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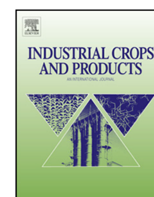

Antioxidant potential of hydrolyzed polyphenolic extracts from tara (*Caesalpinia spinosa*) pods

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ABSTRACT

The antioxidant potential of tara pod extracts rich in gallotannins submitted to chemical hydrolysis was evaluated. The increase in the release of gallic acid from the tara pod extracts during the hydrolysis process reached a maximum ratio of free gallic acid/total phenolics of 94.1% at 20 h, at this point, 100% hydrolysis degree (HD) was obtained. After 4 h of hydrolysis (38.8% of HD) the highest antioxidant capacity was obtained reaching values of 25.9, 23.8 and 8.8 μmol trolox equivalent/mg gallic acid equivalent measured by ABTS, FRAP and ORAC methods. Lipophilicity diminished from 0.8 to 0.3 (log *P* value). In addition, the antioxidant efficacy of 100 ppm total phenolics of hydrolyzates at 9 h (93.7% of HD) and 20 h showed to be significantly more efficient than a similar concentration of the synthetic antioxidant TBHQ to retard soybean oil oxidation. These results indicate that 4 and 9 h of chemical hydrolysis of tara pod extracts under the tested conditions are sufficient to obtain a product with good antioxidant properties to be used as an alternative source of antioxidants.

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1. Introduction

Tannins are phenolic compounds of relative high molecular weight. They are classified as condensed and hydrolyzable tannins. The hydrolyzable tannins are readily hydrolyzed by acids, alkalis or enzymes (tannases) into a sugar or a related polyhydric alcohol (polyol) and a phenolic carboxylic acid (Hagerman, 1998). Depending on the nature of the phenolic carboxylic acid, hydrolyzable tannins are subdivided into gallotannins and ellagitannins. Hydrolysis of gallotannins yields gallic acid while hydrolysis of ellagitannins yields hexahydroxydiphenic acid which is isolated as ellagic acid (Hagerman, 1998).

Hydrolyzable tannins are considered as one of the most potent antioxidants from plant sources. They are ready to form complexes with reactive metals, avoiding free radical generation which results in oxidative damage of cellular membranes and DNA (Khan et al., 2000). Hydrolyzable tannins, in addition, clean free radicals within the body by neutralizing them before cellular damage occurs (Hagerman, 1998). Thus, the *in vitro* antimutagenic and anticarcinogenic activity of tannic acid has been previously reported (Gülçin et al., 2010).

Tara (*Caesalpinia spinosa* (Molina) Kuntze) is a native leguminous tree from South America consisting of red or pale yellow pods of 8–10 cm length. It is spread from the region of Venezuela, Colombia, Ecuador, Peru, Bolivia, until the north of Chile. Tara wildly grows in the Peruvian coast and Andean region at altitudes from 1000 to 2900 m above see level (De la Cruz, 2004). Peru is considered the most important worldwide producer of tara with more than 80% of the world production (Mancero, 2008). Tara infusions have been traditionally and extensively used by the Peruvian folk medicine to treat inflamed tonsils, fever, cold and stomachaches (Bussmann and Sharon, 2006). Tara pods (without seeds) represent approximately 65% (w/w) of the fruit. Ground tara pods concentrate a high tannin content (∼40–60% (w/w)). Tara pods are a good source to produce tannic, gallotannic and gallic acid. Tara tannins are used in the manufacture of leather furniture, plastics and adhesives, as wine clarifier, as malt substitute, as source to obtain the antioxidant gallic acid used in the oil industry (De la Cruz, 2004). Tara tannins are also employed as component of gastroenterological medicaments to cure ulcers and help cicatrization. Astringent, anti-inflammatory, antifungal, antibacterial, antiseptic, antidiarrheal properties have been attributed to tara tannins (Bussmann and Sharon, 2006; De la Cruz et al., 2007).

Tara pods contain gallotannins. Gallotannins are mainly composed of polygalloyl esters of quinic acid. Complete hydrolysis which involves rupture of depside and ester bonds yields quinic and

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gallic acids. Tannins present in other members of the hydrolyzable tannin group contain a galloylated or ellagoylated hexose (Garro et al., 1997). By means of a partial or complete hydrolysis, it is feasible to obtain gallic acid or remaining tannins from tara tannins. Both gallic acid and remaining tannins display higher antioxidant capacity than tannins (Salminen et al., 2002; Wang et al., 2007) due to the exposition of hydroxyl groups of gallic acid released after hydrolysis.

The use of strong acid conditions (2 N sulfuric acid at 100 °C) and prolonged hydrolysis time (26 h) has been previously reported (Inoue and Hagerman, 1988) to completely hydrolyze the gallotannin molecule. The present study, however, aims to demonstrate the feasibility to obtain tara hydrolysis products with high antioxidant capacity without incurring in a complete hydrolysis. Thus, the transformation of tara tannins into phenolic compounds of low molecular weight is an alternative process to obtain high added value extracts. The demand of this alternative product is in rise due to its increasing application as feeding supplement, and the possibility to be employed by the pharmaceutical and food industry. Thus, this work aims: (1) to study the chemical hydrolysis of gallotannin extracts from tara, (2) to evaluate the hydrolysis degree (HD) of tara extracts regarding *in vitro* antioxidant capacity and (3) to evaluate the performance of these extracts by differential scanning calorimetry to retard soybean oil oxidation.

2. Materials and methods

2.1. Material and chemicals

Fresh tara pods were purchased from a local market in Caraz (Ancash, Peru). Tara pods (without seeds) were washed and air-dried at 55 °C until a final humidity of ∼3% was reached. Tara pods were subsequently milled and sieved (#80 mesh) and stored at −20 °C for further analysis.

Gallic acid, pyrogallol, trolox (6-hydroxy-2,5,7,8-tetramethyl chroman-2-carboxylic acid), ABTS (2,2′-azinobis (3-ethylbenzothiazoline-6-sulfonic acid)), TPTZ (2,4,6-tripyridyl-*s*-triazine), AAPH (2,2′-azinobis(2-amidinopropane) dihydrochloride), fluorescein sodium salt, 2 N Folin-Ciocalteu reagent, rhodanine (2-thio-4-ketothiazolidine) and TBHQ (tert-butylhydroquinone) were purchased from Sigma Chemicals Co. (St. Louis, MO, USA). All solvents and other chemicals of analytical grade were purchased from Merck (Darmstadt, Germany) and Fischer Scientific (Fair Lawn, NJ, USA).

2.2. Extract preparation

2.2.1. Extraction

Phenolic compounds were extracted employing 80% (v/v) acetone/water as solvent (Tian et al., 2009) and a material/solvent ratio of 1/100 (w/v). Extraction was carried out at 4 °C for 20 h. Then, the mixture was centrifuged at 10,000 × g for 15 min and the supernatant was vacuum concentrated at 38 °C until dryness. The resulting product was dissolved with Milli-Q water. This aqueous solution was cooled at ∼4 °C for 16 h and then again centrifuged at 10,000 × g for 10 min to obtain a clarified solution. This remaining product is referred as the whole extract.

2.2.2. Hydrolysis of tara extracts

The whole extract was mixed with sulfuric acid until reaching a final concentration of 2 N H_2SO_4 and a phenolic compound concentration of 20 mg gallic acid equivalent/mL. The mixture was let to stand at 100 °C for 0, 0.5, 1, 1.5, 2, 4, 5, 6, 8, 9, 12, 16, 20, 24 and 28 h. The resulting mixture referred as the hydrolyzed extracts were centrifuged at 10,000 × g for 10 min. Then, total phenolic and gallic acid contents were determined by colorimetric methods (Section 2.3.1) in all extracts. The HD was calculated as follows:

$$HD\,(\%) = \frac{GA_{HE} - GA_{I}}{GA_{HC} - GA_{I}} \times 100$$

where GA_{HE}, gallic acid concentration in the hydrolyzed tara extract at certain time (mg GA/mL); GA_I, gallic acid concentration in the initial tara extract (mg GA/mL); GA_{HC}, gallic acid concentration in the totally hydrolyzed tara extract (mg GA/mL).

2.2.3. Clean up of tara hydrolyzed extracts

The tara hydrolyzed extracts were submitted to a liquid–liquid partition using ethyl acetate (González et al., 2004) to eliminate the sulfuric acid present in those extracts. The hydrolyzed extracts were mixed with equal volumes of ethyl acetate. The partition included 10 min agitation in darkness. Then, the mixture was let to stand until two phases were observed and the organic phase was collected. A second partition was performed under the same conditions. The organic phases were assembled; vacuum concentrated at 37 °C until dryness and the remaining pellet was dissolved in absolute ethanol. This extract is referred as tara purified and hydrolyzed extract (TPHEs). The remaining aqueous phase enriched in sulfuric acid was discarded. TPHEs were flushed with nitrogen and stored at −20 °C until analyses. Gallic acid, total phenolics, *in vitro* antioxidant capacity and HPLC-PDA phenolic profiles were determined in the TPHEs.

2.3. Quantitative analysis

2.3.1. Total phenolics, gallic acid, gallotannin content and ratio gallic acid/total phenolics

Total phenolics were determined following the method of Singleton and Rossi (1965) using gallic acid as a standard. Absorbance was measured at 755 nm and the results were expressed as mg of gallic acid equivalents (GAE)/g or mL.

Gallic acid and gallotannin contents were determined using the rhodanine assay reported by Inoue and Hagerman (1988) and Salminen (2003). Briefly, 1.5 mL of 0.667% (w/v) methanolic rhodanine solution was mixed with 1 mL of 0.2 N H_2SO_4 tara extract. After 5 min incubation, 1 mL of 0.5 N KOH was added. After 2.5 min the mixture was diluted to 25 mL with distilled water and the absorbance at 520 nm was measured after 5–10 min. Gallic acid was calculated as mg of GAE/g or GAE/mL from a standard curve developed with gallic acid. Gallotannins were estimated as the difference between the absorbance of the totally hydrolyzed tara extract (20 h hydrolysis) and the initial absorbance of free gallic acid in the initial tara extract. Gallotannin content was calculated as mg of GAE/g or GAE/mL.

The ratio gallic acid/total phenolics was calculated by dividing them and the results were expressed in percentage (%).

2.3.2. In vitro antioxidant capacity and specific antioxidant capacity

The antioxidant capacity was determined with the ABTS, FRAP and ORAC assays. For ABTS assay, the procedure followed was the same procedure described by Campos et al. (2006). Samples (150 μl) were allowed to react with 2850 μl of $ABTS^+$ solution in ethanol until a steady absorbance was reached at room temperature and dark conditions. The decrease in absorbance due to antioxidants was recorded at 734 nm.

The FRAP assay was conducted according to Benzie and Strain (1996) with minor modifications. The FRAP reagent consists of acetate buffer (300 mM, pH 3.6), TPTZ (10 mM in HCl 40 mM) and $FeCl_3{\cdot}6H_2O$ (20 mM) (10:1:1, v/v/v). A total of 2850 μl of FRAP reagent was mixed with 150 μl of sample at 37 °C. Then, the

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