

# Increased Antioxidant Capacity and Inhibition of Lipid Peroxidation in Healthy Adults Consuming an Açai (*Euterpe oleracea*) Fruit-Based Juice

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## Abstract

The *in vitro* and *in vivo* properties of an açai based juice blend (MonaVie Active®, Salt Lake City, UT) were evaluated. Initially, a phenolic profile was determined for the beverage, and a cell-based antioxidant protection (CAP-e) assay (Honzel et al., 2008) was performed, which showed that the antioxidants in the beverage could penetrate erythrocytes and significantly protect the cells from oxidative damage *in vitro* ( $p < 0.001$ ). Polymorphonuclear (PMN) cells exposed to the beverage showed significantly reduced formation of reactive oxygen species (ROS) and also had significantly reduced migration toward three pro-inflammatory chemoattractants.

A randomized, double-blind, placebo-controlled, cross-over study was performed on the beverage using 12 healthy subjects, 19-52 years of age (Jensen et al., 2008). At both one and two hours post consumption, a statistically significant increase in antioxidant capacity within subjects was noted based upon the CAP-e assay performed on serum ( $p < 0.03$  and  $p < 0.015$ ). Additionally, a statistically significant decrease in serum lipid peroxidation was noted using the thiobarbituric acid reactive substances assay (TBARS) at two hours after consumption ( $p < 0.01$ ), together suggesting an *in vivo* antioxidant effect and, hence, bioavailability of the beverage.

## INTRODUCTION

The palm tree *Euterpe oleracea* Mart. grows primarily in the rain forest of the Amazon. British, Portuguese and American botanists of the 18<sup>th</sup> and 19<sup>th</sup> century documented the use of the fruit of *E. oleracea*, known as the açai berry, as one of the primary foods consumed by natives living in the Amazon (Schauss, 2008). The berry has a favorable nutritional composition and a long harvest season (May to December). Up to 7,000 *E. oleracea* palms have been found to grow per acre in the Amazonian flood plains, making the availability of the fruit viable for commercial consumption worldwide. The palm has extraordinary resilience to flooding and wet soil conditions and has the ability to withstand equatorial solar radiation when it serves as the canopy of the rain forest. The palm can grow to a height of 32 meters and fruit for nearly 100 years (Schauss, 2008), although to maximize commercial production of the fruit, the palm stands are thinned at eight to ten years of age.

Each berry is about 2.5 cm in size, appearing almost black at maturity. The berries are not sweet nor do they have a taste familiar to most palates. Yet, in large cities along the Amazon, particularly in Amapa and Para states, Brazil, daily consumption of açai averages up to 2.0 liters of fresh juice a day. The pulp is also added to a wide range of staple foods, such as manioc (*Manihot esculenta*), served as a thick soup, or to fish or meat dishes (Schauss, 2008).

Anthocyanins, proanthocyanidin polymers, and other flavonoids are the predominant phytochemicals in açai (Schauss et al., 2006b). Besides a complement of vitamins, minerals, trace elements, soluble and insoluble fiber, phytosterols (beta-sitosterol, campesterol, and stigmasterol), and a low sugar content (1.3%), the total polyunsaturated, monounsaturated, and saturated fatty acids in açai contribute 13%, 60%, and 26%, respectively, to its total fatty acids content. Oleic acid (56%), palmitic acid (24%) and linoleic acid (13%) are the dominant fatty acids. Nineteen amino acids, including all of the essential amino acids, are found in açai (Schauss et al., 2006b).

Unlike spray-dried açai, which has a comparatively unimpressive ORAC score, freeze-dried (FD) açai has a Total ORAC of 1,027  $\mu\text{mol TE/g}$  (Schauss, 2008; Schauss et al., 2006a). The ORAC-lipophilic scavenging activity of açai, at 29.6  $\mu\text{mol TE/g}$ , is the highest of any known fruit. Açai's superoxide ( $\text{O}_2^-$ ) scavenging capacity, based on the superoxide radical absorbance capacity (SOD) assay, is 1,614 units/g, the highest reported to date for any fruit or vegetable by over 1,000 units/g. FD açai also has high hydroxyl and peroxynitrite radical scavenging activity based on the hydroxyl radical averting capacity (HORAC) and peroxynitrite radical averting capacity (NORAC) assays (Schauss et al., 2006a). The Trolox Equivalent Antioxidant Capacity (TEAC) assay and Ferric Reducing Antioxidant Power (FRAP) assay show similar high antioxidant capacity values for FD açai of 744  $\mu\text{mol TE/g}$  and 249  $\mu\text{mol TE/g}$ , respectively (Schauss, in press).

The total phenolics of açai were calculated as 13.9 mg gallic acid equivalents/g. The ratio between ORAC-hydrophilic scores and total phenolics has been found to vary dramatically in foods. However, the ratio for FD açai is five times greater than that found in other fruits. A possible explanation for this could be that the antioxidants found in açai are unusually strong and not just reliant on its polyphenolics, but rather on higher molecular weight oligomers (Schauss et al., 2006a).

It has been demonstrated that açai's antioxidants can enter human cells and protect these from oxidative damage in the CAP-e cell based antioxidant protection assay using erythrocytes (Honzel et al., 2008). Furthermore, açai-treated human neutrophil cells show a dose-dependent inhibition of  $\text{H}_2\text{O}_2$ -induced ROS formation, at doses down to 0.1 ppt (Schauss et al., 2006a). When comparing the doses at which an antioxidant protection and doses at which an anti-inflammatory effect are seen, the data suggest that anti-inflammatory cellular signaling is responsible for the reduced ROS formation. Lastly, açai has shown mild cyclooxygenase COX-1 and COX-2 inhibitor action and has been found to have a slight inhibitory effect on production of lipopolysaccharide (LPS)-induced nitric oxide (Schauss et al., 2006a).

MonaVie Active is a proprietary juice blend made with açai berries as its primary ingredient. Because of the encouraging anti-inflammatory and antioxidant findings described previously for the açai berry, we were inspired to examine for similar in vivo properties of this beverage in humans in a step-wise fashion.

## **MATERIALS AND METHODS**

A detailed description of all materials and methods can be found in the recent publication by G.S. Jensen and colleagues (Jensen et al., 2008). The methods are briefly described here.

### **Phenolic Analysis**

Phenolic acid analysis was performed on the beverage using the techniques of HPLC (Agilent 1100HPLC system, Agilent Technologies, Palo Alto, CA) and mass spectrometry (4000 Q TRAP mass spectrometer, Applied Biosystems, Foster City, CA).

### **Cell-Based Antioxidant Protection of Erythrocytes Assay (CAPE-e), Polymorpho-Nuclear (PMN) Cell Migration and Reactive Oxygen Species Production In Vitro**

Peripheral human venous blood was obtained for the in vitro studies from healthy volunteers, and PMN and erythrocytes were isolated from the blood. The erythrocytes were utilized in the CAP-e assay, where the cells were treated with the beverage, washed,

and then stained with a fluorescent probe that detects free radical damage. Cells were then treated with the free radical producer hydrogen peroxide, and fluorescence in the cells was measured using flow cytometry.

The PMN cells were treated with the beverage and used to measure ROS formation and migration toward three different pro-inflammatory chemoattractants: bacterial peptide f-Met-Leu-Phe (fmlp), leukotriene B4 (LTB4), or interleukin-8 (IL-8). Fluorescence intensity was also utilized to determine these measurements.

### **Human Clinical Study**

The randomized, double-blind, placebo-controlled, cross-over human clinical study was approved by the Sky Lakes Medical Center Institutional Review Board. Seven healthy subjects participated in a pilot study, and 12 in the succeeding clinical trial. Due to the difficulty in developing a liquid placebo that could not be discerned, an encapsulated placebo was made using potato flakes and purple food coloring. Subjects were purposefully left unsure as to whether the capsules were the placebo or a solid version of the product. Two methods of serum antioxidant evaluation were performed on the subjects' blood and compared in the pilot study, including the ORAC and the CAP-e assays. Because ORAC testing did not result in a trend toward increased antioxidant activity in the subjects, it was not utilized in the following clinical trial. Subjects in the clinical trial arrived for testing after an overnight fast. A baseline blood sample was collected, and then the subjects consumed 120 ml of the juice blend. Blood was drawn again for analysis at one and two hours after consumption. CAP-e assay (as described above) and TBARS assay (utilizing a kit from Cayman Chemical Co, Ann Arbor, MI) were performed on serum samples. Subjects were tested in a randomized fashion after consumption of the beverage or placebo on different days at least one week apart, and data was compared at baseline and after treatment in a within subject design.

### **Statistical Analysis**

Analysis of the data obtained in these studies was performed using the student's *t* test, two-tailed *t* test, and analysis of variance (ANOVA).

## **RESULTS AND DISCUSSION**

### **Phenolic Analysis**

Major phenolics found in the juice blend included anthocyanins, proanthocyanidins, and phenolic acids. The overall profile was expectedly somewhat different than that of previously described freeze dried açai itself, since the beverage contains other fruits besides its main ingredient açai. However, several key phenolics found previously in açai, such as cyaniding 3- glucoside and cyaniding 3-rutinoside (Schauss et al., 2006b) were also major anothocyanins found in the beverage blend.

### **Cell-Based Antioxidant Protection of Erythrocytes Assay (CAPE-e), Polymorpho-Nuclear Cell Migration and Reactive Oxygen Species Production In Vitro**

In vitro, the beverage showed a highly significant dose-dependent antioxidant protection effect in the CAP-e assay (*p* values ranged from  $< 0.001$  at highest beverage concentration of 10 g/L and  $< 0.045$  for lowest concentration of 0.016 g/L). PMN cells which produce ROS very quickly upon stimulation, showed dose-dependently significantly less ROS formation after treatment with the beverage ( $p < 0.003$  at the highest dose). Complex dose effects were seen when looking at migration toward the bacterial peptide fmlp; attraction was enhanced or inhibited depending upon the whether the dose was low or high respectively. However, a more clear dose-dependent inhibitory effect on migration of PMN cells was seen toward LTB4 ( $p < 0.05$ ) and IL8 ( $p < 0.03$ ).

### **Human Clinical Study**

In the clinical study, intake of the beverage rendered a statistically significant increase in serum antioxidant capacity in the subjects blood at both the one hour and two

hour post consumption time points ( $p < 0.03$  and  $p < 0.015$ , respectively) as measured by the CAP-e assay. Consumption of the beverage also resulted in a statistically significant decrease in lipid peroxidation in the blood after two hours ( $p < 0.01$ ), as measured by the TBARS assay. A 45% correlation was calculated at the two-hour time point between the increased antioxidant capacity and the reduction in lipid peroxidation.

It remains unclear whether these statistically significant results found after intake of the juice blend were wholly due to the açai ingredient, as a combination of açai and the other fruit components added to the juice could also be responsible for the results. Further in vivo studies are being carried out to determine possible mechanisms of action. A more detailed description and discussion of all results can be found in the recent publication by G. S. Jensen and colleagues (Jensen et al., 2008).

## CONCLUSIONS

The highly statistically significant antioxidant effect of treatment with MonaVie Active açai-based juice on cells in the CAP-e assay both in vitro and in vivo in human subjects suggests that the antioxidant components from the beverage are able to enter living cells and protect the cells against oxidative insult and are also able to enter serum after consumption and assist in protection in vivo. The significant reduction of lipid peroxidation as measured using the TBARS assay further suggests that consumption of this beverage results in the protection of lipids from oxidative damage. While it is unclear if these results are due solely to the high açai content of the juice, the results correlate well to previous data performed on the freeze-dried açai berry alone.

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