A Thesis

for the Degree of Master of Science

Changes of Flavonoid Contents in Cheju Citrus during Maturation

by

Yung-Chun Kim

DEPARTIMENT OF AGRICULTURAL CHEMISTRY

GRADUATE SCHOOL

CHEJU NATIONAL UNIVERSITY

2000. 12.

제주산 감귤의 시기별, 품종별 Flavonoids의 함량 변화

지도교수 고 정 삼

김 영 천

이 논문을 농학 석사학위 논문으로 제출함

2000년 12월

김영천의 농학 석사학위 논문을 인준함

심사위원장 _____ 인

위 원 _____ 인

위 원 _____ 인

제주대학교 대학원

2000년 12월

Changes of Flavonoid Contents in Cheju Citrus during Maturation

Yung-Chun Kim

(Supervised by professor Jeong-Sam Koh)

A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF AGRICULTURE.

DEPARTMENT OF AGRICULTURAL CHEMISTRY

GRADUATE SCHOOL

CHEJU NATIONAL UNIVERSITY

2000. 12.

Contents

List of Tables

List of Figures

Summary

제주에서 재배되고있는 온주밀감, 만감류 그리고 재래감귤의 flavonoids의 함량 및 구성비와 성숙 중 함량변화를 알아보기 위해 25종의 감귤품종에 대한 12종의 flavonoids함량과 시기별 변화를 측정하였다.

시기별, 품종별 과피율은 유자가 성숙 중 다소 증가되는 경향을 보였고, 나머지 품종들은 변화가 거의 없거나 10% 수준으로 감소되었다. 유자의 과피율의 변화는 거의 없었으며, 지각의 과피율이 54.41%로 가장 높았고, 진귤이 14.08%로 성숙초기 가장 낮은 과피율을 나타내었다.

감귤과피 중의 flavonoids로는 rutin, naringin, hesperidin, neohesperidin, quercetin, naringenin, hesperetin, kaemferol, nobiletin, tangeretin 및 3,5,6,7,8,3′,4′-methoxylated flavone이 검출되었다. rutin은 모두 24종의 감귤품종과피에서 검출되었으며, 9월 초순경 삼보감에서 5.13 ㎎/g으로 가장 많이 검출되었다. naringin은 18종의 과피에서 검출되었으며, 좌등이 9월 초순경 34.02 ㎎/g으로 많이 검출되었다. Hesperidin은 25종의 감귤과피에서 검출되었으며, 8월 하순경 한라에 28.70 ㎎/g이 함유되어 있었다. Neohesperidin은 9월 초순경 좌등에 13.68 ㎎/g으로 가장 많이 함유되어 있었고 13종의 과피에서 검출되었다. Diosmin은 감자와 당유자에서 9월하순과 10월 초순 경까지 0.71~0.01 ㎎/g 정도로 적게 검출되었고, 그 외의 품종의 과피에서는 검출되지 않았다. Quercetin은 23종의 과피에서 검출되었지만 함량은 8월 초순경에도 0.18~0.02 ㎎/g 정도로 적었다. Naringein은 12종의 감귤과피에서 검출되었으며, 8월 하순경 최고 0.66 ㎎/g이 다전금의 과피에 함유되어 있었다. Hesperetin은 8월 하순 및 9월 초순에 11종의 과피에서 검출되었고, 편귤의 과피에서 0.96 ㎎/g이 함유되어 있었다. Kaemferol은 7종의 과피에서 검출되었고 0.34 ㎎/g으로 9월 하순경 편귤의 과피에 가장 많이 함유되어 있었다. Nobiletin은 홍귤의 과피에 4.46 ㎎/g으로 많이 함유되어 있었고 22종에서 검출되었다. 3,5,6,7,8,3′,4′-methoxylated flavone은 17종의 과피에 0.22~0.01 ㎎/g 정도로 함유되어 있었다. Tangeretin은 20종의 과피에서 검출되었고, 8월 하순경 진귤의

과피에 3.28 ㎎/g으로 많이 함유되어 있었다.

재래감귤, 온주감귤, 만감류로 분류해 살펴보았을 때, 재래감귤의 과피인 경우 hesperidin이 거의 없는 지각, 편귤, 당유자는 공통적으로 많은 양의 nagingin과 neohesperidin을 함유하고 있었다. 반대로 naringin과 neohesperidin이 거의 없는 유자, 진귤, 홍귤, 감자, 동정귤, 병귤, 빈귤, 사두감의 과피에는 많은 양의 heperidin이 함유되어 있었다. 온주밀감의 과피에는 주로 hesperidin이 많이 함유되어 있었고, 만감류의 과피에는 naringin을 많이 함유한 품종이 비교적 많았으며, 이런 품종들의 성숙 중 naringin이 감소되는 폭도 좁게 나타났다. 재래귤과 마찬가지로 naringin과 neohesperidin이 많을 경우 hesperidin이 거의 검출되지 않았고, hesperidin이 많을 때에는 naringin과 neohesperidin 함량이 상대적으로 적게 나타났다.

I . Introduction

Citrus can rightly be regarded as a universal fruit with production in over 100 countries in all six continents. Furthermore, citrus 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). Distribution is in a belt spreading approximately 40° latitude on each side of the Equator and is to be found in tropical and sub-tropical regions where favourable soil and climatic conditions occur. The majority of commercial citrus production, however, is restricted to two narrower belts in the sub-tropics roughly between 20° and 4 0° N and S of the Equator(Saunt, 1990).

Cultivation of citrus in the region of Cheju began from AD 476. Although 22 species of Cheju traditional citrus were cultivated at that time, they were later replaced with others because of their poor taste and qualilty. At present, only *Citrus platymama, C. grandis, C. nippokoreana. C. erythrosa, C. tachibana, C. sunki, C. aurantium* and *C. leiocarpe* remain as Cheju traditional citrus(Cheju Farmer Education Center, 1989). The Species of citrus now mostly widely cultivated in the region of Cheju is *Citrus unshiu.* Production per group according to their time of maturity in the Cheju region is that of 36,471 tons(8.1 %) for very early group, 452,563 tons(80 %) for early group, 48,792 tons(9.5 %) for common group and 6,154 tons(2.4 %) for late group of total production(Cheju Citrus Conference, 1999).

The principal ingredients of citrus are sugar, organic acids which are important sitologically, vitamin and flavonoids which are important physiologically. Moreover, flavonoids are widely distributed in plants, and over 4000 flavonoids are considered. Flavonoids are classified as flavonol, flavone, flavanone, flavanol, chalcone, anthocyanin, isoflavone and dihydroflavonol

according to their chemical structure(Herrmann, 1976; Kuhnau, 1976). Flavonoids component 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(Harborn and Mabry, 1982).

Flavonoids can be analysed by the Davis method, UV-Vis Absorption Spectrometry, TLC, HPLC, GC, and so on. However, Davis method is very useful for an approximate quantative anaylsis, but time and expense of analysis in this way are considerable, and it is difficult to provide quantative analysis respectively. The GC method cannot analysis flavonoids precisely, because it requires TMS derivatization reaction. Eventually, the HPLC analysis method is the most accurate for qualitative the analysis of flavonoids(Ting and Rouseff, 1986).

In a physiological function, flavonoids have the following crucial function. Matsubara(1985) confirmed that narirutin and rarcissin extracted from the peel of *Citrus unshiu* by hot water, which have an effect on the depressant of blood pressure, are the flavonoids glucosides. Lio(1984) reported effects of flavonoids on the prevention of dental caries. Han and Yoo(1988) reported antibiotic effects of naringin extracted from the peel of citrus, hesperidin has an effect on the depressant of blood pressure(Son et al., 1988). Flavonoids such as quercetin, kaemferol, myricetin feature in flavonol groups together with apigenin, luteolin, limonin and nomilin involved in flavone groups have an anticancer and antimutation effect(Middleton, 1994; Hertog, 1992). Hertog(1992) quantitativly determinated flavonoids of 28 vegetables and 9 fruits commonly consumed in the Netherlands.

Additionally, flavonoids have a sitological important problem in citrus processing. Due to the bitter taste of naringin(Horowitz and Gentili, 1969), it decreased the quality of citrus. Olson(1979) reported that bitterness in grapefruit

juice can be removed by use of naringinase. Mattews(1990) used styrene - divinylbenzene resin to remove limonin and naringin. Hesperidin, the main ingredient out of flavonoids, aldulterate citrus juice because it causes white turbidity of juice(Masukawa et al., 1985).

Through research of domestic citrus, Koh(1993) reported on the manufacture of processed foods and its quality characteristics from *Kumquats*, a citrus variety produced in Cheju. Koh(1994) reported on the physicochemical properties and sensory evaluation of *Citrus Unshiu* produced in Cheju, and on the physicochemical properties on the quality evaluation of *Citrus unshiu* produced in Cheju(Koh, 1995). Lee(1994) reported seven variety of flavonoids, naringin, hesperidin, neohesperidin, rutin, quercitrin, naringenin and hesperetin, in 11 variety of citrus, Yooja, Iyo, Navel Orange, Hungjin, Sudachi, Meiwa Kumquat, Sankyool, Dangyooja, Natsudaidai, Kinkoji and Sanbogam, were further contained in the flesh of the fruit rather than the rind. Song(1997) reported hesperidin, naringin, rutin and narirutin of the rind of *Citrus unshiu* Marc var. *miyagawa, C. natsudaidai* HAYATA *C. grandis* OSEECK, C. *platymamma*. Hort. SWINGLE, *C. sudachi,* and *C. aurantiun* LINN, were further contained in the flesh of the fruit rather than the rind. Woo(1996) reported the content of naringin and hesperidin of rind of *Citrus unshiu* was much more than that of the flesh of the fruit.

So far, because the research of the flavonoids of citrus has given too much importance to 3~4 variety of flavonoids as well as *Citrus unshiu* which has a vast difference in quantity of production, those of traditional citrus and others by harvest date are insuficient. Therefore, this study is to offer reference about *Citrus unshiu* and traditional citrus gradually vanished for utility of flavonoids in the future by quantitative analyzing flavonoids contained in the rind of *Citrus unshiu* and traditional citrus.

Ⅱ. Materials and Methods

1. Materials

1.1 Sampling

All fruits were havested from two farms located in Aewol and Sogwipo, which were in possession of the Cheju Agricultural Research and Extension Service and Citrus Experiment Station, once a month from August, 1999 to January, 2000. All twenty-five species of citrus, traditional citrus and *C. unshiu,* were harvested (Table 1).

1.2 Standards and Reagent

Some flavonoids standards - rutin(R-5143), naringin(N-1376), hesperidin (H-5254), neohesperidin (N-1887), diosmin (D-3525), quercetin (Q-0125), naringenin (N-5893), hesperetin (H-4125), kaemferol (K-0133), quercitrin (Q-3001) and apigenin(A-3145) - were purchased from Sigma Co., and the others - nobiletin, 3,5,6,7,8,3′,4′ - methoxylated flavone, tangeretin and 3,7,4′ - methoxylated flavone - were supplied from the Faculty of Parmacy, Tokyo University(Ebijuka Yutaka). All standard stock solution for HPLC were prepared from above flavonoids, and the concentrations are seen in Table 2.

Table 1. Place and time for citrus fruits sampling

Sample	August	September	October	November	December	January	Sampling place
Gamja(*Citrus benikoji*)	N	N, K	N, K	K	N, K	N	N, K
Gungchun(*C. unshiu*)	N	N	N				N
Namgam-20(*C. unshiu*)	N	N	N		N		N
Dangyooja(*C. grandis*)	N	N, K	N, K	K	N, K		N, K
Dongjungkyool(*C. erythrosa*)	N	N	N		N	N	N
Dajunkum(*C. junos*)		K	K	K	K		K
Murcott(*smith tangerine*)		K	K	K	K		K
Bungkyool(*C. platymamma*)	N	N	N		N	N	N
Binkyool(*C. leiocarpa*)	N	N, K	N, K	K	N, K	N	N, K
Sadoogam(*C. pseudogulgul*)	N	N	N		N	N	N
Sambogam(*C. sulcata*)		K	K	K	K		K
Singamha(*C. natsudaidai*)		K	K	K	K		K
Seminol(*Dancy tangerine*)		K	K	K	K		K
Yooja(*C. junos*)	N	N	N		N		N
Inchangkyool	N	N	N		N		N
Illnam-1(*C. unshiu*)	N	N	N				N
Jawdung(*C. aurantium*)		K	K	K	K		K
Jikak(*C. aurantium*)	N	N	N		N	N	N
Jinkyool(*C. sunki*)	N	N, K	N, K	K	N, K	N	N, K
Chungdo(*C. unshiu*)	N	N	N		N		N
Punkyool(*C. tangerina*)	N	N, K	N, K	K	N, K	N	N, K
Halla(*C. unshiu*)	N	N	N				N
Hongkyool(*C. tachibana*)	N	N, K	N, K	K	N, K	N	N, K
Hongpalsak(*C. hassaku*)		K	K	K	K		K
Hungjin(*C. unshiu*)	N	N	N		N		N

N : Cheju Agricultural Research and Extension Service,

K : Citrus Experiment Station

Table 2. Concentrations of standard stock solution for HPLC analysis

Flavonoids	Concentration(ppm)
rutin	322.4
naringin	1619.5
hesperidin	2314.2
neohesperidin	2300.7
diosmin	644.8
quercetin	153.1
naringenin	802.2
hesperetin	914.0
kaemferol	300.8
nobiletin	236.2
3,5,6,7,8,3'4'-methoxylated flavone	162.1
tangeretin	237.8

2. Experimental Methods

2.1 Apparatus

The HPLC system for the flavonoids analysis consisted of a Spectrasystem(Spectra-Physics, Model LC-7000160), P4000 pump, UV1000 UV/Vis detector, μBondapak C_{18} Column(300×3.9 ㎜, I.D.) and AS3500 autosampler. An Ultrasonic Bath(J.P. SELECTA) was used for the extraction of flavonoids from the citrus peels. The UV-Visible Spectrophotometer(Hewlett Packard, Model HP 8453) was used for the measurement of absorption spectra.

2.2 Sample preparation

All citrus peel samples were cut into fine small pieces and four 1 g samples were delivered into the test tube for the extractions. At the first test tube, 10 ㎖ of 99.9 % methanol was added and sonicated for several hours without pH control at 60~65 ℃.

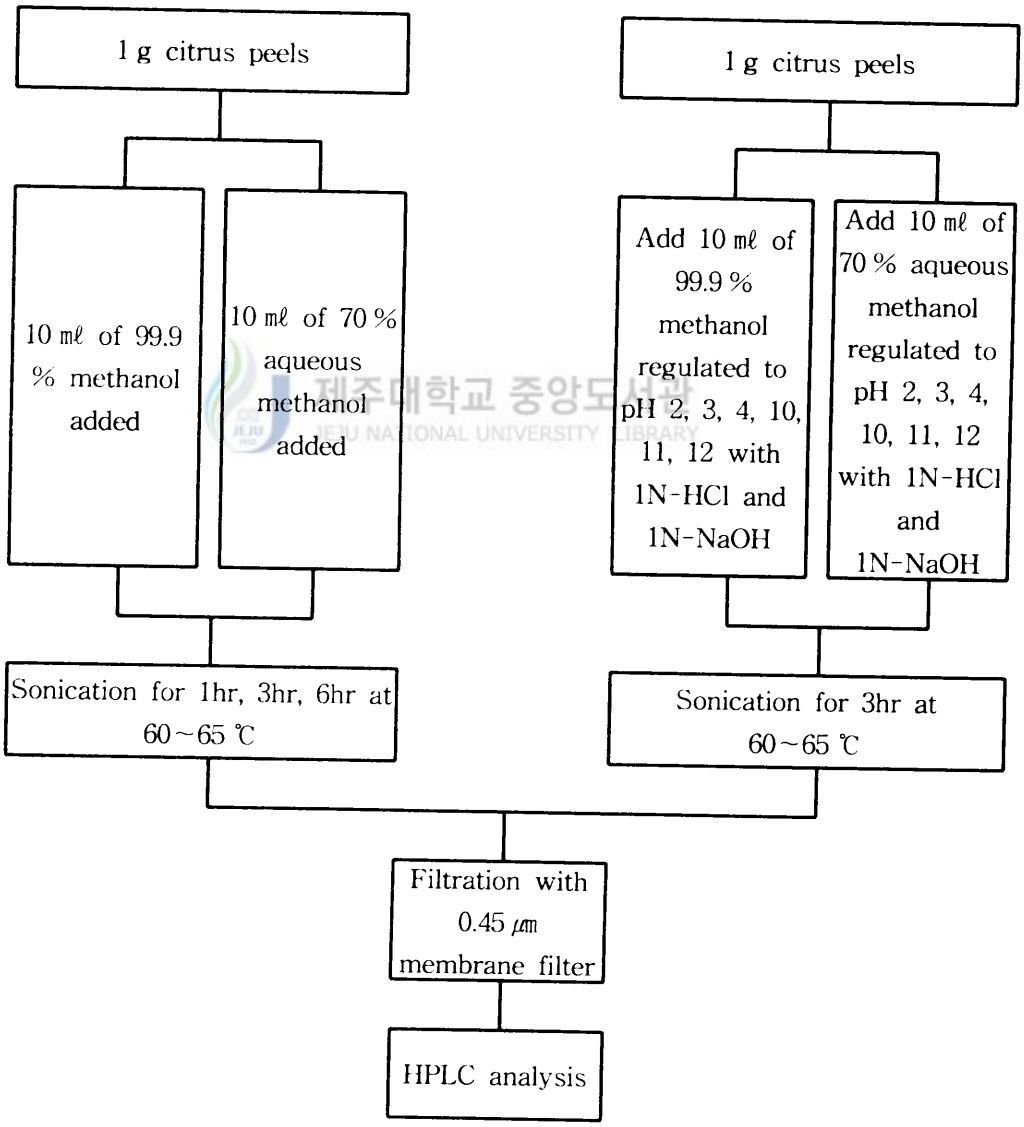

Fig. 1. Procedure for the extraction of flavonoids from citrus peels.

And at the second tube, 10 ㎖ of 70 % aqueous methanol was used with the same condition. At the third tube, 10 ㎖ of 99.9 % methanol was added and sonicated for several hours at 60~65 ℃ with regulating in pH 2, 3, 4, 10, 11, 12 with 1N-HCl and 1N-NaOH. And at the fourth tube, 10 ㎖ of 70 % aqueous methanol was used with the same condition (Lee, 1987; Ting, 1979; Perfetti, 1988; Velloglu and Mazza, 1991). It is summerized in Table 3 and Figure 1. Each extract was diluted to make a total volume 30 ㎖ with extractant. Approximately 1.5 ㎖ of the final extract was filtered using the membrane filter (Whatman, 0.45 ㎛) prior to the injection of the HPLC system.

Table 3. Extraction methods by different time and pH of solvent

Solvent	pH	Extraction time(hr)
99.9 % MeOH	non-treatment	1
	non-treatment	3
	non-treatment	6
	pH 2	3
	pH 3	3
	pH 4	3
	pH 10	3
	pH 11	3
	pH 12	3
70 % MeOH	non-treatment	1
	non-treatment	3
	non-treatment	6
	pH 2	3
	pH 3	3
	pH 4	3
	pH 10	3
	pH 11	3
	pH 12	3

2.3 Determination of analytical wavelength

For the preparation of standard solution, 9 flavonoids (rutin, naringin, quercetin, naringenin, hesperetin, kaemferol, nobiletin, 3,5,6,7,8,3′,4′ - methoxylated flavone, tangeretin, quercitrin, 3,7,4′ - methoxylated flavone) were dissolved in methanol and 4 flavonoids (hesperidin, neohesperidin, diosmin, apigenin), which were not perfectly soluble in methanol, were dissolved in DMSO(Dimethyl sulfoxide). Absorption spectra have been scanned using above standard solutions at a range of 200-400 nm by UV-Visible spectrophotometer.

2.4 Variation of mobile phase composition and Reproducibility

For the HPLC gradient separation, the ratio of water/acetonitrile mixture eluant including acetic acid (0.5 %) was varied to four different compositions according to the elution time as shown in Table 4. In the investigation for the HPLC gradient separations, D condition showed the best result among those four conditions, and also the reproducibility showed the good result.

The final HPLC conditions for the sample analyses are shown in Table 5. Reproducibility was checked three times through analysis of 10 $\mu\ell$ standards solution.

Table 4. Conditions of mobile phase for HPLC analysis

Mobile phase type	Time(min)	Acetonitrile (0.5 % acetic acid)	H_2O (0.5 % acetic acid)
A	0	10	90
	40	10	90
	45	20	82
	65	20	80
	70	30	70
	95	30	70
	100	10	90
	105	10	90
B	0	16	84
	15	16	84
	20	25	75
	35	25	75
	40	40	60
	60	40	60
	65	16	84
	70	16	84
C	0	18	82
	15	18	82
	20	30	70
	35	30	70
	40	40	60
	60	40	60
	65	18	82
	70	18	82
D	0	16	84
	15	16	84
	20	25	75
	30	25	75
	35	35	65
	45	35	65
	50	16	84
	55	16	84

Table 5. Analytical conditions for HPLC

Instrument	Spectrasystem HPLC
Column	μ-Bondapak C_{18}(3.9 ㎜ × 300 ㎜)
Detector wavelength	254 ㎚
Flow rate	1.0 ㎖/min
Injection volume	10 $\mu\ell$
Column temperature	room temperature
Mobile phase	Acetonitrile/Water = 16/84 ~ 35/65

Results and Discussion

1. Analytical methods

1.1 Sample preparation

As the solvent to extract flavonoids 99.9 % methanol was more effective than 70 % methanol, the effect of extraction in acidic and basic solvent were almost the same, and 1hr required time for extraction was sufficient to extract flavonoids(Table 6.). Therefore, 1g of the sample was extracted with 10 ㎖ of methanol in a sonic bath for 1hr at 60~65 ℃ twice, and then the extracts made up to 20 ㎖ by methanol.

1.2 Selection of analytical wavelength

The results of checking absorption maxima of fifteen flavonoids for selection of analytical wavelength were presented in Table 7. Since the analytical wavelength of flavonoids were 254 ㎚(Huh and Kho, 1990), 280 ㎚(Rouseff, 1988) and 313 ㎚(Ting, 1979). In this work, the absorption maxima of 15 flavonoids were shown as follows : 270 ㎚ for apigenin, 322 ㎚ for tangeretin, 284~295 ㎚ for naringin, hesperidin, neohesperidin, quercetin, naringenin and hesperetin, and 247~268 ㎚ for rutin, diosmin, kaemferol, nobiletin, 3,5,6,7,8,3′,4′ - methoxylated flavone, quercitrin and 3,7,4′ - methoxylated flavone. 254 ㎚ was choosen for the analytical wavelength of flavonoids in this work because seven flavonoids have a absorption maxima between 247 to 268 ㎚.

Table 6. Flavonoid content by pretreatment conditions

Flavonoids	Time(hr)	1	3	6	← 3 →					
	Solvent of extraction				pH 2	pH 3	pH 4	pH 10	pH 11	pH 12
rutin	MeOH	0.96	1.00	1.10	1.16	1.06	1.04	0.98	0.97	1.16
	70 % MeOH	0.89	0.99	0.90	0.93	0.95	0.95	0.81	0.96	1.27
hesperidin	MeOH	22.71	20.87	26.55	23.01	20.80	24.43	24.06	22.76	24.01
	70 % MeOH	16.60	14.88	15.37	15.88	19.11	19.06	17.05	20.19	23.85
neohesperidin	MeOH	0.23	0.33	0.21	0.32	0.25	0.26	0.23	0.16	0.00
	70 % MeOH	0.17	0.18	0.11	0.13	0.19	0.23	0.19	0.19	0.00
quercetin	MeOH	0.11	0.11	0.11	0.11	0.11	0.11	0.10	0.10	0.12
	70 % MeOH	0.09	0.10	0.09	0.10	0.11	0.10	0.10	0.11	0.12
nobiletin	MeOH	0.06	0.05	0.05	0.06	0.05	0.05	0.05	0.05	0.06
	70 % MeOH	0.04	0.05	0.05	0.04	0.04	0.05	0.04	0.05	0.05
FL	MeOH	0.02	0.02	0.02	0.03	0.02	0.02	0.02	0.02	0.03
	70 % MeOH	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02
tangeretin	MeOH	0.01	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02
	70 % MeOH	0.01	0.02	0.02	0.01	0.01	0.02	0.02	0.02	0.02

FL: 3,5,6,7,8,3′,4′ - methoxylated flavone

Table 7. UV absorbance of flavonoids

	Max Abs. nm	Abs.(AU)	254 nm Abs.	254 nm Abs./Max Abs. nm
rutin	261	0.712	0.634	0.89
naringin	284	0.535	0.147	0.27
hesperidin	285	0.452	0.118	0.26
neohesperidin	285	0.471	0.090	0.19
diosmin	252	0.358	0.355	0.99
quercetin	295	0.613	0.367	0.60
naringenin	290	0.707	0.147	0.21
hesperetin	288	0.693	0.122	0.18
kaemferol	268	0.663	0.642	0.97
nobiletin	247	0.717	0.655	0.91
FL-1	262	0.789	0.766	0.97
tangeretin	322	0.994	0.456	0.46
quercitrin	262	0.588	0.518	0.88
apigenin	270	0.517	0.299	0.58
FL-2	256	1.069	1.059	0.99

Max Abs.: maximum absorbance

FL-1: 3,5,6,7,8,3′,4′ - methoxylated flavone

FL-2: 3,7,4′ - methoxylated flavone

1.3 Condition of mobile phase

To find appropriate conditions of mobile phase, the four type condition(A, B, C, D) consisted of water/acetonitrile with 0.5 % acetic acid were used . Fig. 1~4 show four chromatogram of fifteen flavonoid according to mobile phase A, B, C and D. In all these conditions, neohesperidin and quercitrin, apigenin and kaemferol, and tangeretin and 3,7,4′ - methoxylated flavone were overlapped and

quercetin and naringenin were overlapped additionally in mobile phase A type, so analysis of quercitrin, apigenin, and 3,7,4′ - methoxylated flavone became difficult.

In the case of mobile phase A type , retention time was so long, B type had broad width between peaks of kaemferol and nobiletin, C type had a narrow width between peaks of diosmin, naringenin, hesperetin and apigenin. Due to the condition of D type being ideal for analysis of flavonoids, we selected D type.

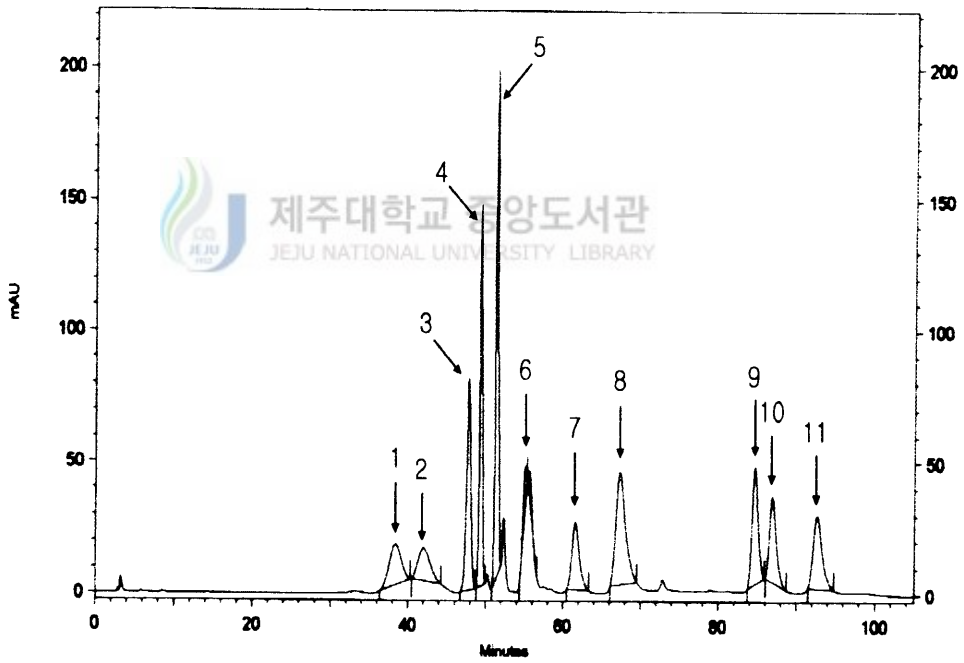

Fig. 2. HPLC chromatogram of standard flavonoids solution with mobile phase A type refered to Table 4.

Peaks: 1, rutin; 2, naringin; 3, hesperidin; 4, neohesperidin, quercitrin; 5, diosmin, 6, quercetin, naringenin; 7, hesperetin; 8, apigenin, kaemferol; 9, nobiletin; 10, 3,5,6,7,8,3′,4′ - methoxylated flavone; 11, tangeretin, 3,7,4′ - methoxylated flavone.

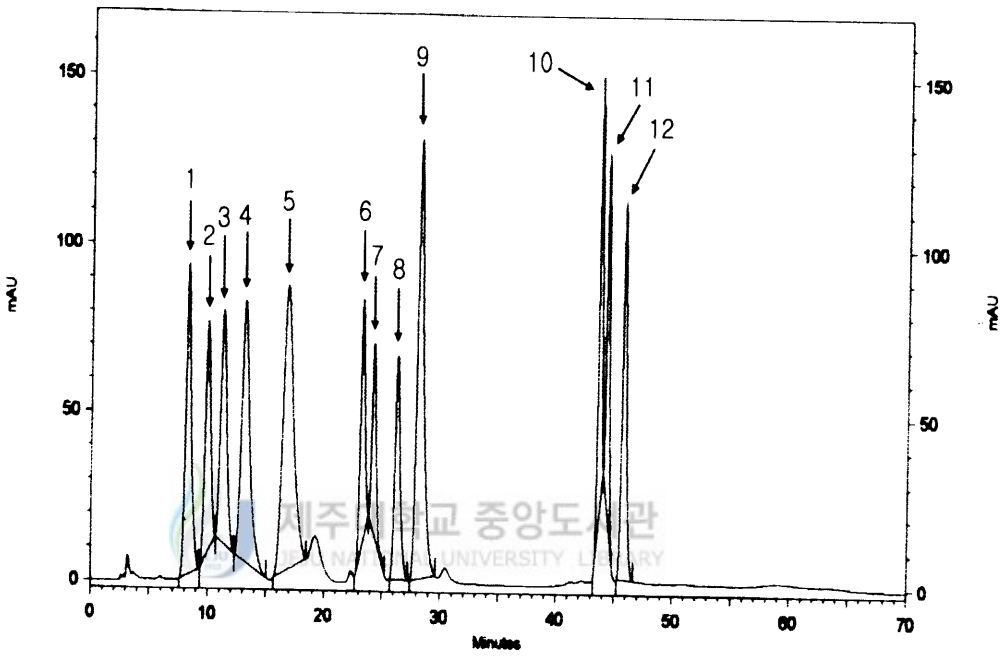

Fig. 3. HPLC chromatogram of standard flavonoids solution with mobile phase B type refered to Table 4.

Peaks: 1, rutin; 2, naringin; 3, hesperidin; 4, neohesperidin, quercitrin; 5, diosmin, 6, quercetin; 7, naringenin; 8, hesperetin; 9, apigenin, kaemferol; 10, nobiletin; 11, 3,5,6,7,8,3′,4′ - methoxylated flavone; 12, tangeretin, 3,7,4′ - methoxylated flavone.

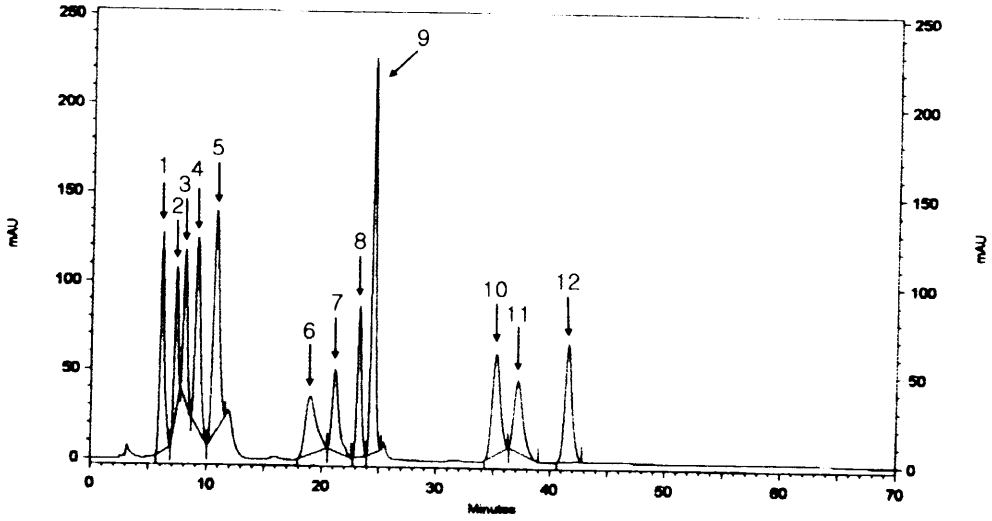

Fig. 4. HPLC chromatogram of standard flavonoids solution with mobile phase C type refered to Table 4. Peaks are refer to Fig. 3.

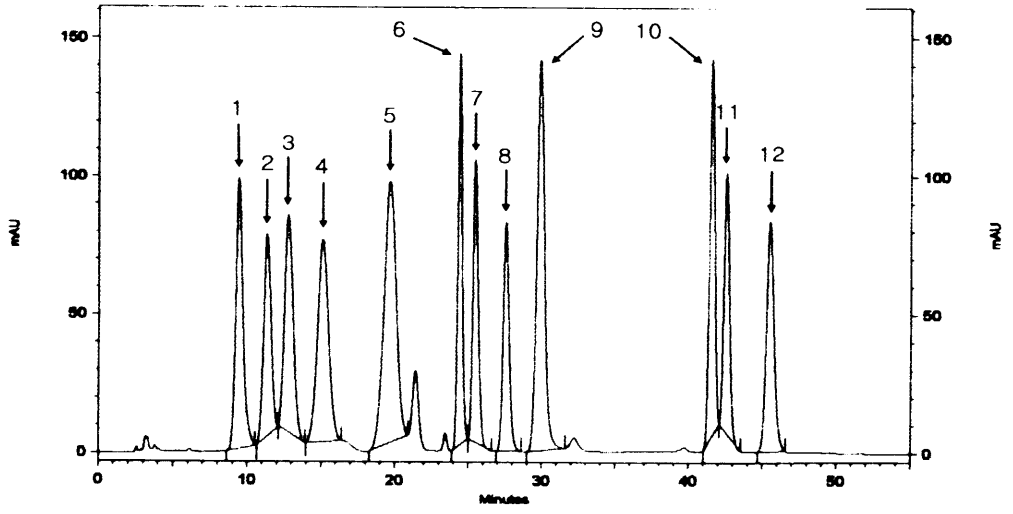

Fig. 5. HPLC chromatogram of standard flavonoids solution with mobile phase D type refered to Table 4. Peaks are refer to Fig. 3.

1.3 Reproducibility

The results of a three time analysis of 10 ㎕ standard solution were presented in Table 8. Rouseff's(1988) Relative Standard Deviation(R.S.D) of the peak area of naringin and neohesperidin were 0.47~1.06 % and 0.40~1.27 %, and those of retention time were 0.24~1.08 % and 0.034~0.92 %. In this work, R.S.D of the peak area of naringin and neohesperidin were 1.62 % and 0.28 %, 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 R.S.D of peak area of quercetin.

Table 8. Mean replicated values of peak area and retention time

	Peak area		Retention time(min)	
	Mean	RSD(%)	Mean	RSD(%)
rutin	3098498	0.04	8.14	1.13
naringin	2243421	1.62	9.81	1.27
hesperidin	2594289	1.92	11.10	1.27
neohesperidin	3842770	0.28	12.96	1.08
diosmin	5456123	0.48	16.52	1.33
quercetin	1925314	2.95	23.06	0.27
naringenin	1420046	1.69	24.05	0.19
hesperetin	2128785	0.41	26.07	0.23
kaemferol	5249230	0.58	27.91	0.31
nobiletin	2677728	0.89	40.78	0.33
FL	2073331	0.73	41.76	0.34
tangeretin	2908775	0.74	44.31	0.41

FL: 3,5,6,7,8,3',4' - methoxylated flavone

2. Peel ratio of citrus fruits by citrus species and the stage of maturation

Fig. 5 and 6 show the Changes of peel ratio of citrus fruits by citrus species and the stage of maturation. Most of the peel ratio of all species decreased during maturation. However, the Changes of peel ratio of Gamja(*Citrus benikoji*), Dangyooja(*C. grandis*), Dongjungkyool(*C. erythrosa*), Illnam-1(*C. unshiu*) harvested at Cheju Agricultural Research and Extension Service, and Dajunkum(*C. junos*) harvested at Citrus Experiment Station were 29.17~29.66 %, 40.84~40.40 %, 47.70~48.39 %, 30.60~30.09 % and 44.17~45.56 %, respectively, and were not so vast. Conversely, the peel ratio of Yooja(*C. junos*) was increased from 41.78 % to 47.17 %. Jikak(*C. aurantium*) and Jinkyool(*C. sunki*) had the highest(50.49 %) and lowest(14.08 %) peel ratio among citrus harvested at Cheju Agricultural Research and Extension Service, and Jwadung(*C. aurantium*) and Binkyool(*C. leiocarpa*) had the highest(58.41 %) and lowest(19.06 %) peel ratio among citrus harvested at the Citrus Experiment Station.

Bark(1968) reported a phenomenon that peel ratio is higher than flesh ratio in the early stages of maturation, was caused by the difference in the division ratio of assimilation between flesh and peel precisely, assimilation supplied for the peel was more abundant than that of the flesh in the early stages of maturation, and then assimilation accumulated in the peel was moved to the flesh. Kim(1996) reported a peel ratio of Hungjin(*C. unshiu*), Hakyool(*C. natsudaidai* HAYATA), Dangyooja and Sudachi(*C. sudachi*) were 27.0 %, 29.7 %, 46.1 % and 34.6 %, respectively, Yang(1967) reported a peel ratio of Dangyooja, Hakyool, *C. unshiu*, Sambogam, 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(1968) reported a peel ratio of Dangyooja, Hakyool,

Gungchun(*C. unshiu*), Sambogam(*C. sulcata*), 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.

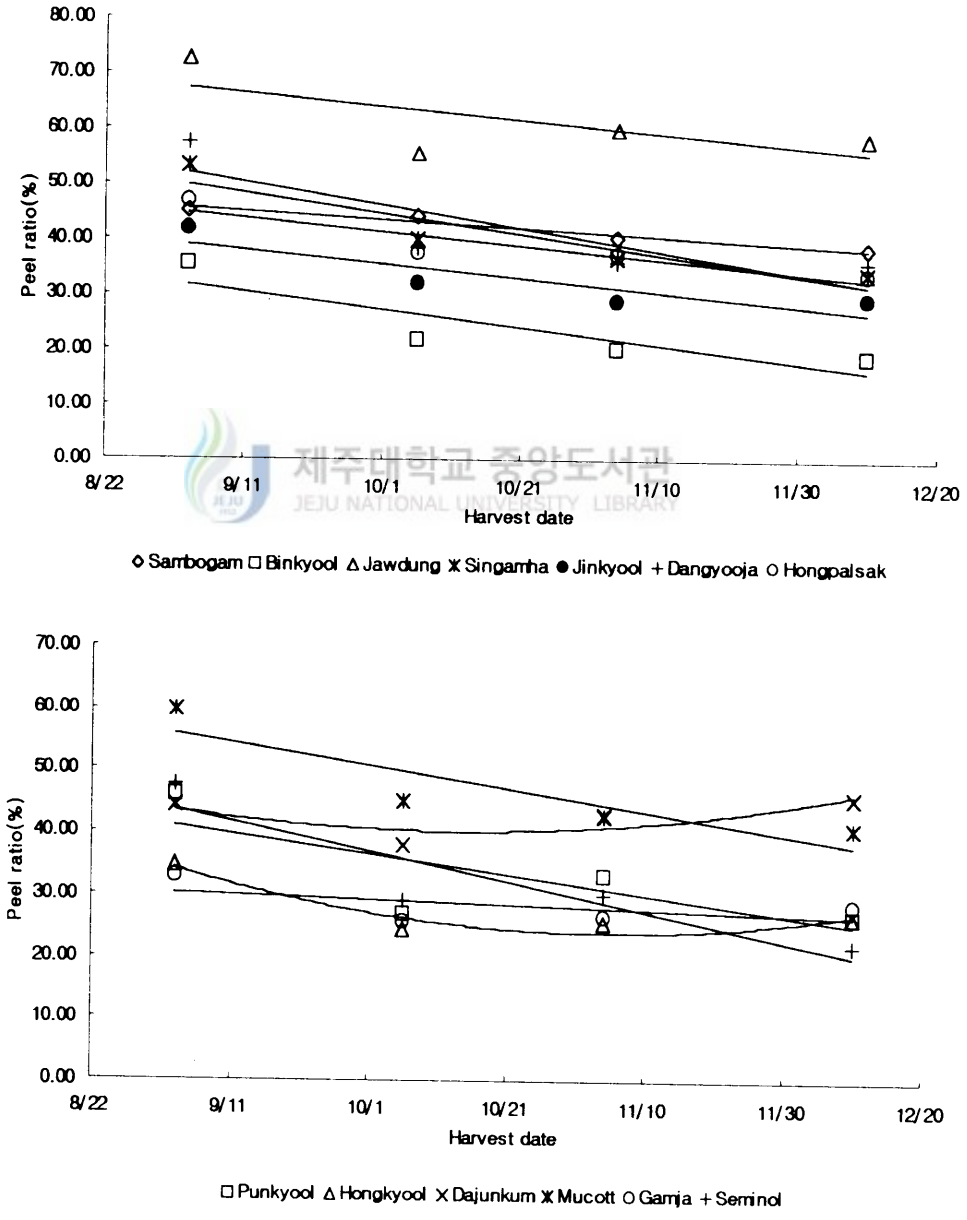

Fig. 6. Peel ratio of citrus fruits sampled at Citrus Experiment Station.

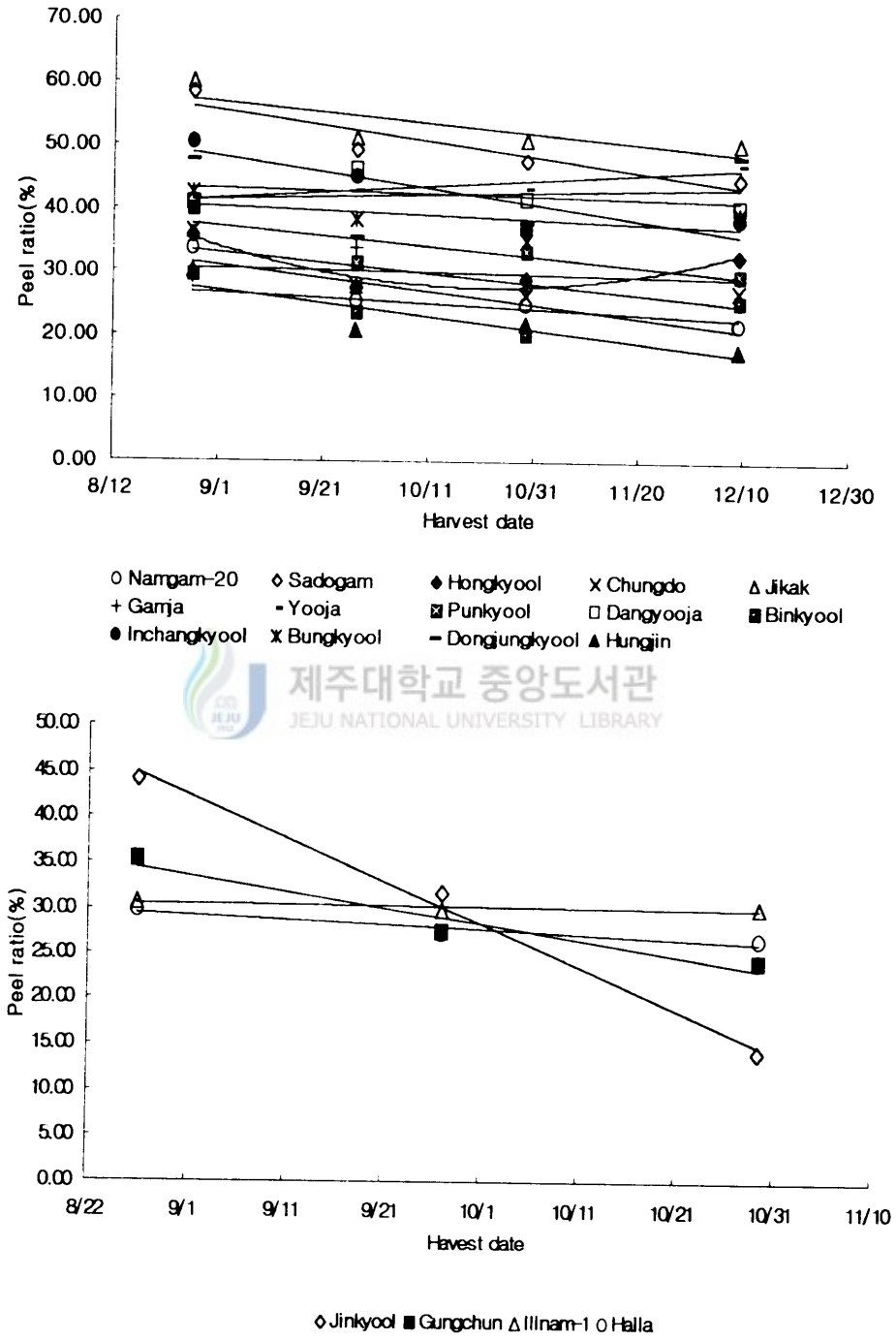

Fig. 7. Peel ratio of citrus fruits sampled at Cheju Agricultural Research and Extension Service.

3. Changes of content by flavonoids

3.1 Change of rutin content

Rutin was found in the peel of all species except for that of Pyunkyool. Changes of rutin content in the peel of citrus harvested at Cheju Agricultural Research and Extension Service and Citrus Experiment Station can be seen in Fig. 7 and 8. Rutin contents in the peel of Sambogam, Sadoogam, Hungjin, Halla, Gungchun, Illnam-1, Chungdo, Murcott and Namgam-20, containing more than 1.00 ㎎/g during the period from late August to early September, were 5.13 ㎎/g, 4.20 ㎎/g, 2.66 ㎎/g, 2.01 ㎎/g, 1.97 ㎎/g, 1.68 ㎎/g, 1.58 ㎎/g, 1.39 ㎎/g and 1.25 ㎎/g in order of content, respectively. Rutin contents in the peel of the above species except for that of Murcott decreased rapidly during the period from late september to early October.

Rutin could be detected less than 1.00 ㎎/g in the peel of Hongkyool (0.09 ㎎/g), Jikak (0.09 ㎎/g), Jinkyool (0.14 ㎎/g), kamja (0.41 ㎎/g), Yooja (0.63 ㎎/g), Dangyooja (0.12 ㎎/g), Binkyool (0.17 ㎎/g), Inchangkyool (0.41 ㎎/g), Byungkyool (0.43 ㎎/g), Dongjungkyool (0.17 ㎎/g), Jwadung (0.16 ㎎/g), Singamha (0.10 ㎎/g), Jinkyool (0.28 ㎎/g), Hongpalsak (0.30 ㎎/g), Dajunkum (0.56 ㎎/g) and Seminol (0.49 ㎎/g) harvested in late August and early September. Changes of content in the peel of the above citrus included Murcott not be shown largely during the stage of maturation.

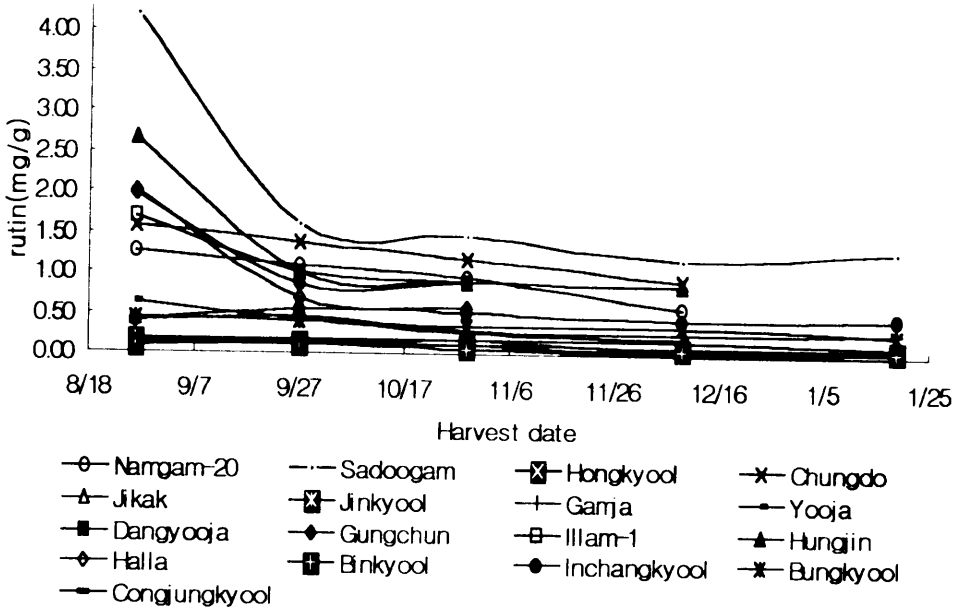

Fig. 8. Changes in rutin of citrus fruits sampled at Cheju Agricultural Research and Extension Service.

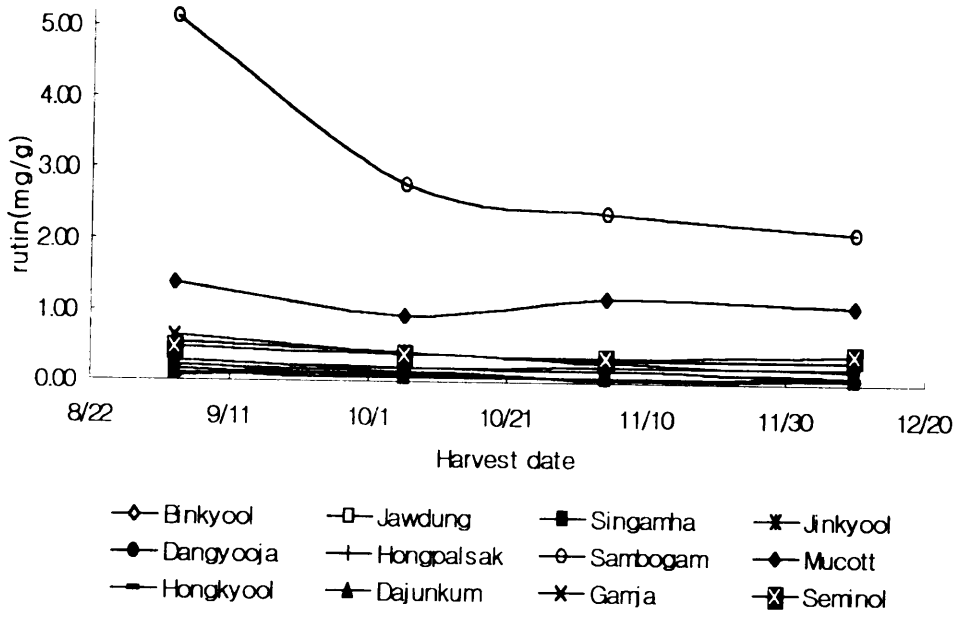

Fig. 9. Changes in rutin of citrus fruits sampled at Citrus Experiment Station.

3.2 Change of naringin content

Change of naringin content is shown in Fig. 9 and 10. Naringin was detected in the peel of fifteen samples. Naringin contents in the peel of Jwadung, Singamha, Jikak, Hongpalsak, Dangyooja, Pyunkyool and Yooja, contained in exeed of 1.00 ㎎/g during the period from late August to early September, were 34.02 ㎎/g, 15.94 ㎎/g, 14.55 ㎎/g, 12.11 ㎎/g, 9.30 ㎎/g, 3.29 ㎎/g and 1.39 ㎎/g in order of content, respectively.

Naringin contents in the peel of Hongpalsak and Pyunkyool were largely unchanged largely during maturation, those in the others were decreased by half during the period from early September to early December. Content in Dajunkum(0.95 ㎎/g) decreased gradually, naringin in peel of Namgam-20(0.26 ㎎/g), Chungdo(0.09 ㎎/g), Gungchun(0.12 ㎎/g), Illnam-1(0.24 ㎎/g), Hungjin(0.23 ㎎/g), Halla(0.12 ㎎/g), Jinkyool(0.19 ㎎/g), harvested in late August and early September, was detected until late of August or late of September.

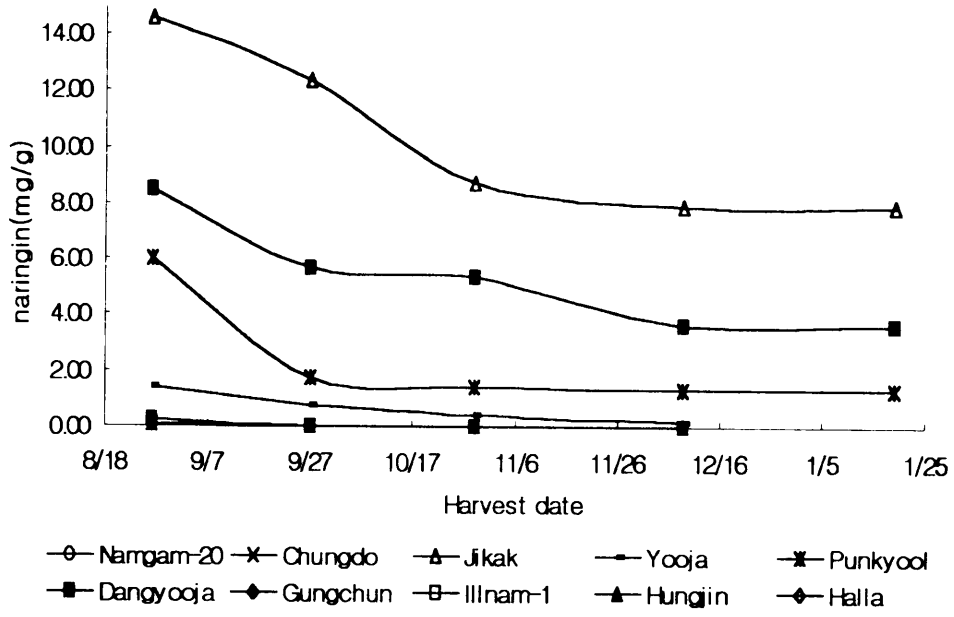

Fig. 10. Changes in naringin of citrus fruits sampled at Cheju Agricultural Research and Extension Service.

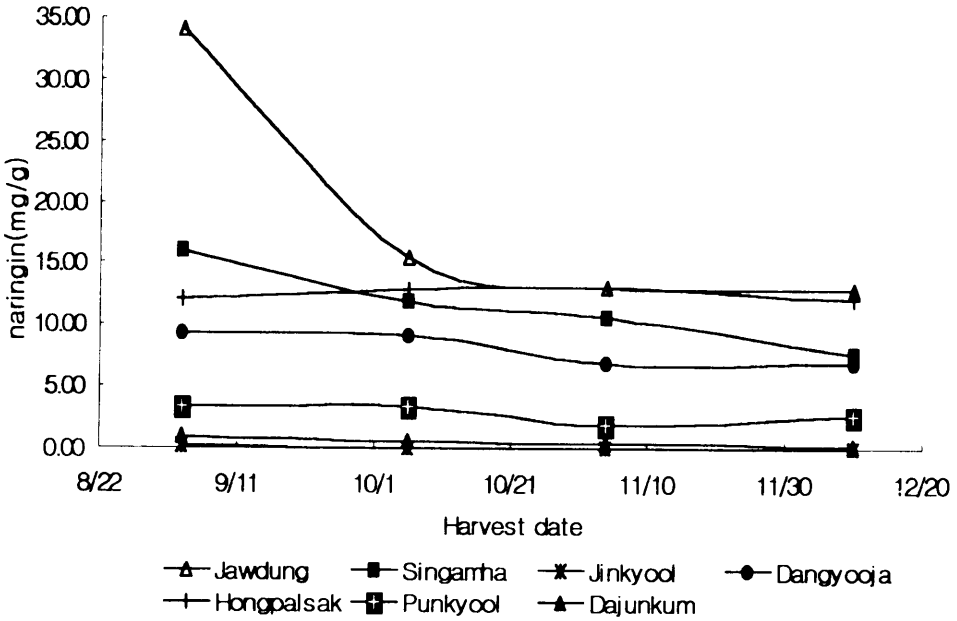

Fig. 11. Changes in naringin of citrus fruits sampled at Citrus Experiment Station.

3.3 Change of hesperidin content

Hesperidin was detected in twenty-two samples. Change of heperidin content during maturation is shown in Fig, 11 and 12. Hesperidin contents in the peel of Halla, Binkyool, Illnam-1, Hungjin, Gungchun, Dongungkyool, Chungdo, Namgam-20, Murcott, Sambogam and Seminol, containing more than 10.00 ㎎/g during the period from late August to early September, were 28.70 ㎎/g, 25.99 ㎎/g, 25.87 ㎎/g, 24.57 ㎎/g, 23.46 ㎎/g, 23.25 ㎎/g, 17.85 ㎎/g, 16.40 ㎎/g, 12.48 ㎎/g, 11.20 ㎎/g and 10.61 ㎎/g in order of content, respectively, and in Halla, Binkyool, Illnam-1, Hungjin, Gungchun and Dongjungkyool decreased rapidly until late September, those in Chungdo, Namgam-20 and Sambogam decreased gradually but in Murcott and Seminol remained regular.

Contents in the peel of Sadoogam, Hongkyool, Jinkyool, Gamja, Yooja, Inchangkyool, Bungkyool, Hongkyool, Dajunkum and Gamja, containing less than 10.00 ㎎/g of hespetidin, were 7.71 ㎎/g, 9.31 ㎎/g, 6.42 ㎎/g, 4.70 ㎎/g, 3.04 ㎎/g, 3.40 ㎎/g, 5.38 ㎎/g, 8.27 ㎎/g, 2.81 ㎎/g and 5.99 ㎎/g, and decreased gradually. Hesperidin in the peel of Pyunkyool, Dangyooja and Hongpalsak was detected until late August or early September.

These results are similar to Masukawa(1985) investing the peel of Satuma mandarin(13.41~21.74 ㎎/g).

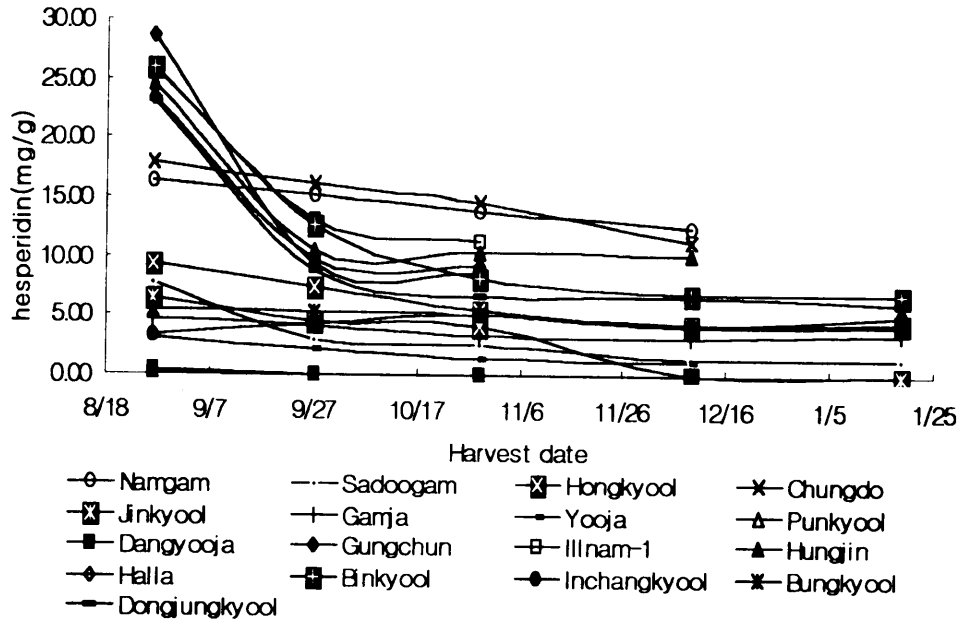

Fig. 12. Changes in hesperidin of citrus fruits sampled at Cheju Agricultural Research and Extension Service.

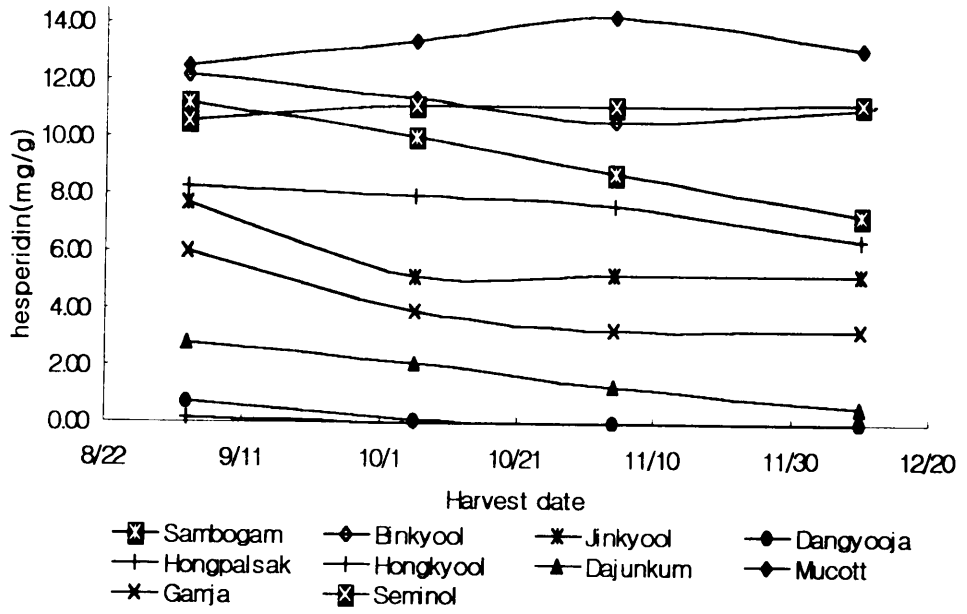

Fig. 13. Changes in hesperidin of citrus fruits sampled at Citrus Experiment Station.

3.4 Change of neohesperidin content

Fig. 13 and 14 show the Change of neohesperidin content, neohesperidin was detected in the peel of thirteen samples. Neohesperidin contents in the peel of Jwadung and Dangyooja, containing more than 10.00 ㎎/g during the period from late August to early September, were 13.68 ㎎/g and 10.06 ㎎/g, and decreased rapidly between late September and early October, in Jikak, Punkyool, Yooja, Inchangkyool, Sambogam, Singamha, Hongpalsak and Gamja, containing more than 1.00 ㎎/g, were 8.06 ㎎/g, 5.97 ㎎/g, 1.19 ㎎/g, 1.07 ㎎/g, 1.61 ㎎/g, 3.87 ㎎/g, 4.10 ㎎/g and 1.14 ㎎/g, respectively. Neohesperidin contents in the peel of Jikak and Punkyool decreased in late October and late September, and those of others decreased gradually during the stage of maturation.

Neohesperidin in Binkyool, Jinkyool, Dajunkum and Seminol, were detected below 1.00 ㎎/g and decreased gradually or detected until late August or early September.

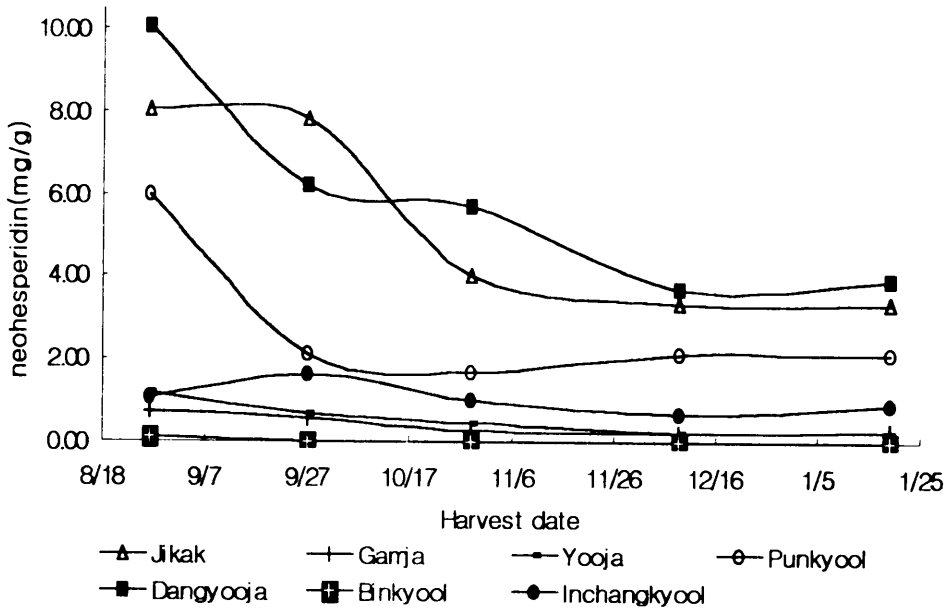

Fig. 14. Changes in neohesperidin of citrus fruits sampled at Cheju Agricultural Research and Extension Service.

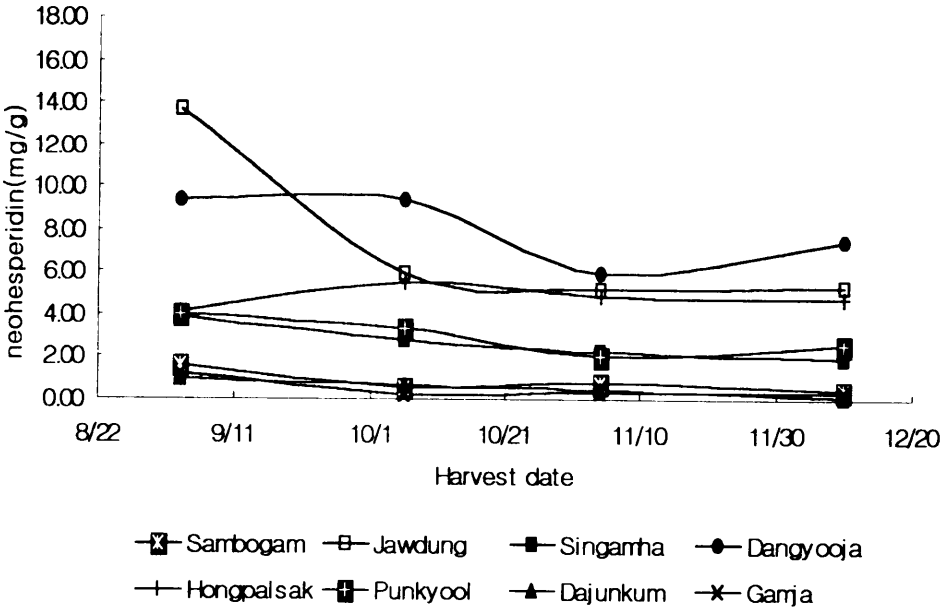

Fig. 15. Changes in neohesperidin of citrus fruits sampled at Citrus Experiment Station.

3.5 Change of quercetin content

Fig. 15 and 16 show the changes of quercetin content in the peel of citrus harvested at Cheju Agricultural Research and Extension Service and Citrus Experiment Station. Quercetin contents in the peel of all samples were not detected or below 1.00 ㎎/g.

Quercetin contents in the peel of Hungjin and Halla were 0.18 ㎎/g, in Gungchun, Illnam-1, Sadoogam, Chungdo and Jikak ranged from 0.14 ㎎/g to 0.11 ㎎/g, in Namgam-20, Binkyool and Jawdung were 0.10 ㎎/g, in Gamja, Semonol, Dongjungkyool and Inchangkyool ranged from 0.08 ㎎/g to 0.05 ㎎/g, in Yooja, Dangyooja, Bungkyool, Samboogam, Singamha, Jinkyool, Hongpalsak, punkyool, Hongkyool, Dajunkum and Murcott were 0.04~0.02 ㎎/g in late August and early September. Quercetin contents in Hungjin, Halla, Sadoogam, Gungchun, Illnam-1, Chungdo, Jikak, Namgam-20, Binkyool, Jawdung and Dangyooja were decreased during the stage of maturation, those in the others were largely unchanged.

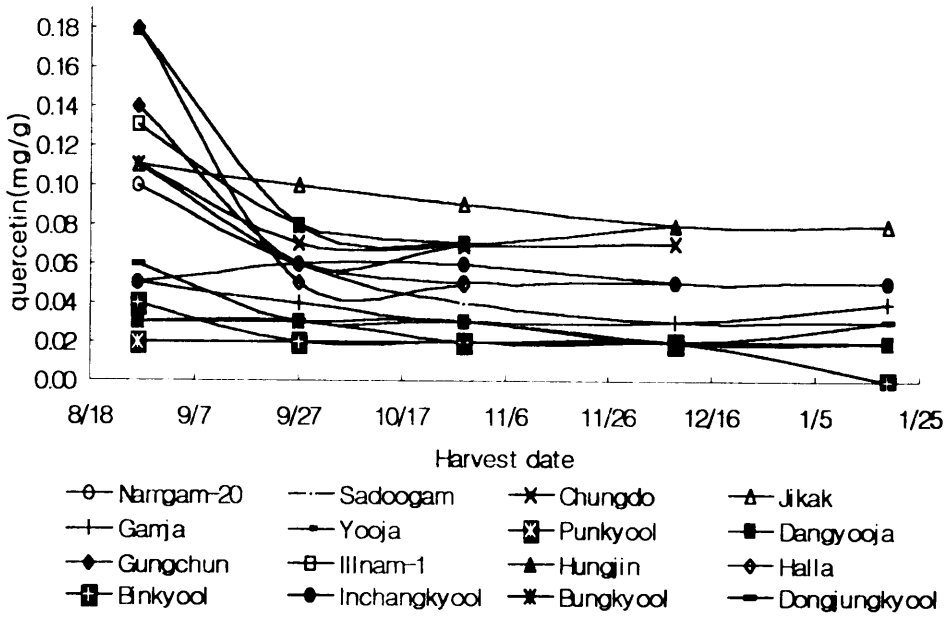

Fig. 16. Changes in quercetin of citrus fruits sampled at Cheju Agricultural Research and Extension Service.

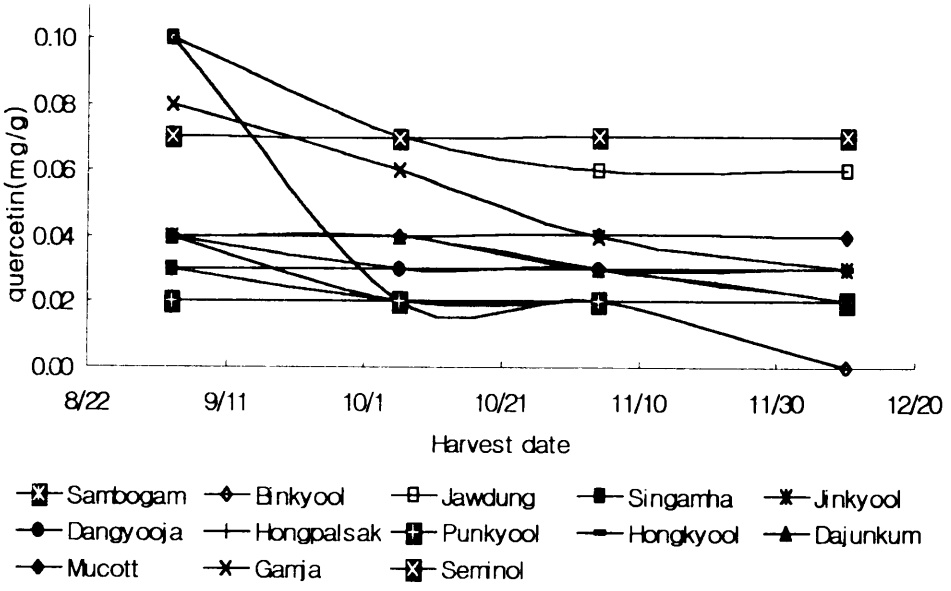

Fig. 17. Changes in quercetin of citrus fruits sampled at Citrus Experiment Station.

3.6 Change of naringenin content

The Change of naringenin content in the peel of citrus harvested at Cheju Agricultural Research and Extension Service and the Citrus Experiment Station were shown in Fig. 17 and 18. Naringenin content in the peel of Dajunkum, Punkyool, Yooja, Dangyooja and Jawdung was 0.66 ㎎/g, 0.41 ㎎/g, 0.30 ㎎/g, 0.14 ㎎/g and 0.10 ㎎/g, respectively. In Gamja, Sanboogam, Binkyool, Murcott and Singamha, it ranged from 0.08 ㎎/g to 0.05 ㎎/g, in Hongpalsak and Seminol from 0.04 ㎎/g to 0.03 ㎎/g in order of content from late August to early September.

Naringenin content in the peel of Punkyool, Yooja and Dangyooja decreased gradually and those in the others were largely unchanged.

3.7 Change of hesperetin content

The Change of hesperetin content in the peel of citrus harvested at Cheju Agricultural Research and Extension Service and the Citrus Experiment Station were shown in Fig. 19 and 20. Hesperetin was detected only in the peel of eleven samples. Hesperetin content in the peel of Punkyool, Seminol, Illnam-1, Hungjin, Namgam-20, Gungchun, Halla, Binkyool, Chungdo, Dangyooja and Gamja was 0.96 ㎎/g, 0.25 ㎎/g, 0.21 ㎎/g, 0.21 ㎎/g, 0.20 ㎎/g, 0.16 ㎎/g, 0.10 ㎎/g, 0.10 ㎎/g, 0.09 ㎎/g, 0.06 ㎎/g and 0.01 ㎎/g in order of content from late August to early September.

Hesperetin content in the peel of Punkyool, Hungjin and Seminol were decreased gradually during the stage of maturation, those in Namgam, Chungdo, Gungchun, Dangyooja, Illnam-1, Halla, Binkyool and Gamja were largely unchangely.

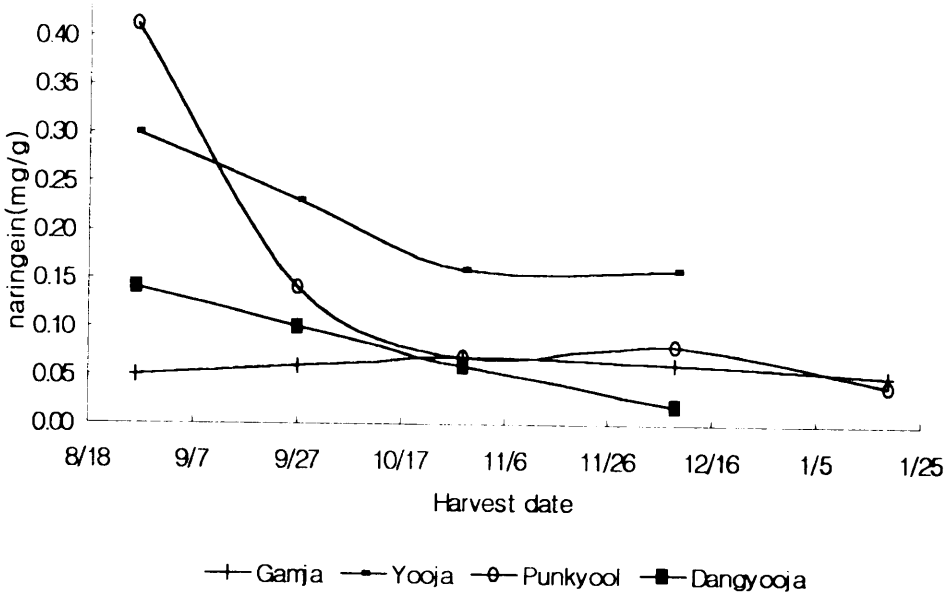

Fig. 18. Changes in naringenin of citrus fruits sampled at Cheju Agricultural Research and Extension Service.

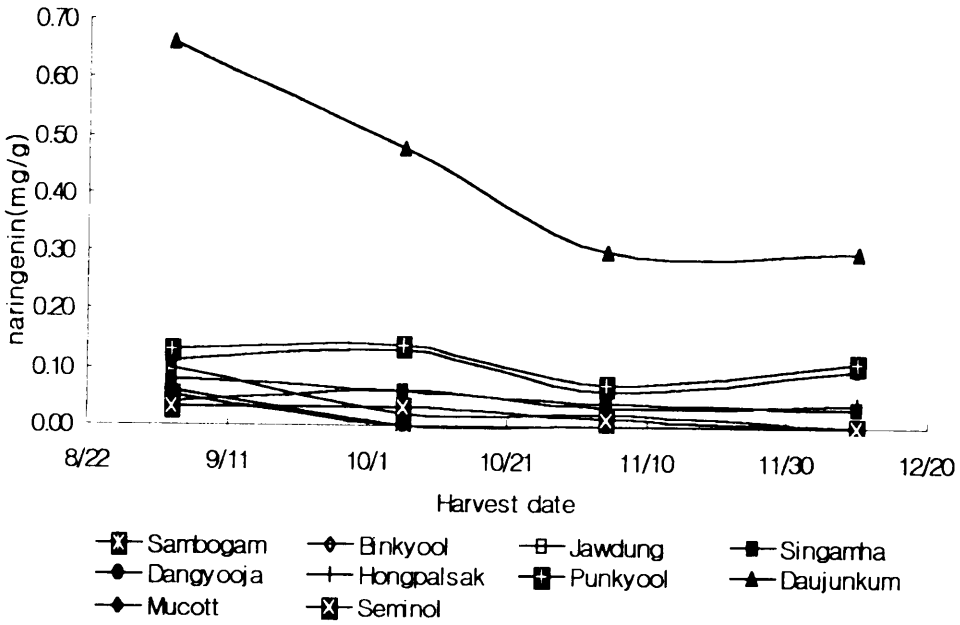

Fig. 19. Changes in naringenin of citrus fruits sampled at Citrus Experiment Station.

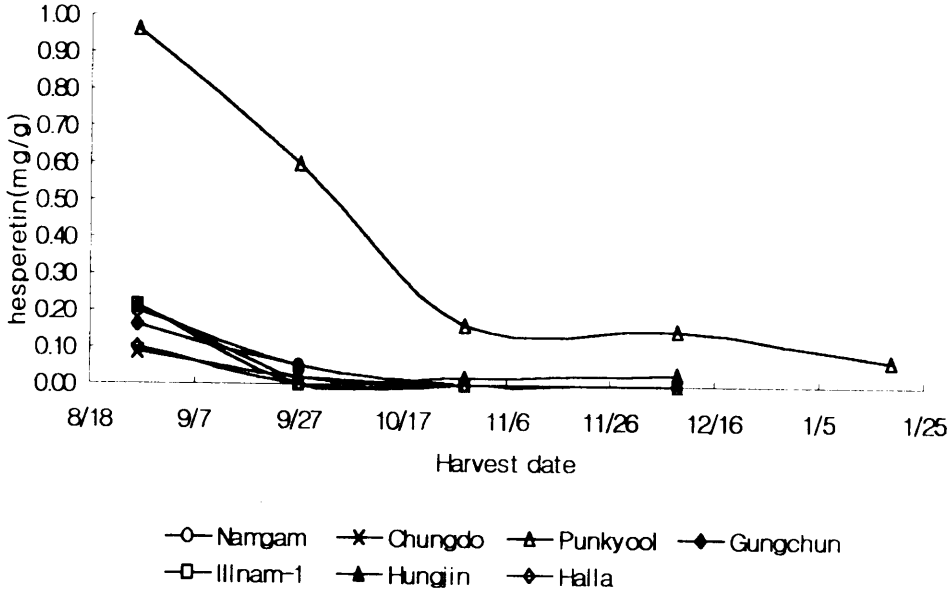

Fig. 20. Changes in hesperetin of citrus fruits sampled at Cheju Agricultural Research and Extension Service.

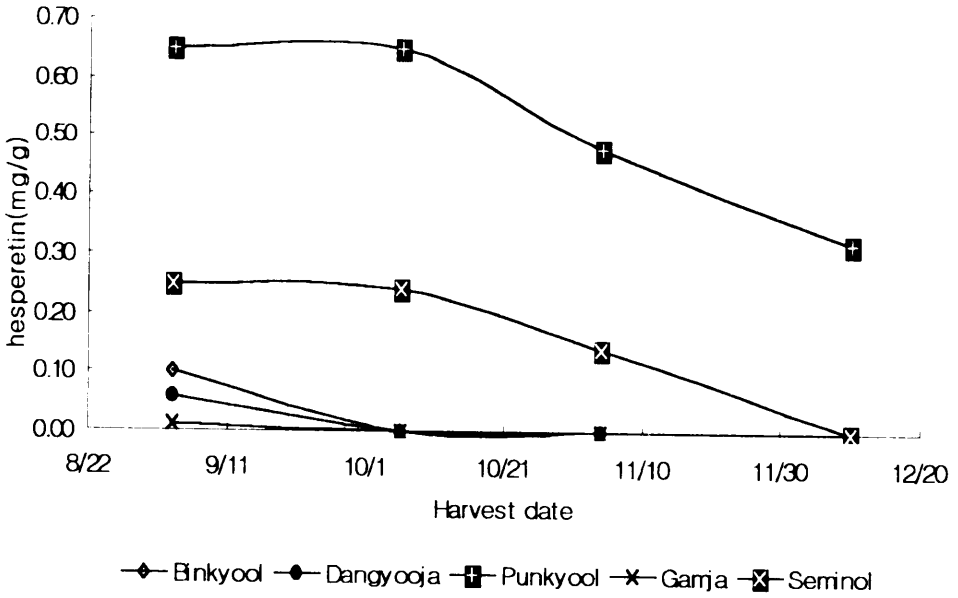

Fig. 21. Changes in hesperetin of citrus fruits sampled at Citrus Experiment Station.

3.8 Change of kaemferol content

Kaemferol was detected only in the peel of seven samples, Fig. 21 and 22 show the Changes of quercetin content in the peel of citrus harvested at Cheju Agricultural Research and Extension Service and the Citrus Experiment Station. Kaemferol content in the peel of Punkyool, Dangyooja and Dajunkum was 0.34 ㎎/g, 0.28 ㎎/g and 0.06 ㎎/g, respectively. In Seminol, Jawdung, Chungdo, and Jikak, it ranged from 0.04 ㎎/g to 0.02 ㎎/g. Content in Punkyool and Dangyooja decreased rapidly from late September to early October, in Chungdo and Dajunkum being found until late August and early October, those in the others were largely unchanged.

3.9 Change of nobiletin content

The Change of nobiletin content of citrus harvested at Cheju Agricultural Research and Extension Service and the Citrus Experiment Station were shown in Fig. 23 and 24. Nobiletin content in the peel of Hongkyool, Jinkyool, Inchangkyool, Bungkyool and Singamha, containing more than 1.00 ㎎/g from late August to early September, was 4.46 ㎎/g, 1.86 ㎎/g, 1.73 ㎎/g, 1.59 ㎎/g and 1.03 ㎎/g in order of content, respectively, and shown the decreasing tendency during maturation. In Namgam-20, Chungdo, Jikak, Gamja, Punkyool, Dangyooja, Gungchun, Illnam-1, Hungjin, Halla, Binkyool, Dingjungkyool, Sambogam, Jawdung, Hongpalsak, Murcott and Seminol, it was 0.11 ㎎/g, 0.06 ㎎/g, 0.15 ㎎/g, 0.24 ㎎/g, 0.41 ㎎/g, 0.26 ㎎/g, 0.10 ㎎/g, 0.11 ㎎/g, 0.13 ㎎/g, 0.10 ㎎/g, 0.71 ㎎/g, 0.40 ㎎/g, 0.06 ㎎/g, 0.08 ㎎/g, 0.11 ㎎/g, 0.16 ㎎/g, 0.08 ㎎/g and 0.16 ㎎/g, respectively, and decreased gradually, but that in the peel of Gungchun was detected until late September.

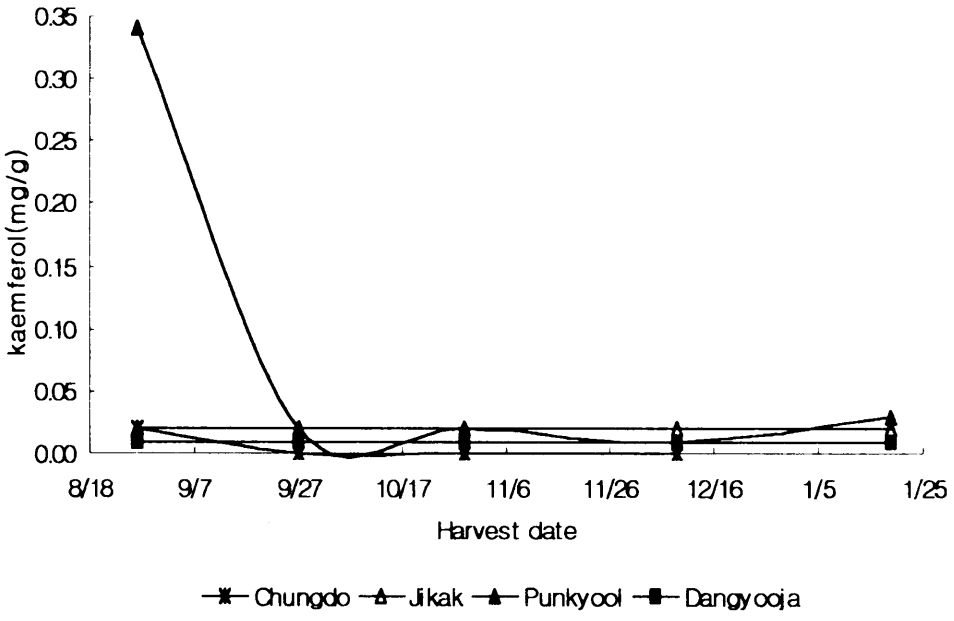

Fig. 22. Changes in kaemferol of citrus fruits sampled at Cheju Agricultural Research and Extension Service.

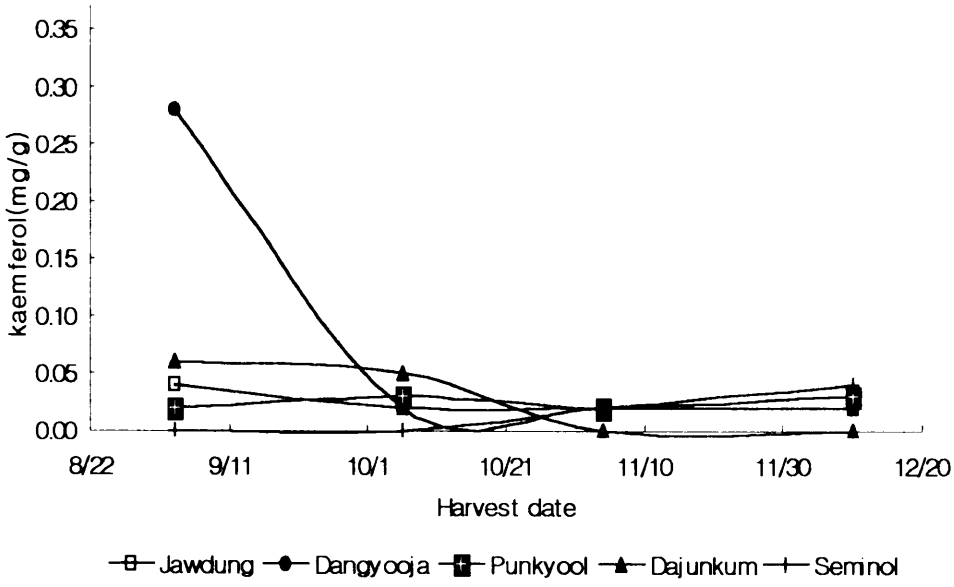

Fig. 23. Changes in kaemferol of citrus fruits sampled at Citrus Experiment Station.

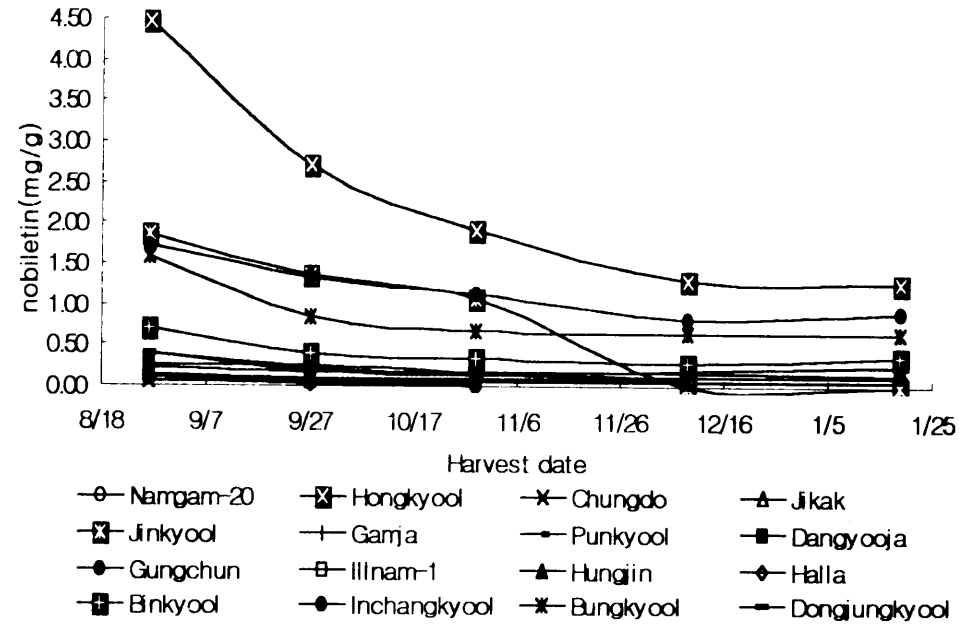

Fig. 24. Changes in nobiletin of citrus fruits sampled at Cheju Agricultural Research and Extension Service.

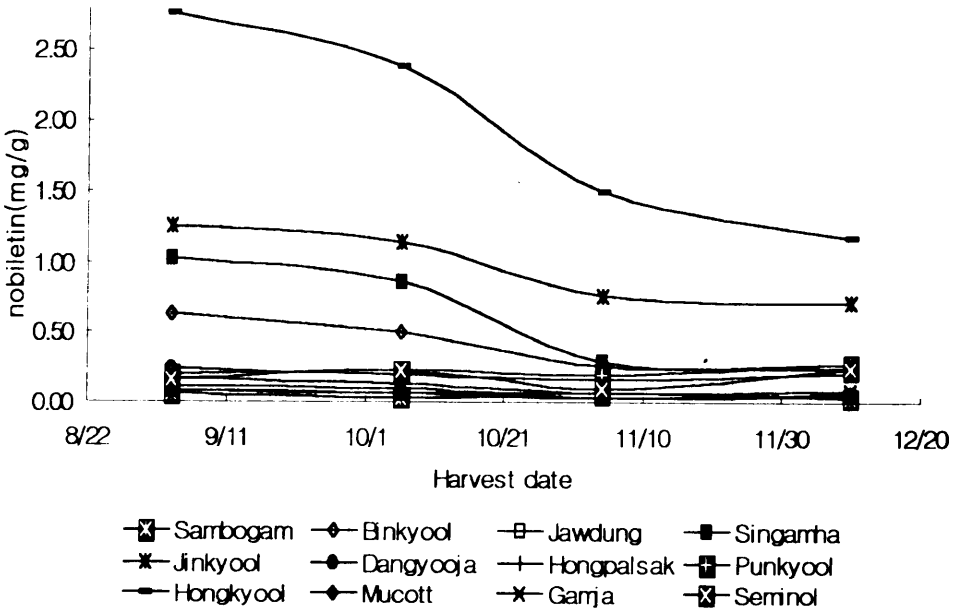

Fig. 25. Changes in nobiletin of citrus fruits sampled at Citrus Experiment Station.

3.10 Change of 3,5,6,7,8,3′,4′-methoxylated flavone(FL-1) content

Fig. 25 and 26 show the Change of quercetin content in the peel of citrus harvested at Cheju Agricultural Research and Extension Service and the Citrus Experiment Station. 3,5,6,7,8,3′,4′ - methoxylated flavone content in the peel of Bungkyool, Gamja and Seminol was 0.22 ㎎/g, 0.20 ㎎/g and 0.16 ㎎/g, respectively, in Jinkyool and Sambogam, it was 0.09 ㎎/g, that in Punkyool and Hungjin was 0.08 ㎎/g, in Hongkyool, Dangyooja, Gungchun, Dongjungkyool and Murcott, it was 0.07 ㎎/g, those in Namgam-20, Chungdo and Illnam-1 was 0.06 ㎎/g, in Hongkyool and Inchangkyool was 0.03 ㎎/g and 0.01 ㎎/g, in order of content. 3,5,6,7,8,3′,4′ - methoxylated flavone was found in peel of Inchangkyool, Halla, Jinkyool, Gungchun and Illnam-1 until the early stages of maturation, those in the others decreased gradually during maturation.

3.11 Change of tangeretin content

The Change of nobiletin content in the peel of citrus harvested at Cheju Agricultural Research and Extension Service and the Citrus Experiment Station were shown in Fig. 27 and 28. Tangeretin content in the peel of Hongkyool, Jinkyool, Inchangkyool, Bungkyool, Binkyool, Dongjungkyool, Punkyool and Gamja was 3.60 ㎎/g, 3.28 ㎎/g, 1.14 ㎎/g, 0.83 ㎎/g, 0.65 ㎎/g, 0.63 ㎎/g, 0.44 ㎎/g and 0.19 ㎎/g, respectively. In Jinkak, Sambogam and Seminol, it ranged from 0.09 to 0.05 ㎎/g, in Namgam-20, Chungdo, Gungchun, Illnam-1, Hungjin, Halla, Jawdung and Murcott, from 0.04 to 0.02 ㎎/g, in order of content. Tangeretin content in the peel of Hongkyool and Jinkyool were decreased rapidly in late of October and that in the others decreased gradually or remained largely unchanged.

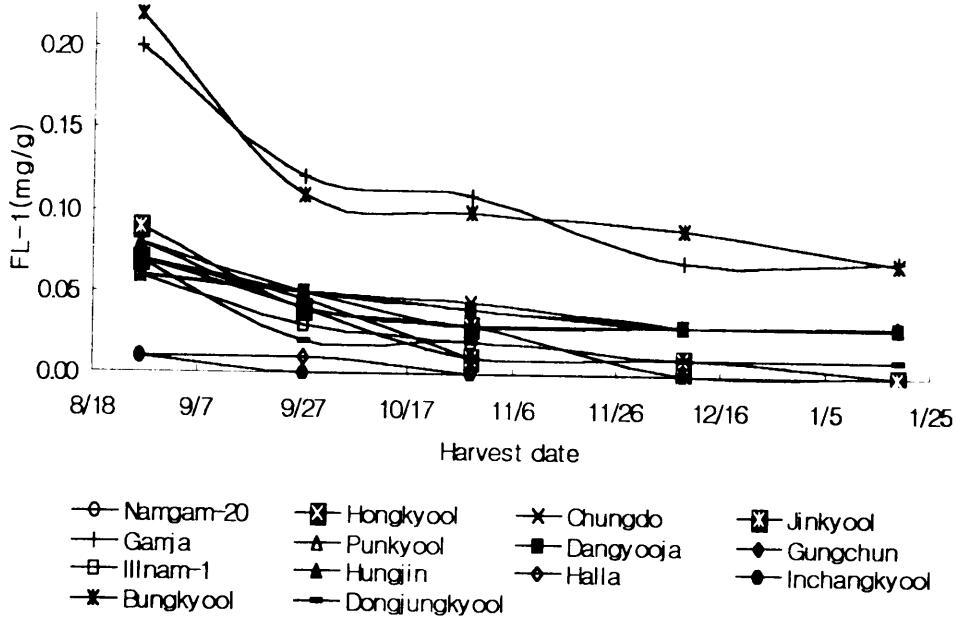

Fig. 26. Changes in flavone of citrus fruits sampled at Cheju Agricultural Research and Extension Service.

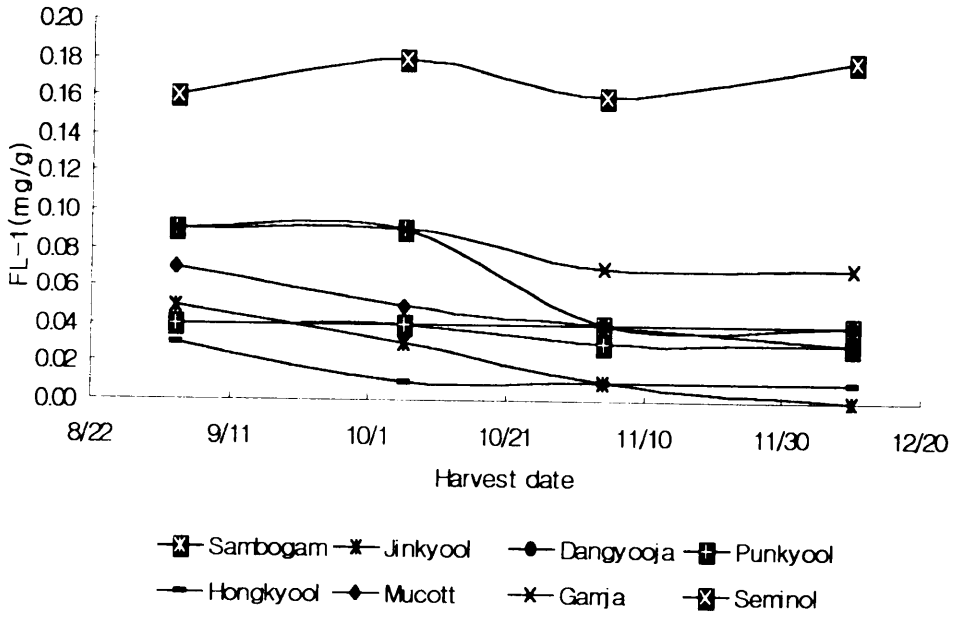

Fig. 27. Changes in flavone of citrus fruits sampled at Citrus Experiment Station.

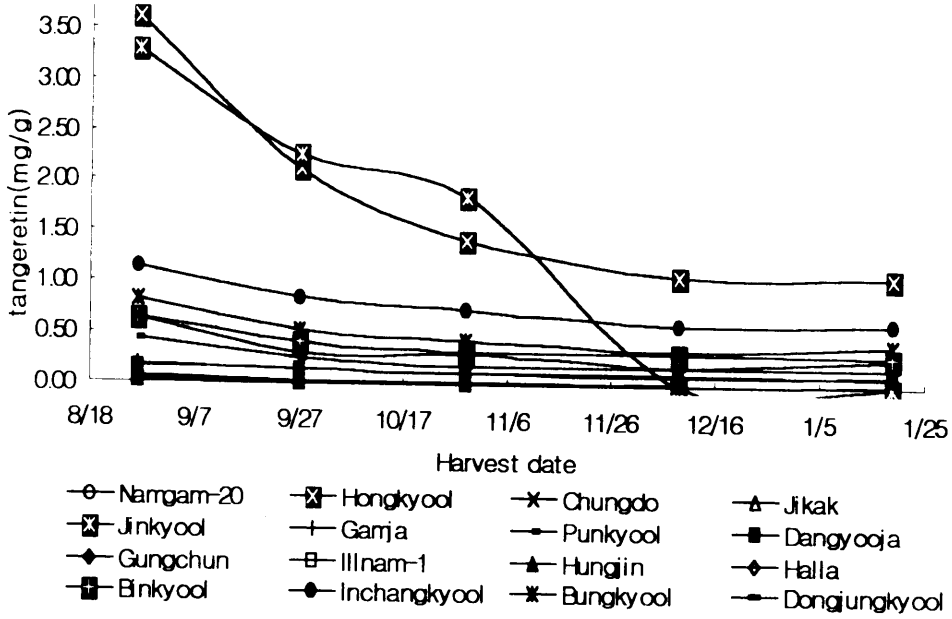

Fig. 28. Changes in tangeretin of citrus fruits sampled at Cheju Agricultural Research and Extension Service.

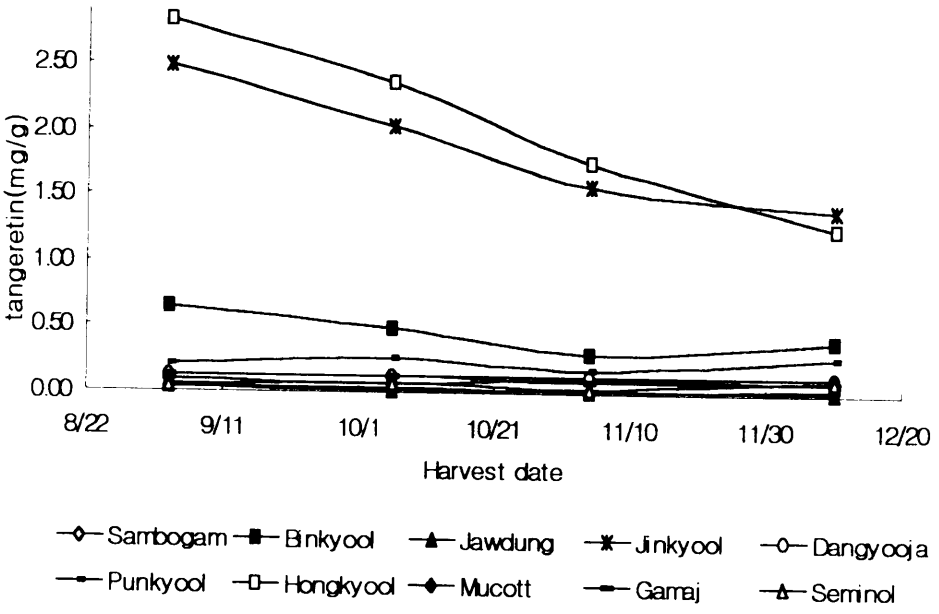

Fig. 29. Changes in tangeretin of citrus fruits sampled at Citrus Experiment Station.

4. Changes of flavonoids content by citrus species

4.1 Change of flavonoids content in Cheju traditional citrus

The Change of flavonoids content in the peel of Cheju traditional citrus by citrus species, are as follows (see Table 9 and 10).

Eight flavonoids, rutin, hesperidin, neohesperidin, quercetin, naringin, nobiletin, 3,5,6,7,8,3',4' - methoxylated flavone and tangeretin, were detected in the peel of Gamja. Hesperidin was contained mainly out of flavonoids, and decreased from 5.99 ㎎/g to 3.28 ㎎/g during maturation.

The peel of Dangyooja had nine flavonoids, rutin, naringin, neohesperidin, quercetin, naringenin, kaemferol, nobiletin, 3,5,6,7,8,3',4' - methoxylated flavone and tangeretin, and the contents of these decreased during maturation.

Neohesperidin and naringin were contained mainly in the peel of Dangyooja and those of content were higher than the others, and decreased from 10.06 ㎎ /g to 3.09 ㎎/g and from 14.55 ㎎/g to 7.95 ㎎/g, respectively.

Rutin, hesperidin, quercetin, nobiletin, 3,5,6,7,8,3',4' - methoxylated flavone and tangeretin were detected in the peel of Dongjungkyool. Hesperidin content was 23.25 ㎎/g, it was high among traditional citrus, and decreased rapidly in late of September.

Six flavonoids, rutin, hesperidin, quercetin, nobiletin, 3,5,6,7,8,3',4' - methoxylated flavone and tangeretin, were detected in the peel of Bungkyool, hesperidin was the highest out of all of them and decreased from 5.38 ㎎/g to 4.19 ㎎/g.

Rutin, hesperidin, quercetin, nobiletin and tangeretin were detected in the peel of Binkyool. Hesperidin content was 25.99 ㎎/g, it was the highest among traditional citrus, and decreased during maturation.

In the peel of Sadoogam, only three flavonoids rutin, hesperidin and quercetin were detected. Hesperidin decreased from 7.71 ㎎/g to 1.43 ㎎/g and rutin content that high compared with the others at 4.20 ㎎/g in early August.

Rutin, naringin, hesperidin, neohesperidin, quercetin and naringenin were detected in the peel of Yooja. naringin, hesperidin and neohesperidin content were 1.39 ㎎/g, 3.04 ㎎/g and 1.19 ㎎/g, respectively, it was similar in content to each other. All of them that were presented in the peel of Yooja decreased during maturation.

Naringin content in the peel of Jikak was 14.55 ㎎/g in early August, it was highest among traditional citrus. Hesperidin content was 8.06 ㎎/g in early August, and decreased during maturation.

Rutin, hesperidin, quercetin, nobiletin, 3,5,6,7,8,3′,4′ - methoxylated flavone and tangeretin was contained in the peel of Jinkyool. Hesperidin content showed high content compared with other flavonoids was 7.71 ㎎/g in early Semtember.

Nine flavonoids naringin, neohesperidin, quercetin, naringenin, hesperetin, kaemferol, nobiletin, 3,5,6,7,8,3′,4′ - methoxylated flavone and tangeretin were detected in the peel of Punkyool. In particular, hesperetin that was not contained in other traditional citrus was found in Punkyool by 0.96 ㎎/g. Naringin and neohesperidin were mainly contained in the peel of Punkyool.

Only five flavonoids, rutin, hesperidin, nobiletin, 3,5,6,7,8,3′,4′ - methoxylated flavone and tangeretin were detected in the peel of Hongkyool. Nobiletin and tangeretin content were 4.46 ㎎/g and 3.60 ㎎/g in late August, it was the highest among traditional citrus, and decreased during maturation.

Rutin, naringin, hesperidin, neohesperidin, quercetin, naringenin and kaemferol were detected in the peel of Dajunkum. Kaemferol was only present in early maturation and hesperidin was mainly contained, the others showed similar contents.

These results show commonly that the peel of Jikak, Punkyool and Dangyooja, not containing hesperidin, contained a high content of naringin and

neohesperidin, conversely, those of Yooja, Jinkyool, Hongkyool, Gamja, Dongjungkyool, Bungkyool, Binkyool and Sadoogam, containing a few of naringin and neohesperidin, contained a high content of hesperidin.

Table 9. Change of flavonoids content in Cheju traditional citrus

	Date	RT	NGI	HD	NHD	DN	QT	NGE	HT	KL	NT	FL	TT
Gamja	9/3	0.65	N	5.99	1.14	N	0.08	N	0.01	N	0.17	0.09	0.13
	10/6	0.43	N	3.94	0.24	N	0.02	N	N	N	0.13	0.09	0.12
	11/4	0.33	N	3.29	0.33	N	0.04	N	N	N	0.07	0.07	0.08
	12/10	0.32	N	3.28	0.28	N	0.03	N	N	N	0.08	0.09	0.08
Dangyooja	8/27	0.12	8.48	0.47	10.06	N	0.03	0.14	N	0.01	0.26	0.07	0.16
	9/27	0.11	5.70	N	6.18	N	0.03	0.10	N	0.01	0.25	0.05	0.13
	10/29	0.17	5.37	N	5.69	N	0.03	0.06	N	0.01	0.18	0.04	0.11
	12/9	0.06	3.61	N	3.68	N	0.02	0.02	N	0.01	0.16	0.03	0.11
	1/9	0.06	3.61	N	3.90	N	0.02	N	N	0.01	0.13	0.03	0.09
Dongjungkyool	8/27	0.17	N	23.25	N	N	0.06	N	N	N	0.40	0.07	0.63
	9/27	0.18	N	8.77	N	N	0.03	N	N	N	0.19	0.02	0.29
	10/29	0.18	N	6.58	N	N	0.03	N	N	N	0.18	0.02	0.29
	12/9	0.18	N	6.68	N	N	0.02	N	N	N	0.12	0.01	0.18
	1/9	0.12	N	5.98	N	N	0.03	N	N	N	0.11	0.01	0.18
Bungkyool	8/27	0.43	N	5.38	N	N	0.03	N	N	N	1.59	0.22	0.83
	9/27	0.40	N	5.18	N	N	0.03	N	N	N	0.84	0.11	0.52
	10/29	0.34	N	5.01	N	N	0.02	N	N	N	0.67	0.10	0.43
	12/9	0.33	N	4.12	N	N	0.02	N	N	N	0.64	0.09	0.34
	1/9	0.25	N	4.19	N	N	0.02	N	N	N	0.65	0.07	0.42
Binkyool	8/27	0.17	N	25.99	0.10	0.06	0.04	N	N	N	0.71	N	0.65
	9/27	0.13	N	12.71	N	N	0.02	N	N	N	0.41	N	0.40
	10/29	0.07	N	8.10	N	N	0.02	N	N	N	0.35	N	0.32
	12/9	0.05	N	6.88	N	N	0.02	N	N	N	0.29	N	0.31
	1/9	0.06	N	6.88	N	N	N	N	N	N	0.38	N	0.30

*N: Not detected, Unit: ㎎/g

RT:rutin, NGI:naringin, HD:hesperidin, NHD:neohesperidin, DN:diosmin, QT:quercetin, NGE:naringenin, HT:hesperetin, KL:kaemferol, NT:nobiletin, FL:3,5,6,7,8,3′4′-methoxylated flavone, TT:tangeretin

Table 10. Change of flavonoids content in Cheju traditional citrus

	Date	RT	NGI	HD	NHD	DN	QT	NGE	HT	KL	NT	FL	TT
Sadoogam	8/27	4.20	N	7.71	N	N	0.11	N	N	N	N	N	N
	9/27	1.60	N	2.90	N	N	0.06	N	N	N	N	N	N
	10/29	1.45	N	2.52	N	N	0.04	N	N	N	N	N	N
	12/9	1.17	N	1.39	N	N	0.03	N	N	N	N	N	N
	1/9	1.28	N	1.43	N	N	0.03	N	N	N	N	N	N
Yooja	8/27	0.63	1.39	3.04	1.19	N	0.02	0.30	N	N	N	N	N
	9/27	0.44	0.76	2.13	0.69	N	0.02	0.23	N	N	N	N	N
	10/29	0.27	0.43	1.43	0.42	N	0.02	0.16	N	N	N	N	N
	12/9	0.20	0.18	1.10	0.24	N	0.02	0.16	N	N	N	N	N
Jikak	8/27	0.09	14.55	N	8.06	N	0.11	N	N	0.02	0.15	N	0.06
	9/27	0.12	12.38	N	7.83	N	0.10	N	N	0.02	0.09	N	0.03
	10/29	0.06	8.73	N	4.02	N	0.09	N	N	0.02	0.08	N	0.03
	12/9	0.04	7.95	N	3.35	N	0.08	N	N	0.02	0.05	N	0.01
	1/9	0.05	7.95	N	3.34	N	0.08	N	N	0.02	0.07	N	0.02
Jinkyool	9/3	0.28	0.19	7.71	0.40	N	0.04	N	N	N	1.26	0.05	2.48
	10/6	0.20	N	5.13	N	N	0.02	N	N	N	1.14	0.03	2.03
	11/4	0.15	N	5.18	N	N	0.02	N	N	N	0.76	0.01	1.58
	12/10	0.11	N	5.23	N	N	0.02	N	N	N	0.72	N	1.39
Punkyool	8/27	N	6.01	0.20	5.97	N	0.02	0.41	0.96	0.34	0.41	0.08	0.44
	9/27	N	1.73	N	2.13	N	0.02	0.14	0.60	0.02	0.23	0.05	0.24
	10/29	N	1.38	N	1.65	N	0.02	0.17	0.16	0.02	0.15	0.03	0.18
	12/9	N	1.32	N	2.12	N	0.02	0.08	0.15	0.01	0.19	0.03	0.19
	1/9	N	1.32	N	2.14	N	N	0.04	0.07	0.03	0.25	0.03	0.28
Hongkyool	8/27	0.09	N	9.31	N	N	N	N	N	N	4.46	0.07	3.60
	9/27	0.12	N	7.45	N	N	N	N	N	N	2.73	0.04	2.13
	10/29	0.12	N	5.36	N	N	N	N	N	N	1.93	0.01	1.42
	12/9	0.09	N	4.37	N	N	N	N	N	N	1.29	0.01	1.08
	1/9	0.08	N	4.43	N	N	N	N	N	N	1.27	N	1.08
Dajunkum	9/3	0.56	0.95	2.81	0.95	N	0.04	0.66	N	0.06	N	N	N
	10/6	0.41	0.61	2.06	0.66	N	0.04	0.75	N	0.05	N	N	N
	11/4	0.30	0.46	1.26	0.39	N	0.03	0.30	N	N	N	N	N
	12/10	0.18	0.21	0.58	0.13	N	0.02	0.30	N	N	N	N	N

Unit: ㎎/g

4.2 Change of flavonoids content in the peel of *Citrus unshiu*.

In the case of *C. unshiu*, heperidin and rutin content were high compared with other flavonoids. Hesperidin content in the peel of Halla, Illnam-1, Hungjin, Gungchun, Chungdo and Namgam in late August was 28.70 ㎎/g, 25.87 ㎎/g, 24.57 ㎎/g, 23.46 ㎎/g, 17.85 ㎎/g and 16.40 ㎎/g in order of content, respectively, and showed decreases during maturation(see Table 11).

Eight flavonoids, rutin, naringin, hesperidin, quercetin, hesperetin, nobiletin, 3,5,6,7,8,3',4' - methoxylated flavone and tangeretin were commonly detected. Naringin and hesperidin were present until late September, but diosmin, naringein and kaemferol were not detected in all the peel of *C. unshiu* havested for this work.

4.3 Change of flavonoids content in the peel of late groups

There are many species that have high content of naringin in late groups. Decreasing ranges of naringin in late groups were not great compared to those of others. Naringin contents in the peel of Jawdung, Singamha and Hongpalsak were 34.02 ㎎/g, 15.94 ㎎/g and 12.11 ㎎/g, respectively. Narigin content in the peel of Jawdung was 34.02 ㎎/g, it was higest among all samples, also neohesperidin was present in higher quantities than other late groups but hesperidin content was not present. Hesperidin contents in the peel of Mucott, Sambogam, Seminol and Inchangkyool were high compared to others(see Table 12).

Table 11. Change of flavonoids in *C. unshiu*

	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
Gungchun	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

Unit: mg/g

Table 12. Change of flavonoids in late group

	Date	RT	NGI	HD	NHD	DN	QT	NGE	HT	KL	NT	FL	TT
Mucott	9/3	1.39	N	12.48	N	N	0.04	0.05	N	N	0.08	0.07	0.04
	10/6	0.93	N	10.40	N	N	0.04	N	N	N	0.07	0.08	0.04
	11/4	1.21	N	14.25	N	N	0.05	N	N	N	0.04	0.04	0.02
	12/10	1.10	N	13.14	N	N	0.04	N	N	N	0.05	0.03	0.03
Singamha	9/3	0.10	15.94	N	3.87	N	0.03	0.08	N	N	1.03	N	N
	10/6	0.07	11.93	N	2.79	N	0.02	0.06	N	N	0.87	N	N
	11/4	0.06	10.56	N	2.22	N	0.02	0.04	N	N	0.30	N	N
	12/10	0.07	7.69	N	1.96	N	0.02	0.03	N	N	0.27	N	N
Sambogam	9/3	5.13	N	11.20	1.61	N	0.02	0.05	N	N	0.06	0.09	0.09
	10/6	2.78	N	10.00	0.58	N	0.02	N	N	N	0.04	0.09	0.07
	11/4	2.38	N	8.70	0.72	N	0.02	N	N	N	0.06	0.04	0.11
	12/10	2.14	N	7.30	0.44	N	0.02	N	N	N	0.03	0.04	0.08
Hongpalsak	9/3	0.30	12.11	0.15	4.10	N	0.03	0.04	N	N	0.11	N	N
	10/6	0.19	12.96	N	5.45	N	0.03	0.06	N	N	0.09	N	N
	11/4	0.23	13.05	0.07	4.88	N	0.03	0.03	N	N	0.03	N	N
	12/10	0.21	12.09	N	4.74	N	0.04	0.04	N	N	0.06	N	N
Seminol	9/3	0.49	N	10.61	0.03	N	0.07	N	0.25	N	0.16	0.07	0.05
	10/6	0.38	N	11.12	0.09	N	0.06	0.03	0.24	N	0.22	0.18	0.07
	11/4	0.34	N	9.56	N	N	0.07	0.01	0.14	0.02	0.10	0.08	0.03
	12/10	0.42	N	11.19	0.12	N	0.07	N	N	0.04	0.25	0.18	0.10
Jawdung	9/3	0.16	34.02	N	13.68	N	0.10	0.10	N	0.04	0.08	N	0.03
	10/6	0.10	15.53	N	5.89	N	0.07	0.02	N	0.02	0.07	N	0.02
	11/4	0.07	12.95	N	5.14	N	0.06	0.02	N	0.02	0.07	N	0.02
	12/10	0.08	12.85	N	5.24	N	0.06	N	N	0.02	0.05	N	0.02
Inchangkyool	8/27	0.41	N	3.40	1.07	N	0.05	N	N	N	1.73	0.01	1.14
	9/27	0.55	N	4.28	1.62	N	0.06	N	N	N	1.33	N	0.84
	10/29	0.51	N	5.08	0.98	N	0.06	N	N	N	1.14	0.01	0.73
	12/9	0.43	N	4.22	0.67	N	0.05	N	N	N	0.82	N	0.60
	1/9	0.46	N	5.10	0.90	N	0.05	N	N	N	0.91	N	0.62

Unit: mg/g

Ⅳ Summary

This study was intended to examinate twelve flavonoid contents and Changes in twenty-five citrus species to identify the flavonoid contents and Changes during maturation in *Citrus unshiu*, late group and Cheju traditional citrus.

The peel ratio according to citrus species, decreased by approximately 10 % during maturation or stable except for Yooja that showed a peel ratio of sligthly increasing tendency. Jikak had the highest peel ratio by 58.41 % and Jinkyool had the lowest peel ratio by 14.08 % in the last harvest .

Rutin, naringin, hesperidin, diosmin, neohesperidin, quercetin, naringenin, hesperetin, kaemferol, nobiletin, 3,5,6,7,8,3′,4′ - methoxylated flavone and tangeretin were detected in citrus peel. Rutin was present in the peel of twenty-four citrus species and contained highest amounts in the peel of Sambogam by 5.13 ㎎/g in early September. Naringin was found in peel of fifteen citrus species and contained highest amounts in the peel of Jawdung by 34.02 ㎎/g in early September. Hesperidin was present in the peel of twenty-two citrus species and contained highest amounts in the peel of Halla by 28.70 ㎎/g in late August. Neohesperidin was present in the peel of thirteen citrus species and contained highest amounts in the peel of Jawdung by 13.68 ㎎/g in early September. Diosmin was detected only in Gamja and Dangyooja by 0.71 ~ 0.01 ㎎/g until late September and October. Quercetin was present in the peel of twenty-three citrus species and contained highest amounts in the peel of Hungjin and Halla by 0.18 ㎎/g in late August. Naringein was present in the peel of twelve citrus species and contained highest amounts in the peel of Dajunkum by 0.66 ㎎/g in early September. Hesperetin was present in the peel of eleven citrus species and contained highest amounts in the peel of

Punkyool by 0.96 ㎎/g in late August. Kaemferol was present in the peel of seven citrus species and contained highest amounts in the peel of Punkyool by 0.34 ㎎/g in late August. Nobiletin contained highest amounts in the peel of Hongkyool by 4.46 ㎎/g and detected in the peel of twenty-two citrus species. 3,5,6,7,8,3′,4′ - methoxylated flavone was contained in the peel of seventeen citrus species and ranged from 0.01 ㎎/g to 0.22 ㎎/g. Tangeretin was contained in the highest quantity in the peel of Jinkyool by 3.28 ㎎/g in late August and detected in the peel of twenty citrus species.

In the case of Cheju traditional citrus, the peel of Jikak, Punkyool and Dangyooja, not containing hesperidin, contained a high content of naringin and neohesperidin, conversely, those of Yooja, Jinkyool, Hongkyool, Gamja, Dongjungkyool, Bungkyool, Binkyool and Sadoogam, containing only a little naringin and neohesperidin, contained a high content of hesperidin. The peel of *Citrus unshiu* mainly contained hesperidin . Much of the peel in the late group contained naringin and showed stable naringin content change during maturation, the peel of the late group that had high naringin and neohesperidin content contained almost no hesperidin, conversely, the peel of the late group that had hesperidin contained low naringin and neohesperidin content just as the *Citrus unshiu*.

V. Reference

Bilyk, A. and Sapers, G.M., Distribution of quercetin and kaemferol in lettice, kale, chive, garlic chive, leek, horseradish, red radish, and red cabbage tissues, *J. Agric. Food Chem.*, 33, 226(1985)

Casteele, K.V., Pooter, H.D. and Van Summre, C.F., Gas chromatograpic separation and analysis of trimethylsilyl derivatives of some naturally occurring non-volitile phenolic compounds and related substances, *J. chromatogr.*, 121, 49-63(1976).

Cheju Famer Education Center, Citrus cultivation, P. 87-117(1989).

Cheju Citrus Conference, The actual condition analysis about circulation disposal of citrus produced in 1999(2000).

Koh, J.S., Koh, J.E. Yang, S.H. and Ahn, S.U., Physicochemical properties and sensory evaluation of *Citrus. unshiu* produced in Cheju, *Korean J. Agric. Chem and Biotechnol.*, 37(3), 161-167(1994).

Koh, J.S., Kim, C.S., Koh, M.S. and Yang, Y.T., Manufacture of kumquats jelly and honey-kumquats slice and its quality characteristics, *Korean J. Food Sci. Technol.*, 25(1), 33-38(1993).

Koh, J.S., Yang, Y.T. and Song, E.Y., Physicochemical properties on the quality evaluation of *Citrus. unshiu* produced in Cheju, *Korean J. Postharvest Sci. Technol.*, 2(2), 251-257(1995).

Kim, B.J., Kim, H.S. and Kang, Y.J., Comparison of physico-chemical components on citrus varieties, *Korean J. Postharvest Sci. Technol.*, 9, 259-268(1995).

Han, S.S. and You, I.J., Sudies on antimicrobial activities and safety of natural naringin in Korea, *Korean J. Mycol.*, 16(1), 1-8(1988).

Huh, I.O. and Koh, K.S., A chemotaxonomic study on the citrus plants. Cheju National University Journal(1990).

Harborne, J.B. and Mabry, T.J., The flavonoids, Chapman and Hall Ltd., New York, p. 189-632(1982).

Hermann, K., Flavonols and flavones in plants. A review, *J. Food. Technol.*, 11(30), 433-448(1976).

Hertog, M.G.L., Hollman, P.C.H. and Katan, M.B., Content of potentially anticarcinogenic flavonoids of 28 vegetables and 9 fruits commonly consumed in the Netherlands, *J. Agric. Food Chem.*, 40(12), 2379(1992).

Hertog, M.G.L., Hollman, P.C.H., and Venema, D.P., Optimization of a quantitative HPLC determination of potentially anticarcinogenic flavonoids in vegetables and fruits, *J. Agric. Food Chem.*, 40(9), 1591(1992).

Horowitz, R.M. and Gentili, B., Taste and structure in phenolic glycosides. *J. Agric. Food Chem.*, 17(4), 696-700(1969).

Kuhnau, J., The flavonoids, A class of semi-essential food components, Their role in human nutrition. *World Rev. Nutr. Diet.*, 24, 117(1976).

Lee, C.H., HPLC analysis of some flavonoids in citrus fruits. Thesis for the degree of master of engineering. Cheju National University(1994).

Lio M., Masaru, M., Iwanami, T. and Yoshikatsu, Y., Flavonoid as a possible preventive of dental carries, *Agric. Biol. Chem.*, 48(8), 2143-2145(1984).

Masukawa, K., Komatsu, H., Fujimura, N., Fukuyama, T., Fukushima, M., and Ishikawa, T., Determination of hesperidin and its enzymatic hydrolysis products in satsuma mandarin by high performance liquid chromatography, *Nippon Shokuhin Kogyo Gakkaishi*, 32(12), 864-869(1985).

Matsubara, Y., Kumamoto, H., Lizuka, Y., Murakami, T., Okamoto, K., Miyake, H. and Yokoi, K., Structure and hypotensive effect of flavonoid glycosides in *Citrus unshiu* peelings, *Agric. Biol. Chem.*, 49(4), 909-914(1985).

Mattews, J.W., Rouseff, R.L., Manlan, M. and Norman, S.I., Removal of limonin and naringin from citrus juice by styrene-divinyl benzene resins, *Food Technol.*, 44(4), 130-132(1990).

Middleton, E.Jr. and Kandaswami, C., Potential health promoting properties of citrus flavonoids, *Food Technol.*, 48(11), 115(1994).

Olson, A.C., Gray, G.M. and Guadagni, D.G., Naringin bitterness of grapefruit juice debittered with naringinase immobilized in a hollow fiber, *J. Food Sci.*, 44, 1358-1361(1979).

Park, H., Kim, Y.S. and Kim, Z.U., Studies on the chemical composition of citrus fruits in Korea(Ⅱ), *Korean J. Agric. Chem and Biotechnol.*, 9, 41-57(1968).

Perfetti, G.A., Joe, F.L., Fazio, T. and Page, S.W., Liquid chromatographic methodology characterization of orange juice. *J. Assoc. Off. Anal. Chem.*, 71(3), 469-473(1988).

Rouseff, R.L., Liquid chromatographic determination of naringin and neohesperidin as a detector of grapefruit juice in orange juice, *J. Assoc. Off. Anal. Chem.*, 7(4), 798-802(1988).

Saunt, J., Citrus varieties of the world, Sinclair International Ltd, Norwich, England, p. 5(1990).

Son, H.S., Kim, H.S., Kwon, B.T. and Ju, J.S., Isolation purification and hypotensive effect of bioflavonoids in citrus sinensis, *J. Korean. Food Nutr.*, 21(2), 136(1992).

Song, E.Y., Quality characteristics of citrus fruits according to harvest date and variety, Thesis for the degree of master of agriculture. Cheju National University(1997).

Tatum, J.H., and Berry, R., Method for determining naringin content in grapefruit juice. *J. Food. Sci.*, 38(2), 340-341(1973).

Ting, S.V., Rouseff, R.L., Dougherty, M.H. and Attaway, J.A., Determination of some methoxylated flavones in citrus juices by high performance liquid chromatography, *J. Food Sci.*, 44(1), 69-71(1979).

Ting, S.V. and Rouseff, R.L., Citrus fruits and their products, Marcel Dekker, New York, p. 109(1986).

Velloglu, Y.S. and Mazza, G., Characterization of flavonoids in petals of rosa damascena by HPLC and spectral analysis, *J. Agric. Food Chem.*, 39(3), 463-467(1991).

Woo, G.J., Eun, J.B. and Jung, Y.M., Identification and determination of dietary fibers and flavonoids in pulp and peel of Korean tangerine, *Korean J. Food Sci. Technol.*, 28(2), 371-377(1996).

Yang, C.B., Park, H. and Kim, Z.U., Studies on the chemical composition of citrus fruits in Korea(Ⅱ), *Korean J. Agric. Chem and Biotechnol.*, 8, 29(1967).

Acknowledgements

I would like to express my deepest appreciation to professor Jeong-Sam Koh and Dr. Kyung-Soo Koh, my adviser during graduate course. I benefited from their guidance, encouragement, and support. I also thank the members of my disseration committe, professor Chan-Shick Kim and professor Chang-Hee Kang for their advice and interest during my work.

I also thank to professor Soon-Suon Kang, professor Zang-Kual U, professor Key-Zung Riu, professor Hae-Nam Hyun for their sincere help. In addition, I wish to express my appreciation to Chul-Hoon Yoo, Ji-Yong Kim, Ja-Hoon Lim and Hae-Lim Kang who made this research possible. I also thank to assistant teacher Chang-Yong Moon and Se-Kwang Koh for their help.

I also extend a most sincere thanks to my mother and family. Finally to my loving wife Eun-Soon Sin for her assistance.