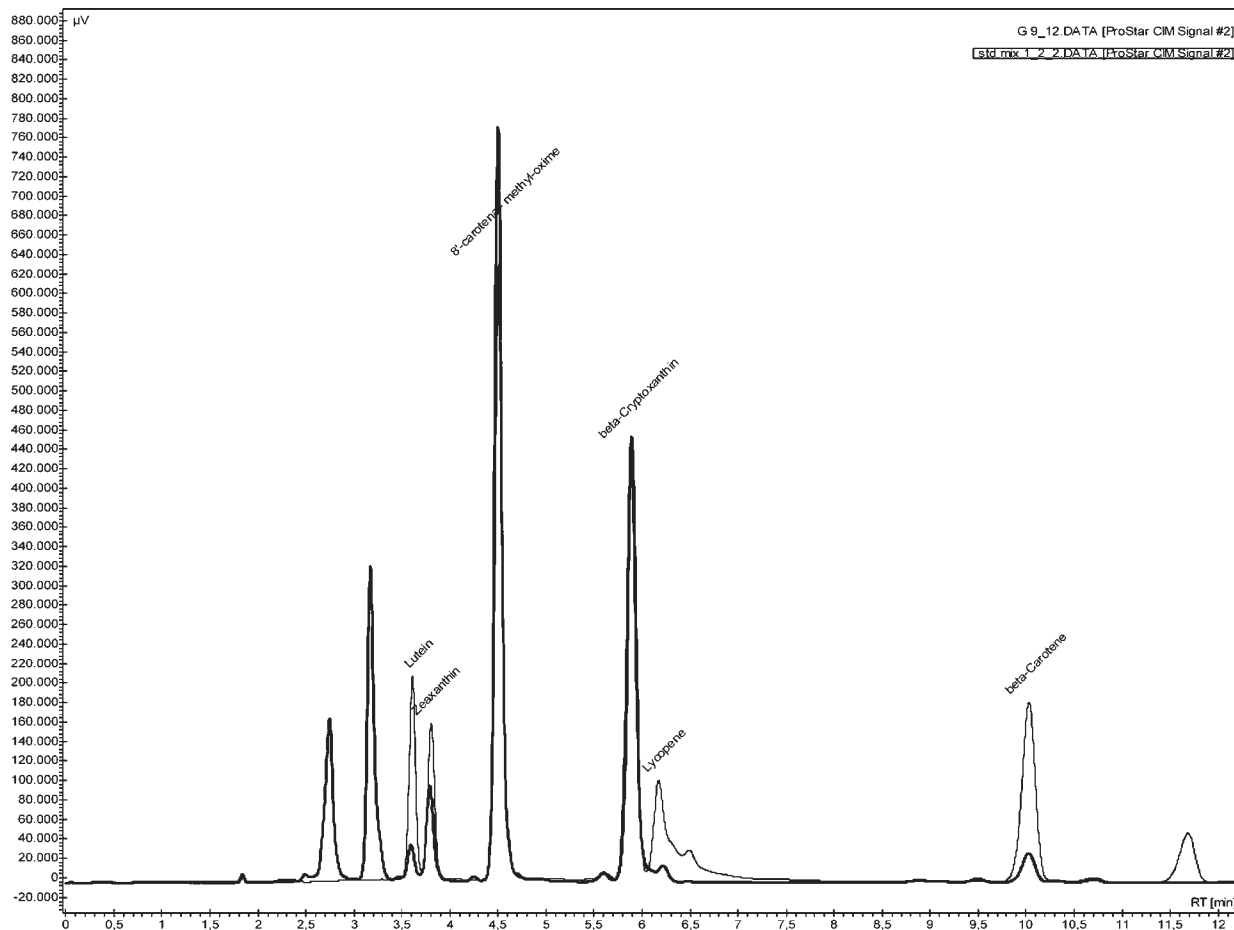
The group comparison of bioactive compounds between the type of agriculture practice revealed higher concentrations of narinutin, hesperidin, and didymin as well as  $\alpha$ -tocopherol in organic than AB and AS grown fruits. The significantly higher concentrations of FGs in both juice and peeled fruit of organic produced fruits are most likely because of the different consistency

**Table 2.** Carotenoids, Ascorbic Acid, and Tocopherols in Tangerine Juice<sup>a</sup>

	AB <sup>b</sup> (n = 12)	AS <sup>c</sup> (n = 13)	organic (n = 5)
lutein (mg/L)	0.412 ± 0.072	0.402 ± 0.114	0.391 ± 0.073
zeaxanthin (mg/L)	1.22 ± 0.14	1.15 ± 0.23	1.19 ± 0.22
$\beta$ -cryptoxanthin (mg/L)	7.04 ± 0.78	6.55 ± 1.68	6.45 ± 1.36
lycopene (mg/L)	0.683 ± 0.171	0.59 ± 0.179	0.614 ± 0.161
$\beta$ -carotene (mg/L)	0.572 ± 0.167	0.496 ± 0.189	0.481 ± 0.179
ascorbic acid (mg/L)	156.1 ± 18.2	161.2 ± 27.6	145.2 ± 11.8
$\alpha$ -tocopherol (mg/L)	1.099 ± 0.241	0.974 ± 0.234	1.324 ± 0.116 <sup>d</sup>
$\gamma$ -tocopherol (mg/L)	0.015 ± 0.007 <sup>e</sup>	0.010 ± 0.002 <sup>f</sup>	0.011 ± 0.004

<sup>a</sup> Values are mean ± SD. <sup>b</sup> AB = agrochemical-based tangerines. <sup>c</sup> AS = agrochemical-safe tangerines. <sup>d</sup> Significant difference between organic and safety grown fruits ( $p < 0.01$ ). <sup>e</sup>  $n = 11/12$ . <sup>f</sup>  $n = 9/13$ .

of fruit pulp (tissues) and peels. Freeze-drying of whole fruits resulted in a higher dry weight for organic grown fruits, which is probably based on a higher content in sugars and fiber, as shown for grapefruits (23). In agreement with the present study, Lester and colleagues (23) described significantly higher concentrations of FGs (naringin, narinutin, and neohesperidin) in the juice from organic than conventional AB grown fruits. The slightly lower concentrations of PMFs in the juice of organic than AB and AS grown Thai tangerines are most likely associated with the thicker peels and, therefore, less effective extraction from organic fruits. However, juice and whole fruit of organic grown tangerines showed significantly higher concentrations of FGs, and juice from organic fruits had significantly higher mean  $\alpha$ -tocopherol than AB and AS produced fruits. No further difference in vitamin concentrations depending upon the type of agriculture was ascertained.



**Figure 3.** Carotenoids (lutein, zeaxanthin,  $\beta$ -cryptoxanthin, lycopene, and  $\beta$ -carotene) in tangerine juice and in comparison to their respective standards.

#### Ascorbic Acid, Carotenoids, and Tocopherols in Tangerine Juice.

Results of ascorbic acid and fat-soluble vitamins are shown in **Table 2**. A typical chromatogram of carotenoids in the juice sample and in comparison to a respective standard mix is shown in **Figure 3**. The predominant carotenoid found was  $\beta$ -cryptoxanthin, followed by zeaxanthin, lycopene,  $\beta$ -carotene, and lutein. The mean ascorbic acid concentration was more than 150 mg/L. Carotenoids and ascorbic acid concentrations were not predicted by different cultivation. In addition,  $\alpha$ -tocopherol was detected in all samples, with the highest mean concentration in juice from organic fruits being significant higher than AS grown fruits.  $\gamma$ -Tocopherol was detected with rather low concentrations in 25 of the 30 juice samples. The present results show that the concentrations of  $\beta$ -cryptoxanthin and  $\beta$ -carotene were higher and the concentrations of lutein and zeaxanthin were similar compared to those reported from the U.S. database (13).  $\beta$ -Carotene and lutein concentrations were lower, while  $\beta$ -cryptoxanthin agreed well with the concentrations given for tangerine juice in a European carotenoid database (24). In contrast to previous studies on carotenoids in tangerines, lycopene was detected with similar levels as analyzed for lutein and  $\beta$ -carotene. Lycopene seemed to be a characteristic for the Sainampung tangerine and needs to be confirmed by HPLC and mass spectrometry. However, lycopene levels were low in comparison to  $\beta$ -cryptoxanthin levels in tangerines and lycopene concentrations reported for pink and red grapefruits (13, 24). Further, the antioxidants  $\alpha$ -tocopherol and ascorbic acid were analyzed with mean concentrations  $\geq 1$  and  $> 150$  mg/L, respectively.

In conclusion, hand-pressed tangerine juice of *C. reticulata* Blanco cv. Sainampung grown in northern Thailand would be an excellent source for the PMFs tangeretin, nobiletin, and sinensetin. Meanwhile,

their peeled fruits offered higher quantities of the FGs narirutin, hesperidin, and didymin but only small amounts of PMFs compared to its juice. Analysis of carotenoids and antioxidants in juice samples confirmed  $\beta$ -cryptoxanthin as the predominant carotenoid in these tangerines and revealed significantly higher levels of  $\alpha$ -tocopherol in organic than juice from AB and AS grown fruits. Consumption of hand-pressed tangerines juice including high concentrations of tangeretin and nobiletin but also being rich in FGs, antioxidants, and  $\beta$ -cryptoxanthin could be able to provide one of the effective sources of food to reduce hypercholesterolemia, incidence of atherosclerosis and cardiovascular disease, and vitamin A deficiency.

We estimate from the present data that 4–5 tangerine fruits ( $\sim 80$  g of each fruit) giving 200 mL of hand-pressed juice (one glass) would provide  $> 5$  mg of nobiletin and tangeretin, 30 mg of hesperidin, 5 mg of narirutin, 1 mg of didymin,  $> 1$  mg of  $\beta$ -cryptoxanthin, 30 mg of ascorbic acid, and 200  $\mu$ g of  $\alpha$ -tocopherol. A total of 1 mg of  $\beta$ -cryptoxanthin ( $\geq 83$   $\mu$ g of retinol equivalents) would reach 17% and 30 mg of ascorbic acid would provide 67% of the daily recommended nutrient intake for an adult of vitamins A and C, respectively (25).

#### ACKNOWLEDGMENT

Dr. Rose McGready (SMRU, Mae Sot District, Tak Province, Thailand) is thanked for the suggestions and the valuable advice in the final phase of the manuscript preparation.

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Received for review January 6, 2010. Revised manuscript received March 15, 2010. Accepted March 30, 2010. We acknowledge the financial support by the Thailand Research Fund (TRF).