カキ‘西条’における新梢長の違いが柿葉茶素材としての葉および新梢の機能性成分に及ぼす影響

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Effects of Shoot Length on Amounts of Functional Components of Leaves and Shoots for Tea from Japanese Persimmon ‘Saijo’

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The functional components of leaves and their shoots classified into four categories according to length were investigated using Japanese persimmon ‘Saijo’. The obtained results are as follows: (1) The total ascorbic acid (T-AsA) content in leaves from long (60~80 cm) shoots (5,310 mg/100 g DW) was about 1.4-fold that in foliating leaves (F-leaves). The highest T-AsA content (4,690 mg/100 g DW) was obtained in medium (40~60 cm) shoots (M-shoots). (2) The isoquercitrin contents in leaves were 240~360 mg/100 g DW and the astragalin content was 200~270 mg/100 g DW. The isoquercitrin and astragalin contents in shoots were significantly lower than those in leaves. (3) The polyphenol as catechin contents in shoots (5,100~6,200 mg/100 g DW) were lower than those in leaves (6,000~9,200 mg/100 g DW). The polyphenol content of F-leaves was the highest (9,200 mg/100 g DW) and decreased with an increase in the length of donor shoots. The polyphenol content of M-shoots was the highest (6,200 mg/100 g DW) among the four shoot categories. In this study, we conclude that shoots as well as leaves are suitable material for health foods as sources of T-AsA, and that F-leaves are suitable sources of functional compounds (Polyphenols).

Persimmon (Diospyros kaki Thumb) originated from China1. Its leaf and calyx are widely used as folk medicines6. Recently, many studies of the function of persimmon leaves have been reported. For example, KUWANA et al.9 confirmed the promotion of hair growth by leaf extract using cultured hair follicle cells. KOTANI et al.6 reported a high anti-allergic activity of the leaf extract when dissolved in hot water. TSURUNAGA et al.5,6 reported that steaming treatment before drying is very effective in inhibiting the decrease in the total ascorbic acid (T-AsA) content in persimmon leaves during the manufacture and storage of persimmon tea. However, there are few reports on an effective method of obtaining highly functional materials from persimmon tea.

In this study, we investigated the effect of the length of donor shoots collected from Japanese persimmon ‘Saijo’ trees on the amounts of functional compounds in persimmon leaves, such as T-AsA, isoquercitrin, astragalin and polyphenol. The functional components of shoots were also investigated to determine the possible use of young shoots in tea preparation.

Materials and Method

1. Plant materials

Shoots about 20~80 cm in length were collected from 14-year-old Japanese persimmon ‘Saijo’ trees in orchard of Shimane Agriculture Experiment Station

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Fig. 1 Typical length of shoots used in the experiment

(Masuda city) on June 14th, 2003. As shown in Fig. 1, the leaves used in this study were classified as follows: ① F-leaves, foliating leaves; ② S-leaves, leaves from short (20～40cm) shoots (S-shoots); ③ M-leaves, leaves from medium (40～60cm) shoots (M-shoots); ④ L-leaves, leaves from long (60～80cm) shoots (L-shoots).

2. Sample preparation

The samples were dried using a vacuum freeze dryer, powdered, and stored at -20℃ until analysis.

3. AsA and DHA analyses

AsA was analyzed following the method of OHTA and HARADA. 200mg of powdered sample was added to 40ml of 2 % metaphosphoric acid and extracted after 1 hr at room temperature. After filling up to 50ml, the extract was filtered through a 0.45μm filter, then reduced ascorbic acid (AsA) was determined by HPLC (LC 10 AT, Shimazu). Dehydroascorbic acid (DHA) in the extract was changed to AsA by adding DDT (threo-1,4-dimercapto-2,3-butanediol) for the determination of total AsA (T-AsA) content. DHA was estimated by subtracting AsA content from T-AsA content.

4. Polyphenol analysis

The amount of total soluble polyphenol was determined according to the Folin method. The Folin method used for the determination of polyphenol content in this study depends on the reducing activity against of what phosphomolybdic acid and phosphotungstic acid during the mixing of the sample extracts and Folin-Ciocalteu reagent. Polyphenols from the samples were extracted with hot water. Each sample (200mg) was added to 20ml of ultra pure water, and an extract was obtained by boiling the mixture for 10 min, after filling up to 50ml. Polyphenol content was represented as catechin equivalent per 100 g DW.

Isoquercitrin (quercetin-3-glucoside) and astragalin (kaempherol-3-glucoside) contents were determined using HPLC (Fig. 2). The extraction method was carried out following the method of polyphenol extraction described above.

5. Sucrose content determination

The samples were extracted using 80% ethanol, and an aliquot of the extract applied to HPLC (LC 10 AT, RID 6 A, Shimazu).

6. Chlorophyll content determination

The samples were obtained by extraction using 80% acetone. The absorbance of the samples was measured at wavelengths of 663 nm, 645 nm and 750 nm according to the Machinney method.

Result and Discussion

1. T-AsA content

As shown in Fig. 3, the T-AsA content in leaves is proportional to the length of shoots attached leaves. The T-AsA content in L-leaves (5.310mg/100g DW) was about 1.4-fold than in F-leaves. The T-AsA content in M-shoots was the highest (4.690mg/100g DW) among the three shoot categories.

The value of chlorophyll meter (SPAD-502, KONICA MINOLTA) in persimmon leaves from longer shoot tended to be higher than what in this
Fig. 3  T-AsA contents of leaves and shoots of different lengths in persimmon 'Saijo'

AsA: ascorbic acid (reduced)  DHA: dehydroascorbic acid
F (foliating leaves), S (short: 20~40cm), M (medium: 40~60cm), L (long: 60~80cm)

Table 1  Four categories of shoots and their leaves used in this experiment

<table>
<thead>
<tr>
<th>Shoot length (cm)</th>
<th>Leaf number</th>
<th>largest leaf (cm)</th>
<th>SPAD[a]</th>
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<tr>
<td></td>
<td></td>
<td>length</td>
<td>width</td>
</tr>
<tr>
<td>F-leaf</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S-leaf</td>
<td>20~40</td>
<td>7.2</td>
<td>9.3</td>
</tr>
<tr>
<td>M-leaf</td>
<td>40~60</td>
<td>10.6</td>
<td>16.6</td>
</tr>
<tr>
<td>L-leaf</td>
<td>&gt;60</td>
<td>14.6</td>
<td>20.5</td>
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[a] The values were measured by SPAD-502 (KONICA MINOLTA).
Mean ± SE (n = 10).

F-leaf: Foliating leaves
S-leaf: leaves detached from short (20~40cm) shoots
M-leaf: leaves detached from medium (40~60cm) shoots
L-leaf: leaves detached from long (60~80cm) shoots

study (Table 1). MA et al.[10] reported that SPAD correlates with photosynthetic rate. Thus, it is suggested that leaves from longer shoots have a higher sugar content. IZUMI[20] confirmed that AsA, glucose and fructose contents in Satsuma Mandarin leaves decrease with a decrease in the degree of shading of leaves. They also described that a higher AsA content in sunleaves coincides with a higher sucrose content. Sucrose is the main sugar and the translocation form of sugar in tea leaves. Thus, chlorophyll and sucrose contents in persimmon leaves and shoots were measured, respectively. The sucrose and chlorophyll contents in leaves from longer shoots were higher (Fig. 4,5). They showed the same trend as T-AsA content.

FUJWARA et al.[20] reported that there is a correlation between AsA content and SPAD in spinach. Their discussion indicated that leaves from longer shoots have a higher T-AsA content owing to an increase in sucrose content induce by an increase in photosynthetic rate.

The highest T-AsA content was obtained in M-shoots (Fig. 3). However, the chlorophyll and the sucrose contents in shoots were almost equivalent regardless of shoot length (Fig. 5). TAKEBE et al.[14] reported that AsA exists in the sieve tube liquid of Ricinus communis L. at high concentrations. It is presumed that T-AsA generated in leaves moves in the sieve tube liquid. For this reason, the T-AsA content in shoots might be high regardless of sucrose content. Further studies must be performed to analyze the sieve tube liquid. The results of our sensory evaluation of tea prepared from shoots show that the taste of such tea was not inferior to
that of tea prepared from leaves (data not shown).

Generally, the manufacture of persimmon tea uses only leaves and disposes detached shoots. In this study, we demonstrated that whole shoots are also suitable materials for tea and other health foods owing to their high T-AsA contents.

2. Isoquercitrin and astragalin content

TOYODA et al.\(^\text{15}\) reported that the main flavonoids in persimmon tea are quercetin glycoside and kaempferol glycoside. Fig. 6 shows the isoquercitrin (quercetin-3-glucoside) and astragalin (kaempferol-3-glucoside) contents in leaves and shoots of ‘Saijo’. The isoquercitrin contents in the leaves and shoots were 240~360 and 20~40 µg/100 g DW and the astragalin contents were 200~270 and 10~20 mg/100 g DW, respectively. The isoquercitrin and astragalin contents in the shoots were significant lower than those in the leaves. The same contents are higher in F-leaves than in L-leaves. Flavonoids are produced in epidermal cells to protect mesophyll cells against UV and stored in vacuoles\(^\text{16}\). The low flavonoid content in leaves from long shoots may be a result of the decreased cell size of the leaves. KURAHASHI et al. found a strong relationship between shoot length and leaf area in ‘Saijo’ (personal communication). Thus, it is suggested that L-leaves with large areas have low flavonoid contents due to the dilution of what in vacuoles.

Flavonoid is a polyphenol with 4,000 components including derivatives, glycosides and polymers. Some flavonoids in foods exist as glycoside and their sugar chains are cut by Bacillus during absorption.
in the small intestines\(^7\). Therefore, the forms of isoquercitrin and astragalin absorbed and metabolized in the human body are quercetin and kaempferol. Quercetin and kaempferol are antioxidants. In addition, some studies have shown that astragalin has antiallergic activities\(^6\)\(^3\). Hence, it is confirmed that persimmon tea with these flavonoids is an excellent health drink. We also demonstrated that leaves with small areas from short shoots have high concentrations of flavonoids.

3. Polyphenol contents

As shown in Fig. 7, the polyphenol (as catechin) content in F-leaves was highest (9,200 mg/100 g DW), and decreased in larger leaves from longer shoots. The polyphenol contents in shoots were lower than those in leaves (5,100~6,200 mg/100 g DW). The polyphenol content was reasonable in shoots in spite of the shoots having lower isoquercitrin and astragalin contents. This means that there are many phenolic compounds aside from isoquercitrin and astragalin in shoots. Rutin is as polyphenol in persimmon leaves\(^8\); however, we could not observe its peak by HPLC. Persimmon leaves have many large-molecular-weight tannins\(^9\), which may be found mainly in shoots; however, this could not be clarified in this study.

Polyphenol content decreased in larger leaves from longer shoots. This suggests the dilution effect of some polyphenol in L-leaves with larger cells, which was not observed in F-leaves and S-leaves with smaller cells. Therefore, polyphenol content per gram dry weight may decrease in what. From the above-mentioned results, we demonstrated that defoliating or small leaves are suitable materials for health foods with high polyphenol and functional flavonoid contents.

**Conclusions**

1) T-AsA is abundant in leaves from long shoots, and is also present at a high concentration in shoots. These imply that the use of shoots is effective for the efficient manufacture of persimmon tea with a high T-AsA content.

2) The isoquercitrin, astragalin and total polyphenol contents are high in leaves from S-shoots and low in shoots. Accordingly, to manufacture persimmon tea containing high concentrations of these compounds, it is desirable not to use shoots, but to use leaves obtained from short shoots.

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**References**


