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Protection from Oxidative Stress in vitro and in vivo in Humans by an Amazonian palm berry,

Euterpe oleracea ("açai")

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Açai grows primarily in the flood plains of the Amazon River and its tributaries, covering an area of over 12 million acres.

Up to 7,000 palms can grow per acre.

In the Amazon flood plains acai palm trees form a canopy that

plains acai palm tree form a canopy that protect plants from equatorial solar radiation from the sun



1768: Açai Fruit Reported by Joseph Banks, botanist on Captain James Cook's, *The Endeavour*. 1791: Acai Fruit documented by Portuguese explorers.





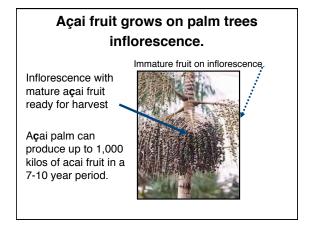
2,400 Palm Species in the World.

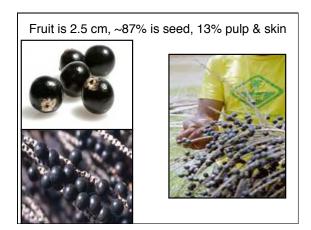
Açai palm (*Euterpe oleracea*) one of 3 species of the Genus, *Euterpe* only found in the Amazon, especially in flood plains.



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Açai palm inflorescence branches being removed 50 meters above ground to strip acai fruit into baskets.







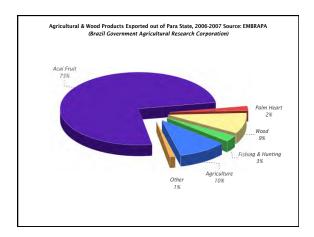


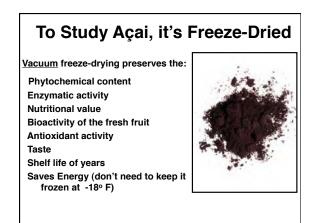


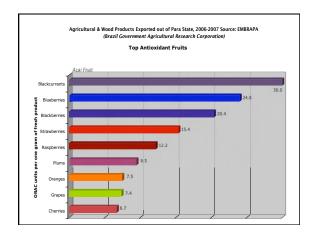


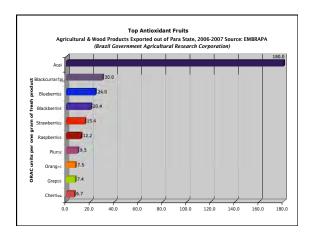


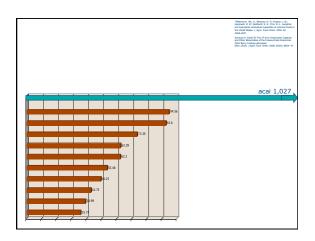


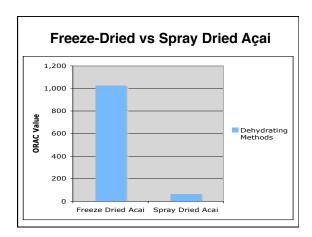












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Published Vitamin/Mineral Composition of Acai

Vitamins Minerals Vitamin A (Beta-carotene) Calcium Vitamin C Magnesium Vitamin E Potassium Vitamin D Sodium Vitamin B-1 Zinc Vitamin B-2 Copper Vitamin B-3 Iron Vitamin B-6 Manganese Vitamin B-12 Selenium Pantothenic acid Boron Biotin Chromium Folic Acid Molybdenum Inositol lodine

Published Amino Acid Composition of Açai

Aspartic acid Tyrosine Threonine Phenylalanine Serine Lysine Glutamic acid Histidine Arginine Glycine **Alanine Proline** Valine Hydroxyproline Methionine Cystine Isoleucine Tryptophan Leucine

Published Phytochemical Composition of Açai

Isovitexin

anthocyanins: Cyanidin-3-glucoside Cyanidin-3-rutoside Cyanidin-3-diglycoside

Cyanidin-3-glucoside-co Cyanidin-3-O-rutinoside

Beta-sitosterol Kaempferol Bela-sitosterol
Campesterol
Catechin
Chrysoeriol
Coumeric acid
Deoxyhexose
Epi-catechin
Eriodictyol
Eriodictyol-7-glucoside
Eurpatorin
Gallic acid
Homoorietin laoquercitin Luteolin
Luteolin-4-glucoside
Methyl-derivative of homo
Myricetin
Orientin
Proanthocyanin Protocatchuric acid Protocateriume acid
Protocatechic acid
Pterostilbene
Quercitin-3-arabino
Resveratrol

Published Nutritional, Lipid, and Fiber Composition of Açai

Fatty acids: 82% monounsaturated and polyunsaturated (palmitic, oleic, linoleic acids) - higher than virgin olive oil or avocado oil

Phytosterol content 1.25%

Very low in sodium (0.25%)

Very low in glucose/dissacharides (0.1 gram/100 grams)

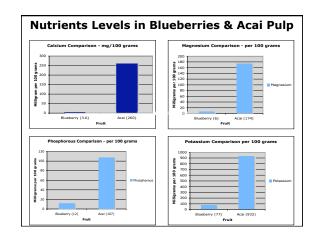
Low in carbohydrates (1.3 gram/100 grams)

High in soluble and water insoluble fibers.

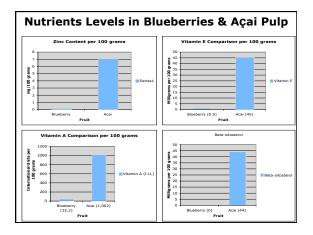
Total Antioxidant Capacity (TAC) Per Serving (Hydrophilic-ORAC + Lipophilic-ORAC) Fruits and Berries, Adjusted % Moisture Content

Vanillic acid

Food	% Moisture Content	TAC umol TE/g	Serving Size (g)	TAC per Serving
A ç ai pulp	60.0	410.80	145	59,566
Blueberry (Cult)	85.0	62.20	145	9,019
Cranberry	87.1	94.56	95	8,983
Blackberry	86.9	53.48	145	7,701
Raspberry	85.8	49.25	123	6,058
Strawberry	91.1	35.77	166	5,938
Apples (Granny) 85.7	38.99	138	5,381
Avocado (Hass	72.0	19.33	173	3,344



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Published Antioxidant Assays of FD Açai

- Total ORAC: 1,027 umole TE/gram.
 Lipophilic ORAC highest of any fruit.
 Peroxyl radical scavenging capacity highest of any fruit or vegetable. A few spices are higher.
 - TEAC and FRAP assay's confirm ORAC assay (744.0 umole TE/gram; 249.0 umole TE/gram.)
- 2) Superoxide ORAC (1,614 SOD units). Highest of any fruit or vegetable.
- 3) High hydroxyl and peroxynitrite scavenging activity (HORAC & NORAC assay)
- 4) Both a slow and fast antioxidant. TAO assay.





Human Pharmacokinetic Study of Açai Juice with Pulp and without Pulp

8 Differences between açai with pulp or without the pulp?

- A large portion of antioxidants are bound to the fiber in the pulp.
- Antioxidants in the fiber of the pulp are released in the gut and then absorbed.
- Juice with pulp had ~45% more antioxidants (total anthocyanins) than juice if the pulp was removed.
- Juice with pulp was significantly higher in plasma antioxidant levels.
- Antioxidant compounds were consistently higher for 4 hours.

Clarified (pulp removed) vs Unclarified Açai Juice Study in Healthy Adults

- 6. Peak antioxidant levels in the blood dropped sooner if pulp is missing.
- 7. The maximum concentration of antioxidants was 105% higher if the pulp was retained.
- Half-life of juice with pulp was 110% longer (6.6 hours) than without pulp (3 hours).

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Brain Health and Oxidative Stress

The brain only weighs 2% of the body's weight, but it uses ~20% of the oxygen we breath.

It is susceptible to oxidative stress.

Does not possess the same antioxidant defenses used by the rest of the body.

Is rich in fatty acids subject to oxidation.

Is high in iron which can act as a catalyst, increases risk of lipid peroxidation.

Oxidative Stress a Risk Factor Against Brain Health

Lipid peroxidation is associated with progressive damage to brain cells.

To protect against oxidative damage, the brain releases two enzymes.

Catalase converts hydrogen peroxide to harmless water and molecular oxygen.

Superoxide dismutase (SOD) converts superoxide to oxygen and hydrogen peroxide (H_2O_2), which catalase turns H_2O_2 into H_2O and oxygen.

Açai Pulp Protects Against Oxidative Stress in the Brain

A Brazilian study found evidence that the pulp in açai might protect the brain from free radical damage.

The study pre-treated brain tissues of rats with açai.

They exposed the tissues to hydrogen peroxide.

The antioxidant polyphenols in acai significantly reduced the levels of lipid and protein damage caused by H_2O_2 .

Authors concluded that "açai could prevent the development of age-related neurodegenerative diseases."

Clearly, more research is needed.

Evaluating Antioxidant Activity of Açai in Human Cells

- Does açai support healthy immune function?
 Studied reactive oxygen species formation using polymorphonuclear cells (ROS PMN) assay
- Does açai provoke cellular signaling between cells?
 Used cell-based antioxidant protection in erythrocyte (CAP-e) assay.

Antioxidant Protection of Human Erythrocytes Using the CAP-e Assay

- Açai was able to significantly protect human cells from oxidative damage.
- 2) Reduce production of free radicals.
- Reduce proinflammatory activity:
- a) fmlp (p<0.001)
- b) Leukotriene B4 (p<0.05)
- c) Interlukin-8 (p<0.03)



Study of a complex Açai/fruit juice drink

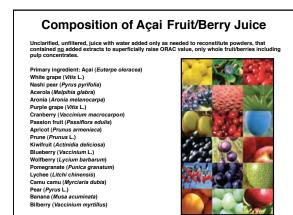
Questions:

- 1) Are the antioxidants in the juice absorbed after consumption?
- 2) Does it enter into circulation?
- 3) Does it enter into cells?
- 4) Does it protect human cells during oxidative stress?

Will 4 ounces a day of the juice enter human cells and protect adults from oxidative stress within 4 hours?

Will adults have less lipid peroxidation in their serum within 4 hours during a state of oxidative stress?

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Cell-based Antioxidant Protection in Erythrocytes (CAP-e) Assay

- A novel assay for evaluation of whether antioxidants in foods are capable of penetrating and protecting cells from oxidative damage.
- Assay is qualitative in principle and does allow for semi-quantitative comparisons to standards such as gallic acid, Trolox, and ascorbic acid.
- Ideally suited for food and natural products research. (Does not use quercitin as standard.)

The CAP-e constitutes a cell-based model for antioxidant testing that neither has the complexity of the PMN/monocytes assay, nor the risk of misinterpretation of a tumor cell-based assay.



In the CAP-e assay, the cells are exposed to test products in physiological saline.

The cells are allowed time to absorb compounds from the test product.

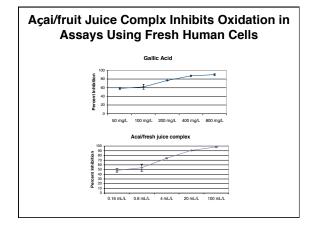
Any compounds not absorbed by the cells are then removed from the assay by centrifugation and washing.

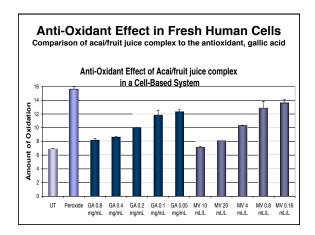
The cells are exposed to a precursor dye that becomes fluorescent if exposed to oxidative damage.

Subsequently, an oxidative challenge (for example H_2O_2) is introduced. The fluorescence intensity equals the amount of oxidative damage.

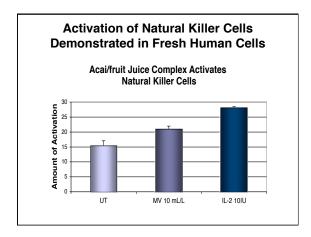
Positive control wells are exposed to oxidative damage without antioxidant protection, and serve as a measure of optimal oxidative damage.

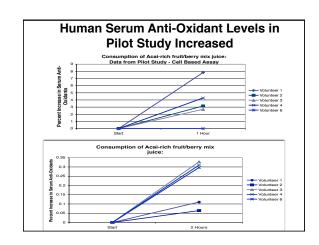
Any reduction of oxidative damage to the cells exposed to the test product is a measure of antioxidant protection.

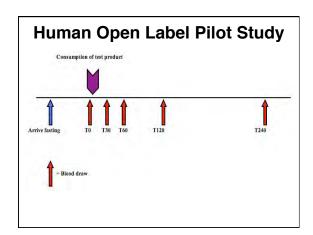


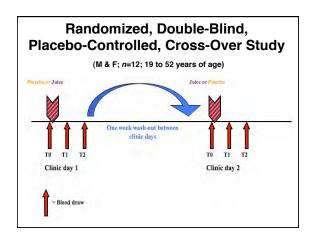


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Pesults Drinking 4 ounces of an açai/fruit juice complex resulted in: 1) Increased antioxidant activity; 2) Increased levels of antioxidant compounds entering cells to protect them from oxidative stress (p<0.03 1st hour; p<0.015 at 2nd hour) 3) 91% of healthy participants had less lipid peroxidation in serum within just 2 hours of consumption compared to placebo (p=0.01). Jersen GS, Wu X, Patterson KM, et al. In vito and in vito antioxidate and anti-influentatory capacity of an antioxidate-rich fout and berry jusco blend. Persults of a plot and rendemized, double-blinded, placebo-controlled, crossover study. J Agricultural Food Cremetry, 2008, 64(16): 6126-61231.

