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Changes of Some Flavonoids in the Peel of Late Maturing Citrus during Maturation

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Abstract

Eleven flavonoids, including rutin, naringin, hesperidin, neohesperidin, quercetin, naringenin, kaempferol, hesperetin, nobiletin, 3,5,6,7,8,3′,4′-methoxylated flavone and tangeretin in the peel of late maturing citrus fruit species of Mucott (*smith tangerine*), Singamha (*C. natsudaidai*), Sambogam (*C. sulcata*), Hongpalsak (*C. hassaku*), Seminol (*Dancy tangerine*) and Jawdung (*C. aurantium*) harvested during from August to December were analyzed with HPLC. The mobile phase consisted of water and acetonitrile with 0.5% acetic acid. Wavelength in UV detector was determined at 254 nm. Naringin and neohesperidin content in the peel of Jawdung harvested at the early stage of maturation were 34.02 mg/g and 13.68 mg/g, respectively, and it was highest among the tested citrus fruits. Hesperidin content in the peel of Mucott harvested at the early stage of maturation was 12.48 mg/g. Rutin content of Sambogam harvested at the early stage of maturation was 5.13 mg/g. Quercetin, naringein, kaempferol, nobiletin, 3,5,6,7,8,3′,4′-methoxylated flavone flavonoids were in trace. Flavonoid contents of Singamha, Sambogam and Jawdung were high in the peel of fruits at the early stage of maturation, after which time they decreased rapidly.

Key words: citrus, flavonoid, maturation

INTRODUCTION

Citrus can be regarded as a universal fruit with production in over 100 countries in all six continents (1). The species of citrus cultivated mainly in the region of Jeju is satsuma mandarin (*Citrus unshiu*). Production per group according to their time of maturity in Jeju island is 33,340 tons (5.9%) for the very early group, 470,164 tons (83.5%) for the early group, 49,220 tons (8.7%) for the common group, and 10,617 tons (1.9%) for the late group of total production in 2000 (2).

Flavonoid components of citrus are naringin, hesperidin, neohesperidin, rutin, naringenin, hesperetin, narirutin, nobiletin, tangeretin, sinensetin, natsudadain, didymin, poncirin, eriocitrin, 5,7,4′-methoxylates flavone, 5′-desmethoxy nobiletin, 4′-methoxylated flavone and 3′,4′-methoxylated flavone (3).

On a physiological level, flavonoids have the following crucial function. Matsubara et al. (4) confirmed that narirutin and rarcissin extracted from the peel of *Citrus unshiu* by hot water, which appear to lower blood pressure, are the flavonoids glycosides. Flavonoids such as quercetin, kaempferol, myricetin, apigenin and luteolin have an anticancer and antimutation effect (5,6). Additionally, flavonoids create a sitologically important problem in citrus processing. Due to the bitter taste of naringin (7), it decreases the quality of citrus juice. Hesperidin, the main ingredient in flavonoids, adulterates citrus juice, because it causes white turbidity in juice (8).

Through research of Jeju citrus, Kim et al. reported on some flavonoids of satsuma mandarin (9), and native citrus fruits (10). Woo et al. (11) reported the content of naringin and hesperidin in the rind of satsuma mandarin was much more than that in the flesh of the fruit. Furthermore, the research on the flavonoids of citrus has given too much importance only to 3~4 varieties of flavonoids as well as satsuma mandarin, which has a vast difference in quantity of production.

Therefore, the objective of this study was to offer the basic data on the changes in flavonoids of late maturing citrus fruits by quantitative analysis, using 12 kinds of authentic flavonoid standards potentially contained in citrus fruits.

MATERIALS AND METHODS

Material

All fruits were harvested once a month from August to December on a farm located in Seogwipo, which was

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part of the Citrus Experiment Station. Flavonoid standards including rutin, naringin, hesperidin, neohesperidin, diosmin, quercetin, naringenin, hesperetin, kaempferol, quercitrin and apigenin were purchased from Sigma Co., and the others including nobiletin, 3,5,6,7,8,3′,4′-methoxylated flavone and tangeretin were supplied from the Faculty of Pharmacy, The University of Tokyo.

Sample preparation

All citrus peels of fresh fruit were cut into fine small pieces, and 1 g of the sample was extracted with 10 mL of methanol in a sonic bath for 1 hr at 60~65°C twice. The extracts were made up to 20 mL by methanol. Approximately 1.5 mL of the final extract was filtered using the membrane filter (Whatman, 0.45 μm) prior to the injection (10 μL) into the HPLC system.

HPLC analysis

HPLC system for flavonoid analysis consisted of a Spectrasystem (Spectra-Physics, LC-7000160, USA), P 4000 pump, UV 1000 UV/Vis detector and AS 3500 autosampler. For the analysis of flavonoids in the citrus peel, μBondapak C_{18} Column (300×3.9 mm, I.D.) was used at room temperature. The mobile phase consisted of acetonitrile with 0.5% acetic acid, and water with 0.5% acetic acid. The gradient of acetonitrile (0.5% acetic acid) was from 16 to 35% for 55 min at flow rate of 1 mL/min. UV spectra were recorded at 254 nm.

RESULTS AND DISCUSSION

Analysis and reproducibility of HPLC

Fig. 1 shows the peaks of flavonoid standards including rutin, naringin, hesperidin, neohesperidin, diosmin, quercetin, naringin, hesperetin, kaempferol, nobiletin, 3,5,6,7,8,3′,4′-methoxylated flavone and tangeretin.

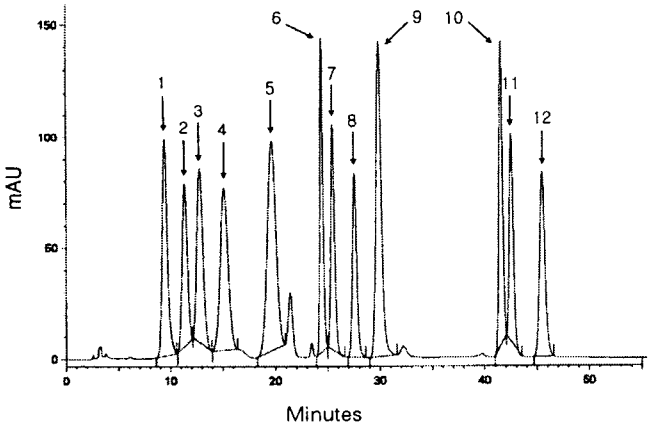

Fig. 1. HPLC chromatogram of standard flavonoids solution with mobile phase (B type).
1: rutin. 2: naringin. 3: hesperidin. 4: neohesperidin. 5: diosmin. 6: quercetin. 7: naringenin. 8: hesperetin. 9: kaempferol. 10: nobiletin. 11: 3,5, 6,7,8,3′,4′-methoxylated flavone. 12: tangeretin.

The results of three time analysis of 10 μL standard solution are presented in Table 1. Rouseff's (12) relative standard deviation (RSD) of the peak area of naringin and neohesperidin were 0.47~1.06 and 0.40~1.27%, respectively. In this work, RSD of the peak areas of naringin and neohesperidin were 1.62 and 0.28%, respectively, and those of the others were between 0.04% rutin and 2.95% quercetin, in the case of the retention time of naringin and neohesperidin were 1.27 and 1.08%, those of the others were between 0.23% hesperetin and 1.33% diosmin. Comparing these results to that of Rouseff, it was adequate except for the RSD of peak area of quercetin.

Peel ratio and total flavonoid of citrus fruits by species and stage of maturation

Fig. 2 shows the changes of the peel ratio and total flavonoid of citrus fruits by cultivars and the stage of maturation. Jawdung had the highest total flavonoid content (48.21 mg/g), and Seminol had the lowest content (11.73 mg/g) in the early stage of maturation. Total flavonoid content in the peels of Mucott (14.15~14.39 mg/g), Seminol (11.73~12.37 mg/g) and Hongpalsak (16.84~17.18 mg/g) were increased somewhat during maturation. The ranges of total flavonoid content of Sambogam, Singamha and Jawdung during maturation were 18.25~10.05, 21.05~9.97 and 48.21~18.32 mg/g, respectively. Jawdung (*C. aurantium*) and Seminol (*Dancy tangerine*) harvested in December had the highest in 58.41% and the lowest in 21.70% peel ratio among samples. The ranges of the peel ratio of Mucott, Sambogam, Singamha, Seminol, Jawdung and Hongpalsak were 56.69~40.68, 44.98~38.60, 53.05~34.33, 47.52~21.70, 72.66~58.41 and 46.81~33.94% during maturation, respectively. The

Table 1. Means and their relative standard deviation of peak areas and retention times by HPLC

	Peak area		Retention time (min)	
	Mean	RSD (%)	Mean	RSD (%)
Rutin	31.0×10^5	0.04	8.14	1.13
Naringin	22.4×10^5	1.62	9.81	1.27
Hesperidin	25.9×10^5	1.92	11.10	1.27
Neohesperidin	38.4×10^5	0.28	12.96	1.08
Diosmin	54.6×10^5	0.48	16.52	1.33
Quercetin	19.3×10^5	2.95	23.06	0.27
Naringenin	14.2×10^5	1.69	24.05	0.19
Hesperetin	21.3×10^5	0.41	26.07	0.23
Kaempferol	52.5×10^5	0.58	27.91	0.31
Nobiletin	26.8×10^5	0.89	40.78	0.33
FL[1)]	20.7×10^5	0.73	41.76	0.34
Tangeretin	29.1×10^5	0.74	44.31	0.41

[1)]FL: 3,5,6,7,8,3′,4′-methoxylated flavone.

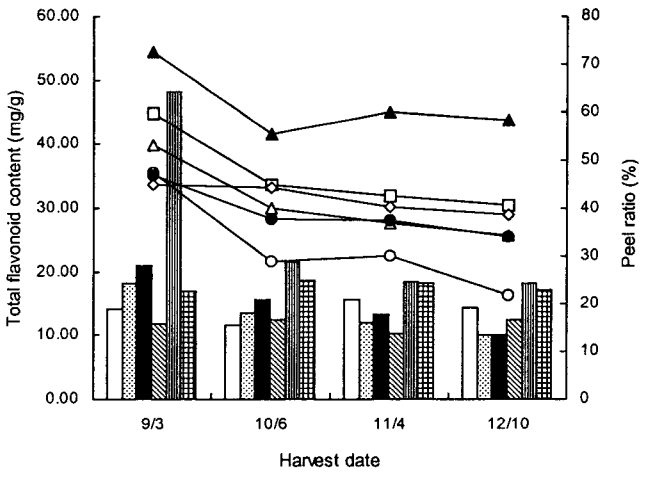

Fig. 2. Peel ratio of citrus fruits sampled at citrus experiment station.
Peel ratio: □, Mucott; ◇, Sambogam; △, Singamha; ○, Seminol; ▲, Jawdung; ●, Hongpalsak. Total flavonoid content: □, Mucott, □, Sambogam; ■, Singamha; ▨, Seminol; ▥, Jawdung; ▦, Hongpalsak.

peel ratio of all cultivars were decreased during maturation.

Bark et al. (13) reported a phenomenon that peel ratio is higher than flesh ratio in the early stages of maturation, and this was derived from the difference in the moving ratio of assimilation metabolites between flesh and peel precisely. The metabolites supplied for the peel was more abundant than that of the flesh in the early stages of maturation, and then the metabolites accumulated in the peel was moved to the flesh. Kim et al. (14) reported the peel ratio of Dangyooja, Hakyool, Hungjin, Sambogam (*C. sulcata*), kumkyoolja (*C. obovoidea* HORT. *ex* TAKAHASHI) and navel orange were 48.6, 35.0, 26.7, 40.0, 36.8 and 28.4%, respectively. Bark et al. (15) reported the peel ratio of Dangyooja, Hakyool, Gungchun (*C. unshiu*), Sambogam, Leeyaegam (*C. iyo* TANAKA), Sankyool (*C. nippokoreana*), Kumkyoolja and washington navel (*C. sinensis*) were 39.4, 33.5, 24.5, 45.8, 39.7, 36.7, 27.7 and 24.5%, respectively.

Change of flavonoids content in the peel of late maturing citrus

Nine flavonoids, including rutin, naringin, hesperidin, neohesperidin, quercetin, hesperetin, nobiletin, 3,5,6,7,8,3′,4′-heptamethoxyflavone and tangeretin in the peels of late maturing citrus fruit were detected. There are some cultivars that have high content of naringin in late maturing citrus. Decreasing ranges of naringin were not great, compared to those of others. Naringin contents in the peel of Jawdung, Singamha and Hongpalsak in September were 34.02, 15.94 and 12.11 mg/g, respectively. Narigin content in the peel of Jawdung was 34.02 mg/g, which it was highest among all cultivars. Also neohesperidin (13.68 mg/g) was presented in higher quantities than other late maturing citrus but hesperidin was not detected. Hesperidin contents in the peel of Mucott (12.48 mg/g), Sambogam (11.20 mg/g) and Seminol (10.61 mg/g) were high, compared to others. Rutin content in the peel of Sambogam (5.13 mg/g) in September was the highest among all cultivars. Quercetin, naringein, kaempferol, nobiletin, 3,5,6,7,8,3′,4′-methoxylated flavone flavonoids in all cultivars were in trace. Flavonoids detected in the

Table 2. Changes in flavonoids of late maturing citrus fruits (mg/g)

	Date	RT[1)]	NGI[2)]	HD[3)]	NHD[4)]	DN[5)]	QT[6)]	NGE[7)]	HT[8)]	KL[9)]	NT[10)]	FL[11)]	TT[12)]
Mucott	9/ 3	1.39±0.41	N[13)]	12.48±1.97	N	N	0.04±0.02	0.05±0.02	N	N	0.08±0.05	0.07±0.03	0.04±0.01
	10/ 6	0.93±0.11	N	10.40±1.55	N	N	0.04±0.02	N	N	N	0.07±0.03	0.08±0.03	0.04±0.02
	11/ 4	1.21±0.52	N	14.25±1.61	N	N	0.05±0.03	N	N	N	0.04±0.02	0.04±0.01	0.02±0.00
	12/10	1.10±0.04	N	13.14±1.87	N	N	0.04±0.01	N	N	N	0.05±0.02	0.03±0.00	0.03±0.01
Singamha	9/ 3	0.10±0.03	15.94±2.15	N	3.87±0.39	N	0.03±0.01	0.08±0.03	N	N	1.03±0.37	N	N
	10/ 6	0.07±0.03	11.93±1.58	N	2.79±0.35	N	0.02±0.00	0.06±0.02	N	N	0.87±0.25	N	N
	11/ 4	0.06±0.01	10.56±1.35	N	2.22±0.49	N	0.02±0.00	0.04±0.02	N	N	0.30±0.06	N	N
	12/10	0.07±0.02	7.69±1.11	N	1.96±0.32	N	0.02±0.00	0.03±0.01	N	N	0.27±0.07	N	N
Sambogam	9/ 3	5.13±0.90	N	11.20±2.15	1.61±0.45	N	0.02±0.01	0.05±0.02	N	N	0.06±0.03	0.09±0.02	0.09±0.03
	10/ 6	2.78±0.24	N	10.00±1.29	0.58±0.12	N	0.02±0.00	N	N	N	0.04±0.02	0.09±0.03	0.07±0.02
	11/ 4	2.38±0.32	N	8.70±0.67	0.72±0.12	N	0.02±0.01	N	N	N	0.06±0.02	0.04±0.02	0.11±0.03
	12/10	2.14±0.27	N	7.30±0.99	0.44±0.18	N	0.02±0.00	N	N	N	0.03±0.01	0.04±0.02	0.08±0.03
Hongpalsak	9/ 3	0.30±0.03	12.11±1.20	0.15±0.05	4.10±0.95	N	0.03±0.01	0.04±0.02	N	N	0.11±0.05	N	N
	10/ 6	0.19±0.02	12.96±1.69	N	5.45±0.04	N	0.03±0.02	0.06±0.03	N	N	0.09±0.03	N	N
	11/ 4	0.23±0.02	13.05±1.25	N	4.88±0.47	N	0.03±0.01	0.03±0.01	N	N	0.03±0.00	N	N
	12/10	0.21±0.04	12.09±1.78	N	4.74±0.27	N	0.04±0.02	0.04±0.02	N	N	0.06±0.03	N	N
Seminol	9/ 3	0.49±0.05	N	10.61±1.58	0.03±0.01	N	0.07±0.03	N	0.25±0.05	N	0.16±0.04	0.07±0.04	0.05±0.01
	10/ 6	0.38±0.04	N	11.12±1.96	0.09±0.03	N	0.06±0.02	0.03±0.01	0.24±0.04	N	0.22±0.06	0.18±0.04	0.07±0.02
	11/ 4	0.34±0.04	N	9.56±1.31	N	N	0.07±0.02	0.01±0.00	0.14±0.03	N	0.10±0.04	0.08±0.05	0.03±0.01
	12/10	0.42±0.06	N	11.19±1.27	0.12±0.05	N	0.07±0.03	N	N	N	0.25±0.06	0.18±0.06	0.10±0.03
Jawdung	9/ 3	0.16±0.02	34.02±2.84	N	13.68±2.40	N	0.10±0.04	0.10±0.03	N	0.04±0.01	0.08±0.03	N	0.03±0.00
	10/ 6	0.10±0.02	15.53±2.28	N	5.89±0.38	N	0.07±0.03	0.02±0.01	N	0.02±0.01	0.07±0.02	N	0.02±0.00
	11/ 4	0.07±0.03	12.95±1.54	N	5.14±0.40	N	0.06±0.03	0.02±0.00	N	0.02±0.00	0.07±0.02	N	0.02±0.00
	12/10	0.08±0.03	12.85±0.79	N	5.24±0.47	N	0.06±0.02	N	N	0.02±0.00	0.05±0.02	N	0.02±0.00

[1)]rutin. [2)]naringin. [3)]hesperidin. [4)]neohesperidin. [5)]diosmin. [6)]quercetin. [7)]naringenin.
[8)]hesperetin. [9)]kaempferol. [10)]nobiletin. [11)]3,5,6,7,8,3′4′-methoxylated flavone. [12)]tangeretin. [13)]Not detected.

peel of Mucott, Hongpalsak and Seminol were not changed during maturation. Flavonoid contents of Singamha, Sambogam and Jawdung were high in the peel of fruits at the early stage of maturation, and then decreased rapidly (Table 2).

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