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Original Research Article

Variation among highbush and rabbiteye cultivars of blueberry for fruit quality and phytochemical characteristics

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ABSTRACT

Variability in soluble solids (SS), titratable acidity (TA), SS/TA, pH, total phenolic content (TPC), ferric reducing ability of plasma (FRAP), total monomeric anthocyanin content (TMAC) and levels of vitamin C were evaluated in a broad array of northern and southern highbush (*Vaccinium corymbosum*) and rabbiteye (*Vaccinium virgatum*) cultivars of blueberry, grown in different locations and years. When cultivars were grouped by decade of release, there were few significant overall trends observed over years in fruit quality and phytochemical content; however, individual cultivars varied significantly for all the traits analysed. Considerable overlap in most quality and phytochemical characteristics were found among cultivars of the three blueberry types, suggesting that genetic barriers do not exist among the various types of blueberries with regard to breeding cultivars with comparable sugar, acid and phytochemical properties. There were significant negative correlations observed between fruit weight and SS, TPC, FRAP and TMAC, suggesting that as breeders have been selecting for larger fruit, they have inadvertantly selected for tarter fruit with lower antioxidant capacity. However, SS was correlated positively with FRAP and TMAC, indicating that cultivars can be developed that have high antioxidant capacity and anthocyanin content, combined with high sugar content.

1. Introduction

Blueberry consumption has steady increased over the last several decades, due to globalisation of the industry and perceived health benefits (Retamales and Hancock, 2012). To a large extent, the quality and storage life of fruit from a blueberry cultivar is related to its soluble solids content, acidity and ratio of soluble solids to acidity (Retamales and Hancock, 2012). Aromatic compounds are also likely to be an important component of fruit quality, but the most important ones associated with flavour have not been identified (Gilbert et al., 2013). Beaudry (1992) suggested that the highest quality blueberry fruit have pH values ranging from 2.25 to 4.25, acidity from 0.3 to 1.3% (w/w), soluble solids > 10% (w/w) and sugar: acid ratios between 10 and 33. Several studies have found considerable variation in these variables among highbush cultivars (Perkins-Veazie et al., 1995; Bremer et al., 2008; Hancock et al., 2008; Saftner et al., 2008).

Fruit quality in blueberries has also become associated with its levels of phenolics, flavonoids and overall antioxidant capacity (AOX). It has been shown that blueberries have among the highest AOX of all the fruits and vegetables, and cultivars vary greatly in their AOX (Prior et al., 1998; Kalt et al., 2001). A number of studies have compared the phytochemical content of blueberries and have found considerable levels of variability (Ehlenfeldt and Prior, 2001; Connor et al., 2002a; Rodrigues et al., 2011). Blueberries also carry significant levels of vitamin C, which have long been associated with healthy fruit. However, only a limited number of studies have compared different blueberry cultivars for vitamin C (Prior et al., 1998; Kalt et al., 1999).

To date, most studies of blueberry chemistry have focused on either the sugars and acids of blueberries, or the AOX properties of blueberry, but not both. Also, few studies have measured the vitamin C content of modern cultivars and compared the fruit biochemistry of the major types of cultivated blueberrries – southern highbush,

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northern highbush and rabbiteye. Herein, we describe cultivar variability for soluble solids, titratable acidity, vitamin C and AOX in a broad range of northern highbush, southern highbush and rabbiteye cultivars, grown in different locations and years. We include several of the newer, most widely planted northern highbush cultivars, which have not been evaluated previously. These comparisons allowed us to identify the cultivars with the most desirable combinations of characteristics, and determine how important environmental variability is in regulating fruit quality and phytochemical content. We were also able to determine whether cultivars can be bred that have both high antioxidant capacity and high sugar content, and whether northern highbush, southern highbush and rabbiteye cultivars can be developed with similar fruit quality attributes.

2. Materials and methods

2.1. Experiment I

This experiment was conducted to evaluate the effect of cultivar and season on fruit quality and phytochemical fruit characteristics of highbush blueberry and determine the correlation between individual components of fruit biochemistry. We were also interested in how these fruit quality and phytochemical characteristics have been altered through the decades by plant breeders. Fruits were harvested from 24 northern highbush blueberry cultivars grown at the Michigan Blueberry Growers (MBG) Marketing research plot near Grand Junction, Michigan, USA from 2010 to 2012. The fruits were picked from 3 to 5 plants of each cultivar when 30–40% of the fruit were fully ripe on the bushes. After harvest, the fruit was stored on ice for several hours and then transferred to a −20 °C freezer. The frozen fruit was then evaluated in the fall of 2012 for soluble solids (SS), titratable acidity (TA), SS/TA, pH, total phenolic content (TPC), ferric reducing ability of plasma (FRAP), total monomeric anthocyanin content (TMAC) and levels of vitamin C. The evaluated cultivars represented a diverse array of releases from all of the North American breeding programs from 1910 to the present.

2.2. Experiment II

In this experiment, we were interested in how much variation there was in the fruit quality and phytochemical fruit characteristics of highbush blueberry grown in Michigan and Oregon, and whether there was a significant genotype (G) × environmental (E) interaction. We were also interested in the correlation between individual components of fruit biochemistry. Fruit was picked in 2013 and stored as described in Experiment I from seven cultivars (*Vaccinium corymbosum* – Aurora, Draper, Duke, Elliott, Legacy, Liberty and Reka) in advanced trials at the MBG Research plots in Michigan and the Fall Creek Nursery and Farm research plots in Lowell, Oregon, and evaluated for the same fruit quality and phytochemical parameters described in Experiment I. The seven cultivars were selected as representatives of the most widely planted northern highbush cultivars.

2.3. Experiment III

This experiment was conducted to determine if rabbiteye and southern highbush cultivars vary significantly in their fruit quality and phytochemical properties. We were also interested in the levels of variation among cultivars within each group and the correlation between individual components of fruit biochemistry. Seven rabbiteye and 11 southern highbush blueberry cultivars were harvested as described above near Manor, Georgia, USA on the farms of Alex and Joe Cornelius, and analysed for the same fruit quality and phytochemical properties as the previous experiments. These cultivars represented some of the most widely planted rabbiteye (*Vaccinium virgatum*) and southern highbush cultivars (*V. corymbosum*), both newly released and established.

2.4. Experiment IV

This experiment was conducted to determine if southern highbush, northern highbush and rabbiteye cultivars varied significantly in their fruit quality and phytochemical properties. We were also interested in the levels of variation among cultivars within each group and the correlation between individual components of fruit biochemistry. Fruits were harvested from seven northern highbush, two rabbiteye and five southern highbush blueberry cultivars at Fall Creek Nursery and Farm research plots in Lowell, Oregon, USA. This site was selected as a location where all three types of blueberries are adapted and can be grown together for direct comparisons. The same fruit quality and phytochemical characteristics were determined as in the previous experiments, using the same harvesting and storage methods. As in Experiment III, these cultivars represented some of the most widely planted rabbiteye, southern and northern highbush cultivars, both newly released and established.

2.5. The analytical protocols

Berry weights were determined on separately collected samples of 50 berries. SS, pH, and TA were measured in each of three replicates, using juice extracted from 100 g fruit samples blended at high speed in a tissue homogenizer (Ultra Turrax T25; Janke and Kunkel Co., Staufen, Germany). SS was determined using a handheld refractometer (Westover Model RHB-32; Southwest United Industries, Tulsa, OK, USA). Results are reported in percent SS (w/w) on a fresh weight (fw) basis. TA was determined from 10 mL of juice diluted to 100 mL with distilled water, titrated with 0.1 N sodium hydroxide (NaOH) to pH 8.2, and expressed as percentage citric acid (w/w) on a fw basis. The SS to TA ratio (SS/TA) was calculated as an indicator of overall sweetness. The pH measurements were made using a digital pH meter.

TPC content was measured according to Singleton and Rossi (1965). Fruit slurries were extracted with buffer containing acetone, water, and acetic acid (70:29.5:0.5, v/v/v) for 24 h in the dark. Then, the extract, Folin–Ciocalteu's phenol reagent and water were incubated for 8 min followed by the addition of 7% sodium carbonate. After 2 h, the absorbance was measured by an automated UV–vis spectrophotometer (Ultrospec II; LKB Biochrom Ltd., Cambridge, UK) at 750 nm. Gallic acid was used as standard. The results were expressed as μg gallic acid equivalent in g fw basis (GAE/g fw).

TMAC was estimated by the pH differential method (Wrolstad, 1976; Giusti et al., 1999) using the UV–vis spectrophotometer. Absorbance was measured at 510 nm and 700 nm in buffers at pH 1.0 and 4.5 using

$$A = (A_{533} - A_{700})_{\mathrm{pH1.0}} - (A_{533} - A_{700})_{\mathrm{pH4.5}}$$

with a molar extinction coefficient of 29,600. Results were expressed as μg of cyanidin-3-glucoside equivalent on a g fw basis.

Total antioxidant capacity (TAC) was estimated by the FRAP procedure, as suggested by Ozgen et al. (2006). FRAP was determined according to the method of Benzie and Strain (1996). The assay was conducted using three aqueous stock solutions containing 0.1 mol/L acetate buffer (pH 3.6), 10 mmol/L TPTZ [2,4,6-tris(2-pyridyl)-1,3,5-triazine], acidified with concentrated hydrochloric acid (1000:3.3, v/v), and 20 mmol/L ferric chloride. These solutions were prepared and stored in the dark