Identification and evaluation of antioxidants in Japanese parsley

Tasuku Ogita*, Rosaly Vallejo Manaoi, Manabu Wakagi, Tomoyuki Oki, Yuko Takano Ishikawa and Jun Watanabe

National Food Research Institute, National Agriculture and Food Research Organization, Tsukuba, Ibaraki, Japan; Kyushu Okinawa Agricultural Research Center, National Agriculture and Food Research Organization, Koshi, Kumamoto, Japan

ABSTRACT

Two cultivars of Japanese parsley were harvested in different seasons; their antioxidant capacities were evaluated by oxygen radical absorbance capacity (ORAC) methods, and the contents of hydrophilic and lipophilic antioxidants were compared. Japanese parsley possessed potent antioxidant capacities both in hydrophilic and lipophilic extracts when evaluated by ORAC methods. LC/MS/MS analyses revealed that chlorogenic acid and four kinds of quercetin glycosides were major antioxidants in the hydrophilic extract. Lutein was the main contributor to the antioxidant capacity of the lipophilic extract. Antioxidant capacities of the hydrophilic extracts of both cultivars tended to be higher in winter because of the increase in the contents of chlorogenic acid and quercetin glycosides. An obvious trend in the lipophilic antioxidant capacities or lutein contents was not observed irrespective of the cultivar.

INTRODUCTION

Japanese parsley (Oenanthe javanica Blume (DC.)) is a perennial plant that grows naturally in the marshlands of Japan, Russia, Southeastern Asia and Oceania. It has been consumed in Japan since ancient times, and it is a familiar vegetable to the Japanese. Japanese parsley contains relatively high amounts of β-carotene and tocopherols (1.6 mg/100 g and 1.9 mg/100 g fresh weight, respectively; Ministry of Agriculture, Forestry and Fisheries 2005). According to the Regional Specialty Vegetables Production Status Study in 2012, Natori City in Miyagi Prefecture has the largest planted area, yield and shipment of Japanese parsley (Ministry of Agriculture, Forestry and Fisheries 2005). Japanese parsley has been also used as medicinal plant, such as efficacious medical for the treatment of jaundice and high blood pressure (Wan-Ibrahim et al. 2010). Anti-inflammatory, anti-hepatic virus infection and anti-oxidative effects of Japanese parsley have been evaluated using in vitro and in vivo models (Wang WN et al. 2005; Jeong 2009; Yang et al. 2013; Lee et al. 2015).

Reactive oxygen species (ROS) damage biological molecules including proteins, lipids and nucleic acids, and chronic and degenerative diseases, such as cancer, heart disease and Alzheimer’s disease are thought to be caused, in part, by ROS produced in the body (Phaniendra et al. 2015). Fruits and vegetables contain large amounts of antioxidants, which inhibit the decomposition of biological molecules by eliminating ROS (Lin 2013). There is thus increasing interest in the relationship between the consumption of fruits and vegetables rich in antioxidants and disease risk (Wang WN et al. 2005; Wang X et al. 2014). In particular, it has been reported that consumption of fruits and vegetables is inversely associated with lung cancer risk among current smokers, and with breast cancer risk in African-American women (Boggs et al. 2010; Vieira et al. 2015). Japanese parsley contains antioxidant components, such as lutein, chlorogenic acid and quercetin glycosides (Aizawa & Inakuma 2007; Khanum et al. 2012). Although, Kameya et al. (2014) reported that Japanese parsley possessed relatively high antioxidant capacity among commonly consumed vegetables in Japan, little is known about the components, contributing to the antioxidant capacity of Japanese parsley.

Herein, antioxidant capacities of Japanese parsley were evaluated by improved oxygen radical absorbance capacity (ORAC) methods. The original ORAC method
developed by Cao et al. (1993) has been widely used because of its advantages related to biological systems (Noguer et al. 2014). Huang et al. (2002) extended the ORAC assay to lipophilic antioxidants using randomly methylated β-cyclodextrin (RMCD) as a solubility enhancer, thereby allowing measurement of the antioxidant capacities of both lipophilic and hydrophilic components in a given sample using the same peroxyl radical source. We improved hydrophilic ORAC (H-ORAC) and lipophilic-ORAC (L-ORAC) methods, and validated improved methods by inter-laboratory studies (Watanabe et al. 2012, 2014a, 2014b).

In this study, we evaluated the antioxidant capacities of hydrophilic and lipophilic extracts of two cultivars Japanese parsley (Natori No.5 and No.6) grown in Natori City by improved ORAC methods, and identified the active antioxidants.

**Materials and methods**

**Japanese parsley samples**

Two different cultivars available of Japanese parsley (Natori No. 5 and No. 6) were used in this study. These cultivars, representing a sample of elite commercial cultivars currently grown in Miyagi Prefecture, were field-grown during the 2014 growing season in Natori City, Miyagi Prefecture, Japan. Japanese parsley samples of both cultivars were harvested on 9 October, 10 November and 10 December 2014, and 19 January and 18 February 2015. Natori No. 5 and No. 6 cultivars were grown in the Simoyoda and Kamiyoda suburbs, respectively. These cultivars were grown under near-identical climate conditions, since the Simoyoda and Kamiyoda suburbs are adjacent to each other and each has an area of less than 3 km². In this study, we were unable to obtain the details about samples harvesting, climate conditions and soil. Therefore, we estimated climate conditions using Japan Meteorological Agency data for Simoyoda and Kamiyoda suburbs.

**Reagents and chemicals**

Fluorescein and Trolox were purchased from Sigma-Aldrich (Milwaukee, WI). 2,2’-Azobis (2-amidinopropane)dihydrochloride (AAPH) was obtained from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Randomly methylated β-cyclodextrin (RMCD) was obtained from Junsei Chemical (Tokyo, Japan). Chlorogenic acid, rutin, isoquercitrin and hyperoside were purchased from Tokiwa Phytochemical (Chiba, Japan). All other chemicals were of reagent grade.

**Preparation of extracts**

Aerial parts of Japanese parsley were cut into small pieces, and about 200 g of each sample was shock-frozen in liquid nitrogen and then freeze-dried. The samples were pulverized using a grinder mill (GM-200; Retsch, Haan, Germany). Freeze-dried samples from each season and cultivar were obtained in triplicate. These samples were extracted with pressurized liquid extraction apparatus (ASE-350; Dionex, San Jose, CA), as described previously (Watanabe et al. 2014a, 2014b). Briefly, the samples (1 g each) were mixed with sea sand (5 g, methanol washed, Wako Pure Chemical Industries, Ltd., Osaka, Japan) and added to a 22 mL extraction cell. The extraction parameters were as follows: temperature 70 °C for n-hexane-dichloromethane (1:1) extraction and 80 °C for MWA (methanol:water:acetic acid =90:9.5:0.5) extraction; static time, 5 min; cycles, 4; rinse volume, 55%. The samples were sequentially extracted with n-hexane-dichloromethane, and then with MWA. It has been reported that lipophilic and hydrophilic antioxidants were effectively extracted from lyophilized food when this extraction method was used (Watanabe et al. 2014a, 2014b).

**H-ORAC measurement**

The MWA extracts of Japanese parsley were made up to 50 mL with MWA, and their H-ORAC values were measured according to the methods described by Watanabe et al. (2012). Fluorescence intensity of each well of a 96-well microplate was measured by a microplate reader (PowerScan HT; DS Pharma Biomedical, Osaka, Japan).

**L-ORAC measurement**

The hexane-dichloromethane extracts of Japanese parsley were dried under nitrogen gas flow, and then dissolved with dimethylsulfoxide (DMSO, 5 mL). L-ORAC values of the extracts were measured according to the methods described by Watanabe et al. (2014a, 2014b) using a microplate reader (PowerScan HT; DS Pharma Biomedical, Osaka, Japan).

**Purification of hydrophilic antioxidants**

Hydrophilic antioxidants in the MWA extracts of Japanese parsley were separated using a Shimadzu UFLC XR liquid chromatography system (Shimadzu, Kyoto, Japan). A YMC-Ultra HT Pro C18 column (2.0 × 75 mm; YMC, Kyoto, Japan) was used at 40 °C. The mobile phase was a gradient of aqueous acetonitrile containing 2% acetic acid at a flow rate of 0.3 mL/min. The gradient was...
as follows: 0–15 min, 0–15% acetonitrile; 15–30 min, 15% acetonitrile; 30–45 min, 15–50% acetonitrile; 45–47 min, 50% acetonitrile. Absorbance at 260 nm was monitored to obtain a chromatogram. Fractions were collected every minute, and the resulting fractions were neutralized with NaOH (1 mol/L, 60 μL). The mixture, Trolox standard solutions and 75 mM phosphate buffer (pH 7.4, 35 μL each) were added to the wells of a 96-well microplate, and 115 μL of fluorescein (110.7 nmol/L) and 50 μL of AAPH (31.7 nmol/L) were added to each well, and then incubated at 37°C. The fluorescence intensity was monitored every 2 min for 90 min by a microplate reader. Net area under the curve (AUC) of the fractions was calculated by subtracting the AUC of fluorescence decay of each fraction from that of phosphate buffer.

**Identification and quantification of hydrophilic antioxidants**

Hydrophilic antioxidants in the MWA extracts were identified on the basis of LC/MS spectral data. LC/MS analysis was performed by electrospray ionization in the negative ion mode using an Esquire 3000 plus mass spectrometer (Bruker Daltonics, Billerica, MA). A Prodigy ODS3 column (4.6 × 250 mm; Phenomenex, Torrance, CA) was used at 40°C. The mobile phase consisted of a gradient of 0.2% (v/v) formic acid aqueous solution (A) and 0.2% (v/v) formic acid in methanol (B). The gradient was as follows: 0–10 min, 15% B; 10–25 min, 15–48% B; 25–30 min, 48–90% B. The flow rate was 1.2 mL/min. The capillary voltage was set at 3.5 kV, and the drying gas flow and temperature were at 8 L/min and 350°C, respectively. Argon was used for fragmentation by collision-induced dissociation in the collision cell.

For the quantification of hydrophilic antioxidants, a Shimadzu UFLC XR liquid chromatography system was used under the same conditions as above, except that a photo diode array detector (PDA-M20A; Shimadzu, Kyoto, Japan) was used at wavelengths between 200 and 400 nm. Standard solutions containing chlorogenic acid, rutin, isoquercitrin and hyperoside were prepared. The concentration of quercetin-3-O-rhamnosyl-galactoside was calculated based on the standard curve of rutin.

**Purification of lipophilic antioxidants**

Lipophilic antioxidants in the hexane-dichloromethane extracts of Japanese parsley were separated using a Shimadzu UFLC XR liquid chromatography system. An YMC-Ultra HT Pro C18 column (2.0 × 75 mm) was used at 40°C. The mobile phase was a gradient of chloroform in methanol. The gradient was as follows:

0–10 min, 0% chloroform; 10–20 min, 0–20% chloroform; 20–25 min, 0% chloroform. The flow rate was 0.4 mL/min. Absorbance at 260 nm was monitored to obtain a chromatogram. Fractions were collected every minute, and the resulting fractions were subjected to H-ORAC measurement. Absorbance of AAPH (31.7 mmol/L) was added to each well, and then DMSO and 7% (w/v) RMCD in 50% acetone (30 μL each) were added. The mixture, Trolox standard solutions, and 7% (w/v) RMCD in 50% acetone as blank (35 μL each) were added to the wells of a 96-well microplate, and 115 μL of fluorescein (77.5 nmol/L) and 50 μL of AAPH (82.4 mmol/L) were added to each well, and then incubated at 37°C. The fluorescence intensity was monitored every 2 min for 120 min by a microplate reader. Net AUCs of the fractions were calculated as described above.

**Quantification of lutein in lipophilic extract**

Lutein in the lipophilic extracts of Japanese parsley was quantified using an Ultimate 3000 rapid separation LC system (Thermo Scientific, Waltham, MA). An YMC-Ultra HT Pro C18 column (2.0 × 75 mm) was used at 40°C. A gradient elution of aqueous methanol was used as follows: 0–1 min, 87.5% methanol; 1–6 min, 87.5–100% methanol; 6–9 min, washing with methanol/chloroform 3:1. The flow rate was 0.4 mL/min. Wavelength at 450 nm was monitored by a photo diode array detector.

**Quantification of α- and γ-tocopherols**

Tocopherols (α, γ) in the lipophilic extracts of Japanese parsley were quantified using a Shimadzu UFLC XR liquid chromatography system. A YMC-Ultra HT Pro C18 column (2.0 × 75 mm) was used at 40°C. The

![Figure 1. Typical HPLC chromatogram of the hydrophilic extract of Japanese parsley and the net AUC values from H-ORAC measurement. Absorbance at 260 nm was monitored to obtain a chromatogram. Fractions were collected every minute, and the resulting fractions were subjected to H-ORAC measurement. Antioxidant capacity of each fraction was shown as the net AUC value from H-ORAC measurement. Four active peaks (I–IV) were observed, and five active compounds were identified as chlorogenic acid (1), quercetin rhamnosyl galactoside (2), rutin (3), hyperoside (4), and isoquercitrin (5).](Image 319x170 to 548x278)
mobile phase was methanol (0–6 min) and then the column was washed with methanol/chloroform 4:1 (6–10 min). The flow rate was 0.4 mL/min. Fluorescence at excitation and emission wavelengths of 298 and 325 nm, respectively, was monitored by a fluorescence detector (RF-10A; Shimadzu, Kyoto, Japan).

Statistical analysis

Statistical analyses were performed with factorial ANOVA with the Tukey-Kramer multiple-comparison test. Data are expressed as means ± SD. The level of significance was defined as \( p < 0.05 \). Correlations were assessed by determination coefficient \( (R^2) \). All the statistical analyses were performed using Excel-Toukei 2015 (Social Survey Research Information Co., Ltd., Tokyo, Japan).

Results

Identification of hydrophilic antioxidants

The MWA extracts of Japanese parsley were subjected to reversed-phase HPLC, and the antioxidant capacities...
of the fractions were compared based on the net AUC values from H-ORAC measurement (Figure 1). A typical HPLC chromatogram of the MWA extracts is shown in Figure 1. Four active peaks (I–IV) were observed on the chromatogram, and peaks I and II occupied about 45% and 30% of the total net AUCs, respectively, suggesting that the antioxidant capacity of the hydrophilic extract of Japanese parsley was derived mainly from these fractions. LC/MS analyses revealed that the molecular weight of component No. 1 in peak I was 354. Compound 1 was identified as chlorogenic acid in reference to the retention time and mass spectrum of the authentic sample. Peak II contained four components (2–5), and all of these showed maximum absorption around 370 nm. Components No. 2 and 3 showed molecular ion peaks at m/z 609, while those of components No. 4 and 5 were at m/z 463. The retention time and mass spectrum of component No. 3 were identical to those of authentic rutin; thus, this component was identified as rutin. Components No. 4 and 5 were suggested to be quercetin mono-glycosides, because these components gave fragment ions at m/z 463 in their MS/MS spectra. These were identified as hyperoside and isoquercitrin, respectively, by comparing the retention times and MS spectra to authentic samples. MS/MS spectrum of the molecular ion peak of component No. 2 was similar to that of component No. 3, and in both spectra a fragment ion was observed at m/z 300.8, suggesting structural similarity. Del Rio et al. (2004) reported the existence of quercetin rhamnosyl-galactoside in tea, and noted that it eluted faster than rutin. Thus, component No. 2 was suggested to be quercetin rhamnosyl-galactoside.

Identification of lipophilic antioxidants

The lipophilic extracts of Japanese parsley were subjected to reversed-phase HPLC (Figure 2A and B), and the antioxidant capacities of the fractions were compared based on the net AUC values from L-ORAC measurement (Figure 2C). Only one active peak was observed on the chromatogram. Active component No. 6 (Figure 2A) possessed maximum absorption around 460 nm, showing the existence of a long conjugated system. Component No. 6 was identified as lutein by comparison of the retention time and absorption spectrum. The highest antioxidant capacity was observed in an earlier fraction than those containing γ- and α- tocopherols (components No. 7 and 8), which possessed potent fluorescence (Figure 2B).

Antioxidant capacities and concentrations of chlorogenic acid and quercetin glycosides in hydrophilic extracts of Japanese parsley

The H-ORAC values of Natori No. 5 were the highest in December, and the samples harvested in December and later showed significantly higher H-ORAC values than the samples harvested in October and November (Figure 3A). Hydrophilic extracts of Natori No. 6 harvested in December and February showed higher H-ORAC values than those harvested in the other months (Figure 3B). Both cultivars showed significant positive correlations between H-ORAC values and the contents of chlorogenic acid, rutin, hyperoside, isoquercitrin and quercetin-3-O-rhamnosyl-galactoside ($R^2 = 0.8070, 0.8830, 0.6228, 0.6134$ and $0.7702$, respectively, Figure 4A–E). Further, total quercetin glycosides showed a significant positive correlation with H-ORAC values ($R^2 = 0.7599$, Figure 4F).
Antioxidant capacities and contents of antioxidants in lipophilic extracts of Japanese parsley

Natori No. 5 showed the highest L-ORAC value in December, while Natori No. 6 showed the highest in October (Figure 5). Lutein contents of Natori No. 5 and No. 6 were positively correlated with the L-ORAC values ($R^2 = 0.4997$, Figure 6A). However, $\alpha$- and $\gamma$-tocopherol contents of Natori No. 5 and No. 6 did not show significant correlations with L-ORAC values ($R^2 = 0.0642, 0.1207$, Figure 6B and C).

Discussion

Chlorogenic acid, rutin, hyperoside, isoquercitrin and quercetin-3-O-rhamnosyl-galactoside were identified as hydrophilic antioxidants in Japanese parsley. In ORAC measurements, the antioxidant capacity of a test sample was calculated from its net AUC value, and is expressed as Trolox equivalent using a Trolox standard curve. The net AUC values of fractions containing these hydrophilic antioxidants occupied more than 75% of the net AUC values of all fractions (Figure 1). In addition, contents of the hydrophilic antioxidants in Japanese parsley showed positive correlations with H-ORAC values (Figure 4). These results suggest that the hydrophilic antioxidant capacity of Japanese parsley was derived mainly from chlorogenic acid and quercetin glycosides.

Fruits and vegetables contain several antioxidants. Accordingly, in contrast to single compounds, synergic effects may arise and modulate antioxidative activity; it has been reported that antioxidative activity depends on antioxidant contents (Vinson et al. 2001; Pinelo et al. 2004). The results of the present study show that
specific antioxidants in Japanese parsley show similar pattern H-ORAC and L-ORAC profiles. Therefore, H-ORAC and L-ORAC values of Natori No. 5 and Natori No. 6 cultivars depend on antioxidant contents.

H-ORAC values of both cultivars tended to be high in winter (December to February, Figure 3). Since H-ORAC values were positively correlated with the contents of hydrophilic antioxidants, higher H-ORAC values accompanied increasing amounts of the antioxidants. Polyphenols concentrations of garden plants were reported to vary according to species, cultivation environment and harvesting season (Imeh & Khokhar 2002; Thomas et al. 2008; Xie et al. 2014). In the case of Japanese parsley, the temperature of the harvesting season might greatly affect the contents of hydrophilic antioxidants. Typically, Japanese parsley is grown via open cultivation in Miyagi Prefecture (Ministry of Agriculture, Forestry and Fisheries 2012). According to the Japan Meteorological Agency database, the mean monthly temperatures of Natori City in December 2014, January 2015 and February 2015 were 2.6°C, 2.4°C and 2.7°C, respectively (Japan Meteorological Agency 2015), which were lower than those in October and November 2014 (14.7°C and 9.2°C, respectively). Thus, a low temperature may enhance the synthesis of polyphenols including chlorogenic acid and quercetin derivatives. It has been reported that the accumulation of anthocyanins in Umbelliferae increased at low temperatures (Hasegawa et al. 2001). In addition, Kirakosyan et al. (2004) reported that chlorogenic acid in the hawthorn plants increased under low temperature (4°C) cultivation compared to that at room temperature (25°C). These reports support the observed increase in hydrophilic antioxidants in

Figure 5. L-ORAC values of two cultivars of Japanese parsley harvested in different months. Two Japanese parsley cultivars (Natori No. 5 (A) and No. 6 (B)) were harvested in different months, and their L-ORAC values were analyzed \((n = 3, \text{mean} \pm \text{SD})\). Different letters denote statistical differences by factorial ANOVA with the Tukey-Kramer multiple-comparison test \((p < 0.05)\).

Figure 6. Correlations between L-ORAC values and contents of three antioxidants in the lipophilic extracts of Japanese parsley. Correlations between contents of lutein (A), \(\alpha\)-tocopherol (B) or \(\gamma\)-tocopherol (C), and L-ORAC values were analyzed.
Japanese parsley during winter (December to February, Supplemental Figure S1). The contents of hydrophilic antioxidants (chlorogenic acid, rutin, isoquercitrin and hyperoside) differed between Natori No. 5 and No. 6 cultivars (Supplemental Figure S1). Natori No. 5 contained the highest amounts of hydrophilic antioxidants in December. On the other hand, hydrophilic extracts of Natori No. 6 harvested in December and February contained more hydrophilic antioxidants than those harvested in October and November (Supplemental Figure S1).

Wu et al. (2004) reported that the ORAC values of fruits and vegetables for the hydrophilic fractions (H-ORAC) were much higher than those for lipophilic fractions (L-ORAC). However, unlike hydrophilic antioxidants, which do not accumulate in the body and are excreted in the urine, lipophilic antioxidants penetrate the lipoprotein cell membrane more easily and therefore reach a higher level of bioavailability. In this study, lutein was identified as a major lipophilic antioxidant in Japanese parsley. It has been widely recognized that tocopherols are typical lipophilic antioxidants, and detectable levels of α- and γ-tocopherols were observed in lipophilic extracts of Japanese parsley. However, the net AUC values of HPLC fractions containing tocopherols showed lower net AUC values than those containing lutein (Figure 2C). In addition, contents of tocopherols did not show a significant correlation with L-ORAC values. These results suggest that the contribution of tocopherols to the lipophilic antioxidant capacity of Japanese parsley was low. The usefulness of umbelliferous plants as lutein sources has been reported (Yoon et al. 2012). Takagi et al. (1990) showed that the lutein content in green leaf increased during the winter season, and was influenced by temperature as well as the duration and intensity of sun exposure. In plant photosynthesis, photoinhibition was accelerated by conditions of high salinity, excess light, drought, and high or low temperature. Further, an excess of active oxygen species enhances photoinhibition in plants (Allakhverdiev & Murata 2004; Takahashi & Murata 2008). On the other hand, photoinhibition by active oxygen species was inhibited by lipophilic antioxidants, such as tocopherol and carotenoids in chloroplasts (Asada 1999; Demmig-Adams & Adams 2002; Vickers et al. 2009). In this study, the lutein content in Natori No. 5 was the highest level in December 2014 (Supplemental Figure S2). The mean temperature of Natori City in December 2014 was 2.6°C (Japan Meteorological Agency 2015). Thus, the increased lutein content of Natori No. 5 in December might be attributable to low temperature-induced photoinhibition. On the other hand, the lutein content of Natori No. 6 was not significantly affected by temperature (Supplemental Figure S2). Differences in lutein contents between Natori No. 5 and No. 6 could also be caused by difference in their sensitivities to low temperatures.

Chu et al. (2012) reported that oral administration of crude caffeine (H-ORAC value: 145 ± 10 μmol TE/g, L-ORAC value: 66 ± 7 μmol TE/g) improved memory and decreased the amyloid-β concentration in Alzheimer’s disease mice model. In addition, Lucchesi et al. (2014) showed that grain cereal powder (ORAC value: 16.77 ± 0.84 μmol TE/g) activated the antioxidative associated pathway in human peripheral blood lymphocytes. Both of the cultivars of Japanese parsley cultivars showed ORAC values of 100 μmol TE/g: accordingly, Natori No. 5 and No. 6 are anticipated to exert possible health-promoting effects in vivo.

Conclusions

Two cultivars of Japanese parsley were harvested in different seasons, and their antioxidant capacities and contents of antioxidants were compared. Chlorogenic acid and four kinds of quercetin glycosides were identified as major hydrophilic antioxidants of Japanese parsley. Lutein was mainly contributed to the antioxidant capacity of the lipophilic extract. Antioxidant capacities of the hydrophilic extracts of both cultivars tended to be higher in winter because of the increase in the contents of chlorogenic acid and quercetin glycosides.

Disclosure statement

The authors report no conflicts of interests. The authors alone are responsible for the content and writing of the manuscript.

Funding information

This work was supported by a grant-in-aid “A Scheme to Revitalize Agriculture and Fisheries in Disaster Area through Deploying Highly Advanced Technology” from the Ministry of Agriculture, Forestry and Fisheries of Japan, by JSPS KAKENHI Grant Number 26282200, and by Kirin Holdings Co., Ltd., Tokyo, during UNU-Kirin Fellowship Programme of RV Manaos at NFRI in 2014-15.

References

Aizawa K, Inakuma T. 2007. Quantitation of carotenoids in consumed vegetables in Japan. Food Sci Tech Res. 21:473–477.

Allakhverdiev SI, Murata N. 2004. Environmental stress inhibits the synthesis de novo of proteins involved in the photodamage-repair cycle of Photosystem II in...
Asada K. 1999. The water-water cycle in chloroplasts: scavenging of active oxygens and dissipation of excess photons. Annu Rev Plant Physiol Plant Mol Biol. 50:601–639.

Boggs DA, Palmer JR, Wise LA, Spiegelman D, Stampfer MJ, Adams-Campbell LL, Rosenberg L. 2010. Fruit and vegetable intake in relation to risk of breast cancer in the Black Women’s Health Study. Am J Epidemiol. 172:1268–1279.

Cao G, Alessio HM, Cutler RG. 1993. Oxygen-radical absorbance capacity assay for antioxidants. Free Radic Biol Med. 14:1–12.

Chu YF, Chang WH, Black RM, Liu JR, Sompol P, Chen Y, Cheng IH. 2012. Crude caffeine reduces memory impairment and amyloid β42 levels in an Alzheimer’s mouse model. Food Chem. 135:2095–2102.

Del Rio D, Stewart AJ, Mullen W, Burns J, Lean ME, Brigent F, Crozier A. 2004. HPLC-M5n analysis of phenolic compounds and purine alkaloids in green and black tea. J Agric Food Chem. 52:2807–2815.

Dennig-Adams B, Adams WW. 2002. Antioxidants in photosynthesis and human nutrition. Science. 298:2149–2153.

Hasegawa H, Fukasawa-Akada T, Okunoz T, Niizeki M, Suzuki M. 2001. Anthocyanin accumulation and related gene expression in Japanese parsley (Oenanthe stolonifera, DC.) induced by low temperature. J Plant Physiol. 78:71–78.

Huang D, Ou B, Hampsch-Woodill M, Flanagan JA, Deemer JA. 2001. Development and validation of oxygen radical absorbance capacity assay for lipophilic antioxidants using randomly methylated beta-cyclodextrin as the solubility enhancer. J Agric Food Chem. 50:1815–1821.

Jeong YH. 2009. The effects of Oenanthe javanica extracts on hepatic fat accumulation and plasma biochemical profiles in a nonalcoholic fatty liver disease model. J Korean Soc Appl Biol Chem. 52:632–637.

Kameya H, Watanabe J, Takano-Ishikawa Y, Todoriki S. 2014. Comparison of scavenging capacities of vegetables by ORAC and EPR. Food Chem. 145:866–873.

Khanam USK, Oba S, Yanase E, Murakami Y. 2012. Phenolic acids, flavonoids and total antioxidant capacity of selected leafy vegetables. J Funct Foods. 4:979–987.

Kirakosyan A, Kaufman P, Warber S, Zick S, Aaronson K, Bolling S, Chang SC. 2004. Applied environmental stresses to enhance the levels of polyphenolics in leaves of hawthorn plants. Physiol Plant. 121:182–186.

Lee CH, Park JH, Cho JH, Kim IH, Ahn JH, Won MH. 2015. Effect of Oenanthe javanica extract on antioxidant enzyme in the rat liver. Chin Med J. 128:1649–1654.

Lin RH. 2013. Health-promoting components of fruits and vegetables in the diet. Adv Nutr. 4:384S–392S.

Lucchesi D, Russo R, Gabriele M, Longo V, Del Prato S, Penno G, Pucci L. 2014. Grain and bean lysates improve function of endothelial progenitor cells from human peripheral blood: involvement of the endogenous antioxidant defenses. PLoS One. 9:e109298.

Ministry of Agriculture, Forestry and Fisheries. 2005. Food Composition Database, based on Standard Tables of Food Composition in Japan, fifth Revised and Enlarged Edition. Available from: http://fooddb.mext.go.jp [Accessed on 17 March 2016].

Ministry of Agriculture, Forestry and Fisheries. 2012. Regional specialty vegetables production status study. Available from: http://www.maff.go.jp/j/tokei/kouhyou/tokusun_yasai/ [Accessed on 2 December 2015].

Noguer M, Cerezo AB, Moyá ML, Troncoso AM, García-Parrilla MC. 2014. Synergism effect between phenolic metabolites and endogenous antioxidants in terms of antioxidant activity. Adv Chem Eng Sci. 4:258–265.

Phaniendra A, Jestadi DB, Periyasamy L. 2015. Free radicals: properties, sources, targets, and their implication in various diseases. Indian J Clin Biochem. 30:11–26.

P fino M, Manzocco L, Nuñez MJ, Nicola MC. 2004. Interaction among phenols in food fortification: negative synergism on antioxidant capacity. J Agric Food Chem. 52:1177–1180.

Takagi S, Kishi F, Nakajima K, Kimura Y, Nakano M. 1990. A seasonal variation of carotenoid composition in green leaves and effect of environmental factors on it. Scientific Reports of the Faculty of Agriculture, Okayama University 75:1–7.

Takahashi S, Murata N. 2008. How do environmental stresses accelerate photoinhibition? Trends Plant Sci. 13:178–182.

Thomas AL, Chen YC, Rottinghaus GE, Malone AM, Byers PL, Applequist WL, Finn CE. 2008. Occurrence of rutin and chlorogenic acid in elderberry leaf, flower, and stem in response to genotype, environment, and season. Acta Hort. 765:197–206.

Vickers CE, Gershenson J, Lerdau MT, Loreto F. 2009. A unified mechanism of action for volatile isoprenoids in plant abiotic stress. Nat Chem Biol. 5:283–291.

Vieira AR, Abar L, Vingelie S, Chan DS, Aune D, Navarro-Rosenblatt D, Stevens C, Greenwood D, Norat T. 2015. Fruits, vegetables and lung cancer risk: a systematic review and meta-analysis. Ann Oncol. 27:81–96.

Vinson JA, Su X, Zubik L, Bose P. 2001. Phenol antioxidant quantity and quality in foods: fruits. J Agric Food Chem. 49:5315–5321.

Wang WN, Yang XB, Liu HZ, Huang ZM, Wu GX. 2005. Effect of Oenanthe javanica flavone on human and duck hepatitis B virus infection. Acta Pharmacol Sin. 26:587–592.

Wang X, Ouyang Y, Liu J, Zhu M, Zhao G, Bao W, Hu FB. 2014. Fruit and vegetable consumption and mortality from all causes, cardiovascular disease, and cancer: systematic review and dose-response meta-analysis of prospective cohort studies. BMJ. 349:g4490.
Watanabe J, Oki T, Takebayashi J, Yamasaki K, Takano-Ishikawa Y, Hino A, Yasui A. 2012. Method validation by interlaboratory studies of improved hydrophilic oxygen radical absorbance capacity methods for the determination of antioxidant capacities of antioxidant solutions and food extracts. Anal Sci. 28:159–165.

Watanabe J, Oki T, Takebayashi J, Takano-Ishikawa Y. 2014a. Extraction efficiency of hydrophilic and lipophilic antioxidants from lyophilized foods using pressurized liquid extraction and manual extraction. J Food Sci. 79:C1665–C1671.

Watanabe J, Oki T, Takebayashi J, Yamasaki K, Takano-Ishikawa Y, Hino A, Yasui A. 2014b. Improvement of the lipophilic-oxygen radical absorbance capacity (L-ORAC) method and single-laboratory validation. Biosci Biotechnol Biochem. 77:857–859.

Wu X, Beecher GR, Holden JM, Haytowitz DB, Gebhardt SE, Prior RL. 2004. Lipophilic and hydrophilic antioxidant capacities of common foods in the United States. J Agric Food Chem. 52:4026–4037.

Xie Y, Zheng Y, Dai X, Wang Q, Cao J, Xiao J. 2014. Seasonal dynamics of total flavonoid contents and antioxidant activity of Dryopteris erythrosora. Food Chem. 186:113–118.

Yang JH, Kim SC, Shin BY, Jin SH, Jo MJ, Jegal KH, Kim YW, Lee JR, Ku SK, Cho IJ, et al. 2013. O-Methylated flavonol isorhamnetin prevents acute inflammation through blocking of NF-κB activation. Food Chem Toxicol. 59:362–372.

Yoon GA, Yeum KJ, Cho YS, Chen CY, Tang G, Blumberg JB, Russell RM, Yoon S, Lee-Kim YC. 2012. Carotenoids and total phenolic contents in plant foods commonly consumed in Korea. Nutr Res Pract. 6:481–490.