Antioxidative Activity of Anthocyanins from Purple Sweet Potato, *Ipomoera batatas* Cultivar Ayamurasaki

Mitsuyoshi Kano, Tomomi Takayanagi, Katsuhisa Harada, Kumiko Makino, and Fumiyasu Ishikawa

Yakult Central Institute for Microbiological Research, 1796 Yaho, Kunitachi, Tokyo 186-8650, Japan

Received December 14, 2004; Accepted March 2, 2005

We evaluated the antioxidative activity of anthocyanins from an extract of the tuber of purple sweet potato (PSP) (*Ipomoea batatas* cultivar Ayamurasaki). Anthocyanins from PSP showed stronger 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical-scavenging activity than anthocyanins from red cabbage, grape skin, elderberry, or purple corn, and eight major components of the anthocyanins from PSP showed higher levels of activity than ascorbic acid. In PSP anthocyanin-injected rats and PSP beverage-administered volunteers, DPPH radical-scavenging activity in the urine increased. The elevation of plasma transaminase activities induced by carbon tetrachloride was depressed in rats administered PSP anthocyanin solution. Two components, cyanidin 3-O-(2-O-(6-O-(E)-caffeoyl-β-D-glucopyranosyl)β-D-glucopyranoside)-5-O-β-D-glucopyranoside and peonidin 3-O-(2-O-(6-O-(E)-caffeoyl-β-D-glucopyranosyl)β-D-glucopyranoside) detected in the plasma, protected low density lipoprotein from oxidation at a physiological concentration. These results indicate that PSP anthocyanins have antioxidative activity in vivo as well as in vitro.

**Key words**: *Ipomoea batatas* cultivar Ayamurasaki; antioxidant; anthocyanin; 1,1-diphenyl-2-picrylhydrazyl; low-density lipoprotein

Recent epidemiological studies have shown that diets rich in fruits and vegetables are associated with a reduction in risk for life-style related diseases. The French paradox has been considered to be caused by the antioxidation of polyphenols contained in red wine. Basic components of wine polyphenols are anthocyanins, and it has been found that proanthocyanidin attenuates the development of aortic atherosclerosis.

Anthocyanins are a widespread source of naturally occurring colorants of foods, but were not recognized as a physiologically functional food factor. During the past decade, anthocyanins have been reported to have antioxidative activity, to scavenge active oxygen radicals, to prevent carcinogenesis, to improve visual functions, to inhibit platelet aggregation, and so on. To date, however, the study of anthocyanins has been confined mainly to grapes and berries.

Anthocyanins of purple sweet potato (PSP) have been noted for their stability, physiological function, and so on. The *Ipomoea batatas* cultivar Ayamurasaki is a variety of PSP, bred in Japan by selecting tubers with a large anthocyanin content. PSP anthocyanins have useful characteristics for food manufacturing, remaining stable after heating and ultraviolet irradiation. Because they have a complex chemical structure compared to other anthocyanins, one report has been published about the effects of these anthocyanins in vivo. To estimate the efficacy of PSP anthocyanins, in this study we investigated their effects in vitro and in vivo on 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity, their effect on hepatopathy induced by carbon tetrachloride (CCL₄), and their effect on the resistance of low-density lipoprotein (LDL) to oxidation.

**Materials and Methods**

**Chemicals.** PSP anthocyanins were isolated from an extract of the tuber of *Ipomoea batatas* cultivar Ayamurasaki. Grape skin and red cabbage anthocyanins were purchased from Kanto Chemical, Tokyo. Elderberry and purple corn anthocyanins were isolated from Kiriyasured ES and Kiriyasured MT-Y (Kiriya Chemical, Osaka, Japan).

All organic solvents were of HPLC grade. The other reagents and chemicals used were commercially available products of extra-pure grade.
PSP beverage and anthocyanin pigment. The PSP beverage was purchased from Yakult Honsha, Tokyo. The anthocyanin pigments from PSP were refined according to the method of Harada et al.\textsuperscript{31)\textsuperscript{31}} Eight major components of the pigments are shown in Fig. 1.

In vitro DPPH radical-scavenging activity. DPPH is a radical-generating substance widely used to monitor the free radical-scavenging abilities of various antioxidants.\textsuperscript{32,33)\textsuperscript{32,33}} Various concentrations of anthocyanin solution (1 ml, 0–100 μM) were mixed with 1 ml of 0.2 mM DPPH in ethanol. The mixture was shaken and left to stand for 30 min. The optical density (OD) of the solution was then measured at 517 nm with a spectrophotometer. DPPH radical-scavenging activity was calculated from the absorption according to the following equation:

Radical-scavenging activity (\%) = (OD control-OD sample)/OD control \times 100

where OD sample represents the absorption of the sample solution, and OD control is that for the control solution (not containing the sample).

Absorption of anthocyanin in rats. Forty-two male Sprague-Dawley rats (\(n = 6\), 10 weeks old, 300–350 g; Japan S.L.C. Shizuoka, Japan) were housed individually in stainless steel wire-bottomed cages in a room with controlled lighting (lights on 08:30–20:30), temperature (24 ± 2 °C), and humidity (60 ± 5%). The rats were given free access to AIN-93,\textsuperscript{34)\textsuperscript{34}} purified diets, and distilled water. They were maintained and treated in accordance with the guidelines of the Ethical Committee for Animal Experiments of the Yakult Central Institute. After a 7-d adaptation period, food was withheld for 16 h. The rats were intragastrically administered the anthocyanin solution (10 ml/kg body). After 5, 15, 30, 60, 120, 180, or 240 minutes, they were anesthetized with diethyl ether. Blood was collected from the hepatic portal vein into tubes containing EDTA. Plasma was obtained by centrifugation at 2000 \(\times\) g for 15 min at 4 °C and stored at −70 °C until analysis. Urine was collected in a dish and also stored at −70 °C until analysis.

Analytical samples were obtained from plasma and urine preparations, and LC/MS conditions were as described in our previous report.\textsuperscript{31)\textsuperscript{31}}

DPPH radical-scavenging activity in rat urine. Male Sprague-Dawley rats (\(n = 6\), 8 weeks old, 300–350 g) were housed and fed as in the absorption study. After a 7-d adaptation period, rats were assigned randomly to two groups (\(n = 6\)), the control and anthocyanin groups. After being deprived of food overnight (16 h), the rats were given sample solutions (10 ml/kg body) intragastrically. The control solution contained 20 g citric acid.
Acid/l. The anthocyanin solution contained 20 g citric acid and 2.7 g anthocyanin pigment/l. Urine was collected in a dish for 2 h after administration and stored at −70 °C until analysis.

**Absorption of anthocyanin in humans.** This study was approved by the Human Studies Committee of the Yakult Central Institute for Microbiological Research, in accordance with the Helsinki Declaration and the Committee’s own guidelines. Six healthy volunteers (five men and one woman aged 37.0 ± 8.0 years) participated in the study. They were deprived of foods rich in anthocyanins for 24 h prior to the experiment. Furthermore, they were served an anthocyanin-free dinner the previous evening. After an overnight fast, they drank a cup of PSP beverage. This beverage (375 ml) contained protein (2.1 g), lipid (0 g), carbohydrates (44.4 g), dietary fiber (1.2 g), and sodium (15 mg), as well as anthocyanins [CyCafSop-Glc (117 mg), CydiCafSop-Glc (33 mg), CyCafPHBSop-Glc (39 mg), PnCafSop-Glc (219 mg), CycafFerSop-Glc (93 mg)], as well as anthocyanins [CyCafSop-Glc (117 mg), CydiCafSop-Glc (33 mg), CyCafPHBSop-Glc (39 mg), PnCafSop-Glc (219 mg), CycafFerSop-Glc (93 mg)], PndiCafSop-Glc (120 mg), PoCafPHBSop-Glc (177 mg), and PnCafFerSop-Glc (135 mg) in a total amount of 933 mg. Blood samples were collected at fixed times (0, 0.5, 1, 2, 3, 4, 6, 8, 12, and 24 h) after feeding. Serum and urine were stored at −4, 6, 8, 12, and 24 h) and micturition times after feeding. Serum and urine preparations and applied LC/MS.31) The resistance of LDL to oxidation. The resistance of LDL to oxidative activity was measured according to Hirano et al.35) Male Golden Syrian hamsters (9 weeks old, Japan S.L.C., Shizuoka, Japan) were housed and fed as in the absorption study for 2 weeks. After an overnight fast, blood was collected from the ventral aorta into EDTA-containing tubes. Plasma was prepared by centrifugation at 2,000 × g for 10 min at 4 °C. LDL was isolated from plasma by ultracentrifugation in saline–EDTA buffer (d = 1.006 g/ml) or saline–EDTA buffer with NaBr (d = 1.182 g/ml) in a Hitachi S120AT2 rotor at 10 °C for 2 h at 120,000 rpm.36) Anthocyanin solutions (10 μl) were mixed with 230 μl of LDL solution (protein 218 μg/ml PBS) and 10 μl of V-70: 2-2’-azobis(4-methoxy-2,4-dimethylvaleronitrile) (Wako) solution (12.5 mM). The kinetics of the oxidation of LDL were determined by monitoring the change in the formation of conjugated diene at 234 nm with a Spectra MAX Pro 190 spectrophotometer (Japan Molecular Devices, Tokyo, Japan). The change in absorbance was recorded at 5-min intervals at 37 °C.

**Statistical analysis.** The results were expressed as the mean and SD. The means of DPPH radical-scavenging activity in urine samples were compared by paired t-test, and CCl₄ hepatopathy data were compared by ANOVA and subsequent Tukey’s honestly significant difference comparisons after logarithmic transformation to stabilize the variance, if the variances differed, using Statistica software (StatSoft, Tulsa, OK).37)

**Results**

**In vitro DPPH radical-scavenging activity**

DPPH radical-scavenging activities of food additive pigments of the anthocyanidin family (red cabbage, grape skin, elderberry, and purple corn) are shown in Table 1. The PSP anthocyanin pigment showed stronger activity than other pigments. All components in PSP showed apparently stronger activity than ascorbic acid (Table 2).

**Absorption of anthocyanin**

Absorption of anthocyanin was examined using blood and urine in rats (Figs. 2, 3) and humans (Figs. 4, 5). Two components (A and D) of PSP anthocyanins were detected in both rat and human (blood and urine). In rat plasma, the peak concentration (A: 1.24 ± 0.65, D: 1.93 ± 0.96 μM) for the anthocyanins appeared 5 min after ingestion of the PSP beverage in the absorption experiment. Plasma was obtained by centrifugation at 2000 × g for 15 min at 4 °C and stored at −70 °C until analysis.

Glutamic oxaloacetic transaminase (GOT) and glutamic pyruvic transaminase (GPT) activities, liver injury markers in plasma were determined using transaminase C II-test Wako (Wako Pure Chemicals, Osaka, Japan).
after administration, but in human serum, the peak concentration (D: 4.48 ± 0.38 μM) appeared after 3 h. But we reported previously, that the concentration of component A in human serum was lower than the limit of quantitation. In rat urine, these anthocyanins were excreted rapidly, and 3 h after administration few anthocyanins were detected in the urine. The urinary excretion of anthocyanins was 0.11%/4 h (calculated for rat urine) and 0.01%/24 h (calculated for human urine) of the amount ingested.

**Urinary DPPH radical-scavenging activity**

To investigate the effect in vivo, we examined DPPH radical scavenging activities in the urine of rats and humans orally administered the anthocyanin pigment solution and the PSP beverage respectively. The rat and human urine showed significantly increased levels of activity compared with the PSP-free urine (Figs. 6, 7).

**Repression of CCl

We investigated the effects of PSP anthocyanins on hepatopathy induced by carbon tetrachloride in rats. The PSP group had lower GOT activity than the control group (Fig. 8).

**Resistance of LDL to oxidation**

For two absorbable anthocyanin components (A and D) in PSP, the lag time of LDL oxidation was examined. Both components showed an increased lag time of LDL oxidation over 0.4 μM with the same pattern (Fig. 9).

<table>
<thead>
<tr>
<th>Pigment</th>
<th>ED50 value (μg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grape skin</td>
<td>89.8 ± 1.3a</td>
</tr>
<tr>
<td>Elderberry</td>
<td>52.2 ± 4.3b</td>
</tr>
<tr>
<td>Red cabbage</td>
<td>48.2 ± 1.4b</td>
</tr>
<tr>
<td>Purple corn</td>
<td>35.1 ± 0.9c</td>
</tr>
<tr>
<td>Ayamurasaki</td>
<td>24.0 ± 0.9d</td>
</tr>
</tbody>
</table>

DPPH solution (at a final concentration of 0.1 mmol/1 ethanol) was mixed with various concentrations of anthocyanins (0-300 μg/ml). After 30 min, the absorption of the solution was measured at 517 nm. DPPH radical-scavenging activity was determined as follows:

Radical-scavenging activity (%) =

\[
\frac{(OD_{control} - OD_{sample})}{OD_{control}} \times 100
\]

Each value is the mean ± SD (according to four individual experiment data).

abcd Mean values not sharing the same letter above the bars are significantly different at \( p < 0.05 \) by Turkey’s test.

### Table 2. 1,1-Diphenyl-2-picryl-hydrazyl (DPPH) Radical-Scavenging Activity by Eight Components (A–H) of Purple Sweet Potato Anthocyanin and Ascorbic Acid

<table>
<thead>
<tr>
<th>Compound</th>
<th>ED50 value (μM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>19.3 ± 0.2d</td>
</tr>
<tr>
<td>B</td>
<td>20.0 ± 1.6d</td>
</tr>
<tr>
<td>C</td>
<td>37.1 ± 1.0d</td>
</tr>
<tr>
<td>D</td>
<td>20.1 ± 1.6d</td>
</tr>
<tr>
<td>E</td>
<td>37.3 ± 2.5b</td>
</tr>
<tr>
<td>F</td>
<td>14.8 ± 1.1e</td>
</tr>
<tr>
<td>G</td>
<td>30.8 ± 2.8e</td>
</tr>
<tr>
<td>H</td>
<td>9.9 ± 0.9d</td>
</tr>
<tr>
<td>Ascorbic acid</td>
<td>55.9 ± 0.8a</td>
</tr>
</tbody>
</table>

Each value is the mean ± SD (according to four individual experiment data).

abcde Mean values not sharing the same letter above the bars are significantly different at \( p < 0.05 \) by Turkey’s test.

---

**Fig. 2.** Anthocyanin Concentration in Rat Plasma after Oral Administration of PSP Extract.

Each value is the mean ± SD (n = 6).
Discussion

PSP anthocyanins are highly stable and have good color tone, so they have been used as pigments in food materials.\textsuperscript{29,30} In this study we showed that the pigments of PSP anthocyanin have higher levels of radical scavenging activity than other pigments: eight components had high-level activity, and the urine of rats and humans that ingested PSP exhibited increased DPPH radical-scavenging activity. Because A and D are absorbable, they would contribute to activity in the urine. The difference in urinary DPPH activity between PSP urine and PSP-free urine was 18% in rats at a 0.05 dilution rate (Fig. 6), and 7% in humans at a 0.12 dilution rate (Fig. 7). If these components affect urinary DPPH activity by themselves separately, the concentration of components A and D, corresponding to the difference of DPPH activity, would be 5.6 and 6.2 $\mu$M in rats, and 1.5 and 2.7 $\mu$M in humans, respectively. These concentrations were calculated from the plots of DPPH activities of A (4.0% at 0.7 $\mu$M, 14.2% at 3.5 $\mu$M, 25.6% at 7.1 $\mu$M), and D (0.9% at 0.7 $\mu$M, 8.9% at 3.6 $\mu$M, 25.6%...
However, the urinary concentration of A and D 2 h after oral administration was 0.56 and 1.2 μM in rats (at a 0.05 dilution rate), and 3.1 and 6.8 nM in humans (at a 0.12 dilution rate), respectively. These results show that the gain in urinary DPPH activity cannot be explained simply by the urinary concentration of components A and D. Other factors must be considered.

It has been reported that pretreatment with PSP juice orally for 5 consecutive prior to CCl₄ treatment reduced GOT and GPT activity. We investigated the effects of PSP anthocyanins on hepatopathy induced by CCl₄ in rats. The elevation in GOT activity was depressed. It is well known that CCl₄ is metabolized to the trichloromethyl radical in the cytochrome P-450 system, and that this radical causes liver cirrhosis. In this experiment, PSP anthocyanins had DPPH radical trapping activity in vivo, presumably with the result that this activity was engaged in suppressing hepatopathy induced by CCl₄.
Much scientific evidence supports the involvement of oxidatively modified LDL in the pathogenesis of atherosclerosis. In order to investigate the nutritional meaning of the radical scavenging activity of PSP anthocyanins, we examined antioxidative activity toward LDL. Components A and D enhanced the resistance of LDL to oxidation. This finding shows that PSP anthocyanins have the potential to contribute to anti-arteriosclerosis.

Previous reports have mentioned that anthocyanins directly absorbed were not metabolized. Our results indicate that orally administered anthocyanins are directly absorbed and excreted, and that their absorptivity is very low compared to that of other flavonoids. These results are compatible with previously published results. We examined PSP anthocyanins in this study. Taking factors other than A and D into consideration, the actual rate of absorption might be higher than in this study. The metabolic products of the anthocyanins absorbed and the mechanism of action of anthocyanins remain to be investigated.

Flavonoids are hydrolyzed and metabolized by intestinal bacteria. For example, the glycosides convert to aglycones, and thereby absorbability and physiological

Fig. 7. 1,1-Diphenyl-2-picryl-hydrazyl (DPPH) Radical-Scavenging Activity by Human Urine, before or 2 h after Ingestion of PSP Beverage. Each value is the mean ± SD (n = 6). Asterisks indicate a significant difference (*p < 0.05, **p < 0.01) between the two groups according to the paired t-test.

Fig. 8. Plasma Transaminase Activities. The rats were injected with carbon tetrachloride (CCl₄) (1 ml of 50% corn oil solution/kg body) (control and PSP groups) or 1 ml corn oil/kg body (untreated group) 4 h after ingestion of PSP extract (including 100 mM citric acid) or 100 mM citric acid (control and untreated groups). Each value is the mean ± SD (n = 6). abMean values not sharing the same letter above the bars are significantly different at p < 0.05 by Tukey’s test.
functions are changed. But few reports about bacterial transformations of anthocyanins have been published. Recently, there was a report that bacterial metabolism of anthocyanins involves the cleavage of glycosidic linkages and breakdown of the anthocyanidin heterocycle, but the effects of intestinal bacteria on anthocyanin are still poorly understood. Hence investigation of intestinal metabolism is very important in anthocyanin studies.

References


Fig. 9. Inhibition of Conjugated Diene Formation in the Oxidation of LDL by Anthocyanin (CyCafSop-Glc and PrCafSop-Glc) and Ascorbic Acid.

Lag time to formation of conjugated diene (component A; component D; ascorbic acid). The oxidation of LDL was monitored continuously over 400 min by measuring the increase in absorbance at 234 nm due to the formation of conjugated diene.
Antioxidative Activity of Purple Sweet Potato Anthocyanin


