Bioactive Compounds and Antioxidant Activity of Blueberry (*Vaccinium ashei* Reade)

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Abstract
The correlation of fruits and vegetables consumption and good health is well established; however, there are many fruits that need to be studied considering the environmental conditions where they grow. Blueberry is one of the most important small fruit studied in the world; however, little is known about the blueberry cultivated in Brazil. There is an incentive to produce blueberry in the southern region of Brazil, mainly cultivars from the rabbiteye group, due to their rusticity and climate adaptation. It is common to observe papers about blueberry and the health benefits in the international journals; however, little is known about the blueberry produced in Brazil. This study had the aim of to determine the content of total phenolic, anthocyanins and antioxidant activity of blueberry cultivars from the rabbiteye group. Four blueberry cultivars were analyzed (‘Climax’, ‘Powder Blue’, ‘Florida’ and ‘Alice Blue’). The blueberry genotypes were harvested at Embrapa Clima Temperado’s field and transported to the Food Science and Technology lab to be analyzed for their total content of phenolics, anthocyanins and antioxidant activity. Total phenolic content was measured using the Folin-Ciocalteau reagent mix. Anthocyanins were measured by the pH difference method. Antioxidant activity was measured using the stable radical DPPH. Regarding the results, the cultivar ‘Alice Blue’ had the highest phenolic content and antioxidant activity among the analyzed cultivars. The anthocyanin content was superior in the ‘Climax’ cultivar. Selected blueberry genotypes had equal or greater phenolic and antioxidant activity than blueberry cultivated in North America. The level of total phenolic did not correlate with the antioxidant activity in the studied blueberry cultivars. The levels of bioactive compounds varies within the blueberry genotypes examined indicating that breeding and selection could be done to develop cultivars with improved levels of these chemical substances.

INTRODUCTION

The red-flesh fruits, such as blueberry, have different groups of phytochemicals that can bring benefits to health if consumed as part of the usual diet. Epidemiological studies have shown evidence that the consumption of these fruits is linked to the prevention of chronic non-transmissible diseases (Hertog et al., 1995), mainly due to the presence of bioactive phytochemical compounds such as phenolic acids, flavonoids and carotenoids (Feldman, 2001; Shahidi and Naczk, 2004). There are several factors that can affect the content of phenolic compounds in blueberries such as the maturity stage of the fruits, genetic differences (cultivars), environmental conditions and processing (Kalt et al., 1999; Deighton et al., 2000;
Hakkinen and Torronen, 2000; Wang and Lin, 2000; Connor et al., 2002; Siriwoharn et al., 2004). In Brazil, mainly rabbiteye cultivars are grown, which are better adapted to the weather conditions, since they are more rustic; however, the fruits are of inferior quality compared to the highbush group cultivars, which is mainly cultivated in the USA. When comparing leaves from the two groups it can be observed that cultivars belonging to the rabbiteye group have higher antioxidant activity, and also, antiviral activity (Akamatsu et al., 2006). Also, there is a good correlation between concentrations of phenolic compounds, anthocyanins and antioxidant capacity in blueberries (Prior et al., 1998; Hakkinen et al., 1999; Taruscio et al., 2004).

The objective of this study was to characterize four blueberry cultivars from the rabbiteye group (‘Climax’, ‘Powder Blue’, ‘Florida’ and ‘Alice Blue’) related to its content of total phenolic, anthocyanins and antioxidant activity.

**MATERIALS AND METHODS**

**Sample Preparation**

The blueberry genotypes were harvested at Embrapa Clima Temperado’s field and transported to the Food Science and Technology lab where they were stored at -18°C until further analysis. Before homogenization of samples, they were selected by size and color. For the phenolic and antioxidant activity analysis five grams were used of samples taken from the equatorial part of the fruit. Samples were always prepared in four replications and homogenized in an ultraturrax homogenizer set to small samples, and centrifuged at 15,000 rpm and temperature of 4°C. After sample preparation, total phenolic compounds and antioxidant activity were assayed.

**Total Phenolic Compounds**

The method used was adapted from Swain and Hillis (1959). Shortly, to each falcon tube were added: 250 ul of sample, 4 ml of ultrapure water and 250 ul of Folin-Ciocalteau (0.25 N) reagent. The sample was agitated vigorously and allowed to react for 3 minutes. 500 µl of sodium carbonate (1 N) was added and samples were incubated at room temperature for 2 hours. Absorbance was measured at a spectrophotometer previously blanked at 725 nm.

**Antioxidant Activity**

The method used was adapted from Brand-Williams et al. (1995). In summary, 200 µl of sample was added to 3800 µl of diluted DPPH in 15-ml Falcon tubes. Tubes were agitated and let to react for 24 h. The spectrophotometer was blanked with methanol and the absorbance was read at 525 nm.

**Total Anthocyanins**

The method used was adapted from Fuleki and Francis (1968). In summary, 5 g of sample was homogenized with acidified ethanol in an ultraturrax homogenizer, and centrifuged at 15,000 rpm and temperature of 4°C. One milliliter of sample was added to 24 ml of acidified ethanol in 15-ml Falcon tubes. The spectrophotometer was blanked with acidified ethanol and the absorbance was read at 535 nm.

**RESULTS AND DISCUSSION**

The relative contents of phenolic compounds among the blueberry cultivars studied varied considerably (Table 1). The cultivar ‘Alice Blue’ showed the highest phenolic content (1,250.29 mg of chlorogenic acid equivalent/100 g fresh weight) among all the studied cultivars. The cultivar ‘Powder Blue’ had a similar content (1,092.60 mg of chlorogenic acid equivalent/100 g fresh weight), however, it was statistically inferior. The cultivars ‘Climax’ and ‘Florida’ presented about half and a third of the phenolic content presented in ‘Alice Blue’, 745.19 and 573.93 mg of chlorogenic acid equivalent/100 g fresh weight, respectively.
The anthocyanin content was superior in ‘Climax’ (1,418.67 mg cyanidin-3-glucose equivalent/100 g fresh weight). The cultivars ‘Powder Blue’ and ‘Alice Blue’ presented half of the anthocyanin content presented in ‘Climax’. The cultivar ‘Florida’ presented the lower anthocyanin content that corresponds to about five times less than ‘Climax’.

The cultivar ‘Alice Blue’ has shown the highest antioxidant activity (13,337.85 µg of trolox equivalent/g fresh sample) followed by the cultivars ‘Florida’ and ‘Climax’. The lower antioxidant activity was observed in the cultivar ‘Powder Blue’ (9,835.74 µg of trolox equivalent/g fresh sample).

Besides the levels of total phenolic did not correlate with antioxidant activity in the studied blueberry cultivars (Fig. 1), the cultivar ‘Alice Blue’ presented both, the highest phenolic content and antioxidant activity. Since there was no correlation between total phenolic content and antioxidant activity, a correlation between total anthocyanin content and antioxidant activity was tried; however it did not correlate well (Fig. 2).

CONCLUSIONS

These results are of great importance to Brazil, where the cultivars of the rabbiteye group are the most produced. It shows that, despite some problems of fruit quality, they have specific characteristics, such as phytochemical contents and antioxidant activity in higher levels than high bush cultivars, which facilitates the “marketing” of the product. Also, the levels of bioactive compounds varies within the blueberry genotypes examined indicating that breeding and selection could be done to develop cultivars with improved levels of these chemical substances.

ACKNOWLEDGEMENTS

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Literature Cited


**Tables**

Table 1. Total phenolic and anthocyanin content and total antioxidant activity of blueberry cultivars. Embrapa Clima Temperado, Pelotas, RS, Brazil, 2010.

<table>
<thead>
<tr>
<th>Cultivars</th>
<th>Phenolic compounds$^1$</th>
<th>Anthocyanins$^2$</th>
<th>Antioxidant activity$^3$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Climax</td>
<td>745.19±75.24 c</td>
<td>1418.67±154.61 a</td>
<td>11514.40±506.62 b</td>
</tr>
<tr>
<td>Powder blue</td>
<td>1092.60±67.90 b</td>
<td>723.61±56.07 b</td>
<td>9835.74±1084.79 c</td>
</tr>
<tr>
<td>Florida</td>
<td>573.93±42.15 d</td>
<td>311.52±71.81 c</td>
<td>11885.08±2026.47 b</td>
</tr>
<tr>
<td>Alice Blue</td>
<td>1250.29±68.88 a</td>
<td>704.74±115.08 b</td>
<td>13337.85±747.00 a</td>
</tr>
</tbody>
</table>

Data are means of four replications ± standard deviation. Different letters in the column shows significant statistical difference (p<0.005).

$^1$ Total phenolic compound in mg chlorogenic acid equivalent/100 g fresh weight.

$^2$ Total anthocyanins in mg cyanidin-3-glucose equivalent/100 g fresh weight.

$^3$ Total antioxidant activity in µg trolox equivalent/g fresh weight.
Figures

Fig. 1. Correlation between total phenolics content and total antioxidant activity of four blueberry cultivars. Embrapa clima Temperado, Pelotas, RS, Brazil, 2010.

Fig. 2. Correlation between total anthocyanin content and total antioxidant activity of four blueberry cultivars. Embrapa clima Temperado, Pelotas, RS, Brazil, 2010.