Effect of antioxidant potential of tropical fruit juices on antioxidant enzyme profiles and lipid peroxidation in rats

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A B S T R A C T

Fruits are a rich source of a variety of biologically active compounds that can have complementary and overlapping mechanisms of action, including detoxification, enzyme modulation and antioxidant effects. Although the effects of tropical fruits have been examined individually, the interactive antioxidant capacity of the bioactive compounds in these formulations has not been sufficiently explored. For this reason, this study investigated the effect of two tropical fruit juices (FA and FB) on lipid peroxidation and antioxidant enzymes in rats. Seven groups, with eight rats each, were fed a normal diet for 4 weeks, and were force-fed daily either water (control), 100, 200, or 400 mg of FA or FB per kg. The results showed that the liver superoxide dismutase and catalase activities (FA200), erythrocytes glutathione peroxidase (FB400) and thiobarbituric acid-reactive substances (FB100, FA400, FB200, FB400) were efficiently reduced by fruit juices when compared with control; whereas HDL-c increased (FB400).

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1. Introduction

Tropical fruits, e.g., camu-camu (Myrciaria dubia), acerola (Malpighia punificifolia), cashew-apple (Anacardium occidentale), yellow mombin (Spondias lutea L) and acai (Euterpe oleracea), are rich sources of water-soluble vitamins, provitamin A, phytosterols and phytochemicals (Müller, Gnoyke, Popken, & Bohm, 2010; Rosso, 2013; Silva et al., 2014). The great interest in the potential health benefits of these particular tropical fruits is due to their antioxidant content and bioactive compounds, and this encourages some food researchers to investigate the in vitro and in vivo effects of these fruits individually (Rosso, 2013 – see also references cited).

However, when the fruits are consumed together, the total antioxidant capacity of mixtures may be modified via synergistic, additive, or antagonism interactions among these constituents, which may alter their physiological impacts (Wang, Meckling, Marcone, Kalkuda, & Tsao, 2011).

Phytochemicals, especially phenolic compounds, exhibit great in vitro and in vivo antioxidant potential, and their beneficial effects are extensively reported in models involving oxidative stresses caused by hypercholesterolemic and atherogenic diets, for example (Auger et al., 2005; Décordé, Teissèdre, Auger, Cristol, & Rouanet, 2008). These bioactive compounds are able to scavenge radical oxygen species (ROS) and consequently reduce oxidative cell damage (Spormann et al., 2008). Lipids, especially polyunsaturated fatty acids, are sensitive to oxidation, leading to the formation of malondialdehyde (MDA). The accumulation of MDA in tissues or biological fluids is indicative of the extent of free radical generation, oxidative stress and tissue damage (Gutteridge, 1995). Thus, antioxidants can alleviate the noxious effects of in vivo oxidative stress, increasing the expression of the genes encoding the antioxidant enzymes involved in the elimination of ROS, such as superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GSH-Px) (Finley et al., 2011; Silva et al., 2012).

Although the in vitro antioxidant capacities of individual tropical fruits have been reported in the literature (Alothman, Bhat, & Karim, 2009; Rosso, 2013; Rufino et al., 2010), especially for acai and acerola (Lichtenthaler et al., 2005; Rufino et al., 2010), the in vivo antioxidant activities of tropical fruits consumed together, in order to investigate a possible synergistic or antagonistic effects, are still unexplored. Therefore, it is important to evaluate the...
in vitro and in vivo antioxidant effects of tropical fruit juices, in order to elucidate and establish their effect on the phytochemicals bioavailability. With this aim, this paper reports the radical scavenging activities of two formulations composed of fruits rich in bioactive compounds, and their effect on antioxidant enzymes and lipid peroxidation.

2. Materials and methods

2.1. Chemicals

The reagents 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2’-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) (ABTS’), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), 2,4,6-tris (2-pyridyl)-s-triazine (TPTZ), fluorescein sodium salt, 2,2’-azobis (2-amidinopropane) dihydrochloride (AAPH), cyanidin-3-glucoside and tridecanoic fatty acid (13:0) were purchased from Sigma Chemical Co. Potassium persulphate was purchased from Acros Organics and ferrous sulphate was from Vetec.

2.2. Tropical fruit juices

Two optimised formulations of juice composed of a mix of tropical fruits, named “FA” and “FB”, were evaluated in this work. The FA formulation was optimized by using a fractional factorial design (2−k, k = 6) coupled with response surface methodology (2k, k = 5), totaling 36 assays in the first step and 46 in the second. The dependent variables used were ascorbic acid, total phenolic content, total antioxidant capacity and sensorial acceptance. The independent variables were the concentrations of each tropical fruit (data not shown). The results indicate an optimised formulation named FA, composed of 10% acerola (Malpighiaceae), 5% acai (E. oleracea), 5% yellow mombin (S. lutea), 5% cashew-apple (Anacardiaceae), 20% pineapple (Ananas comosus L) and 50% water. By using the model to predict the responses in terms of ascorbic acid, total polyphenols (TP) and total antioxidant capacity (TAC), a formulation composed of 10% acerola, 10% acai, 10% yellow mombin, 20% pineapple, and 50% water was prepared, and named “FB”. Both fruit juices were prepared by fresh and non-pausterized frozen pulps, obtained from a local market in the state of Ceará and Pará, Brazil. The juices were adjusted to 12 Brix with sucrose. The tropical fruit juices were prepared, lyophilized and stored at −20 °C prior to use in the in vivo study.

2.3. Total antioxidant capacity (TAC) and total polyphenols (TPs)

Samples were extracted with distilled water or organic solvents to measure TP and TAC by the ABTS, FRAP and DPPH methods. For extraction using organic solvents, the procedure developed by Larrauri, Rupeirez, and Saura-Calixto (1997) was used. The samples were extracted sequentially with 4 mL of methanol/water (50:50, v/v) at 25 °C for 1 h, centrifuged at 25,400g for 15 min and the supernatant recovered. Then, 4 mL of acetone/water (70:30, v/v) was added to the residue at 25 °C, extracted for 60 min and centrifuged with the same condition. Methanolic and acetonic extracts were combined, made up to 10 mL with distilled water and the sample labelled as “EMAc”. For the water extractions, distilled water was added to the samples to make a total volume of 10 mL. The solution was homogenised in a shaker for 1 h at 25 °C and centrifuged at 24,500g for 15 min. The supernatant was filtered and named “EAq”.

Total polyphenols (TPs) in “EAq” and “EMAc” samples were determined by the Folin–Ciocalteu method (Obanda & Owuor, 1997) and the results of TP were expressed as mg GAE (gallic acid equivalent) per 100 g of juice. The total antioxidant capacity (TAC) in “EAq” and “EMAc” was also measured by the DPPH, ABTS and FRAP methods.

The free radical scavenging activity was determined with the DPPH method (Brand-Williams, Cuvelier, & Berzet, 1995), the ABTS− assay was based on a method developed by Miller et al. (1993) and, for the FRAP assay, the procedure described by Benzie and Strain (1996) was used. All the methods used are in accordance with the modifications suggested by Rufino et al. (2010). The results of the DPPH method were expressed as the concentration of antioxidant required to reduce the original amount of free radicals by 50% (EC50), and the values were expressed as g fruit juice per g of DPPH. For the ABTS and FRAP assay, the results were expressed as μM Trolox and μM FeSO4 per g of fruit juice, respectively.

The measurement of antioxidant activity by the ORAC assay was performed as described by Ou, Hampsch-Woodill, and Prior (2001). A Multi-Detection microplate reader (Synergy HT, Bio-Tek Instruments Inc., Winooski, VT) was programmed to record the fluorescence of the diluted samples (25 μL) every minute after incubation of the samples with 150 μL 40 mM fluorescein in 75 mM phosphate buffer (pH 7.4) and the addition of 25 μL AAPH (153 mM in 75 mM phosphate buffer, pH 7.4) for 60 min. The area under the curve of the fluorescence decay was calculated using Gen5 software. The antioxidant activity was measured four times for each sample and the results expressed in μM Trolox/g.

2.4. Fatty acid composition (GC-FID)

Analysis of fatty acids was carried out according to the AOAC 996.06 and AOCS Ce 1j-07 methods. Fatty acid composition was determined by GC-17 A (Shimadzu/Class GC 10) with a flame ionization detector, and a 100 m fused silica SP 2560 capillary column 0.25 μm film (Supelco Park, Bellefonte, PA, USA). The column temperature conditions were 140 °C for 5 min, 140–240 °C at a rate of 4 °C/min and 240 °C for 30 min. Injector temperature: 250 °C; detector temperature: 260 °C; carrier gas: helium (1 mL/min); split 1:50. The internal standard was tridecanoic fatty acid (13:0).

2.5. Anthocyanin detection in tropical fruit juices by LC–DAD–ESI–MS

The tropical juices were analysed on an LC–DAD–ESI–MS system consisting of a Varian 250 HPLC (Varian, CA) coupled to a diode array detector (DAD) and a 500-MS IT mass spectrometer (Varian, CA). The general procedure for screening phenolics in plant materials (Lin & Harnly, 2007) was employed with some modifications. A Symmetry C18 (Varian Inc., Lake Forest, CA) column (3 μm, 250 × 2 mm) was used at a flow rate of 0.4 mL/min. The column oven temperature was set at 30 °C. The mobile phase consisted of a combination of A (0.1% formic acid in water) and B (0.1% formic acid in acetonitrile). The gradient varied linearly from 10% to 26% B (v/v) at 40 min, to 65% B at 70 min, and finally to 100% B at 71 min and held at 100% B for 75 min. The DAD was set at 270 and 512 nm for real-time read-out, and UV/VIS spectra from 190 to 650 nm were continuously collected. Mass spectra were simultaneously acquired using electrospray ionization in the positive and negative ionization modes (PI and NI) at a fragmentation voltage of 80 V for the mass range of 100–1000 amu. Drying gas pressure of 35 psi, nebulizer gas pressure of 40 psi, drying gas temperature of 370 °C, capillary voltages of 3500 V for both PI and NI, and spray shield voltages of 600 V were used. The LC system was coupled to the MSD with a splitting of 50%. Quantification was performed on the basis of DAD data. An external standard curve of cyanidin 3-glucoside was used and concentrations are expressed as cyanidin 3-glucoside equivalents.

2.6. Animals, diets and experimental design

Fifty-six male weanling albino rats of the Wistar strain, weighing 97.8 ± 7.8 g, were provided by the University of São Paulo.
Vivarium. All procedures performed on animals were approved by the Ethics Committee on Animal Experimentation of the Faculty of Pharmaceutical Sciences, University of São Paulo (protocol number 359/2012), according to the guidelines of the Brazilian College on Animal Experimentation. The animals were maintained in clean polypropylene cages, with four rats per cage. The environment was 22 ± 2 °C and 60% humidity, subjected to a 12 h light/dark cycle and food and water ad libitum. The lyophilized tropical fruit juices (FA and FB) and distilled water were administered daily by gavage (0.5 mL/100 g body weight) for 30 days. The rats were distributed into seven groups, with eight rats in each group, treated as follows:

Group 1. Normal control (NC): This group of rats received distilled water.
Group 2. FA100: received 100 mg of FA per kg body weight.
Group 3. FA200: received 200 mg of FA per kg body weight.
Group 4. FA400: received 400 mg of FA per kg body weight.
Group 5. FB100: received 100 mg of FB per kg body weight.
Group 6. FB200: received 200 mg of FB per kg body weight.
Group 7. FB400: received 400 mg of FB per kg body weight.

Nutritional parameters such as weight gain and food consumption were collected three times a week. After 30 days, the animals were deprived of food for 12 h, anesthetized with ketamine (90 mg/kg b.w.) and xylazine (10 mg/kg b.w., Vertbrands, BRA) and sacrificed by extracting blood from the abdominal aorta with a syringe. Serum was obtained by centrifugation at 1500g at 4 °C for 5 min, and the liver were perfused with saline solution (0.9% w/v), collected and immediately frozen at −80 °C. Thiobarbituric reactive substance (TBARS) assay was performed in the serum and liver and the antioxidant enzyme activity was determined in liver and erythrocytes.

2.7. Biochemical parameters and protein quantification

The serum biochemical parameters were determined with a Labtest kit (alanine aminotransferase (ALT), aspartate aminotransferase (AST), HDL-cholesterol, total cholesterol and LDL-cholesterol by difference). A spectrophotometric method (Bradford, 1985) was used to determine the protein content in the serum and tissue analysed.

2.8. Activity of tissue antioxidant enzymes (CAT, SOD and GPx)

The cytoplasmic superoxide dismutase (SOD) activity was determined according to McCord and Fridovich (1969) using a reaction mixture containing cytochrome C (100 mM), xanthine (500 mM), ethylene diamine tetraacetic acid (1 mM), KCN (200 mM) and potassium phosphate buffer (0.05 M – pH 7.8). The results were expressed as units per milligram of protein. One unit of SOD activity was defined as the amount of enzyme required to inhibit the reaction rate by 50% at 25 °C and pH 7.8. Catalase (CAT) activity was determined as described by Beutler (1975). The method is based on the decrease in optical density at 230 nm (molar extinction coefficient – 0.071/mM/cm) as a result of the breakdown of hydrogen peroxide by catalase at 37 °C. The results were expressed as units per mg of protein. One unit of CAT activity was defined as the amount of enzyme required to hydrolyze 1 mol of hydrogen peroxide per minute at 37 °C and pH 8.0. The glutathione peroxidase (GPx) activity in the cytosolic fraction was determined as described by Sies, Koch, Martino, and Boveris (1979). One unit of enzyme activity was defined as the amount of enzyme required to oxidize 1 μmol of NADPH per minute at 30 °C at pH 7.0.

2.9. Thiobarbituric acid reactive substances (TBARS) assay

TBARS levels, as a marker of lipid peroxidation, were measured in liver homogenates and serum using the method of Ohkawa, Ohishi, and Yagi (1979). The results were expressed as nmmol of malondialdehyde (MDA) per mg of protein.

2.10. Statistical analyses

Statistical analyses were conducted using GraphPad Prism 4.0 for Windows (San Diego, CA, USA). A one-way analysis of variance (ANOVA) and Tukey test (P < 0.05) were applied to the results.

3. Results

3.1. Total polyphenols (TPs) and total antioxidant capacity (TAC) in vitro

Table 1 shows the total antioxidant capacity (TAC) and total polyphenols (TPs) in tropical fruit juices (FA and FB) in aqueous (EAq) and methanolic/acetic (EMAc) extracts. The aceton extract (EAc) was used for the ORAC analysis, according to the methodology by Ou et al. (2001). Except for the ORAC analysis, the values obtained using different solvents to extract antioxidant compounds were compared, and TP and ABTS showed a significant (P < 0.05) difference between the solvents for both tropical fruit juices. The values obtained using EMAC as solvent were higher than those using EAq. Comparing FA and FB fruit juices, FA exhibits higher values of TP, ABTS and DPPH, although FRAP and ORAC had no statistical differences (P < 0.05).

3.2. Fatty acids and anthocyanins in the tropical fruit juices

The major anthocyanins detected at 512 nm in the fruit juices FA and FB were cyanidin-3-O-glucoside, cyanidin-3-O-rutinoside, cyanidin-3-O-rhamnoside and pelargonidin-3-O-rhamnoside. Furthermore, the percentage of fatty acids in both fruit juices is presented in Table 2. Fatty acids, from C14:0 to C24:0, were detected and compared between the formulations. As expected, unsaturated fatty acids were in the majority, making up approximately 70% of the total compound. Both fruit juices also contained similar proportions of fatty acids, with oleic [18:1 (n-9)] and palmitic acids [16:0] being the most abundant, representing 49% and 21% of the total compounds detected, respectively. Meanwhile, FA contained half the concentration of fatty acids compared with FB juice (see Table 2).

3.3. Effects of the tropical fruit juices on biochemical parameters in rats

The results obtained in the present study showed no changes in the levels of serum glucose, triglycerides, total cholesterol and LDL-c when comparing all the groups with normal control rats. The activity of the enzymes alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were not affected by the consumption of either tropical juice. Also, no statistical difference was observed in body weight (175.0 g ± 10.5) and food intake (11.6 g ± 0.41) among the groups. However, the levels of HDL-c increased in the groups FA400, FB200 and FB400, although only FB400 is statistically significant (P < 0.05) when compared with the control group (see Table 3).
3.4. Effects of the tropical fruit juices on lipid peroxidation and antioxidant enzyme activity in rats

Fig. 1 shows the TBARS levels in the liver and serum of rats, and the effects of the activity of antioxidant enzymes by the consumption of FA and FB in liver tissue and erythrocytes. TBARS were lower in the liver of rats which consumed FA fruit juice at 100 and 200 mg/kg (19%, for each group) when compared with the control group (P < 0.05). For the serum, rats fed with FA100, FA400, FB200 and FB400 showed beneficial effects, with a reduction of TBARS from literature reports due to differences in protocols, different used. However, it is difficult to compare the current data with that from literature reports due to differences in protocols, different solvents and conditions used.

In addition to the results found in the present study using three methods of extraction, the results have also demonstrated differences between the FA and FB formulations. The fruit juices tested, composed of fruits rich in natural antioxidants, e.g. acerola and acai, offered high values of total antioxidant capacity in all methods tested. Moreover, FA is composed of camu-camu, an Amazonian fruit rich in ascorbic acid and polyphenols (Chirinos, Galarza, Betalléz-Pallardei, Pedreschi, & Campos, 2010). However, although FB does not have camu-camu in its composition, the similar values of TAC and TP were due to the acai concentration, which is almost twice as much as in FA.

Müller et al. (2010) evaluated the total antioxidant capacity of 14 smoothies, composed of 8 fruit purees, 4 fruit concentrates and one juice, mainly from red fruits. The antioxidants were extracted in water, except for ORAC assay, and the results obtained high TAC, with ORAC values from 14.60 to 15.12 μmol Trolox g⁻¹. For ABTS and TP, the extraction with methanol and acetone (EMAc) was more effective than the extraction using water (EAq) as solvent. Alothman et al. (2009) mentioned that the recovery of polyphenols is influenced by the solubility of these compounds in the solvent used for the extraction process. Those authors evaluated the antioxidant capacity and phenolic content of three tropical fruits (honey pineapple, banana and Thai seedless guava), and the results showed that the recovery of polyphenols was dependent on the fruit type and the solvent system used, where acetone (50%) and ethanol (70%) were the most efficient solvents for honey pineapple, acetone (70%) for banana, and acetone (90%) and ethanol (90%) for Thai seedless guava. For DPPH and FRAP, the results in the present study showed no differences in the quantification of antioxidant compounds through the methods of extraction tested. Turkmen, Sari, and Velioglu (2006) evaluated the effects of extraction solvents on concentration and antioxidant activity of black and mate tea polyphenols determined by the DPPH method. Those authors suggest that solvents with different polarity had a significant effect on polyphenol content and antioxidant activity, and the highest extraction of these compounds was found when 50% acetone for black tea, and 50% of ethanol for mate tea were used. However, it is difficult to compare the current data with that from literature reports due to differences in protocols, different solvents and conditions used.

Table 1

<table>
<thead>
<tr>
<th>Solvent</th>
<th>Total antioxidant capacity (TAC)</th>
<th>TP</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>ABTS (μM Trolox/g)</td>
<td>DPPH (μg g⁻¹ g DPPH)</td>
</tr>
<tr>
<td>FA (EAq)</td>
<td>7.64 ± 0.23 c</td>
<td>779.24 ± 29.64 b</td>
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<tr>
<td>FA (EMAc)</td>
<td>10.27 ± 0.22 a</td>
<td>845.87 ± 29.12 b</td>
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<tr>
<td>FB (EAq)</td>
<td>7.53 ± 0.29 c</td>
<td>1217.58 ± 32.40 a</td>
</tr>
<tr>
<td>FB (EMAc)</td>
<td>8.57 ± 0.21 b</td>
<td>1303.88 ± 12.37 a</td>
</tr>
<tr>
<td>FA (EAc)</td>
<td>–</td>
<td>–</td>
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<tr>
<td>FB (EAc)</td>
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Table 2

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<th>Solvent</th>
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<th>TP</th>
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<tbody>
<tr>
<td></td>
<td>ABTS (μM Trolox/g)</td>
<td>DPPH (μg g⁻¹ g DPPH)</td>
</tr>
<tr>
<td>FA (EAq)</td>
<td>182.63 ± 40.77 a</td>
<td>51.63 ± 5.53 a</td>
</tr>
<tr>
<td>FA (EMAc)</td>
<td>156.13 ± 35.89 a</td>
<td>49.75 ± 6.43 a</td>
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<tr>
<td>FA (EAc)</td>
<td>177.75 ± 33.97 a</td>
<td>52.25 ± 4.95 a</td>
</tr>
<tr>
<td>FB (EAq)</td>
<td>176.63 ± 40.60 a</td>
<td>50.00 ± 7.89 a</td>
</tr>
<tr>
<td>FB (EMAc)</td>
<td>176.75 ± 40.81 a</td>
<td>50.00 ± 7.89 a</td>
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<tr>
<td>FB (EAc)</td>
<td>176.63 ± 40.60 a</td>
<td>50.00 ± 7.89 a</td>
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Table 3

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<th>Serum glucose (mg/dl)</th>
<th>ALT (U/mL)</th>
<th>AST (U/mL)</th>
<th>Triglycerides (mg/dl)</th>
<th>Total Cholesterol (mg/dl)</th>
<th>HDL-c (mg/dl)</th>
<th>LDL-c (mg/dl)</th>
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<tr>
<td>Control</td>
<td>197.25 ± 36.02 a</td>
<td>55.75 ± 10.94 a</td>
<td>153.38 ± 23.27 a</td>
<td>51.75 ± 16.07 a</td>
<td>61.75 ± 4.03 ab</td>
<td>26.00 ± 2.51 b</td>
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<tr>
<td>FA 100</td>
<td>177.75 ± 33.97 a</td>
<td>52.25 ± 4.95 a</td>
<td>152.00 ± 11.03 a</td>
<td>49.50 ± 14.18 a</td>
<td>64.13 ± 7.83 ab</td>
<td>25.88 ± 2.36 b</td>
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<td>FA 200</td>
<td>156.13 ± 35.89 a</td>
<td>49.75 ± 6.43 a</td>
<td>147.13 ± 17.85 a</td>
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<td>64.88 ± 4.49 a</td>
<td>26.13 ± 1.46 b</td>
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<tr>
<td>FA 400</td>
<td>182.63 ± 40.77 a</td>
<td>51.63 ± 5.53 a</td>
<td>141.25 ± 24.78 a</td>
<td>58.75 ± 13.38 a</td>
<td>70.38 ± 8.40 a</td>
<td>27.88 ± 2.53 ab</td>
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<tr>
<td>FB 100</td>
<td>211.13 ± 72.20 a</td>
<td>50.50 ± 6.61 a</td>
<td>154.75 ± 31.76 ac</td>
<td>53.63 ± 21.65 a</td>
<td>58.63 ± 5.34 b</td>
<td>26.13 ± 3.00 b</td>
</tr>
<tr>
<td>FB 200</td>
<td>176.75 ± 40.81 a</td>
<td>51.00 ± 7.89 a</td>
<td>124.00 ± 24.15 a</td>
<td>48.63 ± 13.09 a</td>
<td>62.38 ± 8.35 ab</td>
<td>27.75 ± 2.92 ab</td>
</tr>
<tr>
<td>FB 400</td>
<td>176.63 ± 40.60 a</td>
<td>45.63 ± 6.28 a</td>
<td>123.13 ± 13.52 ab</td>
<td>47.75 ± 9.75 a</td>
<td>71.63 ± 6.00 a</td>
<td>31.00 ± 3.02 a</td>
</tr>
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ALT, alanine aminotransferase; AST, aspartate aminotransferase; HDL-c, high-density lipoprotein cholesterol; LDL-c, low-density lipoprotein cholesterol.
ranged from 51.44 to 213.33 mg GAE/100 g for TP, 3.6–19.5 μmol Fe^{2+}/100 g, 3.4–12.8 μmol Trolox/g and 7.9–38.5 μmol TE/100 g. Although the higher results obtained were achieved when the fruit concentrates were used, the values were, for the most part, lower than those obtained in the current work (see Table 1). As mentioned before, the fruits used in the juice (FA and FB) formulations are rich sources of vitamin C and polyphenols (e.g. camu-camu, cashew-apple, acerola and acai), which resulted in higher values of TAC. The fruit juices used also exhibited higher TP content (≈100 mg/100 g GAE) in comparison to 17 fruit juices and skim milk mixture beverages (≈60 mg/100 g GAE) studied by Zulueta, Esteve, Frasquet, and Frígola (2007).

Given the importance of anthocyanins in the antioxidant capacity of tropical fruit juices, their profiles were determined by LC–DAD–ESI–MS. The main compounds detected in both tropical fruit juices were anthocyanins, especially cyanidin-3-O-glucoside and cyanidin-3-O-rutinoside, both previously reported to be the major anthocyanins in E. oleracea (acai) (Brito et al., 2007; Lichtenthaler et al., 2005). Two minor compounds identified as cyanidin-3-O-rhamnoside and pelargonidin-3-O-rhamnoside probably originated from the acerola juice used, since these compounds were previously detected in this fruit (Brito et al., 2007). The concentrations of cyanidin-3-O-glucoside were 0.34 ± 0.02 mg/100 g and 0.88 ± 0.03 mg/100 g for FA and FB, respectively. For cyanidin-3-O-rutinoside, the values were 0.39 ± 0.03 mg/100 g and 0.97 ± 0.05 mg/100 g for FA and FB, respectively. All the other compounds detected were in trace amounts.

Fatty acids were detected in both fruit juices. FB displayed a higher fatty acid concentration than FA due to an almost two-fold higher concentration of acai in this formulation. The formulations

![Fig. 1.](image)
contain fatty acids important to a healthy diet, with oleic acid as the major component of both tropical fruit juices, followed by palmitic [16:0] and linoleic [18:2(n-6)] acids. Through the phytochemical and nutrient component analysis of each pulp fruit isolated, acai was shown to be the major contributor of fatty acids in the formulations. Acai pulp has a low sugar content and is not considered a good source of carbohydrates, but is rich in lipids, with high levels of unsaturated fatty acids (oleic and linoleic acids), phytosterols (β-sitosterol), dietary fibre (Souza, Silva, Silva, Oliveira, & Pedrosa, 2010), and especially phenolic compounds (Lichtenhaler et al., 2005). Moreover, camu-camu, acerola and cashew-apple exhibit important antioxidant activity, not only related to their high ascorbic acid content but also to their phenolic content (Brito et al., 2007; Chirinos et al., 2010). All these compounds could improve the lipid profile and therefore may have beneficial effects on cardiovascular diseases (CVD).

Recently, some studies have shown that the serum concentration of HDL-cholesterol is inversely related to the risk of developing CVD, which does not depend on serum LDL-c and triglyceride concentrations (TC). Qin et al. (2009) demonstrated the beneficial effects of anthocyanin consumption in humans, where the supplementation decreases LDL-c, increases HDL-c concentrations and enhances cellular cholesterol efflux to the serum. In the present investigation, the in vivo assay showed that FB400 was able to increase HDL-c concentrations (P < 0.05), and no differences were observed in LDL-c and TC levels. Apparently, this effect may be attributed to the higher amount of unsaturated fatty acids (400 mg FB/kg body weight) associated with the phenolic composition of this diet. The polyphenols may decrease the micellar solubilization of cholesterol in the digestive tract for an increase in bile flow, bile cholesterol and bile acid concentration, and for a subsequent increase in the fecal excretion of steroids (Afonso et al., 2013). Moreover, the effects of monounsaturated fatty acids in HDL-c and LDL-c levels are extensively reported in the literature (Jenkins et al., 2010; Qin et al., 2009) and are related to the prevention of CVD.

In the current study, no differences (P < 0.05) were observed in serum glucose levels and activities of the enzymes alanine aminotransferase (ALT) and aspartate aminotransferase (AST). Aminotransferases are considered indicators of liver injury, with ALT found primarily in the liver while AST is also found in other tissues, and is therefore a less-specific marker of liver function (Vozarova et al., 2002). Importantly, plasma ALT and AST levels were not affected by FA and FB ingestion in all groups.

In fact, it was observed that the rats showed a sparing effect of catalase (CAT) and glutathione peroxidase (GPx) activities in erythrocytes and liver tissue, and a decrease in liver and serum TBARS levels. These results are consistent with previous investigations reviewed (Auger et al., 2005; Décordé et al., 2008). Furthermore, a decreased activity of antioxidant enzymes (GPx in erythrocytes for FB400; and CAT and SOD in liver for FA200) can be a consequence of the sparing effect of dietary antioxidants, reducing the requirement for enzymatic antioxidant function when high concentrations of exogenous antioxidants are present in the circulatory system (Breinholt, Lauridsen, & Dragsted, 1999). Although some studies suggested that feeding rats with plant materials (e.g. rosemary, seaweed and others) increased the activity of antioxidant enzymes (Afonso et al., 2013; Silva et al., 2012), the results of the present study show a decrease in the antioxidant enzymes, possibly due to the capacity of dietary antioxidants to scavenge oxygen radicals and, consequently, reduce the need for enzymatic endogenous antioxidants. Elsewhere, spontaneous hepatic lipid peroxidation has been shown to decrease with higher levels of dietary antioxidants, and supplementation with tropical fruit juices (FA and FB) effectively decreases lipid peroxidation by decreasing malondialdehyde formation.

Oxygen-derived free radicals, including superoxide and hydroxyl radicals, are involved in the pathogenesis of tissue injury initiating and promoting lipid peroxidation. Polyunsaturated fatty acids of the cellular membranes are degraded by lipid peroxidation with subsequent disruption of membrane integrity, suggesting that lipid peroxidation mediated by oxygen radicals is an important cause of the damage and destruction of cell membranes (Khennouf, Amira, Arrar, & Baghiani, 2010). When the generation of oxygen radicals exceeds the ability of tissue antioxidants to detoxify them, the resultant oxidative stress can cause damage. Because ROS (particularly the hydroxyl radical) are unstable, they tend to react with nearby macromolecules such as lipids and proteins. These reactions damage the macromolecules via peroxidation processes (Yoshikawa, Ueda, Naito, et al., 1989).

The beneficial effects of phenolic compounds in the reduction of lipid peroxidation has been observed in several studies, and the decrease of TBARS levels in liver and serum tissues is also observed in the present investigation. Khennouf et al. (2010) have reported the effect of phenolic compounds and purified tannins on lipid peroxidation in rabbits. Their results showed that phenolic acids and tannins inhibit lipid peroxidation in the brain, at all concentrations tested (10, 25 and 50 µg/ml). Rop et al. (2010) also observed a lipid peroxidation inhibition (12.57–19.81%) in a rat liver slice model by administering black chokeberries. The authors mentioned that this fruit is considered one of the most valuable sources of phenolic substances, with values of total polyphenols (TPs) measured by the Folin–Ciocalteu method in the range of 125.7–198.1 mg GAE/100 g of fresh matter. Moreover, the positive effect shown in the present investigation could be attributed to the antioxidative activity of these formulations and total phenolic compounds, as demonstrated by the TP and TAC assays. Nevertheless, these beneficial effects cannot only be attributed to their phenolic contents, but to the action of different antioxidant compounds present in the fruits (carotenoids, ascorbic acid, phytosterols and others) as demonstrated by ABTS, FRAP, DPPH and ORAC assays. All these components could cause reduction of the peroxides that can damage polyunsaturated fatty acids, thus preventing lipid peroxidation.

In conclusion, the in vitro and in vivo results suggest that the consumption of tropical fruit juices was effective in endogenous antioxidant defense. Furthermore, the beneficial effects could be attributed to high concentrations of antioxidant compounds, especially phenolic compounds. These findings strongly suggest that tropical fruit juices can have significant health benefits and further studies are needed, such as comprehensive bioavailability clinical trials.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.foodchem.2014.01.090.

References

L.) attenuate oxidative stress and reduce blood cholesterol concentrations in diet-induced hypercholesterolemic rats. 


