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Functional Component Contents in Mature Leaves, Young Shoots, and Adventitious Shoots of Japanese Persimmon 'Saijo'

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The mature leaves of Japanese 'Saijo' persimmon (*Diospyros kaki* Thunb.) are consumed as ingredients in health foods and teas. Levels of ascorbic acid and polyphenols were compared between the mature leaf (M-leaf), young shoot (T-shoot; cultured by water-soaking twigs grown the previous year) and adventitious shoot (A-shoot; obtained from orchard trees). The level of total ascorbic acid (T-AsA) was highest in M-leaves, and though the levels of polyphenols were high in M-leaf and A-shoot, they were remarkably low in T-shoot. Isoquercitrin and astragalin levels were highest in A-shoot, followed by M-leaf, and then T-shoot.

Keywords: astragalin, isoquercitrin, persimmon shoot, total ascorbic acid, total polyphenol

Introduction

The bioactivities and functional components of persimmon leaves (*Diospyros kaki* Thunb.) have been reported in several studies. For example, Kuwana *et al.* (1995) reported persimmon leaf extract's hair growth-promoting effects in cultured hair follicle cells. Sakanaka *et al.* (2005) demonstrated increased activity of radical scavenging using persimmon leaf extract, and Kotani *et al.* (1999) reported that the extract had high anti-allergy effects. Persimmon leaves are known to contain large amounts of ascorbic acid (AsA), quercetin glycoside and kaemferol glycoside (Toyoda *et al.*, 1997). We reported effects of steaming treatment on the contents of AsA in persimmon leaf tea before machine-drying (Tsurunaga *et al.*, 2004).

Immature shoots of Japanese angelica trees (*Aralia alata*) and cordate spikenard plants (*Aralia cordata*) have been regarded and used as a food source in Japan for many centuries. Particularly for the angelica tree, a water culturing system by which twigs are cut into sections from a leaf bud is well established (Furuya and Hosoki, 2004). While the mature leaves of persimmon have been used as ingredients for tea and sushi, the young shoot (T-shoot; cultured by water-soaking twigs from the previous year's growth) and adventitious shoot (A-shoot; obtained from trees planted in an orchard) have rarely been used. In this study, we investigated whether these shoots would be appropriate for use as a food or tea. We compared the levels of functional components of young shoots, grown under different conditions, from the Japanese 'Saijo' persimmon, and of mature 'Saijo' persimmon leaves (M-leaf) gathered from an orchard.

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Materials and Methods

Plant material In this study, leaves and shoots from 15-year-old Japanese 'Saijo' persimmon trees at the Shimane Agricultural Experiment Station (Masuda City, Japan) orchard were used. In June 2004, M-leaves were collected from shoots approximately 30 cm long, and A-shoots approximately 10 cm long were collected from the trunks of the same trees from which the M-leaf samples were collected. During the same month, twigs approximately 30 cm long, obtained from trees the previous year were soaked in water for 2 weeks for use as experimental material. Two or three shoots (T-shoot) averaging 10 cm in length were cultured

from every twig (Fig. 1). All samples were freeze-dried and powdered for subsequent analyses.

AsA and DHA analysis The freeze-dried samples (200 mg) were extracted with 40 ml 2% (v/v) metaphosphoric acid and maintained at room temperature for 1 h. The volume was adjusted to 50 ml using 2% metaphosphoric acid, after which the extract was filtered through 0.45-µm filters (Advantec Toyo Kaisha Ltd., Tokyo, Japan).

According to the method used by Ohta and Harada (1996), the reduced ascorbic acid (AsA) content of the extract was analyzed using HPLC (HPLC 10A system; Shimadzu Co., Kyoto, Japan) with an Inertsil ODS-2 (4.6 i.d. × 250 mm) column (GL Sciences Co. Ltd., Tokyo, Japan) and a UV-VIS detector (SPD-10AT; Shimadzu Corp.) set at 254 nm. The column temperature was maintained at 40 ºC. The mobile phase was analyzed using 1% metaphosphoric acid and the flow rate was 1 ml/min.

To estimate the amount of dehydroascorbic acid (DHA) in the extract, DDT (threo-1,4-dimercapto-2,3-butanediol) was added to the above extract to reduce DHA into AsA and determine the amount of total-AsA (T-AsA), from which the AsA was subtracted (Ohta and Harada, 1996).

Polyphenol analysis The total amount of soluble polyphenol was determined according to the method of Folin (Swain and Hillis, 1959). The polyphenol contents of the three sample types (M-leaf, A-shoot, and T-shoot) in both hot water and 80% ethanol extracts were analyzed. For hot water extraction, 20 ml ultra-pure water was added to a 200-mg sample and extracted at 100 °C for 10 min. The extract was adjusted to 50 ml and filtered through a 0.45-µm filter (Advantec Toyo Kaisha Ltd.). For ethanol extraction, a 200 mg sample was mixed with 20 ml of 80% ethanol solution and maintained for 16 h at room temperature. The extract was then filtered through a 0.45-µm filter (Advantec Toyo Kaisha Ltd.) and the polyphenol contents were detected using the method developed by Folin-Denis. Polyphenol amounts were calculated as catechin equivalents per 100 g of dry weight (DW).

The isoquercitrin (quercetin-3-glucoside) and astragalin (kaemferol-3-glucoside) extract levels were analyzed using HPLC (Fig. 2). The HPLC system was composed of an Inertsil ODS-80A (4.6 × 250 mm) column (GL Sciences Co.

Fig. 1. Shoots derived from approximately 30-cm twigs cultured in water for 2 weeks.

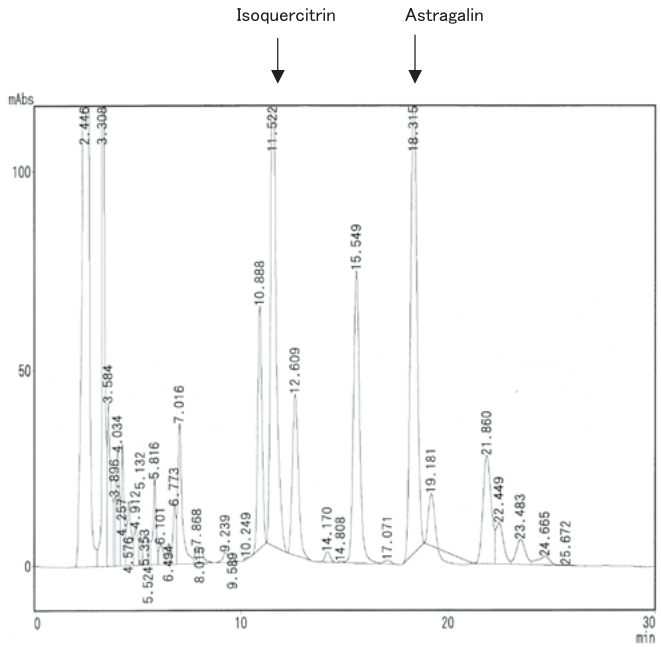

Fig. 2. HPLC chromatogram of isoquercitrin and astragalin. HPLC conditions: column, Inertsil ODS80A; mobile phase, 0.5% H_3PO_4/acetonitrile (82/18, v/v); column temperature, 40 °C; injection volume, 20 µl; detection wavelength, 254 nm; flow rate, 1 ml/min.

Ltd.) and SPD-10AT UV-VIS detector (Shimadzu Corp.) set at 254 nm, with the column temperature maintained at 40 ºC. The mobile phase was analyzed using acetonitrile/0.5% phosphoric acid (18/82, v/v) and a flow rate of 1 ml/min.

Statistical analyses Differences between values were tested using Scheffe's method after ANOVA.

Results and Discussion

AsA and DHA contents The T-AsA level was highest in the M-leaf extracts (3150 mg/100 g DW); followed by A-shoot, and was lowest in T-shoot (Fig. 3). Ascorbic acid is generated from gulonic acid by the breakdown of glucose through the glycolytic and uronic acid pathways. Glucose is stored as starch during dormancy in branches and roots, which is redistributed to form new organs and tissues at the budding stage. This suggests that the starch is necessary for synthesis of T-AsA. It appears likely, therefore, that the low level of T-AsA in the T-shoot as compared to A-shoot and M-leaf is due to the fact that T-shoot is derived from small branches that do not have roots.

A reason for the higher T-AsA level in the M-leaf may be the difference in photosynthetic ability. It is generally believed that the photosynthetic ability of mature leaves of higher plants is greater than that of immature leaves, which is likely to create more glucose for the synthesis of AsA. This suggests that levels of AsA produced from mature leaves would be higher than those of immature leaves.

Polyphenol contents High levels of polyphenol were found in the ethanol extract of M-leaf and A-shoot; 7000 mg and 8000 mg per 100 g DW, respectively (Fig. 4). The polyphenol level for the T-shoot extract was much lower.

Because persimmon leaves are frequently used as a material for tea, we also evaluated polyphenol levels using hot water extraction. Polyphenol levels in the hot water extraction were approximately 30% lower than those found in the 80% ethanol extraction (Fig. 4), most likely because polyphenol was more soluble in ethanol than in hot water. Analysis of the main polyphenol components of persimmon leaves, astragalin and isoquercitrin, showed higher levels in the 80% ethanol extract than in the hot water extract (Fig. 5). These flavonoids are metabolized from phenylalanine or tyrosine. Both components are synthesized from saccharides, organic acids, and saccharic phosphate through the shikimic acid pathway. However, T-shoot has few sources of saccharides, unlike M-leaf and A-shoot, which come directly from the tree. Further, T-shoot cannot absorb nitrogen, as it has no roots. This suggests that T-shoot synthesizes limited amounts of flavonoids due to its limited supply of nitrogen and carbon.

Between M-leaf and A-shoot, A-shoot showed higher levels of polyphenol, isoquercitrin and astragalin, possibly attributable to the correlation between the speed of flavonoid

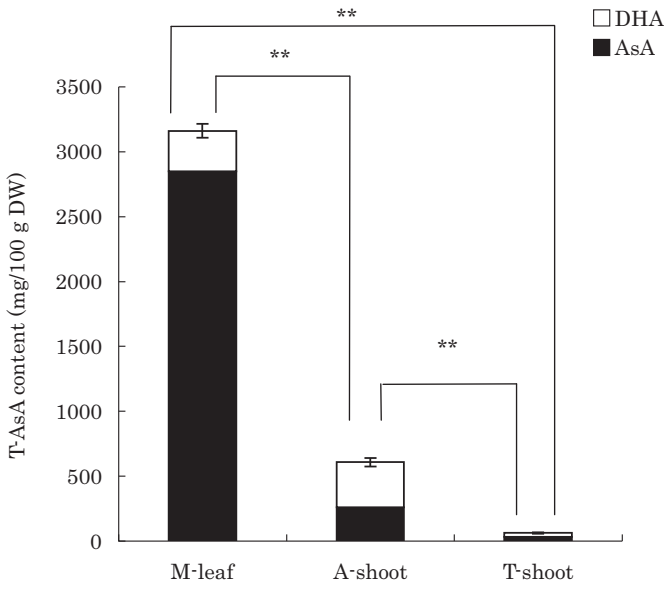

Fig. 3. T-AsA content in persimmon mature leaves and shoots.
M-leaf, mature leaves; A-shoot, adventitious shoots; T-shoot, cultured by water-soaking twigs grown the previous year.
■ AsA: ascorbic acid (reduced)
□ DHA: dehydroascorbic acid
Bar indicates mean ± standard deviation (n = 3).
Statistical significance of difference established by Sheffe's method.
** P < 0.01.

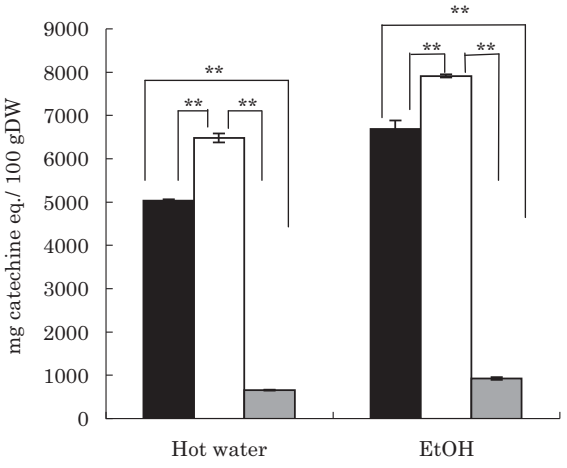

Fig. 4. Polyphenol content in persimmon mature leaves and shoots.
■ M-leaf: Mature leaves
□ A-shoot: Adventitious shoots
■ T-shoot: Cultured by water-soaking twigs grown the previous year.
Hot water: 200 mg of freeze-dried powdered persimmon leaf extracted with 20 ml milli-Q water in boiling water for 10 min, then topped up to 50 ml.
EtOH: 200 mg of freeze-dried powdered persimmon leaf extracted with 20 ml 80% ethanol for 16 h, then topped up to 50 ml.
Bar indicates mean ± standard deviation (n = 3). Statistical significance of difference established by Sheffe's method. ** P<0.01.

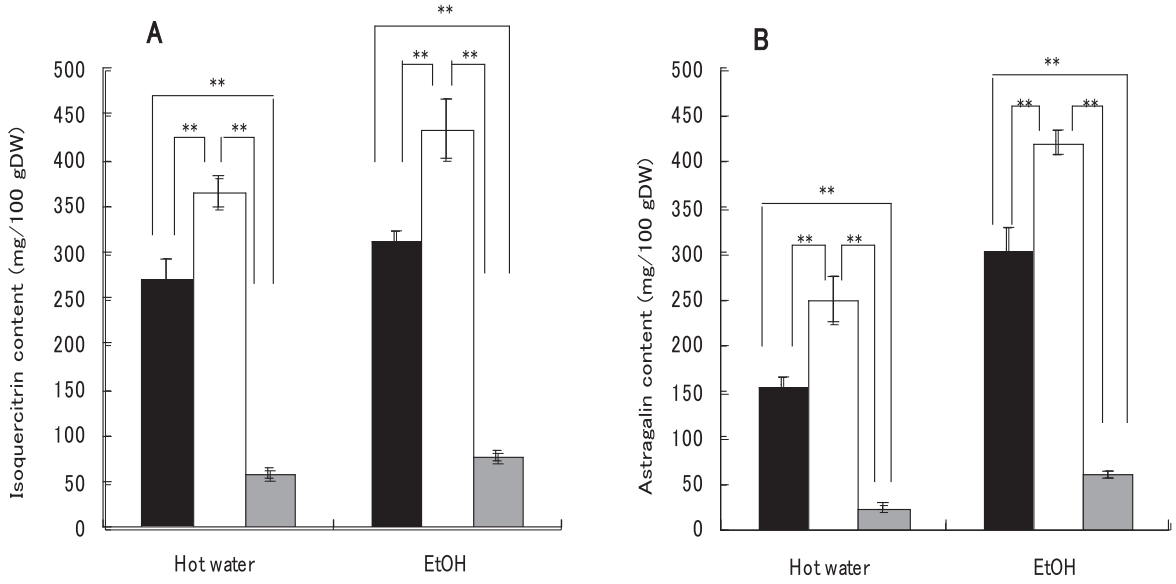

Fig. 5. Isoquercitrin (A) and astragalin (B) content in persimmon mature leaves and shoots.
■ M-leaf: Mature leaves
□ A-shoot: Adventitious shoots
■ T-shoot: Cultured by water-soaking twigs grown the previous year.
Hot water: 200 mg of freeze-dried powdered persimmon leaf extracted with 20 ml milli-Q water in boiling water for 10 min, then topped up to 50 ml.
EtOH: 200 mg of freeze-dried powdered persimmon leaf was extracted with 20 ml 80% ethanol for 16 h, then topped up to 50 ml.
Bar indicates mean ± standard deviation (n = 3).
Statistical significance of difference was established by Sheffe's method ** $P < 0.01$.

synthesis and the speed of leaf growth. Flavonoids are mostly localized in the vacuoles of leaf epidermal cells. Previous reports indicated that flavonoids play a crucial role in filtering ultraviolet rays and reducing UV stress, and flavonoid levels are high at foliation time (Koes *et al.*, 1994; Thomas and Jen, 1980; and Gould, 1995). Therefore, flavonoid content per gram dry weight may be lower in M-leaf, which has larger epidermal cells, than in A-shoot, which has smaller cells.

This study revealed the level of functional components in shoots taken under different conditions and of mature leaves gathered in an orchard of Japanese 'Saijo' persimmon trees. A-shoot contained more isoquercitrin, astragalin and soluble polyphenol, but less T-AsA, than M-leaf. In addition, compared to A-shoot, T-shoot component levels (T-AsA, isoquercitrin, astragalin, soluble polyphenol) were significantly lower. Our results suggest that branches and roots, as storage organs that supply sugar and nutrients, are necessary for generating such functional components. The results also suggest that A-shoot, which contain high concentrations of polyphenol, is suitable for use as food ingredients or as tea.

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