Investigation of Anthocyanin Content and Antioxidant Activity in Blueberry Genotypes.

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Abstract

A concentrated fruit and vegetable diet is associated with lowering the risk of major health disorders, which include coronary heart disease, some forms of stroke and certain types of cancer. Antioxidant (AA) compounds (including flavonoids, phenolic compounds, and vitamins C or E) act as a protective agent in guarding against these health disorders. Anthocyanin (ACV) is a phenolic compound that has significant contribution to the elevated levels of AA in blueberries and they are confined mainly to the fruit skin. In this research, we wanted to evaluate the antioxidant activity among different genotypes of blueberries to find out which genotype has the highest activity of antioxidants. Experiments were conducted with six different blueberry genotypes; fully ripe fruits were collected from each plant and stored at -80°C until use. Genotypes were randomly selected, without prior knowledge of antioxidant activity or anthocyanin content. Fruits were weighed and counted in amounts of 10 grams. Anthocyanin was extracted from each genotype for two extracts using acidified methanol. Acidified methanol is a superior substance used for the extraction of anthocyanin. Extracts were stored at -80°C until antioxidant and anthocyanin assays were done to find out which genotype has higher antioxidants and to evaluate anthocyanin content for each. Our results have showed that genotype two had higher anthocyanin content and that genotype also has smaller fruit size. The research findings from this study will be helpful for developing blueberry varieties with higher levels of antioxidants.

INTRODUCTION

Blueberries (Vaccinium corymbosum) are rich in phenolic acids, mainly chlorogenic acid, and flavonoids due to their high anthocyanin content. They are one of the many fruit sources which have high antioxidant activity (AA) (Kalt et al., 1996; Prior et al., 1998). There is a positive effect of diet on human health and this has become of increasing interest to the scientific community and the public as more individuals are keeping close attention of their health to avoid chronic diseases (Esterbauer et al., 1992). Some studies have proved that one’s health is positively influence by the intake of antioxidants. Dietary antioxidants aid in lowering rates of mortality from coronary heart disease (Hertog et al., 1993, 1997; Knekt et al., 1996), lung cancer (Knekt et al., 1997), stroke (Keli et al., 1996), and nonfatal heart attack in smokers (Hirvenoja et al., 2001). Bioavailability of Anthocyanins may be increased by breeding blueberry fruits with higher amounts of AA. In our study, we examined six genotypes that were grown at DSU Outreach and Research Center in Smyrna DE. Fruits were collected from all six genotypes, the antioxidant and Anthocyanin contents were assayed to find out which genotype has higher AA. Understanding which genotypes have higher AA can aid in breeding blueberries with higher AA.

MATERIALS AND METHODS

i) Blueberry genotypes (1,2,3,4,5,6)
ii) Acidified Methanol (0.1% HCL)
iii) Spectrophotometer (BioTek Synergy HTX, Software: Gen5)
iv) Fruit Harvesting and storage
v) Fruit extraction using acidified methanol (0.1% HCL)
vi) Extraction storage until assayed
vii) Anthocyanin assay
viii) Anthocyanin content evaluation

RESULTS AND DISCUSSION

<table>
<thead>
<tr>
<th>Genotypes</th>
<th># of Fruits, weight: 10grams</th>
<th>Extraction 1</th>
<th>Extraction 2</th>
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<td>10</td>
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Fig 4. Showing six different genotypes and number of berries per 10grams.

Fig 2. Six Blueberry Genotypes before and after extraction.

Fig 3. Mortar and Pestle that was used to homogenize berries.

Fig 5. Graph showing the Anthocyanin content of six different genotypes of Blueberry.

The extracts were diluted 10:90 v/v, that is 10µl extract in 90µl acidified methanol. Total anthocyanin content was determined by measuring spectrophotometric absorbance at 530nm. Calculations were done to evaluate the true absorbance of each sample and the concentration was calculated. The concentration (C) was calculated using the formula:

\[ \text{Absorbance} (A) = \varepsilon \times L \times c \]

Where:
- \( \varepsilon \) is molar extinction coefficient (29,600 of anthocyanin)
- \( L \) is the path length of the cell holder (quivet) (0.1278cm)
- \( c \) is the concentration of the solution (mol dm⁻³)

From the graph above genotype two (2) had the highest concentration (0.019707736 mol dm⁻³) of anthocyanin while genotype four (4) had the lowest concentration (0.001142516 mol dm⁻³).

FUTURE DIRECTIONS

- Repeat the experiment but using heat stress to evaluate other genotypes for Antioxidant activity, Phenolic content and Anthocyanin content.

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