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Changes of Some Flavonoids in the Peel of Satsuma Mandarin (*Citrus unshiu*) Harvested during Maturation

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Eight flavonoids, including rutin, naringin, hesperidin, quercetin, hesperetin, nobiletin, 3,5,6,7,8,3',4'-methoxylated flavone, and tangeretin, in the peels of satsuma mandarin (*Citrus unshiu*) species of Halla, Gungcheon, Hungjin, Namgam-20, Illnam-1, and Chungdo harvested between August and December were analyzed through HPLC. Hesperidin content of Halla harvested during early maturation was 28.70 mg/g, and was the highest among the tested citrus fruits. Rutin content of Hungjin harvested during early maturation was 2.66 mg/g. Naringin in all citrus species and hesperetin in Halla, Gungchun, Namgam-20, and Chungdo were only detected in the peel of fruits harvested during early maturation. Hesperidin and rutin were detected mainly in all citrus species, and other flavonoids in trace. Flavonoid content in the peel of fruits was high during early maturation. Flavonoid contents in the peels of all fruit samples were generally high in the early stage of maturation, which then decreased rapidly.

Key words : Citrus, *satsuma mandarin, flavonoid, hesperidin, naringin.*

Citrus is a universally enjoyed fruit produced by over 100 countries in all six continents. It is the most important tree fruit crop in the world, with current world production far exceeding that of all deciduous tree fruits (apples, pears, peaches, plums, etc).

The principal ingredients of citrus are sitologically important sugar and organic acids, and physiologically important vitamin and flavonoids. Flavonoid components of citrus are naringin, hesperidin, neohesperidin, rutin, naringenin, hesperetin, narirutin, nobiletin, tangeretin, sinensetin, natsudadain, didymin, poncirin, eriocotrin, 5,7,4'-emthoxylates flavone, 5'-desmethoxy nobiletin, 4'-methoxylated flavone, and 3',4'-methoxylated flavone.[1)]

Lio[2)] reported on the effects of flavonoids in preventing dental caries. However, flavonoids have also been found to cause a sitological problem in citrus processing in that the bitter taste of naringin decreased the quality of citrus juice.[3)] In addition, hesperidin, the main ingredient of flavonoids, aldulterates citrus juice by causing white turbidity in the juice.[4)]

Koh[5)] reported on the processed foods made from Kumquats, a citrus variety produced in Jeju, and their quality characteristics. Lee[6)] reported seven types of flavonoids, including naringin, hesperidin, neohesperidin, rutin, quercitrin, naringenin, and hesperetin, in 11 varieties of citrus, Yooja, Iyo, navel orange, Hungjin, Sudachi, Meiwa Kumquat, Sankyool, Dangyooja, Natsudaidai, Kinkoji, and Sanbogam, were contained in the flesh of the fruit rather than the peel. Woo[7)] reported the contents of naringin and hesperidin of *Citrus unshiu* peel were higher than those of the fruit flesh.

Up to now, researches on the flavonoids in citrus have not revealed sufficient data on the satsuma mandarin by harvest date. Therefore, this study was performed to obtain analytical data on the flavonoids contained in the peels of satsuma mandarin through quantitative analysis.

Materials and Methods

Materials. All fruits were harvested from the citrus orchard at Aewol, located in the Jeju Agricultural Research and Extension Service, once a month from August to January.

Some flavonoid standards, including rutin, naringin, hesperidin, neohesperidin, diosmin, quercetin, naringenin, hesperetin, and kaempferol, were purchased from Sigma Co. Nobiletin, 3,5,6,7,8,3',4'-methoxylated flavone, and tangeretin were supplied by the Faculty of Pharmacy, The University of Tokyo (prof. E. Yutaka).

Sample preparation. All citrus peel samples were cut into small fine pieces, and 1 g of samples were added into test tubes for extraction. To the first test tube, 10 m*l* of methanol was added and sonicated for 1 h without pH control at 60~65°C. To the second tube, 10 m*l* of 70 aqueous methanol was added under the same condition. 10 m*l* of methanol was added to the third tube and sonicated for 1 h at 60~65°C, adjusting the pH to 2~4 and 10~12 with 1 N HCl and 1 N NaOH, respectively. To the fourth tube, 10 m*l* of 70% aqueous methanol was added under the same condition as in the third test tube.[8-10)] Approximately 1.5 m*l* of the final extract was fil-

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Table 1. Flavonoid contents in the peel of Illnam-1 havested in August 10th, presented as average valuesstandard deviation (mg/g fresh weigh) under different pretreatment conditions.

Solvent conditions of extraction	RT	HD	NHD	QT	NT	FL	TT	Total flavonoid contents
MeOH (non-treatment)	1.02 ± 0.07	23.38 ± 2.9	0.26 ± 0.06	0.11	0.05 ± 0.01	0.02	0.02	24.78 ± 2.94
acidic	1.09 ± 0.06	22.75 ± 1.83	0.28 ± 0.04	0.11	0.05 ± 0.01	0.02	0.02	24.32 ± 1.84
basic	1.04 ± 0.11	23.61 ± 0.74	0.13 ± 0.12	0.11 ± 0.01	0.05	0.02	0.02	24.98 ± 0.78
70% MeOH (non-treatment)	0.93 ± 0.06	15.62 ± 0.89	0.15 ± 0.04	0.09 ± 0.01	0.05 ± 0.01	0.02	0.02	16.87 ± 0.84
acidic	0.94 ± 0.01	18.02 ± 1.85	0.18 ± 0.05	0.10 ± 0.01	0.04	0.02	0.01	19.32 ± 1.92
basic	1.01 ± 0.22	20.36 ± 3.40	0.13 ± 0.11	0.11 ± 0.01	0.05	0.02	0.02	21.70 ± 3.55

RT: rutin, HD: hesperidin, NHD: neohesperidin, QT: quercetin, NT: nobiletin, FL: 3,5,6,7,8,3',4'-methoxylated flavone, TT: tangeretin.

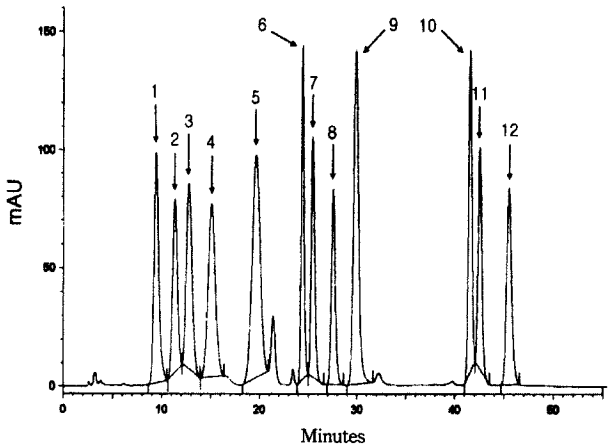

Fig. 1. HPLC chromatogram of standard flavonoid solution. Peaks; 1: rutin, 2: narigin, 3: hesperidin, 5: diosmin, 6: quercetin, 7: narigenin, 8: hesperetin, 9: kaempferol, 10: nobiletin, 11: 3,5,6,7,8,3',4'-methoxylated flavone, 12: tangeretin.

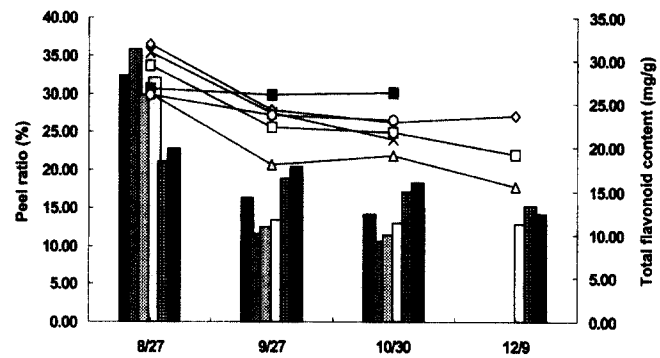

Fig. 2. Peel ratio and total flavonoid content changes. Peel ratio; □ : Namgam-20, ■ : Illnam-1, ◇ : Chungdo, × : Gungcheon, ○ : Halla, △ : Hungjin. Total flavonoid content; ▦ : Namgam-20, ■ : Illnam-1, ▤ : Chungdo, ▩ : Gungcheon, ▨ : Halla, □ : Hungjin.

tered using a membrane filter (Whatman, 0.45 μm) prior to the injection (10 μl) into the HPLC system.

HPLC. HPLC system for the flavonoid analysis consisted of a Spectrasystem (Spectra-Physics, LC-7000160, USA), P4000 pump, UV 1000 UV/Vis detector, and AS 3500 autosampler. For the analysis of flavonoids in the citrus peel, Bondapak C_{18} column (3003.9 mm, I.D.) was used. The mobile phase consisted of acetonitrile and water with 0.5% acetic acid each. The gradient of acetonitrile (0.5% acetic acid) was from 16 to 35% for 55 min at a flow rate of 1 ml/min. UV spectra were recorded at 254 nm. HPLC-grade methanol was purchased from Merck.

Results and Discussion

Sample preparation. As the solvent to extract flavonoids, methanol was more effective than 70% aqueous methanol. The effects of extraction using acidic and basic solvents were almost the same. Therefore, 1 g of the sample was extracted with 10 ml of methanol in a sonic bath for 1 h at 60~65°C twice, and then the extract volume was made up to 20 ml by adding methanol (Table 1).

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

Peel ratio of citrus fruits and total flavonoid contents by species and stages of maturation. Figure 2 shows the changes in the peel ratio and total flavonoid contents by citrus species and the stages of maturation. Chungdo and Halla showed the highest (36.37%) and the lowest (29.78%) peel ratio among citrus fruits examined at the early stage of maturation. However, changes in the peel ratio of Illnam-1 (30.60~30.09%) and Halla (29.78~26.49%) were not large. Total flavonoid contents in the peels of Halla and Namgam-20 were 31.24 and 18.42 mg/g, the highest and the lowest among the citrus fruits at the early stage of maturation, respectively. Most peel ratios and total flavonoid contents of all species decreased during maturation.

Bark[11] reported that ratio of peel is higher than that of flesh at the early stage of maturation, and it was caused by the difference in division ratio of the assimilation metabolites between the flesh and the peel. Assimilation metabolites supplied for the peel was more abundant than that of the flesh during the early stages of maturation, and then the metabolites accumulated in the peel was moved to the flesh. Song[12]

Table 2. Change of flavonoids in the peel of satsuma mandarin.

	Date	RT	NGI	HD	NHD	DN	QT	NGE	HT	KL	NT	FL	TT
Illnam-1	8/27	1.68	0.24	25.87	N	N	0.13	N	0.21	N	0.11	0.06	0.02
	9/27	1.01	N	13.12	N	N	0.08	N	N	N	0.06	0.03	0.01
	10/29	0.89	N	11.39	N	N	0.07	N	N	N	0.05	0.02	N
Halla	8/27	2.01	0.12	28.70	N	N	0.18	N	0.10	N	0.10	0.01	0.02
	9/27	0.68	N	9.33	N	N	0.05	N	N	N	0.04	0.01	N
	10/29	0.58	N	8.63	N	N	0.05	N	N	N	0.02	N	N
Gungcheon	8/27	1.97	0.12	23.46	N	N	0.14	N	0.16	N	0.10	0.07	0.03
	9/27	0.86	0.03	9.78	N	N	0.06	N	0.05	N	0.07	0.08	0.01
	10/29	0.88	N	9.06	N	N	0.07	N	N	N	N	0.01	N
Hungjin	8/27	2.66	0.23	24.57	N	N	0.18	N	0.21	N	0.13	0.08	0.02
	9/27	1.00	N	10.50	N	N	0.08	N	0.02	N	0.06	0.04	0.01
	10/29	0.89	N	10.25	N	N	0.07	N	0.02	N	0.05	0.03	0.01
	12/9	0.87	N	10.19	N	N	0.08	N	0.03	N	0.07	0.03	0.02
Namgam-20	8/27	1.25	0.26	16.40	N	N	0.10	N	0.20	N	0.11	0.06	0.04
	9/27	1.09	0.02	15.11	N	N	0.06	N	0.05	N	0.07	0.05	0.02
	10/29	0.94	N	13.83	N	N	0.05	N	N	N	0.06	0.04	0.01
	12/9	0.57	N	12.53	N	N	0.05	N	N	N	0.08	0.03	0.03
Chungdo	8/27	1.58	0.09	17.85	N	N	0.11	N	0.09	0.02	0.06	0.06	0.02
	9/27	1.37	0.01	16.21	N	N	0.07	N	0.02	N	0.06	0.05	0.02
	10/29	1.17	N	14.56	N	N	0.07	N	N	N	0.08	0.04	0.02
	12/9	0.92	N	11.34	N	N	0.07	N	N	N	0.05	0.03	0.01

N: Not detected, RT: rutin, NGI: naringin, HD: hesperidin, NHD: neohesperidin, DN: diosmin, QT: quercetin, NGE: naringenin, HT: hesperetin, KL: kaempferol, NT: nobiletin, FL: 3,5,6,7,8,3',4'-methoxylated flavone, TT: tangeretin, Unit: mg/g.

reported both naringin and hesperidin contents were significantly high in the immature peel of citrus fruits, and were decreased as peel coloration progressed.

Changes in flavonoid contents in the peel of satsuma mandarin. In the case of satsuma mandarin, heperidin and rutin contents were high compared with other flavonoids. Hesperidin contents in the peel of Halla, Illnam-1, Hungjin, Gungchun, Chungdo, and Namgam in late August were 28.70, 25.87, 24.57, 23.46, 17.85, and 16.40 mg/g, respectively, and decreased during maturation (Table 2). Quercetin, hesperetin, nobiletin, 3,5,6,7,8,3',4'-methoxylated flavone, and tangeretin in all samples were detected in trace amount.

Eight flavonoids, including rutin, naringin, hesperidin, quercetin, hesperetin, nobiletin, 3,5,6,7,8,3',4'-methoxylated flavone, and tangeretin, were commonly detected. Naringin was presented in trace amount until late September, and hesperetin until late September or during maturatiom (Hungjin), while neohesperidin, diosmin, naringenin, and kaempferol could not be detected.

Acknowledgments

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