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Effect of clarified Brazilian native fruit juices on postprandial glycemia in healthy subjects

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ABSTRACT

Brazilian native fruits have been shown as excellent sources of polyphenols which are associated with multiple biological activities including inhibition of carbohydrate-hydrolyzing enzymes, α-amylase and α-glucosidase. Postmeal blood glucose elevations and high glycemic index diets can play a major role in the development of type 2 diabetes, therefore alternative approaches to reduce postprandial hyperglycemia are of growing interest in order to reduce diabetes risk. Here we investigated the effect of six Brazilian native clarified fruit juices from Amazon, Savannah and Atlantic Forest biomes on postprandial glycemia after consumption of a carbohydrate meal. For this, 23 healthy subjects were selected to consume seven meal tests, with a 1-week interval among them, consisting in 50 g white bread plus 300 mL of water (control) or cambuci, cagaita, maracujá-alho, cupuaçu, camu-camu and jaboticaba clarified fruit juices. The results showed that serum glucose concentrations were significantly lower after consumption of cambuci, cagaita, camu-camu and jaboticaba juices, whereas maracujá-alho and cupuaçu juices did not decrease the amount of glucose absorbed, compared to control ($p < 0.05$). In addition, cagaita, cambuci, cupuaçu and jaboticaba juices increased the oxygen radical absorbance capacity in plasma, whereas all juices augmented the ferric reducing ability of plasma, except for cambuci ($p < 0.05$). These results indicate that juices from Brazilian native fruits may be considered as adjuvant treatment for reduction of postprandial glycemia in healthy subjects.

1. Introduction

The prevalence of type 2 diabetes (T2D) is raising exponentially alongside with its co-morbid conditions such as hypertension, dyslipidemia, and cardiovascular diseases (CDC, 2014). Postmeal blood glucose elevations and high glycemic index diets can play a major role in the development of T2D (Cerriello, Colagiuri, Gerich, & Tuomilehto, 2008), therefore adjuvant therapies to reduce glycemic index of foods, and consequently postprandial hyperglycemia, are of growing interest in order to reduce diabetes risk.

Evidence-based studies support the idea that consumption of fruits and plant-based food is inversely correlated with T2D (Anhê et al., 2013). The health properties are partially attributed to phytochemicals such as polyphenols, which can act inhibiting carbohydrate-hydrolyzing enzymes and intestinal glucose uptake through inhibition of glucose transporters (Crozier, Jaganath, & Clifford, 2009; Williamson, 2013).

Recently, it was demonstrated that the acute ingestion of a phlorizin-enriched powder from unripe apples was able to reduce postprandial glucose response by two-fold and to increase urinary glucose excretion by five-fold (Marakova et al., 2015). Besides, differently from soft drink consumption, intakes of 100% fruit and vegetable juices were not associated with risk of T2D (Eshak et al., 2013).

Brazil has different biomes, which results in a large variety of vegetal species, including a natural abundance of native fruits. Although the country is the world's third largest fruit producer (FAO, 2013), few of them are commercialized or have had biochemical and nutritional properties evaluated. Among these several species, some fruits from Atlantic Forest, Amazon and Savannah proved to be excellent sources of polyphenols, with *in vitro* anti-diabetic potential, since these compounds were shown to be effective in inhibition of α-amylase and α-glucosidase enzymatic activities (Gonçalves, Lajolo, & Genovese, 2010). Thus, in the present study we evaluated the effect of Brazilian native clarified fruit juices on postprandial blood glucose response to a

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carbohydrate meal (50 g of white bread), in healthy subjects.

2. Materials and methods

2.1. Subjects

The recruitment of subjects was carried out at the College of Pharmaceutical Sciences and the Chemistry Institute, University of São Paulo (USP). A total of twenty three healthy individuals, 17 females and 6 males, with average age (± SD) of 29 ± 6 years (range, 23–43 years), average body mass index (BMI) (± SD) of 23.7 ± 2.9 kg/m^2 (range, 19.2–30.4 kg/m^2), and average systolic blood pressure of 110 ± 12 mm Hg and diastolic blood pressure of 68 ± 7 mm Hg were selected for participation in this study. All individuals were previously submitted to an oral glucose tolerance test (OGTT) with 75 g of dextrosol after 12 h fasting. Exclusion criteria were: diabetes, obesity, patients undergoing treatment for type 2 diabetes, hormone replacement therapy or to reduce blood viscosity, use of vitamin/mineral supplements or antibiotics, smoking, pregnancy, and patients with gastrointestinal, liver, kidney, or bleeding disorders. This study was approved by the Human Research Ethics Committee of the University of São Paulo Hospital, CEP-HU/USP record: 937/09 - SISNEP and the Ethics Committee in Research of the College of Pharmaceutical Sciences, University of São Paulo (protocol CAAE: 0025.0.018.198-09). Informed consent was obtained from each research participant.

2.2. Test meals

The Brazilian native fruits (jaboticaba, maracujá-alho and cambuci) and frozen pulps (camu-camu, cagaita and cupuaçu) used in the preparation of juices are presented in Table 1. These fruits and pulps were acquired from three commercial establishments: CEAGESP - Companhia de Entrepostos e Armazéns Gerais de São Paulo (São Paulo, SP, Brazil) (jaboticaba); Cupuama® - Cupuaçu do Amazonas Com. Ind. Exp. Ltda (Careiro, AM, Brazil) (camu-camu and cupuaçu) and Sítio do Bello (Paraibuna, SP, Brazil) (cambuci and cagaita). Maracujá-alho fruits were donated by EMBRAPA – Brazilian Agricultural Research Corporation.

Clarification was achieved through homogenization of fresh fruit or commercial frozen pulp followed by centrifugation in Sorvall refrigerated centrifuge model RC-5C, rotor type GSA (22,770*g*/40 min, 4 °C).

Each meal consisted of approximately 25 g of available carbohydrate as white bread (corresponding to one unit of white bread of approximately 50 g), and 300 mL of water (control), or clarified fruit juices, administered after 10–12 h of fasting. All volunteers underwent tests with water (control) and clarified juices with an interval of 7 days among them. They were asked to avoid foods and beverages rich in polyphenols (chocolate, wine, red fruits, coffee, and tea) and alcoholic beverages in this period, especially on the day before the test. The test meals were consumed within 10 min maximum. Participants were not blinded because of the distinct characteristics and sensory properties of these juices.

Table 1
Botanical classification of Brazilian native fruits used in the preparation of juices, administered to volunteers.

Fruit	Origin	Family	Botanical name
Cambuci	Atlantic forest	Myrtaceae	*Campomanesia phaea* (O. Berg.) Landrum
Cagaita	Savannah	Myrtaceae	*Eugenia dysenterica* DC
Cupuaçu	Amazon	Sterculiaceae	*Theoboma grandiflorum* (Wild. Exp. Spreng) Schum
Camu-camu	Amazon	Myrtaceae	*Myrciaria dubia* Mc. Vaugh
Maracujá-alho	Savannah	Passifloraceae	*Passiflora tenuifila* Killip
Jaboticaba	Atlantic forest	Myrtaceae	*Myrciaria cauliflora* Berg.

2.3. Glucose curve and plasma antioxidant capacity

Postprandial blood glucose levels were determined by finger-prick capillary blood sample, using a digital glucometer (Accu-Chek®) at 0 (immediately before consumption of bread), 15, 30, 45, 60, 90, and 120 min after the test meal intake (Josse, Kendall, Augustin, Ellis, & Jenkins, 2007). Blood samples were collected in heparinized tubes at 0 and 120 min to evaluate plasma antioxidant capacity by ferric reducing antioxidant power (FRAP) (Benzie & Strain, 1996) and the oxygen radical absorbance capacity (ORAC) assays (Dávalos, Gómez-Cordovés, & Bartolomé, 2004). To obtain plasma, the samples were immediately centrifuged for 15 min at 1500*g* (4 °C), and stored at − 80 °C. Results were expressed as mean ± standard deviation. The *area under curve* (AUC) for glucose was calculated using the trapezoidal rule to determine the reduction of the glycemic response, expressed in mg/dL. The following variables in the glucose curves were analyzed: *glucose at baseline* (GB), obtained at time zero; *glucose peak value* (GPV), defined as the highest value above the baseline observed after ingestion of the meal, expressed in mg/dL; *absolute increase of glucose* (AIg), defined as the absolute difference between the maximum value obtained after stimulation of glucose GPV and GB, expressed in mg/dL (AIg = GPV − GB); *glucose incremental percentage* (GIP), defined as the ratio between the AIg and GB, expressed in percentage terms (GIP = (AIg / GB) × 100); *glucose incremental velocity* (GIV), defined as the ratio between AIg and the time of maximum blood glucose concentration, expressed in mg/mL minute (GIV = AIg / peak time) (Corrêa, Nogueira, Bevilácquia, & Gomes, 2007).

2.4. Chemical characterization of juices

Titratable acidity, total and soluble solids, total fiber and pH of fruit juices were determined according to the Association of Official Agricultural Chemists (AOAC, 1997). Total sugars were determined according to the method described by Dubois, Gilles, Hamilton, Rebers, and Smith (1956).

The juices were characterized in relation to total phenolics (Singleton, Orthofer, & Lamuela-Raventós, 1999), proanthocyanidins content (Porter, Hrstich, & Chan, 1986), and *in vitro* antioxidant capacity by the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging capacity (Brand-Williams, Cuvelier, & Berset, 1995), the ferric reducing antioxidant power (FRAP) (Benzie & Strain, 1996), and the oxygen radical absorbance capacity (ORAC) assays (Dávalos et al., 2004).

The tentative identification and quantification of the main phenolic compounds in juices were achieved using analytical reversed-phase HPLC in a Hewlett-Packard 1100 system with autosampler and quaternary pump coupled to a diode array detector according to Arabbi, Genovese, and Lajolo (2004). Peak identification was performed by comparison of retention time and diode array spectral characteristics with the standards and the library spectra. All chemicals and solvents were HPLC grade. The standards quercetin, kaempferol, catechin, epicatechin, syringic and ellagic acids, isovitexin and homoorientin were purchased from Sigma Chemical Co. (St. Louis, USA); isoscutellarin, hypoaletin, procyanidin B1 were purchased from Extrasynthese (Genay, France); and cyanidin-3-glucoside was purchased from Apin Chemicals (Abingdon, U.K). Ellagitannins were expressed as mg ellagic acid after subtracting free ellagic acid contents from total ellagic acid contents determined after acid hydrolysis, according to Pinto, Lajolo, and Genovese (2008).

2.5. Inhibitory activities of α-glucosidase and α-amylase in vitro

2.5.1. Polyphenol-rich extracts from Brazilian fruit juices

The polyphenol-rich extracts were prepared according to Arabbi et al. (2004) after solid phase extraction (SPE) of juices in polyamide