Antioxidation Capacities of Extracts from Green, Purple, and White Asparagus Spears Related to Polyphenol Concentration

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Abstract. The antioxidation capacities of green ‘Welcome’, green and white ‘Gijnlim’, and purple ‘Purple passion’ asparagus were investigated. Analyses of rutin and total polyphenols, and assays of DPPH radical absorbing and low-density lipoprotein (LDL) antioxidation were conducted. Varietal differences associated with the colors of spears were observed both in the amounts of rutin, total polyphenols and in DPPH radical absorbing activities, although not in LDL antioxidation activities. DPPH radical absorbing activities seemed to be affected by both rutin and other polyphenolic compounds. However, LDL antioxidation activities were likely to be influenced more by other polyphenolic compounds than by rutin. Total polyphenol content showed a fairly close relationship with rutin content, DPPH radical absorbing activity and LDL antioxidation activity. To determine total polyphenol content using the Folin–Denis’ method seemed to be useful for selecting the breeding lines that show high antioxidative capacities.

Many phytochemicals such as flavonoids, other phenolic compounds and carotenoids have been recently noted to have bioactive (antioxidation, biological, physiological and pharmacological) abilities for human health (Kris-Etherton, 2002). For example, major flavonoid compounds, rutin and its aglycone quercetin, were reported to have beneficial biological effects, such as antagonizing the increase of capillary fragility associated with hemorrhagic disease (Griffith, 1944), reducing hypertension (Hellerstein, 1990), and anti-carcinogenic activity (Deschner, 1991; Yang, 2000). Biological activities of many other flavonoids, polyphenols or phenolic compounds were also reported (Edenharder, 2003; Wenzel, 2000; Yamanaka, 1997).

Some phytochemicals were noted to have preventive effects on atherosclerosis. Previous studies indicated that oxidative modification of low-density lipoproteins (LDL) played an important role in the initial stage of atherosclerosis (Steinberg, 1989). α-Tocopherol and ascorbic acid are known to be the major antioxidants for LDL-oxidation. In addition to these chemicals, it was reported that phytochemicals such as chlorogenic acid, caffeic acid (Vinson, 1995), sulfur-containing compounds existing in onions (Higuchi, 2001), as well as plant extracts such as green tea, black tea (Tijburg, 1997), grape seeds (Vinson, 2001), oats (Handelman, 1999), and Apocynum venetum (Kim, 2000) also have LDL antioxidation activities. Therefore, there is an interest in the effect of dietary plant material consumption in prevention of atherosclerosis.

Recently, a number of breeding programs focusing on the bioactive effects and contents of bioactive phytochemicals—so called functional breeding—have been started for some plants and vegetables. Several high β-carotene (carrot) and rutin (buckwheat) content varieties have already been released on the market.

Asparagus (Asparagus officinalis L.) is known to be a rich source of phytochemicals. Its phytochemicals—flavonoids (e.g., rutin and anthocyanins), allelopathic compounds (e.g., caffeic acids, ferulic acid, other phenolic and polyphenolic compounds), and saponins (e.g., protodioscin)—are reported to have biological and pharmacological activities on human health (Hartung, 1990). Recent research on asparagus has developed methods to determine some phytochemicals, efficient ways to shorten the breeding period and effective methods of seed production, and using these methods, breeding programs focusing on its functional elements have been started (Maeda, 2002; Sonoda, 2003; Wang, 2003). However, fundamental information that is necessary for functional breeding has not been demonstrated. For example, not enough studies have been performed on the varietal differences in the antioxidative chemical content and the relationship between the amounts and constitutions of phytochemicals associated with spear colors and the strength of antioxidative or biological activities.

The objective of this study was to clarify the relationship between the amounts of polyphenols and antioxidative activities of asparagus extracts obtained from green, purple and white spears.

Materials and Methods

Plant materials and sample preparation. Three varieties of green and purple asparagus spears (‘Welcome’ (green, U.S.), ‘Gijnlim’ (green, Netherlands) and ‘Purple passion’ (purple, U.S.)) were harvested from Nagano Vegetable and Ornamental Crops Experimental Station on 14 May 2003. White spears of ‘Gijnlim’ were harvested from a commercial field at Kimobetsu in Hokkaido on 17 June 2003. Spears were cut at the length of 24 cm (white spears; 17 cm) and frozen and stored at −30°C in a plastic bag until extraction. 100 g of frozen spears (5 to 6 spears, each variety) was homogenized with 80% MeOH for 2 min and extracted with 10 times the volume of 80% MeOH for 4 d at room temperature, then filtered and rotary-evaporated to obtain a solid extract. The extracts were weighed respectively, then dissolved with 80% EtOH and mixed to equal 50 mL (2 g fw equivalent/mL). The solvents were stored at 5°C until using for each analysis and assay.

Chemicals. All chemicals used for the HPLC method (rutin, Kanto Chemical Co.), Folin–Denis’ method (Folin–Denis’ reagent, Furuka; quercetin and sodium carbonate, Kanto Chemical Co.), DPPH radical absorbing assay (1,1-diphenyl-2-picrylhydrazyl, Wako Co.; Tris, ICN Biomedicals, Inc.; α-tocopherol, Kanto Chemical Co.) and LDL antioxidation assay (LDL from human plasma, Calbiochem Co.; 2-thiobarbituric acid (TBA), Sigma Co.; Trichloro acetic acid (TCA), Sigma Co.; 1,1,3,3-tetraethoxypropane, Sigma Co.) were purchased commercially.
contents. Rutin content was determined by HPLC following the method of Chin et al. (2002). Each sample solvent (50 µL) was diluted up to 1 mL with 80% EtOH in a glass vial. HPLC analysis was conducted using Waters 2690 system with Waters Symmetry C18 (4.6 × 250 mm) column. The mobile phases consisted of 0.2% phosphoric acid (A) and HPLC grade acetonitrile (B). Each sample analysis run was performed for 35 min at 40 °C of column temperature, using linear gradient system with a flow rate of 1.0 mL·min⁻¹. The gradients were 0 to 5 min, 84% solvent A and 16% solvent B, 35 min, 35% A and 65% B, and postrunning time was 8 min. Each run was monitored at 254 nm with Waters 996 PDA detector. Rutin and quercetin solutions (25, 50, and 100 ppm) were used for external standards.

Total soluble polyphenols content was determined by modified Folin–Denis’ method (Folin, 1915). Each sample solvent was diluted two hundred times with water for the analysis. The sample solution (150 µL) was put into a 96-well microplate, to which 75 µL of 50% Folin–Denis’ reagent and 75 µL of 2.5% sodium carbonate were added to each well. The plate was incubated at room temperature (20 to 25 °C) for 60 min. ABS at 700 nm of each sample solution was measured with BIO-RAD model 550 microplate reader. Quercetin solutions (0, 25, 50, and 100 ppm; 80% MeOH solution) were used as reference standards. Total polyphenols content of the samples was expressed as quercetin-equivalent value (mg quercetin/kg fresh weight).

**Measurement of DPPH radical absorbing capacity.** Each sample solvent (200 µL) was diluted with 800 µL of 0.1 M Tris-HCl buffer (pH 7.4) in a glass cell, then 1.0 mL of 500 µM 1,1-diphenyl-2-picrylhydrazyl (DPPH)–EtOH solution was added to each cell. Test solutions were incubated in the dark at room temperature for 20 min. ABS at 520 nm was measured with Hitachi U-1100 spectrophotometer. As reference standards, 0, 50, 250, and 500 ppm of α-tocopherol (EtOH solution) were used. DPPH radical absorbing activity of each sample was expressed as α-tocopherol equivalent value (ppm α-tocopherol). The assay procedures were replicated three times.

**Measurement of LDL antioxidant activity.** Commercially purchased human LDL solution was dialyzed for 2 days at 5 °C with 0.1 M potassium phosphate buffer solution, pH 7.4, containing 150 mM NaCl (PBS). LDL solution was diluted with PBS up to the concentration of 0.2 mg protein/mL. Each sample solvent was diluted to the appropriate concentration with PBS. LDL-PBS solution (800 µL) and 100 µL of sample-PBS solution or PBS (for the sample blank) were put into an Eppendorf Tube, and incubated for 15 min at 37 °C. Then 100 µL of 50 µM CuSO₄-PBS solution was added to each tube as the oxidative modification inducer, and all tubes were incubated at 37 °C. The test solution consisted of 800 µL of LDL-PBS solution and 200 µL PBS was also prepared for the nonoxidized control. After 1, 3, and 6 hours incubation, 200 µL of test solution was collected, and oxidized LDL in the test solution was measured by the TBARS (thiobarbituric acid–reactive substances) method (Yagi, 1976). The LDL oxidation processes were replicated three times.

The TBARS method was conducted as follows. Test solution (200 µL), 1.5 mL of 0.67% TBA and 20% TCA were mixed in a test tube, and then incubated for 60 min in boiling water. As a reference standard, 1,1,3,3-tetraethoxypropane (10 mmol) was used instead of the test solution. After incubation, test tubes were cooled with tap water. The amount of TBARS in each test solution was measured fluorometrically with a Hitachi F-2000 spectrophotometer, excitation at 515 nm and emission at 535 nm. TBARS values were expressed as nmol of malondialdehyde (MDA). LDL antioxidation activity was calculated with the TBARS values of each sample (MDAs), nonoxidized control (MDAc) and sample blank (MDAb). The calculation formula is shown below, which is expressed with LDL antioxidation inhibition rate (LDL antioxidation activity (%)).

**Relationship between rutin and total polyphenol content and DPPH radical absorbing activity.** Significant differences were observed in both rutin and total polyphenol content among colored and white asparagus spears. Analysis samples were obtained from 100 g of each variety. Means with different letters indicate significant differences (P < 0.05) by Tukey’s test. The bars indicate standard error.

![Fig. 1. Differences in rutin and total polyphenols contents among colored and white asparagus spears.](image-url)
Fig. 2. DPPH radical absorbing activities of asparagus extracts. Means with different letters indicate significant differences ($P < 0.05$) by Tukey’s test. The bars indicate standard error.

Fig. 3. Influence of the doses of asparagus extract on the inhibitory effect of Cu$^{2+}$-induced oxidative modification of LDL (variety: Welcome). Doses of extracts expressed with mg/ml fresh weight equivalent. Means with different letters indicate significant differences ($P < 0.05$) by Tukey’s test conducted independently every 3 and 6 h of incubation. The bars indicate standard error.

two green varieties indicated a definite varietal difference in rutin contents. Previous studies demonstrated that rutin content of green spears differed with varieties (Chin, 2002, Wang, 2003). In breeding asparagus, rutin and/or total polyphenol content seemed to be an important characteristic in selecting breeding lines that show high antioxidant activity.

**LDL antioxidation activity**: The amount of TBARS increased in accordance with the length of the incubation time in the sample blank. This means that Cu$^{2+}$-induced LDL oxidative modification was in progress as the incubation period progressed. However, in the presence of asparagus extract, the oxidation of LDL was significantly inhibited in relation with the density of the doses (Fig. 2). At a dose of 50 mg (fw equivalent), all tested varieties showed strong LDL antioxidation activities (80.3% to 83.5%) after 3 h of incubation. Meanwhile significant differences were observed at a dose of 25 mg (Fig. 3), and no activity was observed at a dose of 10 mg (data not shown). As previously described, recent studies indicated that oxidative modification of LDL seemed to play an important role in the initiation and progression of atherosclerosis. Accordingly, much attention has been focused on the dietary inhibitory materials concerning LDL oxidation. Some naturally occurring flavonoids, phenolic compounds, and plant extracts have been reported to have much stronger LDL antioxidation activities than vitamins such as ascorbic acid and α-tocopherol (Vinson, 1995). The present study demonstrated that colored asparagus spears contained a significant amount of rutin and polyphenolic compounds, and that its extracts had both DPPH radical absorbing activities and LDL antioxidation capacities. These results support the previous studies that suggested the importance of the bioactivities of asparagus (Chin, 2002; Tsushida, 1994).

As was shown in Figs. 1 and 3, white spears showed as strong LDL antioxidation activity as the other colored varieties although it contained very few rutin and a relatively low level of total polyphenols. Vinson (1995) demonstrated that caffeic acid and various other phenolic acids and esters showed much stronger LDL antioxidation activities than rutin and some other flavonoids. On the other hand, Hartung (1990) identified several phenolic acids found in asparagus extract, for example caffeic acid, ferulic acid, and MDC (methylbenzoxy cinnamic acid), as the allelopathic (phytotoxic) compounds. These phenolic acids probably contributed to LDL antioxidation activity in asparagus spears. In the preliminary analyses, much content of polyphenols was distributed at the top section of the spears as compared to the middle and bottom sections. Top sections showed stronger LDL antioxidation activity than the other sections (data not shown). These results suggested that the LDL antioxidation activity of asparagus extracts is caused by the presence of powerful LDL antioxidative compounds, and the activity seemed to be affected not so much by rutin, a major flavonoid, but by some other minor phenolic compounds or antioxidative compounds.

The present study demonstrated clear varietal differences in rutin and total polyphenol content and even in antioxidative capacities associated with spear colors and varieties. As described previously, rutin is a prominent bioactive and antioxidative compound, and DPPH radical absorbing capacity is also important to speculate fundamental antioxidative activities. However, some other compounds seem to play an important role in LDL antioxidation activities. Total polyphenol content clearly correlated with both rutin and DPPH radical absorbing activity, and it seemed to have some relationship with LDL antioxidative activity. Further investigations are needed on the relationship of antioxidative properties between parents and hybrids, and to reveal what compound plays a primary role in LDL antioxidation activity in the white spears and obviously in the colored spears.

The objective of functional breeding of asparagus, which started recently, is mainly the increase of rutin contents, antioxidative capacity and chemoprevention activities.

Fig. 4. LDL antioxidation activities of asparagus extracts after 3 h of incubation. Means with different letters indicate significant differences (P < 0.05) by Tukey’s test conducted independently every dose of extracts. The bars indicate the standard error.

described previously, the determination of rutin or total polyphenol content might make the screening process relatively easy for finding a breeding line that has high antioxidative properties. Measurement of total polyphenols with the Folin-Denis’ method is especially useful to evaluate the antioxidative capacities of breeding lines, because numerous samples can be measured at the same time with a simple, brief procedure in comparison with using HPLC. Total polyphenol content showed a positive correlation both with rutin content and DPPH radical absorbing activities, and thus with LDL antioxidation activities.

Literature Cited


