Journal of Food Composition and Analysis 59 (2017) 105–110

Contents lists available at ScienceDirect

Journal of Food Composition and Analysis

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

Original research article

Inter- and intra-seasonal changes in anthocyanin accumulation and global metabolite profiling of six blueberry genotypes

Michael A. Timmers, Mary H. Grace, Gad G. Yousef, Mary Ann Lila*

Plants for Human Health Institute, North Carolina State University, NCRC, 600 Laureate Way, Kannapolis, NC, 28081, USA

ARTICLE INFO

Article history:
Received 10 November 2016
Received in revised form 17 February 2017
Accepted 26 February 2017
Available online 27 February 2017

Chemical compounds studied in this article:
Delphinidin 3-glucoside (PubChem CID: 443650)
Malvidin 3-glucoside (PubChem CID: 443652)
Cyanidin 3-glucoside (PubChem CID: 92131208)

Keywords:
Anthocyanin
HPLC
PCA
Blueberry
Metabolite profile
Genotype and seasonal variations
Food analysis
Food composition

ABSTRACT

Anthocyanin content in six blueberry genotypes was evaluated across two harvest seasons. Three southern highbush (hybrids of *Vaccinium corymbosum*) genotypes (Legacy, Sampson, SHF2B1-21:3) showed a dramatic and almost linear increase in total anthocyanin content across each harvest season, with an increase of up to 20 mg/g dry weight (DW). The three rabbiteye (*V. virgatum*) genotypes (Ira, Montgomery, Onslow) showed no trend in anthocyanin content, fluctuating by up to 15 mg/g DW. The relative proportions of each individual anthocyanin were also investigated. Rabbiteye genotypes contained higher percentages of cyanidin glycosides than the southern highbush genotypes, while the reverse was found for the levels of delphinidin glycosides present. For southern highbush genotypes, the percentages of malvidin and delphinidin glycosides were inversely proportional across each harvest season. Principal component analysis on the data obtained by HPLC–MS of the crude extracts clearly separated rabbiteyes and southern highbush into two discrete sets based on global metabolite profiling. Within the southern highbush grouping, genotypes were easily distinguished from one another across each harvest season, due to their separation on the scores plot, indicating that each genotype had distinctly different metabolite profile, whereas each of the rabbiteye genotypes overlapped on the scores plot, indicating commonalities in metabolite profiles.

1. Introduction

Seasonal fruits can vary considerably during a harvest season in terms of taste, texture and phytochemical composition, even within a single growing location (Arena et al., 2013; Paterson et al., 2013; Ruiz-Rodriguez et al., 2011; Schwieterman et al., 2014; Voca et al., 2010; Williner et al., 2003). Blueberry genotypes vary by the time at which they start to ripen, the length of harvest season, and can also differ quite dramatically in phytochemical composition (Scalzo et al., 2009). Nearly all blueberries accumulate high levels of anthocyanins, ranging from 13 to 27 different types of anthocyanin (combination of aglycones and sugar moieties and substitutions) present, dependent on genotype (Brito et al., 2014; Grace et al., 2009; Wu and Prior, 2005). This anthocyanin profile is significantly more diverse than other berries, such as blackberries and blackcurrant, which accumulate only 5–8 anthocyanins (Wu and Prior, 2005). Anthocyanins have been studied extensively for their health-promoting benefits, including antioxidant, anti-inflammatory, anti-microbial, anti-diabetic and anti-carcinogenic activity, and cardioprotective and neuroprotective benefits (Esposito et al., 2014; Grace et al., 2014; He and Giusti, 2010; Strathearn et al., 2014).

Anthocyanin biosynthesis has been well documented, following the phenylpropanoid pathway, followed by the flavonoid pathway (Petrussa et al., 2013; Santos-Buelga et al., 2014). This pathway has been well established and all enzyme-catalyzing steps characterized (Jaakola, 2013; Petrussa et al., 2013; Routray and Orsat, 2011). Once anthocyanin formation has occurred (**1**–**2**), methylation can occur at the 3′ or 5′ position (**3**–**5**). The formation of malvidin glycosides (**3**) requires a two-step methylation of delphinidin

* Corresponding author.
E-mail address: mlila@ncsu.edu (M.A. Lila).

http://dx.doi.org/10.1016/j.jfca.2017.02.019
0889-1575/

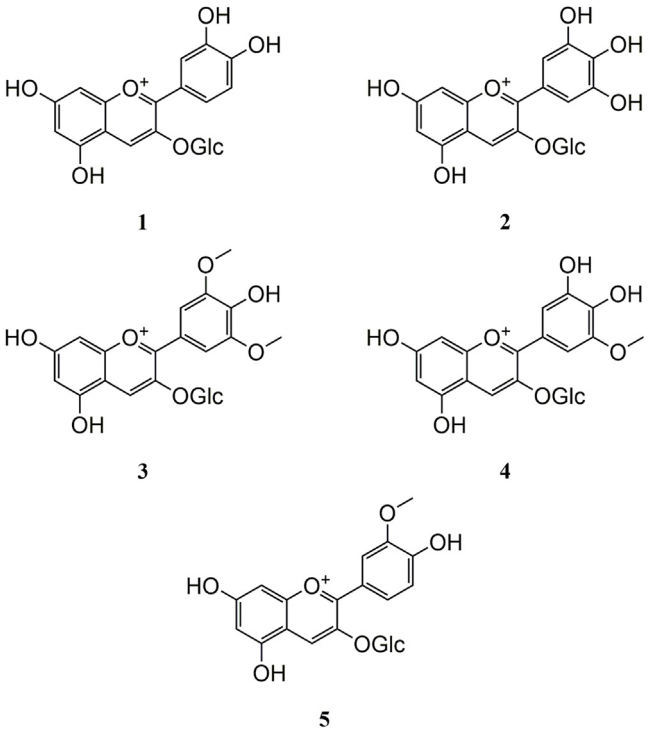

Fig. 1. Five basic types of anthocyanins present in blueberries. Cyanidin 3-glucoside (1); delphinidin 3-glucoside (2); malvidin 3-glucoside; petunidin 3-glucoside (4); peonidin 3-glucoside (5).

glycosides (**2**), while petunidin glycosides (**4**) are intermediate in this process. Acetylation of the sugar moiety occurs as a last step in the biosynthetic pathway, *via* an anthocyanin acetyltransferase enzyme (Nakayama et al., 2003). The five basic anthocyanin structures found in blueberry are shown in Fig. 1.

Total anthocyanin content has been studied in blueberries across harvest seasons (Li et al., 2016; Lohachoompol et al., 2008; Wang et al., 2012; Zoratti et al., 2015); however, the fluctuations between individual anthocyanin moieties has not been examined to our knowledge. This study presents the first in which inter- and intra-seasonal variations in total anthocyanin concentration and individual anthocyanin proportions were examined. The anthocyanin profiles of six blueberry genotypes were assessed approximately weekly over the course of two harvest seasons by extracting ripened berries and analyzing by HPLC. In addition, principal component analysis of global metabolites was performed on the data obtained from non-targeted HPLC-TOF-MS using Progenesis QI software to identify genotype and seasonal variations.

2. Materials and methods

2.1. General experimental

All solvents used were HPLC grade purchased from Fisher Scientific (Pittsburgh, PA) unless specified. HPLC–MS grade solvents – 0.1% formic acid in acetonitrile and 0.1% formic acid in water (Honeywell B&J, Morris Plains, NJ) – were purchased from VWR International (Suwanee, GA). Cyanidin 3-glucoside was purchased from ChromaDex Inc. (Irvine, CA). All HPLC analyses were conducted using a 1200 series HPLC (Agilent Technologies, Santa Clara, CA), equipped with a DAD detector set to 520 nm. HPLC-TOF-MS analysis was performed using an Agilent 6220a TOF-MS, equipped with a 1200 series HPLC (Agilent Technologies).

2.2. Blueberry harvest and extraction

Fresh and ripe blueberries of each genotype were hand-picked weekly from the Piedmont Research Center in Salisbury, North Carolina (35°41′54.5″N: 80°37′22.8″W). Berries were picked when they turned entirely blue and came away easily from the bush, while picking was undertaken early in the morning to limit issues arising from heat. Berries were kept on ice until they were returned to the laboratory before noon.

A total of six genotypes were collected for this study: three rabbiteye genotypes (*V. virgatum*) (Ira, Montgomery and Onslow) and three southern highbush genotypes (hybrids of *V. corymbosum*) (Legacy, Sampson and SHF2B1-21:3). The parentage of the blueberry genotypes evaluated in this study was detailed previously (Yousef et al., 2014). All blueberries picked on a specific harvest date were combined for each genotype and masses recorded. Blueberries were then frozen at −80 °C and lyophilized before further storage at −80 °C. The extraction of lyophilized blueberries was performed as described in Grace et al. (2014). Briefly, for each sample, 2–2.5 g of lyophilized tissue were extracted with 30 mL of 0.5% acetic acid in methanol:water (70:30, v/v) using a homogenizer, centrifuged at 3452*g* for 10 min at 20 °C, and supernatant was collected in a 100-mL volumetric flask. The extracted plant material was subsequently extracted twice, and the collected supernatants in the volumetric flask were brought to volume. About 2 mL of each sample were filtered into an amber HPLC vial using a 0.2-μm PTFE filter.

2.3. HPLC analysis

Quantitative determination of anthocyanin content was performed using HPLC with an RP-Supelcosil-C18 column, 4.6 × 250 mm, 5 μm (Supelco, Bellefonte, PA). The mobile phase consisted of water-formic acid (95:5 *v/v*, solvent A) and methanol (solvent B). Separation was achieved by stepwise gradient elution at 1 mL/min and column temperature of 30 °C. The gradient elution started at 10% B to reach 15% B in 5 min, increased to 20% B at 15 min, to 25% B at 20 min, to 30% B at 25 min, then ramped to 60% B at 45 min, then going back to 10% B at 47 min and maintained at 10% until 60 min for re-equilibration. Anthocyanins were monitored at 520 nm. Cyanidin 3-glucoside (Cy-3-glu) reference at concentrations of 0.03125, 0.0625, 0.125, 0.25 and 0.5 mg/mL was used to form a standard curve and the value of each anthocyanin present was calculated in mg Cy-3-glu equivalents/g dry weight (DW).

2.4. HPLC-TOF-MS analysis

HPLC–MS analysis of the acidified methanol extracts was conducted on the HPLC-TOF-MS system described in Section 2.1 with a Waters XBridge C18 BEH column, 3.0 × 100 mm, 3.5 μm (Waters, Milford, MA). The mobile phase consisted of 0.1% formic acid in water (solvent A) and 0.1% formic acid in acetonitrile (solvent B) at a constant flow rate of 0.6 mL/min. The stepwise gradient started with 2% B for 2 min, ramped to 40% B at 12 min, then to 100% B at 16 min, maintained at 100% B until 18 min, back to 2% B at 20 min, and maintained at this percentage until 24 min. TOF parameters included a drying gas of 12 L/min, nebulizer pressure of 45 psig, capillary voltage 3.5 kV, fragmentor voltage of 80 V and a mass range of *m/z* 100–2000 in positive mode. A standard mix of analytes was used at the start and end of each run sequence to ensure stability across each sample set.