Note

Anthocyanin-Rich Red Potato Flakes Affect Serum Lipid Peroxidation and Hepatic SOD mRNA Level in Rats

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We examined the effects of red potato flakes (RPF) on serum antioxidant potential and hepatic mRNA in rats. The serum thiobarbituric acid-reactive substances concentration and hepatic superoxide dismutase mRNA level in rats fed RPF were significantly lower and higher respectively than those in control rats. These results suggest that RPF might improve the antioxidant system by enhancing hepatic SOD mRNA.

Key words: anthocyanin; antioxidant activity; colored potato; rat; superoxide dismutase mRNA

It is well known that anthocyanin, a natural colorant derived from various edible plants, has a wide range of antioxidant effects in vivo and in vitro.1,2) Furthermore, several studies in human subjects support the conclusion that consumption of anthocyanin-rich plants leads to an increase in serum antioxidant potential.3,4) Like the anthocyanin-rich purple sweet potato, blueberry, elderberry, and red cabbage, colored potatoes are also considered to be important functional food sources of antioxidants,5) but little information on them is available. Recently, we found that purple potato flakes improved antioxidant potential in rats6) but it has not yet been reported that differently colored potato flakes have similar health benefits. Hence, in this study, we examined the antioxidant effects of red potato flakes (RPF) on serum lipid peroxidation and hepatic antioxidant enzyme mRNAs in rats.

Red potatoes (Solanum tuberosum cv. Northern-Ruby), harvested in Hokkaido, Japan in 2004, were a kind gift of the National Agricultural Research Center for the Hokkaido Region of Japan. RPF were prepared according to our previous report.7) Red potatoes were thoroughly washed with water and air dried on filter paper. Then they were peeled, sliced, steam-heated, mashed, dried in a drum dryer, and ground to flour. RPF pigment was extracted with 5% acetic acid and 70% ethanol. Then moisture was removed with a rotary evaporator at 35 °C, and the eluate was diluted with water and lyophilized to give an anthocyanin-rich pigment (approximately 2.26% based on weight). Dietary fibers, protein, lipid, carbohydrate, moisture, and ash in RPF were determined by the AOAC procedure. The total phenolic concentration in the pigment was determined by the Folin-Ciocalteu method.9) The amount of monomeric anthocyanins in the pigment was measured by a pH differential method.10) The flavonoid concentration in the pigment was measured at 510 nm with a (+)-catechin standard.11) The approximate composition of RPF was as follows (in %): moisture, 4.0; protein (N × 6.25), 8.7; lipids, 0.5; carbohydrate, 76.3; dietary fiber, 6.1 (insoluble fiber, 2.5; water-soluble fiber 3.6); and ash, 4.4. The total polyphenol, anthocyanin, and flavonoid in RPF concentrations (in mg/100 g diet) were 460.1, 92.0, and 91.9 respectively. The main pigment in RPF has been identified to be mainly pelargonidin-3-O-[6-O-(4-O-ethylcoumaroyl-1-O-α-thamnopyranosyl)-β-glucopyranoside]-5-O-β-glucopyranoside.7) Male F344/DuCrj rats (8 weeks old) were purchased from Charles River Japan (Yokohama, Japan). The rats were housed individually in plastic cages kept under a 12/12-h light/dark cycle in a room with controlled temperature (22–24 °C) and humidity (55–65%). They were divided randomly into two groups of five. There were no significant differences in body weight at the start of the experiment. The composition of the experimental control diet was as follows (in %): casein, 20; sucrose, 10; α-cornstarch, 55; cellulose powder, 5; soybean oil, 5; l-cystine, 0.3; choline bitartrate, 0.25; t-butyl hydroquinone, 0.0014; and vitamin and mineral mixtures (3.5 and 1, AIN-93G diet12)). RPF-treated rats were administered a 25% RPF plus 30% α-cornstarch diet for 4 weeks. After the rats were anesthetized with pentobarbital, blood was collected between 09.00 and 10.00 h from the vena cava. Then the whole liver was removed, and stored at −80 °C until analysis. All animal procedures conformed to the principles in the Guide for the Care and Use of

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Abbreviations: SOD, superoxide dismutase; TBARS, thiobarbituric acid-reactive substances; GSH-Px, glutathione peroxidase; GSH-R, glutathione reductase; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; TEAC, trolox equivalent antioxidant coefficient
Laboratory Animals. This experimental design was approved by the Animal Experiment Committee of Obihiro University of Agriculture and Veterinary Medicine. Serum total antioxidant capacity was determined using a Total Antioxidant Status kit based on scavenging of 2,2’-azinobis (3-ethylbenzothiazoline-6-sulfonate) radical cations (Randox Laboratories, Antrim, UK). Serum lipid peroxidation was determined using an assay kit (Lipid Hydroperoxide Assay kit; Wako, Tokyo). Serum total cholesterol, triglyceride and phospholipid concentrations were determined enzymatically using commercially available reagent kits (assay kits for the TDX system; Abbott Laboratory, Irving, TX). Lipid peroxidation in the hepatic homogenate, which was composed of 10 volumes (wt/v) of phosphate-buffered saline (pH 7.4), was measured by thiobarbituric acid-reactive substances (TBARS) assay. Protein content was determined by Bradford assay. Total RNA was isolated from the liver using Isogen reagent (Nippon Gene, Tokyo) according to manufacturer’s protocol.

Briefly, mRNAs encoding Mn-superoxide dismutase (Mn-SOD), Cu/Zn-SOD, catalase, glutathione peroxidase (GSH-Px), glutathione reductase (GSH-R), and glyceraldehyde-3-phosphate dehydrogenase (GAPDH, used as an invariant control) were analyzed by semi-quantitative RT-PCR and subsequent Southern hybridization of PCR products with each inner oligonucleotide probe, as previously described. The relative quantity of mRNA was estimated by densitometric scanning with X-ray film. Data are presented as means and standard deviations. The significance of differences between the control and RPF groups was analyzed by Student’s t-test. Differences were considered significant at p < 0.05 and p < 0.01.

There were no significant differences in body weight gain, food intake, feed efficiency, or liver weight between the control and RPF groups (Table 1). The endogenous bioavailability of antioxidants is highly related to absorption, distribution, metabolism, and excretion. Cao et al. reported that consumption of antioxidant-rich foods resulted in the maintenance of higher antioxidant levels in serum.

However, some researchers have suggested that dietary polyphenols are unlikely to contribute significantly to the trolox equivalent antioxidant coefficient (TEAC) value in vivo, since phenolic compounds in the food matrix might interact with other components of the luminal contents and become unabsorbable; interaction of proteins with polyphenols might especially be expected to occur and to affect absorption. In fact, our data showed that the serum TEAC value of rats was enhanced by up to 13% by feeding with RPF, rising from approximately 0.792 mM to 0.894 mM, but there was no significant difference (Table 1). Thus, whether the effect on the serum TEAC value of rats fed RPF is directly attributable to polyphenols containing anthocyanins remains an open question. On the other hand, Natella et al. reported that postprandial elevations of serum lipid peroxidation and oxidative stress are limited when a meal containing oxidized and oxidizable lipids is consumed together with red wine or with proanthocyanins. Recently, Tsuda et al. also reported that anthocyanins exerted an antioxidant effect on serum peroxidation, but did not affect liver peroxidation in rats. Furthermore, they suggested that the decrease in serum peroxidation in the anthocyanin group was not due to the serum lipid concentration. In the present study, we observed that dietary RPF decreased the serum TBARS level but did not affect the hepatic TBARS level in rats fed the RPF diet (Table 1). This might be due to the fact that anthocyanin is not sufficiently abundant in the liver to influence the TBARS level because anthocyanin is poorly absorbed by the intestine. Additionally, there was no significant difference in serum lipids between the control and RPF groups at the end of the 4-week feeding period (Table 1). During gastrointestinal passage, polyphenols might play a key role in limiting potential postprandial oxidative stress by reducing the absorption of hydroperoxides and by chelating autoxidation-promoting heavy metal ions in the gastrointestinal tract.

Therefore, our findings suggest that anthocyanin-rich RPF might contribute to analogous effects on intestinal lipid peroxidation, thereby decreasing serum lipid peroxidation. Unfortunately, in this study we did not analyze intestinal lipid peroxidation. Scavenging of adverse food constituents might be responsible for regulation of the expression of genes related to oxidative stress or antioxidant defense. Yeh and Yen reported that hepatic and intestinal Cu/Zn-SOD and GSH-Px mRNA expression in rats fed phenolic compound supplementation including ferulic acid were greater than those in a control group. Our findings were

### Table 1. Body Weight, Food Intake, Feed Efficiency, Liver Weight and TBARS Level, and Serum TEAC and TBARS Levels of Rats Fed on Experimental Diets for 4 Weeks

<table>
<thead>
<tr>
<th>Dietary groups</th>
<th>Control</th>
<th>RPF</th>
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<tbody>
<tr>
<td>Initial body weight (g)</td>
<td>187 ± 3</td>
<td>188 ± 3</td>
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<tr>
<td>Body weight gain (g/4 week)</td>
<td>67.3 ± 7.0</td>
<td>63.8 ± 4.6</td>
</tr>
<tr>
<td>Food intake (g/4 week)</td>
<td>448 ± 26</td>
<td>454 ± 46</td>
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<tr>
<td>Feed efficiency (g/g food intake)</td>
<td>0.150 ± 0.008</td>
<td>0.142 ± 0.017</td>
</tr>
<tr>
<td>Liver weight (g)</td>
<td>8.70 ± 0.61</td>
<td>8.62 ± 0.49</td>
</tr>
<tr>
<td>Liver TBARS⁎ (nmol/mg protein)</td>
<td>1.07 ± 0.06</td>
<td>1.01 ± 0.15</td>
</tr>
<tr>
<td>Serum TEAC (IU/ml)</td>
<td>0.792 ± 0.082</td>
<td>0.894 ± 0.167</td>
</tr>
<tr>
<td>Serum TBARS (nmol/l)</td>
<td>2.93 ± 0.14</td>
<td>2.38 ± 0.39⁎</td>
</tr>
<tr>
<td>Serum cholesterol (mg/dl)</td>
<td>79.2 ± 8.2</td>
<td>77.2 ± 5.4</td>
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<tr>
<td>Serum triglyceride (mg/dl)</td>
<td>128.6 ± 12.8</td>
<td>117.4 ± 27.5</td>
</tr>
<tr>
<td>Serum phospholipid (mg/dl)</td>
<td>127.2 ± 12.4</td>
<td>122.8 ± 9.4</td>
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Values are expressed as means ± SD for five rats. *p < 0.05 vs. control as determined by analysis by Student’s t-test.

TBARS, thiobarbituric acid-reactive substances; TEAC, trolox equivalent antioxidant coefficient.
similar to the results of Yeh and Yen, indicating that hepatic Cu/Zn-SOD and Mn-SOD mRNA expression in the RPF group was significantly increased as compared to the control group (Fig. 1), but RPF did not affect the expression of catalase, GSH-Px, or GSH-R mRNA (data not shown). Recently, Nara et al. reported that the ferulic acid in potato tubers has an antioxidant effect in vitro. Thus flavonoids such as ferulic acid as well as anthocyanins in the flakes might increase hepatic SOD mRNA expression. The reason for the increased SOD mRNA in the RPF group, although we do not have data to support it, might be related to up-regulation of hepatic multidrug resistance-associated protein and transcription factor, nuclear factor E2-related factor 2. Among several antioxidant enzymes, Mn-SOD in the mitochondria dismutates toxic superoxide radicals (O$_2^-$) into hydrogen peroxide (H$_2$O$_2$), and plays a role in protecting mitochondria against increased oxidative stress, because mitochondria do not synthesize glutathione or contain catalase to reduce H$_2$O$_2$. Although the various signaling routes responsible for Mn-SOD expression are still being identified, it is believed that an increased level of Mn-SOD is cytoprotective. Therefore, it is likely that the present data might support our previous report, which showed that red potato extract protects against D-galactosamine-induced hepatic toxicity in rats.

In conclusion, our results show that a diet containing anthocyanin-rich red potato flakes has an antioxidant effect on serum lipid peroxidation and hepatic SOD mRNA expression in rats, suggesting that consumption of RPF might lead to altered resistance to postprandial oxidative stress. However, further study is needed to investigate how the colored potato diet affects the intestinal circumstances.

Acknowledgments

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References


Fig. 1. Hepatic Mn-SOD and Cu/Zn-SOD mRNA Expression in Rats Fed Control and Red Potato Flake Diets for 4 Weeks.

Each value represents mean ± standard deviation, depicted by vertical bars (n = 5). **p < 0.01 vs. control as determined by analysis by Student’s t-test. The values for Mn-SOD and Cu/Zn-SOD mRNAs are expressed relative to glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNA in all groups. Representative samples illustrating mRNA levels were measured by RT-PCR and Southern blotting.


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