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Research Article

Comparative analysis of nutritional components in various parts of *Hovenia dulcis* Thunbergii

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Abstract In this study, the nutritional compounds present in various parts of *Hovenia dulcis* Thunbergii (*H. dulcis*) were compared. Regarding the free sugar content, fruits exhibited the highest fructose concentration (9.42 g/100 g), whereas branches (2.46 g/100 g) and leaves (5.82 g/100 g) contained the highest glucose levels. The most common types of organic acids were citric and tartaric acids in the fruits, citric and malic acids in the branches, and malic and succinic acids in the leaves. The leaves exhibited the highest total amino acid content of 12,102.91 mg/100 g, whereas vitamin C is predominantly found in branches and leaves at 367.85 mg/100 g and 336.21 mg/100 g, respectively. In *H. dulcis*, β-carotene was present in high concentration in leaves (2.41 mg/100 g), whereas the branches (0.15 mg/100 g) and fruits (0.09 mg/100 g) contain smaller amounts of it. Vitamin E, α-tocopherol, was present in high concentrations in the fruit (11.01 mg/100 g), branches (6.61 mg/100 g), and leaves (11.01 mg/100 g).

Keywords *Hovenia dulcis* Thunbergii, nutritional compounds, vitamin C, β-carotene, vitamin E

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1. Introduction

Hovenia dulcis Thunbergii (*H. dulcis*), known as the oriental raisin tree, is widely distributed throughout Gangwon province and in the hills in some southern coastal areas of South Korea (Park et al., 2009). Similar species of *Hovenia dulcis* Thunb. are found in East Asia including Japan and China, but the Korean raisin tree is recognized as an endemic species because of its unique characteristics such as the size of the gynophore, seed, and flower color (Chung et al., 2004; Seo et al., 2022). This species is mainly found on Mt. Seolak, Mt. Odae, Mt. Jiri, Mt. Halla, and in other mountainous regions, and is more often seen in the southern regions than in the central-northern regions of South Korea. The tree can grow in the shade or in sunlight, and is highly resistant to high humidity but susceptible to drought conditions (Jeong and Shim, 1999). According to the Bon-Cho-Kang-Mok (Kim, 1992; Kim et al., 2010), *H. dulcis* fruit are about 8 mm in diameter, sweet scented,

edible, and active on fermenting alcohol. The bark of the tree is dark gray and branchlets are brown with inconspicuous lenticels. The timber is hard and dense and used as building construction material and to manufacture furniture, containers, and instruments (Kim et al., 2006). In traditional herbal medicine, *H. dulcis* has been used for alleviating alcohol intoxication, inhibited urination, vomiting, thirst and quadriplegia. As folk remedies, tea made from its leaf, stems, and fruit is known to be effective for eliminating alcohol poisoning and protecting the liver function by relieving the side effects of excessive drinking such as jaundice, fatty liver, and liver cirrhosis. In addition, the tea is said to be excellent for preventing gastrointestinal disorders and colitis (Kim, 1992; Kim et al., 2010). The findings of recent studies demonstrated that *H. dulcis* has clinical efficacy in improving liver function and eliminating toxic substances from the liver (Kiyoshi and Toshiko, 1987; Kwon et al., 2020; Na et al., 2004). Yoshikawa et al. (1992; 1993; 1995; 1997) reported that the biologically active components identified in the fruit of *H. dulcis* are found to be effective for alcohol degradation and the restoration of liver function. Mssayuki et al. (1996) suggested that dihydromyricetin isolated from the fruit is effective for promoting the metabolism of alcohol and restoration of normal liver function. A previous study found that the functional compounds of *H. dulcis* contain minerals, fatty acids, and abundant amino acids including glutamic acid, leucine, and arginine (Jeong and Shim, 1999). The fruit and seeds contain flavonoids including ampelopsin, laricetrin, myricetin, and gallocatechin (Ahn et al., 2010; Ding et al., 1997), and flavonols such as hovenitin I, II, and III (Ahn et al., 2010; Yoshikawa et al., 1997). The bark and root contain frangulanine, a peptide alkaloid (Ahn et al., 2010; Hase et al., 1997; Kawai et al., 1977), hovenine, hoveniosides, jujuboside, a saponin compound (Ahn et al., 2010; Yoshikawa et al., 1997), and others. The leaf and stems have been identified to have a variety of useful components such as vanillic acid and ferulic acid (Ahn et al., 2010; Cho et al., 2000). However, these data have not yet been organized systematically according to the parts of *H. dulcis*, and were reported separately; thus, further systematic and comprehensive studies and the establishment of a database is warranted. Therefore, this study compared the useful nutritional components of *H. dulcis* with the aim of conveying accurate information on the compositions of individual components of *H. dulcis* to serve as a reference base for developing various food materials.

2. Materials and methods

2.1. Materials and chemicals

The *Hovenia dulcis* Thunb. trees (age: 10-15 years) used in the experiment were collected from May to August 2013 in Jangdong-myeon, Jangheung-gun, Jeollanam-do and divided into fruit, branch, and leaf. Each of these parts of the trees were dried (final moisture contents: fruit: 13.33±0.26, branch: 4.76±0.95, leaf: 4.66±0.78) in the shade. The materials were stored at -40℃ and subsequently used at room temperature in the frozen state. All the chemical reagents were of analytical grade, and all reagents used in this study were purchased from Sigma-Aldrich (St. Louis, MO, USA).

2.2. Analysis of proximate components

Proximate compositions were determined using the method of Bhat and Riar (2019). The moisture content was assessed by measuring the weight by drying 3 g of the sample, which was placed in a weighing bottle in a dry oven at 105℃ until the

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weight remained constant. The crude ash content was determined by reducing 2 g of the sample to ash by initial heating at 250℃ and direct ashing at 660℃. The crude protein content was evaluated using the Kjeldahl method obtained by multiplying the total nitrogen by the factor of 6.25. The crude fat content was determined using Soxhlet extraction after drying each sample. The crude fiber content was measured after digestion with H_2SO_4-NaOH. The nitrogen free extract (NFE) was calculated by subtracting the sum values of the moisture, crude ash, protein, fat, and fiber from 100.

2.3. Analysis of free sugars and organic acids

The free sugars were analyzed using the method of Wilson et al. (1981). Distilled water was added to a 5 g sample and stirred, after which the volume was increased to 100 mL and the solution was centrifuged at 3,000 rpm for 30 min before the supernatant was filtered. The filtrate was purified with Sep-pak C_{18} and filtered through a 0.45 μm membrane filter (Millipore Co., MA, USA). The resulting solution was analyzed using HPLC and the content was calculated using the external standard method. A carbohydrate column (250×4.6 mm, Agilent, Richardson, TX, USA) was used and the column temperature was maintained at 30℃. Acetonitrile was used for the mobile phase, and the flow rate was 1.0 mL/min. A 30 μL aliquot of the sample was injected and the sample was analyzed using an evaporative light scattering detector (ELSD, Agilent Technologies 1200 Series, Agilent Co., Les Ulis, France).

The organic acids were analyzed using HPLC after the samples were subjected to the same treatment as for the free sugars. This analysis was conducted using an organic acid column (250×4.6 mm, Alltech Co., Deerfield, IL, USA) maintained at 30℃. The mobile phase was a solution of 25 mM KH_2PO_4 with flow rate 1.0 mL/min, and 20 μL of the sample was injected and analyzed using a photodiode-array detector (PDA, Agilent Technologies 1200 Series, Agilent Co., CA, USA).

2.4. Analysis of mineral compositions

The mineral compositions of the samples were determined by pre-treatment using the method of Kang et al. (2021) and the content was determined using an atomic absorption spectrophotometer (AAnalyst 400, Perkin Elmer, Norwalk, CT, USA). Nitric acid was added to 1 g of the sample, and the solution was heated for digestion (Büchi Distillation Unit B-324) at lower temperatures and then at higher temperatures by gradually increasing the temperature until the solution became transparent. This digested solution was frozen and distilled water was added to prepare a 100 mL solution. The filtrate was used for the analysis. The amounts of individual mineral compounds were measured with an atomic absorption spectrophotometer (AAnalyst 400, Perkin Elmer). A standard solution of each element was prepared with the following concentrations: 1, 3, and 5 ppm. Standard calibration curves were constructed for quantification. Analyses were performed at a flow rate of 2.0 mL/min in C_2H_2, a 10 L/min airflow rate, and at the following wavelengths (nm): K, 766.5; Mg, 285.2; Na, 589.0; Ca, 422.7; Fe, 248.3 and Zn, 214.9.

2.5. Analysis of total amino acids and free amino acids

The total amino acids were analyzed with the methods of Daniel and Steven (1993) and Steven and Dennis (1993) after digestion and derivatization processes, whereupon they were identified with HPLC (Agilent Technologies 1200 Series, Agilent Co., CA, USA). A 0.5 g sample was placed in a test tube to which 10 mL 6 N-HCl was added. An ampule was prepared by heating the bottom of the tube over the

flame and the sample was hydrolyzed in the sealed tube at 110℃ for 24 h. Then, the ampule was broken and the content filtered through filter paper. Methanol was added to obtain constant volume of 50 mL. The solution was subjected to a decompression concentration process and mixed with 5 mL of 20 mM HCl to attain constant volume. The sample was passed through a 0.45 μm membrane filter, and the filtrate was analyzed using HPLC after derivatization with AccQ-Tag reagent.

The free amino acids were determined using the same procedure as for the quantification of free sugars. The filtrate obtained by filtration was analyzed by the method of Ohara and Ariyosh (1979). A 5 g sample was pre-treated using the same procedure as the pre-treatment of free sugars. Specifically, 10 mL of the filtrate was reacted by mixing with 25 mg of sulfosalicylic acid and left to stand for 4 h at 4℃. The mixture was centrifuged at 50,000 rpm for 30 min to remove proteins and other components, after which the supernatant was filtered through a 0.45 μm membrane filter. A specific amount of the filtrate was taken for analysis using HPLC after derivatization with AccQ-Tag reagent.

The content was calculated by integration using the external standard method. Analyses were carried out under the following conditions. An AccQ-Tag™ column (150×3.9 mm, Waters Co., MA, USA) was used, and the column temperature was maintained at 37℃. The buffer solutions used were A: AccQ-Tag Eluent A (acetate-phosphate buffer) and B: AccQ-Tag Eluent B (100% acetonitrile). The flow rate was 1.0 mL/min and 5 μL of the sample was injected and analyzed using a fluorescence detector (FLD, Agilent Technologies 1200 Series, Agilent Co., CA, USA).

2.6. Analysis of vitamin C

Vitamin C was analyzed using the method of Lee et al. (2018). About 3 g of the sample was diluted with 50 mL of 2% metaphosphoric acid solution and extracted for 30 min. After centrifugation, the supernatant was passed through a 0.45 μm membrane filter (Millipore Co.), and the filtrate was purified with Sep-pak C_{18} and analyzed with HPLC. An organic acid column (250×4.6 mm, Alltech Co., Deerfield) was used and the column temperature was maintained at 30℃. The mobile phase was composed of 25 mM KH_2PO_4 solution with a flow rate of 1.0 mL/min. The sample (20 μL) was injected and the content was analyzed using the PDA detector (Agilent Technologies 1200 Series, Agilent Co.).

2.7. Analysis of β-carotene

The β-carotene content of *H. dulcis* was analyzed after the sample was homogenized. About 2 g of the sample was weighed in an extraction glass tube covered with a cap and sonicated for 10 min by adding 10 mL of an ethanol solution containing pyrogallol (6%, w/v). Then, 8 mL of 60% potassium hydroxide (KOH) was added and the solution was mixed using a vortex mixer (G560, SI Inc., USA). The oxygen in the tube was replaced with nitrogen gas for a minute and the tube was connected to an air condenser. The sample was shaken in a water bath (HB-205 SW, Hanbaek Scientific Co., Siheung, Korea) at 75℃ and 100 rpm for 1 h for a saponification reaction, and then the tube was cooled in an ice water bath. The sample was thoroughly mixed with 20 mL of 2% sodium chloride (NaCl), after which 15 mL of the extraction solvent (hexane:ethyl acetate= 85:15, v/v, 0.1% 2,6-di-tert-butyl-4-methylphenol, BHT) was added, mixed intensely, and left undisturbed for some time. The isolated supernatant was collected in a 50-mL volumetric flask and passed through a Pasteur pipette containing 2 g of anhydrous sodium sulfate (Na_2SO_4) to remove moisture from the

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extract. This extraction process was repeated three times and the extracted solution was collected in a volumetric flask. The residual extract remaining in the Na_2SO_4 column was collected by washing with extraction solvent several times. All extracts were filled to the volume of 50 mL and thoroughly mixed together. Exactly 10 mL of the extract mixture was taken and the solvent was volatized with nitrogen gas. The residue was remelted with 1 mL of ethanol: chloroform (4:1, v/v) solution and passed through a 0.45 μm membrane filter (Advantec, PTFE, Toyo Roshi Kaisha, Ltd., Tokyo, Japan). The resulting solution was analyzed using HPLC with a Vydac 201TP C_{18} column (250×4.6 mm, GRACE, Santa Clara, CA, USA). In the mobile phase, solvent A (methanol:butanol:water, 60:10:30, v/v/v) and solvent B (methanol:butanol:water, 89.5:10:0.5, v/v/v) were injected through gradient elution (Table 1). The flow rate was 1.0 mL/min and a 20 μL sample dissolved in the mobile phase was injected. The sample was analyzed using a PDA detector (Agilent Technologies 1200 Series, Agilent Co., CA, USA).

2.8. Analysis of vitamin E

Vitamin E was extracted using the same procedure for extracting β-carotene. The vitamin E extract (2 mL) was placed in a test tube and the solvent was volatilized with nitrogen gas. The residue was redissolved in 1 mL hexane and passed through a 0.5 μm membrane filter. The resulting solution was analyzed using HPLC with a LiChrosphere® Diol 100 column (250×4.0 mm, Merck Co., Darmstadt, Germany), with 1% isopropyl alcohol in hexane as the mobile phase. The flow rate was 1.0 mL/min, and 20 μL of the sample was injected into the system. An FLD detector (Agilent Technologies 1200 Series, Agilent Co., CA, USA) was used for detection.

Table 1. The gradient condition of mobile phase for β-carotene analysis by HPLC

Time (min)	Solvent A	Solvent B
	Methanol/Buthanol/Water (v/v/v)	
	60/10/13	89.5/10/0.5
0	75	25
8	75	25
50	90	10
55	90	10
57	75	25
65	75	25

2.9. Statistical analysis

All experiments were repeated at least three times and the gathered data were statistically analyzed using the SPSS statistics program (26, IBM Corp., Armonk, NY, USA). The mean±SD was calculated. The difference between the means was tested using Duncan's multiple range test.

3. Results and discussion

3.1. Content of proximate components

Table 2 presents the analysis results of the *proximate* components in different parts of *H. dulcis*. The moisture content of the fruit, branch, and leaf of *H. dulcis* was 13.33 g/100 g, 4.76 g/100 g, and 4.66 g/100 g, respectively. The fruit had the highest water content. The crude protein content was 2.93 g/100 g (fruit), 6.26 g/100 g (branch), and 22.29 g/100 g (leaf). The crude fat content was 0.92 g/100 g (fruit), 0.55 g/100 g (branch), and 1.84 g/100 g (leaf). The crude ash content of the fruit, branch, and leaf was 4.86 g/100 g, 5.53 g/100 g, and 6.79 g/100 g, respectively. The crude fiber content was 10.53 g/100 g (fruit), 41.47 g/100 g (branch), and 13.15 g/100 g (leaf). The nitrogen free extract (NFE) content was 63.83 g/100 g (fruit), 37.43 g/100 g

Table 2. Proximate compositions in various parts of *Hovenia dulcis* Thunbergii

Composition (g/100 g)	*Hovenia dulcis* parts		
	Fruit	Branch	Leaf
Moisture	13.33±0.26[1)a2)]	4.76±0.95[b]	4.66±0.78[b]
Crude protein	2.93±0.57[b]	6.26±0.71[b]	22.29±0.83[a]
Crude fat	0.92±0.17[b]	0.55±0.14[b]	1.84±0.23[a]
Crude ash	4.86±0.23[c]	5.53±0.18[b]	6.79±0.26[a]
Crude fiber	10.53±1.15[b]	41.47±2.34[a]	13.15±1.49[b]
Nitrogen free extract	63.83	37.43	46.27

[1)]All values are mean±SD (n=3).
[2)]Means with different superscript letters in the same row are significantly different by Duncan's multiple range test ($p<0.05$). a>b>c.

(branch), and 46.27 g/100 g (leaf). Park and Kim (2005) performed a physicochemical study on the fruit, stem, and leaf of the *Hovenia dulcis* Thunb., the Korea raisin tree, and reported that the moisture content was in the range 7.64-10.24 g/100 g, the crude protein content in the range 12.48-19.76 g/100 g, the crude fat content in the range 6.45-12.08 g/100 g, the crude ash content in the range 7.91-10.39 g/100 g, and the NFE content in the range 51.76-61.99 g/100 g. Moreover, Jeong et al. (2000) compared the general components of fermented and roasted tealeaf prepared according to the treatment conditions of *H. dulcis* leaf. Their analysis revealed the content of fermented roasted tealeaf to be in the ranges: 9.79-9.45 g/100 g (water content), 18.31-19.84 g/100 g (crude protein content), 8.78-9.52 g/100 g (crude fat content), 9.91-10.96 g/100 g (crude ash content), and 42.88-45.80 g/100 g (NFE content). The finding that the NFE content was the highest in the fruit and leaf samples was consistent with previous reports. However, in the case of branch samples, the crude fiber content was the highest. These results differed from those of previous studies. This difference is judged to have arisen because of the difference in the experimental items used to derive the NFE content.

3.2. The contents of free sugar and organic acid

Table 3 presents the analysis results of free sugars in the different parts of the tree. The free sugar content of the fruit was high in the order of fructose (9.42 g/100 g), glucose (6.39 g/100 g), sucrose (4.91 g/100 g), and maltose (1.82 g/100 g). The free sugar content of the branch was high in the order of glucose (2.46 g/100 g), fructose (2.13 g/100 g), sucrose (0.63 g/100 g), and maltose (0.52 g/100 g). The free sugar content of the leaf was high in the order of glucose (5.82 g/100 g), fructose (3.07 g/100 g) and sucrose (2.79 g/100 g), whereas maltose was not detected at all. The total sugar content of the fruit was the highest at (22.54 g/100 g), followed by the leaf (11.68 g/100 g) and branch (5.74 g/100 g). Jeong and Shim (1999) analyzed the free sugar content of the leaf and fruit of *H. dulcis*, and found the fructose content of the leaf to be the highest at (1.37 g/100 g), followed by maltose and glucose, whereas the sucrose content of the fruit was the highest (8.83 g/100 g), followed by fructose and glucose. Park and Kim (2005) investigated the sugar content of the fruit, branch, and leaf of *H. dulcis*. Their results showed that the fruit contained high concentrations of glucose, fructose, and sucrose in that order; the leaf and branch samples were

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Table 3. The contents of free sugars and organic acids in various parts of *Hovenia dulcis* Thunbergii

	Hovenia dulcis parts		
	Fruit	Branch	Leaf
Free sugars (g/100 g)			
Fructose	9.42±0.34[1)a2)]	2.13±0.18[b]	3.07±0.18[b]
Glucose	6.39±0.64[a]	2.46±0.23[c]	5.82±0.23[b]
Maltose	1.82±0.14[ns3)]	0.52±0.12	–[4)]
Sucrose	4.91±0.36[a]	0.63±0.17[c]	2.79±0.17[b]
Total	22.54	5.74	11.68
Organic acids (mg/100 g)			
Oxalic acid	13.27±2.69[c]	205.36±26.59[a]	79.56±10.87[b]
Tartaric acid	892.83±27.65[a]	–	130.52±16.94[b]
Malic acid	102.82±8.02[c]	418.87±32.71[b]	1,372.62±38.72[a]
Citric acid	1,260.35±34.67[ns]	1,076.59±45.95	–
Succinic acid	103.95±13.67[b]	–	470.26±28.95[a]
Total	2,373.22	1,700.82	2,052.96

[1)]All values are mean±SD (n=3).
[2)]Means with different superscript letters in the same row are significantly different by Duncan's multiple range test ($p<0.05$). a>b>c.
[3)]Not significant.
[4)]–, trace.

reported to have the highest fructose content. The major organic acids (Table 3) contained in the fruit were citric and tartaric acid at concentrations of 1,260 mg/100 g and 892.83 mg/100 g, respectively. The prevalent organic acids in the branch were citric and malic acid at 1,076.59 mg/100 g each. The major organic acids contained in the leaf were malic and succinic acid at 1,372.62 mg/100 g and 470.26 mg/100 g, respectively. The total organic acid content was the highest in fruit (2,373.22 mg/100 g), followed by the leaf (2,052.96 mg/100 g), and branch (1,700.82 mg/100 g). Park and Kim (2005) found the malic acid content of *H. dulcis* leaf to be 2,137.81 mg/100 g, higher than the concentration determined in our study. In the analysis of Jeong et al. (2000), the major organic acids contained in *H. dulcis* tealeaf were oxalic acid, citric acid, and malonic acid, different from the findings of our study. Wang et al. (2022) reported that intrinsic factors such as the age of the tree and harvest time and extrinsic factors such as the cultivation environment affect the determination of free sugar and organic acid content. The difference between the results was attributed to the influence of these factors.

3.3. Mineral composition

Table 4 contains the results of the analysis of the mineral composition of the different parts of *H. dulcis*. The potassium (K) content was 896.72 mg/100 g, 956.72 mg/100 g, and 970.06 mg/100 g of the fruit, branch, and leaf, respectively. The calcium (Ca) content of the fruit, branch, and leaf was 217.44 mg/100 g, 129.53 mg/100 g, and 189.62 mg/100 g, respectively. The magnesium (Mg) content was 147.59 mg/100 g, 126.74 mg/100 g, and 159.15 mg/100 g of the fruit, branch, and leaf, respectively.

Table 4. The content of minerals in various parts of *Hovenia dulcis* Thunbergii

Minerals (mg/100 g)	*Hovenia dulcis* parts		
	Fruit	Branch	Leaf
Na	89.62±7.69[1)a2)]	47.96±6.54[c]	71.35±11.95[b]
K	896.72±27.51[b]	956.72±21.72[a]	970.06±20.34[a]
Ca	217.44±19.62[a]	129.53±15.28[c]	189.62±25.63[b]
Mg	147.59±4.86[a]	126.74±2.66[b]	159.15±10.27[a]

[1)]All values are mean±SD (n=3).
[2)]Means with different superscript letters in the same row are significantly different by Duncan's multiple range test ($p<0.05$). a>b>c.

The sodium (Na) content was 89.62 mg/100 g, 47.96 mg/100 g, and 71.35 mg/100 g of the fruit, branch, and leaf, respectively. In the study of Park and Kim (2005), who analyzed the mineral composition of *H. dulcis*, K was found to be present in the highest concentrations in the fruit, leaf, stems, and other parts of the tree, followed by Ca, Mg, and Na. Jeong et al. (2000) reported K to be the mineral with the highest concentration of 11,084.6 ppm in roasted tealeaf of *H. dulcis*. In another study, the leaf and fruit of *H. dulcis* had the highest content of K (Jeong and Shim, 1999). The results of our study were in line with those of the previous study in terms of the mineral type and content of the different parts of *H. dulcis*.

3.4. The contents of total and free amino acid

A total of 17 amino acids were detected in the different parts of *H. dulcis* and are listed in Table 5. The most prevalent amino acid in the fruit was phenylalanine at 274.65 mg/100 g, followed by proline (212.51 mg/100 g), arginine (177.47 mg/100 g), and glutamic acid (141.09 mg/100 g). The branch predominantly contained proline at 358.86 mg/100 g, followed by glutamic acid (312.94 mg/100 g), tyrosine (284.39 mg/100 g), and aspartic acid (229.13 mg/100 g). The amino acid present in the highest concentration in the leaf was tyrosine at 1,871.84 mg/100 g, followed by arginine (1,041.17 mg/100 g), isoleucine (963.18 mg/100 g), and glutamic acid (910.86 mg/100 g). Contrary to our findings, Jeong et al. (2000) discovered that the amino acid present in the highest concentrations in *H. dulcis* leaf tea was glutamic acid. This difference is judged to be due to the fact that in the drying process, general drying was conducted in the shade in our study, and fermentation and roasting processes were included in the study by Jung et al. (2000).

Table 6 presents the analysis results of free amino acids by plant part. A total of 14 different amino acids were detected in the fruit. The proline content was the highest at 115.92 mg/100 g, followed by isoleucine (17.02 mg/100 g) and glutamic acid (9.65 mg/100 g). The branch contained a total of 10 amino acids, with proline the most prevalent at 114.05 mg/100 g, followed by arginine (37.07 mg/100 g) and aspartic acid (15.79 mg/100 g). The leaf contained a total of 17 amino acids, with the glutamic acid content being the highest at 510.34 mg/100 g, followed by leucine (367.84 mg/100 g) and proline (359.71 mg/100 g). The total free amino acid content was the highest in the leaf of *H. dulcis* (3,424.36 mg/100 g). In the study of Park and Kim (2005), the free amino acids present in the highest concentrations in *H. dulcis* were phenylalanine and aspartic acid in the fruit, glutamic acid and aspartic acid in the leaf, and glycine and glutamic acid in the branch. This

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Table 5. The content of total amino acids in various parts of *Hovenia dulcis* Thunbergii

Amino acids (mg/100 g)	*Hovenia dulcis* parts		
	Fruit	Branch	Leaf
Aspartic acid	125.11±4.67[4)b5)]	229.13±24.81[b]	851.58±33.97[a]
Serine	68.80±5.39[c]	134.42±17.54[b]	513.27±28.64[a]
Glutamic acid	141.09±12.65[c]	312.94±10.91[b]	910.86±26.48[a]
Glycine	60.54±7.66[c]	129.06±18.62[b]	608.98±15.92[a]
Histidine	44.55±3.92[b]	48.85±7.75[b]	762.55±23.78[a]
Arginine	177.47±15.67[b]	179.65±6.97[b]	1,041.17±24.98[a]
Threonine	59.07±4.33[b]	133.08±14.16[b]	862.85±16.84[a]
Alanine	76.90±6.54[b]	131.21±18.24[b]	451.51±22.38[a]
Proline	212.51±18.96[b]	358.86±30.67[b]	591.83±27.18[a]
Tyrosine	55.93±7.29[c]	284.39±22.42[b]	1,871.84±35.79[a]
Cystine	75.69±8.15[c]	125.29±13.68[b]	538.53±13.57[a]
Valine	7.70±1.34[b]	17.39±4.25[b]	105.24±6.84[a]
Methionine	112.29±5.88[b]	107.26±11.72[b]	564.54±23.44[a]
Lysine	58.08±6.92[c]	112.11±16.57[b]	466.46±18.27[a]
Isoleucine	97.67±11.84[b]	173.60±19.38[b]	963.18±26.88[a]
Leucine	61.51±7.94[b]	110.65±14.77[b]	599.91±21.42[a]
Phenylalanine	274.65±20.42[b]	156.52±13.29[c]	398.61±7.34[a]
TAA[1)]	1,709.56±8.92	2,744.40±14.64	12,102.91±17.71
EAA[2)]	455.04±4.64	828.24±12.14	4,863.26±14.88
EAA/TAA(%)[3)]	26.62	30.18	40.18

[1)]TAA, total amino acid.
[2)]EAA, essential amino acid (Thr+Val+Mct+Ile+Leu+His+Lys).
[3)]EAA/TAA (%), essential amino acid/total amino acid.
[4)]All values are mean±SD (n=3).
[5)]Means with different superscript letters in the same row are significantly different by Duncan's multiple range test ($p<0.05$). a>b>c.

difference is ascribed to the difference that was found between samples grown in Jangheung-gun, Jeollanam-do in our study, and those grown in Yeongcheon-gun, Gyeongsangbuk-do in the study by Park and Kim (2005). In addition, in the sample processing method, reflux extraction was performed in the study by Park and Kim (2005), whereas in our study, the materials were dried in the shade.

3.5. The contents of vitamin C and β-carotene

Table 7 presents the results of the analysis of the different parts of *H. dulcis* to determine the vitamin C content, which was 430.26 mg/100 g (fruit), 367.85 mg/100 g (branch), and 336.21 mg/100 g (leaf). Jeong and Shim (1999) reported vitamin C content of 4.8 mg/100 g and 3.8 mg/100 g for the leaf and fruit, respectively. This difference is thought to be due to the difference that was found using samples grown in Jangheung-gun, Jeollanam-do in our study, and Jinju-si, Gyeongsangnam-do in the study by Jeong and Shim (1999). In addition, in terms of sample preparation, Jeong and Shim (1999) used raw

Table 6. The content of free amino acids in various parts of *Hovenia dulcis* Thunbergii

Amino acids (mg/100 g)	*Hovenia dulcis* parts		
	Fruit	Branch	Leaf
Aspartic acid	7.55±1.54[4)b5)]	15.79±2.59[b]	321.58±13.18[a]
Serine	8.54±2.31[b]	2.39±0.64[b]	126.92±12.96[a]
Glutamic acid	9.65±1.93[b]	2.68±0.57[b]	510.34±18.62[a]
Glycine	2.34±1.19[b]	–	185.69±17.23[a]
Histidine	–[6)]	–	93.85±8.66
Arginine	9.27±2.27[b]	37.07±3.88[b]	228.96±10.73[a]
Threonine	1.64±0.45[b]		134.79±15.29[a]
Alanine	13.85±3.79[b]	6.66±1.06[b]	274.23±21.78[a]
Proline	115.92±10.27[b]	114.05±9.65[b]	359.71±26.49[a]
Tyrosine	2.01±0.59[b]	–	132.58±16.67[a]
Cystine	0.58±0.12[b]	1.09±0.11[b]	62.38±8.42[a]
Valine	2.79±0.21[b]	–	207.27±19.15[a]
Methionine	–	–	67.41±10.02
Lysine	–	–	172.32±17.55
Isoleucine	17.02±3.29[b]	0.45±0.09[c]	178.49±13.24[a]
Leucine	3.34±0.96[b]	0.95±0.22[b]	367.84±20.84[a]
Phenylalanine	2.21±0.76[b]	0.74±0.25[b]	289.74±24.71[a]
TAA[1)]	196.11±2.95	180.78±1.29	3,424.36±11.10
EAA[2)]	26.99±1.27	2.13±1.08	1,511.71±9.11
EAA/TAA(%)[3)]	13.76	1.18	40.70

[1)]TAA, total amino acid.
[2)]EAA, essential amino acid (Thr+Val+Mct+Ile+Leu+His+Lys).
[3)]EAA/TAA (%), essential amino acid/total amino acid.
[4)]All values are mean±SD (n=3).
[5)]Means with different superscript letters in the same row are significantly different by Duncan's multiple range test ($p<0.05$). a>b>c.
[6)]–, trace.

Table 7. The contents of vitamin C and β-carotene in various parts of *Hovenia dulcis* Thunbergii

Component	*Hovenia dulcis* parts		
	Fruit	Branch	Leaf
Vitamin C (mg/100 g)	430.26±16.72[1)a2)]	367.85±14.59[b]	336.21±19.34[b]
β-Carotene (mg/100 g)	0.09±0.02[b]	0.15±0.03[b]	2.41±0.05[a]

[1)]All values are mean±SD (n=3).
[2)]Means with different superscript letters in the same row are significantly different by Duncan's multiple range test ($p<0.05$). a>b.

samples, and in our study we used dried samples. An analysis of vitamin C in *H. dulcis* tea leaf revealed concentrations of 133 mg/100 g for fermented tealeaf and 130 mg/100 g for roasted tealeaf (Jeong et al., 2000). These levels were lower than those determined in our study. Our study findings were comparable to

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those of Park (2005) who reported vitamin C content ranging between 312 and 392 mg/100 g. Furthermore, the vitamin C content of *H. dulcis* leaf was higher than the previous findings of Kim et al. (1999) who suggested the vitamin C content to be 199 mg/100 g (green tealeaf), 117 mg/100 g (black tealeaf), and 39 mg/100 g (mugwort), and of Park and Kim (1995) who reported vitamin C content of 44.46 mg/100 g for the raw juice of *Angelica keiskei*.

Importantly, β-carotene is a precursor of vitamin A and is a functional compound with anticancer, anti-aging, and antioxidant effects (Eom et al., 2019). In the different parts of *H. dulcis*, the β-carotene content (Table 7) was the highest in the leaf (2.41 mg/100 g), followed by the branch (0.15 mg/100 g) and fruit (0.09 mg/100 g). Kim et al. (1999) analyzed the β-carotene content of various plants used as tea materials, and found that β-carotene was contained in high amounts of 8.59 mg/100 g in *Eucommia ulmoides,* 6.22 mg/100 g in persimmon leaf, and 3.65 mg/100 g in green tealeaf. In addition, the β-carotene content of black tealeaf, *Lycium chinense*, medicinal mugwort, green plum, and others ranged between 1.50 and 2.25 mg/100 g. Although the β-carotene content of *H. dulcis* is not as high as that of other resources, the fact that *H. dulcis* contains various functional compounds including β-carotene is expected to lead to its use as material for producing antioxidant products.

3.6. The contents of vitamin E

Table 8 presents the results of the analysis of vitamin E by plant part. The α-tocopherol content was 11.01 mg/100 g, 6.61 mg/100 g, and 11.01 mg/100 g in fruit, branch, and leaf, respectively. The β-tocopherol content of the fruit, branch, and leaf was 0.15 mg/100 g, 0.22 mg/100 g, and 3.57 mg/100 g, respectively. The γ-tocopherol and δ-tocopherol content of the fruit, branch, and leaf were 1.38 mg/100 g, 5.98 mg/100 g, and 7.38 mg/100 g and 0.15 mg/100 g, 0.17 mg/100 g, and 2.78 mg/100 g, respectively. The total tocopherol content was measured to be 24.74 mg/100 g (leaf), 12.98 mg/100 g (branch), and 12.69 mg/100 g (fruit). Tocopherol biosynthesis in plants uses the phytyl moiety of chlorophyll degradation (Muñoz and Munné-Bosch, 2019). The vitamin E content is considered to be the highest as a result of the effect of the chlorophyll in the leaf. In other studies, the α-tocopherol content was found to be the highest in green vegetables at 1.41-0.04 mg/100 g (Gantumar et al., 2013), and α-tocopherol is also contained in various plants used as tea materials such as persimmon leaf (33 mg/100 g) and green tea leaf (16 mg/100 g) (Kim et al., 1999).

Table 8. The content of vitamin E in various parts of *Hovenia dulcis* Thunbergii

Component	*Hovenia dulcis* parts		
	Fruit	Branch	Leaf
α-Tocopherol (mg/100 g)	11.01±0.89[1)a2)]	6.61±0.36[b]	11.01±0.16[a]
β-Tocopherol (mg/100 g)	0.15±0.04[b]	0.22±0.04[b]	3.57±0.18[a]
γ-Tocopherol (mg/100 g)	1.38±0.13[c]	5.98±0.48[b]	7.38±0.25[a]
δ-Tocopherol (mg/100 g)	0.15±0.02[b]	0.10±0.03[b]	2.78±0.17[a]
Total (mg/100 g)	12.69±0.04	12.98±0.12	24.74±0.15

[1)]All values are mean±SD (n=3).
[2)]Means with different superscript letters in the same row are significantly different by Duncan's multiple range test ($p<0.05$). a>b>c.

Furthermore, Kim et al. (2005) confirmed that black soybean contained different tocopherols including α-tocopherol (1.99 mg/100 g), β-tocopherol (0.47 mg/100 g), γ-tocopherol (10.68 mg/100 g), and δ-tocopherol (3.95 mg/100 g). The total tocopherol content of *H. dulcis* leaf and black soybean was similar, whereas the branch and fruit contained lower concentrations compared to black soybean. Therefore, it is thought that the *H. dulcis* leaf can be used as a highly nutritious material.

Conflict of interests

The authors declare no potential conflicts of interest.

Author contributions

Conceptualization: Kim SH, Lee JW, Huh CK. Data curation: Kim SH, Lee JW, Huh CK. Formal analysis: Kim SH, Lee JW, Huh CK. Validation: Kim SH, Lee JW, Huh CK. Writing - original draft: Kim SH, Lee JW, Huh CK. Writing - review & editing: Kim SH, Lee JW, Huh CK.

Ethics approval

This article does not require IRB/IACUC approval because there are no human and animal participants.

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