日本家政学会誌 Vol. 72 No. 10 642～654（2021）

Paper

Antioxidant Activity of the Different Parts of Dokudami (*Houttuynia Cordata* Thunb) and the Effects of Manufacturing Processes on the Polyphenol Content, Antioxidant Activity, and Color Tone of Its Tea

Mina KANOU[1] and Yoko TSURUNAGA[1]*

This study examined the antioxidant activity of the different parts of the Dokudami (*Houttuynia cordata* Thunb) plant and the effects of manufacturing processes on the color tone of its tea. Moreover, the effects of the manufacturing process on the total polyphenol content (TPC), antioxidant activities (by 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity, and hydrophilic oxygen radical antioxidant capacity (H-ORAC) assay) of the Dokudami tea were elucidated. The values of the TPC and antioxidant activities of the different parts of the Dokudami plant decreased in the order of flower > leaf > petiole > stem. Furthermore, the manufacturing process had a greater impact on the a^* value of the Dokudami tea than the L^* and b^* values. TPC and antioxidant activity of the steam treated tea showed higher values than untreated and other-treated tea, while fermentation had the opposite effect. The drying method and roasting treatment were found to have a negligible effect.

Key words：Dokudami, polyphenol content, antioxidant activity, color tone

INTRODUCTION

The Dokudami (*Houttuynia cordata* Thunb) plant has a unique odor, and in Japan, the dried whole Dokudami plant is used to prepare Dokudami tea. In addition, in China and Vietnam, the fresh leaves of Dokudami are consumed in salads and in spring rolls, while the dried whole plant, in addition to the stem and leaf, have been used in Japanese and Chinese folk medicine.

In terms of its biological activity, Dokudami has been reported to exhibit anti-inflammatory properties for the treatment of severe acute respiratory syndrome (SARS) (Lu et al. 2006; Lau et al. 2008), and is also known to prevent infection by the herpes simplex virus (HSV) by blocking the action of NF-κB (Chen et al. 2011). Furthermore, Dokudami has also been shown to exhibit antioxidant properties (Nuengchamnong et al. 2009), more specifically, presenting a high 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity. This is attributed to the fact that it contains a number of antioxidant components, including quinic acid derivatives, caffeic acid derivatives, procyanidin B, neo-chlorogenic acid, catechins, chlorogenic acid, crypto-chlorogenic acid, and quercetin hexoside. Chen et al. (2003) reported that Dokudami showed both antioxidative and antimutagenic properties using an oxidized frying oil-fed model. A number of experiments have been performed in relation to the components and functions of Dokudami.

Various reports on the effect of the manufacturing process on the contents of functional components in healthy teas have also been published. We previously reported that during tea manufacturing, the steaming time (Tsurunaga et al. 2004; Tsurunaga et al. 2006), drying method (Katsube et al. 2009), roasting temperature and time (Tsurunaga et al. 2005), and fermentation process can influence the ascorbic acid, flavonoid, and polyphenol contents, thereby affecting the antioxidative activity. However, no reports currently exist that examine the effect of the tea processing method on the antioxidative activity and functional component content of Dokudami

Affiliation: [1]Shimane university
Received November 30, 2020 Accepted July 1, 2021
* To whom correspondence should be addressed E-mail : ytsurunaga@hmn.shimane-u.ac.jp

Fresh Dokudami (approx. 300 g fresh weight)
↓
Washing (thoroughly in tap water)
↓
Draining
↓
Steaming (0 or 1 min)
↓
Drying (machine drying for 12 h at 60℃ or shade drying for 7 days at approx. 25℃)
↓
Roasting (for 0, 1 or 5 min at 150℃ or 200℃)

Fig.1 Manufacturing process of Dokudami tea

tea. We assumed that the functional component contents of Dokudami tea are affected by the manufacturing process, like other plants. Therefore, the aim of this study is to determine the effect of the manufacturing processes, which includes the steam treatment, drying method, fermentation processing, and roasting treatment, on the total polyphenol content (TPC) and antioxidant activity [by DPPH and hydrophilic oxygen radical antioxidant capacity (H-ORAC) assay] of the final tea sample. In addition, although antioxidant activity of whole Dokudami has been clarified by previous studies (Tian et al. 2011; Lou et al. 2019), antioxidant activity of each part has not been clarified. By measuring the antioxidant activity of each part of Dokudami, we can provide new information on the antioxidant activity of Dokudami as well as enable the production of food products such as Dokudami tea, which is limited to parts with high antioxidant activity. Therefore, initially we measured the TPC and antioxidant activity of the different parts of the Dokudami plant, namely, the leaf, the petiole, the flower, and the stem, and subsequently determined the effects of steam treatment, the drying method, fermentation processing, and roasting treatment on the TPC, antioxidant activity, and color tone of the tea.

MATERIALS AND METHODS

Materials

The Dokudami samples (leaf, petiole, flower, and stem) used for the measurements were collected from the grounds of Shimane University in Shimane Prefecture in Japan, in September 2017. These samples were freeze-dried and powdered for subsequent analyses. Powdering involved grinding of the samples in an Oster blender (Sunbeam Oster, Inc., Boca Raton, FL) for 2 min, and subsequent sifting through a 1 mm mesh sieve. DPPH (95%), Folin-Ciocalteu reagent (2 N), Trolox (97%), 2,2′-azobis (2-amidinopropane) dihydrochloride (AAPH, 95%), and ethanol (99.5%) were obtained from Wako Chemicals, Ltd. (Osaka, Japan). Fluorescein sodium salt (1 mg/mL in pure water) for the ORAC assay were obtained from Sigma-Aldrich Chemie GmbH (Steinheim, Germany).

Manufacturing of Dokudami tea

The whole Dokudami was divided into lots of 300 g and steamed for 0 or 1 min. After steam treatment, the samples were dried using an air-drying machine (for 12 h at 60°C) or using the shade-drying technique (for 7 d at 25°C). After drying, roasting treatment was performed for 0, 1, or 5 min at 150 or 200°C. For fermentation treatment, the samples were shade-dried as described above after massaging for 30 min. The samples were then powdered using an Oster blender (SUNBEAM OSTER) for 2 min prior to sifting through a 1 mm mesh sieve.

Extraction method

The prepared powdered samples (100 mg) were added to pure water (20 mL) and extracted for 10 min using boiling water. After this time, the volume was adjusted to 50 mL using pure water.

TPC

The total soluble polyphenol content of each sample was determined according to the method reported by Folin (Goldstein & Swain 1965). More specifically, the extract (90 µL), the Folin-Ciocalteu reagent (90 µL), and 10% sodium carbonate (90 µL) were mixed in the well of a 96-well microplate. After incubation for 60 min at room temperature, the reaction color was measured using a microplate-reader (SH-9000Lab, CORONA ELECTRIC Co., Ltd., Ibaraki, Japan) at 690 nm. The TPC was expressed catechin (CTN) equivalent per dry weight (mg CTN eq/100 g DW).

1,1-Diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity assay

The antioxidant activity of the crude extract was evaluated using a DPPH radical scavenging assay. More specifically, the previously prepared hot water extract (70 µL), a 99.5% ethanol (70 µL), and a 0.2 M 2-(*N*-morpholino)ethanesulfonic acid (MES) buffer (70 µL) at pH 6.0 were pipetted into a 96-well microplate. The reaction was initiated by adding 200 µM DPPH in ethanol (70 µL). After allowing to stand for 20 min at room temperature, the absorbance of the mixture was measured at 540 nm using a microplate-reader (SH-9000Lab). The DPPH value was expressed as µmol Trolox equivalent per dry weight (µmol TE/g DW).

H-ORAC assay

The extracted samples were diluted with the assay buffer solution (75 mM phosphate buffer solution, pH 7.4), then filtered through a 0.45 µm filter (Advantec Toyo Kaisha Ltd.). A fluorescence plate reader (SH-9000Lab) was used to measure the fluorescence intensity of fluorescein at 37°C after adding the test liquid or a Trolox solution (35 µL) followed by a fluorescein solution (115 µL, 110.7 nmol/L) and an AAPH solution (50 µL, 31.7 nmol/L) to the wells of a 96-well microplate (#3072, Becton-Dickinson). Fluorescence readings were taken at an excitation wavelength of 485 nm and a detection wavelength of 520 nm at 2 min intervals over 90 min. The H-ORAC value was expressed in Trolox equivalent per dry weight (µmol TE/g DW).

Color tone

The L^*, a^*, and b^* values of the powdered samples were measured using a spectrum colorimeter (CR-13, Konica Minolta, Tokyo, Japan).

HPLC analysis

Studies have reported the presence of several antioxidants in Dokudami (Meng et al. 2009; Xu et al. 2006; Yang et al. 2014). Chlorogenic acid, rutin, quercitrin, and quercetin in freeze-dried whole Dokudami and Dokudami tea were analyzed by a quantitative HPLC system (LaChrom, Hitachi, Ltd., Tokyo, Japan), by comparisons with standard compounds using a ODS-80A 5 µm column (4.6 × 250 mm) (GL Sciences Inc. Tokyo, Japan); a solvent, acetonitrile/0.1% formic acid (20:80); UV detection, 280 nm (0–7.5 min) and 370 nm (7.5 min–); and velocity of fluid 1 mL/min.

Statistical analysis

The results of the various measurements are expressed as means ± SE. Data were tested by one-way ANOVA, followed by the Tukey test for multiple comparisons ($p < 0.05$) using SPSS (ver. 25, IBM, Inc., Chicago, USA). In the case of the two treatments, data were tested by the t-test ($p < 0.05$) using SPSS.

RESULTS AND DISCUSSION

TPC and DPPH and H-ORAC values of different parts of the Dokudami plant

The determined TPC and DPPH and H-ORAC values of the different parts of the Dokudami plant are presented in Fig.2. More specifically, for the flower, leaf, petiole, and stem fragments, the TPCs were 9,026 ± 164, 3,963 ± 134, 2,204 ± 108, and 562 ± 71 mg CTN eq/100 g DW, respectively; the DPPH values were 471 ± 5, 192 ± 6, 128 ± 7, and 7 ± 9 µmol TE/g DW, respectively; and the H-ORAC values were 2,411 ± 87, 1,287 ± 9, 962 ± 56, and 325 ± 39 µmol TE/g DW, respectively. Overall, the values decreased in the order flower > leaf > petiole > stem. For comparison, Ksouri et al. (2009) reported that higher TPC and DPPH values were observed in the leaves than in the flowers of *Tamarix gallica* L., while Shabir et al. (2011) reported higher DPPH activity in the flowers and leaves of *Delonix regia* (Bojer ex Hook.) than in the bark. In addition, Deng et al. (2015) compared the TPC, DPPH, and H-ORAC values of the leaves and bark of *Solidago canadensis* L., reporting higher activities in the leaves. Furthermore, Nam et al. (2012) reported that the antioxidant activity of mulberry leaves (*Morus alba* L.) was higher than that of the stem. Lachowicz et al. (2020) reported the same for the antioxidant activity of the flowers and leaves of Great burnet (*Sanguisorba officinalis* L.). These results support our finding that higher antioxidant activities tend to be found in the flowers and leaves.

In our study, we measured the TPC, DPPH value, and H-ORAC value to determine the antioxidant capacities of different parts of the Dokudami plant, and observed similar trends for all three parameters (Fig.2). Therefore, we subsequently decided to examine the correlation between the three assays; positive correlations were observed in all cases (Fig.3). More specifically, the TPC and the DPPH value had a correlation of $R^2 = 0.9712$, the H-ORAC value and the TPC had a correlation of

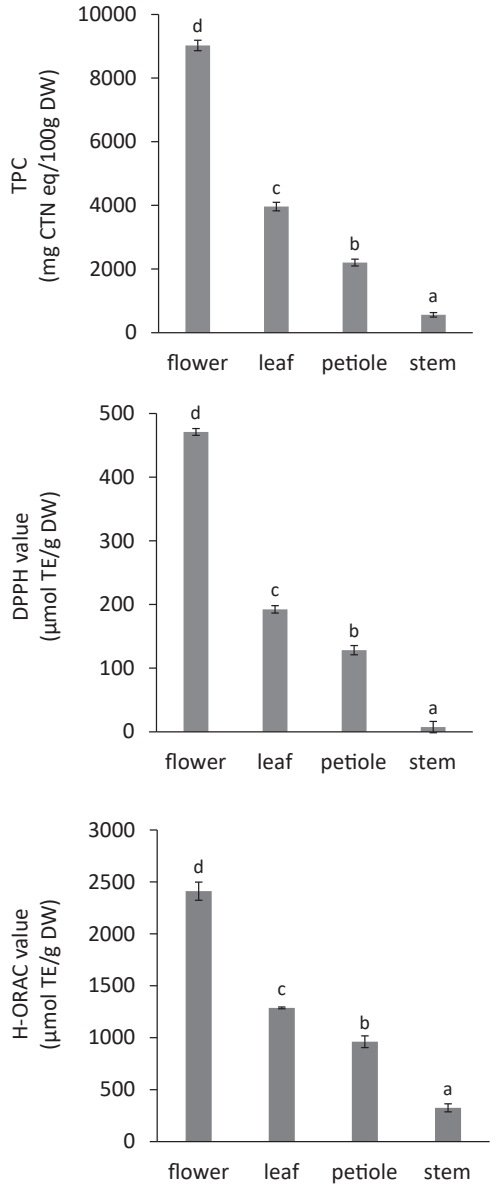


Fig.2 TPC, DPPH radical scavenging activity, and H-ORAC value for the different parts of Dokudami (*Houttuynia cordata* Thunb)

TPC: Total polyphenol content

DPPH value: DPPH radical scavenging activity value

H-ORAC value: Hydrophilic oxygen radical absorbance capacity value

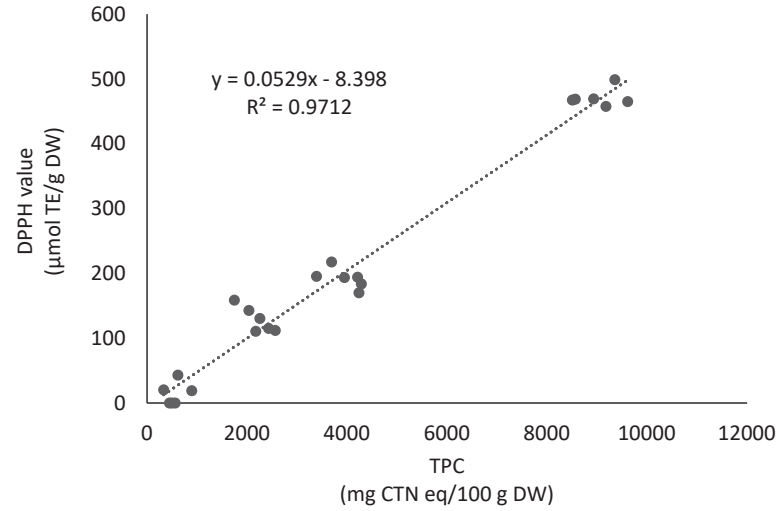
The results were obtained via the Tukey test for multiple comparisons. Different letters signify significant value differences of 5%.

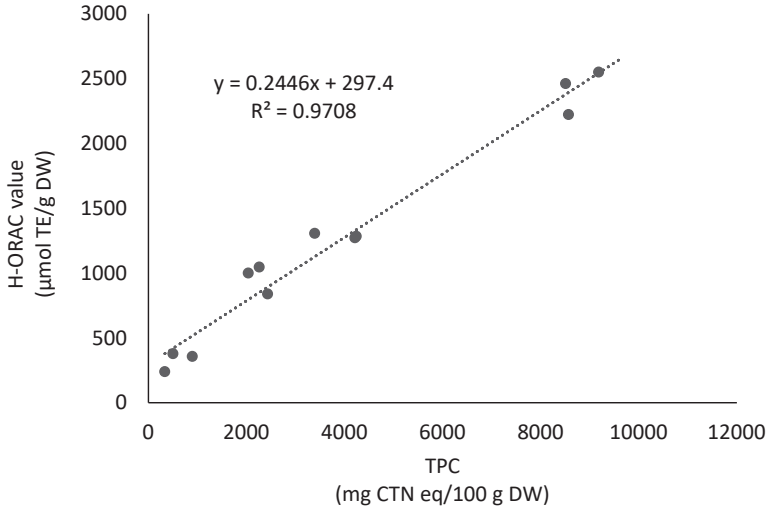
The values were expressed as the standard reagents equivalent per dry weight.

R^2 = 0.9708, and the H-ORAC and DPPH values had a correlation of R^2 = 0.9749. Based on these results, we conceived that high TPC was responsible for the high DPPH and H-ORAC values in the flowers and leaves. In addition, the TPC, DPPH, and H-ORAC values of flowers showed the highest values, which were considered as follows. Fu et al. (2013) reported that the spikes of Dokudami contain flavonoids, such as rutin, hyperin, afzelin, quercitrin, and isoquercitrin. Furthermore, Goto (2019) stated that plants produce antioxidants such as flavonoids that absorb most light in the ultraviolet region of 230–320 nm to protect themselves from oxidative stress caused by ultraviolet rays. Based on this, it was inferred that the TPC, DPPH, and H-ORAC values of flowers, which are essential for reproduction, showed the highest values.

Effect of manufacturing processes on the color tone of Dokudami tea

Table 1 shows the effects of different manufacturing processes on the L^*, a^*, and b^* values of Dokudami tea, representing brightness, green-red tones, and blue-yellow tones, respectively. For machine drying (MD) and shade drying (SD), steaming for 1 min (S area) yielded higher L^* and a^* values than those obtained for the non-steamed areas (NS areas). These results indicate that steam treatment brightens the color of the tea leaves and reduces the degree of green tones. Normally, heat treatment is used to maintain the green color due to the inactivation of chlorophyllase; however, this did not appear to be the case based on our results. It has also been reported that under acidic conditions, chlorophyll forms pheophytin, leading to a reduced degree of green color in the leaves. Dokudami leaves reportedly contain organic acids, such as chlorogenic acid, quinic acid, and caffeic acid, (Fu et al. 2013), which likely contribute to such a transformation. Furthermore, in the NS area, the MD samples showed significantly higher L^*, a^*, and b^* values than the SD samples, although less pronounced differences were observed in the S area. Although Table 1 does not show the L^*, a^*, and b^* values of freeze-dried whole Dokudami, they were 57.0 ± 1.3, −5.8 ± 0.1, and 28.0 ± 0.7, respectively. Regarding the drying method, in MD, the a^* value increased to 3.0 ± 0.0, irrespective of the presence of steam treatment, and in SD, S area was 2.2 ± 0.0 and NS area was 0.0 ± 0.1. In this experiment, we clarified that drying at 60°C increased the a^* value more than drying at 25°C. In order to compare the effect of the drying temperature on the color tone, it was considered necessary to increase the number of treatment groups for the drying temperature in future research. In addition, following roasting, the area heated at 200°C exhibited a significantly lower L^* value and a higher a^* value than





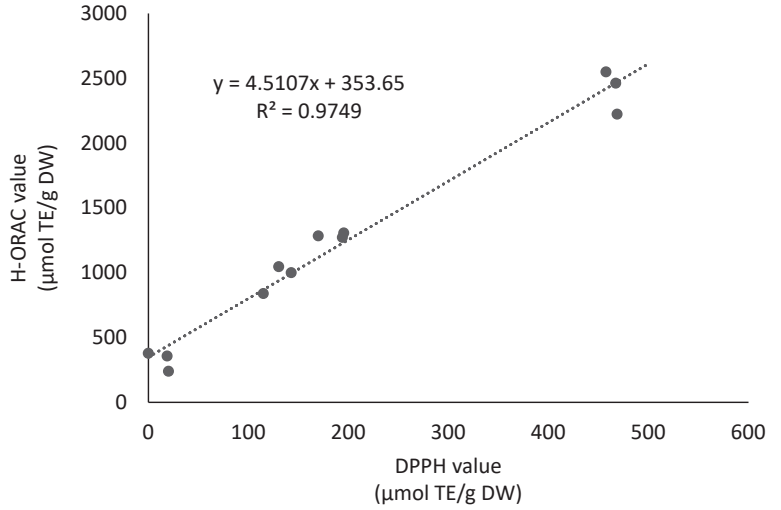


Fig.3　Relationships among the TPC, DPPH radical scavenging activity, and H-ORAC values for different parts of Dokudami (*Houttuynia cordata* Thunb)

TPC: Total polyphenol content
DPPH value: DPPH radical scavenging activity value
H-ORAC value: Hydrophilic oxygen radical absorbance capacity value
The values were expressed as the standard reagents equivalent per dry weight.

Table1 Effects of treatment conditions on the color tone value of Dokudami tea

treatment area name				color		
		Steaming	Drying	L^* value	a^* value	b^* value
Steaming/Drying	NS-MD	non	machine	54.4 ± 0.1 ^{b}	3.0 ± 0.0 ^{c}	30.7 ± 0.0 ^{c}
	S-MD	1 min		58.7 ± 0.2 ^{c}	3.0 ± 0.0 ^{c}	28.0 ± 0.1 ^{b}
	NS-SD	non	shade	50.4 ± 0.7 ^{a}	0.0 ± 0.1 ^{a}	24.0 ± 0.1 ^{a}
	S-SD	1 min		57.3 ± 0.2 ^{c}	2.2 ± 0.0 ^{b}	27.7 ± 0.1 ^{b}
		Roasting	Drying			
Roasting	NR	non	shade	50.4 ± 0.7 ^{bc}	0.0 ± 0.1 ^{a}	24.0 ± 0.1 ^{b}
	R150-1	150°C 1 min		51.0 ± 0.1 ^{c}	0.2 ± 0.1 ^{b}	24.5 ± 0.1 ^{c}
	R150-5	150°C 5 min		50.7 ± 0.2 ^{c}	1.6 ± 0.0 ^{c}	25.5 ± 0.0 ^{e}
	R200-1	200°C 1 min		49.2 ± 0.2 ^{b}	2.5 ± 0.0 ^{d}	25.1 ± 0.0 ^{d}
	R200-5	200°C 5 min		45.3 ± 0.1 ^{a}	7.0 ± 0.0 ^{e}	23.6 ± 0.0 ^{a}
		Fermentation	Drying			
Fermentation	NF	non	shade	50.4 ± 0.7 *	0.0 ± 0.1 *	24.0 ± 0.1 *
	F	massaging 30 min		56.6 ± 0.1	4.1 ± 0.0	24.8 ± 0.0

S: steaming for 1 min
NS: non-steaming
SD: shade-drying
MD: machine-drying
NR: non-roasting
R150-1: after shade-drying roasting treatment at 150°C for 1 min
R150-5: after shade-drying roasting treatment at 150°C for 5 min
R200-1: after shade-drying roasting treatment at 200°C for 1 min
R200-5: after shade-drying roasting treatment at 200°C for 5 min
F: fermentation by massaging for 30 min followed by shade drying
NF: non-fermentation followed by shade drying
Steaming/drying and roasting results were obtained via the Tukey test for multiple comparisons. Different letters signify significant value differences of 5%; fermentation results were obtained via t-tests.

the area heated at 150°C, but no constant trend was observed for the b^* value. We therefore surmised that roasting increases the production of melanoidin by the aminocarbonyl reaction, thereby promoting the browning process and producing a darker color. The L^*, a^*, and b^* values were also significantly higher in the fermented area (F area) than in the non-fermented area (NF area), likely due to enhanced oxidation by polyphenol oxidase upon massage treatment, which in turn facilitated the browning reaction (Kim et al. 2011).

Effect of manufacturing processes on the TPC, DPPH value, and H-ORAC value of Dokudami tea

Fig.4A, 4B, and 4C show the effects of manufacturing processes on the TPC, DPPH value, and H-ORAC value of Dokudami tea. More specifically, the TPC value in the NS area was significantly higher for the SD samples (3,647 ± 105 mg CTN eq/100 g DW) than for the MD samples (3,211 ± 58 mg CTN eq/100 g DW) ($p < 0.05$) (Fig.4A), although no differences were observed in the S area upon changing the drying method. No significant differences in the DPPH and H-ORAC values were observed between the S and NS areas for different drying methods (Fig.4A). Notably, the studies conducted by Tsurunaga et al. on the effects of the manufacturing processes on the properties of bayberry leaf tea and persimmon leaf tea (Tsurunaga et al. 2006; Tsurunaga et al. 2004) showed that the TPC and DPPH value tended to be lower for SD samples than for MD samples. However, we found that the TPC in the NS area was higher for the SD samples (3,647 ± 105 mg CTN eq/100 g DW) than for the MD samples (3,211 ± 58 mg CTN eq/100 g DW). This may be due to

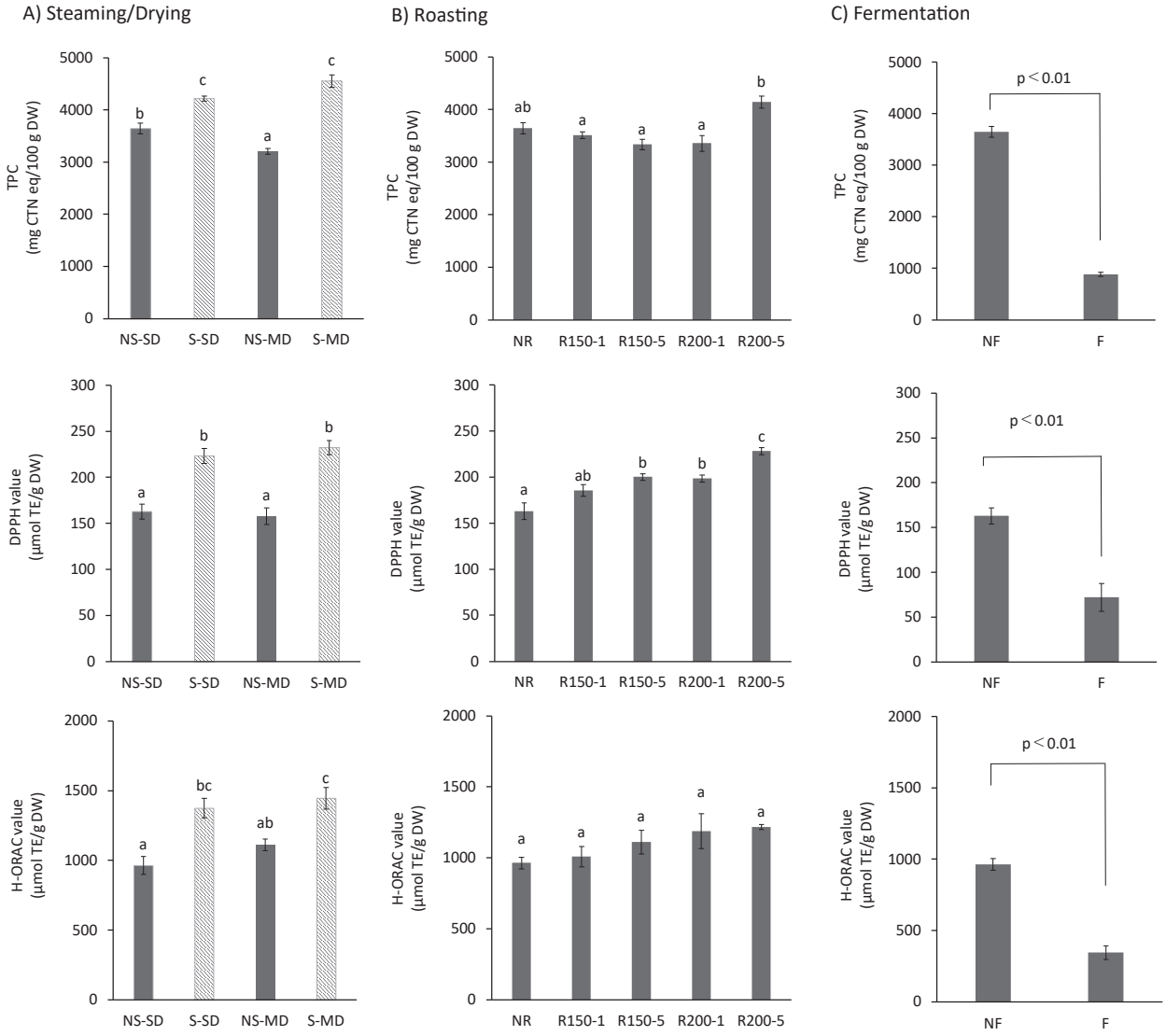


Fig.4　Effect of treatment conditions on the TPC, DPPH radical scavenging activity, and H-ORAC value of Dokudami tea

S: steaming for 1 min
NS: non-steaming
SD: shade drying
MD: machine drying
NR: non-roasting
R150-1: after shade-drying roasting treatment at 150°C for 1 min
R150-5: after shade-drying roasting treatment at 150°C for 5 min
R200-1: after shade-drying roasting treatment at 200°C for 1 min
R200-5: after shade-drying roasting treatment at 200°C for 5 min
F: fermentation by massaging for 30 min followed by shade drying
NF: non-fermentation followed by shade drying
TPC: Total polyphenol content
DPPH value: DPPH radical scavenging activity value
H-ORAC value: Hydrophilic oxygen radical absorbance capacity value
The values were expressed as the standard reagents equivalent per dry weight.

the decomposition of components during the prolonged heating (12 h) at 60°C for the MD method; however, we were unable to clarify this. Although not shown in Fig.4, the TPC, DPPH, and H-ORAC values of freeze-dried whole Dokudami was 3,627.2 ± 54.1 mg CTN eq/100 g DW, 164.8 ± 10.1 μmol TE/g DW, and 1,219.1 ± 71.8 μmol TE/ g DW, respectively. In case of TPC, NS-MD was 3,210.7 ± 57.6 mg CTN eq/100 g DW, which was

significantly lower than FD (3,627.2 ± 54.1 mg CTN eq/100 g DW). However, NS-SD was 3,647.4 ± 104.9 mg CTN eq/100 g DW, showing no significant difference compared to FD. In addition, there was no significant difference between FD, NS-MD, and NS-SD in DPPH and H-ORAC values. From this experiment, it was clarified that during the manufacturing of Dokudami tea, the drying temperature only affected the TPC, and that the TPC when dried at 60°C was significantly lower than that when dried at 25°C. For steam treatment, the TPC, DPPH value, and H-ORAC value were higher in the S areas for both MD and SD samples (Fig.4B). Since steam treatment is known to deactivate various enzymes via protein denaturation, the higher TPC in the S area was likely due to the inactivation of polyphenol oxidase (Ozturk et al. 2016; Xiao et al. 2017; Lin et al. 2012). For roasting treatment, the areas treated at high temperatures for longer periods (200°C, 5 min) presented higher TPC and DPPH value than other treatment areas, while the H-ORAC value was unaffected by roasting. We attributed these results to the fact that the generation of browning substances upon roasting via the promoted non-enzymatic aminocarbonyl reaction was due to the production of melanoidin, which reportedly exhibits antioxidant properties (Anese et al. 1999; Bedinghaus & Ockerman 1995; Hwang et al. 2001). In the color tone, the a^* value of the sample roasted at 200°C for 5 min was large, which suggests that melanoidin was produced. It was suggested that the H-ORAC value was not affected by roasting because the measurement principles of H-ORAC and DPPH assay are different. Ohtani et al. (2007) reported that in natto, melanoidin-related substances produced by aging are mainly involved in the DPPH radical scavenging activity. In addition, the measurement principle of the H-ORAC assay is different from the other two assays. Watanabe et al. (2009a) stated that the measurement principle of the Folin-Ciocalteu method and the DPPH radical scavenging assay is the ET (Electron Transfer) reaction and that the measurement principle of the H-ORAC method is the hydrogen atom transfer (HAT) reaction. In addition, they compared the ORAC values of typical antioxidants with values obtained by several other assays and reported that there was no correlation between the ORAC and DPPH assay. From the above, the melanoidin produced by the aminocarbonyl reaction by roasting in Dokudami tea was thought to be particularly involved in the DPPH radical scavenging activity, and it was considered that the H-ORAC value, a measurement principle different from the DPPH value, was not affected by roasting. Furthermore, Hamauzu (2020) reported the formation of cyanidin and low-molecular-weight phenolic acid, which is considered a decomposition product of cyanidin by heating procyanidins, and increasing in antioxidant activity. Therefore, it was suggested that the TPC and DPPH values of Dokudami tea were higher by roasting treatment was related to the formation of melanoidin and the degradation and molecular weight reduction of polyphenols by heat. Moreover, the aminocarbonyl reaction proceeds further upon increasing the extent of heating, accounting for the differences between roasting treatments at higher and lower temperatures. It was also found that fermentation treatment (F area) led to a significantly lower TPC, DPPH value, and H-ORAC value than when no fermentation was carried out (NF area) ($p < 0.01$) (Fig.4C). More specifically, while the TPC, DPPH value, and H-ORAC value of the NF area were 3,647 ± 105 mg CTN eq/100 g DW, 163 ± 9 μmol TE/g DW, and 964 ± 41 μmol TE/g DW, respectively, lower values were detected in the F area, i.e., 882 ± 41 mg CTN eq/100 g DW, 72 ± 16 μmol TE/g DW, and 345 ± 48 μmol TE/g DW, respectively. For our study, fermentation treatment was conducted by massaging the sample for 30 min. For green tea, massage treatment leads to the polymerization of catechin to produce theaflavin via polyphenol oxidase activity, thereby reducing the soluble polyphenol content and the antioxidant properties (Tanaka et al. 2010; Wang & Ho 2009). Since quercetin, rutin, and isoquercitrin are the main polyphenol components in Dokudami tea (Yang & Jiang 2009), we assumed that these polyphenols were oxidized by polyphenol oxidase, leading to a phenomenon similar to that observed in green tea.

Effect of manufacturing processes on polyphenols of Dokudami tea

Fig.5 shows HPLC profiles of four types of polyphenol (chlorogenic acid, rutin, quercitrin, quercetin) and freeze-dried Dokudami extracts. In freeze-dried Dokudami, chlorogenic acid and quercitrin were detected in large peaks, and rutin and quercetin were hardly detected. Based on this result, we decided to measure the chlorogenic acid and quercitrin contents in the Dokudami tea sample.

Fig.6 shows the effects of manufacturing processes on chlorogenic acid and quercitrin. In the steamed samples,

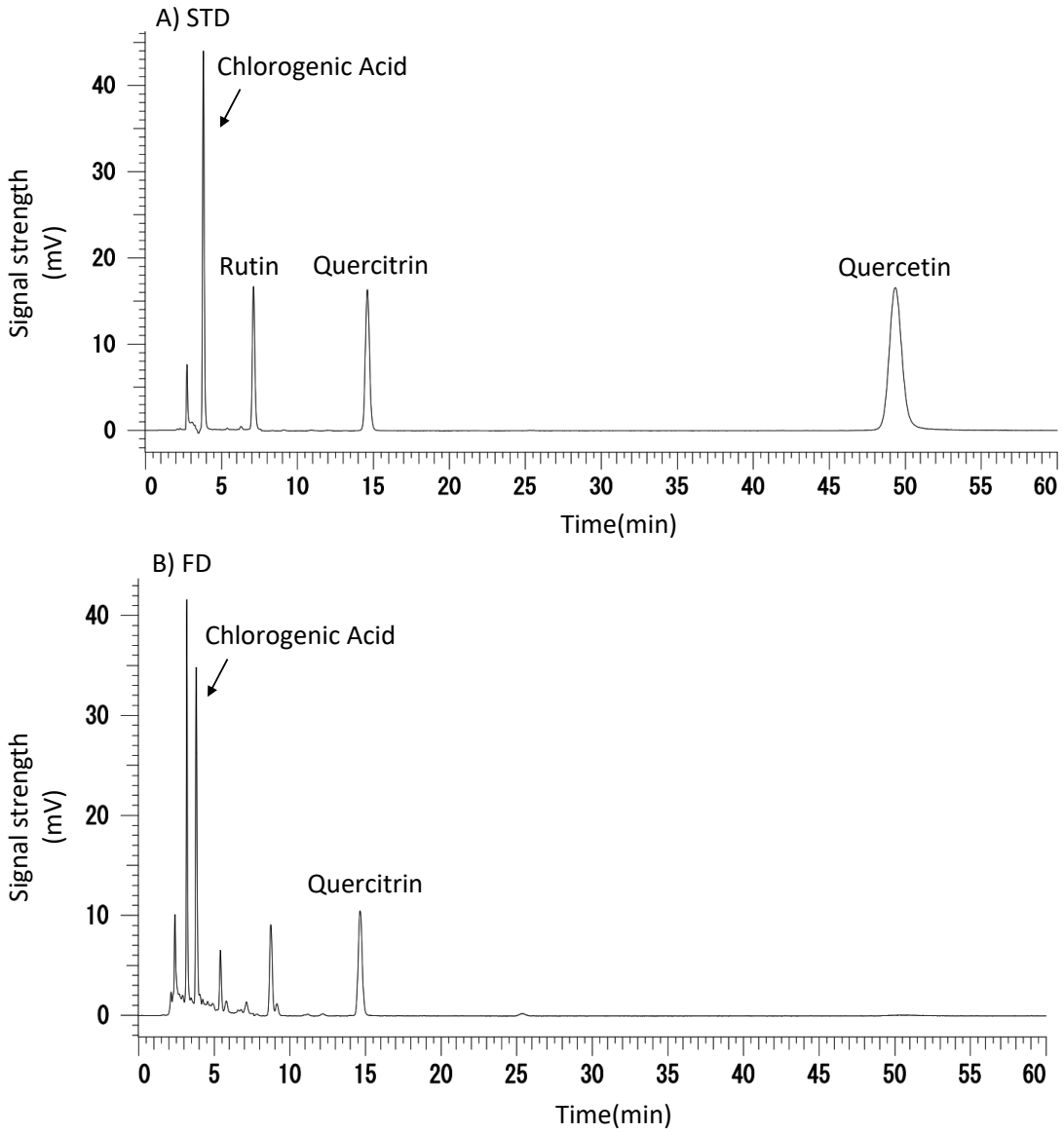


Fig.5　Chromatogram of Dokudami main polyphenol (Chlorogenic Acid, Rutin, Quercetin, Quercitrin) mixture (STD) and Freeze-Dried whole Dokudami (FD)

the chlorogenic acid content in S areas were higher than NS areas. On the other hand, the chlorogenic acid content in F area was lower than NF area. Moreover, compared with chlorogenic acid, quercitrin had a smaller change in content due to manufacturing processes. In addition, both chlorogenic acid and quercitrin were less affected by the roasting treatment than the other treatments. The results of HPLC analysis suggested that chlorogenic acid might affect the TPC, DPPH, and ORAC values.

In this study, it was clarified that the "teaming" process enhances the antioxidant activity, but the effect on the taste, for example, by sensory tests, was not examined. Taste is one of the major factors affecting the sale of healthy tea and thus increases the number of consumers. In this study, fermentation reduced antioxidant activity, but previous studies have reported that fermentation can improve tea flavor and other functionalities (Matsuura et al. 2014; Watanabe et al. 2009b). In the future, we would like to examine and clarify the effect of the manufacturing process on the taste in detail and the positive effect of fermentation.

CONCLUSIONS

This study was performed to determine the antioxidant activities of the different parts of the Dokudami (*Houttuynia cordata* Thunb) plant, to elucidate the effects of manufacturing processes on the color tone of the Dokudami tea, and finally, to clarify the manufacturing process for maintaining high TPC and antioxidant activity

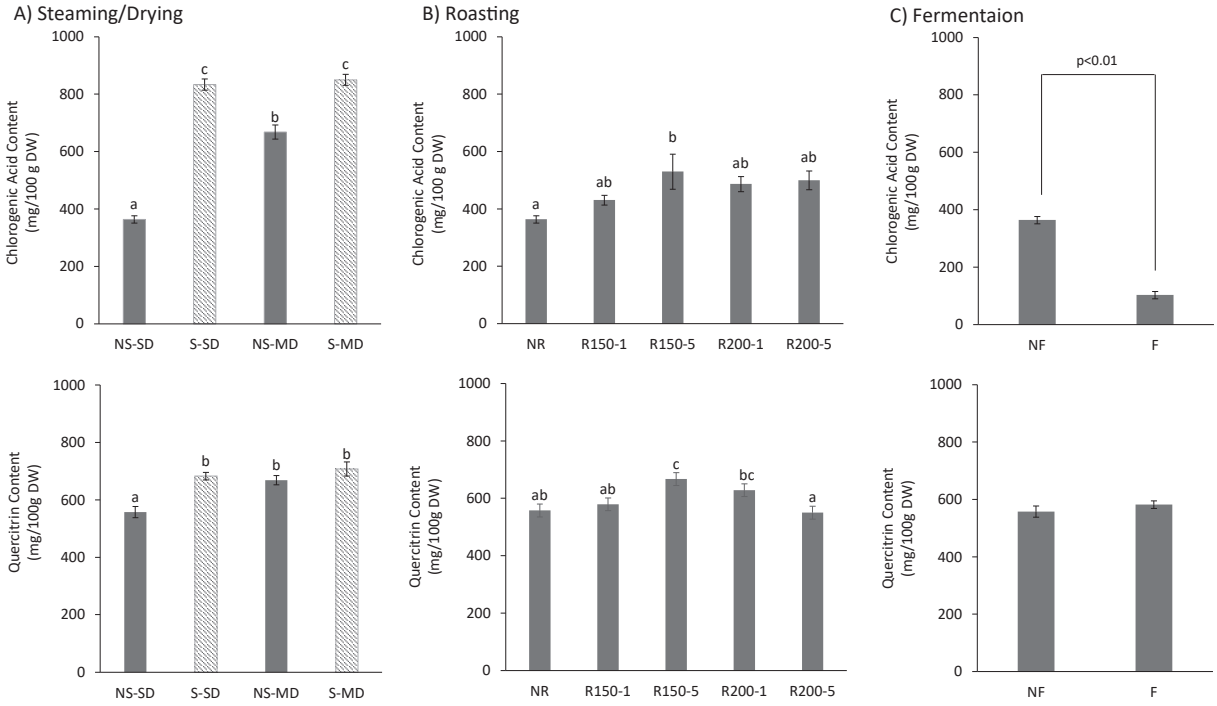


Fig.6 Effect of treatment conditions on Chlorogenic Acid and Quercitrin content of Dokudami tea

S: steaming for 1 min
NS: non-steaming
SD: shade drying
MD: machine drying
NR: non-roasting
R150-1: after shade-drying roasting treatment at 150°C for 1 min
R150-5: after shade-drying roasting treatment at 150°C for 5 min
R200-1: after shade-drying roasting treatment at 200°C for 1 min
R200-5: after shade-drying roasting treatment at 200°C for 5 min
F: fermentation by massaging for 30 min followed by shade drying
NF: non-fermentation followed by shade drying
The values were expressed per dry weight.

(by DPPH and H-ORAC assays) of its tea. The TPC and antioxidant activities of the different parts of the Dokudami plant were found to decrease in the following order: flower > leaf > petiole > stem. In terms of the color tone of the Dokudami tea, the a^* value was influenced the most by the manufacturing processes. Fermentation and steam treatment led to the lowest and highest TPC and antioxidant activity, respectively, of the Dokudami tea, while drying and roasting had less effect on these properties. In this experiment, steam treatment was performed for 1 min, but increasing the steaming time may improve TPC and antioxidant activity further. In the future, it will be necessary to clarify the optimum conditions for improving the TPC and antioxidant activity of Dokudami tea by increasing the duration of the steam treatment.

CONFLICTS OF INTEREST STATEMENT

There are no conflicts of interest to declare.

ACKNOWLEDGMENTS

This work was supported by “Shimane University Grants for Joint Research Project led by Female Researchers” under the MEXT “Initiative for Realizing Diversity in the Research Environment (Collaboration Type), and JSPS KAKENHI Grant Number 21H00808.”

REFERENCES

Anese, M.; Manzocco, L.; Nicoli, M. C.; Lerici, C. R. (1999). Antioxidant properties of tomato juice as affected by heating. *Journal of the Science of Food Agriculture.* Vol. 79, 750–754.

Bedinghaus, A. J.; Ockerman, H. W. (1995). Antioxidative

Maillard Reaction Products from Reducing Sugars and Free Amino Acids in Cooked Ground Pork Patties. *Journal of Food Science*. Vol. 60, 992–995.

Chen, X.; Wang, Z.; Yang, Z.; Wang, J.; Xu, Y.; Tan, Ren-Xiang.; Li, E. (2011). *Houttuynia cordata* blocks HSV infection through inhibition of NF-κB activation. *Antiviral Research*. Vol. 92, 341–345.

Chen, Y.-Y.; Liu, T.-F.; Chen, C.-M.; Chao, P.-Y.; Chang, T.-J. (2003). A study of the Antioxidative and Antimutagenic Effects of *Houttuynia cordata* Thunb. Using an Oxidized Frying Oil-Fed Model. *Journal of Nutritional Science and Vitaminology*. Vol. 49, 327–333.

Deng, Y.; Zhao, Y.; Padilla-Zokour, O.; Yang, G. (2015). Polyphenols, antioxidant and antimicrobial activities of leaf and bark extracts of *Solidago canadensis* L. *Industrial Crops and Products*. Vol. 74, 803–809.

Fu, J.; Dai, L.; Lin, Z.; Lu, H. (2013). *Houttuynia cordata* Thunb: A Review of Phytochemistry and Pharmacology and Quality Control. *Chinese Medicine*. Vol. 4, 101–123.

Goldstein, J. L.; Swain, T. (1965). The inhibition of enzymes by tannins. *Phytochemistry*. Vol. 4, 185–192.

Goto, E. (2019). Environmental Stress and Secondary Metabolites (in Japanese). *Shokubutsu Kankyou Kougaku (Journal of Society of High Technology in Agriculture)*. Vol. 31, 7–20.

Hamauzu, Y. (2020). Studies on Functional Properties of Procyanidins in Fruits and Their Changes during Processing (in Japanese). *Nippon Syokuhin Hozou Kagaku Kaishi (Food Preservation Science)*. Vol. 46, 71–78.

Hwang, J.-Y.; Shue, Y.-S.; Chang, H.-M. (2001). Antioxidative activity of roasted and defatted peanut kernels. *Food Research International*. Vol. 34, 639–647.

Katsube, T.; Tsurunaga, Y.; Sugiyama, M.; Furuno, T.; Yamasaki, Y. (2009). Effect of air-drying temperature on antioxidant capacity and stability of polyphenolic compounds in mulberry (*Morus alba* L.) leaves. *Food Chemistry*. Vol. 113, 964–969.

Kim, Y.; Goodner, K. L.; Park, J.-D.; Choi, J.; Talcott, S. T. (2011). Changes in antioxidant phytochemicals and volatile composition of *Camellia sinensis* by oxidation during tea fermentation. *Food Chemistry*. Vol. 129, 1331–1342.

Ksouri, R.; Falleh, H.; Megdiche, W.; Trabelsi, N.; Mhamdi, B.; Chaieb, K.; Bakrouf, A.; Magné, C.; Abdelly, C. (2009). Antioxidant and antimicrobial activities of the edible medicinal halophyte *Tamarix gallica* L. and related polyphenolic constituents. *Food and Chemical Toxicology*. Vol. 47, 2083–2091.

Lachowicz, S.; Oszmianski, J.; Rapak, A.; Ochmian, I. (2020). Profile and Content of Phenolic Compounds in Leaves, Flowers, Roots, and Stalks of *Sanguisorba officinalis* L. Determined with the LC-DAD-ESI-QTOF-MS/MS Analysis and Their In Vitro Antioxidant, Antidiabetic, Antiproliferative Potency. *Pharmaceuticals*. Vol. 13, 1–23.

Lau, K. M.; Lee, K. M.; Koon, C. M.; Cheung, C. S.-F.; Lau, C. P.; Ho, H. M.; Lee, M. Y.-H.; Au, S. W.-N.; Cheng, C. H.-K.; Lau, C. B.-S.; Tsui. S. K.-W.; Wan, D. C.-C.; Waye, M. M.-Y.; Wong, K.-B.; Wong, C.-K.; Lam, C.W.-K.; Leung, P.-C.; Fung, K.-P. (2008). Immunomodulatory and anti-SARS activities of *Houttuynia cordata*. *Journal of Ethnopharmacology*. Vol. 118, 79–85.

Lin, L.; Lei, F.; Sun, D.-W.; Dong, Y.; Yang, B.; Zhao, M. (2012). Thermal inactivation kinetics of *Rabdosia serra* (Maxim.) Hara leaf peroxidase and polyphenol oxidase and comparative evaluation of drying methods on leaf phenolic profile and bioactivities. *Food Chemistry*. Vol. 134, 2021–2029.

Lou, Y.; Guo, Z.; Zhu, Y.; Kong, M.; Zhang, R.; Lu, L.; Wu, F.; Liu, Z.; Wu, Z. (2019). *Houttuynia cordata* Thunb. and its bioactive compound 2-undecanone significantly suppress benzo(a)pyrene-induced lung tumorigenesis by activating the Nrf2-HO-1/NQO-1 signaling pathway. *Journal of Experimental & Clinical Cancer Research*. Vol. 38, 1–21.

Lu, H. M.; Liang, Y. Z.; Yi, L.Z.; Wu, X. J. (2006). Anti-inflammatory effect of *Houttuynia cordata* injection. *Journal of Ethnopharmacology*. Vol. 104, 245–249.

Matsuura, Y.; Numaguchi, M.; Kai, T.; Kamenaga, K.; Yukizaki, C. (2014). Development of Oxidative Fermented Tea by Using Blueberry Leaves (in Japanese). *Miyazakiken Kougyougijutsu Senta·Miyazakiken Shokuhinkaihatsu Senta Kenkyuuhoukoku (Report of Miyazaki Prefecture Industrial Technology Center & Miyazaki Prefectural Food & R&D Center)*. Vol. 59, 47–51.

Meng, J.; Leung, K.S.-Y.; Dong, X.-p.; Zhou, Y.-s.; Jiang, Z.-h.; Zhao, Z.-z. (2009). Simultaneous quantification of eight bioactive components of *Houttuynia cordata* and related Saururaceae medicinal plants by on-line high performance liquid chromatography–diode array detector–electrospray mass spectrometry. *Fitoterapia*. Vol. 80, 468–474.

Nam, S.; Jang, H. W.; Shibamoto, T. (2012). Antioxidant Activities of Extracts from Teas Prepared from Medicinal Plants, *Morus alba* L., *Camellia sinensis* L., and *Cudrania tricuspidata*, and Their Volatile Components. *Journal of Agricultural and Food Chemistry*. Vol. 60, 9097–9105.

Nuengchamnong, N.; Krittasilp, K.; Ingkaninan, K. (2009). Rapid screening and identification of antioxidants in aqueous extracts of *Houttuynia cordata* using LC–ESI–MS coupled with DPPH assay. *Food Chemistry*. Vol. 117, 750–756.

Ohtani, K.; Nagato, M.; Shibata, M.; Tomita, K.; Sato, K.;

Kawazoe, S. (2007). Changes in the DPPH radical-scavenging activity and the related components of Daitokuji-natto made in Zuihoin Daitokuji during processings (in Japanese). *Nihon Chouri Kagaku Kaishi* (*J. Cookery Sci. Jpn.*). Vol. 40, 239–248.

Ozturk, B.; Seyhan, F.; Ozdemir, I. S.; Karadeniz, B.; Bahar, B.; Ertas, E.; Ilgaz, S. (2016). Change of enzyme activity and quality during the processing of Turkish green tea. *LWT-Food Science and Technology.* Vol. 65, 318–324.

Shabir, G.; Anwar, F.; Sultana, B.; Khalid, Z. M.; Afzal, M.; Khan, Q. K.; Ashrafuzzaman, M. (2011). Antioxidant and Antimicrobial Attributes and Phenolics of Different Solvent Extracts from Leaves, Flowers and Bark of Gold Mohar [*Delonix regia* (Bojer ex Hook.) Raf.]. *Molecules.* Vol. 16, 7302–7319.

Tanaka, T.; Matsuo, Y.; Kouno, I. (2010). Review: Chemistry of Secondary Polyphenols Produced during Processing of Tea and Selected Foods. *International Journal of Molecular Science.* Vol. 11, 14–40.

Tian, L.; Zhao, Y.; Guo, C.; Yang, X. (2011). A comparative study on the antioxidant activities of an acidic polysaccharide and various solvent extracts derived from herbal *Houttuynia cordata. Carbohydrate Polymers.* Vol. 83, 537–544.

Tsurunaga, Y.; Matsuzaki, H.; Mochida, K.; Itamura, H. (2004). Effects of Manufacturing Process on Functionality and Fanctional Component of Persimmon Leaf Tea (in Japanese). *Nippon Shokuhin Kagaku Kogaku Kaishi* (*Journal of the Japanese Society for Food Science and Technology*). Vol. 51, 401–405.

Tsurunaga, Y.; Matsuzaki, H.; Mochida, K.; Matsumoto, T.; Itamura, H. (2005). Effect of Steaming and Roasting Treatment on the Radical Scavenging Activities and Total Ascorbic Acid Content of Persimmon Leaf Tea During Storage (in Japanese). *Nippon Shokuhin Kagaku Kogaku Kaishi* (*Journal of The Japanese Society for Food Science and Technology*). Vol. 52, 391–397.

Tsurunaga, Y.; Matsuzaki, H.; Matsumoto, T.; Tomikawa, Y.; Itamura, H. (2006). Effects of manufacturing process on ascorbic acid contents, polyphenol content and radical scavenging activities of bayberry leaf tea (in Japanese). *Nippon Syokuhin Hozou Kagaku Kaishi* (*Food Preservation Science*). Vol. 32, 221–227.

Wang, Y.; Ho, C.-T. (2009). Polyphenolic Chemistry of Tea and Coffee: A Century of Progress. *Journal of Agricultural and Food Chemistry.* Vol. 57, 8109–8114.

Watanabe, J.; Oki, T.; Takebayashi, J.; Yamasaki, K.; Tsushida, T. (2009a). Approaches to establish the standardized methods for the determination of antioxidant capacities of foodstuffs (in Japanese). *Kagaku to Seibutsu (Kagaku to Seibutsu)*. Vol. 47, 237–243.

Watanabe, Y.; Matsuura, T.; Hayakawa, K.; Ueno, H. (2009b). Post-fermented green tea with yeast and effects on suppression of glucose absorption in rats (in Japanese). *Tyagyou Kenkyuu Houkoku* (*Tea Research Journal*). Vol. 108, 39–49.

Xiao, H.-W.; Pan, Z.; Deng, L.-Z.; El-Mashad, H. M.; Yang, X.-H.; Mujumdar, A. S.; Gao, Z.-J.; Zhang, Q. (2017). Recent developments and trends in thermal blanching – A comprehensive review. *Information Processing in Agriculture.* Vol. 4, 101–127.

Xu, X.; Ye, H.; Wang, W.; Yu, L.; Chen, G. (2006). Determination of flavonoids in Houttuynia cordata Thunb. And *Saururus chinensis* (Lour.) Bail. by capillary electrophoresis with electrochemical detection. *Talanta.* Vol. 68, 759–764.

Yang, L.; Jiang, J.-G. (2009). Bioactive components and functional properties of *Hottuynia cordata* and its applications. *Pharmaceutical Biology.* Vol. 47, 1154–1161.

Yang, Z.-n.; Sun, Y.-m.; Luo, S.-q.; Chen, J.-w.; Yu, Z.-w. (2014). Quality evaluation of *Houttuynia cordata* Thunb. by high performance liquid chromatography with photodiode-array detection (HPLC-DAD). *Pakistan Journal of Pharmaceutical Sciences.* Vol. 27, 223–231.

ドクダミの部位別抗酸化性とドクダミ茶の製造工程によるポリフェノール含量と抗酸化性および色調への影響

加納　己奈[1]，鶴永　陽子[1*]

本研究は，ドクダミ（*Houttuynia cordata* Thunb）の部分別の抗酸化性およびドクダミ茶の色調への製造工程の影響を検討し，最終的に，総ポリフェノール含量（TPC）と抗酸化性（1,1-diphenyl-2-picrylhydrazyl（DPPH）ラジカル捕捉活性および H-ORAC 値（hydrophilic oxygen radical antioxidant capacity））に最も影響を与える工程を明らかにすることを目的として行われた．ドクダミの部位別の TPC と抗酸化性は，花＞葉＞葉柄＞茎の順に低下した．また，ドクダミ茶の色調における a^*値は，L^*値や b^*値に比べて製造工程の影響を最も受けた．ドクダミ茶の TPC と抗酸化性は蒸熱処理によって未処理および他の処理よりも高い値を示したが，発酵は逆の影響を及ぼした．さらに，乾燥および焙煎処理はドクダミ茶の TPC や抗酸化性への影響は少ないことが明らかとなった．

キーワード：ドクダミ，ポリフェノール含量，抗酸化性，色調

所属機関名：[1]島根大学
原稿受付：2020年11月30日　　原稿受理：2021年７月１日
* To whom correspondence should be addressed　　E-mail：ytsurunaga@hmn.shimane-u.ac.jp