An approach towards rapid optical measurements of antioxidant activity in blueberry cultivars*

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Summary
Blueberries are well known for their high antioxidant levels. Compared to bilberries (V. myrtillus) with higher antioxidant activity and more intensive blue colour throughout the whole berry, highbush blueberries have the blue pigments concentrated in the skin. Highbush blueberry skin is found to contain a very high content of phenolic compounds. To measure the total antioxidant activity in blueberries, several methods, mostly destructive, including the FRAP assay, have been used. This work is an initial approach towards a simple and rapid method, combining optical and antioxidant activity measurements.

Materials and methods

Plant material
Highbush blueberry cultivars ‘Bluecrop’, ‘Hardyblue’, ‘Patriot’ (V. corymbosum), and lowbush cultivars ‘Putte’ (a hybrid originated from V. angustifolium) and ‘Aron’ (V. corymbosum x V. uliginosum) were grown at the Norwegian University of Life Sciences (59° 40’N). Berries were harvested at commercial blue-ripe stage of maturity. Fresh berries were cut horizontally and placed on a scanner in order to examine berry size and skin thickness. Berries were weighed, and analysed for antioxidant activity using the FRAP (Ferric Reducing Ability of Plasma) assay. The FRAP assay is a non-specific method based on absorption changes following a reduction of a ferric- to a ferrous-complex in the presence of antioxidants.

Scanning
The scanner combined with statistics and image analysis makes an excellent toolbox to measure interesting physical quality attributes on berries (HAFFNER et al., 2000), and is applied here on highbush blueberries. Fresh berries were cut horizontally and placed on a scanner (Agfa Snapscan 1212u) and scanned using Scanwise software (Scanwise 1.04). Berry and flesh diameter (Fig. 1) were measured at two positions on each berry using an image-processing program (Adobe Photoshop 7.0). Of each cultivar, a total of 10 berries in three replicates were measured. These measurements were used to calculate skin thickness, berry and skin cross-section area of each cultivar.

FRAP assay
The plant material was homogenised, and 3 g homogenate was dissolved in 30 ml methanol. Bottles were flushed with nitrogen before closing, and the samples were mixed and sonicated on a water-bath at 0 °C for 15 min. The extracts were stored at –20 ºC. Samples of 1.5 ml were centrifuged at 12,000 x g for 2 min at 4 ºC. The concentration of antioxidants in the supernatant was measured in triplicate. FRAP values were determined in extracts (BENZIE and STRAIN, 1996), with the exception that the samples were not diluted with water (HALVORSEN et al., 2002). A Technicon RA 1000 system was used for the measurements of absorption changes at 600 nm that appear when the TPTZ-Fe³⁺ complex is reduced to the TPTZ-Fe²⁺ form in the presence of antioxidants. An aqueous solution of 500 mM FeSO₄ x 7 H₂O was used for calibration of the instrument.

Data analysis
One-way analysis of variance (ANOVA) was applied to test differences between the cultivars on the different physical measurements, berry weight, and antioxidant activity. To make a prediction model of antioxidant activity based on physical measurements, a PLS (Partial Least Square)-model was applied using The Unscrambler 9.0 by Camo.

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Results and discussion

The most important result, as evaluated by means of Partial Least Square Regression (PLSR) (Martens and Næs, 1989), is shown in Fig. 2, where ‘Patriot’ and ‘Bluecrop’ have larger skin area and lower FRAP values compared to ‘Hardyblue’ and ‘Putte’. The other variables (berry weight, berry diameter, flesh diameter, berry circumference and berry cross-section area) are clustered at the left side in the PLS plot. This indicates that these are highly correlated. ‘Aron’ did not respond as a significant variable in the PLS model. This is implied by the fact that the variables measured and calculated did not correlate significantly compared to the other cultivars, with the exception of skin thickness and FRAP-values.

The ANOVA showed a great variation between the different cultivars (Tab. 1). All the cultivars were significantly different regarding berry weight. ‘Patriot’ had the largest berries, followed by ‘Bluecrop’ and ‘Hardyblue’, while ‘Putte’ and ‘Aron’ had the smallest. Tab. 2 shows correlations between antioxidant activity and physical measurements. Berry weight, diameter, circumference and cross-section area had significant negative correlations to antioxidant activity. A relationship between berry weight and FRAP-value was calculated to \( r = -0.664 \), indicating that smaller berries have higher FRAP-values. A schematic outline between berry weight and antioxidant activity is shown in Fig. 3. Antioxidant activity (solid bars) and berry weight (lines) are negatively correlated, with a higher antioxidant activity in small berries. Concerning the antioxidant activity, ‘Putte’ had highest values, followed by ‘Hardyblue’, ‘Aron’, ‘Patriot’ and ‘Bluecrop’. This confirms the results by Remberg et al. (2003), where ‘Putte’, ‘Hardyblue’ and ‘Aron’ were found to have significantly higher FRAP-values than ‘Bluecrop’.

Fig. 1: Cultivated blueberries ‘Putte’ (a) and ‘Patriot’ (b) cut horizontally, placed on a scanner and measured at two positions (marked with a black cross in picture b on the top left).

Fig. 2: PLS plots presenting variables and samples. PA=’Patriot’, BL=’Bluecrop’, HB=’Hardyblue’, PU=’Putte’, numbers indicate cultivar replicates. Variables clustered together are indicated by a filled circle (berry weight, berry diameter, flesh diameter, berry circumference and berry cross-section area).
Tab. 1: Effects of blueberry cultivar on berry weight, physical variables in berry cross-sections and antioxidant activity.

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>Berry weight (g)</th>
<th>Berry diameter (mm)</th>
<th>Berry circumference (mm)</th>
<th>Skin thickness (mm)</th>
<th>Berry cross-section area (mm²)</th>
<th>Skin cross-section area (mm²)</th>
<th>FRAP (mmol/100g FW)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aron</td>
<td>0.77e</td>
<td>11.72c</td>
<td>11.31c</td>
<td>0.62</td>
<td>108.85c</td>
<td>7.48b</td>
<td>3.03abc</td>
</tr>
<tr>
<td>Bluecrop</td>
<td>2.94b</td>
<td>19.88a</td>
<td>19.52a</td>
<td>0.54</td>
<td>312.17a</td>
<td>11.14ab</td>
<td>2.28c</td>
</tr>
<tr>
<td>Hardyblue</td>
<td>1.57c</td>
<td>15.52b</td>
<td>15.13b</td>
<td>0.60</td>
<td>190.76b</td>
<td>9.61ab</td>
<td>3.40ab</td>
</tr>
<tr>
<td>Patriot</td>
<td>3.34a</td>
<td>20.70a</td>
<td>20.30a</td>
<td>0.60</td>
<td>337.31a</td>
<td>12.89a</td>
<td>2.81bc</td>
</tr>
<tr>
<td>Putte</td>
<td>1.08d</td>
<td>14.36b</td>
<td>13.97b</td>
<td>0.59</td>
<td>163.31b</td>
<td>8.79b</td>
<td>3.94a</td>
</tr>
</tbody>
</table>

Mean: 1.94 16.44 16.04 51.61 0.59 222.48 9.98 3.09

Level of significance: *** *** *** *** ns *** *** ***

*** = p < 0.001, ns = non significant

Numbers with different letters are significantly different

For most of the physical measurements, ‘Patriot’ had the highest, while ‘Putte’ and ‘Aron’ had the lowest values. ‘Aron’ had slightly thicker skin compared to the other cultivars analyzed. The skin thickness was measured in this experiment using a scanner and an image-processing program. Using light microscopy to study cell walls and epidermal pigment of three *V. corymbosum* cultivars, ALLAN-WOJTAS et al. (2001) found differences between cultivars in pigment distribution. While the epidermis of ‘Burlington’ and ‘Elliot’ consisted of two layers with pigments, ‘Coville’ epidermis consisted of three layers.

‘Aron’ and ‘Putte’ had significantly smaller cross-section skin area than ‘Bluecrop’ and ‘Patriot’ due to lower berry diameter. No literature was found discussing physical measurements in blueberries and antioxidant activity. KALT et al. (2001) analyzed 80 highbush and 135 lowbush blueberry clones for berry weight, anthocyanins and total phenolics, and found no relationship between fruit weight and anthocyanin content. Berry weight and size are in the literature used synonymously (ECK, 1988), and blueberry shape varies between cultivars (KEIPERT, 1981). Predicting a spherical berry shape, this work confirms that berry size measured as diameter is highly correlated with berry weight (r=0.981).

LEE and WROSLAND (2004) found highest antioxidant activity measured as FRAP and ORAC (Oxygen Radical Absorbing Capacity) in blueberry skin compared to flesh and seeds. In our experiments, skin thickness had no influence on antioxidant activity, but high correlations between berry size/weight and FRAP values were found. Scanning was an excellent tool to confirm our findings.

Conclusions

For spherical shaped blueberries, berry weight and/or diameter measurements can be used to estimate antioxidant activity, measured as FRAP. This work indicates that non-destructive measurements can be used to predict health-promoting compounds in cultivated blueberries.

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