Anthocyanins in Mesocarp/Epicarp and Endocarp of Fresh Açai (Euterpe oleracea Mart.) and their Antioxidant Activities and Bioavailability

Sayuri AGAWA1,†, Hiroyuki SAKAKIBARA1,†, Rei IWATA1, Kayoko SHIMO1,2, August HERGESHEIMER3 and Shigenori KUMAZAWA1,*

1 Graduate School of Nutritional and Environmental Sciences, University of Shizuoka, Yada 52-1, Suruga-ku, Shizuoka 422-8526, Japan
2 Global COE Program, University of Shizuoka, Yada 52-1, Suruga-ku, Shizuoka 422-8526, Japan
3 Abios Co. Ltd., 1-1-12-1803, Mita, Minato-ku, Tokyo 107-0062, Japan

Received October 2, 2010; Accepted April 7, 2011

We measured anthocyanin levels in fresh açai (Euterpe oleracea Mart.), a native Amazonian palm fruit; cyanidin-3-O-glucoside (C3G) and cyanidin-3-O-rutinoside (C3R) in mesocarp/epicarp portion were 5.49 and 13.0 mg/g extracts, respectively, and these amounts were remarkably higher than that in endocarp. Hydrophilic ORAC assay suggested that açai mesocarp/epicarp extracts had potent antioxidant activity compared to blueberry extract. Following, absorption and excretion of açai anthocyanins were evaluated. After oral administration of açai extracts (400 mg/kg body weight) to rats, C3G and C3R appeared as intact forms in the plasma as maximum amounts of 101.0 ± 55.6 nmol/L at 60 min and 537.0 ± 99.1 nmol/L at 120 min after administration, respectively. Most of these anthocyanins were excreted in urine by 2 h post-administration time. In conclusion, fresh açai contained hydrophilic antioxidants including C3G and C3R, and therefore has strong antioxidant potency especially in the mesocarp/epicarp portion. Upon consumption, açai anthocyanins appeared as intact forms in plasma.

Keywords: açai, anthocyanin, antioxidant activity, ORAC

Introduction

Anthocyanidins are important plant pigments responsible for red, blue and purple colors. Generally, anthocyanidins widely exist as glycoside derivatives, so-called anthocyanins, in colored fruits and vegetables, such as berries (Sakakibara, et al., 2003; Maatta-Riihinen, et al., 2004; Wu, et al., 2006; Koponen, et al., 2007; Ogawa, et al., 2008). Anthocyanidins and anthocyanins have been shown to exhibit a range of biological effects, including antioxidant activity, anticarcinogenesis, induction of apoptosis, anti-obesity, anti-diabetes, and prevention of DNA damage (Rice-Evans, et al., 1996; Sakakibara, et al., 2002; Hou, 2003; Katsube, et al., 2003; Tsuda, et al., 2003; Cooke, et al., 2006; Duthie, 2007; Sasaki, et al., 2007; Tsuda, 2008). Interestingly, recent research suggests that anthocyanins can also prevent oxidative stress resulting from psychological stress (Rahman, et al., 2008). Consequently, the regular consumption of foods rich in anthocyanins has been considered to be associated with a reduced risk of developing chronic diseases (Harborne and Williams, 2000; Zafra-Stone, et al., 2007).

Açai (Euterpe oleracea Mart.) is a palm plant widely distributed in the Amazonian area, especially Brazil. Açai is a multi-stemmed plant as shown in Fig. 1A, and its fruit looks similar to blueberry in appearance. The size of an individual açai fruit is about 1 to 1.5 cm in diameter. An outer, edible layer composed of mesocarp and very thin epicarp covers a lone, fibrous seed, consisting of the endocarp and endosperm (Fig. 1B). A ripened açai appears dark purple in color due to high amounts of anthocyanins, predominantly cyanidin-3-O-glucoside (C3G) and cyanidin-3-O-rutinoside (C3R) (Fig. 2) (Gallori, et al., 2004), indicating that açai might be one anthocyanin-rich food in addition to berries. Basically, açai has been consumed as a raw açai pulp made of the outer (mixture of mesocarp/epicarp and endocarp), edible layer, which is removed after steeping in cool water. Additionally, freeze-dried açai powder is popularly consumed as an additive in...
juice and ice cream (Sabbe, et al., 2009). Recently, some research groups have reported properties of açai: detailed information of constituents including anthocyanins, of taste, and of antioxidant potency using in vitro and in vivo assay systems (Del Pozo-Insfran, et al., 2004; Gallori, et al., 2004; Chin, et al., 2008; Mertens-Talcott, et al., 2008; Pacheco-Palencia, et al., 2008; Sabbe, et al., 2009; Oliveira de Souza, et al., 2010; Sun, et al., 2010). However, all of those groups used frozen açai pulp or its freeze-dried powder for research materials, not fresh açai fruit. One of the reasons considered is the difficulty in its transport from the place of harvest in the Amazonian area to a laboratory. Anthocyanins have been well documented to decompose during storage (Morais, et al., 2002). Additionally, anthocyanin amounts are largely different in the growth area of the plants, i.e. light conditions (Albert, et al., 2009), and leaves and stalks (Muanda, et al., 2009). In this study, we, therefore, obtained fresh açai fruit through a legal channel and transported it to our laboratory, preserved by dry ice. After separation of the mesocarp/epicarp and endocarp of the açai fruits, their anthocyanins levels and antioxidant activities were analyzed. The data obtained was compared with other typical anthocyanin-rich fruits: blueberry and blackcurrant. Furthermore, absorption and excretion of açai anthocyanins were examined after oral administration of açai extracts into rats.

**Materials and Methods**

**Chemicals**

The standard anthocyanins, C3G and C3R were obtained from Extrasynthèse (Genay, France). Trifluoroacetic acid (TFA), 2,2′-azobis(2-amino-propane) dihydrochloride (AAPH), fluorescein, 2,2′-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), 2,4,6- tripyridyl-1,3,5-triazine (TPTZ), and 2,2-diphenyl-1-picylhydrazyl (DPPH) were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Randomly methylated β-cyclodextrine (RMCD) was obtained from Cyclodextrin Research & Development Laboratory, Ltd (Budapest, Hungary). tert-Butylhydroquinone (BHQ) was obtained from Sigma Aldrich, Inc. (St. Louis, MO, USA). All other reagents were of the highest grade available.

**Materials**

Fresh açai fruits and freeze-dried açai powder were provided from Abios Co. Ltd. (Tokyo, Japan). Blackcurrant (Ribes nigrum) was obtained from SICOLY (St. Laurent d’Agy, France). Blueberry (Vaccinium spp.) was obtained from Life Foods Co., Ltd. (Tokyo, Japan). Fresh açai fruits grown at Murutucú Island (State of Para, Brazil) were harvested in September 2007 and separated to mesocarp/epicarp, endocarp, and endosperm (seed). The mesocarp/epicarp and endocarp of the açai, blackcurrant, and blueberry were homogenized respectively in liquid nitrogen, and were lyophilized using a freeze dryer EYELA FD-5N (Tokyo Rikakikai, Co. Ltd., Tokyo, Japan). All freeze-dried samples were stored at 4°C in a desiccator until analysis.

**Extraction of Anthocyanins and Lipophilic Ingredients**

Anthocyanins from açai and the berries were extracted according to the method reported by Ogawa et al. (2008) with some modifications. Briefly, the stored freeze-dried samples (each 200 mg) were added to 8 mL of 80% ethanol containing 0.5% acetic acid. The solution was allowed to stand in a sonicator for 1 min, and the supernatant was recovered by centrifugation at 4,000 rpm for 10 min at 4°C. After extraction three times, the supernatants were gathered and then dried with a freeze dryer. The dried extracts were kept at 4°C in a desiccator protected from light.

For lipophilic oxygen radical absorbance capacity (L-ORAC<sub>FL</sub>) assay, freeze-dried açai and berries (each 2 g) were extracted with 30 mL of hexane to obtain the lipophilic fraction. The hexane solution was allowed to stand in a sonicator for 1 min, and the supernatant was recovered by centrifugation at 4,000 rpm for 10 min at 4°C. After extraction three times, the supernatants were gathered and then dried with a freeze dryer. The dried extracts were kept at 4°C in a desiccator protected from light.

**HPLC-DAD**

Ten milligrams of each extract was dissolved in 1 mL of 50% methanol containing 0.5% BHQ and 0.5% TFA, and filtered through a 0.45 μm membrane filter (Nacalai Tesque, Inc., Kyoto, Japan) to analyze by HPLC with photo-diode array detector (DAD). The HPLC system employed to analyze anthocyanins was a JASCO system control program HSS-1500 (Tokyo, Japan) equipped with
JASCO-BORWIN chromatography data station, pump PU-1580, autosampler AS-1559, column oven CO-1565, and DAD system MD-1510 for monitoring all wavelengths from 200–600 nm. For the column, Capcell Pak ACR (φ4.6 × 250 mm, 5 μm, Shiseido Co., Ltd., Tokyo, Japan) was used at 40°C. For the analysis of acai anthocyanins, linear gradient elution was performed with solution A (0.5% TFA aqueous) and solution B (acetonitrile 0.1% TFA), delivered at a flow rate of 1.0 mL/min as follows: initially 88% of solution A, and then 85% of solution A for the next 25 min. For the analysis of berry anthocyanins, linear gradient elution was performed with solution A (0.5% TFA aqueous) and solution B (acetonitrile containing 0.1% TFA) delivered at a flow rate of 1.0 mL/min as follows: initially 92% of solution A; for the next 50 min, 85% of solution A; for an additional 10 min, 70% of solution A; and for another 5 min, 40% solution A. The injection volume for the extract was 10 μL.

**Folin-Ciocalteu Assay** Total phenolics analysis was based on the Folin-Ciocalteu method (Singleton, et al., 1999). Gallic acid as the standard compound and samples dissolved in 80% ethanol (each 80 μL) were transferred into 96-well plate, and then 80 μL of 10% Na2CO3 solution, the plate was incubated at room temperature for 1 h without shaking under dark conditions. The supernatants obtained by centrifugation at 2,400 rpm for 10 min were transferred to another 96-well plate, and then absorbance measured at 760 nm. The results were expressed as milligrams of gallic acid equivalents (GAE) per gram extract.

**ORACFL Assay** Hydrophilic ORACFL (H-ORACFL) assay and L-ORACFL assays were conducted separately according to the method reported by Wu et al. (2004a, b) with some modifications. For the H-ORACFL assay, 20 μL of Trolox or test samples diluted in 50% ethanol solution was transferred into a 96-well plate, and then 80 μL of 10% phenol reagent was dissolved in 80% ethanol (each 80 μL) were transferred into 1999). Gallic acid as the standard compound and samples dissolved in 80% ethanol (each 80 μL) were transferred into 96-well plate, and then absorbance measured at 760 nm. The results were expressed as milligrams of gallic acid equivalents (GAE) per gram extract (mg GAE/g extracts).

**DPPH Radical Quenching Assay** The DPPH radical quenching assay was carried out according to the method reported by Blois (Blois, 1958). Each sample extract was dissolved in 50% ethanol at a concentration of 2 mg/mL. The sample (150 μL) was added into 3 mL of 100 μmol/L DPPH in ethanol, and absorbance at 517 nm was measured after 30 min at room temperature. The antioxidant activity of the samples was calculated as the DPPH radical quenching activity (%) compared with the data using only DPPH.

**Superoxide Dismutase Assay (SOD) Assay** The levels of SOD-like activity in the extracts were measured using the SOD Assay Kit-WST according to the technical manual provided by Dojindo Molecular Technologies, Inc. (Tokyo, Japan). Each extract was dissolved in a dilution buffer at a concentration of 1 mg/mL. This assay relies on WST-1 [2-(4-iodophenyl)-3-(4-nitrophenyl-5-(2,4-disulfophenyl)-2H-tetrazolium, monosodium salt], which produces a watersoluble formazan dye upon reduction with O2 −, a reaction inhibited by SOD. In a 96-well microplate, 20 μL of sample solution (Sample well and Blank 2 well) or double distilled water (Blank 1 and Blank 3) was mixed with 200 μL of WST working solution. For Blank 2 and Blank 3, 20 μL of dilution buffer was added. Then, 20 μL of enzyme working solution was added to each Sample well and Blank 1 well. The plate was incubated at 37°C for 20 min, and the absorbance was determined at 450 nm using a microplate reader (Model 550, Bio-Rad Laboratories, Hercules, CA). SOD-like activity (inhibition rate, %) was calculated by the following equation:

SOD-like activity (inhibition rate, %) = \[
\frac{[(A_{Blank\,1} - A_{Blank\,3}) - (A_{Sample} - A_{Blank\,2})]}{(A_{Blank\,1} - A_{Blank\,3})} \times 100
\]

where A_{Blank\,1}, A_{Blank\,2}, A_{Blank\,3} and A_{sample} were the absorbances of Blank 1, Blank 2, Blank 3, and Sample wells, respectively.

**ABTS Radical Quenching Assay** The ABTS radical was generated through a chemical oxidation reaction with potassium persulfate as described by Re et al. (1999). 100 mL of 7 mmol/L ABTS solution and 50 mL of 7.35 mmol/L potassium persulfate solution were mixed and left for 12 h at room temperature. The concentration of the ABTS radical solution was adjusted with ethanol to an absorbance at 734 nm from 0.80–0.90. The sample (2 mg/mL) or 0.1 mg/mL Trolox or...
solvent (100 μL) was added into 3 mL of ABTS radical solution, incubated at room temperature for 5 min, and the absorbance at 734 nm was measured immediately. The percentage inhibition of the radical scavenging activity was calculated.

**Ferric Reducing Activity Power (FRAP) Assay** The FRAP assay was carried out as described by Benzie and Strain (Benzie and Strain, 1996) with a slight modification. FRAP reagent consisted of 10 mmol/L TPTZ solution in 40 mmol/L hydrochloric acid, 300 mmol/L sodium acetate buffer (pH 3.6), and 20 mmol/L ferric chloride (III) solution at the ratio of 10:1:1 (v/v/v), respectively. The sample (2 mg/mL) or solvent (100 μL) was added into 3 mL of FRAP reagent, incubated at room temperature for 3 min, and the absorbance at 593 nm was measured immediately. The results were calculated as mg Trolox equivalent/mL.

**Animal Experiments** Male SD rats (6 weeks; Japan SLC, Shizuoka, Japan) were housed in an air-conditioned room (23 ± 1°C) under 12 h dark/12 h light cycles (light on 08:00 – 20:00) with free access to control diet which contained no anthocyanins (10 kcal% fat, D12450B, Research Diets, Inc., New Brunswick, NJ, USA) and tap water. Animals were acclimated to these conditions for 2 weeks before use in experiments. All experimental procedures were in accordance with the guideline of the University of Shizuoka, Japan, for the Care and Use of the Laboratory Animals, based on those of the American Association for Laboratory Animal Science.

**Protocol-1.** Açai extracts prepared according to the methods described above were dissolved in 0.1% citric acid as amounts of 400 mg/mL just before administration to rats. Açai extracts were administered orally to rats (400 mg/kg body weight; 6 rats per group; body weight, 310.3 ± 10.1 g) based on those of the American Association for Laboratory Animal Science. Female SD rats (6 weeks; Japan SLC, Shizuoka, Japan) were housed in an air-conditioned room (23 ± 1°C) under 12 h dark/12 h light cycles (light on 08:00 – 20:00) with free access to control diet which contained no anthocyanins (10 kcal% fat, D12450B, Research Diets, Inc., New Brunswick, NJ, USA) and tap water. Animals were acclimated to these conditions for 2 weeks before use in experiments. All experimental procedures were in accordance with the guideline of the University of Shizuoka, Japan, for the Care and Use of the Laboratory Animals, based on those of the American Association for Laboratory Animal Science.

**Protocol-2.** After 12 h starvation, açai extracts dissolved in 10% citric acid (400 mg/mL) were orally administered to rats (body weights, 324.7 ± 17.2 g) at a rate of 400 mg/kg body weight. Vehicle controls were given the same volume of 10% citric acid. The rats (6 rats per each group) were anesthetized with ether at individual time points (0, 15, 30, 60, 120, and 240 min), and blood was collected from the abdominal vein using heparinized tubes (Venoject II, Terumo, Tokyo, Japan). The plasma was separated by centrifugation at 3,000 rpm for 10 min and acidified by addition of 20 μL 12 mol/L HCl to 1 mL plasma and stored at –80°C for analysis within one month.

**Extraction of Anthocyanins from Plasma and Urine** The extraction procedure was as previously described (Sakakibara, et al., 2009) with some modifications. Each frozen, acidified plasma or urine sample was thawed, and aliquots (500 μL for plasma or urine) were loaded onto OASIS HLB (10 mg) extraction cartridges (Waters Co., Milford, MA, USA), which were equilibrated with 0.01 mol/L oxalic acid. After washing the cartridge with 2 mL of 0.01 mmol/L oxalic acid, anthocyanins were eluted with 1 mL of methanol containing 0.5% TFA. The eluate was evaporated to dryness using a centrifugal concentrator (VC-96N, Taitec Co., Saitama, Japan). The residue was then dissolved in 150 μL of methanol containing 0.5% TFA, filtered with a 0.45 μm membrane filter, and analyzed by HPLC as described above.

**Results and Discussion**

**Polyphenol and Anthocyanin Amounts in Açai** Total polyphenol and anthocyanin amounts in the edible mesocarp/epicarp and fibrous endocarp, which were separated from fresh açai fruit, were analyzed by HPLC-DAD system, and their levels were compared with commercially available freeze-dried açai powder and anthocyanin-rich blueberry and blackcurrant. Figure 3A shows the typical HPLC chromatogram of the freeze-dried açai powder at 520 nm. Two major peaks appeared and were identified as C3G and cyanidin-3-O-rutinoside C3R, by means of their retention times and spectra as compared to those of the commercially available anthocyanins. Other anthocyanidins and anthocyanins were under the detection limit in the açai samples used in this study. This agrees with the results reported by Gallori (Gallori, et al., 2004). As shown in Table 1, the total amounts of anthocyanins in the edible mesocarp/epicarp and fibrous endocarp were 18.5 mg/g extracts (C3G, 5.49 mg/g; C3R, 13.0 mg/g) and 1.64 mg/g extracts (C3G, 0.39 mg/g; 1.25 mg/g), respectively. Therefore, anthocyanins existing in açai were distributed ten times higher in the edible mesocarp/epicarp than in the fibrous endocarp. On the other hand, total polyphenol levels that included these anthocyanins were within a similar range: 81.2 in skin and 61.9 mg GAE/g extracts.

Commercially processed açai pulp, which contains mesocarp/epicarp and certain amounts of endocarp, has been reported to contain various polyphenols, including apigenin, protocatechuic acid methyl ester, and dihydroconiferyl alcohol (Chin, et al., 2008). Açai endocarp fibers may possibly contain high amounts of certain polyphenols other than the anthocyanins found in the mesocarp. In fact, total polyphenol
and anthocyanin contents of freeze-dried açai powder were higher than that of the mesocarp/epicarp portion itself utilized in this study, although the freeze-dried açai powder was a mixture of mesocarp/epicarp and anthocyanin-poor endocarp.

**Antioxidant Activities of Açai** The antioxidant activities of the mesocarp/epicarp and endocarp portions of açai were evaluated using six types of ordinary antioxidant evaluation methods, H-ORAC<sub>FL</sub>, L-ORAC<sub>FL</sub>, DPPH and ABTS radical quenching assay, FRAP assay, and SOD-like activity (Table 2). ORAC<sub>FL</sub> assay, which is the abbreviation of Oxygen Radical Absorbance Capacity assay with fluorescein, was designed to measure the antioxidant capacity of foods toward peroxyl radical. A wide variety of foods have been tested using this method as Trolox equivalent (TE) (Nutrient Data Laboratory, November 2007), and therefore ORAC is considered to be one of most international, standardized methods for antioxidant potency of foods. In this study, we employed 80% ethanol extracts from individual fruits for evaluation of these antioxidant assays in order to make the sample of everything even in all experiments evaluated in this study. H-ORAC<sub>FL</sub> and L-ORAC<sub>FL</sub> values in freeze-dried açai powder were 6334 and 21.3 μmol TE/g extracts, respectively.

Interestingly, açai mesocarp/epicarp indicated higher results for both H- and L-ORAC<sub>FL</sub> activities than those of freeze-dried açai powder, although mesocarp/epicarp contained less anthocyanins compared to freeze-dried açai powder (Table 1). Trolox at 3.2 μg/mL in the reaction mixture (ca 12.9 μmol/L) quenched DPPH and ABTS radicals by 47% and 20%, respectively, comparable to previous results (Payet, et al., 2005). In the present study, antioxidant potencies by DPPH and ABTS radical scavenging activity, FRAP assay and SOD like activity, in extracts from açai mesocarp/epicarp, endocarp, and freeze-dried açai powder, were compared at the same concentration of 2 mg extracts/mL. In all antioxidant assays except ABTS radical quenching assay, mesocarp/epicarp extracts showed stronger activity than those of endocarp or freeze-dried açai powder. These results obtained in this study indicate that açai, at least the 80% ethanol extract, might exert a strong antioxidant capacity compared with other well known antioxidative plants, blueberry and blackcurrant, because of their relatively higher

### Table 1. Contents of total polyphenols, anthocyanins, and lypophilic amounts in açai, blueberry, and blackcurrant used in this study.

<table>
<thead>
<tr>
<th>Samples</th>
<th>Polyphenols&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Anthocyanins (mg/g extracts)</th>
<th>Lypophilic amounts (mg/g extracts)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total</td>
<td>C3G</td>
<td>C3R</td>
</tr>
<tr>
<td>Açai</td>
<td>91.8</td>
<td>34.1</td>
<td>11.8</td>
</tr>
<tr>
<td>Mesocarp/Epicarp</td>
<td>81.2</td>
<td>18.5</td>
<td>5.49</td>
</tr>
<tr>
<td>Endocarp</td>
<td>61.9</td>
<td>1.64</td>
<td>0.39</td>
</tr>
<tr>
<td>Blueberry&lt;sup&gt;c&lt;/sup&gt;</td>
<td>25.6</td>
<td>10.21</td>
<td>0.03</td>
</tr>
<tr>
<td>Blackcurrant&lt;sup&gt;c&lt;/sup&gt;</td>
<td>47.5</td>
<td>27.53</td>
<td>1.27</td>
</tr>
</tbody>
</table>

<sup>a</sup> The results were expressed as milligrams of gallic acid equivalents (GAE) per gram extract (mg GAE/g extract).

<sup>c</sup> Freeze-dried açai powder was composed of the edible layers of açai including mesocarp and endocarp, removed after steeping in water.

### Table 2. Antioxidant activities of açai, blueberry, and blackcurrant used in this study.

<table>
<thead>
<tr>
<th>Samples</th>
<th>ORAC&lt;sub&gt;FL&lt;/sub&gt;</th>
<th>DPPH</th>
<th>ABTS</th>
<th>SOD</th>
<th>FRAP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Açai</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Freeze-dried açai powder</td>
<td>6334</td>
<td>21</td>
<td>92.0</td>
<td>96.0</td>
<td>81</td>
</tr>
<tr>
<td>Mesocarp/Epicarp</td>
<td>6605</td>
<td>37</td>
<td>85.4</td>
<td>72.0</td>
<td>77.5</td>
</tr>
<tr>
<td>Endocarp</td>
<td>4832</td>
<td>57</td>
<td>57.2</td>
<td>86.9</td>
<td>76.7</td>
</tr>
<tr>
<td>Blueberry</td>
<td>918</td>
<td>253</td>
<td>28.0</td>
<td>14.0</td>
<td>45</td>
</tr>
<tr>
<td>Blackcurrant</td>
<td>610</td>
<td>148</td>
<td>52.0</td>
<td>31.0</td>
<td>68.3</td>
</tr>
<tr>
<td>Trolox</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>47.0</td>
<td>20.0</td>
</tr>
</tbody>
</table>

Each freeze-dried fruit was extracted with 80% ethanol containing 0.5% acetic acid, and then the extracts underwent individual antioxidant assay.

* Hydrophilic ORAC<sub>FL</sub> (H-ORAC<sub>FL</sub>) assay and lipophilic ORAC<sub>FL</sub> (L-ORAC<sub>FL</sub>) assay were expressed as micromoles of Trolox equivalents (TE) per gram extracts of sample (μmol TE/g extracts).

* Açai and berry extracts (2 mg/mL) and Trolox (0.1 mg/mL) were individually used for each method. Antioxidant potencies were indicated as % of radical quenching activity (DPPH and ABTS) and mg TE/mL (FRAP) as described in Materials and Methods. SOD-like activity was indicated as inhibition rate (%).
amounts of anthocyanins. Basically, polyphenol content in açai dramatically changes depending on the time of harvest even when from the same growing area and stage (Rodrigues, et al., 2006). Additionally, amounts of flavonoid in botanical plants were considered to be vastly different among cultivated area (Rodriguez Galdon, et al., 2008). Accordingly, amounts of polyphenols including anthocyanins in fresh açai might be a little less than that of freeze dried one used in this study because of a different cultivated area and/or growing stage. We should, therefore, investigate in our future experiments the seasonal and regional differences of polyphenols and anthocyanins existing in fresh açai (mesocarp/epicarp and endocarp) harvested from the same or different cultivation area in order to conclude that açai might actually be one of the potent antioxidant foods.

Absorption Profiles of Açai Anthocyanins in Plasma and Urine after Single Administration

The concentration profiles of C3G and C3R in rat plasma and urine after consumption of 400 mg açai extracts per kg body weight (26.3 μmol C3G and 37.5 μmol C3R/kg body weight) were investigated by HPLC-DAD. In this experiment, we used açai extracts obtained from freeze-dried açai powder, because adequate amounts of fresh açai fruit could not be procured. Figure 3 shows typical HPLC chromatograms at 520 nm for intact açai extracts (A), rat plasma at 120 min after an oral dose of the extracts (B), and rat urine collected during 2 h and 4 h after an oral dose of the extracts (C). The peak pattern was quite similar during all chromatograms, indicating that açai anthocyanins were mainly absorbed into the body as intact anthocyanins. We could not find detectable amounts of anthocyanins in plasma obtained from rats administered vehicle solvent (data not shown). These results paralleled those reported by Mertens-Talcott et al. (2008) for healthy human volunteers. The plasma concentration of C3R reached a maximum of 537.0 ± 99.1 μmol/L after 120 min of administration and then sharply decreased almost to basal levels after 240 min (Fig. 4). On the other hand, C3G levels in plasma reached a plateau at 30 min after administration, and continued with the same levels until 120 min post-administration (81.7 ± 9.1 μmol/L at 30 min, 101.0 ± 55.6 μmol/L at 60 min, and 86.0 ± 38.4 μmol/L at 120 min). Matsumoto and co-workers reported that when C3G or C3R was independently orally administered to rats, both anthocyanins appeared in the plasma at 15 min after administration (Matsumoto, et al., 2001). After this period, C3G was immediately diminished from plasma, but C3R increased in the period up to 120 min post-administration and then gradually decreased. They hypothesized that these differences between C3G and C3R might be because of sugar moiety conjugated cyanidin: the decrease in the levels of the rutinosides was less than that in the case of the glucosides (Matsumoto, et al., 2001). Our results on C3R existing in açai agree with their results, but the absorption of C3G indicated a somewhat different pattern. Absorption patterns of anthocyanins have been reported to be strongly affected by other ingredients. For example, when phytic acid is administered with anthocyanins, phytic acid enhances gastrointestinal absorption of anthocyanins (Matsumoto, et al., 2007). Hence, açai probably contains some ingredients to enhance and/or change the absorption patterns of anthocyanins, especially C3G.

![Fig. 4. Time-dependent amounts in the plasma of anthocyanins which were orally administrated as a single dose of 400 mg açai extracts/kg body weight (26.3 μmol C3G and 37.5 μmol C3R/kg body weight). Analysis was by HPLC as described in the Materials and Methods. The concentrations of C3G (■) and C3R (○) were calculated and summed. Values were indicated as mean ± S.D (n = 6). C3G, cyanidin-3-O-glucoside; C3R, cyanidin-3-O-rutinoside.](image)

![Fig. 5. Time-dependent excretion of anthocyanins in the urine that were orally administered as a single dose of 400 mg açai extracts/kg body weight (26.3 μmol C3G and 37.5 μmol C3R/kg body weight). Analysis was by HPLC as described in the Materials and Methods. The concentrations of C3G (■) and C3R (○) were calculated and summed. Values were indicated as mean ± S.D (n = 6). C3G, cyanidin-3-O-glucoside; C3R, cyanidin-3-O-rutinoside.](image)
After administration of 400 mg açai extracts per kg body weight to rats, their urine was collected at 2 h intervals 6 times, and then a one time 12 h interval. The urinary anthocyanins were analyzed by HPLC-DAD (Fig. 3C). Açai anthocyanins, C3G and C3R, administered orally appeared as intact forms in the urine. Most of the anthocyanins were excreted into the urine between 0 to 6 h after administration, and only small quantities were excreted after 6 h of administration (Fig. 5). The average quantity of C3G and C3R excreted in the urine during 24 h after administration represented 0.60 ± 0.08% and 1.00 ± 0.11% of the individual anthocyanins ingested. Hence, we guessed that C3R in açai might exert strong bioavailability compared with C3G. Some researchers reported that small amounts of anthocyanin metabolites, for example methylated derivatives, were detected in the urine from rats administered anthocyanin rich berries (He, et al., 2006). However, we could not find detectable amounts of anthocyanin metabolites in urine in this study, indicating that most of C3G and C3R existing in açai might be absorbed immediately into the body as an intact forms but with a slight delay in the case of C3G when compared with consumption of C3G alone. The anthocyanins are circulated in the blood stream, and then immediately excreted into urine up to 6 h after administration.

In conclusion, this study first reported the anthocyanin and total polyphenol levels as well as the antioxidative activities of the mesocarp/epicarp and endocarp portions of fresh açai. The amounts of anthocyanins and total polyphenols in the mesocarp/epicarp were remarkably higher than that of the endocarp. Additionally, these amounts were significantly higher than that of anthocyanin-rich blueberry and blackcurrant. Furthermore, we suggest that intact açai anthocyanins, C3G and C3R, are absorbed and circulated in the blood stream.

References
Morais, H., Ramos, C., Forgacs, E., Cserhati, T. and Oliviera, J.


