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

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Running title: Antioxidant activity and bioavailability of açai

ABSTRACT

Açai (*Euterpe oleracea* Mart.) is a native Amazonian palm fruit, and widely consumed in South America, mainly Brazil. A ripened açai indicates a dark purple color, due to anthocyanins with predominance of cyanidin-3-*O*-glucoside (C3G) and cyanidin-3-*O*-rutinoside (C3R). In this study, we first separated fresh açai to mesocarp/epicarp and endocarp portions and measured their anthocyanin levels. In addition, their antioxidant activities were analyzed by using various assays, hydrophilic and lipophilic oxygen radical absorbance capacity (H-ORAC$_{FL}$ and L-ORAC$_{FL}$), free radical scavenging capacity by 1,1′-diphenyl-2-picrylhydrazyl (DPPH) and 2,2′-azinobis(3-ethybenzothiazoline-6-sulfonic acid) (ABTS), ferric reducing antioxidant power (FRAP), and superoxide dismutase (SOD) like activity. Levels of C3G and C3R in mesocarp/epicarp portion were 5.49±1.52 and 13.0±3.93 mg/g extracts, respectively, and these amounts were remarkably higher than that in endocarp (0.39±0.04 and 1.25±0.06 mg/g extracts). H-ORAC$_{FL}$ value in mesocarp/epicarp was about 150 times higher than L-ORAC$_{FL}$. Açai mesocarp/epicarp extracts showed potent antioxidant activity compared to blueberry extract on every antioxidant assay employed in this study except for L-ORAC$_{FL}$. Next, absorption and exclusion of açai anthocyanins orally administered to rats were evaluated. After oral administration of açai extracts (400 mg/kg body weight), both anthocyanins appeared intact in the plasma. Their plasma concentrations of C3G and C3R reached a maximum of 101.0±55.6 nM at 60 min and 537.0±99.1 nM at 120 min after administration, respectively, and then sharply decreased. Their urinary excretion was highest between 0 h and 2 h after administration, and had ceased by 24 h. The total

quantities of açai anthocyanins excreted into urine represented 0.6±0.1% in C3G and 1.0±0.1% in C3R of consumed anthocyanins. 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.

KEY WORDS

Açai, anthocyanin, antioxidative 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 (*1-5*). 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 (*6-14*). Interestingly, a recent research suggested that anthocyanins can also prevent oxidative stress resulting from psychological stress (*15*). Consequently, the regular consumption of foods rich in anthocyanins has been considered to be associated with a reduced risk of developing chronic diseases (*16, 17*).

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 **Figure 1A**, and its fruit, in appearance, looks similar to blueberry. The size of an individual açai fruit is around 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 (**Figure 1B)**. A ripened açai indicates a dark purple color, due to high amounts of anthocyanins with predominance of cyanidin-3-*O*-glucoside (C3G) and cyanidin-3-*O*-rutinoside (C3R) (*18*), indicating that açai might be one of anthocyanin-rich foods in addition to berries. Basically, açai has been consumed as a raw açai pulp made of the outer, 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 (*19*). Recently, some research groups have reported properties of açai; for example, detailed information of constituents including

anthocyanins, of taste, and of antioxidant potency using *in vitro* and *in vivo* assay systems (*18-25*). However, all of them used for research materials are frozen açai pulp or its freeze-dried powder, 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 (*26*). Additionally, anthocyanin amounts are largely different in the growth area of the plants, i.e. light conditions (*27*) and leaves and stalks (*28*). 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, cyanidin-3-*O*-glucoside and cyanidin-3-*O*-rutinoside were obtained from Extrasynthèse (Genay, France). Trifluoroacetic acid (TFA), 2,2′-azobis(2-amino-propane)dihydrochloride (AAPH), fluorescein, 2,2′-azinobis(3-ethybenzothiazoline-6-sulfonic acid) (ABTS), 2,4,6-tripyridyl-1,3,5-triazine (TPTZ), and 1,1-diphenyl-2-picrylhydrazyl (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,

76 Hungary). *tert*-Butylhydroquinone (BHQ) was from Sigma Aldrich, Inc. (St. Louis, MO).
77 All other reagents were of the highest grade available.
78 **Materials.** Fresh açai fruits and freeze-dried açai powder were provided from Abios Co.
79 Ltd. (Tokyo, Japan). Blackcurrant (*Ribes nigrum*) was obtained from SICOLY (St. Laurent
80 d' Agny, France). Blueberry (*Vaccinium* spp.) was from Life Foods Co., Ltd. (Tokyo, Japan).
81 Fresh açai fruits were separated to mesocarp/epicarp, endocarp, and endosperm (seed). The
82 mesocarp/epicarp and endocarp as shown in **Figure 1**, blackcurrant, and blueberry were
83 homogenized respectively in liquid nitrogen, and were lyophilized using a freeze dryer
84 EYELA FD-5N (Tokyo Rikakikai, Co. Ltd., Tokyo, Japan). All freeze-dried samples were
85 stored at 4ºC in a desiccator until analysis.
86 **Extraction of Anthocyanins and Lipophilic Ingredients.** Anthocyanins from açai and
87 the berries were extracted according to the method reported by Ogawa *et al*. with some
88 modifications (*5*). Briefly, the stored freeze-dried samples (each 200 mg) were added to 8
89 mL of 80% ethanol containing 0.5% acetic acid. The solution was allowed to stand in a
90 sonicator for 1 min, and the supernatant was recovered by centrifugation at 4,000 rpm for
91 10 min under 4ºC. After extraction three times, the supernatants were gathered, and then
92 dried with a freeze dryer. The dried extracts were kept at 4ºC in a desiccator with protection
93 from light.
94 For lipophilic oxygen radical absorbance capacity (L-ORAC$_{FL}$) assay, freeze-dried açai
95 and berries (each 2 g) were extracted with 30 mL of hexane to obtain the lipophilic fraction.
96 The hexane solution was allowed to stand in a sonicator for 1 min, and the supernatant was
97 recovered by centrifugation at 4,000 rpm for 10 min under 4ºC. After extraction three times,

98 the supernatant was dried by evaporator under reduced pressure. The dried extracts were
99 kept at 4ºC in a desiccator with protection from light.
100 **HPLC-DAD.** Ten milligrams of each extract was dissolved in 1 mL of 50% of methanol
101 containing 0.5% BHQ and 0.5% TFA, and filtered through a 0.45 μm membrane filter
102 (Nacalai Tesque, Inc., Kyoto, Japan) to analyze by HPLC with photo-diode array detector
103 (DAD). The HPLC system employed to analyze anthocyanins was a JASCO system control
104 program HSS-1500 (Tokyo, Japan) equipped with JASCO-BORWIN chromatography data
105 station, pump PU-1580, autosampler AS-1559, column oven CO-1565, and DAD system
106 MD-1510 for monitoring at all wavelengths from 200–600 nm. For the column, Capcell
107 Pak ACR (ϕ4.6 x 250 mm, 5 μm, Shiseido Co. Ltd., Tokyo, Japan) was used at 40ºC. For
108 the analysis of açai anthocyanins, linear gradient elution was performed with solution A
109 (0.5% TFA aqueous) and solution B (acetonitrile 0.1% TFA) delivered at a flow rate of 1.0
110 mL/min as follows: initially 88% of solution A then for the next 25 min 85% A. For the
111 analysis of berry anthocyanins, linear gradient elution was performed with solution A (0.5%
112 TFA aqueous) and solution B (acetonitrile containing 0.1% TFA) delivered at a flow rate of
113 1.0 mL/min as follows: initially 92% of solution A; for the next 50 min, 85% A; for another
114 10 min, 70% A; for another 5 min, 40% A. The injection volume for the extract was 10 μL.
115 **Folin-Ciocalteu Assay.** Total phenolics analysis was based on the Folin-Ciocalteu
116 method (*29*). Gallic acid as standard compound and samples dissolved in 80% ethanol
117 (each 80 μL) were transferred into 96-well plate, and then 80 μL of 10% phenol reagent
118 was added in each well. After addition of 80 μL of 10% Na_2CO_3 solution, the plate was
119 incubated at room temperature for 1 h without shacking under a dark condition. The

supernatants obtained by centrifugation at 2,400 rpm for 10 min were transferred to another 96-well plate, and then immediately measured absorbance at 760 nm. The results were expressed as milligrams of gallic acid equivalents (GAE) per gram extract (mg GAE/g extracts).

$ORAC_{FL}$ Assay. Hydrophilic $ORAC_{FL}$ (H-$ORAC_{FL}$) assay and L-$ORAC_{FL}$ assays were conducted separately according to the method reported by Wu *et al*. with some modifications (*30, 31*). For the H-$ORAC_{FL}$ assay, 20 μL of Trolox or test samples diluted in 50% ethanol solution was transferred to a 96-well plate, and then 150 μL of 8.38 nM fluorescein was added. After 10 min of incubation at 39^{o}C, 25 μL of 153 mM AAPH diluted in 75 mM phosphate buffer (pH 7.4) was added in each well, and then immediately measured fluorescence (Ex. 485 nm, Em. 528 nm) at one minute intervals for 60 consecutive measurements using Flex Station II (Molecular Device, Inc., Silicon Valley, CA, USA)

For the L- $ORAC_{FL}$ assay, 10 μL of Trolox and test sample diluted in 7% RMCD solution were transferred to a 96-well plate, and then 100 μL of 7.5 nM Fluorescenin solution was added. After 10 min of incubation at 39^{o}C, 37.5 μL of 63.5 mM AAPH buffer solution was added in each well, and immediately measured fluorescence (ex. 485 nm, em. 528 nm) at one-minute intervals for 60 consecutive measurements.

Individual $ORAC_{FL}$ values were calculated by using a quadratic regression equation between the Trolox or sample concentration and net area under the fluorescence decay curve. Data are expressed as micromoles of Trolox equivalents (TE) per gram extract of sample (μmol TE/g extracts). The area under the curve (AUC) was calculated. The data

were analyzed using a Soft®MaxPro4.7 (Molecular Devices, Inc., Silicon Valley, CA).

DPPH Radical Quenching Assay. The DPPH radical quenching assay was carried out according to the method reported by Blois (*32*). Each sample extract was dissolved in 50% ethanol at a concentration of a 2 mg/mL. The sample (150 μL) was added into 3 mL of 100 μM 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.

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)-2*H*-tetrazolium, monosodium salt], which produce a water-soluble formazan dye upon reduction with O_2^-, 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 37ºC 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, %)

$$= \{[(A_{Blank\ 1} - A_{Blank\ 3}) - (A_{Sample} - A_{Blank\ 2})]/(A_{Blank\ 1} - A_{Blank\ 3})\} \times 100$$

where $A_{Blank\ 1}$, $A_{Blank\ 2}$, $A_{Blank\ 3}$, and A_{Sample} were the absorbance 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* (*33*). 100 mL of 7 mM ABTS solution and 50 mL of 7.35 mM 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 (*34*) with a slight modification. FRAP reagent consisted of 10 mM TPTZ solution in 40 mM hydrochloric acid, 300 mM sodium acetate buffer (pH 3.6), and 20 mM 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 ±1ºC) under 12 h dark/12 h light cycles (light on 8:00–20:00) 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

186 procedures were in accordance with the guideline of the University of Shizuoka, Japan, for
187 the Care and Use of the Laboratory Animals, based on those of the American Association
188 for Laboratory Animal Science.
189 Protocol-1. Açai extracts prepared according to the methods described above were
190 dissolved in 0.1% citric acid as amounts of 400 mg/mL just before the administration to rats.
191 Açai extracts were administered orally to rats (400 mg/kg body weight; 6 rats per group)
192 with prior starvation for 12 h and their urine was collected over six consecutive 2 h periods,
193 and then collected during 12–24 h after administration. Vehicle controls were given same
194 volume of 10% citric acid. The collection bottle, which was protected from light, contained
195 2 mL of 10% citric acid. The volume of urine was measured at each sample time and the
196 urine was stored at –20°C after acidification by addition of 350 μL of 6 M HCl. After 12 h
197 of administration of açai extracts, the control diet which contained no anthocyanins was
198 given to rats.
199 Protocol-2. After 12 h starvation, açai extracts dissolved in 10% citric acid (400 mg/mL)
200 were orally administered to rats at a rate of 400 mg/kg body weight. Vehicle controls were
201 given same volume of 10% citric acid. The rats (6 rats per each group) were anesthetized
202 with ether at individual time points (0, 15, 30, 60, 120, and 240 min) and blood was
203 collected from the abdominal vein using heparinized tubes (Venoject II, Terumo, Tokyo,
204 Japan). The plasma was separated by centrifugation at 3,000 rpm for 10 min and acidified
205 by addition of 20 μL 12 M HCl to 1 mL plasma, and then stored at –80^{o}C for analysis
206 within one month.
207 **Extraction of Anthocyanins from Plasma and Urine.** The extraction procedure was as

208 previously described (*35*) with some modifications. Each frozen, acidified plasma or urine
209 sample was thawed, and aliquots (500 μL for plasma or urine) were loaded onto OASIS
210 HLB (10 mg) extraction cartridges (Waters Co., Milford, MA), which was equilibrated with
211 0.01 M oxalic acid. After washing the cartridge with 2 mL of 0.01 mM oxalic acid,
212 anthocyanins were eluted with 1 mL of methanol containing 0.5% TFA. The eluate was
213 evaporated to dryness using a centrifugal concentrator (VC-96N, Taitec Co., Saitama,
214 Japan). The residue was then dissolved in 150 μL of methanol containing 0.5% TFA,
215 filtered with a 0.45 μm membrane filter, and analyzed by high performance liquid
216 chromatography (HPLC) as described above.
217
218 **RESULTS AND DISCUSSION**
219 **Polyphenol and Anthocyanin Amounts in Açai.** Total polyphenol and anthocyanin
220 amounts in the edible mesocarp/epicarp and fibrous endocarp, which were separated from
221 fresh açai fruit, were analyzed by HPLC-DAD system, and their levels were compared with
222 commercially available freeze-dried açai powder and anthocyanin-rich blueberry and
223 blackcurrant. **Figure 3A** shows the typical HPLC chromatogram at 520 nm of the freeze-
224 dried açai powder. Two major peaks appeared and were identified as
225 cyanidin-3-*O*-glucoside (C3G) and cyanidin-3-*O*-rutinoside (C3R), by means of their
226 retention times and spectra as compared to those of the commercially available
227 anthocyanins. Other anthocyanidins and anthocyanins were under the detection limit in the
228 açai samples used in this study. This agrees with the results reported by Gallori *et al* (*18*).
229 As shown in **Table 1**, the total amounts of anthocyanins in the edible mesocarp/epicarp and

fibrous endocarp were 18.5±5.5 mg/g extracts (C3G, 5.49±1.5 mg/g; C3R, 13.0±3.9 mg/g) and 1.64±0.10 mg/g extracts (C3G, 0.39±0.04 mg/g; 1.25±0.06 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±9.5 in skin and 61.9±6.1 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 (*20*). 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.

Basically, polyphenol contents in açai dramatically changes depending on the time of harvest even when from the same growing area and stage (*36*). Additionally, amounts of flavonoid in botanical plants were considered to be largely different among cultivated area (*37*). 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 different cultivated area and/or growing stage. We should, therefore, investigate in our future experiments the seasonal differences of polyphenols and anthocyanins existing in fresh açai (mesocarp/epicarp and endocarp) harvested from the same cultivation area.

252 **Antioxidant Activities of Açai.** The antioxidant activities of the mesocarp/epicarp and
253 endocarp portions of açai were evaluated using six types of ordinary antioxidant evaluation
254 methods, H-ORAC$_{FL}$, L-ORAC$_{FL}$, DPPH and ABTS radical quenching assay, FRAP assay,
255 and SOD-like activity (**Table 2**). ORAC$_{FL}$ assay, which is the abbreviation of Oxygen
256 Radical Absorbance Capacity assay with fluorescein, was designed to measure the
257 antioxidant capacity of foods toward peroxyl radical. A wide variety of foods have been
258 tested using this method as Trolox equivalent (TE) (*38*), and therefore ORAC is considered
259 to be one of most international, standardized methods for antioxidant potency of foods.
260 H-ORAC$_{FL}$ and L-ORAC$_{FL}$ values in freeze-dried açai powder were 6,334±606 and 21.3±3
261 μmol TE/g extracts, respectively. Interestingly, açai mesocarp/epicarp indicated higher
262 results for both H- and L-ORAC$_{FL}$ activities than those of freeze-dried açai powder,
263 although mesocarp/epicarp contained less anthocyanins compared to freeze-dried açai
264 powder (**Table 1**). Trolox at 3.2 μg/mL in the reaction mixture (*ca* 12.9 μM) quenched
265 DPPH and ABTS radicals by 47% and 20%, respectively, comparable to previous results
266 (*39*). In the present study, antioxidant potencies by DPPH and ABTS radical scavenging
267 activity, FRAP assay and SOD like activity, in extracts from açai mesocarp/epicarp,
268 endocarp, and freeze-dried açai powder, were compared at the same concentration of 2 mg
269 extracts/mL. In all antioxidant assays except ABTS radical quenching assay,
270 mesocarp/epicarp extracts showed stronger activity than those of endocarp or freeze-dried
271 açai powder. These results indicate that the antioxidant capacity of açai may be remarkably
272 higher than that of the other well known, antioxidative plants, blueberry and blackcurrant.
273 Consequently, we suggest in this study that açai has the possibility of being 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 corrected during 2 hr and 4 hr 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 administrated vehicle solvent (data not shown). These results paralleled those reported by Mertens-Talcott *et al*. for human healthy volunteers (*22*). The plasma concentration of C3R reached a maximum of 537.0±99.1 μM after 120 min of administration and then sharply decreased almost to the basal levels after 240 min (**Figure 4**). On the other hand, C3G levels in plasma were reached plateau at 30 min of administration, and continued same levels until 120 min of administration (81.7±9.1 μM at 30 min, 101.0±55.6 μM at 60 min, and 86.0±38.4 μM 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 (*40*). 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

296 differences between C3G and C3R might be because of sugar moiety conjugated cyanidin:
297 the decrease in the levels of the rutinosides was gentler than that in the case of the
298 glucosides (*40*). Our results on C3R existing in açai agree with their results, but the
299 absorption of C3G indicated a somewhat different pattern. Absorption patterns of
300 anthocyanins have been reported to be strongly affected by other ingredients. For example,
301 when phytic acid is administered with anthocyanins, phytic acid enhances gastrointestinal
302 absorption of anthocyanins (*41*). Hence, açai probably contains some ingredients to
303 enhance and/or change the absorption patterns of anthocyanins, especially C3G.
304 After administration of 400 mg açai extracts per kg body weight to rats, their urine
305 was collected at 2 hr interval for 6 times, and then 12 hr for one time. The urinary
306 anthocyanins were analyzed by HPLC-DAD (Figure 3C). Açai anthocyanins, C3G and C3R,
307 administered orally were appeared as intact forms in the urine. Most of the anthocyanins
308 were excreted into the urine between 0 to 6 hr after administration, and only small
309 quantities were excreted after 6 hr of administration (**Figure 5**). The average quantity of
310 C3G and C3R excreted in the urine during 24 hr after administration represented
311 0.60±0.08% and 1.00±0.11% of the individual anthocyanins ingested. Hence, we guessed
312 that C3R in açai might exert strong bioavailability compared with C3G. Some researchers
313 reported that small amounts of anthocyanin metabolites, for example methylated
314 derivatives, were detected in the urine from rats administered anthocyanin rich berries (*42*).
315 However, we could not find detectable amounts of anthocyanin metabolites in urine in this
316 study, indicating that most of C3G and C3R existed in açai might be absorbed to body as
317 their intact forms immediately but a with a slight delay in the case of C3G compared with

318 consumption of C3G singly, circulated in the blood stream, and then immediately excreted
319 into urine until 6 hours after the administration.
320 In conclusion, this study first reported the anthocyanin and total polyphenol levels
321 and the antioxidative activities of the mesocarp/epicarp and endocarp portions of fresh açai.
322 The amounts of anthocyanins and total polyphenols in the mesocarp/epicarp were
323 remarkably higher than that of the endocarp. Additionally, these amounts were significantly
324 higher than that of anthocyanin-rich blueberry and blackcurrant. Furthermore, we suggested
325 that the intact açai anthocyanins, C3G and C3R, were absorbed and circulated in the blood
326 stream.
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FIGURE LEGENDS

Figure 1. Growing açai in Amazonian area, Brazil (**A**), and cross-sectional view of açai fruit (**B**).

Figure 2. Chemical structures of anthocyanins in açai.
(**A**) cyanidin-3-*O*-glucoside and (**B**) cyanidin-3-*O*-rutinoside.

Figure 3. Typical HPLC profiles of the extracts of freeze-dried açai powder, plasma, and urine at 520 nm. (**A**) Extracts of freeze-dried açai powder, (**B**) rat plasma sample collected 120 min after an oral dose of açai extracts, and (**C**) rat urine collected between 2 to 4 h after an oral dose of açai extracts. C3G, cyanidin-3-*O*-glucoside; C3R, cyanidin-3-*O*-rutinoside.

Figure 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.

Figure 5. Time-dependent excretion in the urine of anthocyanins 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.

491 Table 1. Contents of Total Polyphenols, Anthocyanins, and Lypophilic Amounts in Each

492 Sample

Samples	Polyphenols [a]	Anthocyanins (mg/g extracts)			Lypophilic amounts
		Total	C3G	C3R	(mg/g extracts)
Açai					
Freeze-dried açai powder[b]	91.8±6.8	34.1±2.2	11.8±1.0	22.3±1.2	0.330
Mesocarp/Epicarp	81.2±9.5	18.5±5.5	5.49±1.5	13.0±3.9	0.072
Endocarp	61.9±6.1	1.64±0.10	0.39±0.04	1.25±0.06	0.049
Blueberry[c]	25.6±0.2	10.21±0.54	0.03±0.00	u.d.	0.006
Blackcurrant[c]	47.5±0.6	27.53±0.99	1.27±0.03	8.24±0.28	0.006

493 [a]The results were expressed as milligrams of gallic acid equivalents (GAE) per gram

494 extract (mg GAE/g extracts).

495 [b]Freeze-dried açai powder was composed of the edible layers of acai including mesocarp

496 and endocarp, removed after steeping in water.

497 [c]Blueberry and blackcurrant contain other anthocyanins: delphinidin-glycosides,

498 peonidin-glycosides, petunidin-glycosides, peonidin-glycoside, and malvidin-glycosides.

499 C3G, cyanidin-3-*O*-glucoside; C3R, cyanidin-3-*O*-rutinoside; u.d., under the detection

500 limit.

501

502

503 Table 2. Antioxidant Activities of Açai

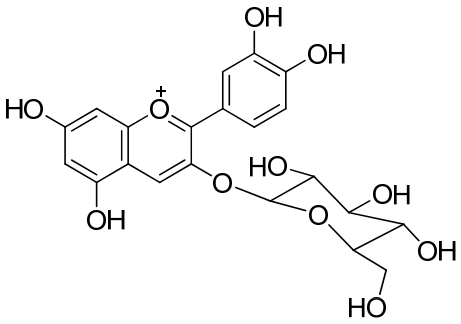
Samples	Antioxidant activity evaluated by					
	$ORAC_{FL}$[a]		DPPH[b]	ABTS[b]	SOD[b]	FRAP[b]
	hydrophilic	lipophilic				
Açai						
Freeze-dried açai powder	6334±606	21±3	92.0±1.69	96.0±1.56	81.0±6.16	298±9.76
Mesocarp/Epicarp	6605±853	37±6	85.4±1.69	72.0±1.56	77.5±6.16	217±9.76
Endocarp	4832±695	57±17	57.2±9.33	86.9±9.40	76.7±9.70	142±23.1
Blueberry	253±16	56±2	28.0±0.36	14.0±0.09	45.0±0.58	56±2.30
Blackcurrant	610±170	148±11	52.0±1.00	31.0±0.10	68.3±2.50	131±4.30
Trolox	-	-	47.0±0.40	20.0±0.10	-	-

504 [a]Hydrophilic $ORAC_{FL}$ (H-$ORAC_{FL}$) assay and lipophilic $ORAC_{FL}$ (L-$ORAC_{FL}$) assay were
505 expressed as micromoles of Trolox equivalents (TE) per gram extracts of sample (μmol
506 TE/g extracts).
507 [b]Açai and berry extracts (2 mg/mL) and trolox (0.1 mg/mL) were individually used for each
508 method. Antioxidant potencies were indicated as % of radical quenching activity (DPPH
509 and ABTS) and mg TE/mL (FRAP) as described in Materials and Methods. SOD-like
510 activity was indicated as inhibition rate (%).

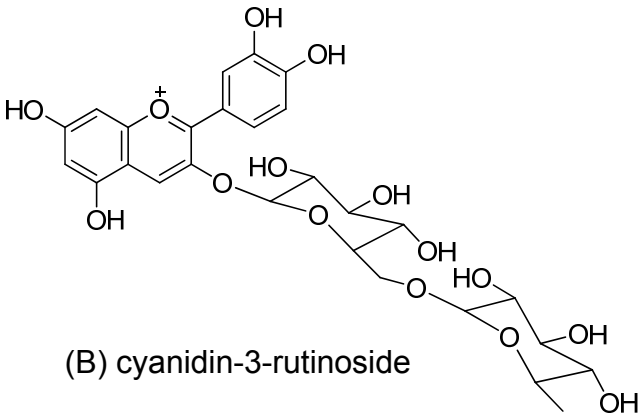
(A)

(B)

Figure 1. Agawa et al



(A) cyanidin-3-glucoside



(B) cyanidin-3-rutinoside

Figure 2. Agawa et al

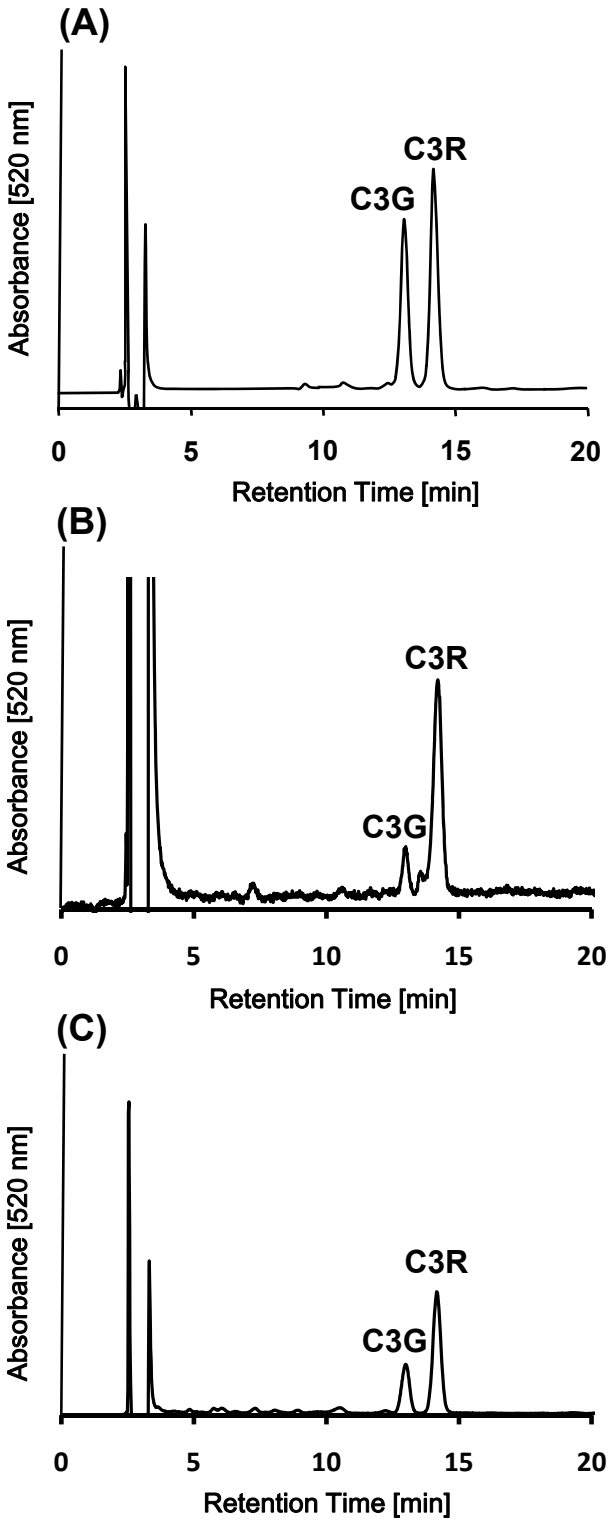


Figure 3. Agawa et al

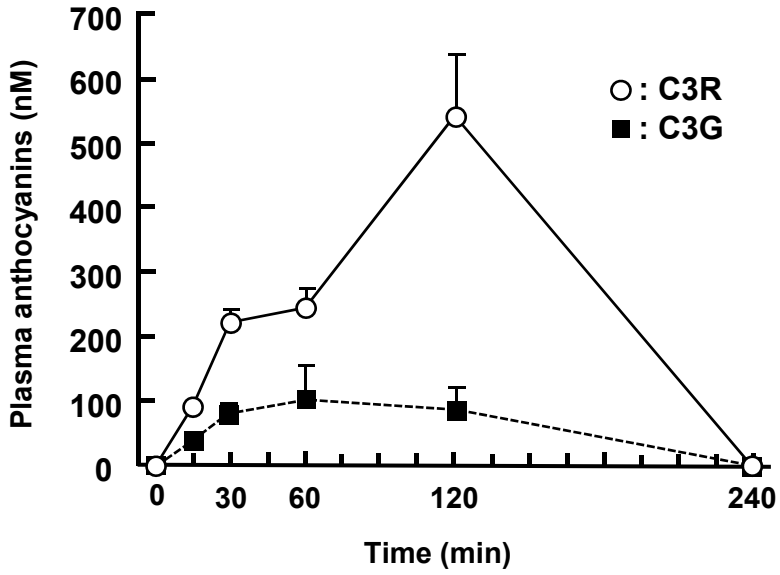


Figure 4. Agawa et al

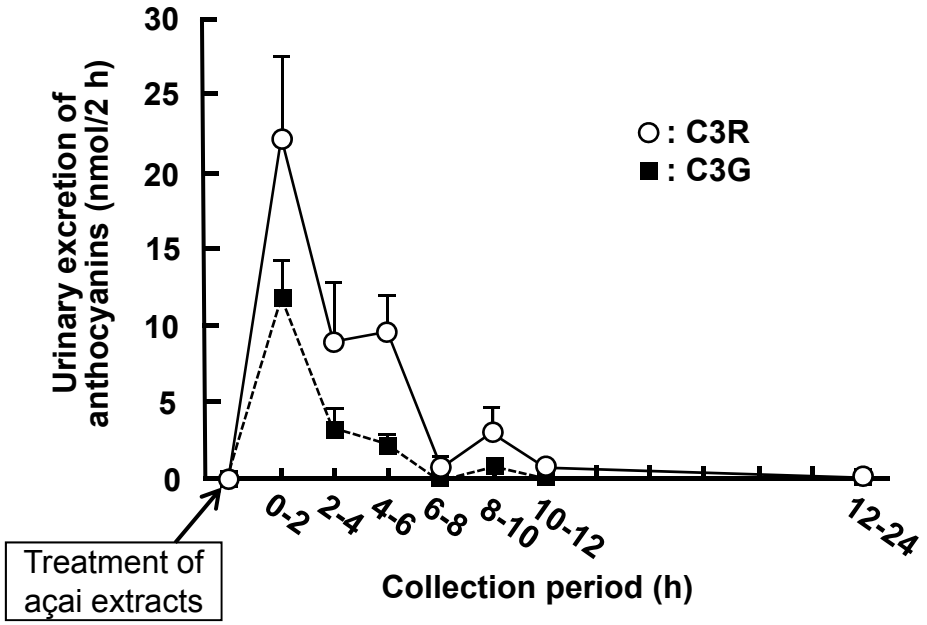


Figure 5. Agawa et al