Food Chemistry 237 (2017) 645–653

Contents lists available at ScienceDirect

Food Chemistry

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

An integrated antioxidant activity fingerprint for commercial teas based on their capacities to scavenge reactive oxygen species

Yu-Jie Guo [a], Li-Qiong Sun [a], Bo-Yang Yu [a,b,*], Jin Qi [a,b,*]

[a] Jiangsu Key Laboratory of TCM Evaluation and Translational Research, China Pharmaceutical University, Nanjing 211198, PR China
[b] State Key Laboratory of Natural Medicines, China Pharmaceutical University, Nanjing 210009, PR China

ARTICLE INFO

Article history:
Received 11 January 2017
Received in revised form 2 May 2017
Accepted 3 May 2017
Available online 5 May 2017

Keywords:
Tea
HPLC
Chemiluminescence
Reactive oxygen species
Quality evaluation

ABSTRACT

An integrated antioxidant activity fingerprint, based on on-line screening methods for three reactive oxygen species (ROS: superoxide anion radical, hydrogen peroxide, and hydroxyl radical) was developed to comprehensively evaluate the quality of 12 batches of commercial tea. High-performance liquid chromatography (HPLC) coupled with a chemiluminescent detector was used to determine the antioxidant characteristics of a selection of teas as bioactivity fingerprints. An HPLC–electrospray ionization–mass spectrometry analysis was used to determine the chemical profiles of the teas in the chromatographic fingerprints. All of the green teas (S01–S08) were better scavengers of the three ROS compared to the oolong teas (S09–S12). The main scavengers of the three ROS in green tea were 5-galloylquinic acid, (−)-epigallocatechin-3-*O*-gallate, and (−)-epicatechin-3-*O*-gallate, whereas in oolong tea, they were (−)-epigallocatechin-3-*O*-gallate and (−)-epigallocatechin. This study demonstrates that comprehensive fingerprinting is a potentially meaningful method for evaluating the quality of food products.

1. Introduction

Modern research has shown that oxidative stress injury in the body accompanies the initiation and progression of many diseases, including cardiovascular diseases, aging, cancer, and so on (Halliwell & Gutteridge, 2007; Valko et al., 2007). Oxidative stress occurs when the levels of reactive oxygen species (ROS) overwhelm the body's antioxidant defense system (Sies, 1997). Many food products have a significant antioxidant capacity because they contain various antioxidants, such as flavonoids and phenolic acids (McKay, Chen, Zampariello, & Blumberg, 2015).

Antioxidant activities have always been used to evaluate the intrinsic quality of functional foods. 1,1-Diphenyl-2-picrylhydrazyl (DPPH) (Chirinos et al., 2009), 2,2′-azinobis-(3-ethyl benzothiazoline-6-sulfonic acid) (ABTS) (Leong & Shui, 2002), the superoxide anion radical ($O_2^{\cdot-}$), hydrogen peroxide (H_2O_2) (Ding et al., 2010), and the hydroxyl radical (·OH) (Wu, Tong, Wu, Liu, & Li, 2016) are the parameters by which antioxidant activity is assessed. The predominant ROS *in vivo*, $O_2^{\cdot-}$, H_2O_2, and ·OH, reflect the antioxidant activities in food products at the physiological level, at least to some extent. In recent years, many researchers have studied bioactivity fingerprints, which can show the bioactivity characteristics of the compounds corresponding to each peak in food plants (Ding et al., 2010). Chromatographic separation coupled to the detection of bioactivity has been used to simultaneously screen for and identify the bioactive compounds in functional foods. High-performance liquid chromatography (HPLC)–DPPH (Bandoniene & Murkovic, 2002) and HPLC–ABTS (Karaçelik et al., 2015) have been used to evaluate antioxidant properties, and HPLC–chemiluminescence (CL) has been used to screen for scavengers of $O_2^{\cdot-}$ and H_2O_2 on-line (Yu, Sun, & Qi, 2014). ·OH is the strongest oxidant present *in vivo* (Halliwell & Gutteridge, 2007). However, to the best of our knowledge, no on-line screening method for ·OH scavengers in natural foods has been published.

Tea, obtained from processed leaves and leaf buds of *Camellia sinensis* (L.) O. Ktze., is one of the most popular nonalcoholic beverages in the world. It can be broadly classified as unfermented tea (green tea), semifermented tea (oolong tea), fully fermented tea (black tea), or postfermented tea (pu-erh tea), according to the processing method used (Zhao, Chen, Huang, & Fang, 2006). Many studies strongly suggest that all tea extracts scavenge ROS and reduce oxidative damage (Toyo'oka, Kashiwazaki, & Kato, 2003; Yokozawa et al., 1998). Therefore, it is important to develop routine methods for evaluating the quality of tea.

In this study, a novel on-line method for screening individual ·OH scavengers in complex matrices was developed. An integrated

* Corresponding authors at: State Key Laboratory of Natural Medicines, China Pharmaceutical University, Nanjing 210009, PR China.
E-mail addresses: boyangyu59@163.com (B.-Y. Yu), qijin2006@163.com (J. Qi).

http://dx.doi.org/10.1016/j.foodchem.2017.05.024
0308-8146/

fingerprint, based on three individual on-line screening methods for scavengers of $O_2^{\cdot-}$, H_2O_2, and ·OH, was also established. The integrated fingerprints, including two chemical fingerprints and three bioactivity fingerprints, provide a comprehensive assessment of the antioxidant activities of commercial teas. This is a meaningful and advanced method for the systematic evaluation of the quality of functional foods.

2. Materials and methods

2.1. Instrumentation and reagents

The Shimadzu LC-2010C system (Shimadzu, Tokyo, Japan), consisting of a binary pump, autosampler, thermostated column compartment, and diode array detector (DAD), and a Zorbax StableBond SB-C18 column (4.6 × 250 mm, 5 μm; Agilent Technologies, Santa Clara, CA, USA) were used for chromatographic separations. The HPLC-mass spectrometry (MS) analysis was performed with this HPLC system interfaced with an Agilent 1100 LC/MSD Trap XCT ESI (Agilent Technologies). The chemiluminescent solutions were injected with a BT-200 peristaltic pump (Huxi Analysis Instrument Factory, Shanghai, China). The chemiluminescent emission was detected with the BPCL system (Academia Sinica Biophysics Institute, Beijing, China).

HPLC-grade acetonitrile (Tedia Company, Fairfield, OH, USA) and analytical grade phosphoric acid (Nanjing Chemical Plant, Jiangsu, China) were used for the chromatographic separations. 1,10-Phenanthroline (Lingfeng Chemical Reagent Corp., Shanghai, China), luminol (Fluka Chemie, Buchs, Switzerland), pyrogallol (Zunyi Second Chemical Corp., Guizhou, China), H_2O_2 (30% in water), ascorbic acid, ethylenediaminetetraacetic acid (EDTA), copper sulfate, sodium carbonate, and sodium bicarbonate (Nanjing Chemical Reagent Corp., Jiangsu, China) were used for CL detection. The HPLC water was purified with a Millipore Water Purification System (Millipore, Bedford, MA, USA).

2.2. Materials

Twelve batches of commercial tea were obtained from supermarkets in various regions of China. Their codes, trade names, and purchased locations are as follows: S01 (Biluochun, Yixing), S02 (Yellow Tea, Huoshan), S03 (Cuibai Cha, Liyang), S04 (Yuhua Cha, Nanjing), S05 (Longjing, Xinchang), S06 (Biluochun, Nanjing), S07 (Xiaohua, Tongcheng), S08 (Biluochun, Suzhou), S09 (Tie Guanyin, Anxi), S10 (Tie Guanyin, Anxi), S11 (Tie Guanyin, Anxi), and S12 (Tie Guanyin, Anxi). S01 to S08 were different types of green tea. S09 to S12, the classic types of oolong tea, were sourced from Anxi, who are the leading tea producers in China. After the commercial teas were procured they were stored at −20 °C until analysis. The reference substance, resveratrol (used as the internal standard), was purchased from Jiangsu Provincial Institute of Material Medical (Jiangsu, China).

2.3. Preparation of chemiluminescent reagent solutions

The chemiluminescent reagent solutions used to determine the H_2O_2- and $O_2^{\cdot-}$-scavenging activities have been described previously (Ding et al., 2009). A stock solution of luminol (1.8×10^{-2} M) was prepared in 0.1 M sodium carbonate and stored in a refrigerator for at least 3 days before dilution. A stock solution of pyrogallol (1.0×10^{-2} M) was prepared in 0.1 mM hydrochloric acid and stored in dark bottles at 4 °C. Reagent solution I (9.0×10^{-6} M luminol with 6.3×10^{-3} M EDTA, diluted with 0.1 M carbonate buffer [pH 10.0]) and reagent solution II (aqueous solution of 8.8×10^{-4} M H_2O_2) were used to determine the H_2O_2-scavenging activities. The reagent solutions used to determine the $O_2^{\cdot-}$-scavenging activities were reagent solution I (5.4×10^{-5} M luminol with 6.3×10^{-3} M EDTA, diluted with 0.1 M carbonate buffer [pH 11.0]) and reagent solution II (aqueous solution of 1.5×10^{-5} M pyrogallol).

The reagent solutions used to determine the ·OH-scavenging activity were reagent solution I (an aqueous 2.0×10^{-3} M copper sulfate solution) and reagent solution II (a mixture solution of 3.0×10^{-3} M phenanthroline and 6.0×10^{-3} M ascorbic acid, prepared in 0.1 M phosphate buffer [pH 6.2] before use). Reagent solution III was an aqueous solution of 2.4×10^{-3} M H_2O_2, diluted from 30% H_2O_2. Reagent solution IV was 0.1 M carbonate buffer (pH 9.0).

2.4. Sample preparation

The 12 commercial teas were milled to powder. The samples (0.200 g) were accurately weighed, extracted with ultrasound in methanol–water (70:30 v/v, 30 mL) for 30 min, and then cooled to room temperature. The supernatants were collected after centrifugation for 10 min at 3000 rpm. The residues were re-extracted with methanol–water (70:30 v/v, 20 mL) under the same conditions and the supernatants were pooled. The sample solutions (4 mg mL^{-1}) were prepared in 50 mL volumetric flasks with methanol–water (70:30 v/v). All the sample solutions were filtered through a membrane filter (0.45 μm). An aliquot (5 μL) of the S01 sample solution was injected into the HPLC apparatus to optimize the conditions for the on-line HPLC–DAD–CL detection of the three ROS-scavenging activities and to validate the HPLC–ESI–MS method.

Because different substances have different ROS-scavenging capacities, different volumes of the sample were injected into the HPLC apparatus to detect the various ROS-scavenging capacities. To determine the H_2O_2-scavenging activity, an aliquot (5 μL) of the sample, including 0.01 mg mL^{-1} resveratrol and 0.5 mg mL^{-1} tea solution, was injected into the HPLC–DAD–CL system. To determine the $O_2^{\cdot-}$-scavenging activity, an aliquot (10 μL) of the sample, including 1 mg mL^{-1} resveratrol and 4 mg mL^{-1} tea solution, was injected for analysis. To determine the ·OH-scavenging activity, an aliquot (5 μL) of the sample, including 0.4 mg mL^{-1} resveratrol and 4 mg mL^{-1} tea solution, was injected.

2.5. HPLC–DAD–CL analysis

The mobile phase was 0.1% (v/v) aqueous phosphoric acid (A) and acetonitrile (B), and the gradient program was: 0–10 min, 3%–5% B; 10–25 min, 5%–10% B; 25–35 min, 10% B; 35–55 min, 10%–15% B; 55–60 min, 15% B; 60–75 min, 15%–20% B; 75–95 min, 20%–35% B; 95–100 min, 35% B. The flow rate was 1.0 mL min^{-1}, the detection wavelength was set at 275 nm, and the column temperature was maintained at 30 °C.

A previously described on-line post-column CL detection system (Ding et al., 2009) was used to establish the antioxidant activity fingerprints for H_2O_2 and $O_2^{\cdot-}$. The chemiluminescent reagent solution I (1.2 mL min^{-1}) and reagent solution II (1.4 mL min^{-1}) were delivered with a dual-pathway peristaltic pump and mixed immediately in the tube. The mixed solution was combined with the split solution, and then arrived at the CL detector.

The HPLC–DAD–CL system was set up to analyze the ·OH-scavenging fingerprint. The chemiluminescent reagent solutions were delivered with a peristaltic pump at a flow rate of 0.45 mL min^{-1} for reagent solutions I and II, and 1.10 mL min^{-1} for solutions III and IV. The CL detector was equipped with a flat glass coil, a detection cell of 80 μL, and a photomultiplier operated at −800 V. All other parts of the HPLC–DAD–CL detection system were interconnected with 0.5 mm I.D. polyether ether ketone tubes and T-type micro mixers (Fig. 1A).