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Short communications

Phytochemical properties and antioxidant capacities of commercial raspberry varieties

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ABSTRACT

The antioxidant capacities of 15 commercial raspberry varieties grown in North China were evaluated and their anthocyanin profiles determined by LC–ESI-MS. Total polyphenol content (TPC), total flavonoid content (TFC), total anthocyanin content (TAC) and antioxidant capacities (AOC) of the 15 raspberries were measured, respectively and the results showed that the TPC, TFC and TAC contents of raspberries correlated well with their antioxidant capacities. Raspberries with higher contents of phytochemicals showed higher antioxidant capacity. The results indicated that the 15 raspberry varieties may be divided into three groups according to their anthocyanin component analysis. The first group was made up of Triple Crown, Shawnee, and Navaho varieties with identical anthocyanin profiles and dark red color. The second group included Canby, Bristol and Mac black varieties, which possessed higher TAC/TPC ratio and contribute more to antioxidant capacity and the rest of the varieties were in the third group with lower antioxidant capacities. The higher phytochemical contents and antioxidant activities of raspberry varieties in the second group indicated that their consumption would be more beneficial to health.

1. Introduction

Raspberry is one of the bramble cultivars with good flavor and attractive color. As an important commercial fruit crop, raspberry is widely distributed in all temperate regions of Europe, Asia and North America. Asia is a major centre of diversity for raspberry and there are over 200 species alone in China (Gu, Zhao, Jin, & Li, 1993). The exploitation of raspberries in diverse areas of food and health products is rapidly increasing. Except that a small amount of raspberries are consumed fresh or frozen, lots of them are processed products such as jams, jellies, syrups and wines (Byamukama, Kiremire, Andersen, & Steigen, 2005).

Raspberry contains numerous bioactive compounds such as phenolics, organic acids, vitamins and minerals (de Ancos, González, & Cano, 2000; Kalt, Forney, Martin, & Prior, 1999; Liu et al., 2002). Compared with other fruits, raspberry is also an excellent source of anthocyanins which provide vegetables and fruits with red, blue, and purple colors. It seems to be promising to use extracts from raspberry as a natural colorant and a potent antioxidant (Espín, Soler-Rivas, Wichers, & García-Viguera, 2000; Francis & Markakis, 1989). *In vitro* studies indicate that anthocyanins and other polyphenols in berries have a range of potential health benefits, including anti-cancer, anti-inflammatory and cell regulatory effects (Rissanen et al., 2003; Seeram, 2008; Tsuda, 2008; Zafra-Stone

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et al., 2007), which are believed to be associated with their antioxidant properties (Wang & Stoner, 2008).

Increasing literature also show that the health-promoting properties and active components of berry plants are affected by cultural, genetic and environmental factors (Anttonen & Karjalainen, 2005). In this regard, the different anthocyanin profiles was characterized and total polyphenol, flavonoid, anthocyanin and antioxidant capacities of the 15 commercial raspberry varieties grown in North China were measured to evaluate the difference among raspberries and the relation between phytochemical properties and antioxidant capacities systematically.

2. Materials and methods

2.1. Chemicals

Ascorbic acid, Folin Ciocalteu's phenol reagent, 2,4,6-tris(2-pyridyl)-1,3,5-triazine (TPTZ), and 2-diphenyl-1-picryhydrazyl (DPPH) were purchased from Sigma–Aldrich Shanghai Trading Co., Ltd. (Shanghai, China). Gallic acid was purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Rutin was purchased from Nanjing Sulang Pharmaceutical Technology Development Co., Ltd. (Nanjing, China). Cyanidin-3-glucoside was purchased from Mansite biological technology Co., Ltd. (Chengdu, China). Methanol and formic acid were of HPLC grade purchased from Honeywell B&J (Beijing, China). All other chemicals were of analytical grade and purchased from Beijing Chemical Works (Beijing, China).

2.2. Plant material

Fifteen raspberry varieties were provided by Institute of Forest Products Industry, Beijing, China, which were cultivated in North China. For each raspberry variety, three set samples of fully mature fruits were harvested by hand from several different plants of the same variety during the period of June–October in 2011 and stored in plastic containers at −40 °C for chemical analysis.

2.3. Extraction procedure

Three replicates of 3 g of fruits samples were thawed at room temperature and immersed in 30 mL of chilled 0.1% (v/v) methanolic HCl. The components were extracted by 5 min of maceration in a Waring blender containing 0.1% (v/v) methanolic HCl. The slurry was centrifuged at 8497 g, and the supernatant fluid was decanted. The extraction procedure was repeated three times. The supernatant fluids from several extractions were combined and evaporated in vacuum to dryness at 30 °C. The residual pigment was resuspended in 0.1% (v/v) methanolic HCl. Samples were filtered through a 0.45 μm cellulose syringe and stored at −20 °C until analysis.

2.4. Purification of anthocyanins

Purification using solid-phase extraction permitted the removal of several interfering components present in the crude extracts (Jackman & Smith, 1996). Raspberry extract was applied to a Bond Elut C18 cartridge (200 mg, Varian, Palo Alto, CA, USA) that had been previously activated with ethyl acetate, followed by acidified methanol (0.01% HCl, v/v) and acidified water (0.01% HCl, v/v). Anthocyanins and other polyphenols were adsorbed onto the Bond Elut column while sugars, acids, and other soluble compounds were removed by washing the cartridge with 2 volumes of acidified water (0.01% HCl, v/v). Less polar polyphenols were subsequently eluted with ethyl acetate. Then, anthocyanins were eluted with acidified methanol (0.01% HCl, v/v). The acidified methanol solution was evaporated to dryness. The dry fraction was taken up in deionized water. Samples were filtered through a 0.45 μm filter before analysis.

2.5. LC-MS analysis of anthocyanins

An Agilent 1200/6120 system (Agilent Technologies Inc., Shanghai, China) equipped with a diode array detector (DAD) and mass spectrometer (MS) was used to confirm the anthocyanins of raspberries (Mullen, Lean, & Crozier, 2002; Wu & Ronald, 2005). The HPLC system consisted of a G1311A solvent delivery unit, a G1379A degasser, a G1315B UV–Vis diode array detector, a sample injection valve (Model 7725i) with a 10 μL loop, and an Agilent HPLC workstation (Agilent, Böblingen, Germany). The column applied in this work was a Zorbax SB-C18 column (250 × 4.6 mm, i.d., 5 mm, Agilent Technologies Inc., Shanghai, China). A linear gradient consisting of methanol and aqueous 5% formic acid solution was employed. The flow rate was 1 mL/min, and the detection wavelength was at 520 nm. All samples were filtered through a 0.45 μm syringe filter before analysis. Electrospray mass spectrometry was performed with an Agilent 6120 single quadruple mass spectrometer (MS). The experiment was operated in positive ion mode, scanning from 250 to 800u.

2.6. Determination of total polyphenol content (TPC)

The total polyphenol content was determined with Folin–Ciocalteu reagent according to the method modified from Singleton (Singleton & Rossi, 1965) using gallic acid as a standard. Total raspberry polyphenol extract was expressed as milligrams of gallic acid equivalence per 100 g of fresh weight (FW) basis. Samples of each extraction were analyzed in triplicate.

2.7. Determination of total flavonoid content (TFC)

The total flavonoid content was determined colorimetrically as described previously (Zhishen, Mengcheng, & Jianming, 1999). The flavonoid content was determined by a rutin standard curve and expressed as mean of milligrams of rutin per 100 g of fresh weight (FW) basis. Triplicate samples were analyzed for each sample.

2.8. Determination of total anthocyanin content (TAC)

Total anthocyanin content was determined according to the pH differential method (Lee, Durst, & Wrolstad, 2005).