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Comparative study of catechin compositions in five Japanese persimmons (*Diospyros kaki*)

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Abstract

In order to study catechin compositions in five Japanese persimmons: Hiratanenashi (HN), Tone-wase (TW), Ishibashi-wase (IW), Maekawa-jiro (MJ) and Matsumoto-wase-fuyu (MF), the contents of epigallocatechin (EGC), catechin (C), and epicatechin (EC) were determined. Total phenolic contents were also determined for the studies of the catechin composition. Identification and quantification of the catechins were performed by HPLC. The EGC contents in three astringent persimmons (HN, TW and IW) were higher than those of two non-astringent persimmons (MJ and MF). The EGC content in HN (astringent) was the highest among the five Japanese persimmons. The EGC content in MF (non-astringent) was the lowest among the five Japanese persimmons. Therefore, astringent persimmons may be better sources of the natural antioxidant, EGC, than non-astringent persimmons, and HN (astringent) may be the best source of EGC among the five Japanese persimmons. This is the first comparative study of catechin compositions in five Japanese persimmons.

Keywords: Catechin; Antioxidant; DPPH; Japanese persimmon; *Diospyros kaki*

1. Introduction

The Japanese persimmon, which is also known as kaki, is among the most popular of fruits in Japan. Although over 100 cultivars are grown in Japan, five Japanese persimmons are well-known as commercial persimmons: Hiratanenashi (HN), Tone-wase (TW), Ishibashi-wase (IW), Matsumoto-wase-fuyu (MF) and Maekawa-jiro (MJ). There are two types of persimmons: astringent and non-astringent. HN, TW and IW are well-known as astringent persimmons. Their pulp is bitter because they contain an astringent substance, tannin, and the soluble tannin concentration is high at maturity. (Macheix, Fleuriet, & Billot, 1990). MF and MJ are known as non-astringent persimmons, and their pulp is sweet because of their low soluble tannin concentration at maturity. Persimmons are rich in polyphenols, such as *p*-coumaric and gallic acid (Gorinstein et al., 1994), and are known to contain proanthocyanidins (Haslam & Lilley, 1988). Matsuo & Itoo (1978) identified 'Kaki-tannin' in a Japanese persimmon and found that the tannin consisted of catechin, catechin-gallate, gallocatechin, and gallocatechin-gallate. Sattar, Bibi, & Chaudry (1992) determined total catechin content and total procyanidin content of persimmon spectrophotometrically. However catechin composition has not yet been determined. Catechins (flavan-3-ols) are polyphenolic compounds which may offer potential benefits to human health. Catechins have been reported to exhibit antioxidant, anticarcinogenic, antimutagenic, and

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cardioprotective effects (Hertlog, Feskens, Hollman, Katan, & Kromhout, 1993; Kondo, Kurihara, Miyata, Suzuki, & Toyoda, 1999; Middleton & Kondaswami, 1992; Renaud & de Lorgeril, 1992), and catechins, as well as persimmon extracts, are known to induce apoptosis of Molt 4B cells (Achiwa, Hibasami, Katsuzaki, Imai, & Komiya, 1997). Catechin composition may vary to a significant extent in different Japanese persimmons. Thus, the purpose of this study is to determine the catechin compositions of five well-known Japanese persimmons.

2. Materials and methods

2.1. Chemicals and reagents

Gallic acid monohydrate, (−)-catechin, (−)-epicatechin, (−)-epigallocatechin, 12 molybdo(VI) phosphoric acid *n*-hydrate, sodium tungstate(VI) dehydrate, sodium carbonate, phosphoric acid, ethyl acetate, chloroform and methanol were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). All chemicals used in this study were of analytical grade or HPLC grade.

2.2. Samples

Five commercial persimmons are used in Japan. Three astringent persimmons, IW, TW and HN were collected from JA Shonai (Japan Agricultural Co-operatives). Two non-astringent persimmons, MF and MJ were collected from the Ninomiya Branch, Field Production Science Center in the Graduate School of Agricultural and Life Sciences, University of Tokyo. All the samples were immediately stored in a −80 °C freezer until extracted.

2.3. Extraction of phenolics

All the samples were peeled before the extraction. Persimmon pulp was boiled in five volumes of distilled water for 5 min to inactivate polyphenol oxidases (Jiménez & García-Carmona, 1999). The pulp was homogenized and boiled again for 30 min. Then the pulp was extracted with distilled water at 80 °C for 90 min. The extract was then dissolved in a water–chloroform (1:1, v/v) solution. The water phase was dissolved in a water–ethyl acetate (1:1, v/v) solution. The ethyl acetate phase was concentrated and freeze-dried to give persimmon extracts (Someya, Yoshiki, & Okubo, 2002).

2.4. Identification and quantification of catechins using HPLC

EGC, C, EC and GA content were identified and quantified by HPLC using an isocratic elution system (Wang, Helliwell, & You, 2000). A DOCOSIL-B (C22) (250 mm × 4.6 mm) column (Senshu Scientific CO., Ltd. Tokyo, Japan) was used for the HPLC analyses. Twenty two percent of methanol in 0.1% phosphoric acid (pH 2.3) was used as a solvent. The flow rate was set to 1.0 ml/min and the wavelength was at 280 nm. The three catechins and GA in the samples were identified by comparison of their retention times with those of standard chemicals. The contents of EGC, C, EC and GA were quantified by comparison of their peak heights in the chromatogram with those of standard chemicals. The identification and quantification were performed 3 times. An epimer of catechins was not determined because the phenolic extracts were extracted by heating at 90 °C, which could lead to epimerization. The total catechin contents were calculated from the sum of the individual amounts of the three catechins.

2.5. Determination of total phenolic contents

The total phenolic contents of each extract were determined using the Folin–Denis method (Swain & Hills, 1959). Three millilitres of extracts and the same volume of Folin–Denis reagent were mixed. After 3 min, 3 ml of 10% Na_2CO_3 were added and the mixture was left at room temperature for 60 min. The absorbance was measured with a spectrophotometer (Shimadzu UV-2200) at 760 nm. The determination was performed 3 times. The results were expressed in mg of (+)-catechin/100 g of dry weight.

3. Results and discussion

3.1. Catechin composition

The catechin compositions of five Japanese persimmons are shown in Table 1. The EGC contents and EC contents in three astringent persimmons (HN, TW and IW) were higher than those of two astringent persimmons (MJ and MF). The EGC content and EC content of HN (astringent) was the highest among the five persimmons. The EGC content and EC content of MF (non-astringent) were the lowest among the five persimmons. However, the C contents in the two non-astringent persimmons (MJ and MF) were higher than those of the three astringent persimmons (HN, TW and IW), and the C content in MJ was the highest among the five persimmons.

3.2. Total catechin contents

Total catechin contents of the five Japanese persimmons are shown in Fig. 1. The total catechin content of HN (5.44 mg/100 g d.w.) was higher than that of TW, IW, MJ and MF because of the higher EGC con-

Table 1
Catechin compositions in five Japanese persimmons

Persimmon sample	EGC (mg/100 g of dry weight)	C	EC	GA
Astringent				
Hiratanenashi (HN)	2.24 ± 0.19	1.71 ± 0.12	1.49 ± 0.17	12.7 ± 0.1
Tone-wase (TW)	1.96 ± 0.08	0.887 ± 0.032	0.900 ± 0.153	9.05 ± 0.09
Ishibashi-wase (IW)	1.30 ± 0.13	1.09 ± 0.09	1.17 ± 0.08	10.8 ± 0.5
Non-astringent				
Maekawa-jiro (MJ)	0.630 ± 0.049	3.33 ± 0.12	0.696 ± 0.057	0.455 ± 0.012
Matsumoto-wase-fuyu (MF)	0.445 ± 0.062	2.08 ± 0.05	0.511 ± 0.016	0.806 ± 0.025

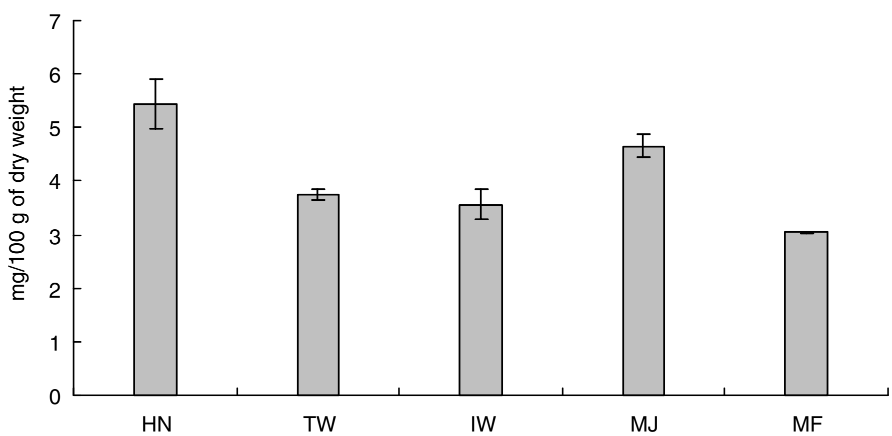

Fig. 1. Total catechin contents of five Japanese persimmons. The results are calculated from the sum of the individual amounts of three catechins: EGC, C, and EC.

tent and EC content in HN. Total catechin content in MF was the lowest (3.04 mg/100 g d.w.) among the five persimmons because of the lower content of EGC and EC in MF. The total catechin content in MJ was higher than those of TW, IW and MF because of the higher C content in MJ.

3.3. Total phenolic contents

Total phenolic contents in five Japanese persimmons are shown in Fig. 2. The total phenolic contents of three astringent persimmons (HN; 84.6, TW; 68.3 and IW; 76.4 mg/100 g d.w.) were 4–6 times higher than those of two non-astringent persimmons (MJ; 18.4 and MF; 14.8 mg/100 g d.w.). These results were apparently not consistent with those of the total catechin content. The higher GA contents in three astringent persimmons may account for the higher total phenolic content (Table 1). The total phenolic content of HN (84.6 mg/100 g d.w.) was higher than that of TW, IW, MJ and MF. The total phenolic content in MF (14.8 mg/100 g d.w.) was lower than that of HN, TW, IW and MJ, and this result was consistent with the total catechin content.

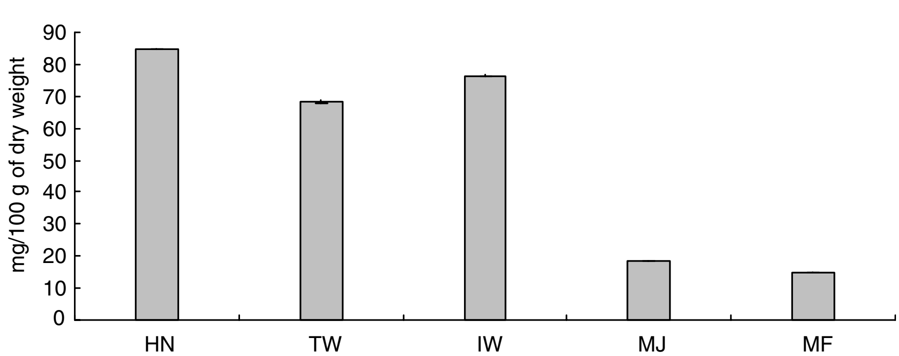

Fig. 2. Total phenolic contents of five Japanese persimmons. The results are expressed in mg of (+)-catechin/100 g of dry weight.

4. Conclusion

Catechins are found in tea (Khokhar & Magnusdottir, 2002), wine (Minussi et al., 2003) and fruit. In fruits, bananas, black grapes, apples, and star apples have been reported to contain catechins (Arts & Hollman, 1998; Luo, Basile, & Kennelley, 2002; Someya et al., 2002).

In this study, the catechin compositions of well-known fruits, five Japanese persimmons were determined. The EGC contents in three astringent persimmons (HN, TW and IW) were higher than those of two non-astringent persimmons (MJ and MF). The EGC content of HN (astringent) was the highest among the five persimmons. Therefore, astringent persimmons may be better sources of natural antioxidants, EGC, than non-astringent persimmons, and HN may be the best source of EGC among the five Japanese persimmons. Currently, we are investigating contents of other polyphenols, such as epigallocatechin-gallate, quercetins, and proanthocyanidin in the Japanese persimmons.

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