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Major Water-Soluble Polyphenols, Proanthocyanidins, in Leaves of Persimmon (*Diospyros kaki*) and Their α-Amylase Inhibitory Activity

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The amounts and compositions of polyphenol in persimmon leaves and persimmon leaf tea were investigated. The predominant polyphenols in fresh leaves were water-soluble, and the contents reached a maximum (2.40% w/w) in June, and then gradually decreased. Separation of them followed by thiolytic degradation revealed that the major components were unique proanthocyanidin oligomers consisting of four heterogeneous extension units, including epigallocatechin-3-*O*-gallate. Persimmon leaf tea also contained similar proanthocyanidins with similar compositional units. Oral administration of starch with polyphenol concentrate of persimmon leaf tea resulted in a significant and dose-dependent decrease in the blood glucose level in Wistar rats. This effect is considered to be due to inhibition of pancreas α-amylase. These results indicate that persimmon leaf tea containing peculiar proanthocyanidins has a significant role in suppressing blood glucose elevation after starch intake, and that the best harvest time is June.

Key words: proanthocyanidin; persimmon leaf; tea; thiolysis; α-amylase

Tea is the most widely consumed beverage in the world and recent studies have revealed numerous beneficial health effects. For example, catechins in green tea have been found to have preventative effects on the major metabolic syndrome conditions of obesity, type-2 diabetes, and cardiovascular risk factors.[1] In addition to green tea, several kinds of herbal tea are traditionally consumed in Japan. The main ingredients include leaves of dietary plants, such as persimmon (*Diospyros kaki*), gutta percha (*Eucommia ulmoides*), guava (*Psidium guajava*), and mulberry (*Morus alba*), which are dried and are commercially available as herbal teas. Traditionally, these teas are believed to be health-promoting beverages, similarly to green tea.

Dietary carbohydrates are major determinants of daily caloric intake, and there is strong evidence of a relationship between obesity and excessive caloric intake. Therefore, reducing or slowing the digestive availability of carbohydrate-derived calories is considered to be useful in the prevention of obesity and diabetes mellitus.[2] In fact, a current recommendation is for the use of inhibitors against digestive enzymes from pharmacological drugs (acarbose and miglitol) to decrease carbohydrate uptake from food.[3] Daily consumption of tea prepared from green tea leaves, guava leaves, and mulberry leaves, is believed also to be effective in reducing available dietary carbohydrates[4] because these have been proven to contain inhibitors against α-amylase activity,[5,6] disaccharidase activity,[7] or intestinal sodium-dependent glucose transport.[8] In the early stage of this study, we found that a hot-water extract of persimmon leaves had α-amylase inhibitory activity correlated with seasonal changes, and the active components appeared to be water-soluble proanthocyanidins (PAs). PAs are flavan-3-ol polymers with high structural diversity. The most usual interflavanol linkages are formed between the C4 of one flavanol unit (named the extension unit) and the C8 of another (named the terminal unit).[9] They are reported to be widespread in foods of plant origin and have been attracting interest for their biological activities. However, there has been neither structural nor biological information on PAs in persimmon leaves and in the tea. We were particularly interested in the structural characteristics and α-amylase inhibitory activity of PAs in persimmon leaves.

In this report, we describe the seasonal changes and structural elucidation of PAs in persimmon leaf extract. We tested α-amylase inhibitory activity, including the effect on blood glucose level, after administration of starch to rats.

Materials and Methods

Materials. Gallic acid and catechin standards of (+)-catechin (CA), (−)-epicatechin (EC), (−)-epicatechin-3-*O*-gallate (ECg), (−)-epigallocatechin (EGC), (−)-epigallocatechin-3-*O*-gallate (EGCg), (−)-catechin-3-*O*-gallate, (−)-gallocatechin, and (−)-gallocatechin-3-*O*-gallate, soluble starch from potato, α-amylase from porcine pancreas, and rat intestinal acetone powders were purchased from Sigma-Aldrich (St. Louis, MO). Chromatographic separation was conducted on phenolic absorbent resin of Amberlite® XAD-7HP (Organo, Tokyo). All the other reagents and solvents used in this study were of analytical or HPLC grade.

Instruments. Analytical high-performance liquid chromatography (HPLC) was carried out on a Shimadzu HPLC system equipped with a SPD-20A photodiode array detector and an Inertsil® ODS-3 column (250 mm × 4.6 mm, 4 μm; GL Science, Tokyo). The separation conditions were as follows: flow rate, 1 ml/min; elution solvent, A (0.5% v/v phosphoric acid in water) and B (0.5% (v/v) phosphoric acid in acetonitrile); and the gradient program, 5% to 10% B (0 to

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Abbreviations: CA, (+)-catechin; EC, (−)-epicatechin; ECg, (−)-epicatechin-3-*O*-gallate; EGC, (−)-epigallocatechin; EGCg, (−)-epigallocatechin-3-*O*-gallate; ExAc, 70% aqueous acetone extract; PaW, water partition; PaEa, ethyl acetate partition; PaW-PP, polyphenol concentrate powders of water partition; BTE, benzylthioether; PA, proanthocyanidin

15 min), 10% to 45% B (15 to 40 min), 45% to 80% B (40 to 55 min), 80% to 100% B (55 to 65 min), and a post-run with 20% B (50 to 60 min); HPLC detection, UV 280 nm. LC-MS analyses were performed using a Shimadzu Prominence UFLC system (Shimadzu) equipped with a triple quadrupole mass spectrometer, API 3200 (Applied Biosystems/MDS SCIEX, Foster City, CA), and a L-column 2 ODS (150 mm × 2.1 mm, 3 μm; Chemicals Evaluation and Research Institute, Tokyo) using gradient solvents of 0.1% v/v formic acid/methanol (solvent B) and 0.1% (v/v) formic acid/H_2O (solvent A). ^{1}H NMR spectral data were measured in acetone-d_6 with a Bruker AC-250 MHz NMR spectrometer (Bruker, Karlsruhe, Germany).

Harvesting of persimmon leaves and polyphenol extracts of persimmon leaves and tea. Fresh persimmon leaves of two astringent cultivars (Hiratanenashi and Tonewase) were harvested at 11 different growing stages from April 23 to November 12, 2007, at Niitsu in Niigata City, Japan. Dried persimmon leaves were prepared by subjecting fresh leaves to a constant stream of 60 °C air for 24 h. For seasonal change analysis of polyphenols in the persimmon leaves, fresh leaves (50 g) were soaked in 70% aqueous acetone (1.0 liter) overnight at room temperature. The liquid phase was collected, and acetone was evaporated *in vacuo* to give a concentrate of 70% aqueous acetone (ExAc). Half of the concentrate was partitioned with ethyl acetate to give an aqueous fraction (water partition; PaW) and an ethyl acetate partition (PaEa). In addition, to mimic traditional tea brewing, dried leaves (50 g) of cultivar Hiratanenashi harvested on June 11 were extracted with hot water (2.5 liters) for 30 min to give a hot-water extract. The PaW and hot-water extract were charged on an Amberlite® XAD-7HP column (0.75 liter). After washing with water (0.75 liter), elution with 100% ethanol (3.0 liters) and evaporation to dryness gave the corresponding polyphenol concentrate powders (PaW-PP).

Total polyphenol contents. The polyphenols were quantified using a colorimetric assay of absorption at 760 nm by Folin–Denis method[10] using a UV-mini240 spectrometer (Shimadzu, Kyoto, Japan). The contents were calculated as gallic acid equivalents based on a gallic acid standard curve.

Thiolysis of persimmon polyphenol concentrates and HPLC analysis. Thiolysis and subsequent HPLC analysis were carried out in quintuplicate according to a modified Guyot method.[11] PaW-PP (8 μg) of persimmon leaves or tea in methanol (90 μl) was mixed with 3.3% v/v hydrochloric acid in methanol (120 μl) and 5% (v/v) benzylthiol in methanol (240 μl), and the mixture was kept at room temperature for 72 h. The resulting thiolytic products were analyzed by direct injection into an HPLC system. The HPLC parameters were the same as those used for the analysis of polyphenol extracts, except for the elution solvents, which were A (2.5% v/v acetic acid in water) and B (100% acetonitrile), and the gradient program was 3% to 11% B from 0 to 3 min, 11% to 30% B from 3 to 7 min, 30% to 50% B from 7 to 25 min, 50% to 100% B from 25 to 30 min, 100% B from 30 to 45 min, and then a post-run with 3% B for 20 min to equilibrate the column for the next injection. The HPLC profiles were monitored at 280 nm.

Identification of thiolytic products. During HPLC analysis, thiolytic products were co-chromatographed with eight authentic catechin standards of CA, EC, ECg, EGC, EGCg, (−)-gallocatechin, (−)-gallocatechin-3-*O*-gallate, and (−)-catechin-3-*O*-gallate. A peak at retention time (t_R) 12.4 min was identified as CA. Compounds in peaks at t_R 19.7, 21.9, 23.3, and 25.2 min were isolated from the reaction mixture, as follow: Thiolysis of persimmon leaf PaW-PP (306 mg, 150 mg gallic acid equivalents of polyphenols) with benzylthiol (550 μl) was carried out in methanol (15 ml) containing 1.1% v/v HCl at 60 °C for 90 min, and the reaction mixture was condensed under a stream of nitrogen. The resulting concentrate was mixed with water (30 ml) and extracted 3 times with ethyl acetate, and the combined pale brown ethyl acetate extract was condensed *in vacuo* to give a red-brown residue (750 mg). The residue (660 mg) was loaded on a silica gel column (230 mm × 20 mm) using a toluene:acetone:formic acid eluting solvent (20:10:1). The isolated components from fraction nos. 38–47 (30 mg), 56–69 (63 mg), 73–93 (40 mg), and 95–100 (68 mg) corresponded to the peaks at t_R 23.3, 19.7, 25.2, and 21.9 min on analytical HPLC, m/z 411, 427, 563, and 579 on LC-MS analysis, and $[\alpha]_D^\circ$ (c %, EtOH) −3.4 (0.06), −45.5 (0.06), −71.3 (0.09), and −101.5 (0.1) [lit.[12] −108 (5.5, acetone)] respectively. In addition, their ^{1}H NMR spectra were in good accordance with the corresponding benzylthioethers (BTEs), as follows: BTE, δ ppm (split, coupling constant Hz) of 2-H, 3-H, 4-H, 5′-H, and galloyl-2,6-H; EC-BTE, 5.28 (s), 4.03–3.97 (m), 4.09 (d, 1.9 Hz), 7.05 (s), and not found; EGC-BTE, 5.21 (s), 4.06–3.94 (m), 4.07 (brs), not found, and not found; ECg-BTE, 5.57 (s), 5.39 (dd, 2.2, 1.2), 4.32 (d, 2.1), 7.13 (s), and 7.10 (s); and EGCg-BTE, 5.50 (s) [lit.[12] 5.50 (s)], 5.44 (dd, 2.2, 1.2) [lit.[12] 5.46 (m)], 4.26 (d, 2.1) [lit.[12] 4.25 (d, 2.2)], not found, and 7.00 (s).

α-Amylase and maltase inhibitory activity. The inhibitory activities of persimmon leaf PaWs and tea PaW-PP on α-amylase and maltase were measured following the methods of Fuwa[13] and Dahlqvist[14] respectively. Porcine pancreatic α-amylase (0.5 unit/ml, 60 μl), 0.04% (w/v) soluble-starch (540 μl), and persimmon leaf PaWs (30 μl, equivalent to 120 μg of fresh leaves) or PaW-PP (0.05–5 mg/ml, 60 μl) were incubated in phosphate buffer (100 mM, pH 7.0) at 37 °C for 30 min. After deactivation in boiling water, the cooled mixtures (40 μl) were mixed with iodine reagent (40 μl, 0.01 M iodine) and water (100 μl), and the remaining amount of starch was measured by the absorbance at 595 nm. Maltase activity was determined as follows: incubation of PaW-PP (0.05–5 mg/ml, 100 μl) and 100 mM maltose (200 μl) with a crude enzyme solution (100 μl) prepared from intestinal acetone powders from rats in phosphate buffer (200 mM, pH 6.0), under same conditions as those for α-amylase, followed by measurement of the liberated glucose by an HPLC system similar to that previously reported.[15] Both inhibitory activities were expressed as the inhibition rate (%) as compared to the corresponding control values without PaWs or PaW-PP.

Oral carbohydrate tolerance test in rats. The experimental animal protocol was approved by the Animal Study Committee of Niigata University of Pharmacy and Applied Life Sciences. Eighteen male Wistar rats (190–230 g) were starved overnight (15 h) and divided into three groups. Two test groups received 1.0 ml of soluble potato starch (2.0 g/kg of body weight) and 1.0 ml of aqueous solution containing PaW-PP (100 and 300 mg/kg of body weight) prepared from persimmon leaf tea. A control group received soluble starch and 1.0 ml of water. Blood samples were collected from the tail vein at 0, 15, 30, 60, 120, and 180 min after oral administration. Blood glucose was measured using Glucose C-II test Wako (Wako Pure Chemical Industries, Osaka, Japan).

Statistical analysis. All results were expressed as means ± SD. Statistical analysis of data were performed by ANOVA, followed by the Tukey test to identify differences among groups.

Results

Seasonal changes in the polyphenol contents of persimmon leaf extracts

The changes in seasonal polyphenol profiles for the various cultivars are shown in Fig. 1 (A and B). The total polyphenol content in ExAcs increased from low levels in April at the leaf-shooting stage, reached their maxima on June 11 (Hiratanenashi, 27.7 ± 1.5; Tonewase, 26.0 ± 1.2 mg/g fresh leaves), and decreased thereafter. The profiles of the two cultivars were found to resemble each other closely throughout the growing season. The majority (more than 80%) of the total polyphenol contents was found to be partitioned into the aqueous phase, except at a very early stage of growth prior to May 28, so that the hydrophilic polyphenol contents in the PaWs showed seasonal changes similar to those of the ExAcs, and also reached their maxima on June 11. The hydrophobic polyphenols in the PaEas showed temporary increases at an early growth stage (May 7), but remained less than 20% of total polyphenols without any

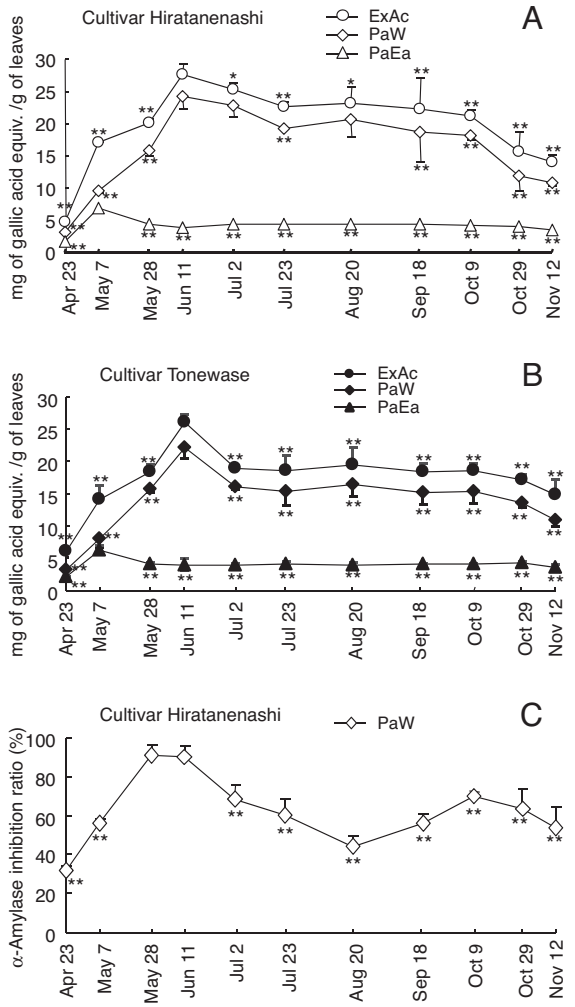

Fig. 1. Seasonal Changes in Polyphenols (A, B) and α-Amylase Inhibitory Activities (C) in Persimmon Leaves of Cultivars Hiratanenashi (A, C) and Tonewase (B).

Extracts of 70% aqueous acetone (ExAc) followed by partitioning between water (PaW) and ethyl acetate (PaEa). Data are expressed as mean ± SD, n = 3. Asterisks indicate values for curves significantly different from the highest value (June 11 for ExAc and PaW, May 7 for PaEa) throughout the growing season ($^{*}p < 0.05$, $^{**}p < 0.01$).

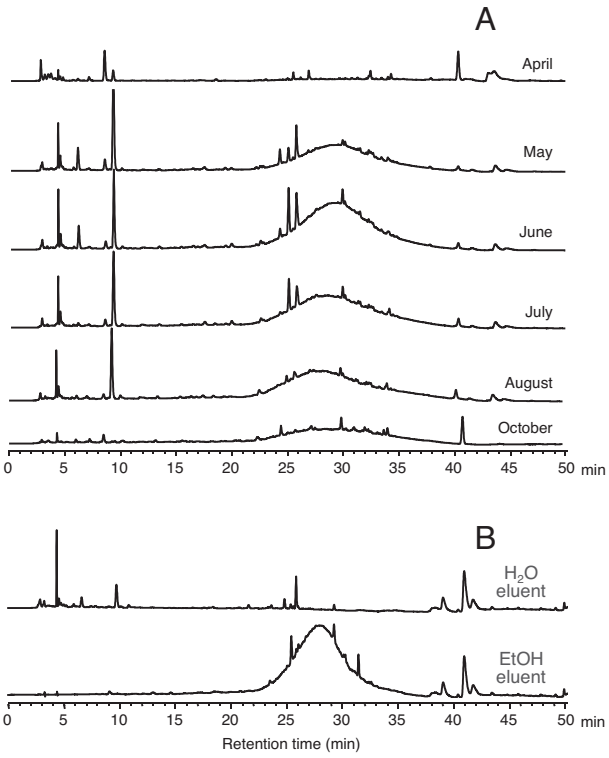

Fig. 2. HPLC Profiles of Water-Soluble Components Extracted from Persimmon Leaves (Hiratanenashi) across the Growing Season (A), and after Amberlite® XAD-7HP Separation (B).

Upper profile in B, H_2O eluent; lower profile, ethanol eluent.

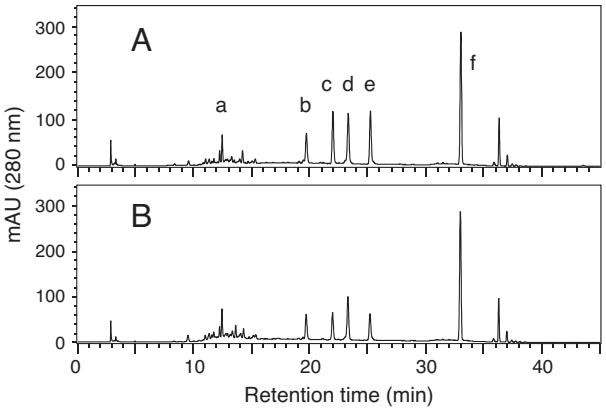

Fig. 3. HPLC Profiles of Thiolytic Degradation Products of Proanthocyanins in 70% Aqueous Acetone (A) and Hot-Water Extract (B).

Terminal unit, catechin (a); extension units, epigallocatechin-4β-BTE (b), epigallocatechin-3-*O*-gallate-4β-BTE (c), epicatechin-4β-BTE (d), and epicatechin-3-*O*-gallate-4β-BTE (e); reaction reagent, benzylthiol (f).

apparent change thereafter. Statistical analyses showed that the maximum values on each curve for the ExAcs, PaWs, and PaEas were significantly different from the values at other growth stages.

HPLC analysis of polyphenols

Polyphenols in the extract were analyzed by HPLC. Representative profiles of ExAcs during growth are shown in Fig. 2A, in which a characteristic broad peak with a retention time from 21 to 35 min had a maximum peak area in June. In addition, the peak moved into PaW after partitioning between PaW and PaEa, and was condensed in an EtOH eluent by Amberlite® XAD-7HP column chromatography (Fig. 2B).

Structural elucidation of persimmon leaf proanthocyanidins by thiolysis

Thiolytic treatment of PaW-PPs of persimmon leaves and tea with benzylthiol gave a mixture of the degradation products with five major new components (Fig. 3). By co-chromatography of eight catechin standards, one with t_R 12.4 min was identified as the CA of the terminal unit. Four components were isolated by silica gel chromatography and analyzed by their spectral data. In LC-MS monitored in the negative ion mode, components with t_R 19.7, 21.9, 23.3, and 25.2 min showed m/z 563, 579, 411, and 427, corresponding to deprotonated molecules $[M - H]^-$ of ECg-, EGCg-, EC-, and EGC-BTE respectively. Furthermore, in their ^{1}H-NMR spectra, the 3-H signals of the four BTEs appeared as multiplets or double doublets having small coupling constants (below 2.2 Hz) with 2-H and 4-H. These small coupling constants indicate that they had an EC-4β-BTE

Table 1. Amounts, Contents, and Compositions of Total Polyphenols and PAs in PaWs and PaW-PPs of 70% Aqueous Acetone and Hot-Water Extracts from Persimmon Leaves[a]

Extracts and analyses		Extraction solvent	
		Aq. acetone[b]	Hot-water
Amounts, g, and contents, % (w/w)			
Fresh leaves, g		100	100
Dried leaves, g		np	38.3
PaW[c], g		16.0	18.5
TPPh, g		2.40 ± 0.08	2.09 ± 0.06
PaW-PP[d], g		3.10	1.71
TPPh, g		1.52 ± 0.01	0.62 ± 0.002
PAs, g		1.43 ± 0.11	0.56 ± 0.045
PAs/TPPh, %		94.1	90.3
Compositions, % (mol), and mDPs of PAs by thiolysis			
Ter	CA	12.0 ± 0.0	16.1 ± 0.0
Ext	EGC	38.2 ± 2.0	39.0 ± 1.1
	EGCg	4.9 ± 0.2	3.2 ± 0.2
	EC	35.6 ± 1.4	36.1 ± 0.3
	ECg	9.2 ± 0.4	5.8 ± 1.4
PD		43.1 ± 2.2	42.2 ± 1.3
Gallation		14.1 ± 0.6	9.0 ± 1.6
mDP		8.3 ± 0.3	6.2 ± 0.1

[a]*Abbreviations*: TPPh, total polyphenols; PAs, proanthocyanidins; Ter, terminal; Ext, extension; CA, catechin; EGC, epigallocatechin; EGCg, epigallocatechin-3-*O*-gallate; EC, epicatechin; ECg, epicatechin-3-*O*-gallate; PD, prodelphinidin; mDP, mean degree of polymerization; np, no performed.
[b]Seventy% aqueous acetone
[c]Water partition (dry matter basis)
[d]Polyphenol concentrate powders of water partition

configuration, because CA-4β-BTE has been reported to have a double doublet 3-H signal with larger coupling constants (9.5 and 4.0 Hz) than the multiplet of EC-4β-BTE.[16] The characteristic signals of galloyl-2, 6-H (7.00 and 7.10 ppm) and B-ring 5′-H (7.05 and 7.13 ppm) gave the structural distinction with and without 3-*O*-gallate and pyrogallol moieties. These results were in good agreement with the reported data for flavanol-BTE structures. For example, all the MS, ^{1}H-NMR, and $[\alpha]_D$ values of the t_R 21.9 min component were in close agreement with those for (−)-EGCg-BTE as reported by Yousef, *et al.*[12] Consequently, thiolytic products with t_R 19.7, 21.9, 23.3, and 25.2 min on HPLC were identified as (−)-EGC-4β-BTE, (−)-EGCg-4β-BTE, (−)-EC-4β-BTE, and (−)-ECg-4β-BTE respectively.

The ratios of each of the isolated extension units to terminal CA were determined by quantitative treatment of the HPLC data, which enabled the structural compositions and amounts of persimmon leaf PAs to be determined. Table 1 summarizes the amounts, contents, and compositions of total polyphenols and proanthocyanidins in 70% aqueous acetone and hot-water extracts from persimmon leaves. In Table 1, the amount and composition of water-soluble polyphenols in the hot water extracts were compared with those of 70% aqueous acetone extracts. The amounts of water-soluble total polyphenols in the hot-water and 70% aqueous acetone extracts represented 2.09 ± 0.06% and 2.40 ± 0.08% (w/w) of the fresh leaves respectively, and there seemed to exist no large difference. However, after Amberlite® XAD-7HP separation, the amount (0.62 ± 0.002%) of hot-water extracts was found to be 40% lower than that (1.52 ± 0.01%) of the 70% aqueous acetone extracts.

Table 2. Inhibitory Activity of Persimmon Leaf Tea PaW-PP against α-Amylase and Maltase

PaW-PP[a] (μg/ml)	Inhibition ratio (%)[b]	
	α-Amylase	Maltase
24	24.6 ± 3.2	1.7 ± 0.3
48	45.2 ± 2.8	2.1 ± 1.3
240	64.3 ± 3.1	5.2 ± 1.7

[a]Polyphenol concentrate powders of water partition
[b]Data are expressed as mean ± SD, n = 3.

The best harvesting time for α-amylase inhibitory activities

Changes in the inhibitory activities in PaW of persimmon leaf extracts against porcine pancreas α-amylase are shown in Fig. 1C. The inhibitory activity increased in their early growing stage (April and May) and reached a maximum at June 11. This profile is closely similar to that of the hydrophilic polyphenol content of PaW (Fig. 1A), and the two on June 11, indicating that that the best harvesting time for polyphenol-rich leaves with high α-amylase inhibitory activity is June. The α-amylase and maltase inhibitory effects of PaW-PP extracted from persimmon leaf tea are shown in Table 2, indicating PaW-PP had considerable α-amylase inhibitory activity in a concentration-dependent manner, but no strong maltase inhibitory activity.

Effects of persimmon leaf tea extract on blood glucose level in Wistar rats

The effects of oral administration of PaW-PP prepared from tea extracts on blood glucose levels were examined in three groups of Wistar rats. When PaW-PPs were orally administered at three different dosages (0, 100, and 300 mg/kg of body weight) in soluble potato starch (2.0 g/kg of body weight), blood glucose levels increased from baseline values of 94 ± 12, 112 ± 9, and 105 ± 15 mg/dl at 0 min to peaks of 155 ± 24, 150 ± 14, and 136 ± 13 mg/dl at 30 min respectively, and then decreased. Since one-way ANOVA among the baseline values showed a significant difference ($p = 0.01$), increasing glucose values for 180 min after administration are shown in Fig. 4, indicating that the values were depressed in a dose-dependent manner by PaW-PP. In comparison with the control group, significant decreases in blood glucose levels were observed 30, 120, and 180 min after administration in the 100 mg/kg test group, and at all measuring time points after administration in the 300 mg/kg group.

Discussion

In the present study, persimmon leaves and tea were found to contain PAs peculiar to persimmon leaves as the major components of water-soluble polyphenols with α-amylase inhibitory activity. In addition, the contents and activity of the polyphenols varied during growth of the leaves, and reached their maxima in June.

Since the major polyphenolic components in the 70% aqueous acetone extracts[17] had characteristic features, including good water-solubility[18] and the appearance of a broad peak on ODS-HPLC analysis,[19] they were assumed to be PA oligomers. It is well known that

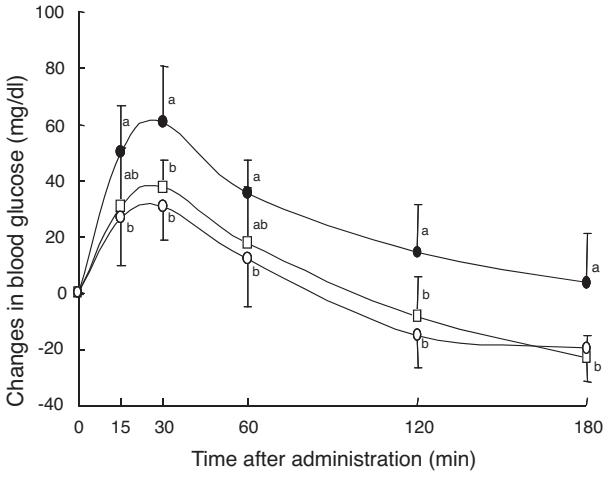

Fig. 4. Changes in Blood Glucose Levels after Oral Administration of Starch with Polyphenol Concentrate Powders (PaW-PP) of Persimmon Leaf Tea to Wistar Rats.

Dosages: 300 (○), 100 (□), and 0 (●, water) mg/kg of body weight. Data are expressed as mean ± SD for six rats. Values with different letters in the curves are significantly different ($p < 0.05$).

thiolysis[9,11] is a reliable method of qualitative and quantitative analysis, because nucleophilic benzylthiol can cleave the interflavanol linkages among terminal and extension units to afford the corresponding terminal flavanols and extention flavanol-benzylthioethers (BTEs) respectively. However, the PaW-PP of persimmon leaves did not easily give expected the degradation products, differently from apple PAs.[11] Many unexpected peaks appeared between t_R 11 and 15 min, as traces of them were still found in Fig. 3. LC-MS analysis of them gave m/z 867, 883, and 1035, corresponding to deprotonated molecules of (EGC-EC)g-BTE (and/or (EC-EGC)g-BTE), (EGC-EGC)g-BTE, and EGCg-EGCg-BTE respectively. We attempted to make these peaks as small as possible, and they fell under the conditions of prolonged reaction time (72 h) at room temperature when we used excess amounts of thiol reagent (data not shown). Our results suggest that the decomposability of PAs in thiolysis is related to their partial interlinkage, which varies from easily decomposable chains with EC units to relatively recalcitrant ones with EGCg. A similar result was reached in a study by Kashiwada *et al.*,[20] who reported that ECg dimers remained as indecomposable products in the thiolysis of rhubarb PAs.

The compositional features of the PAs in both PaW-PPs from the 70% aqueous acetone and hot-water extracts were compared as to thiolytic degradation products. They were found to consist of the same flavan-3-ol units of terminal CA and extensional EGC, EGCg, EC, and ECg. And they showed close compositional similarity in their unit ratios, prodelphinidin %, gallation %, and mean degree of polymerization. Furthermore, the PA contents in the total polyphenols (PA/TPPh) of the aqueous acetone and hot water extract were 94.1 and 90.3% respectively, indicating that PAs are major water-soluble polyphenols in both extracts. These data suggest that there was no large compositional difference in PAs between the persimmon leaves and the tea extract. However, the extractable amounts in the hot-water extracts were 0.56 ± 0.04 g, decreasing to 39.2% of 1.43 ± 0.11 g in the 70% aqueous acetone extracts. These results indicate that about 40% of intact PAs in persimmon leaves is extracted in persimmon leaf tea by hot water brewing.

The PaW-PP of the persimmon tea had α-amylase inhibitory activity, but had no effect on maltase. This was also reflected in the suppression of blood glucose elevation after starch intake in the rats. Compared to the well-studied green tea,[21] the α-amylase and maltase inhibitory strengths of persimmon leaves were almost equal and far less respectively (data not shown). Our studies of the PA oligomers of persimmon leaves have just started, and it remains to be determined whether the oligomers offer any benefits in further studies using diabetic animals and humans. The polyphenols in persimmon leaves can vary quantitatively and/or qualitatively depending on a range of factors, including harvesting time, cultivar, preparation method, and storage condition. However, these results at least indicate that the best harvesting time for polyphenol-rich leaves with high α-amylase inhibitory activity is June.

Recently, PAs have been receiving much attention for their beneficial effects in nutrition and on health.[9] They are widely distributed in food plants, and their biological activities depend on their chemical structures and concentration. PAs have been extracted from cacao,[22] lowbush blueberry,[22] apple,[23] grape,[24] wine,[24] green tea leaves,[25,26] and persimmon fruits.[27] PAs are known to have high structural diversity and complexity, and are distinguished by their structural characteristics, such as flavanol units, stereochemistry of C3 and C4, interflavonol linkages between C4 and C8 or C6, degree of polymerization, and acyl substituents at C3-*O*-. PAs are divided into the sub-families procyanidins and prodelphinidins, consisting of CA, EC, ECg units and GC, EGC, EGCg units respectively. The most common PAs known are the procyanidins in cacao, lowbush blueberry, and apple, and several mixed procyanidin/prodelphinidins have also been reported in grape, green tea leaves, and persimmon fruits.

Structural comparisons of persimmon leaf PAs with previously reported dietary PAs revealed persimmon leaf PAs to have three distinguishing characteristics. First, their extension units consist of 4 different units, EGC, EGCg, EC, and ECg, indicating higher heterogeneity than for other PAs, with 1–3 different units. Second, the prodelphinidin contents, of more than 40%, are considerably higher than those of grape skins (31.2%).[24] Third, the degree of 3-*O*-galloylation was 9–14%, similar to the 12.9% reported for grape seeds.[24] Among these characteristics, we were particularly interested in the EGCg unit, because increases in galloylation have been suggested to lead to the enhanced physicochemical and physiological effects of PAs, *e.g.*, increased affinity to proteins, including enzymes,[28] greater scavenging activities against free radicals,[29] and enhanced cytotoxic activities against tumor cells.[30] The EGCg in green tea has been investigated extensively as the most active component among the four other catechins isolated from green tea.[31] PAs with an EGCg unit are rare in dietary plants, and their preparation methods have suffered from a lack of adequate resources. Our study suggests that persimmon leaves can be a valuable source of PAs containing a considerable amount of EGCg units.

In conclusion, this study indicates that persimmon leaves contain water-soluble polyphenols with α-amylase inhibitory activity, which reaches maximum levels in June during the growing stage of cultivation. The major components were proanthocyanidins, consisting of CA, EGC, EGCg, EC, and ECg. The persimmon leaf tea also contained similar proanthocyanidins with the same units, although the content decreased to 39.2%. This study provides evidence for the best harvesting time in order to optimize the polyphenol constituents and health benefits of persimmon leaf tea, as traditionally consumed in Japan.

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