Food Research International 78 (2015) 378–387

Contents lists available at ScienceDirect

Food Research International

journal homepage: www.elsevier.com/locate/foodres

Impact of in vitro simulated digestion on the potential health benefits of proanthocyanidins from *Choerospondias axillaris* peels

Qian Li [a], Jun Chen [a], Ti Li [a], Chengmei Liu [a,*,1], Xieyi Wang [a], Taotao Dai [a], David Julian McClements [b,c,**,1], Jiyan Liu [d]

[a] State Key Laboratory of Food Science and Technology, Nanchang University, Nanchang 330047, Jiangxi, PR China
[b] Department of Food Science, University of Massachusetts, Amherst, MA 01003, USA
[c] Department of Biochemistry, King Abdulaziz University, Jeddah 21589, Saudi Arabia
[d] Jiangxi Qiyunshan Food Co., Ltd, Ganzhou 341000, Jiangxi, PR China

ARTICLE INFO

Article history:
Received 15 July 2015
Received in revised form 31 August 2015
Accepted 5 September 2015
Available online 10 September 2015

Keywords:
Choerospondias axillaris
Proanthocyanidins
Gastrointestinal tract
Bioavailability
Bioaccessibility
Bioactivity
In vitro digestion

ABSTRACT

The influence of passage through a simulated gastrointestinal tract (GIT) on the stability and bioaccessibility of proanthocyanidins isolated from fruit (*Choerospondias axillaris*) peel was studied. In addition, the effects of the simulated GIT on the antioxidant and α-glucosidase inhibitory activities of proanthocyanidins extracts were evaluated. Gastric digestion had little effect on total polyphenol content (TPC) or mean degree of polymerization (mDP) of crude and purified extracts. However, intestinal digestion led to a significant decrease (about 26% and 19%) in TPC and (about 12% and 7%) in mDP for crude and purified extracts, respectively. The observed reduction in TPC and mDP levels was attributed to interactions of proanthocyanidins with pancreatic enzymes, rather than due to the chemical conditions during digestion. Only small flavan-3-ol molecules (monomers, dimers and trimers) could diffuse into the dialysis tubing used to simulate the intestinal wall. Changes in antioxidant activity during digestion were correlated to changes in TPC. After simulated GIT digestion, over 85% of α-glucosidase inhibitory activity of both extracts was preserved. These results indicate that the majority of the proanthocyanidins maintained their biological activities after passage through the simulated GIT, and were therefore still capable of providing valuable health benefits.

1. Introduction

Proanthocyanidins (PAs), also known as condensed tannins, are oligomers or polymers of flavan-3-ols linked through interflavan bonds. PAs are commonly found in fruits, nuts, cereals, seeds, wine, and chocolate (Mkandawire et al., 2013; Ou & Gu, 2014). Consequently, they are an integral part of the human diet and typically represent over 50% of dietary polyphenols consumed (Gonthier et al., 2003). PAs possess antioxidant properties, and have other physiological actions that may reduce risk factors associated with certain types of disease. For example, PAs have been shown to be more effective than resveratrol or ascorbic acid in scavenging free radicals (Maldonado, Rivero-Cruz, Mata, & Pedraza-Chaverrí, 2005). Procyanidins from cocoa were found to inhibit the growth of human breast cancer and colonic cancer cells (Carnesecchi et al., 2002; Ramljak et al., 2005). Moreover, PAs from plant extracts or fruit juices have been demonstrated to have potential anti-diabetic or anti-hyperglycemic benefits in vitro (Wang, Liu, Song, & Huang, 2012) and in vivo (Quiñones et al., 2013; Roopchand et al., 2012). Consequently, PAs have considerable potential to be utilized as food-grade bioactive components ("nutraceuticals") in functional food products.

Choerospondias axillaris (*C. axillaris*) is a traditional medicinal plant that has been used for the treatment of cardiovascular diseases in China for many years (Wang, Gao, Zhou, Cai, & Yao, 2008). The fruit of this plant is also used in the food industry to produce fruit pastilles and juice (Shuai et al., 2013). During the manufacturing process, a large quantity of peels is produced, which are usually discarded as waste products due to the astringent taste caused by the high levels of PAs they contain. The major PAs in these waste products are epigallocatechin, catechin, epicatechin and their galloylated derivatives (Li, Chen, Li, Liu, Liu, et al., 2015; Li, Chen, Li, Liu, Zhai, et al., 2015), which have considerable potential for utilization as bioactive ingredients in

Abbreviations: PAs, Proanthocyanidins; *C. axillaris*, *Choerospondias axillaris*; TPC, Total polyphenol content; mDP, Mean degree of polymerization; LC–MS^n, Liquid chromatography–mass-spectrometry; TFA, Trifluoroacetic acid; EC, Epicatechin; ABTS, 2, 2′-azino-bis (3-ethylbenzthiazoline-6-sulphonic acid); C, Catechin; DPPH, 2,2-Di(4-tert-octylphenyl)-1-picrylhydrazyl; ECG, Epicatechin-gallate; *p*NPG, *p*-Nitrophenyl-α-D-glucopyranoside.

* Corresponding author.
** Correspondence to: D.J. McClements, Department of Food Science, University of Massachusetts, Amherst, MA 01003, USA.
E-mail addresses: liuchengmei@aliyun.com (C. Liu), mcclements@foodsci.umass.edu (D.J. McClements).

[1] These authors contributed equally to this manuscript.

http://dx.doi.org/10.1016/j.foodres.2015.09.004
0963-9969/

foods, supplements, and pharmaceutical products. Consequently, there is a need to convert these waste products into valuable functional ingredients.

In our previous work, we observed that the polyphenol content of fruit peels was nearly four times greater than that of the flesh, and may therefore serve as a high added-value source of polyphenols (Li, Chen, Li, Liu, Liu, et al., 2015; Li, Chen, Li, Liu, Zhai, et al., 2015). In addition, in vitro and in vivo studies have shown that the polyphenols extracted from *C. axillaris* fruits possess potent antioxidant activities (Wang et al., 2008). However, whether or not these in vitro biological activities are maintained after the polyphenols pass through the harsh environment of the human gastrointestinal tract (GIT) is currently unknown. To achieve any beneficial health effects from these bioactive compounds they must be released in the GIT fluids after ingestion, remain in a bioactive form, be absorbed from the gut into the systemic circulation, and then be delivered to the appropriate location within the body (Liang et al., 2012; McClements & Xiao, 2014; Tenore, Campiglia, Ritieni, & Novellino, 2013; Ting, Jiang, Ho, & Huang, 2014).

It was recently suggested that PAs might be degraded during their transit in the stomach, and degradation into monomer and dimer forms was observed in vitro during cocoa procyanidin incubation with a simulated gastric juice (pH 2–4) (Spencer et al., 2000). However, a study in humans demonstrated that depolymerization did not occur and procyanidins were stable during gastric transit (Rios et al., 2002). Moreover, the bioavailability also differs greatly from one polyphenol to another, and for some compounds it depends on dietary source. The digestive stability and bioaccessibility of PAs from *C. axillaris* peels during GIT digestion are currently unknown.

Polyphenols that are present in plasma at low concentrations may be present in the GIT at much greater concentrations after direct consumption of vegetables, fruit, and their derivatives. Thus, food polyphenols can play an important role in protecting the gastrointestinal tract itself from oxidative damage and inhibiting the activity of α-glucosidase, which is located in the brush-border surface membrane of intestinal cells and could control blood glucose levels in type 2 diabetics after starch-containing meals (Barrett et al., 2013; Lordan, Smyth, Soler-Vila, Stanton, & Ross, 2013). However, so far, there is no investigation about the antioxidant and α-glucosidase inhibitory of PAs from *C. axillaris* peels during gastrointestinal digestion.

Thus, the objectives of the present research were to study the stability, bioaccessibility, and changes in the antioxidant and α-glucosidase inhibitory activity of PAs from *C. axillaris* peels under simulated GIT conditions. Simultaneously, the profiles of the bioaccessible PAs were investigated using liquid chromatography-mass-spectrometry ($LC–MS^n$).

2. Materials and methods

2.1. Materials and reagents

The fruits of *C. axillaris* were collected from Ganzhou, Jiangxi Province (China) in October 26, 2013, and authenticated by Prof. Guowen Zhang (College of Biological Science, Nanchang University, Nanchang, PR China). The acetone, methanol used in extraction and purification were obtained from HuShi (Shanghai, China). The HPLC-grade acetic acid, acetonitrile and trifluoroacetic acid were obtained from Aladdin (Shanghai, China). Standard phloroglucinol, (+)-catechin, (−)-epicatechin, (+)-epicatechin gallate, gallic acid, L-ascorbic acid, α-glucosidase (EC 3.2.1.20), *p*-nitro-phenyl-α-D-glucopyranoside, DPPH, ABTS, pepsin from porcine gastric mucosa, pancreatin from porcine pancreas and bile salts were obtained from Sigma-Aldrich (St. Louis, MO, USA). Sephadex LH-20 was purchased from GE Healthcare Bio-Sciences AB (Sweden). Sep.-Pak cartridges C-18 for sample cleaning were supplied by Waters (1-g capacity, Taunton, USA). Water was purified in the laboratory using a Millipore system (Millipore, Milli-Q plus, USA). All other reagents used were of analytical grade and purchased from Shanghai Chemicals and Reagents Co. (Shanghai, China).

2.2. PAs extraction and purification from C. axillaris peels

The peels of *C. axillaris* fruits were manually separated, oven dried under vacuum at 40 °C for 24 h, then ground finely to give 80-mesh size powder, and stored in darkness at −20 °C until used. A weighed amount of finely ground powdered peel (7 g each) was extracted with 250 mL of 70% aqueous acetone in Erlenmeyer flasks for 20 min that were continuously agitated using an ultrasonic cleaning bath operated at ambient temperature. Then, the extracts were filtered to separate the soluble compounds from the waste solid. The above process was repeated twice to improve the extraction efficiency. The solutions were then combined and rotary-evaporated under vacuum at 40 °C to remove the acetone. The resulting concentrate was then diluted to 300 mL with distilled water and frozen at 4 °C prior to further characterization. This extract was referred to as the "crude extract".

A crude extract solution was pre-purified using a HP2-MGL resin (Mitsubishi Chemical Corp., Japan), after rinsing the column with distilled water, the fraction containing PAs was eluted with 70% acetone. The eluent was concentrated by rotary evaporation to remove acetone and then freeze dried. The obtained powder was dissolved in 50% methanol and then further purified using a Sephadex LH-20 column, which was equilibrated with methanol/water (50:50, *v*/*v*) for 4 h. Then the peel extract was loaded onto the column, and phenolic acids, glycosides, and a part of individual flavan-3-ol monomers were removed with methanol/water (50:50, *v*/*v*; 400 mL). This fraction was discarded and not analyzed. Then, an acetone:water mixture (70:30, *v*/*v*; 250 mL) was used to elute the PAs fraction. The acetone present in the PAs fraction was evaporated at reduced pressure and temperature (<40 °C), then the aqueous extract was diluted to 300 mL with distilled water and stored at 4 °C prior to further analysis. This extract was referred to as the "purified extract". All samples were prepared and processed in triplicate.

2.3. Simulated in vitro gastric and intestinal digestion

The in vitro gastrointestinal digestion procedure was performed with both crude and purified extracts. The procedure used was adapted from a published method (Fernández & Labra, 2013; Gil-Izquierdo, Zafrilla, & Tomás-Barberán, 2002). The method consists of two sequential steps: an initial pepsin/HCl digestion for 2 h at 37 °C to simulate gastric conditions, followed by digestion with bile salts and pancreatin for 2 h at 37 °C to simulate small intestine conditions. Briefly, aqueous solutions (300 mL) containing the peel extracts (crude and purified) were adjusted to pH 2.0 with concentrated HCl, and pepsin was added at 315 U/mL. The samples were then incubated at 37 °C in a water bath for 2 h with shaking at 120 rpm/min. Every 30 min, aliquots (5 mL) of the post-gastric digestion were removed and for further determination of PAs, α-glucosidase inhibition, and antioxidant activity in crude and purified extracts. The remainder of the sample was placed in a 500 mL Erlenmeyer flask, and adjusted to pH 5.5 with 1 M $NaHCO_3$. Then 5 mL of 4 mg/mL pancreatin and 25 mg/mL bile salts were added. The sample was incubated for 2 h at 37 °C. A segment of cellulose dialysis tubing (molecular weight cut off, 12 kDa) containing $NaHCO_3$ equivalent to the titratable acidity measured previously (see below) were placed in the Erlenmeyer flask and the flask was sealed with parafilm. Every 30 min, aliquots (5 mL) of the solution outside the dialysis tubing (referred to as "OUT samples") were taken to represent the material that remained within the gastrointestinal tract (colon-accessible fraction). Aliquots of the solution that had diffused into the dialysis tubing were also taken (referred to as "IN samples") to represent the fraction that was accessible for absorption.

Titratable acidity was determined on a 275 mL aliquot of the pepsin digest adjusted to pH 5.5 and to which 5 mL of the pancreatin-bile