Monthly and Yearly Variations in Oxygen Radical-scavenging Activity, Ascorbic Acid Content, and Degrees Brix of Komatsuna (Brassica rapa var. perviridis) in the Kanto Region, Japan

Ayuchi Takei1,2,*, Yuri Goh1, Gen Hattori1, Masayuki Arii1, Masumi Niwa1,3 and Koki Toyota2

1Delica Foods Co. Ltd., Adachi-ku, Tokyo 121-0073, Japan
2Graduate School of Bio-Applications and Systems Engineering, Tokyo University of Agriculture and Technology, Koganei 184-8588, Japan
3DesignerFoods Co. Ltd., Adachi-ku, Tokyo 121-0073, Japan

It is commonly recognized that vegetables in their best season are delicious and have high nutritional value. We investigated the monthly and yearly variations in radical-scavenging (RS) activity, an indicator of antioxidant activity, ascorbic acid, and degrees Brix of Brassica rapa var. perviridis (komatsuna) cultivated in the Kanto region, Japan. RS activity was significantly higher in December, January, and February than in the other months in all tested years (2010–2015). For two varieties (summer and winter varieties) their RS activity was significantly higher in winter than in summer. The weight proportion of leaf blades and petioles at harvest was similar (leaf blade:petiole = 4:6) both in the summer and winter, indicating that the difference in the RS activity of komatsuna between the two seasons was not due to the weight ratio, but to the increase in the RS activity of leaf blades in the winter. There were no significant differences in the RS activity of komatsuna between summer (30 days) and winter (80 days) cultivation periods, greenhouse and open-field cultivations, and producers. Multiple years of field studies revealed that the antioxidant activity, ascorbic acid content, and degrees Brix of komatsuna in the Kanto region were higher in winter than in other seasons, and suggested that komatsuna with a similar quality level may be produced when grown in regions with similar weather conditions.

Key Words: best season, DPPH radical-scavenging activity, Japanese mustard, seasonal change.

Introduction

Brassica rapa var. perviridis (komatsuna) is a green and yellow Brassicaceae vegetable that is high in ascorbic acid content (Lee and Kader, 2000). The quantity of komatsuna shipped in Japan has increased by 30% in the past 10 years from 74,800 tons in 2006 to 99,100 tons in 2016 (Ministry of Agriculture, Forestry and Fisheries, Japan (MAFF) statistics).

Consumers’ awareness of the key phrase “the best season” is important in the distribution of vegetables because purchasing motivation is affected by the common understanding of the season in which a particular vegetable is most delicious. However, the image of the best season is vague, and its definition is unclear (Nishi, 1980). In general, the best season is explained by many factors, including “deliciousness”, “health benefits”, and “safety” and many consumers require information regarding these factors.

Nowadays, we see many vegetables in shops all year around and it is becoming more difficult for consumers to know the best season for particular vegetables. In addition, consciousness of “diet and health” is growing in consumers’ minds. Social media affects consumer consciousness, and scientific words regarding the quality of vegetables, such as “vitamin C content”, “polyphenol”, “lycopene”, “β-carotene”, and “antioxidant” are increasingly recognized.

Particular attention has been paid to antioxidant capacity, which is evaluated as radical-scavenging (RS) activity. Reactive oxygen species (ROS), such as superoxides, hydrogen peroxide, and hydroxyl radicals, can
cause oxidative damage to cellular structures and functional molecules such as DNA, proteins, and lipids (Finkel and Holbrook, 2000). The intake of natural antioxidants is thought to be one way to protect the body from oxidative stress (Finkel and Holbrook, 2000), and as a result the popularity of antioxidants is growing. The first database on the antioxidant capacity of foods was released in 2002 (Halvorsen et al., 2002) and a lot of data have been accumulated since then (Pellegrini et al., 2019). It is important to define the best season of vegetables based on RS activity and other diet- or health-related indicators, since the best season is an important key phrase when consumers purchase vegetables.

Seasonal variations in vitamins and minerals have been studied in many vegetables (Tsujimura et al., 1997, 1998); however, antioxidant capacity was not tested in these studies. In spinach, it has generally been observed that antioxidant capacity (Howard et al., 2002; Watanabe and Ayugase, 2015a) and its components such as beta carotene (Watanabe and Ayugase, 2015b), vitamin C (Fujiiwara et al., 2005; Sorensen et al., 1994; Tamura et al., 2003), and flavonoid contents (Howard et al., 2002; Watanabe and Ayugase, 2015b) are higher in low temperature periods than in other periods. In leeks, higher vitamin C content is observed in winter, and this may be related to radiation (Kagata et al., 2014).

In general, vegetables are cultivated in many production areas. Consumers need data from different production areas when they purchase food, but there has been little investigation into whether similar trends in antioxidant capacity can be found across several production areas. In addition, few studies have been conducted on yearly differences, despite the fact that antioxidant activity showed large yearly variations in asparagus (Takacs-Hajos and Zsombik, 2015). In komatsuna, the seasonal fluctuations in sugar, vitamin C, and minerals (Acikgöz and Altintas, 2011; Tamura, 2004) and antioxidant capacity (Imahori et al., 2016; Wakagi et al., 2014) have been reported, but these parameters were compared only a few times a year. Data on antioxidant capacity in komatsuna are lacking, particularly regarding seasonal variations throughout the year, yearly variations, and variations in different production areas. Therefore, komatsuna’s best season has not been clarified based on antioxidant capacity, although Imahori et al. (2016) found that it was higher in winter than in summer.

The aims of this study were to investigate monthly variations in RS activity, vitamin C content, and degrees Brix of komatsuna, which is commonly cultivated in Japan all year around, and to clarify whether similar trends could be observed over several years in different production areas. In addition, we focused on the effects of different cultivation factors, such as location, farmer, varieties planted, cultivation days, and parts of the plant, to clarify the effect of the cultivation season on antioxidant capacity. The results obtained in this study will provide consumers and producers with confirmation of komatsuna’s best season in terms of its antioxidant capacity and nutritional value.

### Materials and Methods

**Sampling method of plant materials for monthly and yearly changes in radical-scavenging activity, ascorbic acid and degrees Brix.**

The monthly change in the radical-scavenging activity (RS) of komatsuna (*Brassica rapa var. perviridis*) was evaluated from January to December in 2008 for a producer who cultivated komatsuna all year round in Sakura City, Chiba Prefecture. Since monthly changes can differ depending on the producer, the same investigation as above was also conducted for two production areas (Hokota City, Mito City) in Ibaraki Prefecture. Furthermore, yearly changes were evaluated over four years, from 2010 to 2013 in one Ibaraki production area, and from 2012 to 2015 in another Ibaraki production area, since it was reported that the total polyphenol, flavonoid, and vitamin C contents of asparagus tend to vary greatly from year to year (Takacs-Hajos and Zsombik, 2015). In addition, the same investigation was conducted at the same producer in Chiba Prefecture described above for an additional two years (2012 to 2013).

Table 1 shows the average temperatures, precipitation, and daylight hours for the past 10 years (2008–2018) that were collected from the meteorological stations closest to the three production areas mentioned above. These meteorological data were significantly

| Season                     | Average temperatures (°C) | Average monthly precipitation (mm) | Average monthly daylight hours (h) |
|----------------------------|---------------------------|-----------------------------------|-----------------------------------|
|                            | Chiba (Sakura) | Ibaraki (Mito) | Ibaraki (Hokota) | Chiba (Sakura) | Ibaraki (Mito) | Ibaraki (Hokota) | Chiba (Sakura) | Ibaraki (Mito) | Ibaraki (Hokota) |
| March to November          | 18.5c         | 17.7b           | 17.4a          | 144ab         | 133a          | 149b          | 159a         | 170b          | 167b          |
| December to February       | 4.7c          | 4.3b            | 3.9a           | 66b           | 55a           | 69b           | 174a         | 193b          | 179a          |

Average monthly values were analyzed by paired multiple test and different letters show significant differences of means among the three areas in each season using the Tukey-Kramer method ($P<0.05$).
different among the three areas, but the differences (maximum–minimum) were as small as 1.1°C for average temperature, 15.5 mm for monthly precipitation and 19.5 h for monthly daylight.

Komatsuna was supplied throughout the year by producers who were practicing commercial cultivation. All samples were harvested at commercial harvest maturity (leaf lengths of ca. 25 cm and bunch weights of ca. 50 g fresh weight (FW)). The cultivation period was approximately 50 days in spring, 30 days in summer, 50 days in autumn, and 80 days in winter (2010 to 2015). Komatsuna samples were harvested in the morning and pre-cooled for 14 to 17 h at a refrigeration facility (set at 5°C), to correspond with commercial packaging conditions. They were distributed to a laboratory by a chilled delivery vehicle maintained at 5°C. Then, RS activity, ascorbic acid, and degrees Brix in the harvest--chilled delivery vehicle maintained at 5°C. Then, RS and ‘Hakkei’ (SAKATA SEED Corp.) for spring cultivation; ‘Inamura’ (SAKATA SEED Corp.), ‘Kiyosumi’ (SAKATA SEED Corp.), and ‘Kiyosumi’ (SAKATA SEED Corp.) for summer cultivation; ‘Satokirari’ (Musashino Seed Co., Ltd.) for summer cultivation; ‘Satokirari’ (Musashino Seed Co., Ltd.) for summer and autumn cultivation; and ‘Hisui’ (Watanabe Noji Co., Ltd., Noda, Japan), ‘Inasena’ (KANEKO SEEDS CO., LTD., Maebashi, Japan), ‘Hiromi’ (NOHARA SEED CO., LTD., Kuki, Japan), and ‘Natsu Rakuten’ (TAKII & CO., LTD., Kyoto, Japan) for winter cultivation.

The komatsuna varieties cultivated were ‘Satokirari’ (Musashino Seed Co., Ltd., Tokyo, Japan), ‘Kiyosumi’ (SAKATA SEED CORPORATION. Yokohama, Japan), and ‘Hakkei’ (SAKATA SEED Corp.) for spring cultivation; ‘Inamura’ (SAKATA SEED Corp.), ‘Kiyosumi’ (SAKATA SEED Corp.), and ‘Kiyosumi’ (SAKATA SEED Corp.) for summer cultivation; ‘Satokirari’ (Musashino Seed Co., Ltd.) for spring and autumn cultivation; and ‘Hisui’ (Watanabe Noji Co., Ltd., Noda, Japan), ‘Inasena’ (KANEKO SEEDS CO., LTD., Maebashi, Japan), ‘Hiromi’ (NOHARA SEED CO., LTD., Kuki, Japan), and ‘Natsu Rakuten’ (TAKII & CO., LTD., Kyoto, Japan) for winter cultivation.

**Sampling method of plant materials to assess the effects of different producers, varieties, cultivation areas and cultivation days.**

Differences in cultivation methods by producers can affect RS activity. We selected three producers who have been cultivating komatsuna in Hokota city, Ibaraki Prefecture for more than 10 years (2016–2017). The fields of the three producers were located within an approximately 10 km area. The three producers used ‘Hamatsuzuki’ (SAKATA SEED Corp.) and ‘Natsu no Koshien’ (TOKITA SEED CO., LTD., Saitama, Japan) as winter and summer varieties, respectively. The RS activity of komatsuna was compared among the three producers using the same varieties of komatsuna.

The difference in varieties between summer and winter can be a factor that affects RS activity (Bunning et al., 2010; Howard et al., 2002; Park et al., 2017). RS activity was compared using two varieties in summer in Hokota city, Ibaraki Prefecture. One was ‘Natsu no Koshien’, which is cultivated as a summer variety, and the other was ‘Hamatsuzuki’, which is suitable for winter cultivation. In addition, the same investigation was also conducted in winter. RS activity was compared between ‘Hamatsuzuki’ and ‘Natsu no Koshien’ by cultivating them at the same time.

RS activity was compared for one producer in Hokota city, Ibaraki Prefecture between cultivation areas (a greenhouse and the open-field) by cultivating the same varieties at the same time. In summer, ‘Natsu no Koshien’ and ‘Enka’ (Watanabe Noji Co., Ltd.) were used. ‘Hamatsuzuki’, ‘Inamura’ (SAKATA SEED Corp.), ‘Minami’ (Tohoku Seed Co., Ltd., Utsunomiya, Japan), and ‘Shuto no ace’ (TOKITA SEED CO., LTD.) were used in winter. The distance between the greenhouse and the open-field was about 500 m in a straight line.

In the winter cultivation season (December–February), RS activity was compared using the variety ‘Hamatsuzuki’, which was cultivated for the usual number of days in winter (about 80 days) and in summer (about 30 days). Differences in size due to different cultivation days and those due to the proportion of leaf blades and petioles were investigated.

**Sample preparation**

Komatsuna samples distributed to the laboratory were washed with tap water to remove contaminants. Then, extra moisture was wiped off with paper towels, and the weight of each sample after removing wilting and discolored leaves was measured. The edible part of 5–10 plants was measured as one sample. An edible portion (500 g) from each sample was cut to 1–2 cm with a knife and mixed. Then, 100 g was weighed into a homogenizer (TM835; TESCOM & Co., Ltd., Tokyo, Japan) with 500 mL of distilled water. After homogenization for 1 minute, the homogenate was poured into a 200 mL Erlenmeyer flask and shaded. It was then heated at 80°C for 30 min. After the heat-treatment, the flask was cooled with running tap water, and the homogenate was filtrated through filter paper (Advantec No. 2; Toyo Roshi Kaisha, Ltd., Tokyo, Japan). The filtrate was used for the following analyses. We used the pretreatment methods (homogenization in water and heat treatment at 80°C for 30 min) based on the study by Suda (2000), Fujie et al. (2001), and our preliminary experiment. The method reported by Suda homogenized plant materials in 80% ethanol solution, but ethanol is expensive and its use is not suitable for analyzing a large number of samples. Therefore, we first compared the antioxidant activity of homogenates using 80% ethanol solution and distilled water. The result revealed a highly significant correlation ($R^2 = 0.9198$) between the two methods. We also tested heat processing at 80°C for 30 minutes. There was no significant difference ($P = 0.9495$) in the antioxidant activity of komatsuna samples between heat-treated and non-heated samples. There was no significant difference ($P = 0.606$) in the ascorbic acid content of komatsuna samples between heat-treated and non-heating samples either.
Determination of antioxidant activity (DPPH (1,1-diphenyl-2-picrylhydrazyl) assay)

The antioxidant activity was analyzed by using the 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical-scavenging assay based on the methods of Suda (2000) and Terasawa et al. (2008) with a few modifications. In brief, 1 mL of DPPH solution (500 μM DPPH; Wako Pure Chemical Corp., Osaka, Japan) in ethanol, 0.4 mL of 0.2 M acetate buffer (pH 5.5), and 1.6 mL of ethanol (Wako) were mixed with 2 mL of differently diluted samples (× 2, × 4, × 8, × 16) of the filtrates in a test tube. The mixture was shaken on a vortex and kept in the dark at room temperature (22–25°C) for 30 min. Absorbance at 517 nm was then measured using a spectrophotometer (UV-1800; Shimadzu Corp., Kyoto, Japan), and a 99.5% ethanol solution was used as a blank. The antioxidant activity of the samples was expressed as a percent inhibition of the DPPH radical and calculated by the following equation (Yamaguchi et al., 1998; Shimamura et al., 2007; Topcu et al., 2015). In the calculation, diluted samples that showed 10% to 90% inhibition for DPPH radicals were selected.

\[ I(\%) = \frac{(A_C - A_S) \times 100}{A_C} \]

where \( I \) is the inhibition percentage, and \( A_C \) and \( A_S \) are the absorbance values of the control and test samples, respectively. The concentration providing 50% inhibition (IC50) of the DPPH radical was calculated from the plot of the concentration versus the percent inhibition, and the antioxidant activity was converted to Trolox equivalents (mg TE/100 g FW).

 Extraction and measurement of degrees Brix

Portions (30 g) of the cut samples prepared for the DPPH analysis were used to measure degrees Brix. They were macerated using a juicer mixer (TJ110; TESCOM) for 1 min, and degrees Brix was measured three times for one sample with a Brix meter (PAL-1; Atago Co., Ltd., Tokyo, Japan).

 Extraction and determination of ascorbic acid content

Portions (200 g) of the cut samples prepared for the DPPH analysis were weighed into a cooled homogenizer with 30 mL of 10% metaphosphoric acid (Wako). After homogenizing for 1 min, an additional 70 mL of 10% metaphosphoric acid was added. It was diluted with deionized water to adjust the volume to 200 mL, and the solutions were filtered through filter paper (Advantec No. 2). The ascorbic acid content of the sample was analyzed by using a Reflectoquant Ascorbic Acid Test with RQflex (Merck, Darmstadt, Germany) based on the procedures of Tatebe and Yoneyama (1995). Obtained data were calibrated by a standard curve that was prepared using four different concentrations (50, 150, 200, and 400 mg·L−1) of ascorbic acid (Wako) each time. Each sample was measured three times. The results were expressed as mg ascorbic acid per 100 g FW.

Contribution of ascorbic acid to DPPH radical-scavenging activity

In order to investigate the contribution of ascorbic acid and the other antioxidant components, the DPPH radical scavenging activity of standard solutions of ascorbic acid was measured. Fifty mg of ascorbic acid (Wako) was dissolved in 100 mL of deionized water, and its ascorbic acid content and DPPH radical scavenging activity were measured as described above. Then, based on the DPPH radical scavenging activity per unit g of ascorbic acid, the contribution of ascorbic acid to DPPH radical-scavenging activity was calculated for komatsuna samples in 2012 and 2013, when at least two samples were collected from all the months from all three farms.

Statistical analysis

Using Excel statistics software, a one-way or two-way ANOVA was conducted and then Tukey-Kramer’s multiple comparison test was used to assess DPPH scavenging activity, ascorbic acid, and degrees Brix. To evaluate ascorbic acid to DPPH scavenging activity, the ratios were categorized as the winter season (December to February) and the other season (March to November) and analyzed using paired two-way ANOVA and Tukey-Kramer’s multiple comparison test.

Results

Monthly changes in radical-scavenging (RS) activity

In a survey conducted at a production site (Farm N) in Ibaraki Prefecture from 2012 to 2015, significantly higher (\( P < 0.05 \)) RS activity was observed in months from December to February than in other months (Table S1). In particular, there was a tendency to show the highest RS activity in January. A survey of 2010 to 2013 at another site (Farm A) in Ibaraki Prefecture also showed significantly higher (\( P < 0.05 \)) RS activity from December to January than in other months (Table S1). Also, in the survey of 2008 and from 2012 to 2013 at a production site (Farm T) in Chiba Prefecture, the RS activity was significantly higher (\( P < 0.05 \)) from December to January and again in February than in the other months (Table S1). There were significant differences (\( P < 0.05 \)) in RS activity between the winter season (December to February) and other seasons (March to November) for all combined data (Fig. 1).

Monthly changes in ascorbic acid content and degrees Brix

Ascorbic acid content and degrees Brix at Farm N (2012 to 2015) and Farm A (2010 to 2013) in Ibaraki Prefecture and at Farm T in Chiba Prefecture (2008 and from 2012 to 2013) were significantly higher (\( P < 0.05 \)) in December, January, and February than in other months (Fig. 2A, B; Table S1).
Comparison of RS activity among producers

There were no significant differences in the RS activity of Komatsuna among three producers for the winter variety (Hamatsuzuki) in the 2016 winter cultivation and in the summer variety (Natsu no Koshien) in the 2017 summer cultivation (Fig. 3). In the summer of 2016, there was a significant difference between Farm TH and I; however, the RS activity of all the producers was significantly lower than that in the 2016 winter cultivation.

Comparison of RS activity among varieties

The RS activity of the summer variety (Natsu no Koshien) was significantly higher \((P < 0.05)\) in winter than in summer (Fig. 4). The RS activity of the winter variety (Hamatsuzuki) was significantly lower \((P < 0.05)\) in summer than in winter. No significant difference was found between summer varieties and winter varieties in the same cultivation seasons.

Difference in RS activity between greenhouse cultivation and open-field cultivation

In the summer cultivation of a summer variety (Natsu no Koshien), there was no significant difference in RS activity between nine greenhouses and an open-field. Similar results were obtained for another summer variety (Enka). In the winter cultivation of a winter variety (Hamatsuzuki), the RS activity was significantly higher \((P < 0.05)\) in two out of nine greenhouses than in open-field cultivation (data not shown).

Comparison of RS ability with different cultivation days

When the variety ‘Natsu no Koshien’ (summer variety) was cultivated in winter, there was no significant difference in the RS activity between 30 days and 80 days of cultivation (Fig. 5). On the other hand, significantly higher \((P < 0.05)\) RS activity was obtained from 30 days of cultivation than 80 days of cultivation in the variety ‘Hamatsuzuki’ (winter variety). In both varieties, 30 days and 80 days of winter cultivation showed significantly higher RS activity than 30 days of cultivation in summer.
Comparison of RS activity between leaf blades and petioles and their ratios between summer and winter

The average weight per plant was 6.7 g in ‘Hamatsuzuki’ after 30 cultivation days in winter, and it increased to 42.8 g when the cultivation was extended to 80 days. When ‘Hamatsuzuki’ was cultivated for 30 days in summer, the average weight per plant was 63.6 g. The ratio of leaf blades and petioles was 4.4:5.6 with 80 days of winter cultivation, 4.2:5.8 with 30 days of summer cultivation, and 7:3 with 30 days of winter cultivation. The RS activity was significantly higher (\(P < 0.05\)) in blades than in petioles in all samples cultivated for 30 days and 80 days in winter and for 30 days in summer (Fig. 6).

Contribution of ascorbic acid to RS activity

Standard ascorbic acid solution (50 mg/100 mL) showed 43.5 mg ascorbic acid content measured by RQflex and 51.5 mg TE of RS activity, indicating that 1 mg of ascorbic acid was equivalent to 1.18 mg TE, which is similar to the result of Yamaguchi et al. (1998). According to the conversion factor, the contribution of ascorbic acid to the RS activity of each Komatsuna sample ranged from 49% to 62% in the winter season (December to February, average = 56.2%) and from 57% to 100% in the other months (average = 83.3%) and the difference was significant (\(P < 0.01\)) (Fig. 7).

Discussion

Monthly change in radical-scavenging (RS) activity of komatsuna

The present study revealed that RS activity evaluated with the DPPH method was significantly higher in the winter season (December to February) than in the other seasons (March to November). Previous studies have reported that the antioxidant properties in vegetables and fruit correlate with temperature (Bergquist et al., 2006; Bunning et al., 2010). In this study, DPPH activity was measured with distilled water extraction, not with 80% ethanol extraction as used in the original method of Suda (2000), and the modified method used showed ca. 75% of the original method. Although our method underestimated total antioxidants, high DPPH activity in komatsuna in winter may be also be related to low temperatures. Ascorbic acid is a typical antioxidant in komatsuna. The contribution of ascorbic acid to the RS activity of each Komatsuna sample was as high as 83% in March to November, suggesting that ascorbic acid may be mainly responsible for the RS activity. In contrast, it was ca. 56% in the winter season, indicating
that antioxidants other than ascorbic acid increased in the winter season.

Phenolic compounds may be involved in antioxidant activity, since chilling stress leads to the accumulation of several secondary metabolites including phenolic compounds (Christie et al., 1994). Pennycooke et al. (2005) investigated the relationship between cold acclimation and total phenolic content, and found that pre-treatment at 5°C caused an injury impeding acclimatization and increased phenolic content related to functional protection. Watanabe and Ayugase (2015b) investigated the relationship in cold-set processing of spinach between antioxidant capacity and flavonoids, and suggested that increased antioxidant activity may prevent low temperature stress.

Flavonoids may be also involved, since they are one of the specific metabolites that are biosynthesized in plants in order to respond to environmental stresses. Anthocyanins, a kind of flavonoid, have been reported to promote the removal of ROS under oxidative and dry stresses (Nakabayashi et al., 2013). According to Dixon and Paiva (1995), anthocyanins are induced by low temperatures or high light or uv intensities. Therefore, low temperature stress could increase anthocyanin content and thereby increase RS activity.

Although this study did not measure all antioxidants, these results suggest that the amounts of antioxidants induced by low temperature stress may be larger than those induced by higher solar radiation in summer. The growth rate and metabolic rate of komatsuna may differ depending on the season and low temperatures reduce the rates of enzyme reactions. Such reduced rates due to low temperatures limit the electron transfer of photosystem I and decrease the amount of acceptable light (Kudoh and Sonoike, 2002). Although light stress produces H$_2$O$_2$ (Wingler et al., 2000), Karpinski et al. (1999) showed that Arabidopsis thaliana had more resistance to H$_2$O$_2$ under low temperatures. Decreasing growth and enzyme reaction rates at low temperatures in winter may cause such phenomena and thus increase the antioxidant capacity of plants.

However, the antioxidant synthesizing system in vegetables is too complex to be explained only by differences in daylight intensity and temperature. Further biochemical analysis will provide more accurate explanations for the higher antioxidant activities of komatsuna in winter.

**Monthly change in ascorbic acid content and degrees Brix**

Tamura (1999) investigated the relationship among the amount of solar radiation, temperature and ascorbic acid. They reported that ascorbic acid content increased with low temperature treatment (Tamura, 1999; Tamura et al., 2003). In this study, ascorbic acid content increased in the low temperature period from December to February. Many investigations have revealed the seasonal variation of ascorbic acid content in spinach (Fujiwara et al., 2005; Topcu et al., 2015; Watanabe et al., 1994), and that solar radiation and temperature are the main factors (Fujiwara et al., 2005; Tamura, 2004; Wakagi et al., 2014). Since ascorbic acid is biosynthesized from photosynthetic products, increased solar radiation facilitates photosynthesis and then sugar production, which results in the increased synthesis of ascorbic acid (Tatebe and Yoneyama, 1995). This could be the reason that the ascorbic acid content in this study was the highest in August during the summer period (June to September), when the solar radiation level was the highest. On the other hand, Tamura (1999) reported that the total ascorbic acid content of komatsuna was increased by low temperature conditions under low solar radiation conditions. The present study also showed a similar trend, and the ascorbic acid content may have increased due to low temperatures in winter, contributing to higher RS activity in winter.

Rosette-type plants increase sugar contents to acclimate to cold temperatures by accumulating sugar (Kameno et al., 1990). Indeed, Tamura (2004) found that the sugar contents of komatsuna leaves increased by 5 times when the average lowest temperature at 10 days before harvest decreased from 5°C to −5°C. In this study, the average lowest temperatures were −5.1 to −7.0°C in the three production areas. The cold temperatures in winter may increase the sugar contents of komatsuna leaves.

**Comparison of RS activity by producers**

The RS activity of komatsuna varies depending on the production area (Wakagi et al., 2014), and the variations could be caused by cultivation methods or environmental factors, such as temperature and solar radiation. Therefore, we compared the RS activity of komatsuna cultivated by three producers for the same period using the same varieties. Results showed no significant difference in the RS activity of komatsuna among the three producers, both in a summer variety and a winter variety. This result was reproduced in different years. The fields of three producers are located within a radius of 10 km, and environmental factors such as solar radiation, rainfall, and temperature were considered to be similar. The present study suggests there are few differences in RS activity among different producers with similar environmental conditions.

**Comparison of RS activity by variety**

Many studies have reported differences in RS activity and total polyphenol content among varieties. In this study, ‘Natsu no Koshien’ (a summer variety) and ‘Hamatsuzuki’ (a winter variety) were cultivated in both summer and winter seasons, and the RS activity was significantly higher, even in summer varieties, when it was cultivated in the winter. In addition, when winter varieties were cultivated in the summer, the RS
activity was significantly lower in summer than in winter, suggesting that the difference in RS activity in komatsuna was not due to the variety, but to the cultivation season.

**Difference in RS activity between greenhouse cultivation and open-field cultivation**

In greenhouse cultivation, ultraviolet irradiation, which is considered to increase RS activity, is decreased (Raghuvanshi and Sharma, 2016). In contrast, plants receive more direct sunlight in open-field cultivation than in greenhouse cultivation, and the ascorbic acid content in tomatoes was higher in open-field cultivation (Dumas et al., 2003). Olsson et al. (1998) also reported that the hydroxylation of flavonoids was promoted by stronger light intensity in rapeseed (*Brassica napus*), leading to an increase in antioxidant capacity in open-field cultivation. Similarly, the antioxidant capacity of spinach was stronger with open-field cultivation than with greenhouse cultivation (Kiya et al., 2005). In this study, however, there was no significant difference in the RS activity of three komatsuna varieties between open-field and greenhouse cultivations, as in the study of spinach by Izumi et al. (2008). The exact reasons for the different tendencies remain to be elucidated.

**Difference in RS activity with different cultivation days and between leaf blades and petioles**

In addition to the factors described above, other factors can affect RS activity. These include i) cultivation days, which is related to the temperature during the cultivation period (Bergquist et al., 2006), and ii) the size of a plant, which is also related to cultivation days (Toor et al., 2006). Since the contents of ingredients are different between leaf blades and petioles (Watanabe et al., 1994), the difference in their proportions is another factor affecting RS activity. The relationship between cultivation days and antioxidant components has been investigated in several vegetables. Stino et al. (1973) reported that ascorbic acid content increased with the more cultivation days in spinach. Contrasting results have also been reported: ascorbic acid content tended to decrease with growth in spinach (Bergquist et al., 2006; Watanabe et al., 1994) and in lettuce (Sorensen et al., 1994). Overall, there is no consistent trend in the relationship between the number of cultivation days and antioxidant components. In this study, there was no significant difference in the RS activity between 30 days and 80 days of cultivation in the summer variety ‘Natsu no Koshien’ in winter cultivation. On the other hand, 30 days of cultivation showed higher RS activity than 80 days of cultivation in the winter variety ‘Hamatsuzuki’ in winter cultivation. The ascorbic acid content also showed the same tendency as the RS activity in the winter variety ‘Hamatsuzuki’. It was hypothesized that higher RS activity in winter cultivation was due to the increased cultivation days until harvest, but this may not be the case. In winter cultivation, the RS activity in two varieties, ‘Natsu no Koshien’ and ‘Hamatsuzuki’, was significantly higher with 30 days of cultivation than with 80 days of cultivation. In contrast, the ascorbic acid content was not significantly different in one of the two varieties in terms of the cultivation days, unlike the studies of Stino et al. (1973) and Watanabe et al. (1994). In this study, differences were observed in the RS activity between 30 day and 80 days of cultivation despite no differences in the ascorbic acid content, suggesting that components other than ascorbic acid content may largely contribute to the RS activity of komatsuna in the winter season.

Watanabe et al. (1994) showed that the ascorbic acid and β-carotene contents of spinach were higher in leaf blades than in leaf petioles in both summer and autumn. This study showed that the ratio (4:6) at 80 days of cultivation in winter was the same as that (4:6) at 30 days of cultivation in summer. The RS activity of the blade part was significantly higher than that of the petiole part in the three different cultivations, and the ascorbic acid content also showed similar results. This is in agreement with the findings of Imahori et al. (2016), who reported that komatsuna accumulated more ascorbic acid in leaf tissues to protect them from light and oxidative stress. These results indicated that the factor causing higher RS activity in winter is not likely the difference in the ratio of leaf blades to petioles.

The investigation of monthly and yearly changes in RS activity, degrees Brix, and ascorbic acid in komatsuna revealed that these functionality-related parameters were higher in the winter months (December to February) in every tested year in the Kanto region with similar precipitation amounts, temperatures and daylight hours. This was not because of cultivation methods, varieties, cultivation conditions (greenhouse and open-field), or cultivation days, but because of higher RS activity, ascorbic acid, and degrees Brix in the leaf blades and petioles in winter. As a next step, it is necessary to accumulate data to provide consumers with further information on what is the best season for komatsuna cultivated in regions other than Kanto.

**Acknowledgements**

The authors thank Ibaraki Noryuen CO. LTD, Mr. Tamatsukuri, Mr. Shiga, producers Mr. Takihara, Mr. Isaka, and Mr. Hosoya, who provided komatsuna samples.

**Literature Cited**

Acikgoz, F. E. and S. Altintas. 2011. Seasonal variations in vitamin C and mineral contents and some yield and quality parameters in komatsuna (*Brassica rapa var. pervidis*). J. Food Agric. Environ. 9: 289–291.

Bergquist, S. Å., U. E. Gertsson and M. E. Olsson. 2006. Influence of growth stage and postharvest storage on ascorbic acid and carotenoid content and visual quality of baby spinach (*Spinacia oleracea* L.). J. Sci. Food Agric. 86: 346–
Bunning, M. L., P. A. Kendall, A. B. Stone, F. H. Stonaker and C. Stushinoff. 2010. Effects of seasonal variation on sensory properties and total phenolic content of 5 lettuce cultivars. J. Food Sci. 75: S156–S161.

Christie, P. J., M. R. Alfrnito and V. Walbot. 1994. Impact of low-temperature stress on general phenylpropanoid and anthocyanin pathways—enhancement of transcript abundance and anthocyanin pigmentation in Maize seedlings. Planta 194: 541–549.

Dixon, R. A. and N. L. Paiva. 1995. Stress-induced Phenyl-propanoid metabolism. Plant Cell 7: 1085–1097.

Dumas, Y., M. Dadomo, G. Di Lucca and P. Grolier. 2003. Effects of environmental factors and agricultural techniques on antioxidant content of tomatoes. J. Sci. Food Agric. 83: 369–382.

Finkel, T. and N. J. Holbrook. 2000. Oxidants, oxidative stress and the biology of ageing. Nature 408: 239–247.

Fujie, A., M. Kubota, Y. Umemura and K. Oba. 2001. Vitamin C content, DPPH radical-scavenging activity and polyphenol content in fresh herbs. J. Cookery Sci. Jpn. 34: 380–389 (In Japanese with English abstract).

Fujiwara, T., H. Kumakura, S. Ohta, K. Yoshida and T. Kamo. 2005. Seasonal variation of L-ascorbic acid and nitrate content of commercially available spinach. Hort. Res. (Japan) 4: 347–352.

Halvorsen, B. L., K. Holte, M. C. Myhrstad, I. Barikmo, E. Hvattum, S. F. Remberg, A. B. Wold, K. Haffner, H. Baugerd, L. F. Andersen, Ø. Moskaug, D. R. Jacobs Jr and R. Blomhoff. 2002. A systematic screening of total antioxidants in dietary plants. J. Nutr. 132: 461–471.

Howard, L. R., N. Pandaitian, T. Morelloca and M. I. Gil. 2002. Antioxidant capacity and phenolic content of spinach as affected by genetics and growing season. J. Agric. Food Chem. 50: 5891–5896.

Imahori, Y., K. Kodera, H. Endo, T. Onishi, T. Fujita and S. Naitoh. 2016. The seasonal variation of redox status in komatsuna (Brassica rapa var. perviridis) leaves. Sci. Hortic. 210: 49–56.

Izumi, M., M. Takaya, H. Horie and H. Kiya. 2008. Effect on the organic acid and sugar contents in autumn-grown spinach of the strain, cultivation conditions and growing period, and effect of boiling on the contents and taste. J. Cookery Sci. Jpn. 41: 126–133 (In Japanese with English abstract).

Kagata, E., J. Murakami, M. Tada, Y. Kitajima, M. Kasama and H. Kiya. 2008. Effect on the content, DPPH radical-scavenging activity and polyphenol biosynthesis-related gene expression between summer and winter strawberry cultivars. J. Food Sci. 82: 341–349.

Pellegrini, N., P. Vitaglione, D. Granato and V. Fogliano. 2019. Twenty-five years of total antioxidant capacity measurement of foods and biological fluids: merits and limitations. J. Sci. Food Agric. DOI: 10.1002/jsfa.9550.

Pennycooke, J. C., S. Cox and C. Stushnoff. 2005. Relationship of cold acclimation, total phenolic content and antioxidant capacity with chilling tolerance in petunia (Petunia × hybrida). Environ. Exp. Bot. 53: 225–232.

Raghuvanshi, R. and R. K. Sharma. 2016. Response of two cultivars of Phaseolus vulgaris L. (French beans) plants exposed to enhanced UV-B radiation under mountain ecosystem. Environ. Sci. Poll. Res. 23: 831–842.

Shimamura, T., R. Matsuura, T. Tokuda, N. Sugimoto, T. Yamazaki, H. Matsufuji, T. Matsui, K. Matsumoto and H. Ukeda. 2007. Comparison of conventional antioxidants as says for evaluating potencies of natural antioxidants as food additives by collaborative study. J. Jpn. Soc. Food Sci. Technol. 54: 482–487 (In Japanese with English abstract).

Sorensen, J. N., A. S. Johansen and N. Poulsen. 1994. Influence of growth conditions on the value of crispyhead lettuce 1. Marketable and nutritional quality as affected by nitrogen supply, cultivar and plant age. Plant Food. Hum. Nutr. 46: 1–11.

Stino, K. R., M. A. Abdelfattah and H. Nassar. 1973. Studies on vitamin C and oxalic acid concentration in spinach. Agric. Res. Rev. 51: 109–114.

Suda, I. 2000. Spectroscopic evaluation of antioxidant function. p. 218–220. In: K. Shinohara and S. Uenokawa (eds.). Research methods for food functions. Korin Inc., Tokyo (In Japanese).

Takacs-Hajos, M. and L. Zsomorik. 2015. Total polyphenol, flavonoid and other bioactive materials in different asparagus cultivars. Not. Bot. Hort. Agrobot. 43: 59–63.

Tamura, A. 1999. Effects of low temperature on the sugar and ascorbic acid contents of komatsuna (Brassica campestris L.) under limited solar radiation. J. Japan. Soc. Hort. Sci. 68: 409–413 (In Japanese with English abstract).

Tamura, A. 2004. Effect of air temperature on the content of sugar and vitamin C of spinach and komatsuna. Hort. Res. (Japan) 3: 187–190 (In Japanese with English abstract).

Tamura, A., M. Shinoda and T. Taguchi. 2003. Effect of heat in sulation and cold air introduction in the greenhouse on growth, freezing tolerance, and sugar and vitamin C contents in spinach and komatsuna at the region of limited solar radiation in winter. Bull. Akita. Agric. Exp. Stn. 43: 19–44 (In Japanese).

Tatebe, M. and T. Yoneyama. 1995. An analysis of nitrate and ascorbic acid in crop exudates using a simple reflection pho-
Terasawa, N., N. Imai, M. Nosaka and Y. Kujira. 2008. Effects of position on the plant, ripening stage and water stress due to root-zone restriction on the quality and radical scavenging activity of tomato. J. Jpn. Soc. Food Sci. Technol. 55: 109–116 (In Japanese).

Toor, R. K., G. P. Savage and C. E. Lister. 2006. Seasonal variations in the antioxidant composition of greenhouse grown tomatoes. J. Food Comp. Anal. 19: 1–10.

Tópçu, Y., A. Dogan, Z. Kasimoglu, H. Sahin-Nadeem, E. Polat and M. Erkan. 2015. The effects of UV radiation during the vegetative period on antioxidant compounds and postharvest quality of broccoli (Brassica oleracea L.). Plant Physiol. Biochem. 93: 56–65.

Tsujimura, M., S. Higasa and K. Arai. 1998. Seasonal changes in the contents of vitamins and minerals in vegetables and fruits [2]. Vitamins 72: 613–617 (In Japanese).

Tsujimura, M., H. Komatsubara, K. Arai and T. Fukuda. 1997. Seasonal changes in the content of vitamins and minerals in vegetables and fruits [1]. Vitamins 71: 67–74 (In Japanese with English abstract).

Wakagi, M., J. Watanabe and T. Takano-Ishikawa. 2014. Effects of producing area and harvest season on antioxidant capacities of spinach, komatsuna, tomato, and cucumber. Rep. Natl. Food Res. Inst. 78: 65–71 (In Japanese with English abstract).

Watanabe, M. and J. Ayugase. 2015a. Changes in flavonoids and antioxidant activity of spinach among cultivation periods. J. Jpn. Soc. Food Sci. Technol. 62: 501–507 (In Japanese with English abstract).

Watanabe, M. and J. Ayugase. 2015b. Effect of low temperature on flavonoids, oxygen radical absorbance capacity values and major components of winter sweet spinach (Spinacia oleracea L.). J. Sci. Food Agric. 95: 2095–2104.

Yamaguchi, T., H. Takamura, T. Matoba and J. Terao. 1998. HPLC method for evaluation of the free radical-scavenging activity of foods by using 1,1-diphenyl-2-picrylhydrazyl. Biosci. Biotechnol. Biochem. 62: 1201–1204.

Wingler, A., P. J. Lea, W. P. Quick and R. C. Leegood. 2000. Photorespiration: metabolic pathways and their role in stress protection. Philos. Trans. R. Soc. Lond. Soc. B – Bio. Sci. 355: 1517–1529.

Wingler, A., P. J. Lea, W. P. Quick and R. C. Leegood. 2000. Photorespiration: metabolic pathways and their role in stress protection. Philos. Trans. R. Soc. Lond. Soc. B – Bio. Sci. 355: 1517–1529.