Journal of Applied Botany and Food Quality 80, 36 - 39 (2006)

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

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(Received January 2, 2006)

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.

Highbush blueberry (*V. corymbosum*) cultivars 'Bluecrop', 'Hardyblue', 'Patriot', 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 (59º 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.

Own previous results have shown that antioxidant activity and berry weight varied between cultivars (REMBERG et al., 2003). Small berries had higher antioxidant activity compared to larger berries. In this follow-up project, skin thickness and berry diameter were measured by using an image- processing program. Berry and skin cross-section areas were correlated with the antioxidant activity.

Introduction

Blueberries are good sources for antioxidants (HALVORSEN et al., 2002), and the antioxidant activity has been found to correlate well with the total phenolic and anthocyanin content of the fruits (EHLENFELDT and PRIOR, 2001). The skin of highbush blueberries contains a very high content of phenolic compounds, and in highbush blueberries, pigments are concentrated in the skin (ALLAN-WOJTAS et al., 2001; KALT et al., 2001). Since pigments and phenolic compounds in cultivated blueberries are confined primarily to the skin (LEE and WROLSTAD, 2004), berry size is expected to be an important factor as related to antioxidant activity. Examining highbush (*V. corymbosum*) and lowbush (*V. angustifolium*) blueberry cultivars, KALT et al. (2001) found that the smaller lowbush blueberries were consistently higher in anthocyanins, total phenolics, and antioxidant activity.

The aim of the present investigation was to predict antioxidant activity by measuring physical attributes in blueberry cultivars. 'Bluecrop', 'Hardyblue' and 'Patriot', being important cultivars in Norway, are mainly produced for the fresh marked. The Swedish cultivar 'Putte' and the Finnish cultivar 'Aron' are considered to be especially winter hardy, and therefore suitable for Scandinavia. The berries of these two cultivars are rather small with a dark blue colour.

In previous studies, three methods have been used to assess total antioxidant activity in plants. As concluded in HALVORSEN et al. (2002), the FRAP assay was also chosen in these experiments.

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 (59º 40'N) as a randomized block trial with four replicates of each cultivar. The field was planted in 1990 ('Putte' in 1996). The berries were harvested by hand in the season 2001 at commercial blue-ripe stage of maturity. Berry weight, diameter, skin thickness and antioxidant activity (FRAP) were determined.

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-Fe^{3+} complex is reduced to the TPTZ-Fe^{2+} form in the presence of antioxidants. An aqueous solution of 500 mM $FeSO_4$ x 7 H_2O 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.

* This publication was presented as a poster on the COST Action 924 Working Group Meeting; „Non-Destructive Methods for Detecting Health-Promoting Compounds" (REMBERG et al., 2005).

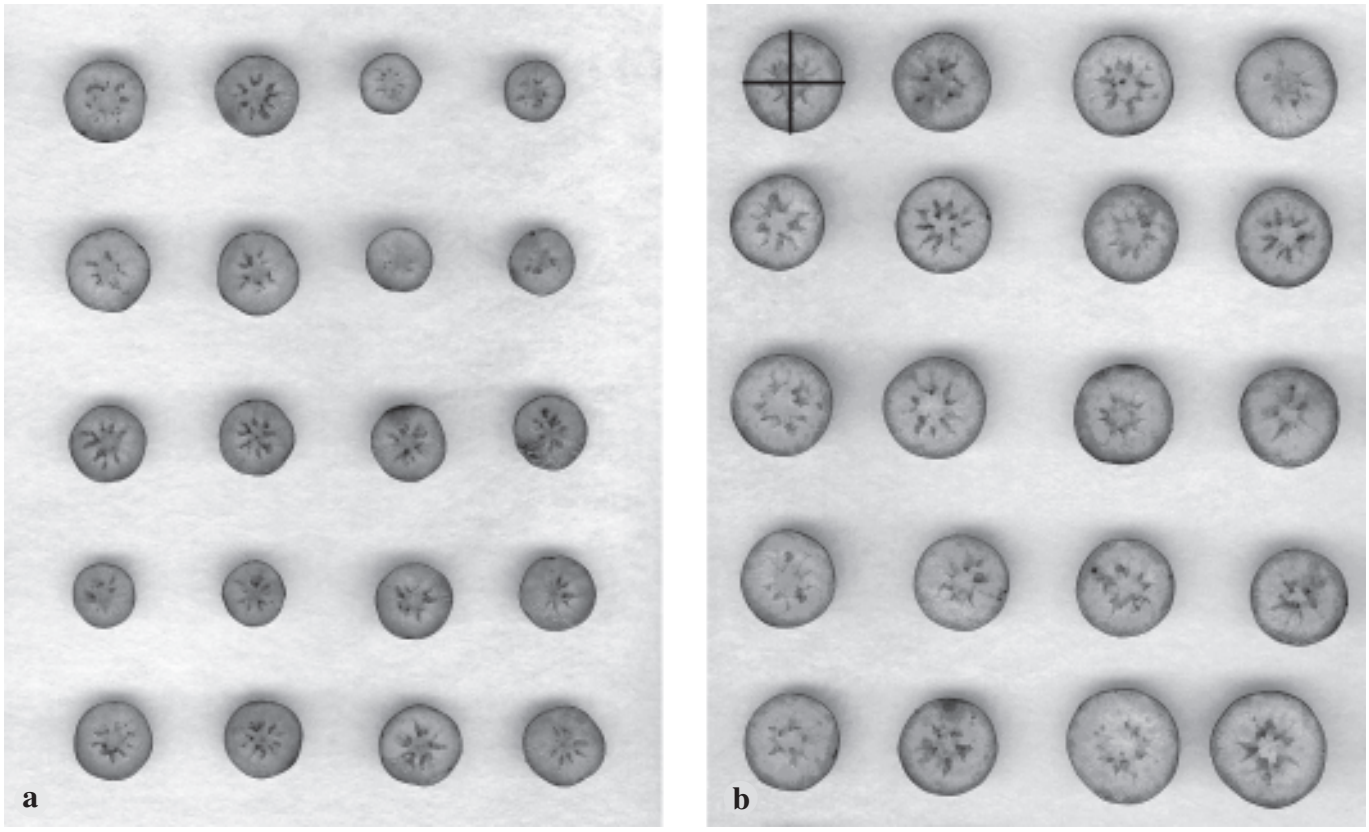

Fig. 1: Cultivated blueberries 'Putte' (a) and 'Patriot' (b) cut horizontally, placed on a scanner and measured at two positions (marked with a black cross in picture b on the top left).

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'.

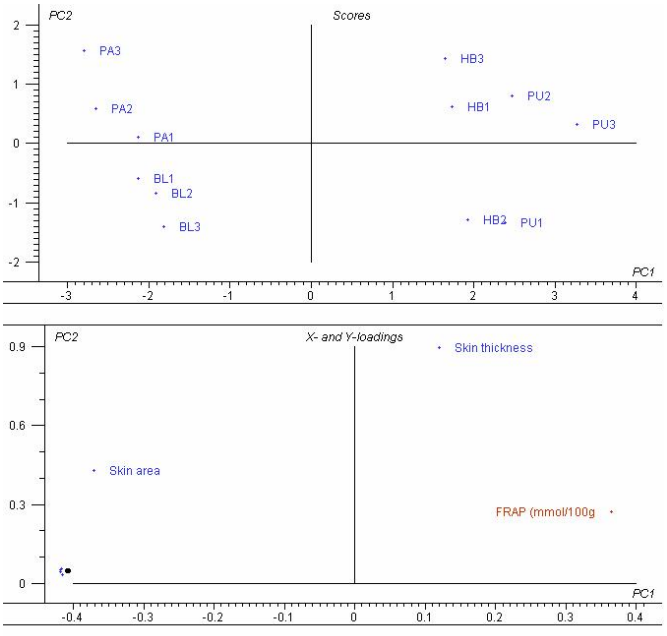

Fig. 2: PLS plots presenting variables and samples. PA = 'Patriot', BL = 'Bluecrop', HB = 'Hardyblue', PU = 'Putte', numbers indicate cultivar replicates. Variables clustered together are indicated by a filled circle (berry weight, berry diameter, flesh diameter, berry circumference and berry cross-section area).

Tab. 1: Effects of blueberry cultivar on berry weight, physical variables in berry cross-sections and antioxidant activity.

Cultivar	Berry weight (g)	Berry diameter (mm)	Flesh diameter (mm)	Berry circumference (mm)	Skin thickness (mm)	Berry area (mm^2)	Skin area (mm^2)	FRAP (mmol/100g FW)
Aron	0.77e	11.72c	11.31c	36.81c	0.62	108.85c	7.48b	3.03abc
Bluecrop	2.94b	19.88a	19.52a	62.41a	0.54	312.17a	11.14ab	2.28c
Hardyblue	1.57c	15.52b	15.13b	48.74b	0.60	190.76b	9.61ab	3.40ab
Patriot	3.34a	20.70a	20.30a	64.98a	0.60	337.31a	12.89a	2.81bc
Putte	1.08d	14.36b	13.97b	45.09b	0.59	163.31b	8.79b	3.94a
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

Tab. 2: Correlations between antioxidant activity (FRAP) and berry weight, berry diameter, berry circumference, skin thickness, berry and skin cross-section area.

	Antioxidant activity (FRAP)	
	r	p-value
Berry weight (g)	-0.664	0.007
Berry diameter (mm)	-0.582	0.023
Berry circumference (mm)	-0.582	0.023
Skin thickness (mm)	0.439	0.101
Berry cross-section area (mm^2)	-0.615	0.015
Skin cross-section area (mm^2)	-0.452	0.090

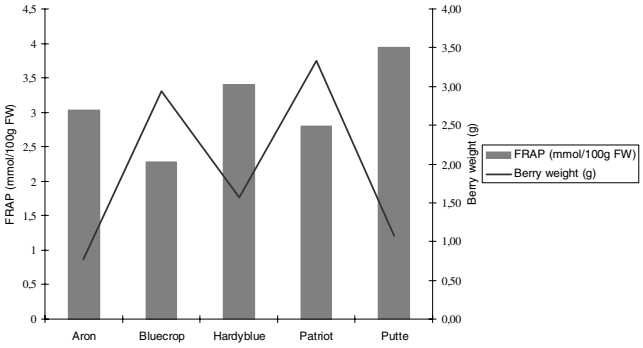

Fig. 3: Relationship between berry weight and antioxidant activity (FRAP).

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 on 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 WROLSTAD (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.

Acknowledgements

The authors wish to thank Signe Hansen and Kari Grønnerød for technical assistance, Sigbjørn Vestrheim for carefully reviewing the manuscript, and The Norwegian Research Council for financing the work.

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