Natural Product Sciences
17(4) : 337-341 (2011)

Simultaneous Determination of Flavanone Glycosides in the Fruit of *Citrus paradisi* and *C. grandis* by HPLC-PDA

Xiang-Lan Piao[1], Qian Wu[1], Saem Han[2], Hyun Young Kim[3,*], and Sanghyun Lee[2,*]

[1]*Institute of Chinese Minority Traditional Medicine, Minzu University of China, Beijing 100081, China*
[2]*Department of Integrative Plant Science, Chung-Ang University, Anseong 456-756, Korea*
[3]*Department of Food Science, Gyeongnam National University of Science and Technology, Jinju 660-758, Korea*

Abstract – An HPLC (high-performance liquid chromatography)-PDA (photodiode array) detection method was established for the determination of naringin, hesperidin and neohesperidin in the fruit of *Citrus paradisi* and *C. grandis*. The flavonoids were separated in less than 20 min using an YMC RP 18 column with isocratic elution using acetonitrile and water (23 : 77, v/v) at a flow rate of 1 ml/min, and a PDA detector. The levels of naringin, hesperidin and neohesperidin were 1345.92, 950.62, and 2078.82 μg/g, respectively, in the peel, and 102.43, 59.13, and 86.68 μg/g, respectively, in the flesh of *C. paradisi*. In *C. grandis*, the levels of naringin, hesperidin and neohesperidin were 3530.56, 80.00, and 5.26 μg/g, respectively, in the peel, and 59.59, 7.43, and 38.41 μg/g, respectively, in the flesh. The total content was highest in the peel, reaching 0.44% and 0.36% in *C. paradisi* and *C. grandis*, respectively, while the flesh contained only 0.025% and 0.011%, respectively. Therefore, the peels of *C. paradisi* and *C. grandis* are necessary for the processing and utilization of flavonoids.
Keywords – *Citrus grandis*, *Citrus paradisi*, Hesperidin, HPLC, Naringin, Neohesperidin

Introduction

Flavonoids are widespread in the plant kingdom and comprise a large group of naturally occurring compounds found in all vascular plants. They are present in *Citrus* fruit as well as in other fruits, vegetables, seeds, tea, and wine (Han *et al.*, 2010; Havsteen, 1983). Over the past few years, several experimental studies have demonstrated the biological and pharmacological properties of many flavonoids, especially their anti-inflammatory (Middleton *et al.*, 2000), antioxidant (Packer *et al.*, 1999) and antitumor (Inoue and Jackson, 1999) effects, which are associated with free radical-scavenging actions. Interest has been focused on the health benefits derived from food with antioxidant activity. Biologically active constituents can be found in fruits and may play a role in reducing the risk of degenerative diseases related to oxidative stress (Gerber *et al.*, 2002; Patel *et al.*, 2004).

These compounds not only play important physiological and ecological roles, but are also of commercial interest because they have a multitude of applications in the food and pharmaceutical industries. For example, naringin, hesperidin and neohesperidin have been isolated from Fructus Aurantii Immaturus and *Citrus* fruits (Miller *et al.*, 2008; Wang *et al.*, 2010). They inhibit oral carcinogenesis (Miller *et al.*, 2008), and possess anticancer, cardiovascular, and anti-inflammatory activity (Benavente-Garcia and Castillo, 2008). They have also been reported to be potent free radical scavengers and iron chelators (Yoo *et al.*, 2009). Naringin and hesperidin may also act as antioxidants and anti-inflammatory agents (Berkarda *et al.*, 1998; Kobayashi and Tanabe, 2006; Zhang *et al.*, 2001). Thus, flavonoids have been described as health-promoting and disease-preventing dietary supplements (Middleton *et al.*, 2000). Recently, dietary supplements and herbal products containing flavonoids have become very popular due to increased public interest in health-promotion and disease-prevention. In general, the content and distribution of flavonoids varies largely across *Citrus* species depending on genetic and environmental factors.

C. paradisi cv. Changshan Huyou (Rutaceae) is the hybrid of *C. grandis* Osbeck and *C. sinensi* Osbeck. *C. paradisi* and *C. grandis* have been cultivated mainly in

*Author for correspondence
Tel: +82-55-751-3277; E-mail: hykim@gntech.ac.kr (H. Y. Kim)
*Author for correspondence
Tel: +82-31-670-4688; E-mail: slee@cau.ac.kr (S. Lee)

Guangdong, Guangxi, Fujian, Zhejiang, Sichuan and Hunan in China (Ren *et al.*, 2009), and are popular autumn and winter fruits in China (Zhang *et al.*, 2001). There are many reports on the isolation of limonoids, coumarins, and flavonoids from *Citrus* spp. (Cho *et al.*, 2000). These components have various effects, including antitumorigenic activity (Song *et al.*, 2006; Wang *et al.*, 2008), cough remedy and expectorant activity (Zheng, 1998), antibacterial activity (Zhao *et al.*, 2003b), and antioxidant activity (Nafisi *et al.*, 2008; Zhao *et al.*, 2003a; Xu *et al.*, 2007).

High-performance liquid chromatography (HPLC) and its coupling with other techniques, particularly photodiode array (PDA) detection, is a convenient, widely used, and powerful approach for the rapid identification of constituents in botanic extracts. Naringin, hesperidin, and neohesperidin are main and important compounds in *Citrus* spp. A previous paper focused on the content analysis of naringin, hesperidin, and neohesperidin in *C. junos* by HPLC and HPLC/MS (Woo *et al.*, 2006). In the present study, we focused on the quantitative determination of naringin, hesperidin and neohesperidin in *C. paradise* and *C. grandis*, and used HPLC-PDA coupled methods for the simultaneous determination of these constituents.

Experimental

Materials and reagents – The fruits of *C. paradisi* and *C. grandis* were purchased from a Chaoshifa Supermarket in Beijing, China and were identified by our laboratory. Voucher specimens (No. CP200911-01, CG200911-01) were deposited at the Isolation and Structure Identification Laboratory in Minzu University, China. Acetonitrile was purchased from Fisher Chemical, USA. Water for the HPLC analysis was purified using the Milli-Q water purification system (Millipore Corporate, France). All other reagents were of analytical grade and were used without further purification.

Isolation of standards – The fruit of *C. junos* (10 kg) was extracted with methanol under reflux. The methanol extracts (980 g) were partitioned between dichloromethane and water. The aqueous layer was further fractionated with *n*-butanol. A portion of the *n*-butanol fraction (20 g) was chromatographed on a silica gel column (No. 7734, 6 × 80 cm) using a gradient solvent system with a chloroform-methanol-water (90 : 20 : 1 → methanol) solvent system to afford 7 sub-fractions. A chromatographic separation of sub-fraction 4 led to the isolation of compounds **1** - **3**. The structures of compounds **1** - **3** were identified as naringin (**1**), hesperidin (**2**), and neohesperidin (**3**) by spectroscopic analysis (Cho *et al.*, 2000).

Compound **1**: $C_{27}H_{32}O_{14}$; FAB-MS: *m/z* 581 $[M + 1]^+$; ^{1}H-NMR (500 Hz, DMSO-d_6): 12.04 (1H, s, 5-OH), 9.62 (1H, s, 4'-OH), 7.32 (2H, d, *J* = 7.8 Hz, H-2',6'), 6.79 (2H, d, *J* = 7.8 Hz, H-3',5'), 6.11 (1H, d, *J* = 2.2 Hz, H-6), 6.08 (1H, d, *J* = 2.2 Hz, H-8), 5.53 (1H, dd, *J* = 11.6, 2.9 Hz, H-2), 5.11 (2H, m, glc-1, rha-1), 3.20 (1H, m, H-3b), 2.72 (1H, dd, *J* = 17.0, 5.3 Hz, H-3a), 1.15 (3H, d, *J* = 6.1 Hz, rha-6); ^{13}C-NMR (125 MHz, DMSO-d_6): 197.7 (C-4), 165.2 (C-7), 163.4 (C-5), 163.2 (C-9), 158.2 (C-4'), 129.0 (C-1'), 128.9 (C-2',6'), 115.6 (C-3',5'), 103.7 (C-10), 100.8 (glc-1), 97.8 (rha-1), 96.7 (C-6), 95.5 (C-8), 79.1 (C-2), 77.5 (glc-3), 77.3 (glc-5), 76.5 (glc-2), 72.2 (rha-4), 70.9 (rha-2), 70.8 (rha-3), 70.0 (glc-4), 68.7 (rha-5), 60.9 (glc-6), 42.4 (C-3), 18.5 (rha-6).

Compound **2**: $C_{28}H_{34}O_{15}$; FAB-MS: *m/z* 581 $[M + 1]^+$; ^{1}H-NMR (500 Hz, DMSO-d_6): 12.02 (1H, s, 5-OH), 9.10 (1H, s, 3'-OH), 6.93 (3H, m, H-2',5',6'), 6.14 (1H, d, *J* = 2.1 Hz, H-6), 6.12 (1H, d, *J* = 2.1 Hz, H-8), 5.50 (1H, dd, *J* = 12.3, 3.0 Hz, H-2), 4.96 (1H, br s, glc-1), 4.52 (1H, br s, rha-1), 3.77 (3H, s, 4'-OMe), 3.25 (1H, m, H-3b), 2.76 (1H, dd, *J* = 17.1, 5.5 Hz, H-3a), 1.08 (3H, d, *J* = 6.2 Hz, rha-6); ^{13}C-NMR (125 MHz, DMSO-d_6): 197.0 (C-4), 165.1 (C-7), 163.0 (C-5), 162.5 (C-9), 147.9 (C-4'), 146.4 (C-3'), 130.9 (C-1'), 117.9 (C-6'), 114.1 (C-2'), 112.1 (C-5'), 103.3 (C-10), 100.6 (rha-1), 99.4 (glc-1), 96.4 (C-6), 95.6 (C-8), 78.4 (C-2), 76.3 (glc-3), 75.5 (glc-5), 73.0 (glc-2), 72.1 (rha-4), 70.7 (glc-4), 70.3 (rha-2), 69.6 (rha-3), 68.3 (rha-5), 66.0 (glc-6), 55.7 (4'-OMe), 42.2 (C-3), 17.8 (rha-6).

Compound **3**: $C_{28}H_{34}O_{15}$; FAB-MS: *m/z* 581 $[M + 1]^+$; ^{1}H-NMR (500 Hz, DMSO-d_6): 12.07 (1H, s, 5-OH), 9.18

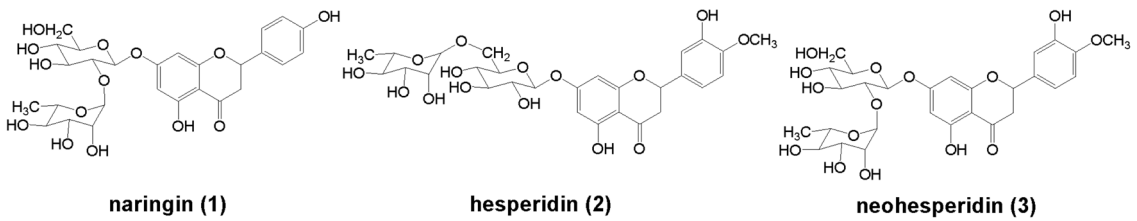

Fig. 1. Chemical structures of flavonoids from *C. junos*.

(1H, s, 3'-OH), 7.00 (1H, m, H-2'), 6.99 (1H, m, H-5'), 6.94 (1H, m, H-6'), 6.17 (1H, d, J = 2.3 Hz, H-8), 5.54 (1H, dd, J = 12.1, 3.0 Hz, H-2), 5.18 (1H, d, J = 3.5 Hz, glc-1), 5.16 (1H, d, J = 3.7 Hz, rha-1), 3.83 (3H, s, 4'-OMe), 3.28 (1H, m, H-3b), 2.80 (1H, dd, J = 17.1, 5.2 Hz, H-3a, 1.22 (3H, d, J = 6.2 Hz, rha-6); ^{13}C-NMR (125 MHz, DMSO-d_6): 197.2 (C-4), 164.8 (C-7), 162.9 (C-5), 162.7 (C-9), 148.1 (C-4'), 146.5 (C-3'), 130.9 (C-1'), 118.0 (C-6'), 114.2 (C-2'), 112.0 (C-5'), 100.5 (glc-1), 103.4 (C-10), 97.0 (rha-1), 96.3 (C-6), 95.2 (C-8), 78.5 (C-2), 77.2 (glc-3), 76.9 (glc-5), 76.2 (glc-2), 71.9 (rha-4), 70.4 (rha-2), 70.4 (rha-3), 69.6 (glc-4), 68.4 (rha-5), 60.5 (glc-6), 55.7 (4'-OMe), 42.2 (C-3), 18.1 (rha-6).

Apparatus and chromatographic conditions – Analysis was performed using a Shimadzu HPLC system (Shimadzu Corporation, Tokyo, Japan), consisting of two model LC-20A pumps coupled with a SIL-20A autosampler, a SPD-M20 photodiode array detector, a CTO-10AS column oven and a CBM-20A system controller. Separations were carried out on an YMC-Pack ODS-A column (250 × 4.6 mm i.d., 5 μm) (Kyoto, Japan). The mobile phase was composed of (A)-water and (B)-acetonitrile with an isocratic elution system (23% B). The flow rate of the mobile phase was 1.0 ml/min, and the temperature was maintained at 25 ºC. The components were quantified based on peak areas at the maximum wavelength in their UV spectrum.

Preparation of standard solutions – Standard stock solutions of naringin, hesperidin and neohesperidin were directly prepared in methanol. Working standard solutions containing the three compounds were prepared and diluted with methanol to appropriate concentrations for the establishment of calibration curves. The standard stock solutions and working solutions were all prepared in dark brown calibrated flasks and stored at 4 ºC. The linearity of the responses was determined for six concentrations. The injection volume for HPLC analysis was 5 μl. LCsolution software was used to prepare the standard curves from the peak area of each compound. The content of these constituents in the test samples was calculated using the regression parameters obtained from the standard curves.

Preparation of sample solutions – The peel and flesh were extracted with pure methanol in 30 min of sonication. The extraction procedure was repeated three times. The three successive extracts were mixed and filtered through a 0.45 μm filter. The final concentration of the fresh peel and flesh was equal to 0.5 g crude/ml methanol. The methanol extracts were sealed in a vial and retained in a refrigerator (4 ºC) until use. The sample injection volume for HPLC analysis was 5 μl.

Recovery test – In order to evaluate the accuracy of the proposed methods, a recovery test was performed by adding known amounts of reference standard solutions to the samples before extraction, followed by analysis using the proposed method. Three concentrations of accurately determined amounts of standard naringin were used to spike the peel position, and then naringin was extracted and analyzed as described in the previous paragraph. The recovery percentage was calculated according to the formula: recovery (%) = (total amount after spiking original amount in sample)/spiked amount × 100%.

Results and Discussion

An HPLC method was successfully developed to analyze naringin, hesperidin and neohesperidin (Fig. 1) in the peel and flesh of *C. paradisi* and *C. grandis* with a PDA detector. With the PDA detector, the UV spectra of the three constituents could be compared with those of authentic standards. The desired compounds from the peel and flesh of *C. paradisi* and *C. grandis* were identified by comparing both the retention times and UV spectra with those of the authentic standards. The identity of the analytes was further confirmed by spiking the actual sample with the standards. The excellent agreement between standard and sample spectra found in the peel and flesh positions of *C. paradisi* and *C. grandis* indicates that under the proposed analytical conditions, the three marker constituents were sufficiently resolved, and separated successfully by isocratic elution in less than 20 min. There was no interference from other components in the matrix.

The assay linearity was determined by the analysis of six different concentrations of the standard solutions. Table 1 shows the regression data and limit of detection

Table 1. Regression data and LODs for the flavonoids (n = 6)

Compound	Regression equation	Correlation coefficient (r)	Linear range (μg/ml)	LOD (μg/ml) (S/N = 3)
1	Y = 5189.7787X + 18495.14	0.9995	3.22-1030	0.00258
2	Y = 522.7624X + 619.97	0.9998	0.59-380	0.05937
3	Y = 3789.0986X + 10934.22	0.9998	0.077-990	0.03867

Table 2. Stability and precision data for the proposed HPLC method

Sample	Position (0.5 g/ml)	Compound	Stability	Precision	
				Intra-day	Inter-day
			RSD (%)	RSD (n = 6, %)	RSD (n = 6, %)
C. paradisi	Peel	**1**	4.18	2.31	3.09
		2	3.94	3.82	1.91
		3	4.33	1.62	3.45
	Flesh	**1**		1.96	4.87
		2		1.89	5.04
		3		5.40	4.73
C. grandis	Peel	**1**	0.29	3.78	1.42
		2	2.03	5.05	2.43
		3	1.91	6.00	1.89
	Flesh	**1**		4.01	5.36
		2		4.64	ND
		3		2.91	ND

*ND, not detected.

(LOD) (S/N = 3) of the components. All calibration curves showed a linear trend (r > 0.9995) within the test ranges. The method was validated for specificity, accuracy, precision, and limits of detection. The naringin, hesperidin and neohesperidin, were well separated, with linear ranges of 3.22 - 1030, 0.59 - 380 and 0.077 -990 μg/ml, respectively. The relative standard deviation (RSD) was considered an indicator of precision and accuracy. Intra- and inter-day precision was determined by assaying sample solutions at concentrations of 0.5 g/ml during a single day and on six different days. As shown in Table 2, the overall intra- and inter-day variations were less than 4% for all 3 analytes. These results demonstrate that the present method is reproducible with good precision. Tests of accuracy were based on analyte recovery. Recovery of naringin was within the range of 95.5%-103.9% and 105.2%-107.2% in the peels of *C. paradisi* and *C. grandis*, with RSDs of 1.54% and 4.96% (n = 3), respectively. These values indicate that the method is acceptable with respect to recovery. A stability test was performed with sample solutions placed under 4 ºC, with analysis at 0, 3, 6, 9, 12, and 24 h. The RSD values of the peak area and retention times were no more than 4.4% and 2.1% in the peel extracts of *C. paradisi* and *C. grandis*, respectively. The solution was therefore considered to be stable for at least 24 h at 4 ºC.

The developed assay was subsequently applied to the simultaneous determination of the three major compounds in *C. paradisi* and *C. grandis*. The quantity of each identified compound is summarized in Table 3. These data indicate that the proposed method is suitable for the simultaneous determination of naringin, hesperidin and neohesperidin in *C. paradisi* and *C. grandis*.

In conclusion, the peel makes up 34.50% and 20.65% by weight of the total fruit of *C. paradisi* and *C. grandis*. The major flavonoids, naringin, hesperidin and neohes-

Table 3. Flavonoid content of the peel and flesh of *C. paradisi* and *C. grandis* (n = 3)

Sample	Position	Compound	Content (μg/g)
C. paradisi	Peel	**1**	1345.92 ± 1.37
		2	950.62 ± 5.37
		3	2078.82 ± 1.86
	Flesh	**1**	102.43 ± 4.65
		2	59.13 ± 1.22
		3	86.68 ± 1.72
C. grandis	Peel	**1**	3530.56 ± 5.81
		2	80.00 ± 2.21
		3	5.26 ± 4.88
	Flesh	**1**	59.59 ± 7.13
		2	7.43 ± 2.19
		3	38.41 ± 5.72

peridin, were separated in less than 20 min by HPLC with isocratic elution using acetonitrile and water (23 : 77, v/v) at a flow rate of 1 ml/min with a PDA detector. The contents were the highest, reaching 0.44% and 0.36%, in the peels of *C. paradisi* and *C. grandis*, while the flesh contained only 0.025% and 0.011% flavonoids, respectively.

There was report on the content of neohesperidin, naringin, and hesperidin in premature Korean citrus fruits harvested at different time (Rhyu *et al.*, 2002). Quantitative analysis of the flavanone glycosides and the peroxynitrite scavenging effect of five Oriental medicinal drugs (Aurantii nobilis Pericarpium, Citrii unshiu Pericarpium, Citrii unshiu Semen, Aurantii Fructus, Poncirii Fructus) was conducted. This report is focused on the relationship between the hesperidin content and the peroxynitrite scavenging effect (Nugroho *et al.*, 2009). Consequently, the relationship between the content of flavonoids and activity is important in the field of pharmaceutical and nutraceutical materials. Therefore, the peels of *C. paradisi* and *C. grandis* are necessary for the processing and utilization of flavonoids.

Acknowledgment

This work was supported financially by the NSFC (No. 30973960; No. 30671522), a grant from the State Ethnic Affairs Commission (No. 09ZY17), HYKJ (No. 2006BAD12B05-10) and a Young Teacher Research Grant from Minzu University of China in 2008.

References

Benavente-Garcia, O. and Castillo, J., Update on uses and properties of citrus flavonoids: new findings in anticancer, cardiovascular, and anti-inflammatory activity. *J. Agric. Food Chem.* **56**, 6185-6205 (2008).

Berkarda, B., Koyuncu, H., Soybir, G., and Baykut, F., Inhibitory effect of hesperidin on tumour initiation and promotion in mouse skin. *Res. Exp. Med.* **198**, 93-99 (1998).

Cho, E.J., Piao, X., Piao, L., Piao, H., Park, M.K., Kim, B.K., and Park, J.H., Chemical constituents of the fruit of *Citrus junos*. *Nat. Prod. Sci* . **6**, 179-182 (2000).

Gerber, M., Boutron-Ruault, M.C., Hercberg, S., Riboli, E., Scalbert, A., and Siess, M.H., Food and cancer: State of the art about the protective effect of fruits and vegetables. *Bull. Cancer* **89**, 293-312 (2002).

Han, S., Kim, H.M., Lee, J.M., Mok, S.Y., and Lee, S., Isolation and identification of polymethoxyflavones from the hybrid *Citrus*, hallabong. *J. Agric. Food Chem.* **58**, 9488-9491 (2010).

Havsteen, B., Flavonoids, a class of natural products of high pharmacological potency. *Biochem. Pharmacol.* **32**, 1141-1148 (1983).

Inoue, T. and Jackson, E.K., Strong antiproliferative effects of baicalein in cultured rat hepatic stellate cells. *Eur. J. Pharmacol.* **378**, 129-135 (1999).

Kobayashi, S. and Tanabe, S., Evaluation of the anti-allergic activity of *Citrus unshiu* using rat basophilic leukemia RBL-2H3 cells as well as basophils of patients with seasonal allergic rhinitis to pollen. *Int. J. Mol. Med.* **17**, 511-515 (2006).

Middleton, E., Jr. Kandaswami, C., and Theoharides, T.C., The effects of plant flavonoids on mammalian cells: implications for inflammation, heart disease, and cancer. *Pharmacol. Rev.* **52**, 673-751 (2000).

Miller, E.G., Peacock, J.J., Bourland, T.C., Taylor, S.E., Wright, J.M., Patil, B.S., and Miller, E.G., Inhibition of oral carcinogenesis by citrus flavonoids. *Nut. Cancer* **60**, 69-74 (2008).

Nafisi, S., Hashemi, M., Rajabi, M., and Tajmir-Riahi, H.A., DNA adducts with antioxidant flavonoids: morin, apigenin, and naringin. *DNA Cell Biol.* **27**, 433-442 (2008).

Nugroho, A., Park, M.G., Jin, S.E., Choi, J.S., and Park, H.J., Quantitative analysis of flavanone glycosides and peroxynitrite scavenging effect of the five Oriental medicinal drugs (Aurantii nobilis Pericarpium, Citrii unshiu Pericarpium, Citrii unshiu Semen, Aurantii Fructus, Poncirii Fructus). *Kor. J. Pharmacogn.* **40**, 370-375 (2009).

Packer, L., Rimbach, G., and Virgili, F., Antioxidant activity and biologic properties of a procyanidin-rich extract from pine (*Pinus maritima*) bark, pycnogenol. *Free Rad. Biol. Med.* **27**, 704-724 (1999).

Patel, A.V., Rojas-Vera, J., and Dacke, C.G., Therapeutic constituents and actions of *Rubus* species. *Curr. Med. Chem.* **11**, 1501-1512 (2004).

Ren, Y.J., Zhang, H.L., and Li, J.Y., Study on the chemical components and pharmacological action of *Citrus paradisi* cv. Changshan Huyou. *J. Anhui Agric. Sci.* **37**, 9976-9977 (2009).

Rhyu, M.R., Kim, E.Y., Bae, I.Y, and Park, Y.K., Contents of naringin, hesperidin, and neohesperidin in premature Korean Citrus fruits. *Korean J. Food Sci. Technol.* **34**, 132-135 (2002).

Song, H., Shi, W.J., Meng, X.Y., and He, L., Research progress in anticancer function of dietary fiber. *Cereals and Oils* **5**, 46-48 (2006).

Wang, C., Pan, Y., Fan, G., Chai, Y., and Wu, Y., Application of an efficient strategy based on MAE, HPLC-DAD-MS/MS and HSCCC for the rapid extraction, identification, separation and purification of flavonoids from Fructus Aurantii Immaturus. *Biomed. Chromatogr.* **24**, 235-244 (2010).

Wang, L.G., Jiang, L.Y., Huang, G.H., and Zhang, X.Q., Extraction and application of dietary fiber from *Pomelo* peel. *J. Anhui Agric. Sci.* **36**, 10492-10494 (2008).

Woo, K.L., Kim, J.I., Kim, M.C., and Chang, D.K., Determination of flavonoid and limonoid compounds in citron (*Citrus junos* Sieb. et Tanaka) seeds by HPLC and HPLC/MS. *J. Korean Soc. Food Sci. Nutr.* **35**, 353-358 (2006).

Xu, G.H., Ye, X.Q., Xu, G.N., Hu, Y.X., and Liu, D.H., Effect of microwave treatment on the phenolic acids and flavanone glycosides contents and antioxidant capacity in Huyou peels. *J. Chinese Inst. Food Sci. Technol.* **7**, 69-74 (2007).

Yoo, K.M., Hwang, I.K., Park, J.H., and Moon, B., Major phytochemical composition of 3 native Korean *Citrus* varieties and bioactive activity on V79-4 cells induced by oxidative stress. *J. Food Sci.* **74**, C462-C468 (2009).

Zhang, T.P., Peng, S.L., Wang, Z.F., and Chen, B.C., Introduction to the study and protection of *Pomelo germplasm*. *Ecol. Sci.* **20**, 8-13 (2001).

Zhao, X.M., Ye, X.Q., and Xi, Y.F., Study on the extraction of flavonoids and antioxidation in Huyou peels. *J. Fruit Sci.* **20**, 261-265 (2003a).

Zhao, X.M., Ye, X.Q., Zhu, D.Y., and Jiang, S.H., Preliminary study on volatile oil chemical composition and antibacterial activity of peel of *Citrus paradise*. *China J. Chinese Mat. Med.* **28**, 1087-1089 (2003b).

Zheng, S.H., Preparation and application of Hu You Pi Ba Cough Syrup. *J. Jiangxi College of Trad. Chinese Med.* **10**, 128 (1998).

Received September 2, 2011
Revised November 30, 2011
Accepted December 10, 2011