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Study of antioxidant capacity and metabolization of quebracho and chestnut tannins through *in vitro* gastrointestinal digestion-fermentation

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ABSTRACT

Quebracho (QUE) and chestnut (CHE) are natural sources of tannins, but there are no connection between QUE, CHE and human health. The study investigated the antioxidant response and metabolization of tannin extracts trough *in vitro* digestion-fermentation. The FRAP assay pointed a higher reducing capacity of CHE than QUE (6.90 vs. 5.07 mmol Trolox/g), in contrast to a stronger scavenging activity of QUE (8.16 mmol Trolox/g vs 6.70 mmol Trolox/g). The results obtained showed a decrease of the antioxidant capacity of tannins after microbial fermentation, but a high prebiotic activity through release of short-chain fatty acids of both CHE (11.14 mmol/g) and QUE (4.79 mmol/g) was observed. The UPLC-MS investigations on digested and fermented tannins gave an identification and semi-quantification of 18 compounds, including hydrolysable and condensed tannins and their metabolites. The results represent a valid basis for further studies on the potential use of these wood extracts in human diet.

1. Introduction

Tannins are secondary metabolites widely distributed in the plant kingdom, extracted from many types of trees and plants and can be present in barks, leaves, wood and also in fruits and roots. These plants generally used for tannin production may contain up to 40% tannin by weight (van Diepeningen et al., 2004). Most raw materials used for industrial production are Chestnut wood (18% of tannin in wood on dry matter), Quebracho hardwood and Mimosa barks (24% in both), Tara pods and Chinese or Turkish gallnut (50% in both). In particular, *Schinopsis lorentzii* Engl. and *Schinopsis balansae* Engl., known as red quebracho, are evergreen tree species widespread in the dense subtropical forests of Gran Chaco (Argentina), Bolivia and Paraguay, while *Castanea sativa* Mill. trees, commonly named chestnut, is found across the Mediterranean region.

The quebracho wood extract (QUE) is among the most industrially produced source of tannins, predominantly composed of oligomers of profisetinidins. It is composed of oligomers in which flavonoid units are condensed together to obtain molecular weight (MW) ranging from 1.000 to 20.000 Da. The composition of QUE was described by Pasch, Pizzi, and Rode (2001) using MALDI-TOF mass spectrometry and Venter, Senekal, et al. (2012), Venter, Sisa, et al. (2012) reported a more detailed description of the linear structures of the polyanthocyanidins oligomers. CHE is characterized by the presence of hydrolysable tannins, composed of a carbohydrate core whose hydroxyl groups are esterified with phenolic acids with a MW ranging from 300 to 5.000 Da (Mueller-Harvey & McAllan, 1992). The chemical composition of CHE has been clarified by using MALDI-TOF mass spectrometry which states that it is an ellagic-type hydrolysable tannin (Pasch & Pizzi, 2002). Castalagin, with the isomer vescalagin, represents around 30% of the product. It has been shown that these substances and their higher oligomers are present in this tannin and are quite stable since they come from the rearrangement of polypentagalloyl glucose, naturally occurring in chestnut wood (Pasch & Pizzi, 2002). The higher oligomers contain repeating units of polygalloyl glucose chain, where galloyl groups can be linked differently to each other (Radebe, Rode,

Abbreviations: QUE, quebracho wood extracts; CHE, chestnut wood extract; SCFAs, short-chain fatty acids; GAR+, global antioxidant response+; MW, molecular weight; FRAP, ferric reducing ability of plasma; ABTS, 2,2-azinobis (3-ethylbenzothiazoline-6-sulfonic acid)

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Pizzi, Giovando, & Pasch, 2013).

QUE and CHE are already commercialized in animal feeding, especially for cattle and poultry (Buccioni et al., 2017; Carrasco et al., 2018). Several studies have reported that addition of QUE and CHE to animal feed improved the nutrition and the animal health in both ruminants and monogastric animals (Buccioni et al., 2017; Diaz Carrasco et al., 2016; Henke et al., 2017; López-Andrés et al., 2013; Redondo, Chacana, Dominguez, & Fernandez Miyakawa, 2014) Due to their chemical composition, these compounds exert antiviral, antimicrobial, scavenging and antimutagenic effects locally in the intestine as unabsorbable complex structures (Serrano, Puupponen-Pimiä, Dauer, Aura, & Saura-Calixto, 2009). However, since a little amount of tannins or their metabolites might be absorbed from the gastrointestinal tract, systemic effects associated to improvement of endogenous antioxidant activity in different organs are observed (Cires, Wong, Carrasco-Pozo, & Gotteland, 2017; Serrano et al., 2009). Tannins may interfere with the digestion of nutrients, binding proteins or delaying the absorption of sugar and lipids (Cires et al., 2017). In particular, complexes with proteins are given by the tannins numerous hydroxyl groups and depend on proline content and size of the proteins (Hagerman & Butler, 1981). Given the antinutrient effect, these compounds could be studied as functional substances. There are studies that reported the possible use of tannins in nutrition for celiac disease, by their potential cross-linking activity of wheat gluten (Girard, Bean, Tilley, Adrianos, & Awika, 2018), or in diabetes mellitus thanks to their anthyperglycemic activity (Cires et al., 2017; Williamson, 2013; Yin et al., 2011).

Even though tannins have shown important biological properties, little is known about the bioavailabilty and biological effects of QUE and CHE extracts in humans. In particular, in order to understand how these extracts determine health benefits, it is crucial to study the metabolization of tannins during digestion and fermentation processes. Several authors have tried to understand the metabolisation of tannins contained in fruits (i.e. apples or grapes) or directly of molecules like proanthocyanidins (Appeldoorn, Vincken, Aura, Hollman, & Gruppen, 2009; Aura et al., 2013; Bazzocco, Mattila, Guyot, Renard, & Aura, 2008; Stoupi, Williamson, Drynan, Barron, & Clifford, 2010), evidencing the production of phenolic acids such as 2-(3,4-dihydroxyphenyl) acetic acid, 2-(3-hydroxyphenyl) acetic acid, 2-(4-hydroxyphenyl) acetic acid and 3-(3-hydroxyphenyl) propionic acid.

Taking all this information into account, the overall aim of the present report was to unravel the possible use of QUE and CHE as potential ingredients for the development of functional foods. In order to reach this goal, three different sub-objectives were defined, after *in vitro* digestion and fermentation of the samples: (i) To study the global antioxidant response of QUE and CHE. (ii) To determine their bioactivity on the gut microbiota, reflected by the production of short chain fatty acids (SCFAs). (iii) To investigate the evolution of the polyphenolic profile of tannins extracts.

2. Material and methods

2.1. Plant material, chemicals and reagents

Tannin extracts (QUE and CHE), obtained by hot water extraction, are commercialized by Silvateam Spa (San Michele di Mondoví, Italia) as powder. QUE, a profisetinidin condensed tannin of 6,25 of degree of polymerization, was previously characterized by Pasch et al. (2001). The composition of CHE, a hydrolysable ellagitannin with 30% of isomers castalagin and vescalagin as representative substances, was described by Pasch & Pizzi (2002). Reagents and inulin were from Sigma-Aldrich (Germany) and Alpha-Aesar (United Kingdom). All chemical reagents used for all the assays, digestion, fermentation, as well as HPLC and UPLC-ESI-MS analysis were of analytical grade.

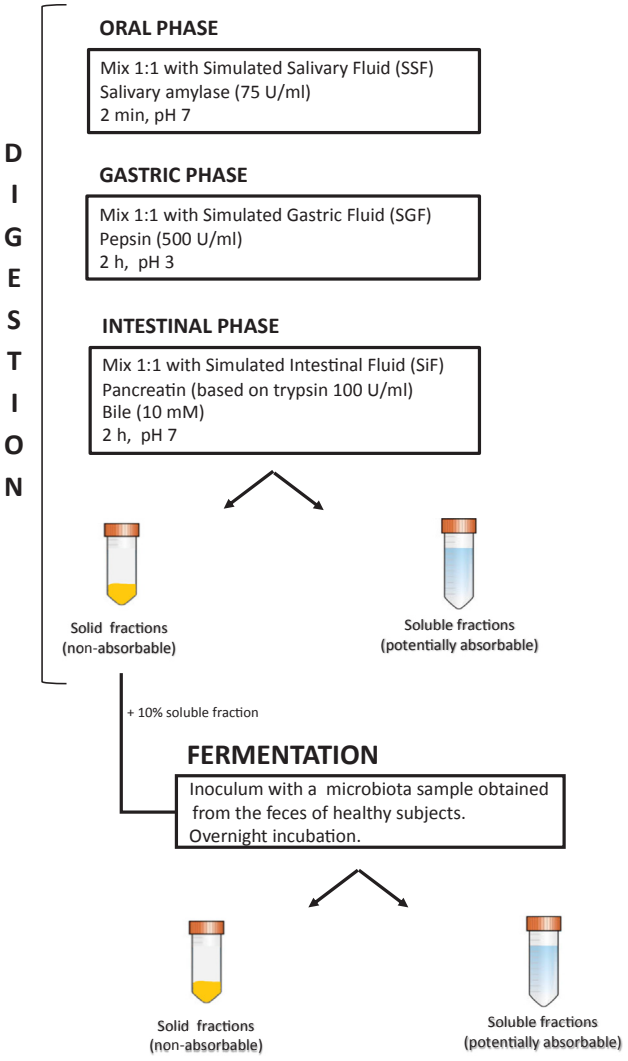

Fig. 1. Schematic description of the *in vitro* digestion and fermentation processes.

2.2. In vitro digestion-fermentation

The *in vitro* digestion-fermentation (Fig. 1) was performed as described by the GAR + method (Pérez-burillo, Rufián-henares, & Pastoriza, 2018a). The samples were subjected to oral, gastric and intestinal digestion and then to a further step of fermentation with the microbiota present in faeces, obtained from healthy donors (Mean Body Index = 21.2). In the oral phase, 5 mL of simulated salivary fluid with α-amylase and 25 μL of $CaCl_2$ were added to 5 g of tannin extract, following incubation at 37 °C for 2 min. Then, 10 mL of simulated gastric fluid with pepsin and 5 μL of $CaCl_2$ were added and the pH was lowered to 3.0 by adding 1 N HCl; the mixture was then incubated at 37 °C for 2 h. Finally, 20 mL of simulated intestinal fluid with bile salts, pancreatin and 40 μL of $CaCl_2$ were added and the pH was raised to 7.0 with 1 N NaOH. The mixture was incubated at 37 °C for 2 h. After that, the tubes were immersed in iced water to stop the enzymatic reactions. A centrifugation of the mixture at 6000 rpm for 10 min at 4 °C enabled the separation of the solid fractions from the supernatants (potentially absorbable solution). Then, 10% of the supernatant was added to the solid residue in order to mimic the fraction that is not readily absorbed after digestion, while the remaining part was stored at −80 °C until further analysis.

The digested wet-solid residues (500 mg) derived from the digestion process were subjected to fermentation (Pérez-burillo, Rufián-Henares,