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Quantitation of bioactive compounds in citrus fruits cultivated in Taiwan

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Abstract

The aim of this study was to determine the levels of bioactive compounds in the edible portions of eight varieties of citrus fruits (Ponkan, Tonkan, Murcott, Wendun, Peiyou, Kumquat, Liucheng, and Lemon) cultivated in Taiwan. The amount of total polyphenol and flavonoid exceeded that of total carotenoid. Hesperidin was the major flavanone, which abounded in Liucheng and Tonkan (5.36 ± 0.145 and 4.13 ± 0.050 mg/g db, respectively). Naringin abounded in Peiyou and Wendun (1250 ± 0.82 and 2205 ± 11.1 μg/g db, respectively). Diosmin was the major flavone, and Kumquat (0.699 ± 0.021 mg/g db) and Lemon (0.323 ± 0.004 mg/g db) had the highest contents. Kaempferol was the most abundant flavanol except in Wendun, Peiyou, and Kumquat, and Murcott had the highest content (1.04 ± 0.007 mg/g db). Chlorogenic acid was the major phenolic acid, and Wendun and Lemon had the highest contents (103 ± 11.5 and 92.6 ± 8.90 μg/g db, respectively). β-Cryptoxanthin was the main carotenoid (0.764 ± 0.031–6.67 ± 0.329 μg/g db), followed by β-carotene (0.435 ± 0.016–3.77 ± 0.154 μg/g db), and these two compounds abounded in Murcott. Tonkan, Wendun, Peiyou, and Lemon had high levels of ascorbic acid. Total pectin levels ranged from 40.4 ± 1.65 to 87.3 ± 3.69 mg/g db.

Keywords: Citrus fruit; Bioactive compound; Quantitation

1. Introduction

Citrus fruit is a major product of Taiwanese agriculture and many varieties are cultivated, such as *Citrus reticulata* Blanco, *Citrus tankan* Hayata, *Citrus reticulate* × *Citrus sinensis*, *Citrus grandis* Osbeck, *Citrus grandis* Osbeck CV, *Citrus microcarpa*, *Citrus sinensis* (L.) Osbeck, and *Citrus limon* (L.) Bur. *Citrus sinensis* (L.) Osbeck is the major variety cultivated in Taiwan, followed in order by *C. reticulata* Blanco, *C. grandis* Osbeck, and *C. tankan* Hayata. *C. reticulate* × *C. sinensis* is the earliest matured variety (January–March) in Taiwan, followed in order by *C. tankan* Hayata (February–March), *Citrus limon* (L.) Bur. (May–June), *C. grandis* Osbeck (September–October), *C. reticulata* Blanco (October–December), *C. grandis* Osbeck CV (October–November), *C. microcarpa* (November–December), and *C. sinensis* (L.) Osbeck (December–January) (Shih, Chen, & Chang, 1982). According to the Agricultural Statistics Year Book (Anonymous, 2003), about 37,000 hectares are dedicated to citrus cultivation in Taiwan, and production is about 500,000 tons annually.

Citrus contains a host of active phytochemicals that contribute to health. In fact, there are more than 170 phytochemicals in an orange. It is commonly accepted that consumption of certain foods can prevent cancer (Stavric, 1994), and flavonoid [including hesperidin (a flavanone), followed abundance in citrus by narirutin, naringin, neohesperidin, eriocitrin, neoeriocitrin, rutin, diosmin, neoponcirin, and nobiletin (Albach, Juarez, & Lime, 1969; Castillo, Benavente Garcia, & Del Rıo, 1992; Jourdan, McIntosh, & Mansell, 1985; Kawaii, Tomono, Katase, Ogawa, & Yano, 1999)] in citrus fruit is among the most prominent cancer-preventing agents. Flavonoid has a wide range of biological effects, such as inhibition of key

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enzymes in mitochondrial respiration, protection against coronary heart disease, and anti-spasmolytic, anti-inflammatory, antioxidative, vascular, estrogenic, cytotoxic antitumor, and antimicrobial activities (Harborne & Williams, 2000).

Approximately 115 different carotenoids have been reported in citrus fruits, and the colour of orange fruit and its peel is due to carotenoid (Stewart & Wheaton, 1973). Pink grapefruit has a high content of β-carotene; other citrus fruits also contain high levels of carotenoid, such as lutein, zeaxanthin, and β-cryptoxanthin (Mangels, Holden, Beecher, Forman, & Lanza, 1993). Red coloration in Red Navel and Valencia oranges is mostly due to lycopene and cryptoxanthin, respectively (Lee, 2001). Numerous studies have demonstrated that a high carotenoid intake or status might decrease risk of cancer, age-related macular degeneration, cataracts, sunburn-induced skin damage, and cardiovascular diseases (Aust, Sies, Stahl, & Polidori, 2001). Addition of lycopene to cell cultures significantly prevented vacuolization in human lens epithelial cells (Mohanty, Joshi, Trivedi, Srivastava, & Gupta, 2002). Higher β-carotene consumption was associated with a lower risk of breast cancer (Fraser & Bramley, 2004). The level of UV-induced lipid peroxidation of human skin fibroblast cells *in vitro* was lowered by addition of β-carotene, lycopene, or lutein (Fraser & Bramley, 2004). Lutein was inversely associated with the incidence of colon cancer in both men and women (Slattery et al., 2000).

Moreover, vitamin C, phenolic acid, and pectin provide various health benefits. Vitamin C, acting as an antioxidant, may reduce the risk of arteriosclerosis, cardiovascular diseases, and some forms of cancer (Diplock, 1994). Hydroxycinnamic acid compounds, such as caffeic acid, ferulic acid, chlorogenic acid, and ρ-coumaric acid, inhibit oxidation of low-density lipoprotein (Meyer, Donovan, Pearson, Waterhouse, & Frankel, 1998) and have anticancer (Kual & Khanduja, 1998) and antimicrobial activities (Kernan et al., 1998). Citrus fruits (both the edible and inedible parts) are a particularly rich source of pectin, which has multiple biological activities, including glycemic and cholesterol level control (Baker, 1994).

The bioactive compound compositions of various varieties of citrus fruit have been studied. Hesperidin was the most important flavonoid, which abounded in *C. reticulata* Blanco (6.76–12.0 mg/g dried matter), *C. sinensis* (L.) Osbeck (6.98–10.8 mg/g dried matter), and *C. limon* (L.) Bur (3.58 mg/g dried matter). Of the other flavonoids, narirutin abounded in *C. tankan* Hayata (6.34 mg/g dried matter) and *C. microcarpa* (2.89–4.60 mg/g dried matter) and naringin abounded in *C. grandis* Osbeck (3.97 mg/g dried matter) (Kawaii et al., 1999). Caffeic acid, ρ-coumaric acid, ferulic acid, and sinapic acid levels in Valencia late juice were 2.1, 8.0, 37.7, 9.0 mg/l, respectively, among which ferulic acid was the main phenolic acid. (Rapisarda, Carollo, Fallico, Tomaselli, & Maccarone, 1998). Lutein, β-cryptoxanthin, and β-carotene levels in Valencia juice were 12.4–49, 33–195, and 12–38 μg/100 ml, respectively (Mouly, Gaydou, & Corsetti, 1999; Mouly, Gaydou, Lapierre, & Corsetti, 1999; Stewart, 1977) and these levels in Murcott were 85.5, 427, and 43.5 μg/100 ml, respectively (Stewart, 1977). In Valencia juice and Murcott, β-cryptoxanthin was a major carotenoid. Vitamic C levels in *C. sinensis* (L.) Osbeck, *C. limon* (L.) Bur, and Kumquat were found to be 47.7–57.7, 47.9, and 51.3 mg/100 g fresh matter, respectively (Gorinstein et al., 2001; Rapisarda et al., 1999; Vinci, Botrè, & Mele, 1995).

The aim of this study was to determine the levels of bioactive compounds in the edible portions of eight varieties of citrus fruits (*C. reticulata* Blanco, *C. tankan* Hayata, *C. reticulate* × *C. sinensis*, *C. grandis* Osbeck, *C. grandis* Osbeck CV, *C. microcarpa*, *C. sinensis* (L.) Osbeck, and *C. limon* (L.) Bur), cultivated in Taiwan and in turn, to establish a database of beneficial compounds in Taiwanese citrus fruits.

2. Materials and methods

2.1. Plant materials and sample preparation

Eight varieties of citrus fruits, of which *C. reticulata* Blanco, *C. tankan* Hayata, *C. reticulate* × *C. sinensis*, *C. grandis* Osbeck, *C. grandis* Osbeck CV, *C. microcarpa*, *C. sinensis* (L.) Osbeck, and *C. limon* (L.) Bur, with the conventional names Ponkan, Tonkan, Murcott, Wendun, Peiyou, Kumquat, Liucheng, and Lemon, respectively, were harvested from trees at local farms between October 2002 and January, 2003. Ponkan, Tonkan, Murcott, and Lemon were collected from Dongshih town; other varieties were collected from various locations, such as Wendun from Ruisuei town, Peiyou from Madou town, Kumquat from Yuanshan town, and Liucheng from Gukeng town. The citrus fruits were separated into edible and inedible portions, and the edible portions were ground into a slurry and homogenized with a homogenizer at low temperature (<5 °C), immediately freeze-dried, and finally stored at −30 °C before use.

2.2. Determination of total polyphenol, flavonoid, and carotenoid contents

The extraction of total polyphenol was achieved using 1% HCl in 80% methanol. In brief, 2 ml of 1% HCl in 80% methanol were added to 200 mg of sample in a centrifuge tube, extracted on a shaker at 200 rpm for 2 h at room temperature, and centrifuged at 10,000 rpm for 15 min at 4 °C. The residue was extracted twice by adding 2 ml of 1% HCl in 80% methanol. All supernatants were combined and made up to 10 ml with extraction solvent. The concentration of total polyphenol was measured by the method described by Singleton and Rossi (1965) with some modification. Briefly, a volume of 0.1 ml was pipetted into a test tube, along with 0.9 ml of water, 5 ml of 0.2 N Folin-Ciocalteau reagent, and 4 ml of Na_2CO_3, and mixed well. The mixture was kept for 2 h at room temperature for col-

our development. Absorbance was measured at 765 nm. Total polyphenol was expressed as gallic acid equivalents; which gallic acid was purchased from Sigma-Aldrich (St. Louis, MO, USA).

For total flavonoid examination, the method of Jia, Tang, and Wu (1999) was used. In brief, 2.5 g of sample placed in a Soxhlet extractor and refluxed with methanol for more than 12 h at 85 °C. The extract was evaporated to dryness in a rotary vacuum evaporator at less than 40 °C and dissolved with methanol. Exactly 0.3 ml of 5% $NaNO_2$ was added to 1 ml of extract in a 10 ml volumetric flask and the mixture was kept for 6 min at room temperature. Addition of 0.3 ml of 10% $Al(NO_3)_3$ to the mixture, which was incubated for 6 min again, was followed by addition of 4 ml of 1 N NaOH and of methanol up to volume. After incubating for 15 min at room temperature for colour development, absorbance at 500 nm was measured. Total flavonoid was expressed as rutin equivalents; rutin was purchased from Acros (NJ, USA).

The method of Lee (2001) was used for total carotenoid quantitation. In brief, 5 g of sample and 50 ml of *n*-hexane–acetone–ethanol (v/v; 50:25:25) were placed in a flask, extracted on a shaker at 200 rpm for 10 min at room temperature, centrifuged at 6500 rpm for 5 min at 4 °C, and the supernatants were collected and made up to 50 ml with extraction solvent. Absorbance was measured at 450 nm. Total carotenoid was expressed as β-carotene equivalents, for which β-carotene was purchased from Acros (NJ, USA).

2.3. HPLC apparatus

Reversed-phase HPLC was used to assay compositions of flavonoid, carotenoid, and ascorbic acid. A Hitachi HPLC system (Tokyo, Japan) consisted of a Model L-7100 pump equipped with a multi-solvent delivery system and a L-7400 ultraviolet (UV) detector. The column type was a LiChrospher®100RP18 e, 5 μm, 4.0 mm internal diameter (i.d.) × 250 mm, which was purchased from Merck (Darmstadt, Germany). The other individual chromatographic conditions for the compounds detection were as described below.

2.4. Analysis of flavonoid composition by HPLC

The method of Schieber, Keller, and Carle (2001) for the analysis of flavonoid composition in citrus fruits was used. The flavonoid standards included: (1) phenolic acids: caffeic acid, ρ-coumaric acid, ferulic acid, sinapic acid, and chlorogenic acid; (2) flavanones: naringin, hesperidin and neohesperidin; (3) flavonols: quercetin, kaempferol and rutin; and (4) flavones: sinensetin, luteolin, and diosmin. All the standards were purchased from Sigma-Aldrich (St. Louis, MO, USA), prepared in methanol–dimethyl sulfoxide (DMSO) (v/v; 50:50), and stored at −18 °C before use.

A mixture of 0.1 g of sample and 1 ml of methanol-DMSO (v/v; 50:50) was placed in a centrifuge tube, stirred for 10 min at room temperature, and centrifuged at 9000 rpm for 15 min at 4 °C. The residues were extracted twice with 2 × 1 ml of the same extraction solvent. All the supernatants were combined and made up to 5 ml with methanol. The sample solution was filtered through a 0.45 μm membrane filter before use.

The mobile phase was composed of (A) 2% acetic acid (aqueous) and (B) 0.5% acetic acid (aqueous)-acetonitrile (50:50 v/v), and gradient elution was performed as follows: 0 min, 95:5; 10 min, 90:10; 40 min, 60:40, 55 min, 45:55; 60 min, 20:80; and 65 min, 0:100. The mobile phase was filtered under vacuum through a 0.45 μm membrane filter before use. The flow rate was 1 ml/min. UV absorbance (for phenolic acid, flavanone and flavonol) was measured at 280 nm and, for flavone, at 340 nm. The operating temperature was maintained at room temperature.

2.5. Analysis of carotenoid composition by HPLC

Carotenoid composition in citrus fruits was determined. The carotenoid standards included lutein, zeaxanthin, β-cryptoxanthin, and β-carotene; all, except β-carotene, which was purchased from Acros (NJ, USA), were purchased from Extrasynthese (Genay, France). Butylated hydroxytoluene (BHT) in chloroform (0.1%) was added to all the standards, and they were diluted with acetonitrile before use.

The sample preparation was according to Pupin, Dennis, and Toledo (1999). In brief, 10 parts 0.004% BHT in ethyl acetate mixed with 1 part of sample powder were stirred for 5 min at room temperature and centrifuged at 9000 rpm for 15 min at 4 °C. The residues were extracted twice with the same extraction solvent. All supernatants were combined and concentrated to dryness in a rotary vacuum evaporator below 40 °C. The concentrate was dissolved with acetonitrile–methanol–dichloromethane (60:35:5 v/v) and filtered through a 0.45 μm membrane filter before use.

The mobile phase was composed of acetonitrile, methanol and dichloromethane, and gradient elution was performed as follows: 0 min, 100:0:0 (v/v/v), with 0.6 ml/min of flow rate; 25 min, 100:0:0, with 1.0 ml/min of flow rate; 30 min, 60:35:5, with 1.0 ml/min of flow rate; and 80 min, 60:35:5, with 1.0 ml/min of flow rate. All mobile phases contained 0.1% BHT, 0.1% triethylamine, and 0.005 M ammonium acetate (in methanol), and were filtered under vacuum through a 0.45 μm membrane filter before use. Absorbance was measured at 450 nm.

2.6. Determination of ascorbic acid content by HPLC

A reversed-phase HPLC method, according to Romero Rodriguez, Vazquez Oderiz, Lopez Hernandez, and Simal Lozano (1992), was used for quantitation of ascorbic acid in citrus fruits. A mixture of 0.1 g of sample and 5 ml of acetonitrile–acetic acid–water (75:2:33 v/v) in a centrifuge tube was stirred for 10 min at room temperature and centrifuged at 9000 rpm for 15 min at 4 °C. The residues were

extracted twice with the same extraction solvent. All the supernatants were combined and made up to 25 ml with the extraction solvent. The sample solution was filtered through a 0.45 μm membrane filter before use. Ascorbic acid standard was purchased from Sigma-Aldrich (St. Louis, MO, USA) and prepared in acetonitrile–acetic acid–water (75:2:33 v/v) at concentrations of 20–50 μg/ml.

The mobile phase was acetonitrile–acetic acid–water (75:2:23 v/v) and eluted at a flow rate of 1 ml/min. Absorbance was measured at 254 nm.

2.7. Determination of pectin content

The method of Yu and Love (1996) was used. A mixture of 5 g of sample powder and 30 ml of hot absolute ethanol was heated in a centrifuge tube for 10 min in a boiling water bath and centrifuged at 10,000 rpm for 10 min at 4 °C. The residues were dried for 24 h at 35 °C, and alcohol-insoluble solids (AIS) was obtained.

One ml of water was added, drop-by-drop with stirring, for 35 min to a mixture of 5 mg of AIS and 2 ml of concentrated sulfuric acid in a test tube until the AIS were dissolved. The mixture was transferred into a 25 ml volumetric flask and made up to volume with distilled water for total pectin examination.

A mixture of 80 mg of AIS and 20 ml of distilled water was stirred in a centrifuge tube for 5 min at room temperature and centrifuged at 10,000 rpm for 10 min at 4 °C. The residues were extracted twice with 2×20 ml of distilled water. All the supernatants were transferred into a 100 ml volumetric flask and made up to volume with distilled water for water soluble pectin examination.

In an ice bath, 1 ml of the above sample solution was added to 6 ml of 0.0125 M sodium tetraborate (in concentrated sulfuric acid) and then heated for 5 min in a boiling water bath. Colour development followed addition of 0.1 ml of 0.15% *m*-hydroxydiphenyl and incubation for 20 min at room temperature. NaOH (0.1 ml) was added instead of 0.15% *m*-hydroxydiphenyl to the control. Both total pectin and water-soluble contents were expressed as galacturonic acid equivalents, for which galacturonic acid was purchased from Fluka, Riedel-de-Haen (Sigma-Aldrich) (St. Louis, MO, USA).

3. Results and discussion

3.1. Total polyphenol, flavonoid, and carotenoid contents

The amounts of total polyphenol, flavonoid and carotenoid in the edible portions of eight varieties of citrus fruits are shown in Table 1. Total polyphenol ranged from 37.3 ± 1.53 to 75.9 ± 3.87 mg/g db (gallic acid equivalents). Lemon contained the most total polyphenol (75.9 ± 3.87 mg/g db [gallic acid equivalents]), followed by Kumquat (52.3 ± 1.55 mg/g db [gallic acid equivalents]); the levels in Tonkan, Wenden, Peiyou, Ponkan, Liucheng and Murcott ranged from 36.9 ± 1.84 to 47.0 ± 0.88 mg/g db (gallic acid equivalents). Total flavonoid varied from 8.41 ± 0.32 to 21.6 ± 0.57 mg/g db (rutin equivalents). Lemon and Peiyou had the highest levels (21.6 ± 0.57 and 19.7 ± 0.48 mg/g db [rutin equivalents], respectively). Liucheng, Wendun, Ponkan, and Murcott had moderate levels (11.1 ± 0.34–15.7 ± 0.43 mg/g db [rutin equivalents]), while Tonkan and Kumquat had the lowest (9.13 ± 0.35 and 8.41 ± 0.32 mg/g db [rutin equivalents], respectively). Total carotenoid content was much lower than those of total polyphenol and flavonoid and ranged from 0.019 ± 0.0003 to 0.336 ± 0.005 mg/g db (β-carotene equivalents). Murcott exhibited the greatest carotenoid content, followed, in order, by Ponkan and Tonkan (0.220 ± 0.007 and 0.198 ± 0.0008 mg/g db [β-carotene equivalents], respectively), Kumquat, Liucheng, Lemon, Peiyou, and Wendun (0.019 ± 0.0003–0.105 ± 0.003 mg/g db [β-carotene equivalents]).

Table 1
Total polyphenol, flavonoid, and carotenoid contents in the edible portions of citrus fruits

Fruit	Total polyphenol (mg/g db[a])[b]	Total flavonoid (mg/g db)	Total carotenoid (mg/g db)
Ponkan	42.6 ± 1.19	11.2 ± 0.32	0.220 ± 0.007
Tonkan	47.0 ± 0.88	9.13 ± 0.35	0.198 ± 0.0008
Murcott	36.9 ± 1.84	11.1 ± 0.34	0.336 ± 0.005
Wendun	46.2 ± 2.54	14.3 ± 0.39	0.019 ± 0.0003
Peiyou	43.1 ± 1.10	19.7 ± 0.48	0.031 ± 0.0008
Kumquat	52.3 ± 1.55	8.41 ± 0.32	0.105 ± 0.003
Liucheng	37.3 ± 1.53	15.7 ± 0.43	0.080 ± 0.002
Lemon	75.9 ± 3.87	21.6 ± 0.57	0.061 ± 0.001

[a] Dried basis.
[b] Data presented are in means ± standard deviation ($n = 3$).

Gorinstein et al. (2001) found that the polyphenol content (chlorogenic acid equivalents) in peeled lemon (*C. limon*) and orange (*C. sinensis*) were 164 ± 10.3 and 154 ± 10.2 mg/100 g fresh fruit, respectively. On the other hand, total polyphenol content in Valencia late juice was 48.8 ± 1.97 mg/100 ml (ferulic acid equivalents) (Rapisarda et al., 1999). Total polyphenol content in orange fruits ranged from 50 to 100 mg/100 g fresh matter (Vinson, 1999). In three commercially available orange juices (orange, Jaffa orange, and Florida orange), total polyphenol ranged from 50.4 ± 1.0 to 75.5 ± 1.8 mg/100 ml (gallic acid equivalents) and total carotenoid ranged from 0.30 ± 0.11 to 0.83 ± 0.20 mg/100 ml (β-carotene equivalents) except for Florida orange which had no detectable carotenoid (Gardner, White, McPhail, & Duthie, 2000). In our study, the total polyphenol and total carotenoid levels in both Liucheng and Lemon were much higher than in the references mentioned above.

3.2. Flavonoid composition

3.2.1. Flavanone compounds

Flavanone is the major flavonoid in oranges. Table 2 shows that, of the three flavanones, hesperidin was the most abundant, except in Wendun and Peiyou. In order

Table 2
Flavanone contents in the edible portions of citrus fruits

Fruit	Naringin (μg/g db[a])[b]	Hesperidin (mg/g db)	Neohesperidin (μg/g db)
Ponkan	14.0 ± 0.82	2.91 ± 0.009	9.07 ± 1.65
Tonkan	12.0 ± 2.56	4.13 ± 0.050	11.1 ± 1.71
Murcott	5.41 ± 0.67	0.545 ± 0.012	6.09 ± 1.35
Wendun	1250 ± 0.82	0.695 ± 0.006	17.3 ± 2.47
Peiyou	2205 ± 11.07	0.964 ± 0.018	6.15 ± 1.23
Kumquat	27.9 ± 3.28	0.366 ± 0.002	ND[c]
Liucheng	60.3 ± 1.71	5.36 ± 0.145	5.69 ± 1.13
Lemon	89.9 ± 2.67	3.43 ± 0.067	4.45 ± 0.89

[a] Dried basis.
[b] Data presented are in means ± standard deviation ($n = 3$).
[c] Not detectable.

of abundance, the other two were naringin and neohesperidin. The fruits listed on the basis of hesperidin content (in order from the highest to lowest) were Liucheng (5.36 ± 0.145 mg/g db), Tonkan (4.13 ± 0.050 mg/g db), Lemon (3.43 ± 0.067 mg/g db), Ponkan (2.91 ± 0.009 mg/g db), Peiyou, Wendun, Murcott, and Kumquat (0.366 ± 0.002–0.964 ± 0.018 mg/g db). In naringin content, Wendun and Peiyou were found to be much higher than others, showing 1250 ± 0.82 and 2205 ± 11.1 μg/g db, respectively; other varieties ranged from 5.41 ± 0.67 to 89.9 ± 2.67 μg/g db. The level of hesperidin varied widely from 0.366 ± 0.002 to 5.36 ± 0.145 mg/g db. Neohesperidin, the least abundant flavonone in citrus fruits, ranged from 4.45 ± 0.89 to 17.3 ± 2.47 μg/g db and was not detectable in Kumquat.

According to the results obtained by Kawaii et al. (1999), hesperidin was the key flavonoid in *C. reticulata* Blanco (6.76–12.0 mg/g, dried matter), *C. sinensis* (L.) Osbeck (6.98–10.8 mg/g, dried matter), and *C. limon* (L.) Bur (3.58 mg/g, dried matter), respectively (Kawaii et al., 1999). On the other hand, naringin was the key flavonoid in *C. grandis* (3.97 mg/g, dried matter). Hesperidin was found in common orange juice (Gil-Izquierdo, Gil, Ferreres, & Tomás-Barberán, 2001), and in 87 authentic orange juices (Ooghe, Ooghe, Detavernier, & Huyghebaert, 1994), Valencia juice (Pupin, Dennis, & Toledo, 1998), *C. sinensis* var. valencia juice (Mouly, Gaydou, & Auffray, 1998), and *C. sinensis* L. Osbeck juice (Caro, Piga, Vacca, & Agabbio, 2004) in the range of 2.33–47.5 mg/100 ml. In our study, hesperidin was the major flavonoid in eight varieties with the exception of Wenden and Peiyou. The hesperidin level in Ponkan in our study was lower than that of Kawaii et al. (1999); in contrast, in Wenden, Peiyou, Tonkan, and Kumquat, the hesperidin levels in our study were higher than that obtained by Kawaii et al. (1999), and in Liucheng and Lemon, the hesperidin levels in our study were similar to their reports. Naringin was the key flavonoid in Wenden and Peiyou in our study, and levels were similar to that found by Kawaii et al. (1999). However, it should be noted that, in contrast to the results obtained by Kawaii et al. (1999), low but detectable levels of neohesperidin were also found in the citrus fruits we examined.

3.2.2. Flavone compounds

Flavone compounds, such as diosmin, luteolin, and sinensetin, contained in the edible portions of eights varieties were also examined. The results in Table 3, show that diosmin was the major flavone and was much more abundant than luteolin and sinensetin. The level of diosmin in Kumquat was the highest (0.699 ± 0.021 mg/g db), followed by Lemon (0.323 ± 0.004 mg/g db), Murcott, Tonkan, Wendun, and Ponkan (0.129 ± 0.002–0.144 ± 0.002 mg/g db), Peiyou and Liucheng were the lowest (0.087 ± 0.002 and 0.084 ± 0.001 mg/g db, respectively). In luteolin content, Lemon was the highest (160 ± 4.45 μg/g db), followed by Murcott, Ponkan, Tonkan, and Liucheng (13.7 ± 0.56–52 ± 1.35 μg/g db); no luteolin was detectable in Wendun, Peiyou, or Kumquat. The level of sinensetin was very low in all eight varieties (1.64 ± 0.08–42.8 ± 0.82 μg/g db).

According to the results obtained by Kawaii et al. (1999), the levels of diosmin in Lemon and luteolin in Kumquat were 0.732 and 0.068–0.138 mg/g dried matter, respectively. With the exception of these results, the two flavones were not detectable in the other seven varieties we tested. Mouly et al. (1998) and Kawaii et al. (1999) found sinensetin in Liucheng at levels of 0.18 mg/100 ml and 40 μg/g dried matter, respectively; however, as reported by Kawaii et al. (1999), the sinensetin was not detectable in the other seven varieties we tested. In our study, the diosmin level found in Lemon (0.323 mg/g db) was similar to that found by Kawaii et al. (1999) but, in the other seven varieties, the diosmin levels in our results ranged from 0.084 to 0.699 mg/g db, while Kawaii et al. (1999) reported a level of zero. In our study, with the exception of Wendum, Peiyou, and Kumquat, luteolin levels in the other five varieties ranged from 13.7 ± 0.56 to 160 ± 4.45 μg/g db, but luteolin was reported as undetectable in the same varieties by Kawaii et al. (1999). Sinensetin level in Liucheng in our results (14.8 ± 0.56 μg/g db) was similar to that found by Mouly et al. (1998) and Kawaii et al. (1999). However, sinensetin level in the other seven varieties in our study ranged from 1.64 ± 0.08 to 42.8 ± 0.82 μg/g db, but it was reported as not detectable by Kawaii et al. (1999).

Table 3
Flavone contents in the edible portions of citrus fruits

Fruit	Diosmin (mg/g db[a])[b]	Luteolin (μg/g db)	Sinensetin (μg/g db)
Ponkan	0.129 ± 0.002	42.9 ± 0.82	9.89 ± 0.24
Tonkan	0.138 ± 0.003	23.9 ± 0.85	12.8 ± 0.34
Murcott	0.144 ± 0.002	52.1 ± 1.35	9.47 ± 0.34
Wendun	0.132 ± 0.002	ND[c]	42.8 ± 0.82
Peiyou	0.087 ± 0.002	ND	24.6 ± 0.37
Kumquat	0.699 ± 0.021	ND	1.64 ± 0.08
Liucheng	0.084 ± 0.001	13.7 ± 0.56	14.8 ± 0.56
Lemon	0.323 ± 0.004	160 ± 4.45	9.80 ± 0.26

[a] Dried basis.
[b] Data presented are in means ± standard deviation ($n = 3$).
[c] Not detectable.

Table 4
Flavonol contents (mg/g db[a]) in the edible portions of citrus fruits

Fruit	Rutin	Quercetin	Kaempferol
Ponkan	0.053 ± 0.001[b]	0.136 ± 0.002	0.503 ± 0.009
Tonkan	0.071 ± 0.003	0.173 ± 0.002	0.870 ± 0.016
Murcott	0.091 ± 0.001	0.141 ± 0.001	1.035 ± 0.007
Wendun	0.090 ± 0.004	0.061 ± 0.001	0.012 ± 0.001
Peiyou	0.073 ± 0.001	0.087 ± 0.007	0.009 ± 0.001
Kumquat	0.043 ± 0.002	0.308 ± 0.003	0.235 ± 0.002
Liucheng	0.042 ± 0.001	0.104 ± 0.001	0.606 ± 0.006
Lemon	0.060 ± 0.003	0.573 ± 0.006	0.611 ± 0.012

[a] Dried basis.
[b] Data presented are in means ± standard deviation ($n = 3$).

3.2.3. Flavonol compounds

Table 4 shows the amounts of flavonols (kaempferol, rutin, and quercetin) present in our eight fruits. Kaempferol (0.009 ± 0.001–1.04 ± 0.007 mg/g db) was the most abundant flavanol in all fruits except Wendun (0.012 ± 0.001 mg/g db), Peiyou (0.009 ± 0.004 mg/g db), and Kumquat (0.235 ± 0.002 mg/g db), followed, in order, by quercetin and rutin. The citrus fruits contained 0.042 ± 0.001–0.091 ± 0.001 mg of rutin/g db. The fruits listed on the basis of their quercetin content from highest to lowest are: Lemon (0.573 ± 0.006 mg/g db), Kumquat (0.308 ± 0.003 mg/g db), Tonkan, Ponkan, Murcott, and Liucheng (0.104 ± 0.001–0.173 ± 0.002 mg/g db), Peiyou (0.087 ± 0.007 mg/g db), and Wendun (0.061 ± 0.001 mg/g db). In kaempferol content, Murcott and Tonkan had the most (1.035 ± 0.007 and 0.870 ± 0.016 mg/g db, respectively), Wendun and Peiyou had the least (0.012 ± 0.001 and 0.009 ± 0.004 mg/g db, respectively), and other varieties ranged from 0.503 ± 0.009 to 0.611 ± 0.012 mg/g db.

According to the results of Kawaii et al. (1999), of the eight varieties we tested, kaempferol was only detected in Kumquat (0.161–0.321 mg/g dried matter). It was not detectable in the other seven varieties. Rutin was only detected in Lemon (0.227 mg/g dried matter), Liucheng (0.035–0.137 mg/g dried matter), and Kumquat (0–0.339 mg/g dried matter). Rutin in the other five varieties was not detectable. In our study, the kaempferol level in Kumquat (0.235 ± 0.002 mg/g db) was similar to the results of Kawaii et al. (1999) (0.161–0.321 mg/g dried matter) but, in the other seven varieties, the kaempferol level ranged from 0.009 ± 0.004 to 1.04 ± 0.007 mg/g db. This differs from the results of Kawaii et al. (1999) who found that it was not detectable. Rutin was found in all varieties in our study, but a low level (0.042–0.090 mg/g db). Here again the results differ from those of Kawaii et al. (1999).

3.2.4. Phenolic acid

Phenolic acid, such as caffeic acid, chlorogenic acid, ferulic acid, sinapic acid, and ρ-coumaric acid, in the edible portions of the eight varieties were quantified. The results are shown in Table 5. Chlorogenic acid was the major phenolic acid and ranged from 18.8 ± 1.14 to 103 ± 11.5 μg/g db. Wendun and Lemon had the most chlorogenic acid (103 ± 11.5 and 92.6 ± 8.90 μg/g db, respectively). Caffeic acid in the eight varieties ranged from 9.39 ± 0.85 to 16.5 ± 1.70 μg/g db; Kumquat was not detectable. The fruits ordered on the basis of ferulic acid content (highest to lowest) were Peiyou (67.7 ± 3.69 μg/g db), Wendun (37.9 ± 1.65 μg/g db), and Liucheng, Lemon, Murcott, Ponkan, Tonkan, and Kumquat (4.92 ± 0.82–16.5 ± 1.14 μg/g db). Ordered on the basis of sinapic acid, they were Lemon (72.1 ± 2.67 μg/g db), Murcott (50.1 ± 7.44 μg/g db), Tonkan, Wendun, Ponkan, Peiyou, and Kumquat (11.5 ± 1.64–32.4 ± 2.56 μg/g db), and Liucheng (9.67 ± 0.57 μg/g db). ρ-Coumaric acid content varied from 13.1 ± 1.71 to 50.3 ± 2.47 μg/g db, and was highest in Ponkan.

The study of Rapisarda et al. (1998) found that ferulic acid (3.77 mg/100 ml) was the main phenolic acid in Liucheng (Valencia late) juices. Caffeic acid (0.21 mg/100 ml), sinapic acid (0.90 mg/100 ml), and ρ-coumaric acid (0.80 mg/100 ml) were also quantified, but chlorogenic acid was not detectable. Manthey and Grohmann (2001) obtained similar results to those of Rapisarda et al. (1998), with ferulic acid, ρ-coumaric acid, and sinapic acid levels at 6.1, 0.7, and 0.7 mg/100 ml, respectively, in Rhode Red Valencia juice. In our study, chlorogenic acid was the main phenolic acid in the eight varieties (8.77 ± 1.14–103 ± 11.5 μg/g db), rather than ferulic acid, as found by Rapisarda et al. (1998) and Manthey and Grohmann (2001). The caffeic acid level (9.39 ± 0.85–16.5 ± 1.70 μg/

Table 5
Phenolic acid contents (μg/g db[a]) in the edible portions of citrus fruits

Fruit	Caffeic acid	Chlorogenic acid	Ferulic acid	Sinapic acid	ρ-Coumaric acid
Ponkan	12.4 ± 0.82[b]	41.2 ± 2.47	14.0 ± 1.65	18.1 ± 1.65	50.3 ± 2.47
Tonkan	9.39 ± 0.85	70.8 ± 8.53	12.0 ± 0.85	32.4 ± 2.56	22.7 ± 5.12
Murcott	10.8 ± 0.68	62.9 ± 2.03	14.9 ± 2.03	50.1 ± 7.44	22.3 ± 1.35
Wendun	11.5 ± 1.65	103 ± 11.5	37.9 ± 1.65	22.2 ± 3.29	20.6 ± 1.65
Peiyou	12.3 ± 2.46	52.9 ± 4.92	67.7 ± 3.69	13.5 ± 1.23	16.0 ± 2.46
Kumquat	ND[c]	50.0 ± 0.82	4.92 ± 0.82	11.5 ± 1.64	22.1 ± 0.82
Liucheng	16.5 ± 1.70	18.8 ± 1.14	16.5 ± 1.14	9.67 ± 0.57	13.1 ± 1.71
Lemon	15.1 ± 1.78	92.6 ± 8.90	16.0 ± 3.56	72.1 ± 2.67	35.6 ± 2.67

[a] Dried base.
[b] Data presented are in means ± standard deviation ($n = 3$).
[c] Not detectable.

g db) in our study was similar to that found by Rapisarda et al. (1998), but the levels of ferulic acid (4.92 ± 0.82–67.7 ± 3.69 μg/g db), sinapic acid (6.67 ± 0.57–72.1 ± 2.67 μg/g db) and ρ-coumaric acid (13.1 ± 1.71–50.3 ± 2.47 μg/g db) in the eight varieties were slightly lower than those reported by Rapisarda et al. (1998) and Manthey and Grohmann (2001).

3.3. Carotenoid composition

The levels of carotenoids, such as lutein, zeaxanthin, β-cryptoxanthin, and β-carotene, in the eight varieties were also examined. The results are shown in Table 6, β-cryptoxanthin was the major carotenoid in the eight varieties except Ponkan and Liucheng. In β-cryptoxanthin content, Murcott was the highest (6.67 ± 0.329 μg/g db), followed, in order, by Ponkan, Tonkan, and Lemon (2.61 ± 0.115–4.02 ± 0.190 μg/g db), Kumquat, Peiyou, Liucheng, and Wenden (0.764 ± 0.031–1.83 ± 0.088 μg/g db). The fruits, ordered on the basis of lutein content, were: Ponkan (4.50 ± 0.191 μg/g db), Tonkan and Liucheng (1.86 ± 0.086 and 1.77 ± 0.079 μg/g db, respectively), Murcott (0.766 ± 0.035 μg/g db), Peiyou, Wendun, Kumquat, and Lemon (0.097 ± 0.003–0.146 ± 0.006 μg/g db). In zeaxanthin content, Murcott had the highest (2.94 ± 0.137 μg/g db), followed in order by Liucheng, Ponkan, Tonkan, and Peiyou (1.20 ± 0.052–1.89 ± 0.033 μg/g db), Wendun, Lemon, and Kumquat (0.104 ± 0.002–0.844 ± 0.037 μg/g db). On the basis of β-carotene content, the order was Murcott (3.77 ± 0.154 μg/g db), Ponkan (2.18 ± 0.088 μg/g db), and the others (0.435 ± 0.016–1.63 ± 0.076 μg/g db).

In the references cited, lutein, β-cryptoxanthin, and β-carotene levels in Valencia orange juice were found to be 36–124, 44–195, and 12–38 μg/100 ml, respectively, reported by Mouly, Gaydou, and Corsetti (1999, 1999), 26.6, 8.6, and 1.9 μg/100 ml, respectively, by Stewart (1977); and 64, 83, and 14 μg/100 g wet weight, respectively, by Hart and Scott (1995). The three carotenoid compounds were found in Tangerine (Tonkan) juice at the levels of 174, 189, and 42.5 μg/100 ml, respectively and in Murcott juice at the levels of 85.5, 426, and 43.5 μg/100 ml, respectively (Stewart, 1977). The zeaxanthin level in Valencia juice was found to be 8 μg/100 ml by Pupin et al. (1999) and 50 μg/100 g wet weight by Hart and Scott (1995), respectively. In these studies, β-cryptoxanthin was found to be the major carotenoid in citrus fruit. In our study, β-cryptoxanthin was found to be the major carotenoid in the eight varieties, with the exception of Ponkan and Liucheng. Both the lutein and β-carotene levels in Liucheng in our study were similar to those found by Mouly et al. (1999, 1999) and Stewart (1977); the β-cryptoxanthin level in Liucheng in our study was similar to that found by Stewart (1977) but lower than that of Mouly et al. (1999, 1999). Both lutein and β-cryptoxanthin levels in Tonkan and Murcott in our study were much lower than those of Stewart (1977), but the β-carotene level we obtained was similar to his results.

3.4. Ascorbic acid and pectin contents

Table 7 shows ascorbic acid levels, which did not vary much (5.97 ± 0.18–14.2 ± 0.21 mg/g db) between the fruits. In the references cited, vitamin C levels in *C. sinensis* (L.) Osbeck, *C. limon* (L.) Bur, and Kumquat were 47.7–57.7, 47.9, and 51.3 mg/100 g fresh matter, respectively (Gorinstein et al., 2001; Rapisarda et al., 1999; Vinci et al., 1995). In our study, the levels in the same varieties (5.97 ± 0.18, 11.3 ± 0.32, and 6.77 ± 0.06 mg/g db, respectively) were higher than those levels.

Table 7
Ascorbic acid and pectin contents in the edible portions of citrus fruits

Fruit	Ascorbic acid (mg/g db[a])[b]	Pectin (mg/g db)	
		Total pectin	Water-soluble pectin
Ponkan	8.09 ± 0.06	40.4 ± 1.65	21.4 ± 0.90
Tonkan	12.5 ± 0.09	54.6 ± 1.71	28.2 ± 1.28
Murcott	7.12 ± 0.09	51.4 ± 2.03	27.7 ± 1.22
Wendun	10.4 ± 0.06	76.5 ± 3.29	39.5 ± 1.81
Peiyou	14.2 ± 0.21	87.3 ± 3.69	38.1 ± 1.72
Kumquat	6.77 ± 0.06	58.2 ± 2.46	27.1 ± 1.36
Liucheng	5.97 ± 0.18	44.9 ± 2.26	25.0 ± 1.02
Lemon	11.3 ± 0.32	60.6 ± 2.67	31.2 ± 1.07

[a] Dried base.
[b] Data presented are in means ± standard deviation ($n = 3$).

Table 6
Carotenoid contents (μg/g db[a]) in the edible portions of citrus fruits

Fruit	Lutein	Zeaxanthin	β-Cryptoxanthin	β-Carotene
Ponkan	4.50 ± 0.191[b]	1.46 ± 0.061	4.02 ± 0.190	2.18 ± 0.088
Tonkan	1.86 ± 0.086	1.33 ± 0.058	3.45 ± 0.235	1.63 ± 0.076
Murcott	0.766 ± 0.035	2.94 ± 0.137	6.67 ± 0.329	3.77 ± 0.154
Wendun	0.113 ± 0.005	0.844 ± 0.037	0.764 ± 0.031	0.679 ± 0.037
Peiyou	0.146 ± 0.006	1.20 ± 0.052	1.39 ± 0.065	0.841 ± 0.045
Kumquat	0.099 ± 0.004	0.104 ± 0.002	1.83 ± 0.088	1.31 ± 0.051
Liucheng	1.77 ± 0.079	1.89 ± 0.033	1.07 ± 0.036	0.435 ± 0.016
Lemon	0.097 ± 0.003	0.458 ± 0.016	2.61 ± 0.115	1.12 ± 0.043

[a] Dried base.
[b] Data presented are in means ± standard deviation ($n = 3$).

Pectins are complex polysaccharides that are classified into three types depending upon their solubility: water-soluble, chelator-soluble, and alcohol-soluble. Table 7 shows the total and water soluble pectin contents of our fruits. Wendun and Peiyou contained the most total pectin and water-soluble pectin: 76.5 ± 3.29 mg/g db and 39.5 ± 1.81 mg/g db, respectively, for Wendun, and 87.3 ± 3.69 mg/g db and 38.1 ± 1.72 mg/g db, respectively, for Peiyou. The levels of total pectin and water-soluble pectin in the other six fruits were not very different: 40.4 ± 1.65–60.6 ± 2.67 and 21.4 ± 0.09–31.2 ± 1.07 mg/g db, respectively. In the peel of Meyer lemon, Marsh White grapefruit, Dancy tangerine, and Marrs orange, pectin was in a range of 1.91 ± 0.11 to 5.29 ± 0.04% of fresh matter; in the segment membrane, it was 3.69 ± 0.30–5.10 ± 0.25% of fresh matter (Liu et al., 2001). These levels were similar to those found in the edible portions of our fruits (Table 7).

4. Conclusion

In this study, bioactive compounds in the edible portions of eight varieties of citrus fruits cultivated in Taiwan have been examined. It was found that the most abundant compounds in the specific varieties were hesperidin in Liucheng and Ponkan, naringin in Peiyou and Wendum, diosmin in Kumquat, quercetin in Lemon, kaempferol in Murcott, chlorogenic acid in Wendum and Lemon, lutein in Ponkan, and β-cryptoxanthin, β-carotene and zeaxanthin in Murcott. In the references cited, diosmin, sinensetinin, and kaempferol were only detectable in Lemon, Liucheng, and Kumquat, respectively. However, these compounds were all detectable at various levels in the eight varieties of citrus fruits we examined.

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