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

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ABSTRACT

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).

1. Introduction

Tropical fruits, e.g., camu-camu (*Myrciaria dubia*), acerola (*Malpighia punicifolia*), 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, Kakuda, & 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

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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 Acrós 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-1}, $k = 6$) coupled with response surface methodology (2^k, $k = 5$), totalling 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 (*M. punicifolia*), 5% acai (*E. oleracea*), 5% yellow mombin (*S. lutea* L.), 5% cashew-apple (*A. occidentale*), 5% camu-camu (*M. dubia*), 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, Rupérez, 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 25,400*g* 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 24,500*g* 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 (galic acid equivalent) per 100 $g^{-1}$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, & Berset, 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% (EC_{50}), 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 Fe_2SO_4 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 nM 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 μMTrolox/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 Symetry 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 97.6 ± 7.8 g, were provided by the University of São Paulo