



Cashew-apple (*Anacardium occidentale* L.) and yacon (*Smallanthus sonchifolius*) functional beverage improve the diabetic state in rats



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ABSTRACT

Cashew-apple and yacon have been widely recognized as an excellent source of bioactive compounds, including prebiotics and antioxidants, which may be beneficial to health. Experimental data indicates that prebiotics and some specific polyphenols could reduce the severity or incidence of degenerative diseases, such as diabetics. The aim of this study was evaluate the hypoglycemic effect of a functional beverage composed of yacon and cashew-apple in alloxan-induced diabetic rats. The growth of lactobacilli in the cecal material, catalase activity in liver and antiproliferative activity using HepG₂ cells were also evaluated. The total antioxidant capacity was determined in the beverage, showing values of $6.45 \pm 0.40 \mu\text{M Trolox} \cdot \text{g}^{-1}$ of fresh matter (FM), $15.58 \pm 0.38 \mu\text{M Fe}_2\text{SO}_4 \cdot \text{g}^{-1}$ of FM and $1780.14 \pm 99.01 \text{ g of functional beverage} \cdot \text{g}^{-1}$ of DPPH, measured by ABTS, FRAP and DPPH assays, respectively. The fructooligosaccharides (FOS) content ($2.97 \pm 0.07 \text{ FOS}/100 \text{ mL}$) and total phenolic were determined ($66.52 \pm 1.17 \text{ mg gallic acid equivalent}/100 \text{ g of FM}$), and the majoritarian phenolic compounds were identified by LC-DAD-ESI-MS. In the antiproliferative assay, HepG₂ was significantly inhibited, in a dose-dependent manner, by exposure to the functional beverage. In addition, the functional beverage was tested *in vivo*. Male Wistar rats were divided into five groups: control, non-treated diabetic, and diabetic treated with 100, 200 or 400 mg of lyophilized functional beverage per kg/day. The results showed a decrease in the glucose levels, a promotion of the growth of lactobacilli in cecal material and an increase in catalase activity in the liver. The results strongly support that yacon and cashew-apple have important hypoglycemic properties that could ameliorate the diabetic state.

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1. Introduction

The cashew (*Anacardium occidentale* L.) is native to Tropical America and has a large socioeconomic importance for the Northeast region of Brazil. Cashew is made up of the developed peduncle (apple) which is attached to the nut (actual fruit composed of shell and kernel). The peduncle, which is also called the cashew-apple, represents the edible portion and is used to prepare juices, pulp and preserves (Rufino et al., 2010). Apart from the usual presence of sugars, minerals and organic acids, one important characteristic of the cashew-apple is its high polyphenols content, such as anacardic acids (Oliveira, Yamada, Fagg, & Brandão, 2012; Tedong et al., 2010). These compounds have received

great attention by researchers and pharmaceutical companies due their involvement in the prevention of important disorders like as cancer, oxidative damage, inflammation and obesity (Hemshkhar, Santhosh, Kemparaju, & Girish, 2011). More recently, hypoglycemic properties of the cashew-apple (Abdullahi & Olatunji, 2010; Domínguez, Henríquez, Sintjago, & Ferrer, 2012; Oliveira et al., 2012) and its constituent – the anacardic acid, were also observed using different models, such as C2C12 muscle cells (Tedong et al., 2010) and streptozotocin-induced diabetic rats (Park, Yang, Hwang, Yoo, & Han, 2009).

Yacon (*Smallanthus sonchifolius*) is an Andean crop that has been used for centuries by the inhabitants of many South American regions in traditional folk medicine, and is particularly known as an abundant source of β -(2 → 1) fructooligosaccharides (FOS) (Campos et al., 2012). Unlike other sources of FOS, yacon is so rich in this component that an effective dose is ensured by consuming only a moderate amount of the root. The yield/ton in the field is also far superior to other conventional sources of FOS (Ojansivu, Ferreira, & Salminen, 2011).

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The available scientific evidence supports the recognition of FOS as dietary fibers with prebiotic properties (Campos et al., 2012; Pedreschi, Campos, Noratto, Chirinos, & Cisneros-Zevallos, 2003). The prebiotic definition according to FAO states that “A prebiotic is a non-viable food component that confers a health benefit on the host associated with modulation of the microbiota” (FAO, 2008), and its use as ingredient in food products were extensively reported in the literature (Cruz et al., 2013; Morais, Morais, Cruz, & Bolini, 2014).

The FOS appears to be a good candidate for modulating the metabolic syndrome, a known predisposing factor in the development of type 2 diabetes. FOS is fermented by specific bacteria promoting the proliferation of bifidobacteria and lactobacilli (Campos et al., 2012), thus improving the intestinal balance. The end products are short-chain fatty acids, such as acetate and propionate, which may influence systemic carbohydrates and the lipid metabolism (Alles et al., 1999). The yacon also has other constituent that may have hypoglycemic effects, the chlorogenic acid (3-O-caffeoyl-D-quinic acid, CGA), an ester formed by caffeic and quinic acids, and is one of the major polyphenolic compounds of yacon. CGA may possibly modulate the plasma insulin concentration and inhibit hepatic gluconeogenesis (Genta et al., 2009; Ojansivu et al., 2011), due the inhibition of glucose-glucose-6-phosphatase, the enzyme that catalyzes the final step of glycogenolysis and gluconeogenesis (Arion et al., 1998; Herling et al., 1998).

Diabetes mellitus (DM) has the highest rates of prevalence and mortality worldwide, that is characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both (American Diabetes Association, 2010). DM is associated with the generation of reactive oxygen species leading to oxidative damage particularly in the liver and the kidney (Mohamed, Bierhaus, & Schiekofer, 1999). Oxidative stress in diabetes coexists with a decrease in the antioxidant status (Picton, Flatt, & Mcclenaghan, 2001), which can increase the deleterious effects of free radicals. Antioxidants — such as catalase — play a major role in protecting biological systems against reactive oxygen species and reflect the antioxidant capacity of the system. The components of the defense system involved reducing the injury from free radical attacks include several enzymes and a few free radical scavenger molecules (Irshad & Chaudhuri, 2002).

The great interest in the potential health benefits of yacon and the cashew-apple is due to their composition in bioactive compounds, more specifically, FOS and phenolic compounds. The bioactive compounds of yacon and cashew-apple present different mechanisms in the diabetes role, and when these compounds were consumed together — in a same product — they could act in an additive or synergism. For this reason, the aim of this study was to elucidate the hypoglycemic effects of a functional beverage prepared with yacon and the cashew-apple in alloxan-induced diabetic rats. The growth of lactobacilli in the cecal material, catalase activity in the liver and antiproliferative activity using HepG₂ cells were also evaluated. As the authors known, this is the first report evaluating a product composed of both materials and their involvement in beneficial effects to health.

2. Material and methods

2.1. Chemical and reagents

The reagents used were potassium persulphate from Acrós Organics and ferrous sulphate from Vetec™. HepG₂ liver cancer cells were obtained from the Rio de Janeiro Cell Bank. HPLC grade water was prepared from distilled water using a Milli-Q system (Millipore Lab., Bedford, MA, USA). All other chemicals were purchased from Sigma-Aldrich Canada Ltd. (Oakville, ON, Canada).

2.2. Preparation of the functional beverage

The raw yacon (*S. sonchifolius*) was obtained in a local market in Fortaleza, Ceará State, Brazil. Then, yacon extracts were processed

as reported previously by Dionísio et al. (2013). Briefly, after washing and sanitizing, the yacon skin was removed manually and the edible portion was cut (1 cm³) and immersed in citric acid solution (2.4% w/v) for 8 min to inactivate enzymes. These small pieces were homogenized and centrifuged, and the aqueous portion was namely “yacon extract”. Cashew-apple (*A. occidentale* L.) non-pasteurized frozen pulp was purchased on the local market in Fortaleza-CE. No preservative was added in the fruit pulp processing. Both materials were stored at -18 ± 1 °C prior to use.

The functional beverage was prepared by mixing cashew-apple pulp (50%) and yacon extract (50%) together with 0.06% of stevioside. The composition of the beverage was based on our previous sensory trials using untrained panelists (Dionísio et al., 2013). An Armfield FT74x unit with a tube heat exchanger was used to pasteurize the functional beverage after preparation. The beverage was fed into a tank and pumped through the heat exchanger to achieve the treatment (85 °C for 90 s), followed by hot-fill in glass bottles, closed with caps, and cooled in an ice/water bath. Then, the functional beverage was stored under 4 ± 1 °C until the chemical analysis. For antiproliferative and *in vivo* assay, the beverage was lyophilized after thermal processing using LP 510 (Liobras) equipment and stored at -18 ± 1 °C prior to use.

2.3. Functional beverage: *in vitro* studies

2.3.1. Total antioxidant capacity (TAC), total polyphenols (TP) and fructooligosaccharides content (FOS)

The total antioxidant capacity was measured by the ABTS, FRAP and DPPH methods. For antioxidant extraction, 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 25,400g for 15 min, and the supernatant was recovered. Then, 4 ml of acetone/water (70:30, v/v) was added to the residue at 25 °C, which was extracted for 1 h, and then centrifuged with the same conditions. Methanolic and acetonic extracts were combined, and then added to 10 ml of distilled water.

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). For all methods, a Varian (CA) spectrophotometer was used. The results of the DPPH method was expressed as the concentration of antioxidants required to reduce the original amount of free radicals by 50% (EC₅₀) and the values were expressed as g functional beverage per g of DPPH. For the ABTS and FRAP assays, the results were expressed as μM Trolox and μM Fe₂SO₄ per g of functional beverage, respectively. Thus, the total polyphenols (TP) was determined by the Folin–Ciocalteu method (Obanda, Owuor, & Taylor, 1997) and the results were expressed as mg GAE (gallic acid equivalent) per 100 g⁻¹ of functional beverage. The fructooligosaccharides (FOS) were determined as described by Horwitz, Latimer, and George (2005), and the results were expressed as g FOS per 100 mL⁻¹ of functional beverage.

2.3.2. Phenolic compound detection in functional beverage by LC–DAD–ESI–MS

The general procedure for screening of phenolics in plant materials (Lin & Harnly, 2007) was employed with modifications. The LC–DAD–ESI/MS instrument consisted of a Varian 250 HPLC (Varian, CA) coupled with a diode array detector (DAD) and a 500-MS IT mass spectrometer (Varian, CA). A Symmetry C18 (Varian, 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) and flow rate of 0.4 mL · min⁻¹ that was used with a linear gradient from 10% to 26% B (v/v) in 40 min, then to 65% B at 70 min, and finally to

100% B at 71 min and was maintained up to 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. A drying gas pressure of 35 psi, a nebulizer gas pressure of 40 psi, a drying gas temperature of 370 °C, capillary voltages of 3.5 kV for PI and NI, and spray shield voltages of 600 V were used. The LC system was coupled to the MSD with a 50% splitting.

2.3.3. Glucose, fructose, and sucrose determination

The glucose, fructose and sucrose of the functional beverage were determined as described by Pinto, Honorato, Rabelo, Gonçalves, and Rodrigues (2007) with modifications. After dilution in water (1:10), the samples were filtered through a 0.45 µm Millipore filter. Briefly, the compounds were separated using a Hiplax Pb (8 µm, 300 × 7.7 mm) column and a 50 × 7.7 mm guard column on a Varian 3380 Separation Module (Varian, Milford, MA) equipped with an auto-injector and a 355 infrared detector (ID). The mobile phase was composed of water at a flow rate of 0.6 mL/min and 65 °C.

2.3.4. Measurement of inhibition activity on HepG₂ cell proliferation

The antiproliferative activity of the functional beverage was measured by the MTS assay (MTS-based cell titer 96 non-radioactivity cell proliferation assay), as described by Sun, Chu, Wu, and Liu (2002). HepG₂ cells were maintained in Dulbecco's Modified Eagle's Medium (DMEM, Sigma, CA, USA)/F12 (Sigma, St. Louis, MO) supplemented with 10% fetal bovine serum (FBS, Cultilab, São Paulo, Brazil) and maintained at 37 °C in 5% CO₂ in an incubator. A total of 1.0 × 10⁴ HepG₂ cells in growth media were placed in each well of a 96-well flat-bottom plate. After 24 h, the growth medium was replaced by media containing 1 to 10 mg · mL⁻¹ of the functional beverage. Control cultures received the extraction solution minus the functional beverage, and blank wells contained 100 µL of growth medium with no cells. After 72 h, cell proliferation was determined by colorimetric MTS assay. Cell proliferation (percent) was determined from the MTS absorbance (490 nm) reading for each concentration compared to the control. Three replications for each sample were used to determine the cell proliferation.

2.4. Functional beverage: in vivo studies

2.4.1. Animals and experimental design

The study started after approval by the Research Ethics Committee on the use of animals at the Federal University of Alfenas/UNIFAL-MG (Protocol no. 528/2013). Thirty male rats weighing 90 ± 5 g were kept under standard laboratory conditions of temperature (22 ± 2 °C), relative humidity (52 ± 5%), and 12 h light–dark cycle. Diet and water were provided *ad libitum*. Diabetes was induced in overnight fasted rats by intraperitoneal injection of 120 mg/kg of alloxan monohydrate (Verma, Khatri, Kaushik, Patil, & Pawar, 2010). After four days, the glycemic levels were measured, and the rats that presented glucose levels higher than 200 mg/dL were used in the experiment. The lyophilized functional beverage and saline solution (0.9% w/v NaCl, for the control groups) were administered daily by gavage (0.5 ml/100 g of body weight) for 30 days. The rats were distributed into five groups, with six rats in each group, treated as follows:

- Negative control: Healthy animals received a saline solution.
- Positive control: Diabetic animals received a saline solution.
- Group 1. Diabetic animals received 100 mg of functional beverage per kg of body weight (b.w.).
- Group 2. Diabetic animals received 200 mg of functional beverage/kg of b.w.
- Group 3. Diabetic animals received 400 mg of functional beverage/kg of b.w.

Nutritional parameters such as weight gain and food consumption were collected three times a week. The blood glucose levels were measured nine times. After 30 days, the animals were deprived of food for 12 h, anesthetized by halothane inhalation (2 L · min⁻¹) and sacrificed by extracting blood from the abdominal aorta with a syringe. Serum was obtained by centrifugation at 1900 g for 10 min, and the liver was perfused with saline solution (0.9% w/v), collected and immediately frozen at –80 °C.

2.4.2. Catalase activity

The catalase activity from rat liver extract was measured according to the Hugo and Lester (1984) method. The disappearance of hydrogen peroxide was observed using a spectrophotometer (240 nm, 1 min, 25 °C). One unit of activity corresponds to the mmol of H₂O₂ destroyed/min/mg protein.

2.4.3. Lactobacilli analysis of cecal material

One gram of cecal material was transferred into a sterile tube and mixed with 9 ml of sterile saline phosphate solution (PBS, Sigma Aldrich) and sequent dilutions (from 10⁻¹ to 10⁻⁷) and then inoculated in a Rogosa Agar SL medium (Becton Dickinson, USA). Incubation was performed at 30 °C under anaerobic conditions using the anaerobic jar with Anaerocult A (Merck, Darmstadt, Germany). The number of cells was recorded as a log₁₀ cfu · g⁻¹ of wet samples after 48 h of incubation.

2.5. Statistical analysis

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

3. Results and discussion

3.1. Functional beverage: in vitro studies

Diabetes mellitus is a chronic metabolic disease that is rapidly increasing around the world (American Diabetes Association, 2010). The higher levels of glucose in the blood due to non-secretion of insulin or insulin insensitivity cause disturbances in carbohydrates, lipids and protein metabolism. Hyperglycemia induces auto-oxidation of lipids and glycation of protein/glucose, resulting in the formation of free radicals of oxygen (ROS) and nitrogen (RNS) (Chaudhry, Ghosh, Roy, & Chandra, 2007). Foods contain many different antioxidant components that can neutralize these free radicals, and several methods have been developed to determine their antioxidant potential. However, to measure the antioxidant activity of food extracts, at least two test systems have been recommended (Rufino et al., 2010). For this reason, the antioxidant activity of the functional beverage was measured using the ABTS, FRAP and DPPH methods and the results are shown in the Table 1, expressed in fresh matter (FM).

The total antioxidant activity of the functional beverage was 6.45 ± 0.40 µM Trolox · g⁻¹ of FM and 15.58 ± 0.38 µM Fe₂SO₄ of FM, for the

Table 1

Total antioxidant capacity (TAC), total polyphenols (TP) and fructooligosaccharides content (FOS) in the functional beverage.

In vitro analyses of functional beverage	Mean	S.D.	C.V.
Total phenolic (mg · 100 g ⁻¹)*	66.52	1.17	1.76
Fructooligosaccharides (g · 100 mL ⁻¹)*	2.97	0.07	2.00
FRAP (µM Fe ₂ SO ₄ · g ⁻¹)*	15.58	0.38	2.46
ABTS (µM Trolox · g ⁻¹)*	6.45	0.40	6.18
DPPH (functional beverage · g ⁻¹ of DPPH)*	1780.14	99.01	5.56

All values are shown as mean (n = 3). S.D. (standard deviation). C.V. (coefficient of variation).

* The results are expressed in fresh matter (FM).

ABTS and the FRAP method, respectively. Also, the value obtained by the DPPH method was 1780.14 ± 99.01 g of functional beverage \cdot g⁻¹ (EC₅₀), measured by the molar ratio of antioxidant to DPPH radical required for 50% reduction in DPPH radical concentration. It is well known that the polyphenols represent one of the most important classes of compounds that can influence the antioxidant capacity of the yacon and the cashew-apple (Brito et al., 2007; Yan et al., 1999). Some specific polyphenols, such as chlorogenic acid (CGA) (Arion et al., 1998; Herling et al., 1998) and anacardic acids (Park et al., 2009; Tedong et al., 2010) have recently been reported as hypoglycemic agents. Due to this previous statement, we measured the phenolic contents of the functional beverage (66.52 ± 1.17 mg GAE/100 g of FM), and identified the majoritarian phenolic compounds through LC–DAD–ESI–MS.

Chromatograms at 340 nm showed the CGA, 1-O-*trans*-cinnamoyl-beta-D-glucopyranose and anacardic acids as the majoritarian compounds. This was consistent with previous investigations that reported CGA as the main phenolic identified in yacon (Campos et al., 2012), as well as the 1-O-*trans*-cinnamoyl-beta-D-glucopyranose and anacardic acids as some of a major phenolic components reported in the cashew-apple (Michodjehoun-Mestres, Amraoui, Fulcrand, & Brillouet, 2009; Trevisan et al., 2006). Although phenolic compounds are important as therapeutic complements for diabetes, these compounds also exhibit antioxidant properties and consequently, may be involved in the prevention of different types of cancer, including liver cancer. Thus, we evaluate the antiproliferative activity of the functional beverage on the growth of HepG₂ human liver cancer cells *in vitro*, and the results are summarized in Fig. 1. The results showed that the functional beverage exhibits antiproliferative activity in a dose-dependent manner. The antiproliferative activity was expressed as the relative cell viability (%) after 72 h of beverage exposition, where a lower value indicates a higher antiproliferative activity under the experimental conditions tested. Many studies have linked yacon consumption, and its constituent, the CGA, to a wide range of health benefits, including antitumor activity. Thus, the literature reports that the anacardic acid could be considered as a therapeutic agent in the treatment of the most serious pathophysiological disorders like cancer (Hemshkhar et al., 2011), and exhibit moderate cytotoxic activity against both BT-20 breast and HeLa epithelioid cervix carcinoma cells (Kubo, Ochi, Vieira, & Komats, 1993). These authors suggest that consuming the cashew-apple and/or its products continuously for long durations may be advantageous in controlling tumors. Therefore, the identification of phytochemicals that have antiproliferative activity in the functional beverage investigated in the present paper is worthy of investigation.

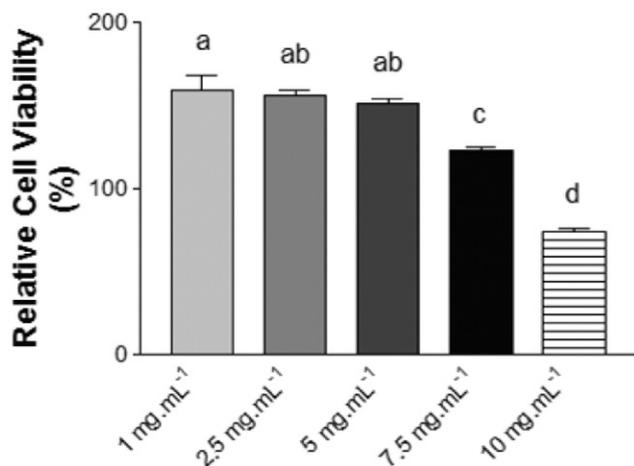


Fig. 1. Antiproliferative activity of the functional beverage against HepG2 cells. Bars with no letters in common are significantly different ($P < 0.05$).

3.2. Functional beverage: *in vivo* studies

Alloxan is widely used to induce experimental diabetes in animals. The action mechanism in the pancreas beta-cells is mediated by reactive oxygen species. The action of reactive oxygen species with a simultaneous massive increase in cytosolic calcium concentration causes rapid destruction of beta-cells (Szkudelski, 2001). In diabetic animals, symptoms of marked hyperglycemia include polyuria, polydipsia, weight loss, and polyphagia (American Diabetes Association, 2010). In the present experiment, the alloxan-induced diabetic rats exhibited a significant increase ($P < 0.05$) in diet consumption when compared with normal rats, as shown in Fig. 2a and no statistical differences ($P > 0.05$) were observed in all groups tested with different concentrations of the functional beverage, when compared with the positive control. However, a loss of body weight after 30 days of treatment was similar in all groups (normal and diabetics rats) (Fig. 2a).

Interestingly, the data presented in Fig. 3a shows that the administration of the functional beverage (G1) for 30 days caused a significant decrease in blood glucose levels ($P < 0.05$) when compared with untreated diabetic rats. Although no statistical difference ($P > 0.05$) was observed in the treatments that receiving the functional beverage (G1, G2 and G3), the results shows that the G1 presents the lowest values of blood glucose levels (see Fig. 3a) when compared with all the diabetic groups, with no difference among glucose values

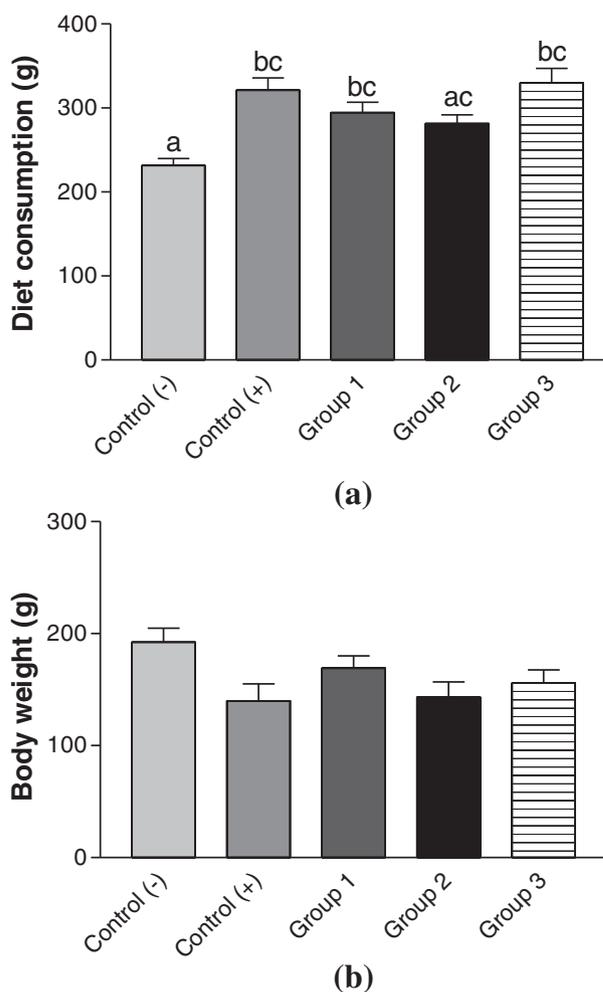


Fig. 2. Effect of the 30 day administration of functional beverages on alloxan-induced diabetic rats on (a) body weight and (b) diet consumption. (-) and (+) control: healthy and diabetic animals received a saline solution; G1, G2 and G3: diabetic animals received 100, 200 and 400 mg of functional beverage/kg/b.w., respectively. Bars with no letters in common are significantly different ($P < 0.05$).

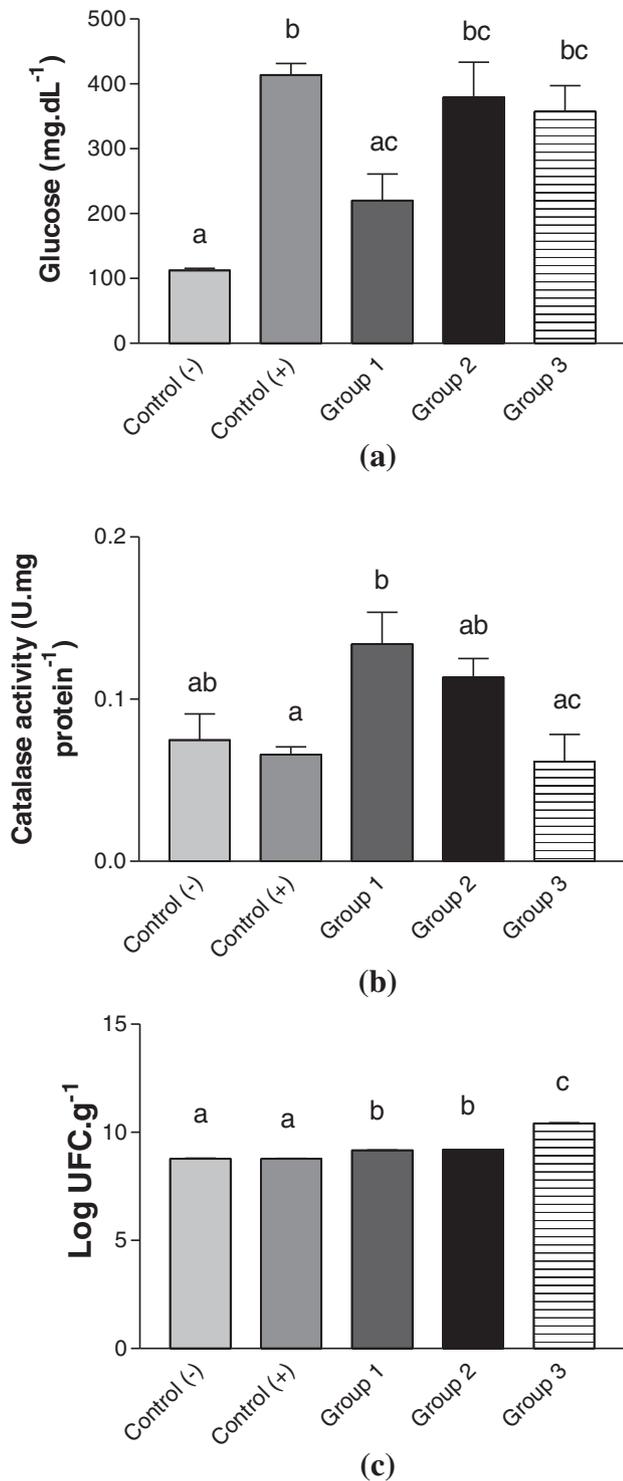


Fig. 3. Effect of 30 day administration of the functional beverage on alloxan-induced diabetic rats on (a) the plasma glucose levels, (b) liver catalase activity and (c) lactobacilli concentration in the cecal material. (-) and (+) control: healthy and diabetic animals received a saline solution; G1, G2 and G3: diabetic animals received 100, 200 and 400 mg of functional beverage/kg/b.w., respectively. Bars with no letters in common are significantly different ($P < 0.05$).

($P > 0.05$) when compared with the negative control, that represents the healthy animals. G2 and G3 could be affected negatively by the higher concentration of monosaccharide (0.47 ± 0.001 and 0.28 ± 0.001 g/100 ml for glucose and fructose, respectively) presented in the beverage and, for this reason, the effect was more pronounced in the G1 animals group. In order to compare these results, the literature

reports a decrease in blood glucose in diabetic rats that received yacon or cashew-apple, singly. However, as the authors known, it is the first report that describes the positive effects when these two products are offered together, as a functional beverage. Recently, the results obtained by Oliveira, Braga, and Fernandes (2013), Genta et al. (2009) and Satoh, Nguyen, Kudoh, and Watanabe (2013) raise the possibility that yacon intake has beneficial effects in treating obesity-related insulin resistance and type 2 diabetes mellitus. For cashew-apple, there are only a few reports of hypoglycemic effects in animal and human models (Abdullahi & Olatunji, 2010; Domínguez et al., 2012; Oliveira et al., 2012).

As mentioned before, the oxidative stress in diabetes coexists with a decrease in the antioxidant status (Picton et al., 2001). In the present experiment, we have also observed a decrease, although with no statistical significance ($P > 0.05$), in the catalase activity in the liver of diabetic (positive control) and non-diabetic (negative control) animals (see Fig. 3b). Moreover, the administration of the functional beverage for 30 days caused a significant increase in the antioxidant enzyme activity, and, in agreement with blood glucose levels, the G1 was the treatment with the best results. This means that this functional beverage can reduce the potential glycation of enzymes or it may reduce reactive oxygen free radicals and improve the activity of this antioxidant enzyme. These results are consistent with previous studies showing that yacon and cashew-apple exhibits antioxidant activity (Campos et al., 2012; Rufino et al., 2010; Yan et al., 1999).

Finally, the results from the bacteriological analysis of cecal material are presented in Fig. 3c. The functional beverage contains 2.97 ± 0.07 of FOS/100 ml, about four times higher than that the amount of FOS required by Brazilian regulation for products with functional properties (Anvisa, 2014). The permitted claim is “FOS as prebiotics contribute to a balance/equilibrium of the intestinal flora. Their consumption should be associated with a balanced diet and a healthy life-style”. The presence of a relatively higher concentration of FOS in the beverage is associated with the dose-dependent results of lactobacilli in the cecal material. Lactobacilli concentrations (\log_{10} cfu g^{-1} wet weight) were significantly higher ($P < 0.05$) in samples obtained from diabetic animals that received the functional beverage *via* gavage compared to both control groups, diabetic and non-diabetic animals, that received only saline solution. These results are consistent with others studies, showing that the FOS from the yacon is efficiently metabolized by lactobacilli *in vitro* (Pedreschi et al., 2003) and *in vivo* using a mouse and a guinea pig model (Bibas Bonet et al., 2010; Campos et al., 2012).

In conclusion, the present investigation showed that the functional beverage of yacon and cashew-apple have considerable concentrations of bioactive compounds, such as FOS and phenolic compounds, which may be directly or indirectly responsible for its hypoglycemic properties. Furthermore, the functional beverage promoted the growth of lactobacilli in the cecal material in all concentration tested (100, 200 and 400 mg/kg b.w.), increased the catalase activity in the liver, especially in the group receiving 100 mg/kg b.w., and showed antiproliferative activity, in a dose-dependent manner. Further studies should cover a consumer test and sensory profiling according to Morais et al. (2014) and Pimentel, Cruz, and Prudêncio (2013). Therefore, this beverage should be considered as a potential candidate for future studies on diabetes.

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