Journal of Functional Foods 20 (2016) 317–331

Available online at www.sciencedirect.com

ScienceDirect

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

Nutraceutical potential and antioxidant benefits of selected fruit seeds subjected to an *in vitro* digestion

Guan-Lin Chen [a,1], *Song-Gen Chen* [a,1], *Fu Chen* [a], *Ying-Qing Xie* [a], *Men-Di Han* [a], *Chun-Xia Luo* [a], *Ying-Ying Zhao* [a], *Yong-Qing Gao* [b,*]

[a] *Foshan Institute of Occupational Disease Prevention and Control, Foshan 528000, China*
[b] *School of Food Science, Guangdong Pharmaceutical University, Zhongshan 528458, China*

ARTICLE INFO

Article history:
Received 20 July 2015
Received in revised form 26 October 2015
Accepted 3 November 2015
Available online

Keywords:
Antioxidant capacity
In vitro digestion
Phenolic content
Fruit seeds

ABSTRACT

In order to find new sources of natural antioxidants, total phenolic contents and antioxidant capacities of 11 fruit seeds before and after *in vitro* digestion were evaluated by Folin–Ciocalteu method, 2,2-diphenyl-1-picrylhydrazyl radical scavenging activity assay (DPPH), ferric reducing antioxidant power assay (FRAP), and $ABTS^{\bullet+}$ scavenging activity (ABTS) assay, respectively. The correlations between DPPH, FRAP, ABTS values and total phenolic contents were also evaluated. The results showed that 11 fruit seeds had diverse antioxidant capacities and the variation was very large. Furthermore, the main compounds of 11 fruit seeds were identified and quantified. Four fruit seeds, *Dimocarpus longgana* Lour, *Vitis vinifera* Linn (red), *Vitis vinifera* Linn (black), and *Litchi chinensis* Sonn showed the strongest antioxidant activities whether before or after digestion, which implied that these fruit seeds are important natural sources for preventing oxidative stress diseases.

1. Introduction

Free radicals are generated in our body during the normal metabolic processes and during exposure to adverse pathophysiological conditions (Van Langendonckt, Casanas-Roux, & Donnez, 2002). Free radicals are unstable species; they can induce cellular damage in several ways. The most deleterious effects of free radicals are damage to DNA, which is associated with the process of carcinogenesis (Nagala, Yekula, & Tamanam, 2013). Antioxidants have great importance in terms of reducing oxidative stress, which could cause damage to biological molecules (Tepe, Sokmen, Akpulat, & Sokmen, 2005). Although synthetic antioxidants such as tertiary butylated hydroxytoluene, butylated hydroxyanisole, gallic acid esters and tertiary-butylhydroquinone have the potential to neutralise free

Abbreviations: FRAP, ferric reducing antioxidant power; DPPH, 2, 2-diphenyl-1-picrylhydrazyl; ABTS, 2, 2-azinobs-(3-ethylbenzothiazoline -6-sulfonic acid); UPLC, Ultra performance liquid chromatography

* Corresponding author. School of Food Science, Guangdong Pharmaceutical University, Zhongshan 528458, China. Tel.: +86 60 88207955; fax: +86 60 88207956.
E-mail address: yongqingg@163.com (Y.-Q. Gao).

[1] These authors contributed equally to this work.

Chemical compounds: Gallic acid (PubChem CID: 370); (+)-Catechin (PubChem CID: 107957); Epicatechin (PubChem CID: 182232); Protocatechuic acid (PubChem CID: 72); Caffeic acid (PubChem CID: 689043); Vanillic acid (PubChem CID: 8468); Ferulic acid (PubChem CID: 445858); *p*-Coumaric acid (PubChem CID: 637542); Quercetin (PubChem CID: 5280343); Isoquercitrin (PubChem CID: 5280804); Rutin (PubChem CID: 5280805); Quercitrin (PubChem CID: 5280459); Syringic acid (PubChem CID: 10742).

http://dx.doi.org/10.1016/j.jff.2015.11.003
1756-4646/

radicals, they have been criticised due to their possible toxic effects and low solubility. Hence there is a need to discover new potential natural sources of antioxidants (Parejo et al., 2002; Piconi, Quagliaro, & Ceriello, 2003). Previous studies have indicated that fruit, vegetable, cereal, barley, millet grains, wheat, Brazil nut, macro-fungi and microalgae are rich in natural antioxidants (Chandrasekara & Shahidi, 2010, 2011; Deng et al., 2012; Guo et al., 2012; John & Shahidi, 2010; La Vecchia, Altieri, & Tavani, 2001; Lako et al., 2007; Li et al., 2007; Liyana-Pathirana & Shahidi, 2006a, 2006b; Liyanapathirana & Shahidi, 2004). The best health and nutrition results can be achieved not only from the consumption of vegetables, fruits and macro-fungi, but also from plant and fruit seeds. Fruit seeds have not generally received much attention as antioxidant sources, and this could be due to their lack of popularity and lack of commercial applications (Soong & Barlow, 2004); thus, millions of tons of fruit seeds were wasted every year. Previous studies have indicated that fruit seeds are other important source of antioxidants (Deng et al., 2012; Soong & Barlow, 2004); if they could be used as source of natural antioxidants, it would be beneficial in improving the complete utilisation of the seeds. Several studies have shown that some fruit seeds possess more potent antioxidant activity than common fruits and vegetables (Soong & Barlow, 2004). Antioxidant compounds of the citrus (Bocco, Cuvelier, Richard, & Berset, 1998), grape (Jayaprakasha, Singh, & Sakariah, 2001), mango (Puravankara, Boghra, & Sharma, 2000), and lupin (Tsaliki, Lagouri, & Doxastakis, 1999) in the seeds have been identified, and the antioxidants of some tropical and subtropical fruit seeds were also determined. Not too many studies have investigated the antioxidant activity of fruit seeds, especially those used in an *in vitro* digestion model. The objectives of the present study were to determine the phenolic contents and total antioxidant capacities of 11 selected fruit seeds by an *in vitro* digestion model, to investigate the relationships between antioxidant activity and phenolic content before and after the *in vitro* digestion, and to investigate the relationships between the antioxidant activity assays before and after the *in vitro* digestion. In addition, polyphenols in selected fruit seeds were also identified and quantified by UPLC.

2. Materials and methods

2.1. Materials

Acetonitrile was HPLC grade and purchased from Merck (Darmstadt, Germany). Formic acid was HPLC grade and purchased from CNW Technologies GmbH. Gallic acid, (+)-catechin, epicatechin, protocatechuic acid, caffeic acid, vanillic acid, ferulic acid, *p*-coumaric acid, quercetin, isoquercitrin, rutin, quercitrin and syringic acid were bought from Yanshengshiye CO., LTD (Shanghai, China) and National Institutes for Food and Drug Control of China. The 2,4,6-Tri(2-pyridyl)-S-triazine (TPTZ), 2, 2-diphenyl-1-picrylhydrazyl radical (DPPH•), porcine pepsin, taurodeoxycholate, taurocholate, glycodeoxycholate and pancreatin were purchased from Aladdin Industrial Inc. The 6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (Trolox) and the Folin–Ciocalteu's phenol reagent were purchased from Sigma-Aldrich (St. Louis, MO, USA). All chemicals used in the experiments were of analytical grade, and deionised water was used.

2.2. Sample preparation

Eleven fruits were studied, namely *Amygdalus persica* Linn, *Annona squamosa* L, *Citrullus lanatus* (Thunb.) Matsum and Nakai, *Dimocarpus longgana* Lour, *Durio zibethinus* L, *Eriobotrya japonica* (Thunb.) Lindl, *Litchi chinensis* Sonn, *Prunus salicina* Lindl, *Prunus salicina* Lindl (san hua), *Vitis vinifera* Linn (red), and *Vitis vinifera* Linn (black). The fruits were collected from the local markets in Fo-shan, China. Fruit seeds were separated from pulp and washed with deionised water. Then, the fruit seeds were placed in an oven at 60 °C until dry. The dry seeds were ground to a fine powder with a special grinder for herbal medicine and passed through a 20-mesh sieve. The particle size was 0.8 mms. Fifty millilitres of 30% ethanol was added to 1.0 g of the powder, and then samples were ultrasound for 15 min to extract phenolic content at room temperature. The mixture was centrifuged at 1540 g for 10 min to obtained free phenolic acids. The supernatant containing free phenolic acids was recovered for the evaluation of antioxidant capacity and total phenolic content.

2.3. In vitro *digestion procedure*

The *in vitro* digestion model was carried out according to the procedure described in the literature (Ryan, O Connell, O Sullivan, Aherne, & O Brien, 2008). Samples were transferred to clean amber bottles and mixed with saline to create a final volume of 20 mL. The samples were acidified to pH 2.0 with 1 mL of a porcine pepsin preparation (0.04 g pepsin in 1 mL 0.1 mol/L HCl) and incubated at 37 °C in a shaking water bath at 95 rpm for 1 h. After gastric digestion, the pH was increased to 5.3 with 0.9 mol/L sodium bicarbonate, followed by the addition of 200 μL of bile salts glycodeoxycholate (0.04 g in 1 mL saline), taurodeoxycholate (0.025 g in 1 mL saline), taurocholate (0.04 g in 1 mL saline) and 100 μL of pancreatin (0.04 g in 500 μL saline). The pH of each sample was increased to 7.4 with 1 mol/L NaOH. Samples were incubated in a shaking water bath (95 rpm) at 37 °C for 2 h to complete the intestinal phase of the *in vitro* digestion process. After the intestinal phase, 2 mL of each sample was extracted and stored at –20 °C; samples were analysed within 2 weeks.

2.4. FRAP

The ferric reducing antioxidant power (FRAP) assay before and after the gastric and duodenal phases of digestion was carried out according to the procedure described in the literature (Benzie & Strain, 1996). Briefly, the FRAP reagent was prepared from 20 mmol/L iron(III) chloride solution, 10 mmol/L TPTZ solution in 40 mmol/L HCl and 300 mmol/L sodium acetate buffer (pH 3.6) in a volume ratio of 1:1:10, respectively. FRAP reagent should be prepared fresh daily and warmed in a water bath at 37 °C before use. Then 100 μL of the diluted sample was added to 3 mL of the FRAP solution and the absorbance was determined at 593 nm after 2 h using a Rui Li U9600 UV–vis spectrophotometer (Beijing, China) (Ozgen, Reese, Tulio, Scheerens, & Miller, 2006). Trolox was used as a reference