

Antioxidative Substances and Their Changes in the Leaves of Persimmon (*Diospyros kaki*) during Growth

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감잎의 성장 중 항산화물질의 함량 변화

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Abstract

Changes in antioxidative substance levels in eleven different cultivars of persimmon leaves during growth were investigated. In general, the contents of soluble phenols, L-ascorbic acid and flavonoids in astringent persimmon leaves(APL) were higher than those of nonastringent persimmon leaves(NAPL). The soluble phenol contents in APL and NAPL showed a tendency to decrease throughout leaf growth. L-ascorbic acid content in APL decreased rapidly during growth, whereas its content in NAPL reached its highest value at the late of July, and then decreased rapidly. Major flavonoids in APL and NAPL were quercetin and kaempferol which were present in conjugate forms. Before acid hydrolysis, the contents of kaempferol and quercetin in APL and NAPL remained at a relatively constant level until the late of July, and then decreased slightly. After acid hydrolysis, kaempferol contents in APL and NAPL varied significantly by cultivar and growth stage, while quercetin contents decreased slowly until the late of July, and then increased drastically, reached a maximum at the early of August, afterward continuously decreased. These results suggest that APL harvested at the early of June may be useful as potential sources of natural antioxidants.

Key words : persimmon leaf, soluble phenols, L-ascorbic acid, flavonoids.

Introduction

The leaves of a persimmon, *Diospyros kaki* Thunb. (Ebenaceae) are good sources of important dietary antioxidants, such as vitamin A & C, flavonoids and tannins. In addition, persimmon leaves also contained several kinds of minerals and dietary

fibers, thereby widely used them as a tea in oriental countries.

The above antioxidant substances in persimmon leaves have been reported to possess many biochemical and pharmacological effects, including anticancer [1,2], antitumour [3,4] and antioxidation [5-7]. A persimmon tannin and leaf have been traditionally used for treatment of hypertensive diseases [8-10], and especially the flavonol glucosides, such as astragaloside and kaempferol 3-O-

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(2''-*O*-galloyl)-glucoside were recently found to be effective inhibitors against angiotension-converting enzyme (ACE) [11]. In addition, several reports linked flavonoid intake to reduced cancer risk, and also inhibited enzymes, such as prostaglandin synthase, lipoxygenase, cyclooxygenase, closely related to inflammation and tumorigenesis [12].

Several researches are available on the quantification of antioxidative compounds from persimmon leaves [13-15], and it was reported that persimmon leaves have condensed tannins, vitamin C, and flavonoids. Their contents varied dramatically, especially as influenced by factors, such as growth, genotype and processing [13,16]. However, there is few report on the systematic analysis of antioxidative compounds, such as L-ascorbic acid and flavonoids in persimmon leaf using by HPLC, and on the changes of their compounds during leaf growth.

The purpose of present study was to determine the contents of antioxidative substances in eleven cultivars of persimmon leaves and to investigate the change of their levels during growth.

Materials and Methods

Materials and reagents

Six astringent persimmon (*Diospyros kaki*) leaf cultivars, Bansi, Kojongsi, Dungsí, Bongok, Tabe, and Weulhasi, were harvested from various parts of the young trees at the farm of Chungdo, Sangju, Kimcheon, and Youngdong, respectively. Five nonastringent persimmon leaf cultivars, Fuyu, Sunsahwan, Sechonjosang, Idu, and Jiro were also harvested from the farm at Kimhae. All persimmon leaves were obtained on a continuing basis at 15 days intervals after flowering, from June to September 1997. All samples were harvested in early morning and maintained below 15°C until arrival at the laboratory. Quercetin, kaempferol, L-ascorbic acid, and gallic acid was purchased from Sigma Chemical Co., (St Louis, MO, USA). All other chemicals used for this study were of analytical grade.

Souble phenolic compounds

The extraction and measurement of total soluble phenolics was performed according to the method of Swain and Hillis [17]. Fresh persimmon leaf (5g) was homogenized with distilled-H₂O for 1min, filtered through Whatman No. 2 filter paper, and finally filled up to 100ml with distilled-H₂O. Sample (5ml) was mixed with 5ml of 0.2N Folin-Ciocalteu reagent and throughly shaken for 3min. Further 5ml of saturated sodium carbonate (75g/l) was added to the mixture and then shaken. The aborbance of the solution at 765nm was measured after 1hr with a spectrophotometer (Spectronic Genesys, Milton Roy, USA). Quantification was based on the standard curve of 100, 200, 300, 400, and 500mg/l of gallic acid prepared as the same time.

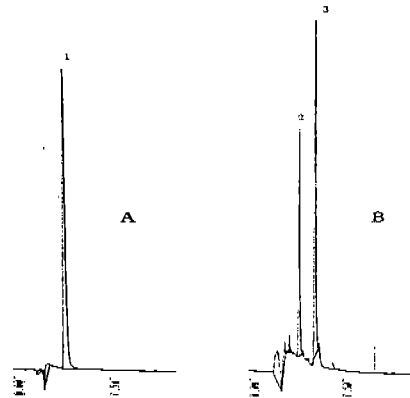


Fig. 1. Typical HPLC chromatograms of L-ascorbic acid(A) and flavonoid aglycones(B) isolated from the extracts of persimmon leaves. 1, L-ascorbic acid; 2, quercetin; 3, kaempferol. HPLC conditions were the same as illustrated in the Materials and Methods.

L-Ascorbic acid analysis

L-Ascorbic acid extraction was performed according to the method of Wimalasiri and Wills [18]. Fresh persimmon leaf (5g) was homogenized in 80ml of 3% citric acid, filtered, and then filled up to 100ml with the same solvent. The extract (4ml) was passed through Sep-Pak C₁₈ cartridges to remove

Table 1. Changes of soluble phenol contents in eleven different persimmon leaves during growth
(mg/g, fresh weight)

Type	Cultivar	Date							
		6/4	6/20	7/4	7/20	8/4	8/20	9/4	9/20
APL ¹	Kojongsi	11.74	8.13	6.43	3.97	5.05	6.77	7.48	4.58
	Dungsi	11.23	10.42	8.33	6.20	7.10	8.52	9.10	7.69
	Bongkok	11.37	10.74	8.69	7.74	6.82	7.59	6.83	5.01
	Weulhasi	10.63	8.88	6.24	6.15	5.73	5.79	5.91	5.25
	Taba	11.27	6.88	5.24	4.63	5.23	6.20	4.21	3.89
	Bansi	12.01	9.70	9.11	5.43	6.25	6.98	5.76	5.44
NAPL ²	Sunsahwan	9.22	8.80	8.17	7.08	7.41	6.45	6.20	5.52
	Jiro	9.45	7.04	5.79	4.98	4.16	4.78	4.10	3.90
	Idu	10.66	9.58	8.39	6.62	5.10	5.32	4.55	4.30
	Fuyu	9.54	8.01	7.41	6.81	5.54	5.62	5.44	5.37
	Sechonjosang	9.11	7.87	7.39	5.42	5.39	6.61	7.10	6.24

The data were means of duplicate determination.

¹Astringent persimmon leaf.

²Nonastringent persimmon leaf.

interfering compounds prior to HPLC analysis. The first 3ml was discarded and next 1ml was used for analysis. HPLC analysis was performed using a Bondapak-NH₂ column (3.6mm x 30cm, Waters Associates, Milford, MA, USA) with a mobile phase of CH₃CN/H₂O (70:30) with 0.01M ammonium dihydrogen phosphate (pH 4.3) at a flow rate of 1ml/min with monitoring at 254nm (Fig.1). Recovery rate for ascorbic acid was 99%.

Flavonoid analysis

The procedure for the extraction and analysis of flavonoids was slightly modified from the method of Hertog et al. [19]. Flavonoid aglycones were analyzed before and after acid hydrolysis of flavonoid conjugates. HPLC chromatograms were compared with quercetin and kaempferol standards.

Fresh persimmon leaf (5g) was homogenized in 80ml of 60% aqueous methanol, filtered through filter paper, washed with same solvent and filled up to 100ml. Extract (5ml) was passed through Sep-Pak C₁₈ cartridges, and used for quercetin and kaempferol quantitation by HPLC. The other extract (5ml) was hydrolyzed with 10ml of 2N HCl in 50% methanol at 90°C for 1hr, and then concentrated to a small volume *in vacuo*. The flavonoid aglycones were

extracted with ethyl acetate (20ml), and then dried with anhydrous sodium sulfate and concentrated to dryness. The resulting residue was redissolved in 5ml of 50% acetonitrile, and then quantified kaempferol and quercetin by HPLC using a ODS-5 column (4.6mm x 25cm, Nomura Chem., Saito, Japan), with a solvent system of CH₃CN/H₂O (50:50), pH 3.0 with phosphoric acid at 1ml/min monitoring at 375nm (Fig.1). Recovery rates were: quercetin 96%, kaempferol 97%. Duplicate analyses of all sample were conducted on duplicate samples.

Results and Discussion

Quantification of total soluble phenolic compounds

The changes of total soluble phenol contents in eleven cultivars of persimmon leaves during growth are shown in Table 1. At the early stage of growth, most of APL had higher the soluble phenol contents than NAPL. Their contents of APL were the highest at the early of June, decreased rapidly until the late of July, and then slightly increased, and thereafter continuously decreased with development of fruit. Their contents of NAPL also showed the same pattern of change. Thus, the decrease of total soluble

Table 2. Changes of L-ascorbic acid contents in eleven different persimmon leaves during growth
(mg/g, fresh weight)

Type	Cultivar	Date							
		6/4	6/20	7/4	7/20	8/4	8/20	9/4	9/20
APL ¹	Kojongsi	7.78	5.40	4.56	3.16	3.96	2.58	1.74	0.74
	Dungsi	6.56	4.42	4.77	4.80	5.84	4.98	3.31	1.12
	Bongok	8.10	7.66	6.53	5.30	1.99	0.99	0.78	0.92
	Weulhasi	8.04	7.68	6.52	5.35	3.53	2.94	1.21	0.97
	Tabe	5.90	4.18	2.86	1.55	2.66	1.25	0.33	0.47
	Bansi	6.97	6.82	4.67	2.94	0.65	0.98	0.67	0.34
NAPL ²	Sunsawhan	1.23	0.82	1.53	4.36	2.19	0.41	0.35	0.25
	Jiro	1.53	1.24	1.75	1.81	2.12	1.42	0.91	0.67
	Idu	4.58	4.79	5.89	6.21	2.47	0.90	0.61	0.43
	Fuyu	1.13	1.68	2.73	3.01	2.04	1.34	1.20	0.96
	Sechonjosang	2.32	2.22	3.92	5.71	2.30	2.11	1.64	1.33

The data were means of duplicate determination.

¹Astringent persimmon leaf.

²Nonstringent persimmon leaf.

phenol contents after flowering may mainly be caused by movement of soluble phenol in leaf to persimmon fruit with development of persimmon fruits [20]. This result support an earlier report that the soluble phenol content in persimmon leaves have a tendency to decrease and increase during growth [13,20].

Quantification of L-Ascorbic acid

The changes of L-ascorbic acid content in eleven cultivars of persimmon leaves during growth are given in Table 2. At the early stage of growth, a large cultivar variation in L-ascorbic acid level, ranging from 1.13mg/g to 8.10mg/g, was noticed. Most of APL had higher the L-ascorbic acid contents than NAPL. Its contents in APL was the highest at the early of June, rapidly decreased until the late of July, and then slightly increased again, afterward decreased drastically. An exception was "Weulhasi", "Bansi" and "Bongok" persimmon leaves, which were decreased sharply throughout growth. Meanwhile, its content in NAPL increased rapidly during the leaf development stage (between June and July), reached maximum value at the late of July or the early of August, and then continuously decreased. This result support that ascorbic acid content in fruits and vegetables has varied greatly among cultivars and growth[21]. In

particular, these ascorbic acid concentrations were considerably lower than previous results from similar persimmon leaf cultivars[13]. Differences may be due to experimental conditions, i.e. HPLC analyses is useful method for simultaneous determination of L-ascorbic acid and dehydroascorbic acid, whereas a 2,4-dinitrophenyl- hydrazine (DNP) method used for measurement of a total vitamin C can not be differentiated L-ascorbic acid from dehydroascorbic acid [13,18].

Quantification of flavonoids

It is well-known that persimmon leaves contained major flavonol aglycones, such as kaempferol, quercetin and their glycosides together [6,9]. So, we determined flavonol aglycone contents in APL and NAPL during growth before and after acid hydrolysis, respectively (Table 3). In the previous report [22], it was found that a 30min hydrolysis with 2N HCl in 50% aqueous MeOH at 90°C was found to be the most effective acid hydrolysis conditions for analysis of flavonoid aglycones.

Before acid hydrolysis, the contents of kaempferol and quercetin in APL and NAPL remained at a relatively constant level until the late of July, and then decreased slightly. In contrast, after acid hydrolysis, the contents of two flavonoids in persimmon leaves were very variable according to

Table 3. Changes of Kaempferol contents in eleven different persimmon leaves during growth
(mg/kg, fresh weight)

Type	Cultivar	Date								
		6/4	6/20	7/4	7/20	8/4	8/20	9/4	9/20	
APL ¹	Kojongsi	561.12 (200)	324.23 (150)	372.45 (170)	382.46 (180)	313.29 (93)	275.67 (86)	216.46 (72)	301.27 (61)	
	Dungsi	420.43 (150)	483.57 (130)	464.37 (130)	453.83 (140)	354.43 (82)	432.49 (51)	404.67 (51)	369.53 (49)	
	Bongok	414.48 (120)	315.78 (110)	484.46 (110)	582.92 (130)	475.67 (110)	488.34 (56)	312.67 (52)	221.84 (50)	
	Weulhasi	407.45 (98)	267.38 (77)	301.46 (89)	436.23 (100)	378.28 (79)	334.26 (48)	278.48 (44)	267.57 (40)	
	Tabe	338.24 (97)	493.48 (190)	694.25 (210)	871.39 (230)	575.69 (210)	378.95 (130)	312.86 (110)	264.35 (90)	
	Bansi	445.23 (97)	373.57 (110)	324.39 (84)	314.84 (70)	384.39 (49)	328.39 (38)	267.58 (32)	243.67 (29)	
	Sunsahwan	245.56 (81)	225.87 (88)	210.34 (69)	189.57 (59)	446.37 (39)	412.8 (36)	321.9 (32)	256.65 (29)	
	Jiro	534.36 (130)	519.52 (120)	428.51 (110)	329.54 (120)	310.35 (110)	281.04 (75)	243.63 (71)	220.34 (68)	
	NAPL ²	Idu	229.56 (92)	210.45 (59)	189.98 (80)	178.94 (47)	374.35 (75)	270.87 (53)	238.49 (50)	258.52 (41)
		Fuyu	208.67 (81)	186.58 (57)	278.73 (68)	345.67 (86)	334.14 (100)	334.07 (70)	275.84 (69)	302.43 (58)
Sechonjosang		306.92 (110)	304.53 (99)	256.59 (87)	228.84 (93)	543.67 (48)	456.95 (45)	339.24 (40)	289.72 (38)	

The data were means of duplicate determination.

¹Astringent persimmon leaf.

²Non-astringent persimmon leaf.

() : Before acid hydrolysis.

cultivars. At the early stage of growth, kaempferol contents in APL were higher than those of NAPL, but the contents of quercetin in APL were not significantly different from those of NAPL. The contents of kaempferol and quercetin in APL remained at a relatively constant level throughout growth, except "Tabe", which has the highest content at the late of July. Their contents in NAPL decreased slightly until the late of July, and then rapidly increased, reached a maximum value at the early of August, afterward decreased rapidly, except "Jiro", which have a tendency to decrease during growth. Thus, persimmon leaves have high kaempferol and quercetin levels when compared to other fruits and vegetables [23], thereby can play an important role in protection against risk of coronary heart disease and cancer [24]. This result also indicated that flavonoid glycosides may be mainly contributed to the change of flavonoid contents in

two types of persimmon leaves during growth. From above these results, it was found that the levels of antioxidative substances in persimmon leaves varied greatly according to cultivars and growth. Although the best quality of persimmon leaf cultivars did not speculate, our data suggest that APL harvested at the early of June may be useful as potential sources of natural antioxidants due to higher soluble phenols, L-ascorbic acid and flavonoid levels. Further research on the antioxidative activity of APL harvested at the early of June using a rat liver microsomal lipid peroxidation system is now in progress.

요 약

천연항산화물질의 원료로 적합한 감잎을 선별하기 위해, 한국에서 자생하는 11종 (뽕은감잎 6종, 단감잎 5종)의 감잎을 6월초부터 9월말까지 수확

Table 4. Changes of quercetin contents in eleven different persimmon leaves during growth
(mg/kg, fresh weight)

Type	Cultivar	Date								
		6/4	6/20	7/4	7/20	8/4	8/20	9/4	9/20	
APL ¹	Kojongsi	189.45 (89)	110.35 (51)	167.92 (62)	234.98 (62)	80.82 (27)	142.11 (31)	119.53 (30)	100.05 (28)	
	Dungsi	128.65 (30)	186.99 (62)	239.52 (75)	267.84 (83)	143.67 (26)	179.22 (40)	159.54 (36)	169.74 (35)	
	Bongok	130.34 (39)	115.46 (30)	285.94 (68)	356.66 (82)	199.94 (38)	225.72 (43)	208.51 (40)	204.72 (39)	
	Weulhasi	128.53 (40)	91.43 (19)	184.72 (47)	284.91 (66)	157.53 (23)	144.23 (12)	110.52 (11)	104.15 (10)	
	Tabe	72.43 (40)	163.56 (59)	456.55 (140)	630.54 (180)	246.67 (73)	184.35 (64)	143.9 (60)	120.35 (59)	
	Bansi	185.93 (41)	184.19 (31)	168.53 (38)	167.92 (90)	287.55 (53)	194.32 (24)	167.54 (20)	148.51 (17)	
	Sunsawhan	110.21 (60)	90.12 (52)	79.12 (54)	69.73 (55)	338.49 (24)	247.88 (22)	218.91 (20)	189.62 (17)	
	Jiro	307.52 (130)	318.34 (120)	278.72 (120)	210.43 (120)	200.93 (72)	178.5 (53)	156.72 (50)	145.98 (41)	
	NAPL ²	Idu	95.82 (30)	100.04 (34)	100.04 (41)	113.56 (45)	294.78 (66)	230.03 (34)	200.04 (31)	157.91 (24)
		Fuyu	135.67 (31)	113.57 (68)	122.05 (64)	138.35 (61)	238.56 (62)	201.42 (47)	189.43 (42)	117.11 (31)
Sechonjosang		157.97 (83)	143.87 (79)	129.65 (74)	117.62 (75)	373.44 (30)	256.83 (28)	208.94 (21)	188.83 (13)	

The data were means of duplicate determination.

¹Astringent persimmon leaf.

²Nonstringent persimmon leaf.

() : Before acid hydrolysis.

하여 항산화성분인 수용성페놀화합물, 비타민 C 및 플라보노이드(quercetin & kaempferol)를 정량하였다. 대부분의 뽕은감잎은 단감잎보다 수용성페놀화합물, 비타민 C 및 플라보노이드의 함량이 높았다. 뽕은감잎 및 단감잎의 수용성페놀화합물의 함량은 성장중기에 약간 증가하였으나, 대체적으로 성장과 더불어 감소하는 경향을 나타내었다. 뽕은감잎의 비타민 C 함량은 성장중 급격히 감소하였으나, 단감잎에서는 7월말기에 최고로 높았으나 그 이후 급격히 감소하였다. 감잎에 존재하는 주된 플라보노이드인 kaempferol과 quercetin은 주로 배당체로서 존재하였으며, 산가수분해전 감잎에서 두가지 플라보노이드 함량은 7월말까지 거의 일정하였으나, 그 이후 약간 감소하였다. 한편 산가수분해후 감잎의 kaempferol 함량은 품종 및 성장시기별 상당히 차이를 보였으나, quercetin 함량은 7월말까지 감소하다가 그 이후 크게 증가하여 8월초에 최대에 도달한 후 다시 감소하였다. 이러한 결과를 미루어 볼 때 뽕은감잎을 6월초에 수확

하여, 천연항산화물질의 원료로서 사용하는 것이 적합하다고 생각된다.

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