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Anthocyanins characterization and antioxidant activities of sugarcane (*Saccharum officinarum* L.) rind extracts

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ABSTRACT

Anthocyanins in rind extracts of three sugarcane cultivars, ROC 22, Haitang 22 and Guitang 21, were characterized using ultra performance liquid chromatography (UPLC) combined with electrospray ionization quadrupole-time-of-flight tandem mass spectrometry (ESI-QTOF-MS/MS). A total of thirteen anthocyanins were identified and quantified. Except for cyanidin-3-glucoside, twelve anthocyanins were reported for the first time from sugarcane. The total anthocyanin content (TAC) varied significantly from 10.8 (Haitang 22) to 132.0 μg/g dry rind weight (DW) (ROC 22). Regarding single anthocyanins, ROC 22 contained 79.8 μg/g DW cyanidin-3-glucoside but this anthocyanin was not identified in the other two cultivars. Instead, Guitang 21 contained 94.6 μg/g DW malvidin-3-*p*-coumaroyl-rhamnoside-5-glucoside. The rinds of red sugarcane cultivars ROC 22 and Guitang 21 had higher total antioxidant activities than green sugarcane Haitang 22, which was attributed to much higher contents of free and total phenolics. This study provides useful information for the production of valuable nutraceuticals from sugarcane.

1. Introduction

Sugarcane (*Saccharum officinarum* L.), a world widely grown cash crop, is the main feedstock for sugar production. Nowadays, sugarcane also becomes a potential source for the production of health promoting substances (Duarte-Almeida et al., 2011). A number of human health beneficial compounds, such minor steroids (campesterol, barassicasterol and 6-oxygenated steroids) and phenolic compounds have been identified in sugarcane (Zhao et al., 2015; Zheng et al., 2017b). Among the phenolic compounds, anthocyanins (cyanidin and petunidin), flavonoids (naringenin, tricin, apigenin and luteolin derivatives) and phenolic acids (chlorogenic acid, coumaric acid, and ferulic acid) are the main natural antioxidant compounds (Duarte-Almeida et al., 2007; Zhao et al., 2013) and their antioxidant activities have been assessed and reported (Abbas et al., 2014; Chen et al., 2015; Zheng et al., 2017a).

Anthocyanins, a group of glycosylated phenolic compounds, are the most important pigments in flowers, fruits and vegetables. Natural anthocyanins generally include cyanidin, peonidin, malvidin, pelargonidin, petunidin and delphinidin. The molecular structure of anthocyanins mainly consists of an aromatic ring [A], a heterocyclic ring [C] and another type aromatic ring [B] as shown in Fig. 1 (Iacobucci and Sweeny, 1983). The main differences of anthocyanins are the types of functional groups (*i.e.*, OH and OCH_3 on positions 3′–5′, 3, 5 and 7 as shown in Fig. 1) as well as the types of bonded sugar moieties (on positions 3 and 5 as shown in Fig. 1), such as glucose, galactose, arabinose and rhamnose, as well as natural acylated acids, such as caffeic acid, *p*-hydroxybenzoic acid and ferulic acid. Presently, more than 600 kinds of anthocyanins are identified in plants (Huang et al., 2009). These anthocyanins are not only used as a group of water-soluble phenolic pigments for food additives (Pazmino-Duran et al., 2001), but also enhance the health-promoting qualities of foods (Konczak and Zhang, 2004). Anthocyanins and their derivatives have anti-oxidation (Ramirez et al., 2015), anticancer (Bontempo et al., 2015), antidiabetic activities (Hong et al., 2013), and the potential to prevent memory loss and neurodegenerative diseases (Gutierres et al., 2014).

Sugarcane also contains anthocyanins. Many previous studies were focused on the development of methods for extraction and determination of total anthocyanins (Li et al., 2011). Only limited studies

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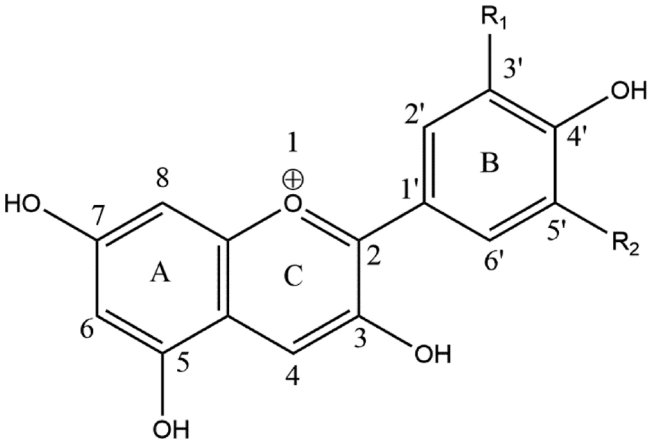

Anthocyanin	R_1	R_2
Pelargonidin	H	H
Cyanidin	OH	H
Delphinidin	OH	OH
Peonidin	OCH_3	H
Petunidin	OCH_3	OH
Malvidin	OCH_3	OCH_3

Fig. 1. General structure of anthocyanins (modified from Iacobucci and Sweeny, 1983).

determined the structures of some anthocyanins in sugarcane using high-performance liquid chromatography with tandem mass spectrometry (HPLC–MS/MS). Two anthocyanins, cyanindin-3-*O*-glucoside and petunidin-3-*O*-(6"-succinyl)–rhamnoside were identified in sugarcane using HPLC–MS/MS by Li et al. (2010). Recently, a more efficient method, ultra-performance liquid chromatography-electrospray ionization-quadrupole time-of-flight tandem mass spectrometry (UPLC-ESI-QTOF-MS/MS) was developed to characterize anthocyanins. With this method, thirteen acylated anthocyanins were identified in purple fleshed sweet potato cultivars (He et al., 2016). In addition, seven anthocyanins in a domestic *Perilla* cultivar were characterized (He et al., 2015). UPLC-ESI-QTOF-MS/MS, an alternative method for characterizing structures of anthocyanins or isomers which have similar retention times and spectroscopic characteristics with HPLC-PDA/DAD analysis, has unique advantages in anthocyanin identification and quantitation.

In this study, a rapid and reliable method was developed to characterize anthocyanins in three sugarcane cultivars based on UPLC-ESI-QTOF-MS/MS. With this method, a total of thirteen anthocyanins were identified and twelve were found for the first time from sugarcane. Furthermore, antioxidant activities of free and bound phenolics of the three cultivars sugarcanes were assessed for the first time using 2,2-diphenyl-1-picrylhydrazyl (DPPH) agent to determine radical scavenging capacity, by rapid peroxyl radical scavenging capacity (PSC) assay and by oxygen radical absorption capacity (ORAC) assay.

2. Materials and methods

2.1. Plant materials

The sugarcane cultivars of ROC 22 (red-rind) and Guitang 21 (red-rind) and Haitang 22 (green-rind) were manually harvested and provided by Guangzhou Sugarcane Industry Research Institute (Guangzhou, China) in 2015. The collected rind samples of ROC 22, Haitang 22 and Guitang 21 were separated from the inner pith and cut into small pieces by sharp knives, respectively. Frozen dried samples were crushed into powder with a pulverizer (FW100) at 24000 rpm (Tianjin Taisite Instrument Co., China) and passed through a sieve of 40 meshes. The powder was kept dry at −20 °C for later use.

2.2. Chemicals and reagents

Cyanidin-3-glucoside chloride was obtained from MUST Bio-Tech Co. (Chengdu, China). Folin–Ciocalteu reagent, 2,2-Diphenyl-1-picrylhydrazyl (DPPH), 2′,7′-Dichlorofluorescin diacetate (DCFH-DA), fluorescein disodium salt, 2, 2′-azobis (2-amidinopropane) dihydrochloride (ABAP), 6–hydroxy–2,5,7,8–tetramethylchroman–2–carboxylic acid (Trolox), gallic acid and ascorbic acid of analytical grade or above were purchased from Sigma-Aldrich Ltd. (St. Louis, MO, USA). Other analytical reagents were obtained from Guangzhou Reagent Co. (China). Trifluoroacetic acid (HPLC grade) was purchased from Aladdin Co. (Shanghai, China). Methanol and acetonitrile (all HPLC grade) were obtained from Anpel Ltd. (Shanghai, China).

2.3. Extraction of anthocyanins

The anthocyanins were extracted according to the method described in a previous report (Li et al., 2010) with slight modification. The rind powder (10 g) was extracted twice with 1.5 L 40% acetone (v/v) and 40% methanol (v/v) in water (5% of 6 mol/L hydrochloric acid) in dark for 12 h at room temperature. Subsequently, the suspension was combined and centrifuged at 3500 rpm for 20 min. The supernatant was collected and concentrated by a rotary evaporator at 40 °C in vacuum to remove solvents. An AB-8 adsorptive resin column was used to remove sugars, formic acid, and other interfering substances from the concentrated extracts. Distilled water with 0.5% hydrochloric acid was used to wash anthocyanins adsorbed to resin. Afterwards, anthocyanins were eluted with 60% ethanol containing 0.5% hydrochloric acid. The collected eluent was evaporated at 40 °C to dryness. All samples were stored at −40 °C until further analysis.

2.4. Extraction of phenolic compounds

Free phenolic compounds were extracted from rind powder according to a previously reported method (Wen et al., 2015) with some modification. The rind powder (100 g) was extracted twice with 1.5 L 40% acetone (v/v) and 40% methanol (v/v) in water (5% of 6 mol/L hydrochloric acid) in dark for 12 h at room temperature. Subsequently, the suspension was combined and centrifuged at 3500 rpm for 20 min. The supernatant was then collected and concentrated to less than 5 mL by a rotary evaporator at 40 °C under vacuum. The solid residue was collected for the extraction of bound phenolics. The extracts were made up to a final volume of 25 mL with the addition of 20% methanol solution and stored at −40 °C until further analysis.

Bound phenolics were extracted from the residues after extraction of free phenolics according to a method reported previously (Adom et al., 2003; Wang et al., 2013) with modification. The residues were digested in a 50 mL bottle containing 20 mL of 2 M sodium hydroxide with an agitation speed of 60 rpm at room temperature for 1.5 h under nitrogen. Afterwards, the mixture was neutralized with 1 M hydrochloric acid and hexane was used to remove the lipids from the mixture. Ethyl acetate was used to extract bound phenolics from the remaining mixture, and then the solvents were evaporated at 45 °C. The bound phenolics were reconstituted to 10 mL with the addition of 20% methanol solution. The extracts were stored at −40 °C until further analysis.