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TOTAL PHENOLICS AND ANTIOXIDANT ACTIVITY OF A FUNCTIONAL GEL BASED ON AÇAÍ (Euterpe oleracea Martius) PULP

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ABSTRACT

The beneficial effects attributed to açaí and its derivatives are associated with its significant content of phenolic compounds, which has stimulated multiple/many researchers to identify these compounds and demonstrate their pharmacological properties, which include anti-inflammatory and antioxidant activities. The aim of this study was to determine the concentration of total phenolic compounds and the antioxidant activity of lyophilized pulps and functional gels of açaí. The antioxidant activity was the determining variable for the choice of the best formulation for the supplement. Pulp L exhibited higher antioxidant activity than the others according to the DPPH method, as well as higher content of total phenolic compounds. For the antioxidant activity, gels 3 and 4 did not differ significantly for the DPPH method, whereas in the ORAC method, gels 1 and 3 showed greater Trolox equivalents. It was concluded that gels 3 and 4 which had higher concentrations of lyophilized açaí pulp, showed higher concentrations of total phenolic compounds and consequently higher antioxidant capacity as demonstrated by the two methods applied and by the results obtained in the statistical analysis. Functional açaí gel may be used by adults who are involved in physical activities to reduce oxidative stress.

Indexing terms/Keywords:

açaí, functional gel, antioxidant capacity, Euterpe oleracea

Academic Discipline: Food Science and Technology of Fruits

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Antioxidant activity analyses of fruits and vegetables. Reduction of free radicals production. Reactive oxygen species (ROS). ORAC, DPPH and others merhods.

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INTRODUCTION

Energy drinks, gels and dairy products enriched with bioactive compounds have become popular because of claims that they aid in maintaining health, have anti-aging properties, increase energy and physical and mental performance in addition to helping improve cardiovascular conditions (FLUEGEL et al., 2010; BALDISSERA et al., 2011)[1,2].

Currently, there is great interest in the study of oxidative stress (OS) due primarily to the constant action of reactive oxygen species (ROS) and reactive nitrogen species generated in inflammatory processes, through certain biological dysfunctions or from foods (HALLIWELL et al., 1995)[3].

Physical exercise is one of the factors that can increase the production of free radicals and reactive oxygen species as part of the metabolic reactions due to muscle training-induced OS (BLOOMER, 2005 and 2006)[4,5]. According to Pietta (2000)[6], excess free radicals in the body can be combated with antioxidant substances produced by the body or absorbed from the diet (α-tocopherol, β-carotene, ascorbic acid and phenolic compounds such as flavonoids and polyflavonoids), which when present at low concentrations compared to the oxidizable substrate, significantly regenerate or prevent their oxidation. Diet and supplementation with antioxidants are preventive measures that can supply the needs of an organism with an imbalance between the production of ROS and endogenous or exogenous antioxidants induced by OS (MORILLAS-RUIZ, 2006)[7].

To be considered a functional supplement for athletes, a product must control at least two physiological or biochemical parameters as assessed by clinical studies that may be associated with benefits to the health of the group studied but without offering risk to the probable consumers (EUSSEN et al., 2011)[8]. Açaí is a viable option for enriching these formulations considering its composition rich in flavonoids in addition to being a fruit that is widely cultivated in Brazil (Pará, Amazonas, Maranhão and Amapá states) and sold in the form of frozen pulp (DEL POZO-INSFRAN et al., 2004)[9].

The beneficial effects attributed to this fruit and its derivatives are associated with its significant content of phenolic compounds, which has stimulated many researchers to identify these compounds and assess their pharmacological properties, which include anti-inflammatory, antioxidant and cardioprotective activities (HOGAN et al., 2010; HEINRICH, DHANJI & CASSELMAN, 2011)[10,11].

The widely available drinks prepared from tropical fruits and enriched with antioxidant nutrients are gaining great popular interest due to the desire of consumers to incorporate various healthier drinks and foods into their diet with exotic flavors and aromas. At the same time, the industry has focused on producing drinks with health claims as a market differentiator (FLUEGEL et al., 2010; MUÑOZ et al., 2010)[1,12].

The objective of this study was to determine the concentration of total phenolic compounds and the antioxidant activity of lyophilized pulps and functional açaí gels.

MATERIALS AND METHODS

Selection and lyophilization of açaí pulp

Açaí pulps were provided by the Brasfrut company. After adequate evaluation of its antioxidant activity, nutritional composition and microbiological quality, the pulp that exhibited the best characteristics for subsequent lyophilization was selected.The pulp was lyophilized for 3 days in a Labconco Freeze-Dryer 8 lyophilizer according to the capacity of the equipment (approximately 12 hours) and frozen at – 40°C.The lyophilized pulp was packed into high-density aluminum-coated polyethylene bags to block light incidence and thus preserve the constituents. The bags were then vacuum-sealed and stored at - 10°C until the performance of the experiments and analyses.

Açaí gel formulation

Based on a 2^2 factorial experimental design (MONTGOMERY & RUNGER, 2003)[13] four gels were formulated with concentrations of 8, 12, 16 and 20% lyophilized açaí pulp and sugars. The ingredients used were locust gum, filtered water and citric acid in sufficient quantities for the desired effect.

The antioxidant activity was the determining variable for the choice of the best formulation for the supplement. The gels were codiffied as G1, G2, G3 and G4.

Total phenolic compounds

The total phenolic compounds in the lyophilized açaí pulp samples selected based on the highest total solids content and of the gels were determined by the spectrophotometric method, using the Folin-Ciocalteau reagent by Singleton and Rossi (1965)[14]. Briefly, a 1.0 mL sample of gel was weighed, transferred to a 100 mL volumetric flask and its volume was completed with distilled water. For the preparation of aqueous açaí extract, 1.0 g of lyophilized açaí pulp from the samples was weighed and suspended in a 200 mL volumetric flask with the final volume adjusted with distilled water. After this procedure, the samples were placed in an ultrasound bath for 15 minutes, filtered twice and stored at approximately 4ºC for 12 hours. The reactions occurred in 5.0 mL test tubes using 2.5 mL of the reagent (10%), 0.5 mL aqueous extract from the sample (or 0.5 mL distilled water in place of the sample for the blank) and 2.0 mL of 7.5% Na_2CO_3 solution. The samples (in triplicate) were placed in a dark room for 2 hours to complete the reaction prior to reading the absorbance at 760 nm in a spectrophotometer (UV mini 1240-UV-vis, Shimadzu®). The results are expressed in µggallic acid equivalents (GAE) per 100 g of fresh weight for the lyophilized pulp samples and for the gel they are expressed in GAE/100 mL of sample.

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Total anthocyanins

To determine the total concentration of anthocyanins in the selected samples of lyophilized açaí pulp and in the gels, the differential pH method described by Wang & Lin (2000)[15]. The analyses were performed in triplicate and the results expressed in mg.100 mL^{-1} or g.100 g^{-1} of the standard.

Initially, 0.025 M potassium chloride (pH 1.0) and 0.4 M sodium acetate (pH 4.5) buffer solutions were prepared. Next, 1.0 mL of gel was weighed and transferred to a 200 mL volumetric flask and the volume was completed with each of the buffers separately. Before being read, the samples were incubated at room temperature for 30 minutes. The samples prepared with potassium chloride and sodium acetate buffer were read at 520 nm and 700 nm, respectively, in a spectrophotometer.To calculate the concentration of monomeric anthocyanins (TMA) from the absorbance of the diluted sample (A) the following equations were used:

$A = (A_{\lambda max} - A700)\ pH\ 1.0 – (A_{\lambda max} - A700)\ pH\ 4.5$

$TMA\ (mg/L) = (A \times MW \times 1000)/(\varepsilon \times 1)$

Where: MW = molecular weight of the predominant anthocyanin in the sample.

Antioxidant activity

DPPH

The ability to scavenge DPPH free radicals was determined based on the method of Rufino et al. (2007)[16]. In this method, the stable radical 2.2-diphenyl-1-picrylhydrazyl (DPPH·) is reduced, which by fixing H· (removed from the antioxidant in the sample) leads to a decrease in absorbance.The preparation of extracts from the samples of lyophilized açaí and from the gel occurred in the same manner as in the determination of phenolic compounds. A 0.1 mL aliquot of aqueous extract from the sample was added to 3.9 mL of DPPH solution.The aqueous extract from the sample was substituted with the same quantity of methanol for the blank. The samples were kept in a dark room for 1 hour prior to the absorbance readings in a spectrophotometer at 517 nm. The results were calculated according to the following equation and expressed in percent capacity to scavenge DPPH free radicals in GAEs.

$$AA\ \% = 100 - \frac{[(Abs.C - Abs.S) \times 100]}{Abs.\ NC}$$

Where: Abs.S = absorbance of the sample solution;

Abs.C = absorbance of the control;

Abs. NC = absorbance of the negative control.

ORAC (Oxygen Radical Absorbance Capacity)

The methodology consists of measuring the loss of fluorescence of proteins as a consequence of the loss of their conformation due to oxidative damage following the use of AAPH (2.2'-azobis'2-amidino-propane) as a free radical-generating system (DÁVALOS et al., 2003)[18]. For the aqueous extract of lyophilized açaí and for the gel a 0.02 mL aliquot was filtered and removed, and 0.12 mL of fluorescein and 0.06 mL of AAPH were added to the wells.The readings were performed immediately in a FLUO star Omega spectrofluorimeter. The fluorescence of each sample (ζ excitation: 485 ηm and ζ emission: 520 ηm) was determined every minute for 80 minutes. Trolox was used as a standard and buffer solution (pH = 7.4) was used as a blank under the same conditions described for the samples, in triplicate. The results were expressed in relative ORAC values (Trolox equivalents mg/100 g) as indicated by equations (1) and (2) below:

1) $AUC = 1 + f1/f0 + \ldots + fi/f0 + f30/f0$

Where: f_0 represents the fluorescence obtained at time 0 and fi represents the fluorescence obtained at times between 0 and 80 minutes.

2) ORAC value = $[(AUC_{sample} - AUC_{blank}) / (AUC_{Trolox} - AUC_{blank})] \times (molarity_{Trolox} / molarity_{sample})$

Statistical analys

The statistical analysis was performed using analysis of variance (ANOVA) at a significance level of $P < 0.05$. Duncan's test was used to determine the differences between the means. The analyses were performed with Microsoft Excel.

RESULTS AND DISCUSSION

DPPH, ORAC, total phenolics and total anthocyanins in açaí pulps

Pulp L showed higher antioxidant activity than the others according to the DPPH method (Table 1), as well as higher content of total phenolic compounds, whereas pulp M showed higher activity than the other pulps using the ORAC method and lower total phenolic compound content.

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Table 1. Antioxidant activity (DPPH and ORAC), total phenolics and, total anthocyanins (TA) in açaí pulps E, L and, M

Analyses	Açaí Pulp E	Açaí Pulp L	Açaí Pulp M
DPPH µg. g^{-1} GAE	0.73[a] (± 0.33)	1.43[b] (± 0.15)	1.03[c] (± 0.08)
Total phenolics µg. g^{-1} GAE	2921.53[a] (± 0.16)	5869.82[b] (± 0.22)	2202.28[c] (± 0.23)
ORAC µg. g^{-1} GAE	3781.00[a] (± 0.28)	4851.00[b] (± 0.18)	5969.83[c] (± 0.04)
Total anthocyanins (AT) mg. g^{-1}cyd-glu	7.9[a] (± 0.32)	9.9[b] (± 0.30)	7.1[a] (± 0.46)

Different letters in the same column differ significantly (P < 0.05)

In a study performed by Spada et al. (2008)[19], it was shown that strawberry, acerola, orange and açaí are rich in carotenoids, ascorbic acid and phenolic compounds, which confer important antioxidant activity to these fruits. Rufino et al. (2007 and 2010)[16,17] and Schauss et al. (2006)[20] also state that the total content of phenolic compounds and anthocyanins is associated with the powerful antioxidant activity of different fruits.Table 2 shows the values obtained with regard to the nutritional evaluation, total phenolic compounds, total anthocyanins and antioxidant activity of the lyophilized açaí pulp for the preparation of a functional energy gel.

Table 2. Total phenolics, total anthocyanins (TA) DPPH, ORAC and, nutritional composition in freeze-dried açaí pulps

Components	g. 100 g^{-1}
Carbohydrates	36.61
Linoleic Acid	6.24
Oleic Acid	28.69
Total solids	13.14
Moisture	86.86
Zinc	3.08
Iron (mg /Kg)	16.32
Aminoacids	8.52
Glutamic Acid	0.13
Isoleucine	0.06
Leucine	0.10
Valine	0.10
Metionin	0.01
Histidin	0.02
Phenilalanine	0.07
Cafein	10.55
Vit A (U/L)	0.78
Vit E (mg/Kg)	17.00
Omega 6	0.82
Fibers	1.83
Proteins	8.30
Kcal/100g	84.04
Total lipids	6.72
ORAC (mcg/g GAE)	3558.00
DPPH (% scavenge DPPH free radicals)	1.03
Total anthocyanins (mg/g ms)	0.71
Total phenolics (mcg/g GAE)	2202.28

*GAE- Galic Acid Equivalent

* dm = dry matter

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Spada (2010)[21] analyzed the concentration of minerals from 23 frozen fruit samples using the PIXE (Particle Induced X-ray) method. All of the fruits, including açaí pulp, contained Fe, Mg, Cl, P, K and Na. The concentration of total phenolic compounds varied from 2202.28 to 5869.82 μg.g^{-1}, which can be considered a good concentration of total phenolic compounds. Total anthocyanins varied from 7.1 to 9.9 mg.100 g^{-1} of dry matter, which is a good concentration of anthocyanins according to an analysis of previous studies (RUFINO et al., 2010)[17].The gallic acid standard curve (Figure 1) showed a coefficient of determination R^2 equal to 0.967, which was adequate for the concentrations of the samples, and R^2 for the gallic acid curve for the gels was equal to 0.9976.

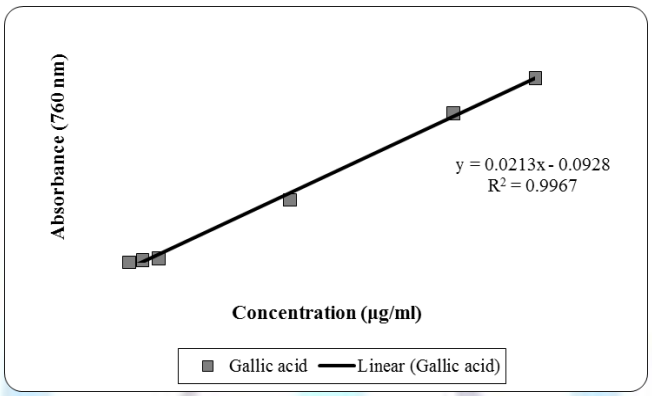

Figure 1. Gallic acid calibration curve for lyophilized açaí pulp total phenolic analysis

Good correlation was observed between the ORAC and total anthocyanins results for the lyophilized açaí pulp (R^2 = 0.9921) as well as between the total phenolic compounds method and the ORAC method (R^2 = 0.9493).The free radical sequestration capacities of the L, E, and M samples by the DPPH (% FRS) method were 1.426, 0.726 and 1.030 and the results from ORAC (mg g^{-1} GAE) were 4851, 3781 and 3568, respectively (Table 3).

Table 3. Apparent viscosity of comercial gels and açaí gels whit different locust gum contents

Açaí based gels	Aparent viscosity (m Pa.s)
Commercial 1	3700.00^a
Commercial 2	300.00^b
Gel 3 (2.0 g of gum)	735000.00^c
Gel 3 (1.0 g of gum)	9500.00^d
Gel 3 (0.4g of gum)	2950.00^e
Gel 3 (0.5 g of gum)	4250.00^f

Viscosity: measured in a Rotational Analogyc viscosymeter (Quimis, Q860A24).

Different letter in the same column differ significantly ($P < 0.05$).

The samples of lyophilized açaí pulp with high total solids concentrations therefore had high concentrations of total phenolic compounds and anthocyanins. However, pulp L had the highest values for these determinations.

The phenolic compounds and total anthocyanins in the lyophilized açaí samples were found to be strongly correlated when the ORAC method was used, demonstrating that the greater the concentration of phenolic compounds and anthocyanins, the greater the antioxidant activity by the ORAC compared to the DPPH method.

Formulation and evaluation of a gel supplemented with lyophilized açaí pulp

The initial basic gel formulation for the preparation of the final product was composed of sugars at concentrations of 29.75 (G1), 31.30 (G2), 29.75 (G3) and 31.30 (G4). The ingredients used were locust gum, filtered water and citric acid in sufficient quantities for the desired effect. For inclusion of the lyophilized açaí pulp in the gel, adjustments were made to the sugar concentration and to the water, and four gels with different concentrations lyophilized açaí pulp were prepared.

The gum content in gels was fixed in 0.4 g.100^{-1}g based on a previous analysis of the viscosity of the products existing on the market (Table 3), and that of citric acid was fixed at 0.2 g.100^{-1}g.

Total phenolic compounds, anthocyanins and antioxidant activity of the functional açaí gel

The antioxidant activity was the determinant in the choice of the formulation that best met the expectations for the final product.

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Total phenolic compounds and total anthocyanins

Table 4 shows the concentrations of total phenolic compounds (TPC) and total anthocyanins (TA) of the açaí. As expected, gels 3 and 4, which had the highest concentrations of lyophilized açaí pulp, showed the highest concentrations of TPC. The total phenolic compound concentrations of the four açaí gels analyzed were expressed in mg GAE/100 g. The TPC values varied from 230.56 to 346.67 mg GAE/100 g. Samples G3 and G4, which contained the highest lyophilized açaí concentrations, had the highest TPC values.

Table 4. Total phenolics and total anthocyanins (TA) in the four gels containing lyophilized açaí pulp

Gel	Total phenolics (EAG/ 100 g sample)	AT (mg/100g)
G1	230.56[a] (± 3.69)	21.33[a] (± 1.19)
G2	203.67[b] (± 8.86)	24.19[b] (± 0.24)
G3	322.03[c] (± 3.48)	33.11[c] (± 0.33)
G4	346.67[d] (± 8.24)	27.34[d] (± 0.32)

Means = ±; SD = Standard deviation an, n = 3

Different letter in the same column differ significantly (P < 0.05).

Lower values were found by Kuskoski et al. (2006)[22] analyzing frozen pulps of blackberry, açaí, strawberry and grape. The authors found TPC concentrations of 118.9; 136.8; 132.1 and 117.1 mg GAE/100 g for these fruits, respectively. Santos et al. (2008)[23] reported TPC values between 182.95 GAE/100 g and 598.95 GAE/100 g in twelve açaí pulps. The results revealed higher TA contents in samples G3 and G4 compared to the other samples, demonstrating good content for these compounds. According to Kuskoski et al. (2005)[24], the amount of total anthocyanins in whole pulp of strawberry and açaí were found to be 23.7 and 22.8 mg/100 g, respectively. In a study performed by Cruz (2008)[25], the concentration of total anthocyanins in fine mature açaí pulp was 80.4 mg of cyanidin-3-glucoside/100 g, a value close to that obtained by Menezes (2005)[25] of 78.8 mg/100 g in crude açaí. Furthermore, this value is more than double the concentration found by Kuskosky et al. (2006)[22] for grape pulp (30.9 mg/100 g) and almost double the value of blackberry pulp (41.8 mg/100 g), indicating that açaí is a good source of these pigments. According to Schauss et al. (2006)[20] anthocyanins, the pigment responsible for the violet color of the fruit, and other flavonoid compounds compose the majority of the phytochemicals present in açaí. These authors reported a total anthocyanins value in açaí of 3.19 mg/g in dry weight. Some authors emphasized that anthocyanins appear to be the components that most contribute to the antioxidant activity of açaí (POZO-INSFRAN, BRENES & TALCOTT, 2004; CHIN, 2008)[26, 27]. Yuyama et al. (2011)[28] observed variation from 128.4 to 868.9 mg/100 g on a dry basis, in anthocyanins in açaí fruit. Considering an average moisture content of açaí juice of 88%, the upper and lower limits in açaí would be on the order of 104.3 mg/100 g and 15.4 mg/100 g, respectively. Phenolic compounds present in extract of crude oil from açaí fruit were identified for the first time by Pacheco-Palencia et al. (2008)[29]. The stability of açaí oil containing three different concentrations of TPC were evaluated under short and long term storage for the oxidation of lipids and the impact on the antioxidant capacity of the TPC.

DPPH and ORAC

Table 5 shows the percentage sequestration of free radicals from DPPH of the formulated gel samples. The gallic acid calibration curve for the DPPH analysis of the lyophilized açaí gels showed an adequate coefficient of determination (R^2) of 0.9974, and that of the lyophilized açaí pulp was 0.9919. The values found by the ORAC method for the antioxidant capacity in lyophilized açaí pulp gel samples G1, G2, G3 and G4 were 61.27, 52.56, 82.34 and 54.26 Trolox equivalents, respectively.

Table 5. DPPH values of the four açaí based gels

Açaí based gels	DPPH (%)
G1	8.86[a] (± 0.12)
G2	9.10[b] (± 0.14)
G3	15.10[c] (± 0.31)
G4	15.03[c] (± 0.10)

Means = ±; SD = Standard deviation an, n = 3

Different letter in the same column differ significantly (P < 0.05).

Based on the results, gel 3 showed the highest antioxidant activity among the four gels evaluated. Moreover, the statistical analysis revealed that antioxidant activities of the four gels differed significantly from one to another (P < 0.05). Data from the literature have demonstrated the significant antioxidant action of lyophilized açaí pulp against superoxide radicals in a test with superoxide dismutase (SOD) and against peroxyl radicals in tests based on the capacity to absorb reactive

species through fluorescence reactions. In addition to its antioxidant activity, lyophilized açaí pulp has shown an important role as an inhibitor of the COX-1 and COX-2 enzymes, which mediate inflammatory processes (SCHAUSS et al., 2006)[20]. Analysis of the pulps from fruits such as blackberry, açaí, grape and guava with regard to their antioxidant activity by the DPPH method has shown that phenolic compounds contribute to their antioxidant activity and are directly correlated with it (HEIM et al., 2002)[30]. The number of DPPH molecules that are reduced is correlated with the presence of hydroxyl groups (MENSOR, 2001)[31]. In a study with condensed tannins from the leaves of Maytenus ilicifolia performed by Pessuto (2009)[32], the greater the number of phenolic hydroxyls, the greater the capacity for sequestration of free radicals. In the literature review performed by Bernaud & Funchal (2011)[33] involving data from recent studies investigating the antioxidant effects of açaí, the authors are unanimous in attributing antioxidant potential to the fruit of the açai palm, from its pulp to the extracted oil.

CONCLUSION

As expected, the gels three and four, which contained higher concentrations of freeze-dried acai pulp showed higher total phenolic content and therefore higher antioxidant activity. According to the results obtained in the determination of antioxidant activity, total phenolics and anthocyanins in the pulp and the acai lyophilized gels it can be concluded that lyophilization may be considered as an excellent alternative for pulp preservation due to the presence of important nutritional components found therein. It is also an excellent option for to be incorporated into meals for individuals with low weight, particularly child age, to be highly caloric. Furthermore, the use of functional açaí gel can be added to individual practitioners of physical activity aimed at reducing oxidative stress.

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REFERENCES

[1] Fluegel, S.M., Shultz, T.D., Powers, J.R., Clark, S., Barbosa-Leiker, C., Wright, B.R., Freson, B.R., Fluegel, T.S., Minch, J.D., Schwarzkopf, L.K., Miller, A.L. and Di Filippo, M.M. 2010. Whey beverages decrease blood pressure in prehypertensive and hypertensive young men and women.

[2] Baldissera, A.C., Betta, F.B, Penna, A.L.B. and Lindner, J.D.D. 2011. Functional Foods: A new frontier for the development of protein-based whey beverage.

[3] Halliwell, B., Aeschbach, R., Loliger, J., and Arouma, O.I. 1995. The characterization of antioxidants.

[4] Bloomer, R.J. 2005. Effects of acute aerobic and anaerobic exercise on blood markers of oxidative stress.

[5] Bloomer, R.J. 2006. Effects of acute aerobic and anaerobic exercise on blood markers of antioxidant supplements.

[6] Pietta, P.G. 2000. Flavonoids as antioxidants**.**

[7] Morillas-Ruiz, J.M 2006. Effects of polyphenolic antioxidants on exercise-induced oxidative stress.

[8] Eussen, S.R.B.M, Hans, V,Klungel, O.H,Garssen, J, VanLoveren, H., VanKranen, H.J. and Rompelberg, C.J.M. 2011. Functional foods and dietary supplements: Products at the interface between pharma and nutrition.

[9] Del Pozo-Insfran, D., Brenes, C.H., Talcott, S.T. and Rynes, L.D. 2004. Phytochemical composition and pigment stability of Açai (Euterpe oleracea Mart.).

[10] Hogan, S., Chung, H., Zhang, L., Li, J., Lee, Y., Dai, Y. and Zhou, K. 2010. Antiproliferative and antioxidant properties of anthocyanin-rich extract from açaí.

[11] Heinrich, M., Dhanji, T. and Casselman, I. 2011. Açai (Euterpe oleracea Mart.) A phytochemical and pharmacological assessment of the species' health claims.

[12] Muñoz, M.E., Galan, A.I., Palacios, E., Diez, M.A, Muguerza, B,, Cobaleda, C., Calvo, J.I., Aruoma, O.I, Sanchez-Garcia and Jimenez, R. 2010. Effect of an antioxidant functional food beverage on exercise-induced oxidative stress: A long-term and large-scale clinical intervention study.

[13] Montgomery, D.C. and Runger, G.C. 2003. Applied statistics and probability for engineer**.** LTC.

[14] Singleton, V.L. and Rossi, J.A. 1965. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagent

[15] Wang, S.I. and Lin, H.S. 2000. Antioxidant activity in fruits and leaves of blackberry, raspberry and strawberry varies with cultivar and developmental stage.

[16] Rufino, M.S.M., Alves, R.E., Brito, E.S., Morais, S.M, Sampaio, C.G., Pérez-Jiménez, J. and Saura-Calixto, F.D. 2007. Determination of total antioxidant activity in fruits by capturing the free radical ABTS. [17] Rufino, M.S.M., Alves, R.E., Brito, E.S., Pérez-Jiménez, J., Saura-Calixto, F.D. and Mancini-Filho, J. 2010. Bioactive compounds and antioxidant capacities of 18 non-traditional tropical fruits from Brazil.

[18] Dávalos, A., Bartolomé, B., Suberviola, J. and Gómez-Cordovés, C. 2003. ORAC- Fluorescein as a model for evaluating antioxidant activity of wines.

[19] Spada, P.D.S., Souza, G.G.N.,Bortolini, G.V.,Henriques, J.A.P. and Salvador, M. 2008. Antioxidant, mutagenic, and antimutagenic activity of frozen fruits.

[20] Schauss, A., Wu, X., Prior, R., Ou, B., Patel, D., Huang, D. and Kababick J. 2006. Phytochemical and nutrient composition of the freeze-dried amazonian palm berry Euterpe oleracea Mart. (açaí).

[21] Spada, P.D.S. 2010. Macro and microminerals: are frozen fruits a good source?

[22] Kuskoski, E.M., Asuero, G.A., Morales, M.T. and Fett, R. 2006. Wild fruits and pulps of frozen fruits: antioxidant activity, polyphenols and anthocyanins

[23] Santos, G.M. 2008. Correlation between antioxidant activity and bioactive compounds of commercial açaí pulp (Euterpe oleracea Mart).

[24] Kuskoski, E.M., Asuero, G.A., Troncoso, A.M., Mancini-Filho, J. and Fett, R. 2005. Aplicación de diversos métodos químicos para determinar a atividad antioxidante em pulpa de frutos.

[25] Cruz, A.P.C. 2008. Avaliação do efeito da extração e da microfiltração do açaí sobre sua

composição e atividade antioxidante. Universidade Federal do Rio de Janeiro: Instituto de Química, Rio de Janeiro. Thesis. 104p.

[26] Del Pozo-Insfran, D., Brenes, C.H. and Talcott, S.T. 2004. Phytochemical composition and pigment stability of Açai (Euterpe oleracea Mart.).

[27] Chin, Y.W., Chai, H.B., Keller, W.J. and Kinghorn, A.D. 2008. Lignans and other constituents of the fruits of Euterpe oleracea (Açai) with antioxidant and cytoprotective activities.

[28] Yuyama, L. K. O., Aguiar, J. P. L., Silva Filho, D. F., Yuyama, K., Varejão, M. J., Fávaro, D. I. T., Vasconcellos, M. B. A., Pimentel, S. A. and Caruso, M. S. F. 2011. Physicochemical characterization of acai juice ofEuterpe precatoria Mart. from different amazonian ecosystems.

[29] Pacheco-Palencia, L.A., Mertens-Talcott, S. and Talcott, S.T. 2008. Chemical composition, antioxidant properties, and thermal stability of a phytochemical enriched oil from açai (Euterpe oleracea Mart.).

[30] Heim, K.E., Tagliaferro, A.R. and Bobilya, D.J. 2002. Flavonoid antioxidants: chemistry, metabolism, and structure-activity relationships. Reviews: current topics.

[31] Mensor, L. L. 2001.Screening of brazilian plant extract for antioxidant activity by the use of DPPH free radical method.

[32] Pessuto, M.B., Costa, I.C., Souza, A.B., Nicoli, F. M. and Mello, J. C. P., Petereit, F. and Luftmann, H. 2009. Atividade antioxidante de extratos e taninos condensados das folhas de Maytenus ilicifolia Mart. ex Reiss.

[33] Bernaud, F. S. R.and Funchal, C. D. (2011). Atividade antioxidante do açaí. Nutrição Brasil, 10(5), 310-316.