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Effect of *Stevia rebaudiana* addition on bioaccessibility of bioactive compounds and antioxidant activity of beverages based on exotic fruits mixed with oat following simulated human digestion

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ABSTRACT

In order to determine the impact of *Stevia rebaudiana* (*SR*) addition on bioactive compounds bioaccessibility of a new developed functional beverage based on exotic fruits (mango juice, papaya juice and açaí) mixed with orange juice and oat, an *in vitro* gastrointestinal digestion was performed. Ascorbic acid, total carotenoids, total phenolics, total anthocyanins, total antioxidant capacity and steviol glycosides were evaluated before and after a simulated gastrointestinal digestion. Salivary and gastric digestion had no substantial effect on any of the major phenolic compounds, ascorbic acid, total antioxidant capacity and steviol glycosides, whereas carotenoids and anthocyanins diminished significantly during the gastric step. All analysed compounds were significantly altered during the pancreatic-bile digestion and this effect was more marked for carotenoids and total anthocyanins. However, phenolic compounds, anthocyanins, total antioxidant capacity and steviol glycosides bioaccessibility increased as did *SR* concentration. Ascorbic acid bioaccessibility was negatively affected by the *SR* addition.

1. Introduction

Current trends and worldwide developments on new food products with functionality aim to demonstrate a significant bioactivity of exotic fruits with positive impact in several chronic disorders (Costa, García-Díaz, Jimenez, & Silva, 2013). In this sense, research has focused on combinations of exotic fruits with other ingredients in beverages (Carbonell-Capella, Barba, Esteve, & Frígola, 2013). Fruit juice blends with other ingredients are gaining importance in the market probably due to public perception of juices as a healthy natural source of nutrients and increased public interest in health issues.

Additionally, the use of *Stevia rebaudiana* (*SR*) leaves is increasing as a natural sweetener 300 times sweeter than sucrose without caloric value, allowing consumers to enjoy sweet taste without concerns about weight gain. They do not replace the sugar naturally present in foods, but they can be an excellent substitute for added sugars and thus an effective aid in weight management. The European Commission granted final regulatory approval for the use of stevia extracts in foods and beverages on 11 November 2011. Stevia leaves contain a mixture of diterpene glycosides (steviosides) and is considered a good source dietary fibre, minerals and essential amino acids (Kim, Yang, Lee, & Kang, 2011). Stevia leaf extract shows a high level of antioxidant activity, as well as a variety of phytochemicals such as phenolic compounds, directly associated with the removal of free electrons and superoxide radicals (Geuns, Hajihashemi, & Claes, 2012). Due to its chemical structure and health-promoting phytochemical components, stevia is suitable as a replacement for sucrose in beverages and for the production of functional food ingredients (Šic Žlabur et al., 2013). The sweetening power of steviol glycosides differ between them, with rebaudioside A being 400 times sweeter than sugar and stevioside about 300 times sweeter (Ceunen & Geuns, 2013). As a result, determination of the steviol glycoside profile is of great interest to industry.

Despite the enormous research on antioxidant properties of fruit beverages, studies investigating the effect of gastrointestinal digestion on dietary antioxidants are scarce. Only phytochemicals released from matrices become bioaccessible and are potentially available for absorption by the gastro-intestinal tract, and, therefore, able to exert their beneficial effects in the human body. Under gastrointestinal conditions, transformations (degradation, epimerisation, hydrolysis and oxidation) and interactions between phytochemicals and food components may also occur, modifying therefore the biological activity of the bioactive compounds (Carbonell-Capella, Buniowska, Barba, Esteve, & Frígola, 2014). Therefore, it is important, before concluding on any potential health effect, to assess how the digestion process affects bioactive compounds and their stability, as this, in turn, will affect their

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bioavailability for uptake, as well as their possible beneficial effects.

Previous studies have confirmed that an *in vitro* digestion model system simulating human digestion could support reliable prediction of bioaccessibility of bioactive compounds and total antioxidant capacity in plant products (Rodríguez-Roque, Rojas-Graü, Elez-Martínez, & Martín-Belloso, 2013). However, the effect of *SR* extracts on the stability and bioaccessibility of phytochemicals in beverages typically consumed with adjuncts or as formulated products has not yet been reported in the literature data so far. The extent to which formulation may modify the bioactive compound profile of exotic fruit-oat beverages or influence their bioavailability is critical to understanding ultimate physiological effects elicited by these beverages. Furthermore, available knowledge on the digestibility of steviol glycosides is limited. Therefore, at this stage of development, it is necessary to study the impact of digestive conditions when a new specific formulation of commercial ready-to-drink matrix is designed in order to better design future studies focused on assessment of specific biological outcomes.

The objective of the current study was to investigate the bioaccessibility of phenolic compounds, anthocyanins, carotenoids, ascorbic acid, steviol glycosides and antioxidative effect in exotic fruit-oat beverages with (1.25% and 2.5%) and without *SR*. The effect of *SR* extract addition on the bioaccessibility of bioactive compounds and total antioxidant capacity was evaluated with an *in vitro* physiological approach simulating human digestion in the upper gastrointestinal tract, with the inclusion of a salivary, gastric and duodenal step with a dialysis membrane. The release of bioactive compounds as well as the total antioxidant capacity of the beverages were determined in aliquots collected at the end of each digestion step.

2. Materials and methods

2.1. Samples

Cultivars of papaya (*Carica papaya*), mango (*Mangifera indica*), oranges (*Citrus sinensis*, cultivar Navel) and oat beverage (Santiveri, Lérida, Spain) were purchased from a local supermarket. Papaya, mango and orange juices were extracted after appropriate washing of the fruits and the pulp was removed. Açaí provided by Nature's Way Products Inc. (Utah, USA) (containing 450 mg of açaí berries extract, with 10% of polyphenols) was added to the beverage.

S. rebaudiana leaves were supplied by company Anagalide, S.A. (Barbastro, Huesca, Spain) and stored at room temperature. A stock solution (8.33%, w/v) of *S. rebaudiana* was prepared in order to formulate the beverage (Carbonell-Capella et al., 2013). For this purpose, 100 mL of bottled water at 100 °C were added on the dried leaves (8.33 g) and were kept for 30 min. The infusion was vacuum filtered using filter paper (Whatman No. 1) and the filtrate obtained was stored for the duration of the experiment at −40 °C.

The fruit juice mixture was prepared by mixing 32.5% (v/v) of papaya juice, 10% (v/v) of mango juice, 7.5% (v/v) of orange juice, 20% of oat beverage, 1% of açaí powder (w/v) and water to 100%. To obtain final stevia concentrations of 1.25% and 2.5% (w/v), different volumes of stevia stock solution (30 and 60 mL) were added to prepare 200 mL of beverage instead of water. The higher stevia concentration (2.5%, w/v) was selected, taking into account the sucrose concentration of commercial fruit-based beverages and the sweetness equivalence of stevia and sucrose.

2.2. Chemicals and reagents

Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), as a standard substance (2 mM) to measure TEAC, 2,2′-azobis (2-methylpropionamidina)dihydrochloride (ABTS), 2,2-diphenyl-1-picrylhydrazyl (DPPH), fluorescein sodium salt, 2,2′-azobis (2-amidinopropane)dihydrochloride (AAPH), disodium metabisulfite, Folin–Ciocalteu (ammonium molibdotugstat) reagent, rebaudioside A, stevioside hydrate, steviol hydrate, α-amylase from *Bacillus*, mucin from porcine stomach, pepsin from porcine gastric mucosa, pancreatin from porcine pancreas, bile extract porcine and EDTA Na_2 were purchased from Sigma (Steinheim, Germany). Gallic acid 1-hydrate in distilled water, as a standard (10 mg/mL) for phenolic compounds, was purchased from UCB (Brussels, Germany). Oxalic acid, acetic acid, chlorhidric acid, acetone, sodium acetate, potassium persulphate ($K_2S_2O_8$), sodium di-hydrogen phosphate (anhydrous) (NaH_2PO_4) and di-potassium hydrogen phosphate (K_2HPO_4) were purchased from Panreac (Barcelona, Spain), while di-sodium hydrogen phosphate anhydrous (Na_2HPO_4) and potassium dihydrogen phosphate (KH_2PO_4) from Scharlau (Barcelona, Spain). Ethanol, methanol, acetonitrile, hexane, sodium chlorure, sodium carbonate anhydrous (Na_2CO_3), trichloroacetic acid and sodium sulphate proceeded from Baker (Deventer, The Netherlands). Ascorbic acid and sodium dodecyl sulphate were obtained from Merck (Darmstadt, Germany) and rebaudioside C and rebaudioside F from Wako (Osaka, Japan).

2.3. Simulated digestion

A three-stage *in vitro* digestion model was performed based on the previously described procedure by Rodríguez-Roque et al. (2013), with the addition of a salivary step. Briefly, 50 mL of each beverage (in triplicate) was transferred to an Erlenmeyer flask, and a saliva solution (5 mL, pH 6.75 ± 0.2) containing 2.38 g Na_2HPO_4, 0.19 g KH_2PO_4, 8 g NaCl, 100 mg of mucin and α-amylase (200 U/L of enzyme activity) in 1 L of distilled water was added. This mixture was kept in a shaking water bath (37 °C, 90 rpm) for 10 min. Salivary digested aliquots were taken for analysis. Afterwards, 13.08 mg of pepsin from porcine stomach was added and pH was adjusted to 2 by addition of HCl (12 M). This mixture was incubated in darkness in a water bath at 37 °C with continuous stirring (90 rpm) for 2 h. At the end of the gastric digestion, aliquots were taken for analysis and 20 mL were used for titration with NaOH (0.5 M) to pH 7.5 after adding 5 mL of pancreatin (4 g/L) – bile (25 g/L) mixture.

Dialysis membrane was prepared by soaking it with 0.01 M EDTA Na_2, 2% $NaHCO_3$ and 0.1% sodium dodecyl sulphate at boiling point, rinsing it with distilled water and cutting it into segments of 30 cm. Dialysis membrane segments were filled with 25 mL of water–$NaHCO_3$ mixture, with the amount of $NaHCO_3$ (0.5 N) used in the previous titration. 20 mL of the gastric digest were placed into a beaker and the dialysis membrane was immersed in that digest until reaching pH 5.0. This process allows gradual pH adjustment, mimicking intestinal conditions. After 30 min, 5 mL of pancreatin (4 g/L) – bile (25 g/L) mixture was added and the incubation continued for further 2 h (37 °C, 90 rpm). The dialysate (fraction inside the dialysis sac), consisting of soluble compounds of low molecular weight, and the retentate (fraction outside the dialysis sac), consisting of soluble and insoluble compounds of low and high molecular weight, were collected and placed in a cold water bath for 10 min.

2.4. Bioactive compounds analysis

2.4.1. Polarographic determination of ascorbic acid

The method used was in accordance to Barba, Cortés, Esteve, and Frígola (2012). Beverage (5 mL) was diluted to 25 mL with the extraction solution (1% w/v oxalic acid, 2% w/v trichloroacetic acid and 1% w/v sodium sulphate). After vigorous shaking, the solution was filtered through a folded filter (Whatman No. 1). 1%