Light Condition Influences Rutin and Polyphenol Contents in Asparagus Spears in the Mother-fern Culture System during the Summer–Autumn Harvest

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We investigated the variation in rutin and total polyphenol contents in asparagus spears under different conditions of mother-fern culture. Rutin and polyphenol contents were highest at early spring harvest at all test sites. The contents gradually decreased during the harvest season. In particular, a considerable decrease was observed after the growth of mother ferns. The absorption of the 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical was weaker in summer. Rutin content in the spears of plants grown outdoors was generally higher than in those grown in a greenhouse, except at early spring harvest. Rutin contents decreased in the spears of plants grown under a shading net or an ultraviolet (UV) radiation-filtering film. These results suggest that cultivation conditions, especially light conditions, have a great influence on rutin and polyphenol contents. We evaluated two new methods to increase rutin content by improving light conditions. Light conditions in asparagus rows were improved by using light-reflecting sheets and net screens; however, a significant increase in rutin content was observed only when net screening was used, implying that this method is better than light reflecting sheets to increase the rutin content.

Key Words: antioxidant, Asparagus officinalis, HPLC, phenolic compounds, UV.

Introduction

Asparagus (Asparagus officinalis L.) has been widely grown in Japan since the 1960s, and conventional spring harvest was the most popular method of harvesting asparagus during that period. Since the 1980s, the mother-fern culture method has been used widely, especially in the south-western part of Japan because it leads to high yields and is suitable for a long harvest season (9 to 10 months a year) (Inoue, 2008). The total annual yield obtained using this method sometimes reaches more than 40 tons per hectare (Inoue et al., 2008).

Asparagus is a popular vegetable and is a rich source of compounds beneficial for health. Some compounds from asparagus such as flavonoids, phenolic acids, and saponins have been reported to show biological and pharmacological activities (Deschner et al., 1991; Hartung et al., 1990; Wang et al., 2003). Rutin is one of the major flavonoids present in asparagus (Chin et al., 2002; Deeds and Couch, 1948; Maeda et al., 2005) and has been reported to exert biological effects such as suppressing the weakening of capillaries associated with hemorrhagic disease (Griffith et al., 1944) and reducing hypertension in humans (Hellerstein et al., 1951). The anthocyanins, caffeic acid, and other phenolic compounds from asparagus were also reported to show biological and/or pharmacological activities (Vinson et al., 1995; Yamanaka et al., 1997).

In the mother-fern culture method, asparagus spears grow and are harvested mostly in the shadows of mother ferns during the summer–autumn season. This culture method sometimes results in problems such as low quality of spears; in particular, spears harvested during the summer–autumn harvest are paler than those harvested during spring (Inoue et al., 2008). Therefore, the content of rutin and phenolic compounds may differ significantly among summer–autumn spears cultivated by the mother-fern culture method and those cultivated by the conventional method. Further, even differences
in the cultivation conditions within a method, such as whether the plants are grown outdoors or in a greenhouse, may also affect the chemical contents of asparagus. However, few studies have been conducted to determine the effects of these different conditions on spear quality, especially the contents of phenolic compounds. The objective of this study was to study seasonal changes in the levels of rutin and phenolic compounds under different conditions of mother-fern culture, and to estimate the effects of light intensity on the contents of these compounds. In addition, we evaluated the effectiveness of 2 methods that were aimed at improving light conditions during the summer–autumn harvest in increasing rutin and polyphenol contents.

Materials and Methods

Sample preparations and analysis

Green asparagus spears were harvested in an experimental field at Hokkaido University (conventional method, outdoors), Nagano Vegetable and Ornamental Crops Experimental Station (outdoors and in a greenhouse), Fukushima Agricultural Research Center (outdoors), and Nagasaki Agricultural and Forestry Experiment Station (in a greenhouse). Uniform-sized spears (bottom diameter ranging from 14 to 20 mm) were cut at a length of 24 cm, immediately frozen and sent to Hokkaido University, and then stored at −30°C until extraction. The frozen spears were cut into 3 sections of equal length (8 cm). The top section of 3 to 5 spears was used for analysis.

Rutin and total soluble polyphenol were extracted from 5 g of chopped frozen spears or from 20 mg of freeze-dried powder of the spears by using 20 ml (for frozen spears) or 1 ml (for freeze-dried powder) of 80% methanol. The extraction was conducted for three hours at room temperature. Sample solutions were then centrifuged (10,000 rpm, 10 min) and filtered (0.45 μm). These extraction procedures were repeated 3 times. Rutin content was determined by high-performance liquid chromatography (HPLC) as described by Maeda et al. (2005). HPLC analysis was conducted using a Waters 2690 system (Waters Corp. Milford, USA) equipped with a Waters Symmetry C18 (4.6 × 250 mm) column. The mobile phases consisted of 20 mM potassium phosphate buffer (pH 5.0; A) and acetonitrile (B). Analysis was performed by running each sample for 35 min at a column temperature of 40°C, using a linear gradient system at a flow rate of 1.0 ml·min⁻¹. The gradients were 0–5 min, 84% solvent A and 16% solvent B and 35 min, 35% solvent A and 65% solvent B, and the post-running time was 8 min. Each run was monitored at a wavelength of 354 nm using a photodiode array detector.

The total soluble polyphenol content was determined by modified Folin-Denis method (Folin and Denis, 1915; Maeda et al., 2005). The sample solution (150 μl) was transferred into a 96-well microplate, to which 75 μl of 50% Folin-Denis reagent and 75 μl of 2.5% Na₂CO₃ were added per well. The plate was incubated at room temperature (20–25°C) for 60 min. The absorbance (ABS) of each sample solution was measured at a wavelength of 700 nm by using a plate reader (Model 550, Bio-Rad Lab. Japan Inc., Tokyo, Japan). Quercetin was used as the reference standard. The polyphenol content of each sample was expressed as quercetin-equivalent value (mg Q/100 g FW). The antioxidant activities of the samples were determined by measuring the ability to absorb the 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical. Each sample solution (200 μl) was diluted with 800 μl of 0.1 M Tris-HCl buffer (pH 7.4) in a glass cell, after which 1.0 ml of 500 μM DPPH-ethanol solution was added to each tube. The test solutions were incubated in the dark at room temperature for 25 min, and then ABS was measured at a wavelength of 520 nm. Alpha-tocopherol was used as the reference standard.

Seasonal changes in rutin and polyphenol contents under various conditions of mother-fern culture

The spears were sampled as follows for the investigation of seasonal changes in the total polyphenol content under different conditions of the mother-fern culture. Cultivar ‘UC157’ (4-year-old plants) were grown in the outdoor experimental field in Fukushima and harvested from late April until the end of October. Spears harvested on April 22, May 21 (spring harvest), July 7, July 23, and August 26 (summer–autumn harvest), 2003, were collected for the determination of rutin content, total polyphenol content, and DPPH radical-absorbing activity. Mother ferns were cultivated after May 21 harvest and had grown well by July 7.

‘UC157’ and ‘Gijnlim’ plants were grown outdoors and in a greenhouse in an experimental field in Nagano and were harvested from April to October. Spears harvested on April 30, May 24 (spring harvest), June 28, and July 26 (summer–autumn harvest) in 2004 were collected for rutin analysis. Mother ferns had grown in June both outdoors and in the greenhouse. Mother stalks at both the sites were kept luxuriant until November.

Effect of reduction in the light intensity and ultraviolet radiation on rutin content

The experiment was conducted in an experimental field at Hokkaido University (Sapporo, Japan). On May 25, 2005, a row of ‘MW500W’ plants (age, 15 years) was tunnel-covered with 2 types of shade net (Tairen #30 and Tairen #5; Daiho Chemical Co., Osaka, Japan) and a film filtering ultraviolet (UV) radiation (Skycoat UV Tekinashi; C.I. Kasei Co., Tokyo, Japan) in order to reduce the intensity of sunlight or UV irradiation. Spears previously grown under tunnels were cut at ground level on the same day. Light intensity in each tunnel was monitored every 5 minutes from 0:00 on May 26 to 0:00 on June 10 using an MES-136 radiation meter with an IKS-27-10/136 photon flux sensor (Koito Industries Ltd., Yokohama, Japan). Spears that grew
under the tunnels were harvested daily from May 31 to Jun 17.

Effect of the improved cultivation method on rutin content
We used two types of improved cultivation method for improving light intensity in the asparagus field. The first experiment was conducted outdoors in an experimental field at the Nagano Vegetable and Ornamental Crops Experimental Station in 2006. Each furrow in the field where ‘NJ953’ and ‘Purple Passion’ plants (age, 6 years) were grown was covered with a high-light-reflecting material, namely a Tyvek sheet (DuPont-Asahi Flash Spun Products Co., Ltd., Tokyo, Japan) throughout the harvest season (from April to the end of September). Part of the field was not covered, and plants in this part served as the uncovered control. The Tyvek sheet reflects more than 90% of incident light rays according to the manufacturer’s data. For rutin analysis, we collected spears harvested on May 8, 28, and 29 (spring harvest); August 9 and 11 (mid-summer harvest); and September 19, 20, and 25 (autumn harvest). From May 31 to June 12 (spring harvest; no mother fern grown) and from August 31 to September 7 (summer–autumn harvest; mother fern fully grown), the intensity of incidental light on the rows of test and control fields was measured once in a day (at 13:00) with a MES-136 radiation meter using the IKS-27-10/136 photon flux sensor (Koito Industries Ltd.).

The second experiment was conducted in a greenhouse in an experimental field at Nagasaki Agricultural and Forestry Experiment Station in 2007. The test plot was prepared as follows. On June 25, net screens (width 100 cm, mesh size 10 cm) were arranged along both sides of each row of greenhouse-cultivated ‘UC157’ plants (age, 10 years old) at a height of 50 to 150 cm above ground level. These nets restricted the growth of ferns within a width of 80 to 90 cm above each row so as to improve light intensity and to secure enough working space in furrows (Fig. 1A). The plot with no net treatment (conventional mother-fern cultivation) was used as a control (Fig. 1B). Light intensity in each plot was measured using an ANA-F11 illumination photometer (Tokyo Koden Co., Tokyo, Japan) on July 23 and August 20 (both days had fine weather). The observed value (lux) was converted to PPF value (μmol·m⁻²·s⁻¹), using the formula described by Inada (1984). The summer–autumn harvest was conducted from May until the end of October. Spears harvested on July 1 and August 1 were collected for rutin analysis.

Results
Seasonal changes in the content of antioxidative phenolic compounds under various conditions of mother-fern culture
In Fukushima, both rutin and total polyphenol contents were the highest (67.6 mg/100 g FW and 56.4 mg Q/100 g FW, respectively) in late April, in the early period of the harvest season, and then gradually decreased throughout the summer. In particular, they decreased to a large extent after the growth of the mother ferns in July (Fig. 2). DPPH radical-absorbing activity was relatively high during spring, and subsequently decreased. The pattern of changes in DPPH radical-absorbing activity was similar to that observed in the case of total polyphenol and rutin contents (Fig. 3).

In Nagano, the rutin content of the two tested cultivars was highest in April and then gradually decreased in both plants grown outdoors and in a greenhouse, as was observed in Fukushima. Rutin content varied widely during the early spring harvest. Spears cultivated outdoors tended to have higher rutin content than those cultivated in the greenhouse, except during the early spring harvest (Fig. 4).

Fig. 1. Use of a net screen during the summer–autumn harvest. (A) Conventional method (control). (B) Method using a net screen (greenhouse at Nagasaki Agricultural and Forestry Experiment Station)
Effect of light intensity and reduction in UV radiation intensity on rutin content in spears

Compared to light intensity in the control field, the relative average light intensity in plots covered with shade nets Tairen #30 and Tairen #5, and that covered with a UV-filtering film was reduced to 21.1%, 5.1%, and 88.6%, respectively (Fig. 5A). The average rutin content in spears harvested in the control plot was 8.4 mg·g$^{-1}$ DW, while those in spears harvested in plots covered with Tairen #30, Tairen #5, and a UV-filtering film were 3.2, 2.2, and 5.4 mg·g$^{-1}$ DW, respectively. Rutin contents decreased with the decrease in light intensity (Fig. 5B). In plot covered with the UV-filtering film, the rutin content was relatively low although light intensity was not affected in this plot.

Effect of the improved method on rutin content

In 2006, we tested a sheet of the light-reflecting material Tyvek in Nagano to assess whether it can improve light intensity during the summer–autumn harvest when the mother fern culture method is used. Light intensity was slightly higher in the plot where Tyvek was used than in the control plot (Table 1); however, no significant difference was observed in the rutin content of spears grown in the plot where Tyvek was used and the control plot at any period during the harvest season (Fig. 6).

In 2007, we tested the utility of the net screen to limit the area available for the growth of mother ferns in order to improve light intensity and a secure working space in the Nagasaki greenhouse. Light intensity in plots where the net screen was used was significantly higher than that in the control plot (Table 2). Rutin contents in spears harvested in these plots were significantly higher.

![Fig. 2. Seasonal changes in the contents of rutin and total polyphenolic compounds in ‘UC157’ spears. Spears were harvested at Fukushima. Bars indicate SD (n=3).](image)

![Fig. 3. Seasonal change in DPPH radical-absorbing activity (‘UC157’). Spears were harvested in Fukushima. Means with different letters indicate significant differences ($P < 0.05$) as calculated by Tukey’s test. Bars indicate SE (n=3).](image)

![Fig. 4. Seasonal changes in the rutin content of ‘UC157’ and ‘Gijnlim’ spears under outdoor/greenhouse conditions. Spears were harvested in Nagano. Bars indicate SD (n=3).](image)
than those harvested in control plots on both July 1 and August 1 (Fig. 7); however, the rutin contents in spears cultivated in these plots were lower than those of spears harvested in spring or cultivated outdoors.

**Discussion**

We observed seasonal change of rutin and polyphenol contents in asparagus spears in this study, and also tried to evaluate the effect of light conditions on rutin biosynthesis in asparagus spears. The results of our observation showed that rutin content decreased in the summer–autumn season, especially after the growth of mother ferns both in Fukushima and Nagano. The changes in rutin content in asparagus spears well reflected changes in the total polyphenol content and antioxidative activity; therefore, changes in rutin content in asparagus spears may reflect changes in the total polyphenol content and antioxidative activity. These results supported previously reported findings which indicated that rutin is one of the major phenolic

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**Table 1.** Effect of light-reflecting material on light intensity in a row of asparagus plants cultivated by the mother-fern culture method (Nagano, Outdoors).

<table>
<thead>
<tr>
<th>Date of measurement</th>
<th>Light intensity (µmol·m⁻²·s⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
</tr>
<tr>
<td>Spring harvest</td>
<td></td>
</tr>
<tr>
<td>(No mother fern)</td>
<td></td>
</tr>
<tr>
<td>Fine</td>
<td>May 31, Jun 1, Jun 7</td>
</tr>
<tr>
<td>Cloudy</td>
<td>Jun 8, Jun 9, Jun 12</td>
</tr>
<tr>
<td>Rain</td>
<td>Jun 6</td>
</tr>
<tr>
<td>Summer–autumn harvest</td>
<td></td>
</tr>
<tr>
<td>(Full-grown mother fern)</td>
<td></td>
</tr>
<tr>
<td>Fine</td>
<td>Aug 8, Aug 9, Aug 10</td>
</tr>
<tr>
<td>Rain</td>
<td>Sep 1, Sep 6, Sep 7</td>
</tr>
</tbody>
</table>

* Measurement was conducted at 13:30.

**Table 2.** Effect of using a net screen on light intensity in a row of asparagus plants growing under mother fern (Nagasaki, greenhouse).

<table>
<thead>
<tr>
<th>Date of measurement</th>
<th>Light intensity (µmol·m⁻²·s⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Outdoor</td>
</tr>
<tr>
<td>July 23 (Fine)</td>
<td>1829.5 ± 5.0</td>
</tr>
<tr>
<td>August 20 (Fine)</td>
<td>1849.7 ± 8.4</td>
</tr>
</tbody>
</table>

* Measurement was conducted at 13:00.

* Means ± SD (n = 3).
compounds in asparagus (Maeda et al., 2005; Tsushida et al., 1994; Wang et al., 2003).

In the summer–autumn season, spears mostly grow in the shadows of mother ferns; therefore, light intensity around spears is relatively low compared to in the spring season, and light intensity in a greenhouse is lower than outdoors because of the covering film (Whittle and Lawrence, 1959) and the vigorous growth of mother stalks (Motoki et al., unpublished data). Rutin contents of spears grown under a shade net significantly decreased with the decrease in light intensity. These results suggested that the shadows of mother ferns and/or greenhouse film caused a reduction in light intensity, and thus, rutin content in asparagus spears fell during the summer–autumn season. Moreover, the results of our experiment suggested that UV radiation has a significant effect on the biosynthesis of rutin in asparagus spears. Flavonoids, including rutin, are considered to be sensitive to certain components of light, especially UV light (Stapleton and Walbot, 1994), and flavonoid biosynthesis in plants is thought to be strongly influenced by UV radiation. Many previous reports indicated that the gene expression of key enzymes mediating the flavonoid biosynthesis pathway, such as chalcone synthase, is regulated by light, especially blue light or UV radiation (Christie and Jenkins, 1996; Fuglevand et al., 1996; Zhou et al., 2007). Inoue et al. (2008) reported that using a net screen improved the color of the spears. Additionally, the yield and working efficiency in this plot were higher than in the control plot.

The results of the present study suggested that spears harvested in the summer–autumn period showed lower rutin and polyphenol contents than spears harvested in spring because of low levels of light and UV radiation; however, the light conditions and rutin content improved when a net screen was used. Thus, the use of net screens seemed to be a preferable method for producing spears with relatively high quality—in terms of both appearance and antioxidative ability—during the summer–autumn harvest by the mother-fern culture method.

### Literature Cited


