Antioxidant Capacity and Other Bioactivities of the Freeze-Dried Amazonian Palm Berry, *Euterpe oleraceae* Mart. (Acai)

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The fruit of *Euterpe oleraceae*, commonly known as acai, has been demonstrated to exhibit significantly high antioxidant capacity *in vitro*, especially for superoxide and peroxyl scavenging, and, therefore, may have possible health benefits. In this study, the antioxidant capacities of freeze-dried acai fruit pulp/skin powder (OptiAcai) were evaluated by different assays with various free radical sources. It was found to have exceptional activity against superoxide in the superoxide scavenging (SOD) assay, the highest of any food reported to date against the peroxyl radical as measured by the oxygen radical absorbance capacity assay with fluorescein as the fluorescent probe ($ORAC_{FL}$), and mild activity against both the peroxynitrite and hydroxyl radical by the peroxynitrite averting capacity (NORAC) and hydroxyl radical averting capacity (HORAC) assays, respectively. The SOD of acai was 1614 units/g, an extremely high scavenging capacity for $O_2^{\bullet-}$, by far the highest of any fruit or vegetable tested to date. Total phenolics were also tested as comparison. In the total antioxidant (TAO) assay, antioxidants in acai were differentiated into "slow-acting" and "fast-acting" components. An assay measuring inhibition of reactive oxygen species (ROS) formation in freshly purified human neutrophils showed that antioxidants in acai are able to enter human cells in a fully functional form and to perform an oxygen quenching function at very low doses. Furthermore, other bioactivities related to anti-inflammation and immune functions were also investigated. Acai was found to be a potential cyclooxygenase (COX)-1 and COX-2 inhibitor. It also showed a weak effect on lipopolysaccharide (LPS)-induced nitric oxide but no effect on either lymphocyte proliferation and phagocytic capacity.

KEYWORDS: *Euterpe oleraceae*; acai; reactive oxygen species (ROS); antioxidant; $ORAC_{FL}$; NORAC; HORAC; superoxide; SOD; TAO; cyclooxygenase (COX); macrophage phagocytosis assay; nitric oxide assay; lymphocyte proliferation assay

INTRODUCTION

High intake of fruits and vegetables was found to positively associate with lower chance of many diseases by epidemiologi-cal studies and clinical trials. Antioxidant capacity was believed to be one of the possible mechanisms, though others are also involved. Acai, fruits of *Euterpe oleraceae* Martius, is consumed in a variety of beverages and food preparations in the native land in Brazil, Colombia, and Suriname and used medicinally as an antidiarrheal agent (*1*, *2*). Recently, much attention has been paid to its antioxidant capacity and possible role as a "functional food" or food ingredient (*3−6*). *Euterpe oleraceae* fruit pulp has been reported to quench peroxyl radicals, peroxynitrite, and *in vitro* hydroxyl radicals by the TOSC assay (*4*). In another study, the antioxidant activity of acai frozen pulp was determined on the basis of the inhibition of copper-induced

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peroxidation of liposome and the inhibition of the co-oxidation of the linoleic acid and β-carotene system (5). Phytochemical composition and nutrient analysis of acai have been presented in our former paper (7). Here, we focus on its antioxidant capacities evaluated by different assays with various free radical sources to further enhance our knowledge of this fruit's health potential.

Free radicals are consistently formed as byproducts of aerobic metabolism in the human body (8). They are generally reactive oxygen or nitrogen species (ROS or RNS). The most common ROS and RNS *in vivo* are superoxide ($O_2^{\bullet-}$), hydroxyl radical ($OH_2^{\bullet}$), peroxyl radical ($RO_2^{\bullet}$), nitric oxide ($^{\bullet}NO$), and peroxynitrite ($ONOO^-$). These ROS have been associated with many chronic and degenerative diseases including vascular diseases, diabetes, cancer, and overall aging (9−11). Dietary antioxidants are believed to be good external sources to counteract free radicals in the body (12). A large number of methods have been developed to evaluate total antioxidant capacity (TAC) of food samples. Nevertheless, few of them have been used widely due to the difficulty of measuring TAC owing to limitations associated with methodological issues and free radical sources (13). In this study, the TAC of acai was evaluated by a series of oxygen radical absorbance capacity assay with fluorescein as the fluorescent probe ($ORAC_{FL}$) based assays, including hydrophilic $ORAC_{FL}$ (H-$ORAC_{FL}$), lipophilic $ORAC_{FL}$ (L-$ORAC_{FL}$), peroxynitrite radical averting capacity (NORAC), and hydroxyl radical averting capacity (HORAC). As a comparison, total phenolics was also measured by the Folin−Ciocalteu method. Moreover, several novel antioxidant capacity assays including the superoxide scavenging (SOD) assay, total antioxidant (TAO) assay, and inhibition of ROS formation in a functional, cell-based assay using freshly purified human neutrophils from healthy donors were also performed. Results from these assays are expected to provide additional information to help us better understand the antioxidant capacity of acai.

Bioactivities based on mechanisms other than antioxidant activities may also contribute to the overall health benefits of acai. In this study, we conducted several assays related to anti-inflammation and immune functions, including the cyclooxygenase (COX-1 and COX-2) inhibitor assay, macrophage phagocytosis assay, nitric oxide assay, and lymphocyte proliferation assay.

MATERIALS AND METHODS

Plant Material. Freeze-dried acai (*Euterpe oleracea*) fruit pulp/skin powder (OptiAcai) was obtained from K2A LLC (Provo, UT). The berries were collected in Belem, Brazil. Within hours of harvesting, acai berries were frozen and stored at −20 °C until transferred for freeze drying. The freeze-dried acai powder was kept at −20 °C until analyzed.

Chemicals and Standards. *ORAC-Based Assays and Total Phenolics.* 2,2′-Azobis(2-amidinopropane) dihydrochloride (AAPH) was purchased from Wako Chemicals USA (Richmond, VA). 6-Hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), fluorescein (sodium salt) (Fl), cobalt(II) fluoride tetrahydrate, and picolinic acid were obtained from Aldrich (Milwaukee, WI). Randomly methylated β-cyclodextrin (Trappsol, Pharm Grade, RMCD) was obtained from Cyclodextrin Technologies Development Inc. (High Springs, FL). Folin−Ciocalteu's phenol reagent, sodium carbonate, sodium hydrogen carbonate (NaHCO$_3$), dihydrorhodamine-123 (DHR-123), caffeic acid, and gallic acid were all purchased from Sigma (St. Louis, MO). Potassium phosphate dibasic (K$_2$HPO), potassium phosphate monobasic (KH$_2$PO$_4$), and hydrogen peroxide were obtained from VWR (West Chester, PA). 3-Morpholinosydnonimine hydrochloride (SIN-1) was provided by Toronto Research Chemicals (North York, Ontario, Canada). Other solvents were purchased from Fisher (Fair Lawn, NJ).

SOD Assay. Hydroethidine was from Polysciences, Inc. (Warrington, PA). Xanthine oxidase (from butter milk, catalog number X4875),

xanthine, and superoxide dismutase (from bovine erythrocytes, catalog number S 2515) were purchased from Sigma-Aldrich (St. Louis, MO). Manganese(III) 5,10,15,20-tetra(4-pyridyl)-21*H*,23*H*-porphine chloride tetrakis(methochloride) was obtained from Aldrich (Milwaukee, WI,).

TAO Assay. TAO iodine reagent was provided by Shanbrom Technologies, LLC (Ojai, CA). A 710A+ basic ion selective meter was obtained from Thermo-Electron Corp (Waltham, MA).

Inhibition of ROS Formation in Human PMN Cells. Histopaque 1119 and 1077 are both from Sigma-Aldrich (St. Louis, MO). DCF-DA is from Molecular Probes (Eugene, OR).

COX-1 and COX-2 Inhibitor Assay. Arachidonic acid and COX-1 and COX-2 enzymes are all purchased from Cayman Chemical (Ann Arbor, MI).

Macrophage Phagocytosis Assay, Nitric Oxide Assay, and Lymphocyte Proliferation Assay. RPMI-1640 media was purchased from Invitrogen (Carlsbad, CA) and phosphate-buffered saline (PBS) from Hyclone (Logan, UT). Lipopolysaccharide (LPS), 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl-tetrazolium bromide (MTT) and naphthylethylene diamine dihydrochloride (NEDD) were obtained from Sigma (St. Louis, MO). Other reagents were of analytical grade (Bangalore, India).

Total Phenolic Analysis. The acetone/water/acetic acid (AWA) extracts were subjected to total phenolics measurement by Folin−Ciocalteu reagent according to the method of Wu et al. (14). The results were expressed as milligrams of gallic acid equivalents per 100 g of fresh weight (mg of GAE/100 g of FW).

$ORAC_{FL}$-Based Assays. Freeze-dried acai (0.035 g) was extracted with 20 mL of acetone/water (50:50 v/v) for 1 h at room temperature on an orbital shaker. The extracts were centrifuged at 5900 rpm, and the supernatant was ready for H-$ORAC_{FL}$, HORAC, and NORAC analysis. An acai sample (1 g) was extracted with hexane/dichloromethane two times (10 mL × 2). The supernatants were combined for L-$ORAC_{FL}$ analysis (15).

The H-$ORAC_{FL}$ assay was conducted on the basis of a report by Ou and co-workers (16), modified for the FL600 microplate fluorescence reader (Bio-Tek Instruments, Inc., Winooski, VT). The FL600 microplate fluorescence reader was used with fluorescence filters for an excitation wavelength of 485 ± 20 nm and an emission wavelength of 530 ± 25 nm. The plate reader was controlled by software KC4 3.0. For L-$ORAC_{FL}$, a sample solution was prepared according to a previous paper (17). Then the L-$ORAC_{FL}$ was also measured in the same plate reader based on a published procedure (17).

The HORAC assay is based on a report by Ou and co-workers and modified for the FL600 fluorescence microplate plate reader (Bio-TeK Instruments, Inc., Winooski, VT) (18).

$ONOO^-$ scavenging was measured by monitoring the oxidation of DHR-123 according to a modification of the method of Chung et al (19). Briefly, a stock solution of DHR-123 (5 mM) in dimethylformamide was purged with nitrogen and stored at −80 °C. A working solution with DHR-123 (final concentration, fc, 5 M) diluted from the stock solution was placed on ice in the dark immediately prior to the study. The buffer of 90 mM sodium chloride, 50 mM sodium phosphate (pH 7.4), and 5 mM potassium chloride with 100 M (fc) diethylenetriaminepentaacetic acid (DTPA) was purged with nitrogen and placed on ice before use. $ONOO^-$ scavenging by the oxidation of DHR-123 was measured with a microplate fluorescence reader FL600 with excitation and emission wavelengths of 485 and 530 nm, respectively, at room temperature. The background and final fluorescent intensities were measured 5 min after treatment with or without SIN-1 (fc 10 M) or authentic $ONOO^-$ (fc 10 M) in 0.3 N sodium hydroxide. Oxidation of DHR-123 by decomposition of SIN-1 gradually increased, whereas authentic $ONOO^-$ rapidly oxidized DHR-123 with its final fluorescent intensity being stable over time.

SOD Assay. An acai sample (0.02 g) was extracted with 20 mL of an acetone/water mixture on a shaker for 1 h. The mixture was centrifuged at 5900 rpm and 20 °C for 10 min. The supernatant was used for SOD assay. SOD assay was carried out on the basis of an in-house protocol (Brunswick Labs, Wareham, MA) on a Precision 2000 eight channel liquid handling system and Synergy HT microplate UV−vis and fluorescence reader, both from Bio-Tek Inc. (Winooski, VT).

TAO Assay. Acai powder (4 g) was placed in 40 mL of 10% (w/v) soluble polyvinyl pyrrolidone (PVP; BASF, Kollidon 17PF) and

incubated for 2 h at 37 °C. The extract was centrifuged at 4000 rpm for 10 min, and the supernatant was decanted from the tube and serially diluted 2-fold to 1:8. Iodine reagent (0.1 mL) was mixed well with diluted supernatant. The iodide level (μg/mL) in the sample was determined by an Orion Iodide Sure-Flow solid-state combination electrode (Waltham, MA) at 30 s and 30 min after addition of iodine reagent. The TAO value is equivalent to ppm (μg/mL) of iodide formed.

Inhibition of ROS Formation in Human PMN Cells. Heparinized blood samples were obtained from healthy volunteers upon informed consent. The blood was immediately layered on top of a double gradient of Histopaque 1119 and 1077 (both from Sigma-Aldrich, St. Louis, MO), and centrifuged for 25 min at room temperature. The top layer of mononuclear cells was removed. The second layer of cells between the two gradients, which represents almost 100% neutrophils, was harvested and used for the evaluation of ROS formation. Cells were washed twice in phosphate-buffered saline without calcium or magnesium.

An extract of the test product was prepared by adding acai powder (0.5 g) to 5 mL of phosphate-buffered saline, pH 7.4. This mixture was vortexed repeatedly and allowed to sit at room temperature for 1 h. Prior to use, insoluble particles were removed by centrifugation and subsequent filtration using a 0.22 μm cellulose−acetate syringe filter. This liquid was used to prepare a series of 100-fold dilutions in phosphate-buffered saline without calcium or magnesium. Freshly purified human neutrophils were preincubated with acai extracts over a wide range of dilutions and then incubated at 37 °C for 90 min. Following a wash to remove compounds within the extracts that could interfere with the oxidation marker, cells were loaded with 0.5 μM DCF-DA (Molecular Probes, Eugene, OR) for 1 h at 37 °C. All samples, except for the untreated control samples, were then exposed to 167 mM H_2O_2 for a period of 45 min to induce oxidative stress. Samples were washed to remove the peroxide, transferred to cold RPMI, and stored on ice in preparation for immediate acquisition by flow cytometry, using a FACSCalibur cytometer (Becton-Dickinson, San Jose, CA). Untreated samples were run before and after running all other samples, to verify that spontaneous oxidation was minimal.

Intracellular levels of DFC-DA fluorescence intensity in untreated versus challenged cells in the presence versus absence of the test product were analyzed by flow cytometry. A standard curve of DCF-DA fluorescence intensity as a result of treatment with known amounts of hydrogen peroxide was used to produce an estimation of the effectiveness of a given natural product in terms of quenched hydrogen peroxide molecules. Data was collected in triplicate for controls and duplicate for each sample concentration. Dose levels are reported in volumetric parts per billion. Statistical significance was determined using Student's t-test.

COX Assay. Acai powder (2 g) was extracted with 50% acetone and tested directly without further dilution. The sample was incubated at 37 °C with Tris buffer (0.5 mL) in the reaction chamber followed by 5 μL of 100 μM heme in DMSO. To the solution, 5 μL of COX-1 (or 10 μL of COX-2) enzyme solution was added (used as received from supplier). The mixture was incubated for 1 min. A 5 μL sample (in DMSO or ethanol) was added and incubated for 1 min. Arachidonic acid (5 μL) was added, and the reaction rate was monitored. The oxygen concentration was monitored in real time by an Oxytherm (Hansatech Instrumental, Norfolk, England). The initial oxygen consumption rate is obtained from the kinetic curve. In the presence of inhibitors, the initial rate decreased. The IC_{50}, the concentration at which the initial oxygen consumption rate decreased by 50%, was used to express the COX-1 and -2 inhibition activity.

Nitric Oxide Assay, Macrophage Phagocytosis Assay, and Lymphocyte Proliferation Assay. Acai was diluted in media containing 5% DMSO to a concentration of 10 mg/mL. It was subjected to sonication at 35 kHz for 10 min in a Bandelin Sonorex sonicator. The supernatant was collected, sterilized though a 0.22 μm filter, and used immediately for the assay.

Mouse splenocytes were isolated according to previously published procedures with minor modifications (20).

The lymphocyte proliferation assay was performed per previously established protocols (20, 21). Splenocytes were plated in 96-well plates on day 0 (0 h) at 5×10^5 cells/well (200 μL per well) in RPMI-1640 media containing 10% fetal bovine serum (FBS). The plates were incubated for 2 h at 37 °C in a CO_2 incubator (5% CO_2) to allow the cells to recover. A Trypan blue dye exclusion test was performed at the time lymphocytes were seeded in the well. Cell viability was >95%. Acai extracts were added to the wells at the requisite concentrations, and the plates were incubated again for 24 h at 37 °C in a CO_2 incubator (5% CO_2). LPS (5 μg/mL) was also added to some wells as a positive control for the assay. Cell proliferation was checked at 24 h using the MTT assay.

The nitric oxide assay was performed as per previously established protocols (22−24). Briefly, J774A.1 cells were plated in 96 well plates on day 0 (0 h) at 5×10^5 cells/mL (200 μL per well) in RPMI-1640 medium containing 10% FBS. The plates were incubated overnight (for about 16 h) at 37 °C in a CO_2 incubator (5% CO_2). The old media was removed from the wells after 16 h, and fresh media was added onto the cells. LPS or herbal extract at different concentrations was added to the wells. The cells were incubated for 48 h at 37 °C in a CO_2 incubator (5% CO_2). At the end of 48 h, the supernatants were collected and used for the nitric oxide (NO) assays.

In order to perform the NO assay, 100 μL of the sample supernatant was added in a 96-well plate. Greiss reagent (100 μL) was added to each well, and the samples were incubated at room temperature for 10 min. After 10 min incubation, the absorbance was measured at 540 nm. A standard curve was made with different concentrations of $NaNO_2$, and the data was expressed in terms of micromoles of $NaNO_2$. The experiment was run three times with six replicates per data point.

The macrophage phagocytosis assay was performed per previously established protocols (25). Briefly, J774A.1 cells were plated in 35 mm Petri dishes on day 0 (0 h) at 4×10^5 cells/dish (2 mL per 35 mm Petri dish) in RPMI-1640 medium containing 10% FBS. The plates were incubated for 6 h at 37 °C in a CO_2 incubator (5% CO_2) to allow the cells to adhere to the plates. The herbal extracts were added at different concentrations and the plates were incubated overnight (for about 16 h) at 37 °C in a CO_2 incubator (5% CO_2). The old media was removed from the plates after 16 h, and fresh media was added onto the cells. Yeast cells were added onto the plates at a 1:8 (macrophage/yeast) ratio, and the cells were incubated again at 37 °C in a CO_2 incubator (5% CO_2) for 1 h. At the end of 1 h, the supernatant was discarded, and the cells were washed twice with phosphate-buffered saline to remove unattached yeast cells. The cells were then fixed with methanol, stained with Geimsa stain, and observed under the oil immersion lens of the microscope for calculating the phagocytotic index.

Cells were counted in different fields, and a minimum of 100 macrophages were observed per sample. The phagocytotic index was expressed in two sets of parameters: percentage infected macrophages and average number of yeast per 100 infected macrophages. Attached but noninternalized yeast were not counted. The experiment was run three times with three replicates per data point.

The statistical analysis was performed using the GraphPad Prism program. A one-way analysis of variance (ANOVA) was performed on the data to analyze for significance, followed by a Neuman−Keuls test to compare multiple samples. A value of $P < 0.05$ was considered to be significant.

RESULTS

$ORAC_{FL}$, HORAC, NORAC, SOD, and Total Phenolic (TP) Content of Acai. $H-ORAC_{FL}$, $L-ORAC_{FL}$, HORAC, NORAC, SOD, and total phenolics (TP) content of freeze-dried acai are reported in **Table 1**. Total antioxidant capacity (TAC) was calculated as sum of $H-ORAC_{FL}$ and $L-ORAC_{FL}$.

Antioxidant Capacity from TAO. Antioxidant capacity of freeze-dried acai from the TAO assay is shown in **Figure 1**. In this assay, the antioxidant values of "slow-acting" (measured at 30 min) and "fast-acting" (measured at 30 s) were differentiated.

Inhibition of ROS Formation. Pretreatment of human neutrophils with freeze-dried acai extracts prior to induction of ROS by H_2O_2 treatment resulted in a significant reduction in

Table 1. Antioxidant Capacity from ORAC Assay with Different Free Radicals, SOD Assay, and Total Phenolics (TP) of Freeze-Dried Acai and Other Acai Products[a]

sample	H-ORAC$_{FL}$[b] (μmol TE/g)	L-ORAC$_{FL}$[c] (μmol TE/g)	TAC[d] (μmol TE/g)	NORAC (μmol TE/g)	HORAC (μmol GAE/g)	SOD (unit/g)	TP[e] (mg GAE/g)
freeze-dried acai	997	30	1027	34	52	1614	13.9

[a] Data was expressed as mean of duplicate measurements. [b] Hydrophilic ORAC$_{FL}$. [c] Lipophilic ORAC$_{FL}$. [d] Total antioxidant capacity, calculated as the sum of H-ORAC$_{FL}$ and L-ORAC$_{FL}$. [e] Total phenolics.
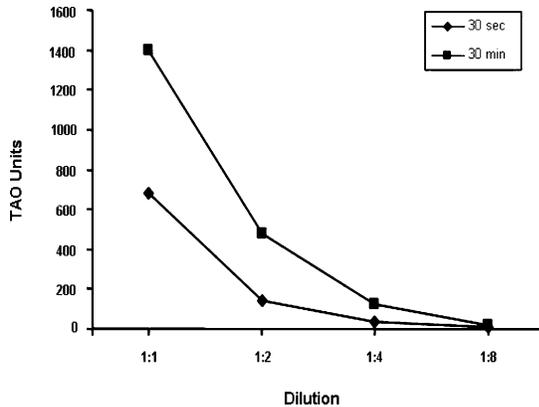
Figure 1. Total antioxidant (TAO) activity of freeze-dried acai, in which TAO assay differentiates antioxidant into "slow-acting" (30 min) and "fast-acting" (30 s) components.
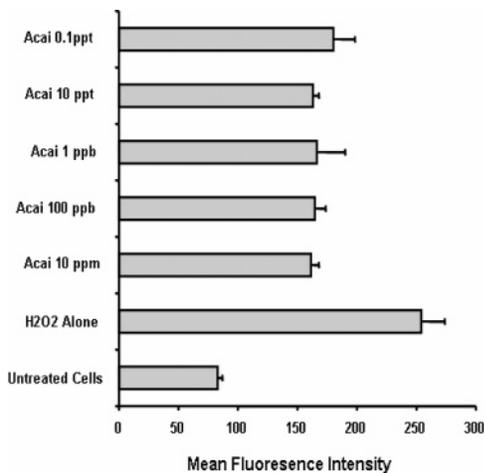
Figure 2. Freeze-dried acai reduced the H_2O_2-induced formation of reactive oxygen species (ROS) in freshly purified human neutrophils.

Table 2. Results from COX Assay of Freeze-Dried Acai

sample	IC$_{50}$ of COX-1[a] (mg/mL)	IC$_{50}$ of COX-2[a] (mg/mL)	IC$_{50}$ ratio (COX-1 vs COX-2)
freeze-dried acai	6.96	12.50	0.56

[a] The result is reported as the IC$_{50}$ (50% enzyme activity inhibition concentration).

ROS production. The formation of ROS was significantly inhibited, even at extremely low doses of freeze-dried acai. (**Figure 2**).

COX Inhibition Effects. Inhibition of COX-1 and COX-2 by freeze-dried acai is shown in **Table 2**. The IC$_{50}$ ratio of COX-1 vs COX-2, which indicates the selectivity of the sample in inhibition of COX enzymes, is also presented (**Table 2**).

Lymphocyte Proliferation Activity. Freeze-dried acai did not show any effect on lymphocyte proliferation at the concentrations tested (5−1000 μg/mL) at a 24 h assay point. LPS (5 μg/mL), the positive control for the assay, demonstrated a 1.55-
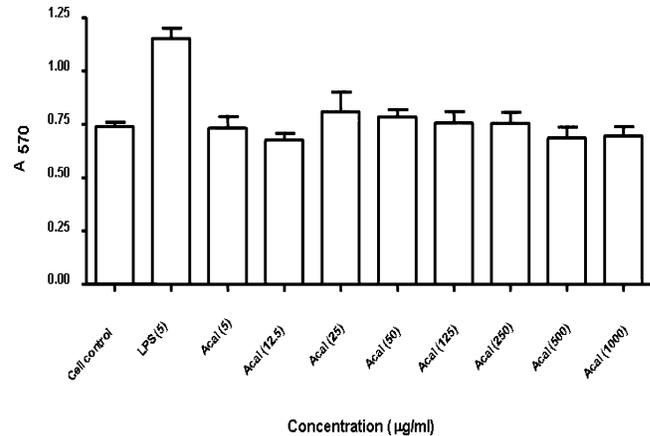
**Figure 3.** Effect of freeze-dried acai on lymphocyte proliferation.

fold increase in lymphocyte proliferation over cell controls. The latter result is in keeping with the data usually obtained with LPS in this assay (**Figure 3**).

Nitric Oxide Assay. The freeze-dried acai did not show any effect on NO release by J774A.1 macrophages at the above concentrations (250−2500 μg/mL) at a 48 h assay point. LPS (5 μg/mL), the positive control for the assay, demonstrated a 15.66-fold increase in NO release over cell controls. Freeze-dried acai at the above concentrations demonstrated a significant dose-dependent inhibition of LPS-induced nitric oxide in this assay (**Figure 4**).

Macrophage Phagocytosis Activity. Freeze-dried acai at 5−250 μg/mL increased macrophage infection by about 1.4−1.5-fold over control values. However, this effect did not appear to be dose dependent, and infection levels came down to control values with 500 μg/mL acai. There was a significant increase in the number of yeast engulfed per macrophage at 5 μg/mL acai, but the effect was not evident at higher concentrations (**Figure 5**).

DISCUSSION

An Internet search using the words "acai and antioxidant" entered into Google.com resulted in over 200 000 hits. Other than its antioxidant capacity, it was interesting to note how many health benefits were reported for acai. Yet, little research has been reported in the literature, while even less existed to support its claimed health benefits. In this study, antioxidant capacities of acai were investigated by different assays in an effort to fully understand the scope of its antioxidant capacities. Moreover, other possible bioactivities of acai related to inflammatory processes and its effect on markers related to immune function were also performed. Due to the complexity of the antioxidant defense system and involvement of many different types of free radicals in the body, a single antioxidant assay cannot provide us a complete picture of the antioxidant capacity of a given food *in vitro*, much less *in vivo*. Thus, several different antioxidant assays were used to study the antioxidant capacity of acai *in vitro*.
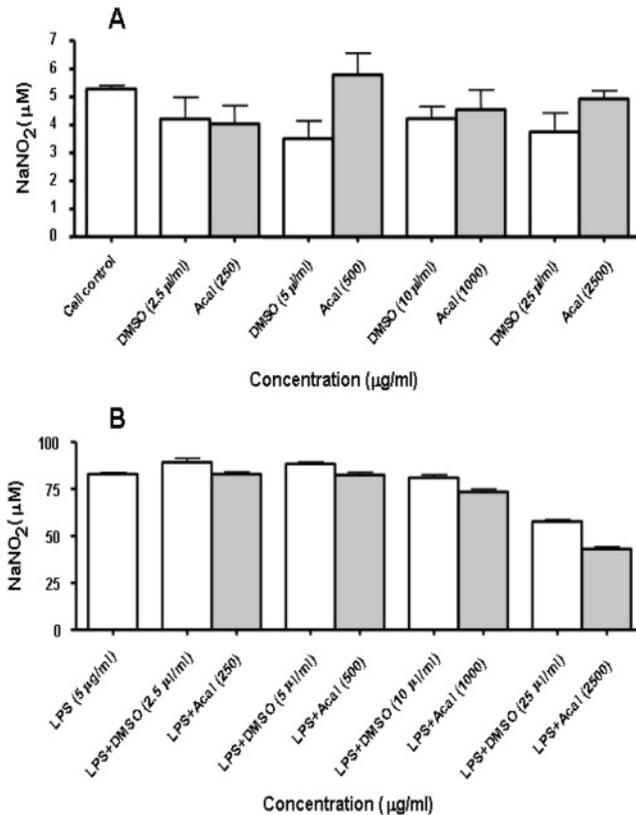
Figure 4. Effects of freeze-dried acai on nitric oxide release by J774 cells (**A**) and on LPS-stimulated nitric oxide release by J774 cells (**B**).
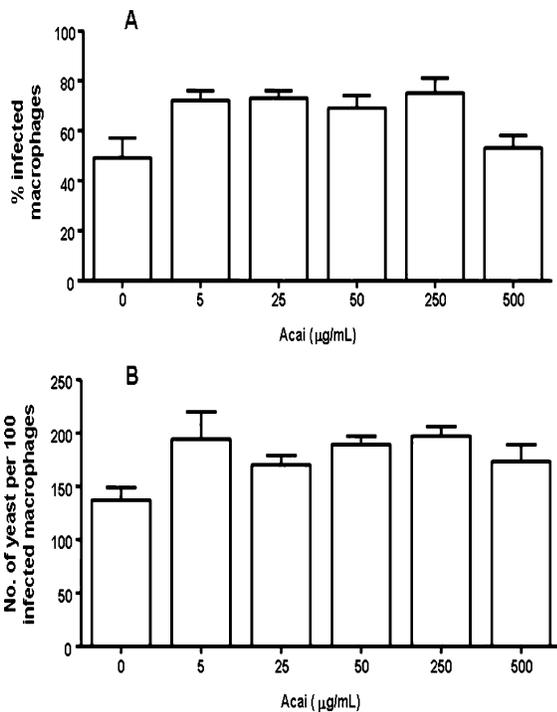
Figure 5. Effects of freeze-dried acai on J774 macrophage infection by yeast cells (**A**) and number of yeast engulfed by J774 macrophage (**B**).

The original ORAC$_{FL}$ assay was designed to measure the antioxidant capacity of foods toward peroxyl radicals, and it can be conducted to measure both hydrophilic and lipophilic antioxidants (16, 17). From our results, the H-ORAC$_{FL}$ of freeze-dried acai was 996.9 μmol TE/g, which is significantly higher than that of most dark colored berry or any fruit or vegetable

tested to date when appropriately converting fresh weight to dry weights (14). The L-ORAC$_{FL}$ of freeze-dried acai was 30 μmol TE/g, thereby yielding a total ORAC of 1026.9 μmol TE/g. Contradictorily and surprisingly, the contents of anthocyanins, proanthocyanidins, and other polyphenol compounds in this freeze-dried product were found to be much lower than those found in blueberry or any other berries with elevated H-ORAC$_{FL}$ values. To make things even more confusing, the total phenolics in acai was found to be only 13.9 mg/g GAE. In a recent paper, the ratio between hydrophilic ORAC$_{FL}$ and total phenolics was found to vary dramatically from less than 2 to more than 100 for different groups of foods (14). For most fruits and vegetables, this ratio is about 10. However, the ratio in acai is 50, five times greater than that found for any other fruit. This "unusual" ratio raises questions whether acai contains much stronger antioxidants than those found in other berries on an equal weight basis. Determining which antioxidants contributed to this unusual ratio warrants further work.

Freeze-dried acai has an oily feel when rubbed between the fingers, suggesting that acai contains fairly large amounts of lipophilic compounds. The L-ORAC$_{FL}$ is 29.6 μmol TE/g, which is higher than any berry samples tested to date (14).

HORAC and NORAC, two assays developed from ORAC, were adopted to measure antioxidant capacity of acai toward OH$^\bullet$ and ONOO$^-$, two of the major cell-killing ROS in the human body (26). The HORAC value of freeze-dried acai was 52 μmol GAE/g, which is similar to that of grapes but lower than that of dark colored berries (18). From our limited data (unpublished data), the NORAC value of freeze-dried acai is among the average of other fruits.

Superoxide (O$_2^{\bullet-}$) is believed to be the cause of other ROS formations such as hydrogen peroxide, peroxynitrite, and hydroxyl radicals. Therefore, O$_2^{\bullet-}$ scavenging capacity in the human body is the first line of defense against oxidative stress. It has been reported that overexpression of superoxide dismutase and catalase in transgenic flies extended life-span by as much as one-third, perhaps, due to decreased oxidative stress reflected by lower protein carbonyl contents (27). Superoxide scavenging capacity in blood is considered very important in maintaining antioxidant status. The most studied SOD from any natural source is wheat sprout SOD, ranging from 160 to 500 units/g for different samples (unpublished data). The SOD of acai was 1614 units/g, meaning acai has extremely high scavenging capacity to O$_2^{\bullet-}$, by far the highest of any fruit or vegetable tested to date.

The total antioxidant (TAO) assay was developed to permit rapid and simple determination of a sample's antioxidant capacity. The TAO assay is based on the iodine−iodide oxidation−reduction (redox) reaction, with the formation of iodide in the sample proportional to the antioxidant (or reducing) capacity of the sample. The TAO assay also differentiates antioxidants into a "slow-acting" component, which includes complex organic antioxidants (e.g., phenolics) and a "fast-acting" or "vitamin-C-like" component. The "fast-acting" antioxidants were measured at 30 s, whereas the "slow-acting" antioxidants were measured at 30 min. The combination of these values is the total antioxidant capacity (28). The TAO assay results for freeze-dried acai clearly showed that the antioxidant capacity of "slow-acting" antioxidants was stronger than that of "fast-acting" antioxidants (**Figure 1**).

Freeze-dried acai was also assayed for inhibition of ROS formation in freshly purified human neutrophils. Freeze-dried acai demonstrated a substantial inhibitory effect on the ROS formation in human neutrophil cells (**Figure 2**). Freeze-dried

acai displayed a maximum effect at a concentration of 1−10 parts per trillion (ppt, v/v). A nonmonotonic dose response was observed, which is typical in this type of *in vitro* cell-based assay, where a complex blend of active ingredients results in multiple factors contributing to intracellular oxidative stress. The ROS inhibition by the freeze-dried acai extract was effective at extremely low doses. The level of ROS formation was not brought back to the level of the positive control at any of the acai dilutions tested, including 0.1 ppt. This data indicates that the active antioxidant compounds in the freeze-dried acai are able to enter human cells in a fully functional form and perform oxygen quenching at extremely low doses.

Other Bioactivities of Acai. Antioxidants are surely not the only reason that we should eat fruits and vegetables. There are hundreds and even sometimes thousands of compounds in foods. Many of them contribute to health benefits through mechanisms other than antioxidant activity. Proteins in acai pulps have been found to show high antitryptic activity and considerable inhibition activity toward human salivary α-amylase (*29*). The effects of acai polyphenolics on the antiproliferation and induction of apoptosis in HL-60 human leukemia cells have also been investigated (*30*). Hence, we primarily focused on the possible effects of freeze-dried acai on immune parameters and some anti-inflammatory markers.

The lymphocyte proliferation assay (LPA) is a measure of immune activation/stimulation. It measures the ability of lymphocytes placed in short-term tissue culture to undergo clonal proliferation when exposed to a foreign substance/ mitogen. This assay helps evaluate the immunostimulatory/ immunosuppressive activity of a mitogen. Freeze-dried acai fruit demonstrated no significant effect on lymphocyte proliferation across a very wide concentration range (5−1000 μg/mL) in this assay (**Figure 3**) (*31*).

Nitric oxide (NO) is an inorganic free radical that functions as an intracellular messenger and effector molecule. It is produced during the conversion of arginine to citrulline and its production is catalyzed by the enzyme nitric oxide synthase (NOS) (*32*). NOS has three isoforms: NOS I, II, and III. Out of these three isoforms, only NOS II is inducible and is produced during macrophage activation (*33*). Macrophage activation is thus accompanied by the induction of inducible nitric oxide synthase and sustained release of NO (*34*). Synthesis of NO endows macrophages with cytostatic or cytotoxic activity against viruses, bacteria, fungi, protozoa, helminths, and tumor cells (*35*, *36*). Freeze-dried acai fruit did not affect nitric oxide release by J774A.1 macrophages at a concentration of 250−2500 μg/ mL (**Figure 4**).

Neutrophils/macrophages play a major role in phagocytosis of microorganisms and other foreign entities that enter the body. Compounds that increase the phagocytic capacity of these cells are potent immunostimulators. Thus, this assay can be used to gauge the potential immunostimulatory effect of a substance. Freeze-dried acai was found to increase macrophage activity slightly (1.4−1.5-fold over control values) at concentrations of 5−250 μg/mL (**Figure 5**). However, this effect did not appear to be dose dependent, and activity levels came down to control values at 500 μg/mL. There was also a significant increase in the number of yeast engulfed per macrophage at 5 μg/mL, but the effect was not statistically significant at higher concentrations. This suggests that lower concentrations of freeze-dried acai may be activators of macrophage phagocytosis but possibly not at higher concentrations. Thus on the whole, it appears that freeze-dried acai probably possesses minimal immunostimulatory properties at concentrations higher than 5 μg/mL. Lower

concentrations (less than 5 μg/mL) might be immunostimulatory, but additional study for this is needed. We also observed an inhibition in nitric oxide levels within J774A.1 macrophages with acai treatment. Since increased nitric oxide is associated with increased killing of microorganisms, and we have examined only initial macrophage phagocytosis and not yeast killing at later time points, it is probable that acai does not significantly enhance immunity *in vitro*.

Interestingly, the freeze-dried acai at 250−2500 μg/mL demonstrated a significant dose-dependent inhibition of LPS-induced nitric oxide (**Figure 4**). Since inhibition of LPS-induced nitric oxide has been correlated with anti-inflammatory activity (*37*), this result suggests that the freeze-dried acai may be used as a potent anti-inflammatory substance and thus may find applications in allergic and autoimmune disorders.

Only recently has the mechanism of botanicals been investigated at the molecular biology level by using COX-1 and COX-2 inhibitory assays to measure the pain-relieving and anti-inflammatory potential of herbal supplements (*38*). Freeze-dried acai showed mild inhibition capacity *in vitro* based on the COX-1 and COX-2 assays (**Table 2**). The IC_{50} ratio of COX-1 vs COX-2 indicates the selectivity of the sample in inhibition of COX enzymes. When the ratio is one, there is no selectivity. If the ratio is smaller than one, the sample inhibits COX-1 better than COX-2. If the ratio is larger than one, the sample inhibits COX-2 better. Therefore, freeze-dried acai inhibits the COX-1 enzyme more efficiently than the COX-2 enzyme.

Conclusion. In this study, freeze-dried acai fruit pulp/skin powder has been shown to be extremely powerful in its antioxidant properties against superoxide ($O_2^{\bullet-}$) by SOD assay. The freeze-dried acai fruit pulp/skin powder was also shown to be excellent against the peroxyl radical ($RO_2^{\bullet}$), with the highest reported total ORAC (1026.9 μmol TE/g) of any fruit or vegetable, and mild against both peroxynitrite ($ONOO^-$) and hydroxyl radical ($OH^{\bullet}$) by $ORAC_{FL}$-based assays. In addition, this freeze-dried acai was found to be a potential COX-1 and COX-2 inhibitor. These findings may have significant value as to this fruit's antioxidant role in aging and disease. Although this study proved that antioxidants in freeze-dried acai are able to enter human cells in a fully functional form *in vitro*, more studies are warranted to determine safety and efficacy of acai *in vivo*.

LITERATURE CITED

(1) Strudwick, J.; Sobel, G. L. Uses of *Euterpe oleraceae* Mart. in the Amazon estuary. *Braz. Adv. Econ. Bot.* **1988**, *6*, 225−253.

(2) Plotkin, M. J.; Balick, M. J. Medicine uses of South American palms. *J. Ethnopharm.* **1984**, *10*, 157−179.

(3) Arruda, D. C.; Felippi, R.; Mantovani, I. S. B.; Santos, G. B.; Gabriel, F. T.; de Sa, A. C.; Fernández, S. B. de O.; Ribeiro-do-Valle, R. M.; Menezes, F. S.; Ckless, K. Antioxidant activity and levels of phenolics in *Euterpe oleracea* Mart extract (Acai). *Rev. Bras. Plant. Med.* **2004**, *6*, 5−10.

(4) Lichtenthäler, R.; Rodrigues, R. B.; Maia, J. G. S.; Papagiannopoulos, M.; Fabricius, H.; Marx, F. Total oxidant scavenging capacities of *Euterpe oleraceae* Mart. (Acai) fruit. *Int. J. Food Sci. Nutr.* **2005**, *56*, 53−64.

(5) Hassimotto, N. M. A.; Genovese, M. I.; Lajolo, F. M. Antioxidant activity of dietary fruits, vegetables, and commercial frozen fruit pulps. *J. Agric. Food Chem.* **2005**, *53*, 2928−2935.

(6) Coïsson, J. D.; Travaglia, F.; Piana, G.; Capasso, M.; Arlorio, M. *Euterpe oleraceae* juice as functional pigment for yogurt. *Food Res. Int.* **2005**, *38*, 847−853.

(7) Schauss, A. G.; Wu, X.; Prior, R. L.; Ou, B.; Patel, D.; Huang, D.; Kababick, J. Phytochemical and nutrient composition of the freeze-dried Amazonian palm berry, *Euterpe oleraceae* Mart. (Acai). *J. Agric. Food Chem.* **2006**, *54*, http://dx.doi.org/10.1021/jf060976g.

(8) Davies, K. J. A. Oxidative stress, antioxidant defenses, and damage removal, repair, and replacement systems. *IUBMB Life* **2000**, *50*, 279−289.

(9) Diaz, M. N.; Frei, B.; Vita, J. A.; Keaney, J. F. Mechanism of disease: antioxidants and atherosclerotic herat disease. *N. Engl. J. Med.* **1997**, *337*, 408−416.

(10) Dröge, W. Free radicals in the physiological control of cell function. *Physiol. Rev.* **2002**, *82*, 47−95.

(11) Finkel, T.; Holbrook, N. J. Oxidants, oxidative stress and the biology of ageing. *Nature* **2000**, *408*, 239−247.

(12) Kaur, C.; Kapoor, H. C. Antioxidants in fruits and vegetables − the millennium's health. *Int. J. Food Sci. Nutr.* **2001**, *36*, 703−725.

(13) Prior, R. L.; Wu, X.; Schaich, K. Standardized methods for the determination of antioxidant capacity and phenolics in foods and dietary supplements. *J. Agric. Food Chem.* **2005**, *53*, 4290−4302.

(14) Wu, X.; Beecher, G.; Holden, J.; Haytowitz, D.; Gebhardt, S. E.; Prior, R. L. Lipophilic and hydrophilic antioxidant capacities of common foods in the U.S. *J. Agric. Food Chem.* **2004**, *52*, 4026−4037.

(15) Wu, X.; Gu, L.; Holden, J.; Haytowitz, D.; Gebhardt, S. E.; Beecher, G.; Prior, R. L. Development of a database for total antioxidant capacity in foods: A preliminary study. *J. Food Compos. Anal.* **2004**, *17*, 407−422.

(16) Ou, B.; Hampsch-Woodill, M.; Prior, R. L. Development and validation of an improved oxygen radical absorbance capacity assay using fluorescein as the fluorescent probe. *J. Agric. Food Chem.* **2001**, *49*, 4619−4626.

(17) Huang, D.; Ou, B.; Hampsch-Woodill, M.; Flanagan, J. A.; Deemer, E. K. Development and validation of oxygen radical absorbance capacity assay for lipophilic antioxidants using randomly methylated β-cyclodextrin as the solubility enhancer. *J. Agric. Food Chem.* **2002**, *50*, 1815−1821.

(18) Ou, B.; Hampsch-Woodill, M.; Flanagan, J.; Deemer, E. K.; Prior, R. L.; Huang, D. Novel fluorometric assay for hydroxyl radical prevention capacity using fluorescein as the probe. *J. Agric. Food Chem.* **2002**, *50*, 2772−2777.

(19) Chung, H.; Choi, H.; Park, H.; Choi, J.; Choi, W. Peroxynitrite scavenging and cytoprotective activity of 2,3,6-tribromo-4,5-dihydroxybenzyl methyl ether from the marine alga *Symphyocladia latiuscula*. *J. Agric. Food Chem.* **2001**, *49*, 3614−3621.

(20) Ho, C. Y. Differential effect of *Coriolus versicolor* (Yunzhi) extracts on cytokine production by murine lymphocytes in vitro. *Int. Immunopharmacol.* **2004**, *4*, 1549−1557.

(21) Gooi, H. C., Chapel, H., Eds. *Clinical Immunology: A Practical Approach*; Oxford University Press: Oxford, U.K., 1990.

(22) MacMicking, J.; Xie, Q. W.; Nathan, C. Nitric oxide and macrophage function. *Annu. Rev. Immunol.* **1997,** *15*, 323.

(23) Stuchr, D. J.; Nathan, C. F. Nitric oxide: a macrophage product responsible for cytostasis and respiratory inhibition in tumor target cells. *J. Exp. Med.* **1989**, *169*, 1543−1555.

(24) Hibbs, J. B., Jr.; Taintor, R. R.; Varin, Z.; Rachlin, E. M. Nitric oxide: a cytotoxic activated macrophage effector molecule. *Biochem. Biophys. Res. Commun.* **1988**, *157*, 87−94.

(25) Campbell, P. A. In *Current Protocols in Immunology*; Coligan, J. E., Kruisbeek, A. M., Margulies, D. H., Shevach, E. M., Strober, W., Eds.; John Wiley & Sons: New York, 1995; pp 14.6.1−14.6.13.

(26) Halliwell, B. Reactive oxygen species in living systems − source, biochemistry, and role in human disease. *Am. J. Med.* **1991**, *91*, S14−S22.

(27) Orr, W. C.; Sohal, R. S. Extension of life-span by overexpression of superoxide dismutase and catalase in *Drosophila melanogaster*. *Science* **1994**, *263*, 1128−1130.

(28) Owens, W. J.; Shanbrom, E. TAO: a standard, simple measure of antioxidant capacity. Presented at the 2nd International Congress of Antioxidant Methods [abstract], June 22−24, 2005, Orlando, FL.

(29) Araújo, C. L.; Bezerra, I. W. L.; Dantas, I. C.; Lima, T. V. S.; Oliveira, A. S.; Miranda, M. R. R. A.; Leite, E. L.; Sales, M. P. Biological activity of proteins from pulps of tropical fruits. *Food Chem.* **2004**, *85*, 107−110.

(30) Del Pozo-Insfran, D.; Percival, S. S.; Talcott, S. T. Acai (*Euterpe oleracea* Mart.) polyphenolics in their glycoside and aglycones forms induce apoptosis of HL-60 leukemia cells. *J. Agric. Food Chem.* **2006**, *54*, 1222−1229.

(31) Harput, U. S.; Saracoglu, I.; Ogihara, Y. Stimulation of lymphocyte proliferation and inhibition of nitric oxide production by aqueous *Urtica dioca* extract. *Phytother. Res.* **2005**, *19*, 346−348.

(32) Knowles, R. G.; Moncada, S. Nitric oxide synthases in mammals. *Biochem. J.* **1994**, *298*, 249−258.

(33) Knowles, R. G. Nitric oxide biochemistry. *Biochem. Soc. Trans.* **1996**, *24*, 875−878.

(34) MacMicking, J.; Xie, Q. W.; Nathan, C. Nitric oxide and macrophage function. *Annu. Rev. Immunol.* **1997**, *15*, 323−350.

(35) Nathan, C. F.; Hibbs J. B., Jr. Role of nitric oxide synthesis in macrophage antimicrobial activity. *Curr. Opin. Immunol.* **1991,** *3*, 65−70.

(36) Tohyama, M.; Kawakami, K.; Saito, A. Anticryptococcal effect of amphotericin B is mediated through macrophage production of nitric oxide. *Antimicrob. Agents Chemother.* **1996**, *40*, 1919−1923.

(37) Shin, K-M.; Kim, I-T.; Park, Y-M.; Ha, J.; Choi, J-W.; Hee-Juhn Park, H-J.; Lee, Y. S.; Lee, K-T. Anti-inflammatory effect of caffeic acid methyl ester and its mode of action through the inhibition of prostaglandin E2, nitric oxide and tumor necrosis factor-a production. *Biochem. Pharmacol.* **2004**, *68*, 2327−2336.

(38) Wu, D.; Nair, M. G.; DeWitt, D. L. Novel compounds from *Piper methysticum* Forst (kava kava) roots and their effect on cyclooxygenase enzyme. *J. Agric. Food Chem.* **2002**, *50*, 701−705.