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ALPHA-GLUCOSIDASE INHIBITORY ACTIVITY AND TOTAL PHENOLIC COMPOUNDS OF ARAÇÁ (*Psidium cattleianum* SABINE) FRUIT EXTRACT CHANGES ALONG THE DIGESTIVE PROCESS[1]

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INTRODUCTION

The alpha-glucosidase (alpha-Gluc) is an enzyme present mainly in the intestine, which catalyzes the digestion of complex carbohydrates, converting them into easily digestible monosaccharides. Inhibitors of this enzyme are used in individuals with type 2 diabetes mellitus (T2DM) in order to promote a decrease in glucose uptake and consequently a reduction in blood sugar levels (LI et al., 2010). In recent years a growing demand for alpha-Gluc inhibitors from natural sources as an alternative for the treatment of T2DM has growth, mainly because current drugs shows undesirable side effects. As part of an ongoing research, ethanolic extract of yellow Araçá, also known as lemon guava, has demonstrated a strong inhibitory activity against alpha-Gluc, with a half maximum inhibitory concentration (IC_{50}) of the enzyme of 25.4±0.7 µg/mL (VINHOLES et al., 2015). This value is 16 times lower than the IC_{50} obtained for the drug (acarbose) used by T2DM patients. Araçás are rich in vitamin C and phenolic compounds, being epicatechin and gallic acid their main constituents (MEDINA et al., 2011). These compounds can exert health benefits for humans by preventing diseases such as cancer, cardiovascular and neurodegeneratives diseases, and diabetes. Nevertheless, to achieve the desirable enzymatic inhibitory effect under physiological conditions, it is necessary that the compounds, responsible for the activity do not lose their activities along the gastrointestinal digestion. In this context, the study of the stability of the bioactive compounds is important since, depending on the studied natural matrix, a significant change in the composition and activities can occur after the digestive process (SIRACUSA et al., 2011). Thus, the present work aims to determine the changes on alpha-Gluc inhibitory activity and the total phenolic composition of yellow Araça ethanolic fruit extract along the digestive process.

MATERIAL AND METHODS

Standards and reagents

Reagents were purchased from different suppliers. Alpha-glucosidase (alpha-Gluc) (type I from baker's yeast), 4-nitrophenyl alpha-D-glucopyranoside (PNP-G), Phosphate Buffer pH 7, Folin-Ciocalteu reagent, sodium carbonate and chlorogenic acid were obtained from Sigma-Aldrich (St. Louis, MO, USA). Ethanol was purchased from Synth (Diadema, SP, Brazil).

Samples and preparation of fruit extract

Samples of Araçá selections were obtained from the Active Germplasm Bank of native fruits at Embrapa Clima Temperado. The fruits were sampled searching for a mixture of completely ripe fruits. All fruits were harvested in 2015, between the months of March and April. Fruits were selected considering the absence of visible injury and infections and also color and size uniformity and were frozen (-20 °C) until analysis.

Fruit extracts were prepared from the edible portions of fruits (skin, pulp and seeds). Fruit samples, at least 10 fruits, were thawed at room temperature sliced and the extraction was

performed with 95% ethanol (1:4, w/v) during 5 min using an Ultra-Turrax homogenizer (Ika, Artur Nogueira, São Paulo, Brazil). Homogenates were filtered through paper filter and further evaporated under pressure at 40 ºC. Ethanolic extract yield was 8.55 % (±0.42 %). Extract were prepared in triplicate, reconstituted in ethanol/water (3:1 v/v) and stored at -20 ºC until analysis.

In vitro simulated digestion

In vitro digestion procedure was carried out on ethanolic extract of yellow Araçá to evaluate the bioaccessibility of phytochemical compounds according to Gião et al. (2012), with a few modifications. The method reproduced three physiological steps of the digestion process: α-amylase digestion to stimulate mouth conditions (Mouth digesta); digestion with pepsin/HCl to simulate gastric conditions (Gastric digesta); and digestion with bile salts/pancreatin to simulate small intestine conditions (Intestinal digesta). Extracts were evaluated for each digestive process separately and a total digestive process was also carried out (Completa digesta). Briefly, 0.9 mL of ethanolic extract was diluted in 10 mL of water and mixed with freshly prepared α-amylase solution (0.60 mL, 100 mU/mL), incubated at 37 °C for 1 min in water bath under shaking (200 rpm). The gastric digestion was performed by adjusting the pH to 2.0 (HCl, 1M) and the mixture was incubated under shaking (130 rpm) with an amount (0.5 mL) of freshly prepared pepsin solution (25 mg/mL in 0.1 M HCl). For the intestinal digestion samples were adjusted at pH 6.0 with $NaHCO_3$ (1M) before addition of 2.5 mL of freshly prepared pancreatin-bile salts solution (2g/L of pancreatin plus 12 g/L of bile salts in $NaHCO_3$ (0.1 M)) and incubated for 1 h at 37ºC, 45 rpm. Enzymatic inactivation was carried out by emerging samples in water (100 ºC) during 1 minute. Samples were then filtered through a 0.45 µm membrane and frozen until analysis (alpha-Gluc inhibition and TPC). Controls of sample with adjusted pH for each step, in the absence of enzymes, were run in parallel (Control mouth digesta, Control gastric digesta and Control intestinal digesta). Extract diluted in ethanol (Extract) at the same concentration of those diluted in water was used for comparison of activity and TPC. One control was run for each extract (n=3) and extract with added enzymes were run in duplicate (n=6).

Alpha-gluc inhibition and TPC determination

For the evaluation of the alpha-Gluc inhibition and the total phenolic compounds of araçá extract, *in vitro* assays were performed applying spectrophotometric methods using a Amersham, Modelo UV Vis Ultrospec-3100 Pro Amersham Bioscience spectrophotometer.

alpha-Gluc inhibitory activity

The effect on alpha-Gluc was assessed using a procedure previously reported (FERRERES et al., 2013) slightly modified. Briefly, 20 µL of fruit extract or ethanol (control) was added to a vial with 100 µL of PNP-G (3.25 mM) in phosphate buffer (pH 7.0). The reaction was initiated by the addition of 100 µL of enzyme (9.37 U/mL in phosphate buffer, pH 7.0) and vials were incubated at 37 ºC for 10 min. The reaction was stopped by adding 0.600 mL of Na_2CO_3 (1M) and the absorbance at 405 nm was measured. Analysis were carried out in duplicate, controls (n=6), samples (n=12).

Total phenolic compounds. (TPC)

TPC content was measured according to the Folin–Ciocalteu method adapted from Swain and Hillis (SWAIN; HILLIS, 1959). Analysis were carried out in duplicate, controls (n=6), samples (n=12).

Statistical analysis

Results are expressed as means ± standard error of the mean (±SEM) and statistical significance of the difference between the results observed for the extract and each treatment was evaluated by one-way analysis of variance (ANOVA) followed by a comparison of means by Tukey test. Differences were considered to be significant when $P < 0.05$.

RESULTS AND DISCUSSION

Araçá extract at a concentration of 485 µg/mL was able to inhibit 90% of the alpha-Gluc enzyme (Figure 1A). At the same concentration, the extract diluted in water (Control mouth digesta) showed an inhibitory property of 54% (Figure 1A). This result indicates a loss of activity that can be probably due to instability of compounds in water. No significant difference was observed between Controls and treatments of mouth and gastric digesta. Nevertheless, a significant increase on the inhibitory properties over alpha-Gluc was observed for the Control intestinal digesta (extract adjusted to pH6) and also with addition of enzymes (Intestinal digesta) treatment. Control complete digesta and Complete digesta showed similar results to the Control intestinal digesta and Intestinal digesta, indicating that this last digestive step can be responsible for the release or changes of bioactive compounds leading to an increase on the inhibitory activity, fact that was also observed by Lee et al. (2016). The increase on activity observed at this step varied from 31 to 41 %, reaching almost 95% of inhibition, values similar to the initial Extract (Figure 1A).

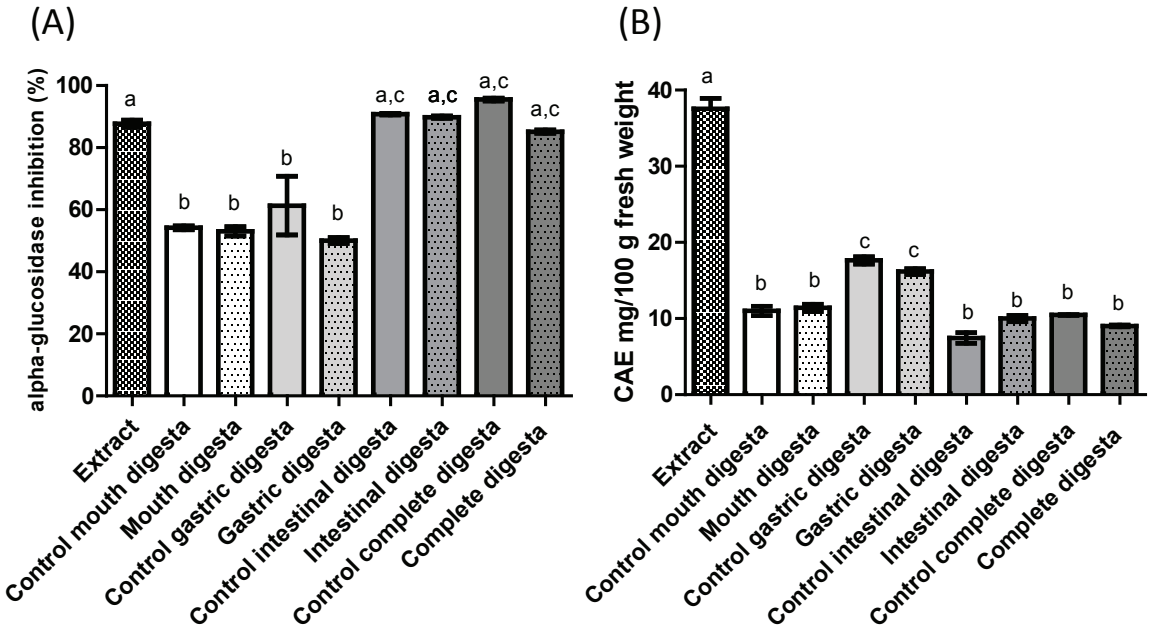

Figure 1. Changes on alpha-glucosidase inhibitory activity (A) and total phenolic compounds (B), expressed as mg of chlorogenic acid equivalents per 100 g of fresh weight (CAE mg/100g of fresh weight) of Araçá fruits ethanolic extract along the digestive process. Values show mean ± SEM. Means without a common superscript, are significantly different from each other (P <0.05)

Different biological activities have been attributed to phenolic compounds, including the alpha-Gluc inhibitory activity. It is well stablished by different studies that the TPC of a matrix gives an idea of how rich this product is in antioxidants, since these parameters are closely related. In the present study, the TPC of Araçá ethanolic fruit extract were determined along the digestive process (Figure 1B) in order to relate its enzymatic inhibitory activity with possible changes in the chemical composition. The Araçá ethanolic extract diluted in ethanol (Extract) showed a concentration of almost 40 mg of CAE/100g of fresh weight, however, when this extract, at the same concentration, was diluted in water (Control mouth digesta) the TPC value was almost three times lower. This result is in accordance with the loss of activity observed over the enzyme (Figure 1A). Nevertheless, a significant increase on the TPC was observed for the Control gastric digesta e Gastric digesta (Figure 1B) fact that can be attributed to a decrease in the pH of Araçá extract after the incubation at pH 2.0, which may favor the bioaccesibility of some compounds (WONG et al., 2014). The TPC content at the final step of the digestion (intestine) do not correlate with the increase on the inhibitory activity of the extract over the enzyme. However, as stated before, changes on the chemical structures may occurs, leading to compounds with increased inhibitory activity (LEE et al., 2016).

CONCLUSIONS

Bioactive compounds present in the Araçá ethanolic extract are not stable in water with consequent loss of activity.

The simulated *in vitro* digestion of Araçá ethanolic extracts indicates that the bioactive compounds reaches the target organ, the intestine, with increased inhibitory properties over the alpha-Gluc enzyme.

Araçá ethanolic extract is a promising source of bioactive compounds to be used in the prevention, control and treatment of T2DM.

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