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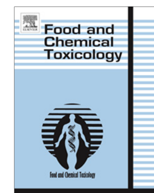

Evaluation of antioxidant and mutagenic activities of honey-sweetened cashew apple nectar

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ABSTRACT

In vitro chemical properties and antioxidant potential and *in vivo* mutagenic activity of honey-sweetened cashew apple nectar (HSCAN), a beverage produced from the cashew pseudo-fruit (*Anacardium occidentale* L.) and of its constituents were assessed. Analytical procedures were carried out to investigate the honey used in the HSCAN preparation, and the results observed are in accordance with Brazilian legal regulations, except for diastase number. HSCAN and pulp were investigated for ascorbic acid, carotenoid, anthocyanin and total phenolic contents, and both showed high acid ascorbic concentrations. Antioxidant capacity using 2,2-diphenyl-1-picrylhydrazyl radical (DPPH) and/or β-carotene/linoleic acid systems were applied and demonstrated a weak antioxidant capacity of honey and HSCAN, but cashew apple pulp demonstrated high antioxidant capacity. A weakly positive mutagenic effect of cashew pulp 20% was observed using the somatic mutation and recombination test (SMART) in *Drosophila melanogaster* only in the high-bioactivation (HB) cross. On the contrary, HSCAN was not mutagenic in both standard and high bioactivation crosses. HSCAN exhibited slight antioxidant activity, which could be associated with the high amount of ascorbic acid found in the samples evaluated. The beverage prepared did not induce DNA damage in somatic cells of *D. melanogaster*, which means that it is neither mutagenic nor recombinagenic in this test system.

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1. Introduction

Agribusiness development has been heavily influenced by the needs of consumers. Compared to fresh fruits, the consumption of processed fruits has increased worldwide (Hawkes et al., 2010). This growth has sparked the interest of the beverage industry in the production of new products, mainly influenced by consumers' search for healthy and functional foods (Smith, 2006; Betoret et al., 2011).

The overall market for beverages based on fruits has also grown substantially in recent years. There has been a combined effort among scientists and the industry to explore and utilize varying food sources and combinations of different elements to develop new products (Kraak et al., 2011). In this sense, the development of blended drinks that afford an array of new tastes and that at the same time improve nutrition quality currently stands as an important research field (Akinwale, 2000; Jain and Khurdiya, 2004).

Cashew fruit (*Anacardium occidentale* L.) is considered one of the crops of greatest economic importance in northeastern Brazil, grown mainly in the states of Ceará, Rio Grande do Norte and Piauí (Pommer and Barbosa, 2009). However, this crop is grown in other countries also, especially Mali, Madagascar, Guyana, Vietnam, Nigeria, India, Côte d'Ivoire, Indonesia and the Philippines (FAO, 2010). The fruit of the cashew tree, popularly known as the cashew

Abbreviations: BHT, butylated hydroxytoluene; DA, diastase activity; DCPIP, 2,6-dichlorophenolindophenol; DN, diastase number; DPPH, 2,2-diphenylpicrylhydrazyl; EC50, 50% of radicals scavenging activity; GAE, gallic acid equivalent; HCl, hydrochloric acid; HMF, hydroxymethylfurfural; HSCAN, honey-sweetened cashew apple nectar; IS, insoluble solids; SCE, sister chromatids exchange; SMART, somatic mutation and recombination test; TP, total phenolics.

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apple, is composed of two parts: the fruit itself, chestnuts, and the pseudo-fruit, which is its juicy part. Compared with the chestnuts, the pseudo-fruit is less important commercially, though there is considerable potential for the exploration of this structure of the fruit. With this in mind, a number of processes have been developed to use the pseudo-fruit in products like juices, jams, syrups and beverages (Trevisan et al., 2006).

As far as its nutritional value is concerned, the cashew apple is considered an excellent source of vitamin C, whose contents on average are around 269 mg per 100 mL juice, five times higher than the vitamin's levels detected in orange juice (Maciel et al., 1986; Contreras-Calderón et al., 2011). Furthermore, it is a rich source of vitamin A precursors, and has been shown to present resorcinol lipids and phenolic compounds with proved antioxidant activity (Kozubek et al., 2001).

According to Melo Cavalcante et al. (2003), the cashew apple juice is able to sequester the peroxyl radical in dilutions of up to 10 times in *in vitro* studies, and is considered an excellent antioxidant. In addition, other studies have revealed that tannic acid, one of the compounds present in cashew apple juice, has antimutagenic effects on *Salmonella thyphimurium* TA 98 (Chen and Chung, 2000). Tannic acid has also been shown to reduce the frequency of sister chromatids exchange (SCE) in ovary cells of Chinese hamster (Kuo et al., 1992).

The aim of the present study was to evaluate the antioxidant potential and chemical composition of honey-sweetened cashew apple nectar (HSCAN) and cashew apple pulp and the physical–chemical properties of the honey used in its preparation using *in vitro* tests. Additionally, considering the importance of studies involving the interaction of dietary components with human genetic material, the mutagenic activity of HSCAN, as well as of its individual components (cashew apple pulp, honey, sodium benzoate and sodium metabisulfite) was analyzed using the somatic mutation and recombination test (SMART) in *Drosophila melanogaster.*

2. Material and methods

2.1. Chemical compounds

The solvents and reagents used were: gallic acid (Sigma, Steinheim, Germany), DPPH (Sigma, Steinheim, Germany), isopropilic alcohol (Vetec Brazil), hexane (Vetec Brazil), 2,6-dichlorophenolindophenol (DCPIP; Sigma, Steinheim, Germany), HCl (P.A, Vetec, Brazil), ethanol (99.5 wt%, Vetec, Brazil), β-carotene (Type I, approx. 95% UV, Sigma), polyoxyethylene 40 sorbitan monooleate (Tween 40, Merck), linoleic acid (puriss. p.a. standard for GC ⩾ 99%, Sigma–Aldrich, Steinheim, Germany), chloroform (Sigma standards, Steinheim, Germany) and urethane (URE, CAS No. 51-79-6; Sigma–Aldrich Company, St. Louis, MO, USA).

2.2. Formulation of honey-sweetened cashew apple nectar (HSCAN)

The processing of HSCAN was performed with cashew pulp frozen at −20 °C, obtained from a pulp mill located in the rural area of Teresina, Piauí, Brazil. Local monofloral *Apis mellifera* honey from the floral source vine-grape (*Serjania sp*., Sapindaceae) was purchased at the local market in the city of Picos, Piauí, Brazil.

The HSCAN was prepared according to Silva et al. (2008), with 20% of pulp, sweetened with honey until it reaches 11°Brix (measure of total soluble solids), containing sodium metabisulfite (40 ppm) and benzoate sodium (200 ppm) as preservatives, obtained from Vetec Química Fina Ltda., Duque de Caxias, RJ.

After the addition of preservatives and adjusting °Brix to 11, the formulation was subjected to heat treatment at 90 °C for 1 min in a stainless steel open tank and hot-bottling, using a semi-automatic filler. HSCAN was stored in 250 mL glass bottles sealed with plastic caps, which were subsequently cooled with chlorinated water until 35 °C.

2.3. Physical–chemical properties and antioxidant activity by the DPPH scavenging method of honey used in the HSCAN

The physical–chemical analysis of the honey were carried out according to the Codex Alimentarius standards (CAC, 1990) and Association of Official Agricultural Chemists (AOAC, 1998): soluble solids (brix),hydroxymethylfurfural (HMF), diastase activity (DA), insoluble solids (IS), free acidity, ash, total phenolics (TP), reducing sugars and sucrose.

TP in honey was estimated by the Folin–Ciocalteu spectrophotometric method based on the procedure of Singleton et al. (1999). In this method, 5 g of honey was diluted in 50 mL of deionized water and 0.5 mL of this dilution was mixed with 2.5 ml of 0.2 N Folin–Ciocalteu phenol reagent. After standing for 5 min at room temperature, 2 mL of sodium carbonate solution (75 g/mL) was added to the mixture, shaken thoroughly. The mixture was allowed to stand for 2 h at room temperature and the absorbance was measured at 760 nm, using a UV–visible spectrophotometer (Spectronic® 20 Genesys®, USA). A calibration curve was prepared, using a standard solution of gallic acid (20, 40, 60, 80 and 100 mg/l, r^2 = 0.995). Results were expressed as fresh weight basis (fw) as mg gallic acid equivalents/100 g of sample.

The antioxidant activity of honey was evaluated by the 2,2-Diphenylpicrylhydrazyl (DPPH) assay according to Brand-Williams et al. (1995). Briefly, 1.7 mL of DPPH solution (6×10^{-5} mol/L methanol) was incubated with 0.3 mL of honey fractions at four concentrations (12.5, 25, 37.5 and 50 mg/L). The control was prepared as above, but without honey. The mixture was then kept at room temperature in the dark for 30 min and the reduction in the DPPH radical was measured at 517 nm using a UV–visible spectrophotometer. DPPH radical-scavenging activity was calculated as follows:

$$\text{Scavenging effect}(\%) = [(\text{Absorbance of control} - \text{Absorbance of sample}) / \text{Absorbance of control}] \text{x}100$$

The EC50 value (concentration of sample required to scavenge 50% of free radicals) was also calculated.

2.4. Determination of bioactive substances and antioxidant activity in the pulp and HSCAN

The ascorbic acid level (mg.100 g^{-1} of pulp or HSCAN) was determined according to the Cox and Pearson (1976) method, which is based on the reduction of 2,6-dichlorophenolindophenol (DCPIP) by ascorbic acid. Anthocyanins level (mg.100 mL^{-1}) was measured according to Francis (1982), by immersing the samples in ethanol extract solution 95% HCl (1.5 N) – 85:15v/v for 12 h at 4 °C. The samples were then filtered, properly diluted in a volumetric flask and absorbance was measured at 535 nm using a spectrophotometer. A blank with ethanol extract solution instead of the sample was also included. Total carotenoids were determined according to Higby (1962) by extracting these pigments with isopropilic alcohol and hexane, and spectrophotometric readings were made at 450 nm. The results were expressed as mg.100 mL^{-1}.

The antioxidant activity of pulp and HSCAN was measured by two *in vitro* assays: DPPH scavenging method and co-oxidation of β-carotene/linoleic acid. The DPPH assay was performed as previously described for honey, and the following concentrations of pulp and HSCAN were tested: 100, 200, 300 and 400 μg/mL.

The β-carotene linoleate model system was applied according to the method described by Miller (1971). A solution of β-carotene (20 mg/mL chloroform) was prepared and 0.02 mL was mixed with 0.04 mL of linoleic acid and 530 mg of Tween 40 emulsifier and 1 mL of chloroform. After chloroform was removed under vacuum, 100 mL of aerated distilled water were added to the flask with vigorous shaking. Aliquots (5 mL) of this emulsion were transferred to different test tubes containing different concentrations of pulp and extracts. As soon as the emulsion was added to each tube, the zero time absorbance was measured at 470 nm using a spectrophotometer. Subsequent absorbance readings were recorded over a 2 h period at 15 min intervals by keeping the samples in a water bath at 50 °C. Blank samples, devoid of β-carotene, were prepared for background subtraction. The results are expressed as an oxidation inhibition percentage (%*I*), which is calculated considering the decay of the control's optical density (Dc) as 100% oxidation, according to the equation:$\%I = [(Dc - Dam)/Dc] \times 100$, where the decrease in optical density (Dam) is given by Dam = Absinitial – Absfinal (absorbance variation in the sample) and of the control Dc = Absinitial – Absfinal (absorbance variation in the control, i.e., without the substance that inhibits oxidation).

Based on these data, a comparative study of pulp and HSCAN kinetic behaviors with a synthetic antioxidant BHT was performed. This study is important in order to provide information on how the natural antioxidants present in the pulp and HSCAN curb the oxidative process in the β-carotene/linoleic acid system.

All spectrophotometric analyses were performed using a Spectronic® 20 Genesys™ (Spectronic Instruments Inc., Rochester, NY, USA) spectrophotometer.

2.4.1. Kinetic study of antioxidant activity in the β-carotene/linoleate model system

Using the method of tangents in two parts of the kinetic curves, the efficiency of extract antioxidant activity was estimated. This method was initially described by Yanishlieva and Marinova (1995) and subsequently modified by Moreira and Mancini Filho (2003).

In the first part of the curve (between 15 and 45 min after initiating the reaction), the antioxidant efficiency in blocking the chain reaction through interaction with the peroxide radicals was measured. This efficiency was measured through the ratio between the tangents of the kinetic curves for the pulp or HSCAN

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