

## METHODS TO DETERMINE ANTIOXIDATIVE CAPACITY IN FRUIT

Eivind Vangdal<sup>1</sup> and Rune Slimestad<sup>2</sup>

<sup>1</sup> Planteforsk Ullensvang Research Centre, NO-5781 Lofthus, NORWAY  
e-mail: eivind.vangdal@planteforsk.no

<sup>2</sup> Plantchem, P.O. Box 3082, Ganddal, NO-4392 Sandnes, NORWAY  
e-mail: rune@plantchem.org

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### A B S T R A C T

Anthocyanins content, total phenolics content and antioxidative capacity were measured in nine sweet cherry cultivars and nine plum cultivars. In the sweet cherry cultivars, the mean anthocyanins content was 35 mg/100 g FW, total phenolics content ranged from 23 to 168 mg/100 g FW, and antioxidative capacities (FRAP) ranged from 0.44 to 2.67 mmol/100g FW. In the plum cultivars, anthocyanins content ranged from 0.7 to 10.8 mg/100 g FW with a mean of 3.5 mg/100 g FW, total phenolics content ranged from 27 to 54 mg/100 g FW, and antioxidative capacity ranged from 0.61 to 1.28 mmol/100 g FW. There were strong correlations between antioxidative capacity (FRAP) and anthocyanins content and between antioxidative capacity and phenolics content. Antioxidative capacity is an important fruit quality parameter. However, the methods used to measure antioxidative capacity vary widely and often give conflicting results. A large part of the discrepancy is due to how the samples are prepared and extracted before testing.

**Key words:** plums, *Prunus domestica*, sweet cherries, anthocyanins, phenolics, antioxidants, FRAP

### INTRODUCTION

Consumer attention has recently been focused on the health-promoting properties of fruits and vegetables, which reduce the risk of several serious chronic diseases, such as cancer, coronary heart disease and type II diabetes (World Cancer Research Fund, 1997). Antioxidants from dietary plants play

an important role. As can be seen in Table 1, fruits and berries are an important source of antioxidants in the Norwegian diet (Halvorsen et al., 2002).

Table 1. The contribution of different plant foods of the total intake of plant antioxidants in a Norwegian diet (Halvorsen et al., 2002)

Plant food	Contribution [%]
Fruits	44
Berries	27
Cereals	12
Vegetables	9

A biological antioxidant has been described as any substance that, when present in low concentrations when compared to the concentration of an oxidizable substrate, significantly delays or prevents oxidation of that substrate (Halliwell, 1990). The chemical and biological mechanisms by which antioxidants act have been reviewed by Frankel and Meyer (2000).

A lot of studies have been carried out to show that particular compounds in fruits possess antioxidant properties, some successful, others not (Halvorsen et al., 2002). Nevertheless, the advertising media constantly bombard us with the message that our bodies need antioxidants to battle damaging free radicals.

Antioxidants are also relevant in post-harvest research because they play an important role in the natural defence of fruit (De Gara et al., 2003; Hodges et al., 2004). After all, plants do not produce antioxidants to improve the health of the humans eating them, but to protect themselves from pests and diseases.

Even though horticulturists are not specialists in chemistry and nutrition, the content of antioxidants in horticultural products should be an important part of horticultural research (Tomas-Barberan and Espin, 2001). Fruit quality researchers have to add a new quality parameter, antioxidative capacity, to the list of quality parameters such as size, color, flavour, soluble solids content and titratable acidity.

Unfortunately, different methods are used to determine antioxidative capacity in fruit. Frankel and Meyer (2000) state: 'There is a great need to standardize antioxidant testing to minimize the present chaos in the methodologies used to evaluate antioxidants'.

## MATERIAL AND METHODS

### Plant material

Fruit samples were picked in the experimental orchard at the Planteforsk Ullensvang Research Center in Western Norway in 2002. Samples of three to five fruits were immediately frozen after harvest at -20°C, and the frozen samples were transported to the laboratory. Anthocyanins content, total

phenolics content and antioxidative capacity were measured in nine sweet cherry cultivars and nine plum cultivars.

### **Extraction**

Samples of approximately 20 g of fruit were weighed. Each sample was extracted with 100 ml 0.5% HCl in methanol for twenty four hours at room temperature in the dark. For some sweet cherry cultivars, five single fruits were extracted to determine the magnitude of variation between individual fruits of the same cultivar. The fruits were extracted whole after carefully removing the stones. After extraction, the fruits were crushed and the juice was filtered and mixed with the extract. In plums, three fruits of each cultivar were analyzed.

### **Total anthocyanins**

The pH-differential method is described by Guisti and Wrolstad (2001). 500  $\mu$ l of the extract was diluted in 4.5 ml of two buffers, pH 1.03 and pH 4.52. Absorption at 520 nm and 700 nm were measured, and anthocyanins content was recorded as equivalents of cyanidin-3-glucoside according to the equation:

$$A = (A_{520 \text{ nm}} - A_{700 \text{ nm}})_{\text{pH } 1.03} - (A_{520 \text{ nm}} - A_{700 \text{ nm}})_{\text{pH } 4.52}$$

### **High-performance liquid chromatography (HPLC)**

HPLC was used to identify individual components of the total anthocyanins. A liquid chromatograph (Agilent 1100-system, Agilent Technologies) equipped with an autosampler and a photodetector was used to fractionate anthocyanins and phenolics. An Eclipse XDB-C8 (4.6 x 150 mm, 5  $\mu$ m) column (Agilent Technologies) was used with a binary solvent system consisting of (A) 0.05% TFA in water and (B) 0.05% TFA in acetonitrile. The gradient of B in A was linear from 5 to 10 in 5 min, from 10 to 20 for the next 5 min, from 20 to 85 in 8 min, from 85 to 5 in 2 min, and finally recondition of the column by 5% in 2 min. The flow rate was 0.8 mL/min. Sample size was 10  $\mu$ L. Fractionation was carried out at 30°C. Neochlorogenic acid and *p*-coumaroylquinic acid were detected at 320 nm, and anthocyanins were detected at 520 nm. All HPLC-samples were filtered through a 13 mm syringe filter (Nylon 0.45  $\mu$ m, VWR International) before injection. Standards were supplied by PlantChem, Norway.

### **Total phenolics**

Analysis was performed according to Price and Butler (1977), Graham (1992) and Hagerman (2002). 100  $\mu$ l of extract was diluted with 3 ml water and 1 ml of each reagent was added. The reaction was terminated after 15 min. Gallic acid served as the standard.

### Antioxidative capacity (FRAP)

The FRAP method (Ferric Reducing Ability Plasma) was used to determine the antioxidative capacity in the extracts according to Benzie and Strain (1996). Results were expressed as equivalents of Fe(II).

### Statistical analysis

Data were statistically elaborated using Microsoft Excel.

## RESULTS AND DISCUSSION

### Anthocyanins content

In the sweet cherry cultivars, anthocyanins content ranged from 0.6 to 120.6 mg/100 g FW (Tab. 2). In the plum cultivars, anthocyanins content were lower and ranged from 0.7 to 10.8 mg/100 g FW. Anthocyanin content was highest in cultivars with dark red juice and lowest in cultivars with pale yellow juice. All of the plum cultivars included in this study had yellow flesh.

Table 2. Anthocyanins content, total phenolics content and antioxidative capacity (FRAP) in sweet cherries and plums

	Sweet cherries		Plums	
	mean of nine cultivars	range	mean of nine cultivars	range
Anthocyanins [mg/100 g FW]	35.0	0.56-120.6	3.5	0.7-10.8
Total phenolics [mg/100 g FW]	71	23-168	38	27-54
Antioxidative capacity (FRAP) [mmol/100 g FW]	1.34	0.44-2.67	0.85	0.61-1.28

The sweet cherry cultivar ‘Van’ was the only cultivar included both in this study and in the study by Gao and Mazza (1995). Anthocyanins content in ‘Van’ was 31 mg/100 g FW in our study and 150 mg/100 g FW in their study. The difference may be due to differences in growing conditions and extraction methods. Mozetic et al. (2002) used an extraction procedure similar to the method we used and found that anthocyanins content ranged from 29 to 62 mg/100 g FW, which agrees well with our results.

Four different methods of extracting anthocyanins are presented in Table 3. Different homogenation methods are used. Extraction time ranges from eight seconds to twenty four hours. Extracts are clarified by filtration or centrifugation. Connor et al. (2002) extracted for 24 hours in a dark room. In

most of the methods described, light is not mentioned, so the extractions were presumably performed in normal room light. Data from different studies are difficult to compare because the extraction methods used were very different. Standardized methods need to be worked out.

Table 3. Four different methods for sample preparation and extraction of compounds with antioxidant properties in fruit

Storage of fruit samples	Homogenization method	Extraction medium	Extraction time/temp.	Reference
Freezer (-18°C)	Whole fruit crushed just before analysis after carefully removing the stone	80% acid MeOH	24 h /room temperature	Vangdal et al. (2005)
Freezer (-38°C)	Waring Blender	70% acid MeOH	6 min /15°C	Gao and Mazza (1995)
Cold storage	Food Processor (water added if necessary)	90% MeOH	15 min /0°C	Halvorsen et al. (2002)
Liquid nitrogen (-196°C)/Freezer (-10°C)	Frozen sample ground in a Bosch coffee mill for 15 seconds	Acid MeOH	24 h /room temperature	Slimestad and Verheul (2005)

### Total phenolics content

In the sweet cherry cultivars, total phenolics content ranged from 23 to 168 mg/100 g FW (Tab. 2). The main colourless phenolics were neochlorogenic acid and coumaroylquinic acid.

The phenolics contents in this study were low compared to those reported by Goncalves et al. (2004). 'Van' was the only cultivar common to both studies. Total phenolics content in 'Van' was 75 g/100 g FW in our study and 124 mg/100 g FW in their study. Phenolics content is also affected by weather conditions, and therefore varies widely from year to year (Goncalves et al., 2004).

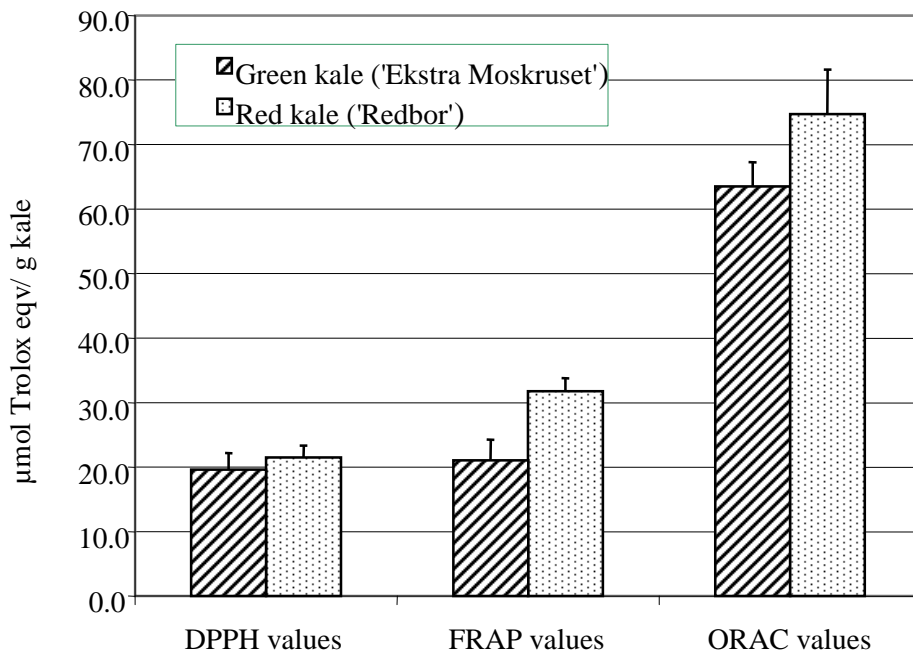
In the plum cultivars, total phenolics content ranged from 27 to 54 mg/100 g FW (Tab. 2). Gil et al. (2002) reported that phenolics content ranged from 42 to 109 mg/100 g FW in five plum cultivars, which is slightly higher than our results. Even higher results were reported by Kim et al. (2003), who reported that total phenolics content ranged from 174 to 375 mg/100 g FW in six plum cultivars. Different extraction methods were used in these studies.

### Antioxidative capacity

In the sweet cherry cultivars, antioxidative capacity (FRAP) ranged from 0.44 to 2.67 mmol/100 g FW. Antioxidative capacity was higher in cultivars with dark fruits. Halvorsen et al. (2002) reported that antioxidative capacity was 0.62 mmol/100 g FW for three samples of sweet cherry grown in the USA, and 1.42 mmol/100 g FW for three samples grown in Norway, which agrees well with our results.

In the plum cultivars, antioxidative capacity ranged from 0.61 to 1.28 mmol/100 g FW. Halvorsen et al. (2002) reported that overall mean antioxidative capacity for plums was 1.06 mmol/100 g FW, and that antioxidative capacity was 1.42 mmol/100 g FW for 'Red beauty' (*Prunus salicina*) and 1.02 mmol/100 g FW for 'Herman' (*Prunus domestica*). This agrees well with our results. Kim et al. (2003) used the VCEAC method to determine antioxidative capacity in six plum cultivars. The highest value they measured was about twice the lowest value they measured, which agrees well with our results.

Different assays have been used to measure antioxidative capacity. Borge



**Figure 1.** Antioxidant capacity in green and red kale. Comparison of three different methods; DPPH, FRAP and ORAC

et al. (2005) compared three of these methods, FRAP, ORAC and DPPH, and found differences between them (Fig.1). Antioxidative capacity as determined

by ORAC was three times higher than antioxidative capacity as determined by DPPH. Connor et al. (2002) used FRAP, ORAC and MeLO (methyl linoleate oxidation) to measure antioxidative capacity in blueberries. There was a strong correlation between the values obtained with FRAP and ORAC ( $P < 0.01$ ), but the correlations with MeLO were weaker.

There were strong correlations between antioxidative capacity and anthocyanins content and between antioxidative capacity and phenolics content, especially in the sweet cherry cultivars (Tab. 4). In the cultivars in this study, total phenolic content was a better predictor of antioxidative capacity than anthocyanin content. This agrees well with earlier results for blueberries (Connor et al., 2002).

Table 4. Correlations between FRAP values and contents of anthocyanins and total phenolics in sweet cherries and plums

	R-square	P
Sweet cherries:		
FRAP – total phenolics	0.978	<0.001
FRAP – anthocyanins	0.952	0.001
Plums:		
FRAP – total phenolics	0.833	0.005
FRAP – anthocyanins	0.698	0.036

## CONCLUSIONS

Antioxidative capacity is an important fruit quality parameter. Related parameters include anthocyanins content, total phenolics content, tannins content, vitamin C content and phenolic acids content. In recent years, antioxidative capacity has been reported under several names, including antioxidants content and antioxidant effect. However, the methods used to measure antioxidative capacity vary widely and often give conflicting results. A large part of the discrepancy is due to how the samples are prepared and extracted before testing.

Anthocyanins content and total phenolics content vary widely among different sweet cherry and plum cultivars. There were strong correlations between antioxidative capacity (FRAP) and anthocyanins content and between antioxidative capacity and phenolics content.

## REFERENCES

- Benzie I.F.F., Strain J.J. 1996. The ferric reducing ability of plasma (FRAP) as a measure of “antioxidant power”: The FRAP assay. *ANAL. BIOCHEM.* 239: 70-76.
- Borge G.I., Bengtsson G., Skrede G. 2005. Unpublished results. Norwegian Food Research Institute.

- Connor A.M., Luby J.J., Tong C.B.S. 2002. Variability in Antioxidant Activity in Blueberry and Correlations among Different Antioxidant Activity Assays. J. AMER. SOC. HORT. SCI. 127: 238-244.
- De Gara L., de Pinto M.C., Tommasi F. 2003. The antioxidant systems vis-a-vis reactive species during plant-pathogen interaction. PLANT PHYS. BIOCHEM. 41: 863-870.
- Frankel E.N., Meyer A.S. 2000. The problems of using one-dimensional methods to evaluate multifunctional food and biological antioxidants. J. SCI. FOOD AGRIC. 80: 1925-1941.
- Gao L., Mazza G. 1995. Characterization, quantitation and distribution of anthocyanins and colourless phenolics in sweet cherries. J. AGRIC. FOOD CHEM. 43: 343-346.
- Gil M.I., Tomas-Barberan F.A., Hess-Pierce B., Kadar A.A. 2002. Antioxidant capacities, phenolic compounds, carotenoids, and vitamin C contents of nectarine, peach, and plum cultivars from California. J. AGR. FOOD CHEM. 50: 4976-4982.
- Goncalves B., Landbo A.K., Knudsen D., Silva A.P., Moutinho-Pereira J., Rosa E., Meyer A.S. 2004. Effect of ripeness and postharvest storage on the phenolic profiles of cherries (*Prunus avium* L.). J. AGR. FOOD CHEM. 52: 523-530.
- Graham A.D. 1992. Stabilization of the Prussian blue color in the determination of polyphenols. J. AGRIC. FOOD CHEM. 40: 801-805.
- Guisti M., Wrolstad R.E. 2001. Current Protocols in Food Analytical Chemistry, unit F1. 2.
- Hagerman A.E. 2002. The Tannin Handbook, pp. 45-46.
- Halliwell B. 1990. How to characterize a biological antioxidant. FREE RADICAL RES. COMMUN. 9: 1-32.
- Halvorsen B.L., Holte K., Myhrstad M.C.W., Barikmo I., Hvattum E., Remberg S.F., Wold A-B., Haffner K., Baugerød H., Andersen L.F., Moskaug J.Ø., Jacobs Jr. D.R., Blomhoff R. 2002. A systematic screening of total antioxidants in dietary plants. J. NUTR. 132: 461-471.
- Hodges D.M., Lester G.E., Munro K.D., Toivonen P.M.A. 2004. Oxidative stress: Importance for postharvest quality. HORTSCIENCE 39: 924-929.
- Kim D.-O., Jeong S.W., Lee C.Y. 2003. Antioxidant capacity of phenolic phytochemicals from various cultivars of plums. FOOD CHEM. 81: 321-326.
- Mozetic B., Trebse P., Hribar J. 2002. Determination and quantification of anthocyanins and hydroxycinnamic acids in different cultivars of sweet cherries (*Prunus avium* L.) from Nova Gorica region (Slovenia). FOOD TECHNOL. BIOTECHNOL. 40: 207-212.
- Price M.L., Butler L.G. 1977. Rapid visual estimation and spectrophotometric determination of tannin content of sorghum grain. J. AGR. FOOD CHEM. 25: 1268-1273.
- Slimestad R., Verheul M.J. 2005. Seasonal variations in the level of plant constituents in greenhouse production of cherry tomatoes. J. AGRIC. FOOD CHEM. 53: 3114-3119.
- Tomas-Barberan F.A., Espin J.C. 2001. Phenolic compounds and related enzymes as determinantes of quality in fruits and vegetables. J. SCI. FOOD AGRIC. 81: 853-876.
- Vangdal E., Slimestad R., Sekse L. 2005. Phenolics and other compounds with antioxidative effect in stone fruit – Preliminary results. Presentation at The 8.



International Plum and Prune Symposium at Lofthus, Norway, 2004. (accepted for publication in Acta Hort.).  
World Cancer Research Fund / American Institute for Cancer Research 1997. Food, Nutrition and the Prevention of Cancer: A Global Perspective. American Institute of Cancer Research, Washington, DC.

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## METODY OZNACZANIA ANTYOKSYDACYJNYCH WŁAŚCIWOŚCI OWOCÓW

Eivind Vangdal i Rune Slimestad

### S T R E S Z C Z E N I E

Analizowano próbki 9 odmian czereśni i 9 odmian śliw. Średnia zawartość antocyjanów w czereśniach wynosiła 35 mg/100 g świeżej masy. Ogólna zawartość fenoli wynosiła od 23 do 186 mg/100g św.m., a antyoksydacyjne właściwości (FRAP) od 0,44 do 2,67 mmol/100 g św.m. W śliwkach zawartość antocyjanów wynosiła od 0,7 do 10,8 mg/100 g św.m., przy czym średnia wartość – 3,5 mg/100 g św.m. Ogólna zawartość fenoli wynosiła od 27 do 54 mg/100 g św.m., a wartości FRAP wynosiły od 0,6 do 1,28 mmol/100 g św.m. Wykazano korelację pomiędzy właściwościami antyoksydacyjnymi a zawartością antocyjanów i ogólną zawartością fenoli.

Antyoksydacyjne właściwości owoców są ważnym parametrem ich jakości. Jednak metody stosowane do oznaczania właściwości antyoksydacyjnych są różne, co utrudnia porównanie wyników różnych badań. Podczas wykonywania analiz szczególną uwagę należy zwrócić na przygotowanie i ekstrakcję próbki.

**Słowa kluczowe:** śliwki, *Prunus domestica*, czereśnie, antocyjany, fenole, antyoksydanty, FRAP