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Changes in polyphenol composition and bioactivity of the native Chilean white strawberry (*Fragaria chiloensis* spp. *chiloensis f. chiloensis*) after in vitro gastrointestinal digestion

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ABSTRACT

The Chilean white strawberry (*Fragaria chiloensis* spp. *chiloensis f. chiloensis*) is a semi-domesticated strawberry with high polyphenol content and antioxidant activity occurring in southern Chile. The aim of this work was to compare the composition and bioactivity of the polyphenol-enriched fruit extract (PEE) before and after simulated gastrointestinal digestion (GID). Results show a decrease by > 50% in the total phenolic (TP) content at the end of the GID, compared to the non-digested PEE. A reduction in the antioxidant capacity of the PEEs was observed after GID by means of DPPH, FRAP, TEAC and anion superoxide assays. After simulated GID the PEE significantly inhibited α-glucosidase with an IC_{50} value of 3.13 μg/mL. The inhibition of pancreatic lipase was reduced by 95% after GID. All the PEEs did not show inhibitory effect towards α-amylase throughout the GID. In the same way, the PEEs did not significantly protect human gastric adenocarcinoma (AGS) cells against H_2O_2-induced stress. Thirty eight compounds were tentatively identified in the non-digested PEE. The compounds that were more affected by the simulated GID were simple phenolics. After the GID, only 33 and 25 compounds were detected, in the gastric and intestinal steps, respectively. These results evidence the changes elicited by GID on the bioactivity and polyphenolic composition of the white strawberry.

1. Introduction

Polyphenolic compounds are known to be useful as nutraceuticals or supplements for the prevention of several diseases due to their antioxidant capacity which may help to slow down the oxidative stress generated during physiological processes (López-Alarcón & Denicola, 2013; Tomé-Carneiro & Visioli, 2016). Wild or cultivated berries are recognized as health-promoting fruits due to their high content of phytochemicals, including polyphenols, such as flavonols, anthocyanins, proanthocyanidins and phenolic acids (Li et al., 2016; Zhang & Tsao, 2016).

Polyphenols are able to inhibit metabolic syndrome-associated enzymes such as α-amylase, α-glucosidase and pancreatic lipase at concentrations that are achievable in the gut after intake of a small amount of fruits (Boath, Grussu, Stewart, & McDougall, 2012). Inhibitors of α-amylase and α-glucosidase retard carbohydrate digestion, thus resulting in a reduction of the glucose absorption rate (Bhandari, Nhilubon, Gao, & Kawabata, 2008), while the inhibition of pancreatic lipase is efficient in weight management and obesity control (Birari & Bhutani, 2007). Some polyphenol extracts from berries, including red strawberry, raspberry, blueberry, murta berry, maqui berry and wild currants have shown inhibitory activity against the above mentioned enzymes (McDougall et al., 2005; Rubilar et al., 2011; Burgos-Edwards, Jiménez-Aspee, Thomas-Valdés, Schmeda-Hirschmann, & Theoduloz, 2017).

Strawberries are one of the most consumed berries. Besides the popular red strawberry, the native Chilean white strawberry (*Fragaria chiloensis* spp. *chiloensis f. chiloensis* (L.) Mill) stands out for its white

Abbreviations: AGS, human gastric adenocarcinoma cells; CE, catechin equivalents; DPPH, 2,2-diphenyl-1-picrylhydrazyl radical; FRAP, ferric reducing-antioxidant power; GAE, gallic acid equivalents; GD, gastric digestion; GID, gastrointestinal digestion; HCA, hydroxycinnamic acids; ID, intestinal digestion; LF, lyophilized fruits; ND, non-digested; PEE, polyphenol-enriched fruit extract; TF, total flavonoid content; TP, total phenolic content

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color, pleasant taste and aroma (Retamales, Caligari, Carrasco, & Saud, 2005). Several studies have been carried out to characterize the metabolomic profile of this native species (Cheel, Theoduloz, Rodríguez, Caligari, & Schmeda-Hirschmann, 2007; Simirgiotis & Schmeda-Hirschmann, 2010). The main phenolic compounds of the white strawberry were identified as ellagitannins, ellagic acid, quercetin, kaempferol and isorhamnetin derivatives (Simirgiotis & Schmeda-Hirschmann, 2010). The antioxidant activity of the fruit extracts by means of the DPPH and superoxide anion assays has been previously reported (Cheel et al., 2007).

Single in vitro chemical methodologies are not enough and should be complemented with other strategies to study the bioactivity and functional properties of food plants (López-Alarcón & Denicola, 2013). In this sense, the study of antioxidant activity can be further studied using cell cultures (Cheli & Baldi, 2011). The human gastric adenocarcinoma cells (AGS) are considered to be a good model to evaluate antioxidant activity from berries (Jiménez-Aspee, Theoduloz, et al., 2016; Jiménez-Aspee, Thomas-Valdés, et al., 2016).

In vitro models of gastrointestinal digestion (GID) are powerful tools to simulate the physiological conditions occurring in human digestion. They are currently used as a first approach to evaluate the changes in stability and biological activity of food plants (Bohn et al., 2017; Lucas-Gonzalez et al., 2016; Burgos-Edwards et al., 2017). Along with the avoidance of ethical issues, these methods present advantages such as low cost, better control of variables and rapid obtainment of results (Minekus et al., 2014).

The aim of this study was to evaluate the changes in the phenolic composition and bioactivity of the Chilean white strawberry polyphenol-enriched fruit extract (PEE), before and after in vitro GID. To the best of our knowledge, this is the first report on the effect of simulated GID on this native species.

2. Experimental

2.1. Chemicals and reagents

ABTS (2,2′-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt), Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxilic acid), potassium persulfate, sodium carbonate, neocuproin, Folin-Ciocalteu reagent, acetic acid, $FeCl_3x6H_2O$, acetonitrile (ACN), methanol (MeOH), potassium sodium tartrate, formic acid and hydrogen peroxide were purchased from Merck (Darmstadt, Germany). Sodium chloride, potassium phosphate monobasic, sodium hydroxide were purchased from Scharlau (Barcelona, Spain). Pepsin A (P3271; EC 3.4.23.1) was acquired from USBiological (Salem, MA, USA). Acarbose, DPPH (2,2-diphenyl-1-picrylhydrazyl radical), 3,5-dinitrosalicylic acid, hypoxanthine, 4-nitrophenyl-α-D-glucopyranoside, nitroblue tetrazolium salt (NBT), 4-nitrophenyl palmitate, starch, Triton X-100, TPTZ (2,4,6-tri(2-pyridyl)1,3,5-triazine), potassium chloride, catechin, aluminum trichloride, amberlite XAD-7 HP resin, pancreatin from porcine pancreas (P3292), lipase from porcine pancreas (L3126; EC 3.1.1.3), bile extract porcine (B8631), α-amylase from porcine pancreas (A3176; EC 3.2.1.1), amyloglucosidase from *Aspergillus niger* (10115; EC 3.2.1.3), α-glucosidase from *Saccharomyces cerevisiae* (G5003; EC 3.2.1.20), and trizma maleate buffer, L-glutamine, 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) and sodium bicarbonate were obtained from Sigma-Aldrich (St. Louis, MO, USA). Orlistat was from Laboratorio Chile (Santiago, Chile). Ultrapure water was obtained from a Barnstead EasyPure water system (Thermo Scientific, Marietta, OH, USA). Culture media, antibiotics and fetal bovine serum (FBS) were from Invitrogen Corp. (Grand Island, NY, USA).

2.2. Fruit material and sample preparation

Ripe fruits of white strawberry were purchased from producers in Contulmo, Región del Bio-Bio, Chile, in December 2015. Fruits were washed, homogenized in a blender (Thomas TH-501 V; Thomas Elektrogeräte, Germany) and extracted three times with MeOH:formic acid (99:1 v/v) in a 1:3 v/v homogenate:solvent ratio in the dark under sonication (15 min each). The combined extracts were taken to dryness under reduced pressure at 40 °C in a rotary evaporator (Laborota 4001, Heidolph, Schwabach, Germany). Methanol extracts were adsorbed in Amberlite XAD-7 resin to obtain a phenolic-enriched extract (PEE). This methodology has shown to be efficient for the recovery of hydroxycinnamic acids (117%), anthocyanins (85%) and flavonoids (78.5%) (Jiménez-Aspee, Thomas-Valdés, et al., 2016). Briefly, the MeOH extract was dissolved in water, filtered and mixed with Amberlite XAD-7 in a 1:5 (extract:resin) ratio and kept under stirring for 40 min. The resin was filtered, washed with water and phenolics were desorbed with MeOH. The extract was evaporated under reduced pressure at 40 °C and freeze-dried to obtain the PEE. A small portion of the fresh fruits was freeze dried to obtain a lyophilized fruit powder for comparison purposes.

2.3. In vitro gastrointestinal digestion

The in vitro digestion was performed as described by Minekus et al. (2014). The digestion procedure was carried out using 250 mg of PEE, in triplicate, in a water bath at 37 °C, under constant shaking and in the dark. Gastric-simulated fluid was prepared with NaCl, pepsin (0.32% w/v), HCl and ultrapure water (pH 1.2). Samples re-suspended in gastric fluid were incubated during 30 min, and then, the pH was adjusted to 4.5 with NaOH. An amyloglucosidase solution (12% w/v, in buffer Trizma-maleate) was added and samples were further incubated for 30 min. Then, pH was adjusted to 6.9 with NaOH and an α-amylase solution (12% w/v, in buffer Trizma-maleate) was added. The samples were further incubated for 45 min. After the gastric step, an aliquot of the supernatant was removed and kept at − 80 °C for subsequent analyses.

The remains of the gastric digested sample were submitted to intestinal digestion (ID). Simulated intestinal fluid was prepared with K_2HPO_4, NaOH, pancreatin (1% w/v) and ultrapure water (pH 7.5). Samples re-suspended in intestinal fluid were incubated for 30 min. Then, a lipase solution (0.63% w/v, in PBS buffer) was added and samples were further incubated for 30 min. Polyphenols were recovered from the gastric and intestinal solutions by solid-phase extraction using HF Bond Elut C18 cartridges (Agilent Technologies, Santa Clara, CA, USA) (Burgos-Edwards et al., 2017). This procedure yielded a polyphenol-enriched fruit extract after gastric digestion (GD-PEE) and the polyphenol-enriched fruit extract after intestinal digestion (ID-PEE). Controls with lyophilized fruits (LF) and heat-inactivated enzymes were performed in parallel.

2.4. Total phenolic (TP) and total flavonoid (TF) content

The total phenolic (TP) and total flavonoid (TF) content were determined by the Folin-Ciocalteu and $AlCl_3$ methods, respectively (Jiménez-Aspee, Theoduloz, et al., 2016). For TP, the results are expressed as g of gallic acid equivalents per kg of PEE (g GAE/kg sample). For TF, results are expressed as g of catechin equivalents per kg of PEE (g CE/kg sample).

2.5. Antioxidant assays

The free radical scavenging activity of the samples was determined with the DPPH and superoxide anion radicals as previously described (Burgos-Edwards et al., 2017). Samples were assayed at concentrations ranging between 0 and 100 μg/mL and results are expressed as the amount of extract that is able to scavenge 50% of the radicals (SC_{50}, μg/mL). The Trolox-equivalent antioxidant capacity (TEAC) was determined using the ABTS radical (Jiménez-Aspee, Theoduloz, et al.,