Antioxidant Capacity and Polyphenol Content of Extracts from Crops Cultivated in Japan, and the Effect of Cultivation Environment

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Received October 29, 2011; Accepted October 14, 2012

Oxygen radical absorbance capacity (ORAC) value and polyphenol contents of extracts from 71 crops cultivated in Japan were determined. Hydrophilic ORAC values (H-ORAC) ranged from 194 − 29,830 μmol TE/100 g fresh weight (FW), whereas lipophilic ORAC values (L-ORAC) ranged from 6 − 2,076 μmol TE/100 g FW. High total ORAC value was indicated in the following vegetables and fruits: mulukhiya (\textit{Corchorus olitorius}), perilla (\textit{Perilla frutescens}), water spinach (\textit{Ipomoea aquatica}), edible burdock (\textit{Arctium lappa}), blueberries (\textit{Vaccinium corymbosum}) and hardy kiwifruit (\textit{Actinidia arguta}) (6,562 − 31,802 μmol TE/100 g FW). These extracts showed a strong positive correlation between ORAC value and polyphenol content (\(r = 0.98\)). In addition, to gain a better understanding of ORAC variability in Japanese crops, we also discussed the influence of environmental factors on the ORAC value of stem mustard (\textit{Brassica juncea}) “Umino” grown in three different regions. Regional variations of ORAC in stem mustard likely result from different levels of ultraviolet radiation and fertilizer application regimens specific to those regions.

Keywords: ORAC, polyphenol content, stem mustard (\textit{Brassica juncea}) “Umino”, environmental effects

Introduction

There is increasing evidence that oxidative stress is associated with the development of many chronic and degenerative diseases, as well as the aging process (Awika et al., 2003; Bacchiocca et al., 2006; Cho et al., 2005; Miller et al., 2008; Samaniego Sánchez et al., 2007). Although the human body has the ability to eliminate free radicals, such ability is commonly insufficient (Seeram et al., 2008). Therefore, dietary antioxidants, such as in fruits and vegetables, can help to decrease the adverse effects of reactive oxygen species in humans. With the aging of Japanese society, food, especially fruits and vegetables, is increasingly viewed as a means of disease prevention and good health maintenance. According to studies of food consumption performed by the Ministry of Agriculture, Forestry, and Fishery in 2004 (i), many Japanese consumers are seeking information about the effects of agricultural products on their health. Therefore, the government has established several programs, “local production for local consumption” and “information which is useful for food selection and food safety” (ii), aimed at improving the health of the Japanese through promoting the production of high-quality foods by local farmers. These programs also contribute to the economic development of local agriculture in Japan by revitalizing various high-quality agricultural products.

Determination of the antioxidant capacity of locally produced food is important for assessing its ability to induce effects on people’s health. The ORAC assay is a commonly adopted and relatively standardized method to determine antioxidant capacity in foods (Cao et al., 1993; Huang et al., 2002; Prior et al., 2003; Wu et al., 2004a). The ORAC method measures scavenging capacity against the peroxyl radical, which is the predominant free radical involved in lipid oxi-
vation in foods and biological systems (Mikami et al., 2009; Takebayashi et al., 2010; Wu et al., 2004b). In 2010, the USDA released the second database of the antioxidant capacity of 326 selected foods determined using ORAC methodology (iii). Furthermore, drinks and supplements marketed in the USA are labeled with the ORAC value. However, in Japan, various antioxidant assay methods have been used for a prolonged period. Therefore, unification and standardization of the antioxidant assay is needed and is currently being developed (Watanabe et al., 2010). Several studies on ORAC values of local farm produce have recently been performed (Kitazume et al., 2011; Oki et al., 2009; Sato et al., 2010). The term “Functional foods” is an established food products category in Japan (Arai, 1996), and has strong consumer acceptance (iv). In addition, a program to ensure the quality of local health-related products is currently being carried out in Japan (Tsushida, 2009). However, ORAC values for most Japanese farm products have not yet been determined. Therefore, constructing a database of various food products, each labeled with its corresponding ORAC value, will be required.

The objective of this study is to further explore the ORAC values of various crops produced in Japan. We examined 71 samples of locally grown agricultural products in Ibaraki prefecture in Japan. In addition we investigated the role of various factors, such as growing conditions, which affect the antioxidant capacity of the food, in order to gain a better understanding of ORAC variability. In particular, we focused on the influence of environmental factors, ultraviolet radiation, and fertilizer application, on ORAC values. For this study, we employed stem mustard (Brassica juncea), which has a comparably high ORAC value, and was grown in three different regions.

Materials and Methods

Plant materials Seventy samples of different crops were purchased from retail stores in Ibaraki prefecture, Japan in 2006-2011 (Table 1). All samples (except for samples No. 60, 61, 65 in Table 1) were separated into three 50 – 100 g units of edible parts, lyophilized, then powdered using a mill and stored at −30°C until used. Rice (No. 60 and 61) and Kinako (No. 65, roasted and ground green soybeans) were kept at −30°C in their original form until analysis.

As shown in Fig. 1, stem mustard (Brassica juncea) “Umino” was cultivated by six farmers in three prefectures: Ibaraki [36° 5’ N, 140° 6’ E], Tsukuba region, two farmers (A-1, 2), open field plot, direct-seeded], Yamagata [37° 58’ N, 140° 10’ E], Takahata region, one farmer (B), tunnel plot, planted 2 – 5 week transplants] and Kanagawa [35° 9’ N, 139° 39’ E], Miura region, three farmers (C-1, 2, 3), open field plot, direct-seeded, and were harvested in 2009 – 2011. Each farm’s growing period was as follows: (A) Ibaraki; 24 Sep. 2009 – 14 Dec. 2009, 17 Sep. 2010 – 5 Jan. 2011, (B) Yamagata; 12 Aug. 2009 – 12 Dec. 2009, 12 Sep. 2010 – 24 Jan. 2011, (C) Kanagawa; 5 Oct. 2009 – 10 Feb. 2010, 12 Sep. 2010 – 24 Jan. 2011. The basal (I) and additional (II) fertilizer used in this study were as follows: Tsukuba farm [(I) (II)]; Silver Almighty (composted animal manure; 8-10-10, N-P-K, Fukuei Hiryō Co., Hyogo, Japan)], Takahata farm [(I); Sun-ace (12-8-10, N-P-K, Taiyo fertilizer Co., Ibaraki, Japan), (II); Shikishima-6 (6-8.5-6, N-P-K, Taki chemical Co., Hyogo, Japan)], Miura farm [(I) (II); Ainakasei (14-16-10, N-P-K, Katakuragawa Chikkarin Co., Tokyo, Japan)]. And at Tsukuba farm, (I) and (II) fertilizer were applied at 80 and 48 kg N ha⁻¹, respectively. At Takahata farm, (I) and (II) were applied at 240 and 120 kg N ha⁻¹, respectively. At Miura farm, (I) and (II) were applied at 42 and 112 kg N ha⁻¹, respectively. After harvesting, 50 – 100 g of bulk sample from of the three different plants was used for lyophilization. Each part of the plants was separately lyophilized (leaf, petiole, stem, flower bud, and leaf of flower bud), powdered using a mill, and stored at −30°C until used.

Reagents 6-Hydroxy-2,5,7,8-tetramethyl chroman-2-carboxylic acid (Trolox), fluorescein (sodium salt) (FL), and gallic acid were purchased from Sigma-Aldrich (St. Louis, MO). Methyl-β-cyclodextrin (MCD) was obtained from Junsei Chemical Co Ltd (Tokyo, Japan). Folin-Ciocalteu’s phenol reagent was purchased from MP Biomedicals, LLC (Illkirch, France). 2,2’-Azobis (2-amidinopropane) dihydrochloride (AAP, extra pure reagent), sodium carbonate and other reagents were purchased from Wako Pure Chemical Industries (Osaka, Japan). Except for AAPH, special grade chemicals were used in this experiment.

Fig. 1. Distribution of the three regions where stem mustard “Umino” was cultivated in this study. Takahata city is about 30 km away from Yamagata city. Miura city in Kanagawa prefecture is about 30 km away from Yokohama city.
Sample preparation Approximately 0.5 or 1 g of freeze-dried sample, milled rice (No. 60, 61) and Kinako (No. 65) were applied to the extraction by hexane/dichloromethane (1:1) using an extraction system (ASE200, Dionex Co., Osaka, Japan) as described by Wu et al. (2004b) and Oki et al. (2008). Then, the hexane/dichloromethane was evaporated using nitrogen gas and the remaining sample was dissolved in 2 to 4 mL of acetone. This solution was then used for the lipophilic ORAC (L-ORAC) assay after being appropriately diluted with 7% MCD solvent (w/v) in a 50% acetone-water mixture (v/v) (Huang et al., 2002). Methanol/water/acetetic acid (90:9.5:0.5; MWA) extracts were obtained from the remaining residue after obtaining hexane/dichloromethane (1:1) extracts using ASE200 (Kitazume et al., 2011). The volume of the MWA extracts was fixed at 25 mL and used for hydrophilic ORAC (H-ORAC) assay. For the Folin-Ciocalteu assay, hexane/dichloromethane (1:1) extracts and MWA extracts prepared by ASE200 were used (Kitazume et al., 2011). Hexane and dichloromethane solvent was substituted with dimethyl sulfoxide (DMSO) by evaporation with nitrogen gas before the assay (Suda et al., 2005).

ORAC assay The ORAC value of the extracts was evaluated according to the method of Oki et al. (2008) and Huang et al. (2002) with slight modifications. In summary, each 35 μL of diluted sample or Trolox standard was mixed with 115 μL of fluorescein solution [(110.7 mM/assay buffer (75 mM KH2PO4−K2HPO4 at pH 7.4)] in a clear, 96-well microplate (Falcon #353072) and incubated at 37°C for 10 min. Next, a reading was taken using a plate reader (Powerscan HT, DS Pharma Biomedical Co., Osaka, Japan) before the addition of AAPH solution. Then, 50 μL of AAPH solution (31.7 mM/assay buffer) was rapidly added to each well using a multi-channel pipette. Immediately following the addition of AAPH, the plate was agitated for 5 s prior to the first reading and for 3 s before each subsequent reading. Readings were done at 2 min intervals for 90 min for H-ORAC assay, and for 120 min for L-ORAC assay. Excitation and emission filter wavelengths were set at 485 nm and 528 nm, respectively. Data were expressed as μmol Trolox equivalents (TE) per 100 g of fresh weight (FW). Instead of using AAPH solution (31.7 mM/assay buffer) for H-ORAC assay, AAPH solution (63.4 mM/assay buffer) was used for L-ORAC assay. Total ORAC value was calculated by combining L-ORAC and H-ORAC values.

Total phenolics (TP) analysis The TP analysis was based on the Folin-Ciocalteau method (Sun et al., 2005), slightly modified by Kitazume et al. (2011) and Tsuruta et al. (2007). Concentrated Folin-Ciocalteau reagent was diluted two-fold with water. Water (60 μL) and 10 μL of sample extracts were mixed in a clear, 96-well microplate. Next, 15 μL of diluted Folin-Ciocalteau reagent was mixed and incubated for 5 min at room temperature and 75 μL of 2% sodium carbonate solution was added. After incubation for 15 min at room temperature, absorbance of the solution was determined at 750 nm using a plate reader (TERMO max, Molecular Devices, Sunny vale, CA). Gallic acid was used to prepare the standard curve. Results were expressed as mg gallic acid equivalents (GAE) per 100 g of FW. TP value was calculated by combining polyphenol content from hexane/dichloromethane (1:1) extracts (LTP) and MWA extracts (HTP).

Results and Discussion ORAC and TP values of selected crops in Japan As shown in Table 1, the extracts from 70 specimens of crops cultivated in Ibaraki prefecture in Japan were prepared by sequential extraction with hexane/dichloromethane (1:1) and MWA using freeze-dried samples, and their ORAC value and TP content were measured. Total ORAC value was calculated by combining L-ORAC and H-ORAC values. TP value was calculated by combining polyphenol content from hexane/dichloromethane (1:1) extracts (LTP) and MWA extracts (HTP).

H-ORAC values ranged from 194 to 29,830 μmol TE/100 g FW, whereas L-ORAC values ranged from 6 to 2,076 μmol TE/100 g FW. TP values varied in the range of 7 to 951 mg GAE/100 g FW. High total ORAC value was mainly indicated in leaf and stem vegetables: mulukhiya (No. 40 in Table 1, Corchorus olitorius, 10,168 μmol TE/100g FW), water spinach (No. 49, Ipomoea aquatica, 5,373 ± 1,474 μmol TE/100 g FW), purple cabbage (No. 34, Perilla frutescens, 28,879 ± 2,287 μmol TE/100 g FW), water spinach (No. 49, Ipomoea aquatica, 5,373 ± 1,474 μmol TE/100 g FW), water spinach (No. 49, Ipomoea aquatica, 5,373 ± 1,474 μmol TE/100 g FW), purple cabbages (No. 34, Brassica oleracea, 3,470 μmol TE/100 g FW), saltwort (No. 47, Salsola komarowii, 4,586 μmol TE/100 g FW), and radish leaf (No. 56, Raphanus sativus, 3,632 ± 1,142 μmol TE/100 g FW). High total ORAC values were also indicated in the following: edible burdock (No. 51, Arctium lappa, 6,747 μmol TE/100 g FW) in the root crop, blueberry (No. 1, Vaccinium corymbosum, 6,562 μmol TE/100 g FW) and hardy kiwi-fruit (No. 3, Actinidia arguta, 9,476 μmol TE/100 g FW) in the fruit, small hot pepper (No. 15, Capsicum annum, 3,848 μmol TE/100 g FW) in the fruit vegetable, uguisukinako (No. 65, roasted and ground green beans, Pisum sativum, 9,677 μmol TE/100 g FW) in pulse (Table 1). A rather high L-ORAC value was found in the following: edible burdock (No. 51, Arctium lappa, 6,747 μmol TE/100 g FW). High total ORAC values were also indicated in the following: edible burdock (No. 51, Arctium lappa, 6,747 μmol TE/100 g FW) in the root crop, blueberry (No. 1, Vaccinium corymbosum, 6,562 μmol TE/100 g FW) and hardy kiwi-fruit (No. 3, Actinidia arguta, 9,476 μmol TE/100 g FW) in the fruit, small hot pepper (No. 15, Capsicum annum, 3,848 μmol TE/100 g FW) in the fruit vegetable, uguisukinako (No. 65, roasted and ground green beans, Pisum sativum, 9,677 μmol TE/100 g FW) in pulse (Table 1). A rather high L-ORAC value was found in the following: mulukhiya (No. 40, Corchorus olitorius, 817 μmol TE/100 g FW), perilla (No. 43 − 45, Perilla frutescens, 1,537 ± 318 μmol TE/100 g FW), ginger (No. 52, 53, Zingiber officinale, 1,787 ± 289 μmol TE/100 g FW) and uguisukinako (No. 65, roasted and ground green beans, Pisum sativum, 1,094 μmol TE/100g FW). H-ORAC values were much higher than...
L-ORAC values, with the exception of ginger.

The USDA database containing antioxidant capacities of 326 selected foods measured using ORAC methodology (iii) demonstrates high ORAC values in herb, berry and cereal grain. However, in this study, leafy vegetables (such as mulukhiya and perilla), edible burdock and hardy kiwi showed higher ORAC values.

The term “functional foods” indicates the “tertiary” function of foods, and differs from the conventional “primary” and “secondary” functions that are related to nutrition and preference, respectively (Arai, 1996). Food has various functions, and consumption of each agricultural product differs in terms of its effects on the human body. The products from the six farms examined constitute a large part of agricultural output of Ibaraki prefecture (v), and present a good source of calories, dietary fiber or minerals. However, their ORAC value per gram is at best moderate: brown rice (No. 60, 61, *Oryza sativa*, 1,592 ± 157 μmol TE/100 g FW), sweet potato (No. 64, *Ipomoea batatas*, 872 μmol TE/100 g FW), melon (No. 16 – 21, *Cucumis melo*, 285 ± 40 μmol TE/100 g FW), lettuce (No. 39, *Lactuca sativa*, 491 μmol TE/100 g FW), tomato (No. 29, *Lycopersicum esculentum*, 418 μmol TE/100 g FW), and welsh onion (No. 32, *Allium fistulosum*, 561 ± 138 μmol TE/100 g FW). Although examination of food intake is not being carried out at this time, a balanced intake of various agricultural products is important.

Figure 2 shows the correlation between ORAC value and polyphenol content of the crop extracts assessed in this study. The results demonstrate that the ORAC value of these extracts has a strong positive correlation with polyphenol content, suggesting that antioxidant capacity of these crops appear to result mainly from the polyphenol content.

In this study, we performed multiple measurements of ORAC level in samples of the same products purchased from different farmers: water spinach (No. 49 – 1 and 2: 6,847 and 3,898 μmol TE/100 g FW) and radish (No. 56 – 1 and 2: root; 2,175 and 2,511, leaf; 4,794 and 2,470 (μmol TE/100 g FW)). These variations in ORAC value may be explained by variations in the strains, growth stages or the environment.

Genotype is the principal factor contributing to variations in antioxidant capacity of fruits and vegetables (Howard et al., 2002; Pandjaitan et al., 2005; Reyes-Carmona et al., 2005). However, the cultivation environment is also an important factor that determines variability of antioxidant capacity (Zhao et al., 2007; Piergiovanni et al., 2010). Antioxidant capacity of fruits and vegetables grown organically versus that of conventionally grown produce has also been the subject of multiple studies, due to an increased demand for information on organic produce (Mitchell et al., 2007; Wang et al., 2008; Zhao et al., 2007). Zhao et al. (2007) compared ORAC values of leafy vegetables at different development stages, grown in different environments and with different fertilization regimes. They indicated that differences in cultivation practices result in significant changes in the antioxidant properties of each plant.

For a better understanding of ORAC variability, we examined several factors that may affect ORAC values.

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**Fig. 2.** Correlation between ORAC value and poly-phenol content of crop extracts from Ibaraki prefecture.

ORAC value and total polyphenol content of crop extracts correspond to Table 1. A; MWA (methanol/water/acetic acid (90:9.5:0.5) extracts, B; hexane/dichloro-methane (1:1) extracts, C; total value of two solutions (A and B extracts).
**ORAC value of stem mustard "Umino" extract**

The ORAC value of stem mustard leaf (5,475 ± 2,055 μmol TE/100 g FW) was almost the same as that of water spinach (No. 49). Water spinach had the third highest ORAC value of the examined vegetables (Table 1) after perilla (No. 43−45) and mulukhiya (No. 40). A part of expanded stem of stem mustard is utilized as Zha cai; however, stem mustard leaf is currently an underutilized resource, at least in Japan. However, by measuring ORAC values of each part of stem mustard, stem mustard leaf was found to have a high ORAC value and can be considered a good-quality material. For these reasons, here we used each part of the stem mustard, cultivated in three different regions, for the following evaluation of ORAC variation.

Stem mustard (Brassica juncea (Linn.) Czern. et Coss. var. tumida Tsen et Lee) “Umino” is a stable cold resistant inbred strain bred by Nakanishi and Murakami. It was registered in 2007 (vi) and is one of the special local crops in Ibaraki prefecture. However, there are no reports about its antioxidative capacity. This strain has an expanded stem, wide leaf and thick petiole. Its expanded stem is ideal for lightly pickled vegetable, which is well known as Zha cai. It has been cultivated only in Ibaraki prefecture, but was recently adapted to grow in three different regions (Ibaraki, Yamagata and Kanagawa prefectures). Therefore, interest in the evaluation of the strain against various cultivation conditions has increased.

First, we compared ORAC values of each part of the stem mustard plant [leaf (L), petiole (P), stem (S), flower bud (F) and leaf of flower bud (LF)]. As shown in Fig. 3, remarkable differences in ORAC value were observed in different parts of the stem mustard in all three prefectures; ORAC value of the leaf was 7−12, 10−30 times higher than that of stem and petiole, respectively. In addition, ORAC value showed a strong positive correlation with polyphenol content (Fig. 4), suggesting that antioxidative capacity of the stem mustard results mainly from the polyphenol content.

Second, we focused on the “growth stage”, and compared ORAC values of stem mustard extracts (Fig. 3). As a result, ORAC value of the leaf harvested at the “flower bud (LF) differentiation stage” tended to be higher than that of “vegetative growth (L) stage”. This suggests that variations in the phenolic level and antioxidant capacity of stem mustard are dependent on its growth stage.

With respect to “growth stage”, Pandjaitan et al. (2005) and Zhao et al. (2007) showed that the influence of maturation on hydrophilic and total antioxidant capacities was dependent upon the vegetable type and production environment. For example, significant elevated values at the mature head stage were only observed in pac choi (Brassica rapa) from high tunnel fields and spinach from open fields. But in other types of vegetables (Red leaf lettuce and Romaine let-
tuce), production environment did not affect the difference in ORAC value according to harvest stage (Zhao et al., 2007). At the same time, mid-maturity spinach (Spinacia oleracea) leaves had higher levels of total phenolics, total flavonoids, and antioxidant capacity than immature and mature leaves (Pandjaitan et al., 2005). In this case, spinach genotypes should be harvested at the mid-maturity stage for consumers to benefit from the elevated levels of health-promoting flavonoids present in the leaves.

Third, we examined differences in cultivation areas and compared ORAC values of the stem mustard extracts from three prefectures. As a result, variability of ORAC value was observed (Fig. 3); ORAC value of leaf, petiole and stem in Tsukuba (Ibaraki) was two times, 1.5 – 3.1 times, 1.7 – 3 times higher than those of other regions, respectively, in season I (2009 – 2010). Although differences in ORAC value among season II (2010 – 2011) samples were smaller than those of season I, the tendency for high ORAC values in samples grown in Ibaraki was observed.

Effects of cultivation environment on ORAC of stem mustard “Umino” As shown in Figs. 3 and 4, the ORAC and TP values of the stem mustard extracts from Ibaraki were higher than those of the other two regions. Therefore, we examined effects of cultivation environment by comparing the following data of the three regions: UV index, day length, vegetative periods, rainfall, temperature and fertilizer (Figs. 5 and 6). We also investigated several hypotheses to elucidate the factors that affect ORAC values in stem mustard in these regions. As a result, we paid special attention to UV radiation (Fig. 5) and fertilizer (Fig. 6) as the main cultivation factors affecting ORAC value of stem mustard in these regions.

In plants, the biosynthesis of flavonoids (polyphenolic compounds) produced through the phenylpropanoid pathway is known to be activated by environmental stresses, such as nutrient deficiency, wounding, pathogens, and UV radiation (Dixon and Paiva, 1995; Mitchell et al., 2007). Connor et al. (2002) showed that differences in antioxidant capacity may reflect differences in cultivation practices among locations, including differences in water stress, mineral nutrient availability, and UV radiation.

![Fig. 5. Graphic representation of UV index, day length (I), rainfall, maximum and minimum temperatures (II) during cultivation of stem mustard “Umino” in 2009 – 2010 (Left of I and II), and 2010 – 2011 (Right of I and II).](image-url)
UV index (vii) is an international standard measurement of the strength of the UV radiation from the sun, and is categorized into five exposure levels: minimal (1 − 2), low (3 − 4), moderate (5 − 6), high (7 − 9), and very high (10 +). As shown in Fig. 5-I, when we take account of each growing period [Tsukuba (Ibaraki); 24 Sep. 2009 − 14 Dec. 2009, 17 Sep. 2010 − 5 Jan. 2011, Takahata (Yamagata); 12 Aug. 2009 − 12 Dec. 2009, 12 Sep. 2010 − 24 Jan. 2011, Miura (Kanagawa); 5 Oct. 2009 − 10 Feb. 2010, 12 Sep. 2010 − 24 Jan. 2011], the effect of the UV radiation in Ibaraki was the strongest among the three regions. The averages of monthly UV index during each growing period (I) 2009-2010 and (II) 2010-2011 in the three regions were as follows: Ibaraki; (I) 8.25, (II) 8.76, Kanagawa; (I) 7.02, (II) 6.60, Yamagata; (I) 7.15, (II) 5.88. The plants in Yamagata were grown in a tunnel plot, which may reduce the amount of UV radiation and sunshine compared to plants grown in the open field. The day length in Ibaraki was 9 − 10% shorter than that of Kanagawa; however, the effect of UV radiation on ORAC value was unclear.

Solar UV-B radiation elicits a variety of acclimation responses, which typically include increased activity of antioxidant enzymes, increased DNA repair capacity, and accumulation of phenolic compounds that serve as “sunscreens” or UV filters (Caldwell et al., 2007). Exposure to increased levels of UV radiation during cultivation caused the leaves of red leaf lettuce to redden and an increase in ORAC values and concentrations of total phenols and main flavonoids, such as quercetin and cyanidin glycosides, as well as luteolin conjugates and phenolic acids (García-Macías et al., 2007). Reyes-Carmona et al. (2005) compared values of ORAC, ferric reducing antioxidant power (FRAP), total phenolic and anthocyanin contents among a range of blackberry genotypes produced in different climatic regions, and showed that wild blackberry exhibited the highest values. They concluded that high concentrations of protective polyphenolic compounds had resulted from greater exposure of the unsheltered wild plants to extreme conditions. However, Josuttis et al. (2010) reported that effects of UV radiation differed depending upon the types of phenolics; the contents of anthocyanin (cyanidin 3-glucoside) and flavonols (quercetin 3-glucuronide and kaempferol 3-glucoside) were decreased in the fruits grown under UV blocking film compared to open-field grown strawberries. At the same time, other phenolics were not consistently affected by UV radiation. Therefore, in future, further analysis using high-performance liquid chromatography (HPLC) is required to clarify not only the contributors to stem mustard antioxidant capacity, but also to explain ORAC variations among plants grown in different regions.

Another stress factor is temperature regime. It is known that stem mustard is ruined by frost. Therefore, Kanagawa, where temperatures do not fall below the freezing point, is considered to be favorable for its cultivation (Fig. 5-II). In Ibaraki, temperatures drop to minus five degrees or less during late January and plants are normally harvested before that period. Temperatures in Yamagata did not greatly affect the plants, since they were grown in tunnel plots. According to the literature (Josuttis et al., 2010; Wang and Zheng, 2001), the content of kaempferol (3-glucoside- malonate) could be sensitive to temperature and UV, similar to the UV sensitivity of anthocyanin levels in strawberries. Therefore, significant differences in temperature regime may affect stem mustard antioxidant capacity. Precipitation does not affect stem mustard strongly, as it is a winter crop.
Figure 6 displays the amount of fertilizer used at each farm. Ibaraki and Yamagata farmers relied on chemical fertilizer containing organic manure while purely chemical fertilizers were used at the Kanagawa farm. Depending on the farm, the amounts and the sources of the basal and additional fertilizers differed greatly. As previously reported, the increase in total phenolic content seems to be stimulated by low nitrogen supply; the amount of nitrogen fertilizer used in Ibaraki was the lowest among the three farms. The amount of nitrogen fertilizer used in Kanagawa (154 kg N ha\(^{-1}\)) and Yamagata (360 kg N ha\(^{-1}\)) farms was 1.2 and 2.8 times larger than in Ibaraki (128 kg N ha\(^{-1}\)), respectively.

Dixon and Paiva (1995) attributed the accumulation of phenolic compounds to nitrogen deficiency. They showed that low nitrogen activates flavonoid and isoflavonoid nod gene inducers and chemoattractants for nitrogen-fixing symbionts in soybean and white lupin (Lupinus albus) plants. Elevation of total phenolic content stimulated by low nitrogen supply was also reported in other plants and apple leaves (Lester and Treutter, 2005). Norbaek et al. (2003) applied 0, 50, 100 and 150 kg N ha\(^{-1}\) of “farrowyard manure” or “cattle slurry” to barley (Hordeum vulgare) and showed increases of flavones and soluble phenolic acids in barley leaves with decreased application rates of animal manure. Zhao et al. (2007) compared pac choi (B. rapa) grown under three application rates of organic and conventional fertilizers: low, medium and high rates corresponding, respectively, to 0, 156 and 312 kg N ha\(^{-1}\) for organic fertilization and 0, 78, and 156 kg N ha\(^{-1}\) for conventional fertilization. They showed that the total ORAC value of pac choi decreased as the fertilizer rate increased, especially under conventional fertilization. They also reported an interesting tendency demonstrated by ORAC values in response to fertilizer rates; in general, H-ORAC and total ORAC values of pac choi tended to decrease at higher fertilization, while L-ORAC reached the highest levels at a medium fertilizer rate. Meanwhile, Toor et al. (2006) reported that different nutrient sources (mineral nutrient solutions containing NH\(_4\)\(^+\) and NO\(_3\)\(^-\), chicken manure, and gross-clover mulch) affect the levels of antioxidants in tomatoes.

As mentioned above, stem mustard responded to high N-supply with increased crop yield (data not shown) and reduced leaf accumulation of total phenolic compounds (Figs. 3 and 6), consistent with previous reports (Lester and Treutter, 2005; Asami et al., 2003; Norbaek et al., 2003). With regard to the effect of different sources of manure (animal, plant, mineral, etc.) on stem mustard antioxidant capacity, the effect is unclear throughout this study. Although all of the environmental variables that affect ORAC values were not controlled for in this study, we compared three cultivation environments and found that differences in UV radiation and nutrient availability are important factors that determine ORAC values. In future work, comparison on the same background will be necessary to address the issues of nutrient availability.

In this study, we showed the wide range of ORAC and TP values of 71 types of farm products in Japan. In addition, we discussed growing conditions as factors that affect the antioxidant capacity of crops. We expect that our data on ORAC values will help familiarize the Japanese population with indicators of crop quality that are connected with health outcomes. In addition, elucidating the causes behind high ORAC values in crops will help local farmers ensure a steady supply of high-quality farm produce to the market.

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