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Comparison and screening of bioactive phenolic compounds in different blueberry cultivars: Evaluation of anti-oxidation and α-glucosidase inhibition effect

Ying Wu[b,c], Qing Zhou[b,*], Xiao-yong Chen[d], Xing Li[a], Yi Wang[b], Jiu-liang Zhang[a,e,**]

[a] College of Food Science and Technology, Huazhong Agricultural University, Wuhan 430070, China
[b] Department of Pharmacy, Wuhan City Central Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan 430014, China
[c] College of Pharmacy, Hubei University of Chinese Medicine, Wuhan 430065, China
[d] Hubei Institute for Food and Drug Control, Wuhan 430064, China
[e] Key Laboratory of Environment Correlative Dietology (Huazhong Agricultural University), Ministry of Education, Wuhan 430070, China

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ABSTRACT

This study was aimed to investigate antioxidation and α-glucosidase inhibition of the bioactive compounds in three cultivars of blueberry ('Northland' (NL) from the hybrid blueberry (*V. corymbosum* L. × *V. angustifolium* Aiton), 'Britewell' (BW), and 'Gardenblue' (GB) from the rabbiteye blueberry (*Vaccinium ashei* Reade)). A total of eighteen anthocyanins were identified and quantified in Anthocyanins (ACNs), among which four acylated anthocyanins were exclusively found in Northland. The blueberry anthocyanin extracts (BAEs) were further measured the antioxidant activity and α-glucosidase inhibition effect. Northland BAEs exhibited significantly superior antioxidant activity compared with BAEs of other cultivars, and the antioxidant activity was correlated with the content of anthocyanins. However, α-glucosidase inhibition test showed that Britewell BAEs had the strongest inhibitory effect. BAEs were further separated into anthocyanin fraction (AF) and copigment fraction (CF). Fifteen phenolic acids and four iridoids were identified in CF. In terms of α-glucosidase inhibition effects, the CF from three cultivars could inhibit α-glucosidase in a dose-dependent manner, while the AF did not show significant inhibitory effects. The blueberry exhibits excellent antioxidant activity and α-glucosidase inhibition.

1. Introduction

As a kind of important fruit crop, blueberries (*Vaccinium spp.*) have gained worldwide and particular interests of people due to their excellent sensory properties and the presence of healthy constituents. Several studies have confirmed that the blueberry extracts have pharmacological effects against ophthalmologic disorders (Calo & Marabini, 2014) and bone protection (Shen et al., 2012), and can decrease blood pressure and cholesterol (Basu et al., 2010; Johnson, Wallig, Vital, & de Mejia, 2016; Stull, Cash, Johnson, Champagne, & Cefalu, 2010). Besides, blueberries have been reported to be characterized by anti-diabetic properties, protection of pancreatic β-cells from glucose-induced oxidative stress and improvement insulin sensitivity (Al-Awwadi et al., 2005; DeFuria et al., 2009; Johnson, de Mejia, Fan, Lila, & Yousef, 2013; Johnson et al., 2016; Sánchez-Villavicencio et al., 2017). The bioactivity was mainly attributed to phenolics, such as anthocyanins, phenolic acids and proanthocyanidins, which exist ubiquitously in plants as secondary metabolites (Giovanelli, Brambilla, & Sinelli, 2013). Antioxidant capacity has become a desirable and essential quality characteristic of blueberries and a predictor for selecting blueberry cultivars.

Type 2 diabetes originates from the imbalance of hormones (insulin and glucagon), which could lead to glucose uptake or glycogenolysis or gluconeogenesis, and finally up-regulate glucose concentration in the blood. Thus, glucose uptake could be a therapeutic target, such as the inhibition of α-glucosidase in mammals, which could reduce the hydrolyzation of starch into glucose, decrease glucose uptake and normalize postprandial blood glucose concentration. Meanwhile, dietary phenolic compounds were reported to participate in the digestion, absorption and metabolism of starch and sucrose (Hanhineva et al., 2010). Several studies have been focused on the reduction of absorption of glucose by berries and their extracted compounds through inhibiting the activity of α-glucosidase in vitro (Figueiredo-González, Grosso, Valentão, & Andrade, 2016; Johnson, Lucius, Meyer, & Gonzalez de

* Corresponding author.
** Correspondence to: J. L. Zhang, College of Food Science and Technology, Huazhong Agricultural University, Wuhan 430070, China.
E-mail addresses: qingz_zqing@aliyun.com (Q. Zhou), zjl_ljz@mail.hzau.edu.cn (J.-l. Zhang).

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Mejia, 2011; Johnson et al., 2013; Wan, Yuan, Cirello, & Seeram, 2012; Wang, Camp, & Ehlenfeldt, 2012a). However, there is still a great lack of comprehensive profiling of the bioactive compounds in various blueberry cultivars.

Three blueberry cultivars were studied in this paper, including the hybrid blueberry (*V. corymbosum* L. × *V. angustifolium* Aiton) named 'Northland' (NL), the rabbiteye blueberry (*Vaccinium ashei* Reade) named 'Britewell' (BW), and 'Gardenblue' (GB). There have been few reports (Samoticha, Wojdyło, & Golis, 2017; Wang, Wu, Wang, Li, & Zheng, 2015) on the comparison of bioactive compounds in rabbiteye blueberry and hybrid cultivars. Most of the bioactive compounds were reported to be anthocyanins without taking copigment into account, which is primarily phenolic acids extracted in an acidic environment simultaneously. Besides, few studies (Esatbeyoglu, Rodríguez-Werner, Schlösser, Winterhalter, & Rimbach, 2017) were focused on the inhibitory activity of copigment against α-glucosidase.

Thus, the objective of the present study was to identify and compare the bioactive compounds in three blueberry cultivars. All samples were separated into two fractions: anthocyanins fraction (AF) and copigment fraction (CF, primary phenolic acids), and were further characterized by LC-MS. In addition, their antioxidant activities were evaluated using reducing power, 1,1-diphenyl-2-picryhydrazyl (DPPH) radical scavenging and oxygen radical absorbance capacity (ORAC). Moreover, we screened the purified components for α-glucosidase inhibition in vitro. This study provides new and important insights into the correlation between the bioactivity and compounds of blueberries.

2. Materials and methods

2.1. Plant materials

The blueberries (*Vaccinium spp.*) were obtained from Cowherd Blueberry Technology Co., Ltd. (Wuhan, China). Ripe half-high bush blueberries (*V. corymbosum* L. × *V. angustifolium* Aiton.) 'Northland' were harvested at commercial maturity in the last ten days of July from Enshi blueberry orchard, Hubei Province, China. Rabbiteye blueberries (*V. ashei* Reade) 'Britewell' and 'Gardenblue' were harvested also in the last ten days of July from Mulan Lake blueberry orchard, Wuhan, China. The samples were stored at − 20 °C until use.

2.2. Chemicals

HPLC-MS grade acetonitrile and methanol were obtained from J.T. Baker (Deventer, the Netherlands). HPLC grade formic acid eluent additive was purchased from Guangfu Fine Chemical Research Institute (Tianjin, China). Ultrapure water was purified with a Milli-Q system supplied by Millipore (Billerica, Massachusetts, USA). The α-glucosidase from *Saccharomyces cerevisiae* (EC 3.2.1.20) was obtained from Sigma-Aldrich (St. Louis, Missouri, USA). The *p*-nitrophenyl α-D-glucosidase (*p*NPG) (purity > 99%), acarbose (purity > 95%), cyanidin 3-O-glucoside (purity > 95%), Trolox, fluorescein disodium, 1,1-diphenyl-2-picrylhydrazyl (DPPH), and 2,2′-azobis (2-amidinopropane) dihydrochloride (AAPH) were purchased from Yuanye Biological Technology (Shanghai, China). AB-8 macroporous adsorption resin (0.3–1.25 mm particle size) was purchased from Nankai Hecheng Science & Technology Co. (Tianjin, China).

2.3. Preparation of phenolic compounds

2.3.1. Extraction of BAEs

The extraction was performed following the method in previous studies (Xu et al., 2016). Samples of frozen fresh blueberries of three cultivars (10 g for each cultivar) were individually homogenized and mixed with 150 mL 60% ethanol acidified with 0.1 M HCl solution to pH 3.0, then incubated in 40 °C water bath for 2 h. After filtering under vacuum, the primary extract was concentrated by rotary evaporation under 40 °C and then defatted by hexane solvent. The procedure was repeated three times and the resulting extracts were combined, evaporated and lyophilized. The extracts of three cultivars (100 mg) were respectively dissolved in pH 3.0 buffer containing citric acid and disodium hydrogen phosphate, and individually loaded onto an AB-8 resin column (25 mm × 100 mm). After that, the column was washed by distilled water acidified with 0.1% HCl and the phenolic compounds were desorpted by 60% (*v*/v) aqueous ethanol. Then, the eluent was evaporated and freeze-dried. Finally, the blueberry anthocyanin extracts (BAEs) were obtained and then stored at − 20 °C until use.

2.3.2. Purification of ACNs from BAEs by HS-CCC

The preparative high-speed counter current chromatography (HS-CCC) was performed using a model TBE-300B HS-CCC (Tauto Biotechnique Company, Shanghai, China). The two-phase solvent system and the partition coefficient (K) for HS-CCC separation were determined using a previously described method (Jin et al., 2016). The solvent system was water: n-butanol: methyl *tert*-butyl ether: acetonitrile: trifluoroacetic acid (6: 5: 2: 1: 0.02, *v*/v), which was shaken and stabilized for two phase solvent systems, and the partition coefficients ranged from 0.5 to 2.5. Northland BAEs (100 mg), Britewell BAEs (100 mg), and Gardenblue BAEs (100 mg) were dissolved in 10 mL of the lower phase of the two phase solvent system, respectively, followed by filtration with a 0.22 μm syringe filter for the HS-CCC procedure. The upper phase was used for HS-CCC separation with a flow rate of 5 mL/min. The instrument was rotated at 800 r/min for equilibration of the solvent and the preparative separation process. The effluent was continuously monitored by EasyChrom-1000 chromatography workstation with a UV 2000D detector (Shanghai Sanotac Scientific Instrument Co. Ltd., Shanghai, China) at 520 nm and the selected peaks were collected manually according to the chromatographic profile. Then the fluid was evaporated and freeze-dried, and the purified ACNs by HS-CCC were obtained and then stored at − 20 °C until characterization.

2.3.3. Separation of AF and CF from BAEs by preparative HPLC

To separate anthocyanin fraction (AF) and copigment fraction (CF), preparative high-performance liquid chromatography (HPLC) was carried out by H & E QuikSep system equipped with a ODS column (10 mm × 250 mm, 5 μm; YMC, Kyoto, Japan). Gradient elution was carried out with solvent system A (methanol) and B (formic acid/water, 5:95, *v*/v) at a flow rate of 3 mL/min. The linear gradient was as follows: 0 min, 85% B; 5 min, 80% B; 10 min, 80% B; 25 min, 75% B; 30 min, 75% B; 46 min, 40% B; 52 min, 40% B. Approximately 90 mg of BAEs was dissolved in 9 mL of ultrapure water, and was then filtered using 0.22 μm syringe filter. The injection volume was 900 μL, and the column temperature was maintained at 25 °C. The absorbance of AF and CF was monitored at 520 nm and 280 nm by HPLC-DAD, respectively. The collected eluent of CF and AF was evaporated and freeze-dried.

2.4. Identification and quantification by LC-MS

2.4.1. Characterization of ACNs by HPLC-ESI-MS/MS.

The ACNs were analyzed by HPLC-ESI-MS/MS consisting of a Waters module 2695 equipped with a 2998 photodiode array detector (Milford, Massachusetts, USA). The separation was carried out on a C_{18} column (4.6 mm × 250 mm, 5 μm; Dikma Technologies Inc., Beijing, China). The eluent and elution gradient were the same as the preparative HPLC but at the flow rate of 1.0 mL/min. The volume of injection was 10 μL. The purified ACNs from HS-CCC were dissolved in ultrapure water to form a solution at a concentration of 1 mg/mL and filtered.

For MS detection, ESI conditions were: positive ionization mode, dry gas flow at 10 mL/min, dry gas temperature at 325 °C, nebulizer pressure at 40 psi, capillary at 3500 V, MS full scans in the mass range from *m/z* 100 to 1500.