DIETARY SUPPLEMENTS

Simultaneous Quantification of Adrenergic Amines and Flavonoids in *C. aurantium*, Various *Citrus* Species, and Dietary Supplements by Liquid Chromatography

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An analytical method was developed for the simultaneous quantitative analysis of 6 amines and 20 flavonoids in fruits and extracts of 30 *Citrus* species, including *C. aurantium*, near-*Citrus* relatives, and dietary supplements by liquid chromatography with photodiode array detection. The separation was achieved with a Phenomenex Synergi Hydro reversed-phase column using gradient mobile phase of sodium acetate buffer (pH 5.5) and acetonitrile. Elution was run at a flow rate of 1.0 mL/min and UV at 254, 280, and 330 nm. Among the amines analyzed, synephrine, the main component, was present in the levels from 0.11 to 2.0 mg/g dry weight in 21 *Citrus* species and 0.07 to 18.62% in dietary supplements claiming to contain *C. aurantium*. The flavanones and flavones were analyzed in the same *Citrus* samples and were species-specific. The levels of flavones were very low compared with those of flavanones. The method facilitated the simultaneous quantification of 6 amines and 20 flavonoids in various *Citrus* species, the distinction between the different *Citrus* species, and the analysis of dietary supplements containing *C. aurantium*.

Citrus species are known for the accumulation of various physiologically active compounds such as flavonoids, adrenergic amines, coumarins, and limonoids (1). Synephrine and other adrenergic amines in the fruits/peels of *C. aurantium* or in *Citrus* species stimulate lipolysis and elevate metabolic rate. The oxidation of fat through increased thermogenesis may reduce the fat mass in obese humans (2–4). Due to the recent ban of *Ephedra* species in dietary supplements by the U.S. Food and Drug Administration, concerning its association with stroke, heart attacks, hypertension, and psychiatric problems (5), manufacturers have begun replacing ephedra with *C. aurantium*. Many of these weight-loss preparations contain *C. aurantium* standardized to 6 or 8% synephrine. In a recent study in which an extract of *C. aurantium* (6% synephrine) was administered for 6 weeks, there was no marked effect on blood pressure in humans (5). The antidepressant-like effect of (+)-synephrine was also demonstrated (6).

With respect to the *Citrus* flavonoids, 3 different classes occur, i.e., flavanones, flavones, and flavonols. They are present either in free-form or as their sugar conjugates. In *Citrus* species, including *C. aurantium*, flavanones are present in large quantities, whereas the amounts of flavones and flavonols are very small (7). Their chromatographic pattern is specific to each species (8). The flavonoids in fruits and peels of *Citrus* species have well-documented pharmacological activities, including anticancer, antiviral, anti-oxidant, anti-inflammatory, anti-allergic, analgesic, and antimicrobial activities, as well as the ability to reduce capillary fragility and to inhibit human platelet aggregation (9).

Several researchers have reported a number of analytical methods in the literature for the analysis of synephrine alone or synephrine with other amines (3, 10–13). Likewise, there are methods reported for the *Citrus* flavonoids (3, 7–8, 12–20) by high-performance liquid chromatography (LC). However, no method has been developed for the simultaneous analysis of 6 adrenergic amines and 20 flavonoids in *Citrus* species by LC with photodiode array (PDA) detection.

This study reports a simple LC method that detects and quantifies 6 amines: octopamine (**1**), synephrine (**2**), tyramine (**3**), *n*-methyl tyramine (**4**), hordenine (**5**), and methoxy synephrine (**6**) and 20 flavonoids: 6,8-di-*C*-glucosyl apigenin (**7**), rutin (**8**), neoeriocitrin (**9**), 5,7,4′ trihydroxy-8, 3′-dimethoxyflavone-3-*O*-[3-hydroxy-3-methylglutaryl(1-6)]-β-D-glucoside (**10**), narirutin (**11**), naringin (**12**), hesperidin (**13**), neodiosmin (**14**), neohesperidin (**15**), neoponcirin (**16**), poncirin (**17**), naringenin (**18**), apigenin (**19**), hesperitin (**20**), sinensetin (**21**), 5,7,8,4′-tetramethoxy flavone (**22**), nobiletin (**23**), 3,5,6,7,8,3′,4′-heptamethoxyflavone (**24**), tangeretin (**25**), and 5-*O*-demethylnobiletin (**26**) (Figure 1). The compounds

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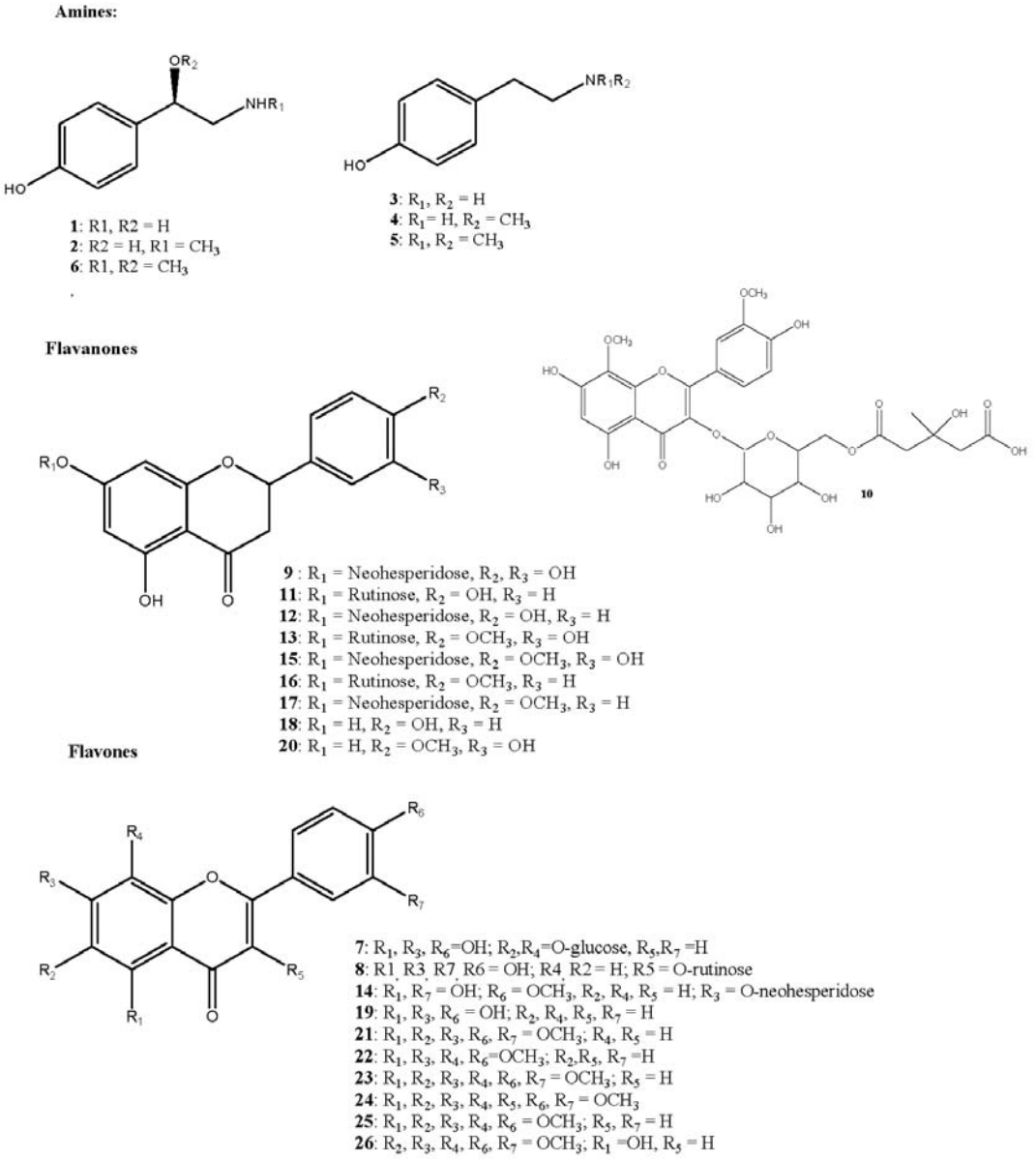

Figure 1. Structures of amines and flavonoids.

were numbered by the order of elution. The analytical method was applied for fruits/peels of various *Citrus* species/hybrids, near-*Citrus* relatives and dietary supplements claiming to contain *Citrus* species.

The present work had 4 objectives: to characterize the most suitable agent for the extraction of flavonoids and amines from the plant material; to optimize the analytical method used for all the flavonoids and adrenergic amines; to simultaneously determine the flavonoids and amines present in various *Citrus* species, near-*Citrus* relatives, and dietary supplements; and to contribute to the better understanding of the species differences in *Citrus* and near-*Citrus* relatives by their amine and flavonoid pattern.

METHOD

Instrumentation and Chromatographic Conditions

(**a**) *LC system*.—Waters (Waters Corp., Milford, MA) models were as follows: 6000A pumps, U6K injector, 680 automated gradient controller, 996 photodiode array detector, and a computerized data station equipped with Waters Millennium software. Separation was achieved on a Synergi Hydro-RP 80A column (Phenomenex, Torrance, CA; 250 × 4.6 mm id, 4 μm particle size) and operated at 30°C. The column was equipped with a 2 cm LC18 guard column (Supelco, Bellefonte, PA).

(**b**) *Mobile phase*.—Consisted of 0.1 M sodium acetate buffer with pH adjusted to 5.5 with glacial acetic acid (A) and acetonitrile (B), which were applied in the following gradient

elution: 0 min, 100% A held for 8 min; in the next 10 min to 80% A: 20% B; then for 17 min 70% A: 30% B; then for 10 min it is adjusted to 35% A: 65% B; finally in the next 5 min to 100% B and held at that composition for another 5 min. Each run was followed by a 5 min wash with 100% acetonitrile and an equilibration period of 15 min. The flow rate was adjusted to 1.0 mL/min. The wavelengths used for quantification of amines and flavonoids with the diode array detector were 254 nm for **8**, **14**, and **24**; 280 nm for compounds **1–7**, **9–13**, **15–20**, **22**, **23**, **25**, and **26**; and 330 nm for **21**. The total run time for analysis was 49.6 min.

Chemicals

(**a**) *Standard compounds*.—Compounds **1–3**, **5**, **8**, **9**, **15, 17**, and **19** were purchased from Sigma (St. Louis, MO); **11–13**, **16**, **20**, **21**, and **25** were purchased from Chromadex (Santa Ana, CA); **14** and **18** were purchased from Indofine (Belle Mead, NJ); and compounds **4**, **6**, **7**, **10**, **22–24**, and **26** were isolated at National Center for Natural Products Research (NCNPR). Their identity and purity were confirmed by thin-layer chromatography and LC methods and compared with published spectral data (infrared, nuclear magnetic resonance, and high-resolution mass spectrometry.

(**b**) *Acetonitrile, glacial acetic acid, and sodium acetate.*—LC grade, purchased from Fisher Scientific (Fair Lawn, NJ). Water for the LC mobile phase was purified in a Milli-Q system (Millipore, Bedford, MA).

(**c**) *C. aurantium products*.—Obtained online. Different species of *Citrus* and hybrids were obtained from Missouri Botanical Garden (St. Louis, MO). *C. aurantium* extracts standardized to contain 4, 6, 10, 30, 90, and 95% synephrine were obtained from different commercial sources. The populations of single *Citrus* species of *C. aurantium* (CA1–CA13), *C. sinensis*, *C. limon*, *C. paradisi*, *C. reticulata*, *C. grandis*, and *C. medica* were obtained from different locations in the United States, People's Republic of China (PRC), India, and Sri Lanka. The peel of *Fortunella* spp. and mature fruit of *C. nobilis* were obtained from local stores. The unripe fruits of *C. aurantium* L. var. *amara* and *C. sinensis* were obtained from the PRC. Voucher specimens of all samples are deposited at the NCNPR, University of Mississippi.

Table 1. Calibration data [regression equation and correlation coefficient (R^2)] and limit of detection (LOD) for compounds 1–26

Analyte	Regression equation	LOD, μg/mL
1	$y = 2.67 \times 10^3 x$	0.02
2	$y = 3.51 \times 10^3 x$	0.03
3	$y = 4.02 \times 10^3 x$	0.02
4	$y = 4.35 \times 10^2 x$	0.05
5	$y = 2.96 \times 10^3 x$	0.05
6	$y = 1.75 \times 10^3 x$	0.30
7	$y = 1.18 \times 10^4 x$	0.05
8	$y = 9.86 \times 10^3 x$	0.05
9	$y = 2.30 \times 10^4 x$	0.04
10	$y = 5.77 \times 10^3 x$	0.30
11	$y = 1.62 \times 10^4 x$	0.05
12	$y = 2.01 \times 10^4 x$	0.03
13	$y = 1.89 \times 10^4 x$	0.02
14	$y = 1.53 \times 10^4 x$	0.05
15	$y = 1.89 \times 10^4 x$	0.02
16	$y = 1.86 \times 10^4 x$	0.07
17	$y = 1.21 \times 10^4 x$	0.01
18	$y = 4.70 \times 10^4 x$	0.02
19	$y = 1.45 \times 10^4 x$	0.20
20	$y = 5.34 \times 10^4 x$	0.02
21	$y = 4.28 \times 10^4 x$	0.03
22	$y = 7.79 \times 10^3 x$	0.20
23	$y = 3.99 \times 10^4 x$	0.03
24	$y = 2.75 \times 10^4 x$	0.01
25	$y = 2.03 \times 10^4 x$	0.05
26	$y = 2.66 \times 10^4 x$	0.03

Plant Materials

The following mature fruits were studied: *C. aurantium* (CS1), *C. karna* (CS2), *C. tachibana* (CS3), *C. sunki* (CS4), *C. maxima* (CS5), *C. reshni* (CS6), *C. natsudaidai* (CS7), *C. pennivesculata* (CS8), *C. unshiu* (CS9), *C. bergamia* (CS10), *C. depressa* (CS11), *C. taiwanica* (CS12), *C. tangeriana* (CS13), *C. hystrix* (CS14), *C. sulcata* (CS15), *C. deliciosa* (CS16), *C. volkameriana* (CS17), *C. macroptera* (CS18), *C. wilsonii* (CS19), *C. reticulata* (CS21), C. *paradisi* (CS22), C. *nobilis* (CS23), *C. medica* (CS24), *C. limon* (CS25), *C. grandis* (CS26), *C. aurantifolia* (CS27), *C. meyeri* (CS28), *C. amblycarpa* (CS29), *C. jambhiri* (CS30), *C. aurantium* L. var. *amara* (CSV), *Poncirus trifoliatus* (PS), mature hybrid fruits of *C. nobilis* X (CH1), *C. reticulata* X *Poncirus* (CH2), *C. aurantium* X *Poncirus* (CH3), *C. aurantium* X *C. myrtifolia* (CH4), *C. paradisi* X (CH5), *C. aurantium* X *Fortunella* (CH6), *C. reticulata* X *C. paradisi* (CH7), unripe fruits of *C. sinensis* (CS20) and *C. aurantium* L. var. *amara* (CSV), various populations within a single *Citrus* species (ripe and unripe fruits), mature peel of *Fortunella* spp. (FS), mature fruit of unknown *Citrus* species (CSU), and *C. aurantium* extracts standardized to contain 4, 6, 10, 30, 90, and 95% synephrine (CE1-CE6).

Standard Solution

Individual stock solutions of amines and flavonoids were prepared at a concentration of 0.5 mg/mL in methanol. The quantification was performed using 7 levels of external standards. The ranges obtained were 0.9–10.0 to 80–30 μg/mL, depending on the concentration of each stock solution. Table 1 shows the calibration data and calculated limit of detection (determined by serial dilution based on a

signal-to-noise ratio of 3:1) and calibration curves generated by linear regression based on peak area.

Sample Preparation

Finely powdered dried fruits or peels of *Citrus* species (0.3 g) or an average weight of 5 powdered tablets or capsules were extracted twice (2.0 mL each) with a mixture of methanol–DMSO (1 + 1) by sonication for 20 min, followed by centrifugation for 15 min at 9000 rpm. The supernatant was transferred to a 10 mL volumetric flask. The extraction was repeated 3 times with water, and the respective supernatants were combined. The final volume was adjusted to 10 mL with water. All solutions were centrifuged for 10 min at 9000 rpm, and the clear supernatant was collected in an LC sample vial. Each sample solution was injected in triplicate.

Results and Discussion

Chromatographic Conditions

Developing an LC method for the analysis of 6 adrenergic amines and 20 flavonoids (flavanones and polymethoxylated flavones) in *Citrus* species, *Citrus* hybrids, near-*Citrus* relatives, and dietary supplements in one run was difficult to achieve because the compounds of interest exhibited a wide range of polarity. The adrenergic amines **1**–**6** are all rather polar compounds; on the other hand, compounds from **7** to **26** are much less polar and can be eluted with a high percentage of organic solvent in the mobile phase. The glycosylated flavanones eluted first, followed by their aglycones, which are less polar and hence eluted later.

Optimal chromatographic conditions were obtained with a reversed-phase C18 column. Composition of the mobile phase contained an acidic buffer system to improve the peak symmetry of all compounds. The buffer system also increased the retention of the compounds (especially **1** and **2**). Hence, 0.1 M acetate buffer and acetonitrile were used as the mobile phase. The different columns tried were Synergi 4 μ Max-RP 80A, Aqua 5 μ C18 200 A, Luna 5 μ C18(2) 100A, Lichrospher 5 RP18, Synergi 4 μ Hydro-RP 80A, Synergi 4 μ Polar-RP 80A, Capcell PAK SCX UG80A 5 μ, and Supelcosil LC-SCX 5 μ. However, most of the column materials tested could not resolve compounds satisfactorily. The standards **1**–**6** showed very short retention times and resolution of flavonones, and flavones were not complete with Aqua, Luna, Lichrospher, Capcell, Synergi-Polar RP, Synergi-MAX RP, and Supelcosil columns. The best separations were obtained with the Synergi Hydro-RP column using sodium acetate buffer (pH 5.5) and acetonitrile as the mobile phase. Solvents other than acetonitrile or the addition of modifiers such as tetrahydrofuran or methyl *t*-butyl ether did not improve the separation. Variation of the column temperature between 25° and 40°C did not cause a significant change in the resolution; however, changes in the retention time were observed. Thus, an optimum temperature of 30°C at a flow rate of 1.0 mL/min was chosen. The method allowed separation of 6 amines and 20 flavonoids in less than 50 min. A chromatogram of the amines and flavonoids is shown in Figure 2.

Optimization of the Amine and Flavonoids Extraction Solvent in Citrus Samples

The standard compounds exhibited a wide range of polarity. The amines were much more polar than the flavonones and flavones. To determine the most suitable solvent for flavonoids and amine extraction in the *Citrus* species (fruit/peel), the relative content of compounds **2**, **12**, and **15** in *Citrus* extracts with different solvents was studied: water; methanol; water–methanol (1 + 1); water–methanol (2 + 1); water–methanol (1 + 2); and methanol–DMSO (1 + 1) and water. Solvents with organic nature gave the best results for both the flavonoid compounds (**12**, **15**); water gave the best results for compound **2**. Hence, the most effective solvent for the flavonoids and amines extraction was methanol–DMSO (1 + 1) and water.

Accuracy, Precision and Linearity

The calibration curve showed a linear correlation between sample concentration and peak area. Intra- and interday variation were determined with standards. It was performed 3 times on 3 different days, and each concentration point was injected in triplicate. Purity of the standards was confirmed by the PDA data of all peaks of interest. To determine the accuracy of the method, one sample was spiked with a known

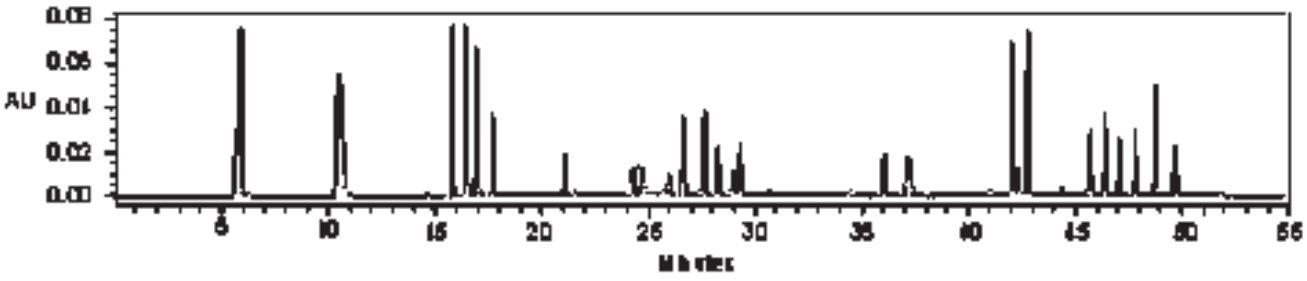

Figure 2. Typical HPLC chromatogram of pure amine and flavonoid standards (1–26) at 280 nm.

Table 2. Division of genera *Citrus*, *Poncirus*, and *Fortunella* species according to the amine and major flavonoids

Species code	Species name	Compounds	
		Amines	Major flavonoids
CS1	*C. aurantium*	1, 2, 3, 4	9, 12, 15
CS2	*C. karna*	1, 2, 3, 4	11, 13
CS3	*C. tachibana*	1, 2, 3	11, 13, 21, 23, 25
CS4	*C. sunki*	1, 2, 3, 4	11, 13, 16, 22, 23, 25
CS5	*C. maxima*	3, 4	9, 11, 12, 17
CS6	*C. reshni*	1, 2	11, 13, 16, 22, 23, 25
CS7	*C. natsudaidai*	1, 2	11, 12, 15, 17
CS8	*C. pennivesculata*	1, 2	8, 9, 11, 13, 15, 22, 24
CS9	*C. unshiu*	1, 2	11, 13, 16
CS10	*C. bergamia*	2	12, 15
CS11	*C. depressa*	1, 2, 3, 4	13, 22, 23, 25
CS12	*C. taiwanica*	2, 3, 4	12, 13, 14, 15, 17, 21
CS13	*C. tangerina*	2	11, 13, 16, 25
CS14	*C. hystrix*	3	13, 15
CS15	*C. sulcata*	2	11, 13, 24
CS16	*C. deliciosa*	1, 2	13, 16, 23, 25
CS17	*C. volkameriana*	2, 3	13, 23
CS18	*C. macroptera*	—	11, 13
CS19	*C. wilsonii*	—	11, 12, 13, 14, 15, 17
CS20	*C. sinensis*	2	11, 13, 16, 21, 23
CS21	*C. reticulata*	2	11, 13, 16, 23, 25
CS22	*C. paradisi*	—	11, 12, 17, 21, 24
CS23	*C. nobilis*	2, 3	11, 13, 16, 23, 25
CS24	*C. medica*	—	11, 12, 21
CS25	*C. limon*	—	13
CS26	*C. grandis*	—	12, 21
CS27	*C. aurantifolia*	—	11, 13, 21
CS28	*C. meyeri*	1	11, 13
CS29	*C. amblycarpa*	2	11, 13, 15, 21, 23, 24
CS30	*C. jambhiri*	2	11, 13
CSV	*C. aurantium* var *amara*	2	9, 12, 15, 17, 21
PS	*Poncirus trifoliatus*	—	11, 12, 16, 17
FS	*Fortunella* spp.	—	8, 9, 12, 16, 17
CH1	Hybrid (*C. nobilis* X)	1, 2, 4	11, 13, 16, 22, 23, 25
CH2	Hybrid (*C. reticulata* X *Poncirus*)	1, 4	11, 13, 16, 24, 25
CH3	Hybrid (*C. aurantium* X *Poncirus*)	1, 2	11, 12, 15, 17
CH4	Hybrid (*C. aurantium* X *C. myrtifolia*)	2	11, 12, 14, 15
CH5	Hybrid (*C. paradisi* X)	1, 3	11, 12, 13, 15, 17
CH6	Hybrid (*C. aurantium* X *Fortunella*)	2	8, 9, 13, 16, 17
CH7	Hybrid (*C. reticulata* X *C. paradisi*)	2, 3	11, 13, 16, 22, 24
CSU	Unknown *Citrus* species	2, 3	9, 11, 13, 23, 25

Table 3. Amine or flavonoid content in various *Citrus* species, hybrids, and related genera[a]

Compound	CS1	CS2	CS3	CS4	CS5	CS6	CS7	CS8	CS9	CS10
1	0.02	0.05	0.08	0.04	ND[b]	0.03	0.05	0.04	0.02	ND
2	0.5	0.5	1.3	0.5	ND	1.94	0.11	0.75	0.95	0.30
3	0.03	0.02	0.03	0.02	0.02	DUL[c]	DUL	DUL	ND	ND
4	0.17	3.26	DUL	0.13	0.36	ND	ND	DUL	ND	ND
5	ND	DUL	ND	ND	ND	ND	ND	ND	ND	ND
6	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
7	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
8	DUL	ND	ND	ND	ND	0.16	ND	5.48	ND	0.04
9	4.9	ND	0.04	ND	0.26	ND	0.21	9.78	ND	0.08
10	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
11	0.40	3.60	0.71	0.12	2.20	0.65	0.42	0.73	2.44	0.39
12	9.40	ND	ND	0.02	21.5	ND	7.54	ND	ND	4.77
13	0.08	28.20	12.70	13.3	DUL	23.2	0.09	29.8	11.9	DUL
14	0.53	ND	ND	ND	ND	0.01	ND	ND	ND	0.83
15	10.3	ND	ND	ND	ND	ND	2.47	1.32	ND	4.37
16	0.02	0.07	0.40	0.11	0.05	0.43	DUL	DUL	0.47	ND
17	0.13	ND	ND	ND	0.54	ND	0.29	ND	ND	0.06
18	0.02	DUL	ND	0.002	0.001	ND	0.001	ND	DUL	DUL
19	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
20	0.005	0.002	ND	0.002	0.006	0.009	0.003	ND	DUL	0.003
21	0.1	ND	0.5	0.08	0.09	0.06	0.05	0.03	DUL	0.07
22	0.03	DUL	DUL	0.49	ND	0.33	0.02	4.06	0.02	0.02
23	0.09	0.01	2.02	0.42	DUL	0.39	0.03	0.74	0.03	0.04
24	0.02	ND	ND	ND	0.07	0.07	0.02	6.67	0.31	DUL
25	0.06	0.02	3.90	1.37	0.01	0.94	0.08	0.99	0.03	0.03
26	0.027	0.005	0.16	0.042	ND	0.009	0.008	0.02	ND	0.005

Compound	CS11	CS12	CS13	CS14	CS15	CS16	CS17	CS18	CS19	CS20
1	0.02	DUL	ND	ND	DUL	0.03	DUL	ND	DUL	DUL
2	2.0	0.38	1.11	ND	0.49	0.42	1.07	ND	ND	1.64
3	0.02	0.03	ND	DUL	ND	ND	0.01	ND	DUL	DUL
4	0.05	0.03	ND	ND	DUL	ND	DUL	DUL	DUL	DUL
5	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
6	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
7	DUL	DUL	DUL	DUL	ND	ND	ND	DUL	ND	ND
8	ND	0.007	ND	DUL	ND	ND	0.003	ND	ND	0.008
9	ND	ND	ND	0.05	ND	0.02	DUL	ND	ND	DUL
10	DUL	0.01	ND	ND	ND	ND	ND	ND	DUL	0.01
11	0.03	0.79	2.83	0.43	11.70	0.16	0.05	1.66	2.34	6.55
12	ND	12.4	ND	0.02	0.06	0.03	ND	0.05	48.59	DUL
13	10.1	1.04	5.46	4.12	21.2	16.4	11.06	8.47	3.43	29.3
14	ND	4.68	ND	0.09	ND	ND	ND	ND	11.6	ND
15	ND	23.5	0.02	5.29	DUL	ND	ND	ND	57.3	0
16	0.14	ND	0.43	DUL	ND	0.3	0.02	0.1	0.13	0.41
17	ND	12.0	ND	ND	ND	ND	DUL	ND	1.94	ND
18	ND	ND	0.001	ND	ND	ND	0.001	ND	0.02	ND
19	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND

Table 3. (*continued*)

Compound	CS11	CS12	CS13	CS14	CS15	CS16	CS17	CS18	CS19	CS20
20	0.005	0.011	DUL	ND	ND	0.001	0.001	ND	DUL	ND
21	0.31	0.47	0.03	DUL	0.01	0.04	DUL	0.03	0.05	1.23
22	1.51	0.05	0.03	DUL	0.09	0.08	ND	DUL	0.02	0.07
23	1.87	0.01	0.02	DUL	0.28	0.38	0.19	DUL	0.08	1.11
24	DUL	0.14	0.02	ND	1.23	0.07	0.09	ND	0.13	0.21
25	1.87	0.14	0.19	DUL	0.29	0.55	0.07	DUL	0.10	0.27
26	0.07	0.004	0.006	0.03	0.003	0.005	0.06	DUL	0.01	0.011

Compound	CS21	CS22	CS23	CS24	CS25	CS26	CS27	CS28	CS29	CS30
1	ND	ND	DUL	DUL	DUL	ND	ND	0.04	ND	ND
2	1.75	ND	0.95	ND	ND	ND	ND	ND	0.19	0.33
3	DUL	DUL	0.12	ND	ND	ND	ND	ND	ND	ND
4	DUL	DUL	DUL	ND	ND	ND	ND	DUL	DUL	DUL
5	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
6	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
7	ND	DUL	ND	ND	ND	ND	ND	ND	ND	ND
8	ND	ND	ND	0.08	ND	ND	ND	ND	ND	ND
9	DUL	DUL	0.01	ND	DUL	DUL	ND	ND	ND	ND
10	ND	ND	0.02	ND	ND	ND	ND	ND	0.01	ND
11	8.70	2.39	6.49	4.60	0.12	DUL	1.82	4.0	1.42	0.10
12	ND	17.65	DUL	22.80	ND	16.45	DUL	ND	0.49	ND
13	59.0	DUL	0.79	DUL	9.92	DUL	16.29	10.3	4.84	6.57
14	ND	ND	ND	ND	ND	ND	ND	ND	1.34	ND
15	ND	DUL	DUL	ND	ND	ND	ND	ND	7.66	ND
16	0.29	0.16	3.04	0.08	ND	0.03	0.03	0.13	0.14	0.03
17	ND	2.06	ND	ND	ND	DUL	ND	ND	DUL	ND
18	DUL	DUL	ND	0.01	ND	0.01	ND	ND	ND	DUL
19	ND	DUL	ND	ND	ND	ND	ND	ND	ND	ND
20	DUL	DUL	ND	0.02	ND	DUL	ND	DUL	DUL	0.001
21	0.89	0.17	0.39	0.32	DUL	0.34	0.15	0.002	0.32	ND
22	0.91	0.04	0.31	ND	ND	ND	DUL	0.05	0.05	0.01
23	5.42	0.12	2.19	DUL	DUL	ND	0.05	0.006	0.41	0.08
24	0.12	0.23	0.53	ND	ND	DUL	ND	DUL	0.24	0.05
25	4.27	0.09	1.78	0.01	DUL	DUL	DUL	0.01	0.05	0.03
26	0.94	DUL	0.10	0.08	ND	ND	DUL	DUL	0.15	0.06

Compound	CSV	CH1	CH2	CH3	CH4	CH5	CH6	CH7	CSU	FS	PS
1	DUL	0.06	0.017	0.02	DUL	0.03	ND	ND	ND	DUL	ND
2	1.59	0.5	ND	0.19	1.72	ND	DUL	0.35	0.68	DUL	ND
3	DUL	DUL	DUL	ND	DUL	0.05	ND	0.02	0.03	DUL	DUL
4	DUL	0.45	0.46	DUL	DUL	DUL	ND	DUL	DUL	DUL	DUL
5	DUL	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
6	DUL	ND	ND	DUL	ND	ND	ND	ND	ND	ND	ND
7	DUL	ND	ND	DUL	DUL	ND	ND	ND	ND	ND	ND
8	0.13	ND	ND	0.02	0.21	ND	0.85	ND	ND	3.03	ND
9	2.67	DUL	ND	DUL	0.51	ND	1.93	0.08	6.59	0.66	DUL
10	0.11	ND	ND	0.039	DUL	ND	ND	ND	ND	DUL	ND
11	0.81	0.39	3.59	1.47	1.67	2.77	0.14	0.41	0.26	0.01	1.18

Table 3. (*continued*)

Compound	CSV	CH1	CH2	CH3	CH4	CH5	CH6	CH7	CSU	FS	PS
12	40.74	ND	ND	6.56	19.56	7.47	DUL	0.01	0.03	0.59	7.11
13	0.64	28.23	4.40	0.08	0.04	0.96	1.29	8.11	1.21	0.04	DUL
14	1.51	ND	DUL	0.05	3.15	1.28	ND	ND	ND	DUL	DUL
15	38.06	ND	ND	2.87	16.88	6.44	DUL	ND	DUL	DUL	DUL
16	0.07	2.03	12.26	0.63	0.04	ND	0.94	0.20	0.008	0.79	1.98
17	1.13	ND	DUL	4.09	0.06	0.41	0.29	ND	ND	0.20	16.81
18	0.09	0.001	0.001	ND	0.005	0.001	DUL	DUL	DUL	DUL	DUL
19	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
20	0.07	0.006	0.001	ND	0.01	0.003	ND	DUL	DUL	DUL	DUL
21	0.34	0.07	0.02	0.18	0.14	0.05	DUL	DUL	0.05	ND	ND
22	0.08	0.42	DUL	0.11	0.05	0.006	0.009	0.40	0.05	0.002	ND
23	0.19	0.66	0.014	0.06	0.14	0.02	ND	0.07	0.88	0.002	ND
24	0.03	0.13	0.29	0.06	0.02	0.064	ND	0.14	0.02	0.003	DUL
25	0.19	0.88	0.52	0.09	0.13	0.04	DUL	0.12	0.67	0.01	DUL
26	0.01	0.013	0.03	0.016	ND	DUL	DUL	DUL	DUL	DUL	DUL

[a] All values are given in mg/g of dried sample for $n = 3$.
[b] ND = Not detected.
[c] DUL = Detected under limit of quantification.

amount of the standard compounds and recovery rates were between 98.0 and 103.0%. An indicator for precision is the standard deviation (δ). All samples were injected in triplicate, and the standard deviation of standard compounds was 3.0%. Calibration data (Table 1) indicated the linearity of the detector response for all standard compounds from 0.90 to 130.0 μg/mL. The 5-point calibration curves were linear; least-squares regression gave a good correlation coefficient of 0.9999. The limit of detection (LOD) was between 0.01 and 0.05 μg/mL for the standard compounds with exception of compounds **10**, **19**, and **22**, which were between 0.2 and 0.3 μg/mL.

Citrus Samples Analysis

Identification of the compounds in *Citrus* samples was based on the retention times and the comparison of UV spectra with those of authentic standards. The LC analysis of the mature fruit of various *Citrus* species, hybrids, and near–*Citrus* relatives had revealed the characteristic patterns of amines, flavanones, and polymethoxylated flavones. Flavanones predominated among the *Citrus* flavonoids; amines, flavanone glycosides, and flavones unique to 30 different *Citrus* species, including *C. aurantium*, were examined (Table 3). The method was validated by testing a large number of populations within a single species of *Citrus*. Thirteen populations from unripe and ripe fruits of *C. aurantium* (CA1–CA13; Table 4), 5 from unripe and ripe fruits/peels of *C. sinensis* (CSin1–CSin5; Table 5), 3 from ripe fruits of *C. limon* (CL1–CL3; Table 5), 6 from ripe and unripe fruits/peels of *C. reticulata* (CR1–CR6; Table 6), 4 from ripe and unripe fruits of *C. grandis* (CG1–CG4; Table 6), 2 from ripe peels of *C. paradisi* (CPara1–CPara2; Table 7), and 2 populations from ripe fruits of *C. medica* (CG1–CG2; Table 7) obtained from different locations in the PRC, the United States, India, and Sri Lanka were analyzed. The chromatographic pattern/profile in the different populations of single species looked the same. Their content of amines and flavonoids varied due to several factors, including location, time of harvest, and part of plant used. Immature fruit/peel (CSin2, CA6, CA7, CA9, CR3) contained higher concentration of amines and flavonoids than the fully ripe fruit/peel (CA1, CSin1, CR6).

Analysis of Amines in Citrus Samples and Dietary Supplements

Tables 3 and 8 show the variations in concentrations of amines and flavonoids in 30 *Citrus* species (CS1–CS30), 7 hybrids (CH1–CH7), 1 *Citrus* variety (CSV), 1 unknown *Citrus* species (CSU), and 8 dietary supplements (CP1–CP8; Figure 3). The amines that are present in trace amounts or absent in the various species or hybrids are not shown in Figure 3A. The content of compound **2** was present in the levels from 0.11–2.0 mg/g dry weight in 21 *Citrus* species and 4 hybrids. Highest concentration of compound **2** was present in CS11 (2.0 mg/g dry weight) and lowest concentration in CS7 (0.1 mg/g dry weight). The range of compounds **1** and **3** for the *Citrus* species/hybrids detected was between 0.02–0.08 mg/g dry weight and 0.01–0.12 mg/g dry

Table 4. Amine and flavonoid contents in 13 populations of *C. aurantium* (CA1–CA13)[a]

Compound	CA1	CA2	CA3	CA4	CA5	CA6	CA7	CA8	CA9	CA10	CA11	CA12	CA13
1	DUL[b]	DUL	DUL	0.01	DUL	DUL	DUL	DUL	0.01	DUL	DUL	DUL	DUL
2	0.76	0.81	0.38	1.35	1.11	2.71	4.07	3.8	2.77	0.64	1.0	1.59	1.65
3	0.06	0.09	0.01	0.05	0.05	DUL	0.49	0.12	0.09	0.11	0.10	0.03	0.04
4	0.86	0.74	0.01	0.65	0.5	DUL	1.77	DUL	DUL	DUL	DUL	DUL	DUL
5	ND[c]	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
6	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
7	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
8	0.06	0.06	0.02	0.79	0.02	0.31	DUL	0.16	0.02	DUL	DUL	DUL	DUL
9	1.17	1.15	0.85	2.83	1.04	2.83	10.24	0.33	4.08	0.63	1.14	2.63	1.58
10	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
11	1.81	2.08	1.24	5.74	3.6	10.54	5.26	5.69	6.32	1.86	4.48	3.78	4.12
12	22.6	25.5	21.5	23.9	26.7	40.81	43.59	27.06	32.69	21.59	27.11	20.74	25.61
13	1.37	1.63	2.14	0.3	1.3	5.37	10.47	8.96	2.97	1.37	1.78	0.64	0.65
14	0.16	0.02	0.08	2.0	0.22	0.31	1.12	1.08	0.34	0.42	0.27	1.51	0.02
15	25.1	30.7	33.7	30.1	30.2	51.91	51.8	35.38	44.8	29.02	38.5	28.0	33.42
16	0.11	0.15	0.19	0.24	0.25	0.02	0.46	0.31	0.31	0.15	0.20	0.24	0.25
17	1.6	0.54	1.66	0.19	3.1	10.4	1.59	7.18	7.79	2.83	3.96	1.13	1.76
18	0.03	0.03	0.03	0.04	0.03	0.06	0.1	0.10	0.05	0.03	0.05	0.05	0.05
19	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
20	DUL	DUL	0.4	0.02	0.08	0.17	0.27	0.09	0.13	0.06	DUL	DUL	DUL
21	0.12	0.1	0.04	0.59	0.13	ND	DUL	0.02	0.02	0.01	0.08	0.34	0.08
22	0.20	0.17	0.20	0.22	0.20	0.25	0.31	0.27	0.27	0.15	0.09	0.25	0.25
23	0.15	0.08	0.19	0.4	1.02	0.81	0.49	0.47	0.83	0.44	0.94	0.19	0.05
24	0.04	0.04	0.16	0.07	0.11	0.49	0.03	0.02	0.16	0.12	0.3	0.03	0.002
25	0.17	0.17	0.29	0.35	2.52	1.02	0.67	0.76	1.31	0.45	1.26	0.19	1.32
26	DUL	DUL	0.3	0.13	0.13	0.25	0.17	0.08	0.12	0.12	0.11	0.07	0.99

[a] All values are given in mg/g of dried sample for $n = 3$.
[b] DUL = Detected under limit of quantification.
[c] ND = Not detected.

weight, respectively. Compound **1** in CH1–CH5, CS12, CS15, CS17, CS19, CS20, CS23–25, and CSV; compound **3** in CH1, CH2, CH4, CH5, CH7, CS6–CS8, CS14, CS19–CS22, and CSV; and compound **4** in CS1–CS5, CS8, CH2, CS11–CS12, CH3–CH5, CSU, CS15, CS17–CS23, CSV, and CS28–CS30 were present in trace amounts. The contents of compounds **5** and **6** were present in trace amounts in CSV. In *Citrus* species/hybrids CS5, CS14, CS18, CS19, CS24–CS26, CS27, CS28, CH2, and CH5, compound **2** was not detected. Compounds **1** and **3**–**6** were not detected in all other *Citrus* species.

A study of dietary supplements (tablets and capsules claiming to contain *C. aurantium* extract or plant material) revealed a significant difference in their composition, which may be due to the time of harvest or plant part used. Six of the 8 products listed the quantity of *C. aurantium* herb/extract in each dosage form (range, 85–900 mg). It was observed that among the amines analyzed, compound **2** was the major component. Five products made a label claim for the actual content of compound **2**. It was observed that the quantity of *C. aurantium* herb/extract in products did not appear to correlate with compound **2**; its highest concentration was present in CP5 (18.62%) and lowest concentration was in CP4 (0.073%). Trace amounts of compounds **1** and **3** were detected in CP1 to CP4 and in products CP1 and CP3, respectively. Compound **4** was detected in the range of 0.13–0.16% in all the products except CP3 and CP4; compound **5** was detected in trace amounts in products CP1, CP5, and CP8; compound **6** was not detected in CP1–CP8 products.

Flavanone and Polymethoxylated Flavone Content

Figures 4 and 5 show the variations in *Citrus* species (CS1–CS30) or hybrids (CH1–CH5) or unknown

Table 5. Amine and flavonoid contents in *C. sinensis* and *C. limon* species[a]

Compound	CSin1	CSin2	CSin3	CSin4	CSin5	CL1	CL2	CL3
1	ND[b]	ND	ND	ND	ND	ND	ND	ND
2	0.57	0.97	1.23	1.05	0.73	ND	ND	ND
3	0.03	0.06	DUL[c]	0.06	DUL	ND	DUL	ND
4	DUL	DUL	DUL	0.03	DUL	ND	ND	ND
5	ND	ND	ND	ND	ND	ND	ND	ND
6	ND	ND	ND	ND	ND	ND	ND	ND
7	ND	ND	ND	ND	ND	ND	ND	ND
8	DUL	DUL	DUL	DUL	DUL	ND	ND	ND
9	DUL	0.03	DUL	DUL	DUL	ND	ND	ND
10	ND	ND	ND	ND	ND	ND	ND	ND
11	1.03	3.88	1.17	3.15	5.27	0.15	0.3	0.45
12	0.3	0.02	0.1	0.02	DUL	ND	ND	ND
13	25.8	38.3	27.9	27.4	40.3	9.12	12.9	22.9
14	ND	ND	ND	ND	ND	ND	ND	ND
15	ND	ND	ND	ND	ND	ND	ND	ND
16	0.34	0.45	0.41	0.39	0.47	ND	ND	ND
17	ND	ND	ND	ND	ND	ND	ND	ND
18	ND	ND	ND	ND	ND	ND	ND	ND
19	ND	ND	ND	ND	ND	ND	ND	ND
20	ND	ND	ND	ND	ND	ND	ND	ND
21	0.82	0.56	2.1	0.78	0.52	DUL	DUL	DUL
22	0.05	0.09	0.05	0.06	0.09	ND	ND	ND
23	0.68	0.44	1.04	0.66	0.31	DUL	DUL	DUL
24	0.11	0.11	0.05	0.11	0.05	ND	DUL	ND
25	0.15	0.11	0.15	0.19	0.05	DUL	DUL	DUL
26	0.07	DUL	0.09	DUL	DUL	ND	ND	ND

[a] All values are given in mg/g of dried sample for $n = 3$.
[b] ND = Not detected.
[c] DUL = Detected under limit of quantification.

species (CSU) and 8 dietary supplements (CP1–CP8) for the major flavanone and polymethoxylated flavone contents. Table 2 shows the division of genera *Citrus*, *Poncirus*, *Fortunella* species and hybrids depending on the major compounds. Differences between species in terms of the flavanone glycoside content, particularly compounds **12** and **13**, were observed. Horowitz (21), investigated the structure of *Citrus* flavanones and found that their glycosides have mainly 2 types, rutinoside and neohesperidoside, the flavanones with rutinoside (**11**, **13**, **16**) were tasteless, and the flavanones with neohesperidoside (**9**, **12**, **15**, **17**) were bitter. It was considered likely that most *Citrus* species contained either all rutinoside or neohesperidoside, so that all *Citrus* could be distinguished according to their glycoside form.

The highest compound **13** or rutinoside-containing species are CS2–CS4, CS6, CS8–CS9, CS11, CS13-CS18, CS20, CS21, CS25, and CS27–CS30. The highest concentration of compound **13** was found in CS21 (59.0 mg/g dry weight). On the other hand, the highest **12** or neohesperidoside-containing species are CS1, CS5, CS7, CS10, CS12, CS19, CS22, CS24, and CS26. The highest amount of compound **12** was found in CS19 (48.6 mg/g dry weight). There are some exceptions that contained a considerable amount of both compounds **12** and **13**. These species have mixed glycosylation patterns and are mostly CS1, CS4, CS7, CS12, CS14, CS15, CS18–19, CS29, CSV, CSU, and CH3–CH7. Compound **13** (detected in all 30 *Citrus* species) was the most abundant flavonoid in all the *Citrus* samples analyzed followed by compounds **12** (detected in 19 *Citrus* species), **9** (detected in 16 *Citrus* species), and **15** (detected in 13 *Citrus* species). The contents of compounds **16** and **17** were also helpful in characterization of different *Citrus* species. Both compounds **16** and **17** were

Table 6. Amine and flavonoid contents in *C. reticulata* and *C. grandis* species[a]

Compound	CR1	CR2	CR3	CR4	CR5	CR6	CG1	CG2	CG3	CG4
1	ND[b]	ND	ND	ND	ND	ND	ND	ND	ND	ND
2	2.72	0.92	2.65	0.94	2.73	0.46	ND	ND	ND	ND
3	0.13	0.05	0.35	DUL[c]	0.24	0.03	ND	ND	ND	ND
4	0.43	0.23	DUL	DUL	DUL	DUL	ND	ND	ND	ND
5	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
6	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
7	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
8	ND	ND	ND	ND	ND	DUL	ND	ND	ND	ND
9	DUL	DUL	DUL	DUL	DUL	DUL	DUL	DUL	DUL	DUL
10	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
11	4.06	9.54	13.87	8.67	4.35	3.9	ND	ND	ND	ND
12	ND	ND	ND	ND	ND	DUL	40.59	42.64	18.45	25.44
13	33.83	49.08	69.0	42.72	38.9	27.99	DUL	DUL	DUL	DUL
14	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
15	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
16	0.23	0.25	0.33	0.23	0.21	0.22	0.02	0.04	0.03	0.05
17	ND	ND	ND	ND	ND	ND	DUL	DUL	ND	DUL
18	ND	DUL	ND	ND	DUL	DUL	DUL	DUL	DUL	DUL
19	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
20	ND	DUL	ND	ND	DUL	DUL	DUL	DUL	DUL	DUL
21	0.15	0.03	0.41	0.53	0.42	0.11	DUL	DUL	DUL	DUL
22	0.57	0.63	0.89	0.62	0.55	0.41	ND	ND	ND	ND
23	4.89	1.99	5.22	4.12	3.84	1.34	DUL	DUL	ND	ND
24	DUL	0.77	0.08	DUL	0.1	0.07	DUL	DUL	ND	ND
25	3.68	0.53	4.27	3.01	2.96	0.45	DUL	DUL	DUL	DUL
26	0.53	0.01	0.84	0.31	1.17	0.02	ND	ND	ND	ND

[a] All values are given in mg/g of dried sample for $n = 3$.
[b] ND = Not detected.
[c] DUL = Detected under limit of quantification.

present in CS1, CS5, CS7, CS17, CS19, CS22, CS26, CS29, CSV, and CSU and near-*Citrus* relatives *Fortunella* spp. and *Poncirus trifoliatus*. The content of compound **17** was more than that of compound **16**. *Citrus* species CS2–4, CS6, CS8–9, CS11, CS13–14, CS16, CS18, CS20–21, CS23–24, CS27–28, and CS30 showed the presence of compound **16** only, and species CS10 and CS12 showed only the presence of compound **17**. The highest concentrations of compounds **16** and **17** were found in CS23 and CS22, respectively. The other flavonoids occurred in small amounts in both *Citrus* samples and dietary supplements.

The amounts of polymethoxylated flavones present were very low in CS1, CS2, CS5, CS7, CS9, CS10, CS12–14, CS17–19, CS22, CS24–30, CSV, and CH2–8. The content of compounds **23** and **25** in 10 *Citrus* species, CS3–4, CS6, CS8, CS11, CS15–16, CS20–21, and CS23 and in one hybrid (CH1) were in the ranges of 0.03–0.54% and 0.03–0.43%, respectively. The highest concentrations of compounds **23** and **25** were observed in CS21. In the CSV sample, all 25 compounds were detected except for compound **19**. Fruits of CSV are a good source of compound **12** (40.7 mg/g dry weight) and **15** (38.1 mg/g dry weight).

It is a well-established fact (22) that the nature of a hybrid can be demonstrated by the presence of parent–specific compounds in the hybrid. Amines and flavonoids are important markers for the detection of hybridization of *Citrus* plants. Seven hybrids (CH1–CH7) have been characterized by using amine, flavanone, and polymethoxylated flavone patterns, which were the key feature to detect hybridization of *Citrus* plants. For example, analysis of CH7, a hybrid between *C. reticulata* and *C. paradisi*, showed that while *C. paradisi* (CS22) contained **12** as the major compound, *C. reticulata* (CS21) contained compound **13** as the predominant flavonoid. Both compounds **12** and **13** were present in

Table 7. Amine and flavonoid contents in *C. paradisi* and *C. medica* species[a]

Compound	CPara1	CPara2	CM1	CM2
1	ND[b]	ND	DUL[c]	ND
2	ND	ND	ND	ND
3	DUL	DUL	ND	ND
4	DUL	DUL	ND	ND
5	ND	ND	ND	ND
6	ND	ND	ND	ND
7	DUL	DUL	ND	ND
8	ND	ND	0.22	0.1
9	DUL	DUL	DUL	ND
10	ND	ND	ND	ND
11	2.25	2.73	2.12	1.78
12	15.75	0.78	21.3	18.85
13	DUL	DUL	DUL	DUL
14	ND	ND	ND	ND
15	0.35	0.45	ND	ND
16	1.23	1.54	0.14	DUL
17	2.34	2.65	ND	ND
18	DUL	DUL	0.03	DUL
19	DUL	ND	ND	ND
20	DUL	ND	ND	ND
21	DUL	DUL	DUL	DUL
22	DUL	DUL	ND	ND
23	0.11	0.15	DUL	DUL
24	0.22	0.17	ND	ND
25	0.06	0.015	DUL	DUL
26	DUL	DUL	DUL	DUL

[a] All values are given in mg/g of dried sample for $n = 3$.
[b] ND = Not detected.
[c] DUL = Detected under limit of quantification.

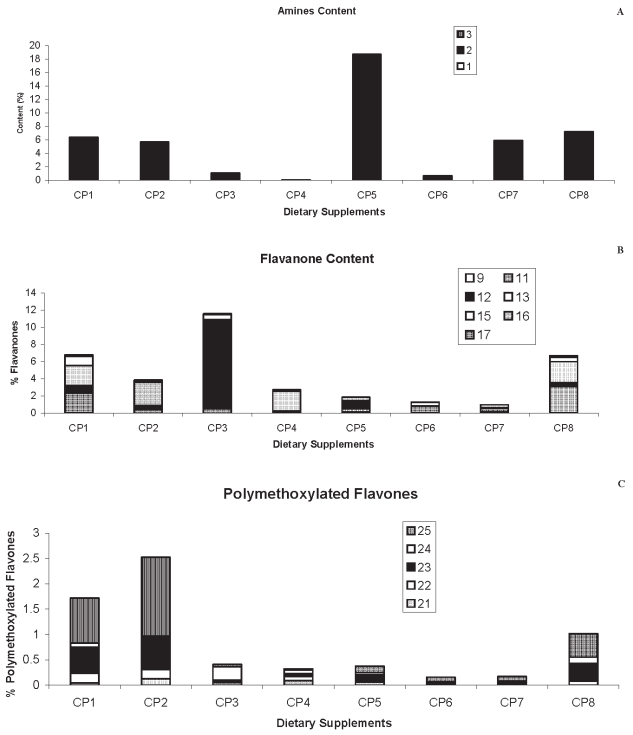

Figure 3. Variations in the concentrations of amines (A), major flavanones (B), and flavones (C) in dietary supplements claiming to contain *C. aurantium*.

substantial amounts in the hybrid (CH7). Compounds **1**, **5**–**8**, **10**, and **14** were not present in either plant and were also absent in the hybrid.

The contents of other compounds present in either plant are seen in the hybrid. *C. aurantium* extracts standardized to contain 4, 6, 10, 30, 90, and 95% synephrine (CE1-CE6) were analyzed. The contents of compound **2** were 4.6, 6.95, 10.99, 34.31, 90.33, and 97.51%, respectively. Other amines were present in trace amounts. CE1–CE3 contained considerable detectable amounts of flavonoids, and CE4-CE6 contained trace amounts of flavonoids and polymethoxylated flavones.

Fortunella and *Poncirus* are considered to be near-*Citrus* relatives, as confirmed by the presence of amines and flavonoids. The peel of *Fortunella* spp. showed the presence of compounds **8**, **9**, **11**–**13**, **16**, **17**, and **25** in detectable amounts and compounds **1**–**4**, **10**, **15**, **18**, **20**, and **26** in trace amounts. The fruits of *P. trifoliatus* showed trace amounts of compounds **3**, **4**, **9**, **13**, **15**, **18**, **20**, and **24**–**26** and detectable amounts of compounds **11**, **12**, **16**, and **17**. The compound **12** was the predominant flavonoid in both the genera *Fortunella* and in *P. trifoliatus*.

Our analysis and literature studies (3, 7–8, 16–18) showed that compound **12** was the predominant flavonoid in *C. aurantium* species (CS1). Products CP1, CP2, and CP4 contained one herbal (*C. aurantium*), CP3 is a combination of 3 herbals (*C. aurantium*, Ma Huang, and Guarana extract), and CP5–CP8 contained various herbals. The products CP3 and CP5 contained compound **12** as the predominant flavonoid. Flavanone compounds **9**, **11**, **13**, and **15** were present in all the dietary supplements. In products CP1, CP2, CP4, and CP6–CP8, compounds **11** and **13** were the major flavonoids. Considerable amounts of polymethoxylated flavones were detected in CP1, CP2, and CP8. The content of compound **13** was more than that of compound **12** in products CP1, CP2, CP4, and CP6–CP8. Similarly polymethoxylated flavone contents were higher in products CP1, CP2, CP4, and CP7–CP8 that in *C. aurantium* species. Results of analysis showed that dietary supplements in which the content of hesperidin was more than the naringin content might be adulterated with other *Citrus* species.

Table 8. Amine and flavonoids in dietary supplements claimed to contain *C. aurantium*[a]

Compound	CP1, %	CP2, %	CP3, %	CP4, %	CP5, %	CP6, %	CP7, %	CP8, %
1	0.024	0.04	DUL[b]	DUL	0.10	0.10	DUL	DUL
2	6.36	5.64	1.12	0.073	18.62	0.58	5.93	7.23
3	0.01	ND[c]	DUL	ND	ND	ND	ND	ND
4	0.11	0.15	ND	ND	0.13	DUL	0.15	0.20
5	ND	ND	ND	ND	DUL	ND	ND	DUL
6	ND	ND	ND	ND	ND	ND	ND	ND
7	ND	ND	ND	ND	ND	ND	ND	ND
8	ND	ND	ND	ND	ND	ND	ND	ND
9	0.07	DUL	DUL	DUL	0.15	0.05	0.16	0.04
10	ND	ND	ND	ND	ND	ND	ND	ND
11	2.31	0.43	0.53	0.22	0.37	0.77	0.43	3.05
12	0.88	0.49	10.42	0.04	0.96	0.05	0.03	0.46
13	2.29	2.66	0.55	2.34	0.38	0.42	0.36	2.47
14	ND	ND	ND	ND	ND	ND	ND	ND
15	1.05	0.19	0.05	0.02	0.02	DUL	0.006	0.52
16	0.17	0.09	DUL	0.12	DUL	0.11	0.09	0.19
17	0.02	DUL	0.08	DUL	DUL	DUL	DUL	DUL
18	0.04	0.06	0.03	DUL	0.22	0.005	0.004	0.06
19	ND	ND	ND	ND	ND	ND	ND	ND
20	0.03	0.01	0.005	0.001	0.65	0.02	DUL	0.14
21	0.04	0.13	0.05	0.09	0.06	0.012	0.012	0.08
22	0.19	0.18	DUL	0.09	DUL	DUL	DUL	DUL
23	0.51	0.66	0.06	0.06	0.15	0.05	0.05	0.35
24	0.09	0.009	0.26	0.07	0.04	0.01	0.01	0.12
25	0.89	1.56	0.05	0.02	0.13	0.09	0.09	0.47
26	0.18	0.29	DUL	0.004	0.02	0.02	0.02	0.12

[a] All values are given in mg/g of dried sample for $n = 3$.
[b] DUL = Detected under limit of quantification.
[c] ND = Not detected.

There was a report (13) on the detection of m-synephrine (phenylephrine) in *C. aurantium* species. Using our method we analyzed for the confirmation of m-synephrine in 2 samples of *C. aurantium*, and our analysis of 3 dietary supplements claiming to contain *C. aurantium* revealed its absence.

This method was applied on the blind sample (CSU) to prove the concept of its usage in identification of various amines and flavonoids in *Citrus* species. The sample CSU showed the presence of compounds **2**, **3**, **9**, **11**–**13**, **15**, **16**, and **21**–**26**. Compound **13** was the predominant flavonoid and exhibited the identical chemical fingerprint with that of CS21.

Conclusions

The developed method permitted the simultaneous quantitative analysis of amines and flavonoids in *Citrus* samples as well as in dietary supplements. It is a useful analytical tool for establishing the quality of fruits/peels of *C. aurantium* preparations and providing distinction between different *Citrus* species.

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