Available online at www.sciencedirect.com

ScienceDirect

Food Chemistry 110 (2008) 156–160

FOOD CHEMISTRY

www.elsevier.com/locate/foodchem

Organ-specific distribution of phenolic compounds in bilberry (*Vaccinium myrtillus*) and 'northblue' blueberry (*Vaccinium corymbosum x V. angustifolium*)

Kaisu Riihinen [a,*], Laura Jaakola [b], Sirpa Kärenlampi [a], Anja Hohtola [b]

[a] *Department of Biosciences, University of Kuopio, P.O. Box 1627, FIN-70211 Kuopio, Finland*
[b] *Department of Biology/Botany, University of Oulu, P.O. Box 3000, FIN-90014 Oulu, Finland*

Received 14 November 2007; received in revised form 16 January 2008; accepted 23 January 2008

Abstract

Blueberries and bilberries are recognized as some of the best sources of flavonoids, especially anthocyanins. The contents of flavonoids (anthocyanins, proanthocyanidins, flavonols) and hydroxycinnamic acids in the flower, fruit skin and pulp, leaf and rhizome of bilberry and the blueberry cultivar 'Northblue' were analyzed using high-performance liquid chromatography combined with diode-array detection. The most striking difference in the fruits was the predominance of hydroxycinnamic acids in blueberry, whereas in bilberry the anthocyanin content was much higher, particularly in the pulp. Differences in flavonoid contents of fruits were already apparent at the flower stage. Bilberry and blueberry leaves both contained high amounts of proanthocyanidins, flavonols and hydroxycinnamic acids. Blueberry rhizomes accumulated high amounts of hydroxycinnamic acids. All plant parts of bilberry and blueberry are potential sources of phenolic compounds for use either as dietary botanicals or by the pharmaceutical industry.

Keywords: Food analysis; Berries; Phenolic compounds; HPLC; Anthocyanins

1. Introduction

Blueberries belong to the genus *Vaccinium*, a widespread genus with over 200 species of evergreen and deciduous woody plants varying in size from dwarf shrubs to trees. Blueberries include several closely related small fruit species. The main species are the North-American highbush (*Vaccinium corymbosum*) and lowbush (*Vaccinium angustifolium*) blueberries together with the native European blueberry, also called bilberry (*Vaccinium myrtillus*). In Northern Europe, bilberry is one of the most important wild berries.

There is a great interest worldwide in the fruits of bilberry and blueberry because of their high anthocyanin content. Anthocyanins are flavonoids as are flavonol glycosides, flavan-3-ols and proanthocyanidins, whereas hydroxycinnamic acids are classified as phenolic acids (Fig. 1). Anthocyanins are valued as pigments but are also widely used in natural health products due to their suggested positive effects on night vision, even though firm evidence from clinical trials is still lacking (Canter & Ernst, 2004; Ghosh & Konishi, 2007). Flavonoids and other phenolic compounds are reported to have multiple biological effects including antioxidant, antimutagenic, anticarcinogenic, anti-inflammatory, antiproliferative and antimicrobial activities (Baliga & Katiyar, 2006; Heinonen, 2007; Morton, Abu-Amsha Caccetta, Puddey, & Croft, 2000; Tapiero, Tew, Ba, & Mathe, 2002).

Flavonoids can be found in all plant species and in different organs where they play several important roles. Flavonoids have functional roles in fruit-bearing plants as colourful attractants for birds helping in seed dispersal and as cellular support materials. They can serve as signal

* Corresponding author. Tel.: +358 17 163103; fax: +358 17 163322.
E-mail address: Kaisu.Riihinen@uku.fi (K. Riihinen).

0308-8146/$ - see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodchem.2008.01.057

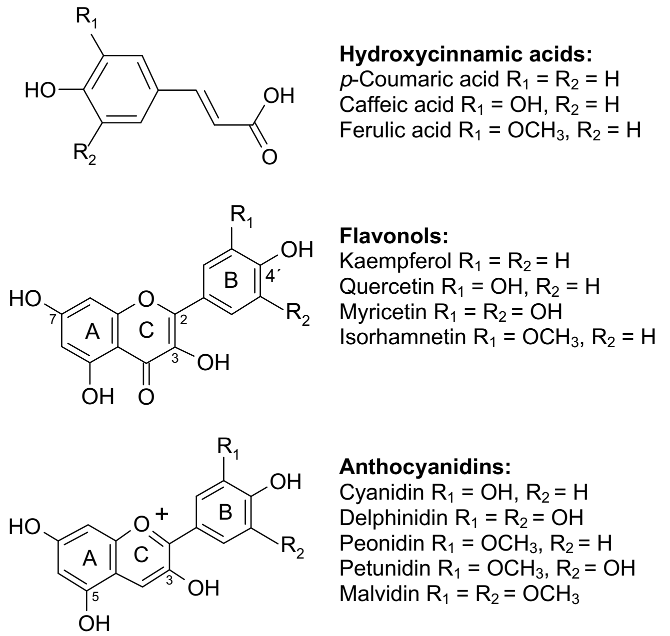

Fig. 1. Chemical structures of hydroxycinnamic acids, flavonols and anthocyanidins.

molecules in sexual reproduction and protect the plant against UV radiation, as well as participate in plant–microbe interactions and defense responses (Dixon & Paiva, 1995; Treutter, 2005). In general, evolutionally more advanced plants contain more complex flavonoid profiles.

Members of all groups of phenolic compounds are present in the fruits and leaves of bilberry and blueberry (Häkkinen, Kärenlampi, Heinonen, Mykkänen, & Törrönen, 1999; Jaakola et al., 2002 Määttä-Riihinen, Kamal-Eldin, Mattila, González-Paramás, & Törrönen, 2004; Taruscio, Barney, & Exon, 2004). The composition of phenolic compounds in the leaf, stem, root and fruit extracts of *V. angustifolium* was reported recently (Harris et al., 2007). However, limited information is available about the flavonoid and phenolic acid composition of the different parts of blueberry and bilberry plants. The aim of this study was to compare the contents of flavonoids and hydroxycinnamic acids in different parts of bilberry and 'Northblue' blueberry and to evaluate their potential as sources of phenolic compounds.

2. Materials and methods

2.1. Plant material

Samples of leaves, flowers, fruits and rhizome of bilberry and 'Northblue' (*V. corymbosum x V. angustifolium*) blueberry were obtained from the test field of the Botanical Garden of the University of Oulu, Finland, during the growing season 2002. Ripe fruits and leaf and rhizome samples were collected at the same time. Flower samples were collected in June 2002. The samples were immediately frozen in liquid nitrogen, with the exception that fruit skin was separated from pulp before freezing. The samples were stored at −70 °C.

2.2. Analysis of flavonoids and hydroxycinnamic acids

A simple suspension method was developed for the analysis of leaf and fruit samples (Jaakola, Määttä-Riihinen, Kärenlampi, & Hohtola, 2004; Jaakola et al., 2002). Frozen samples (0.25–2 g) were ground using a mortar and pestle to a fine powder in liquid nitrogen and macerated in 10 ml of acidified (0.6 M HCl) methanol. After removing 1 ml of the fruit-solvent suspension for the analysis of anthocyanins and hydroxycinnamic acids, the rest of the samples were hydrolysed with acid for the analysis of flavonols as aglycones and proanthocyanidins as anthocyanidins as described previously (Määttä, Kamal-Eldin, & Törrönen, 2003).

All samples were filtered through a 0.45-m syringe filter before their injection into the HPLC. The separation of the phenolic compounds was achieved on a (125 × 3 mm i.d., 5 m) LiChroCART Purospher RP-18e column (Merck, Darmstadt, Germany) with one gradient for anthocyanins and another one for other phenolic compounds. A 20-min linear gradient of acetonitrile in 1% formic acid was used to separate flavonols, hydroxycinnamic acids, and anthocyanidins. A step-gradient of acetonitrile in 5% formic acid was used to separate anthocyanins as follows: 5–10% acetonitrile (0–5 min), 10% acetonitrile (5–10 min), 10–40% acetonitrile (10–25 min), and finally 40–90% acetonitrile (25–35 min). For further details of the method see Määttä, Kamal-Eldin, and Törrönen (2001) and Määttä-Riihinen et al. (2004).

Diode array detection was used for UV–Vis spectral analysis and quantification. Identification of the conjugated and free forms of phenolic compounds in the chromatograms was based on retention times and on the comparison of their UV–Vis spectra, wavelengths of maximum absorption and wavelengths of shoulders (sh) with those of representative standards as described by Määttä et al. (2001) and Määttä-Riihinen et al. (2004). Individual compounds were quantified within the linear range using standard curves of representative compounds. The response factors were determined from freshly prepared solutions in the following concentration ranges of aglycones: anthocyanins 1.5–85 mg/l and other phenolic compounds 2–250 mg/l. The response factors of anthocyanidins and anthocyanins were determined in acidified methanol (0.6 M HCl).

3. Results

The composition of flavonoids and phenolic acids varied markedly between the different parts of the bilberry and blueberry plants (Table 1). The missing anthocyanin pigment of blueberry pulp is visually shown in Fig. 2. The highest content of anthocyanins and myricetin were found in fruit skins, whereas the contents of proanthocyanidins,

Table 1
Contents (μg/g) of anthocyanins, proanthocyanidins, flavonols and hydroxycinnamic acids in plant segments of blueberry and bilberry

	Anthocyanins[a]	Proanthocyanidins		Flavonols			Hydroxycinnamic acids		Sum
		Prodelphinidins	Procyanidins	Myricetin	Quercetin	Kaempferol	p-Coumaric	Caffeic or Ferulic	
Blueberry									
Flower	13	6	41	ND[b]	1553	198	223	2847	4881
Berry peels	6223	NA	NA[b]	31	531	ND	19	715	7519
Berry pulps	19	6	369	ND	ND	ND	7	522	923
Green leaves	ND	468	364	ND	1784	191	490	7537	10,834
Red leaves	62	485	272	ND	3530	505	3060	19,870	27,784
Rhizome	ND	ND	433	ND	ND	ND	167	2314	2914
Bilberry									
Flower	86	ND	50	ND	130	ND	396	587	1244
Berry peels	20256	NA	NA	47	159	ND	32	175	20,669
Berry pulps	1040	NA	NA	3	12	ND	100	63	1218
Green leaves	ND	25	962	ND	3369	171	2989	7808	15,324
Red leaves	882	36	402	ND	10369	244	6007	16,249	34,193
Rhizome	ND	ND	336	ND	ND	ND	778	273	1387

[a] Quantified anthocyanins were cyanidin glycosides in flower and leaves and delphinidin-, cyanidin-, petunidin-, peonidin-, and malvidinglycosides in peels and pulps of berries.
[b] NA = not analyzed, ND = not detected.

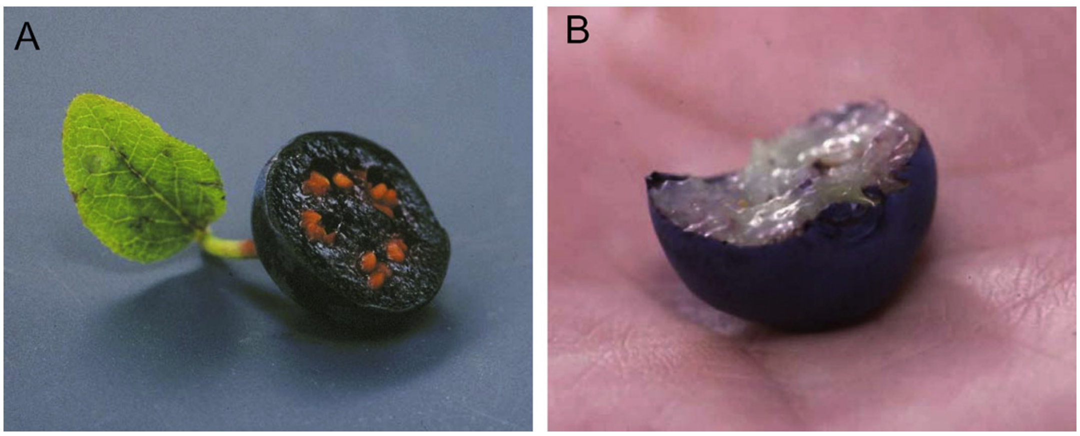

Fig. 2. (A) Cross section of bilberry fruit shows the intense coloration of the pulp. (B) Cross section of blueberry fruit.

kaempferol, quercetin and hydroxycinnamic acids were highest in the leaves. In the rhizomes, no anthocyanins, prodelphinidins or flavonols were found; instead, procyanidins and hydroxycinnamic acids were the main phenolic compounds in these plant parts. The fruits of bilberry and blueberry were clearly differentiated by their content of anthocyanins and hydroxycinnamic acids. Procyanidins were the major phenolic compounds in the blueberry pulp where the content of prodelphinidins and anthocyanins was very low. The pulp and flower of bilberry contained much higher amounts of anthocyanins and p-coumaric acid than those of blueberry, whereas the reverse was true for caffeic or ferulic acid. Interestingly, both the flower and the fruit peel of blueberry had higher quercetin contents than those of bilberry. In the leaves, the most striking differences were found in anthocyanin (higher in red bilberry leaves) and prodelphinidin (higher in both red and green blueberry leaves) contents. While procyanidins were the main proanthocyanidins in bilberry leaves and rhizomes, the content of prodelphinidins in blueberry leaves was higher than that of procyanidins. The anthocyanin profiles of bilberry fruits and leaves were very different, only cyanidin-glycosides being present in the red leaves (Fig. 3).

4. Discussion

Anthocyanins are the most interesting phenolic compounds in bilberry and blueberry due to the use of their fruit extracts and fractions in pharmaceutical products. Food items with the highest contents of anthocyanidins are easily recognizable by their deep red or bluish-black colour. Bilberry is one of the best natural sources of anthocyanins (Heinonen, 2007; Määttä-Riihinen et al., 2004). Anthocyanins are present both in the peel and pulp of bilberry but mainly in the peel of blueberry. Therefore, the content of anthocyanins is clearly lower in blueberry than in bilberry on a fresh weight basis. Anthocyanins constitute 2% of the fresh weight in bilberry peel, indicating that the

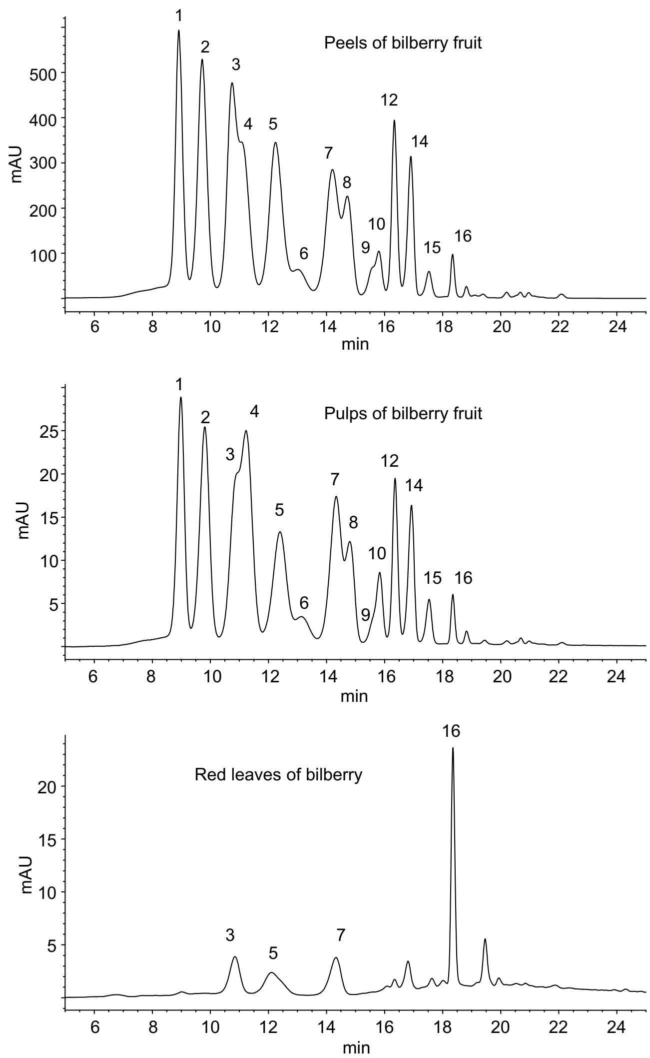

Fig. 3. HPLC–DAD profile of anthocyanins in bilberry peels, pulps and leaves at 520 nm. Peak identity in the order of retention time is: (1) delphinidin 3-galactoside, (2) delphinidin 3-glucoside, (3) cyanidin 3-galactoside, (4) delphinidin 3-arabinoside, (5) cyanidin 3-glucoside, (6) petunidin 3-galactoside, (7) cyanidin 3-arabinoside, (8) petunidin 3-glucoside, (9) peonidin 3-galactoside, (10) petunidin 3-arabinoside, (11) minor peonidin 3-glucoside overlapped, (12) malvidin 3-galactoside, (13) minor peonidin 3-arabinoside overlapped, (14) malvidin 3-glucoside, (15) malvidin 3-arabinoside, and (16) cyanidin.

peel residues remaining after juice extraction would still be an excellent source of anthocyanins. Compared to the fruits, the red leaves contain low amounts of anthocyanins. Moreover, the profiles of anthocyanins in both bilberry and blueberry fruits are composed of delphinidin-, cyanidin-, petunidin-, peonidin-, and malvidin-glycosides (Jaakola et al., 2002; Määttä-Riihinen et al., 2004), whereas only cyanidin-glycosides are found in the red leaves (Fig. 3). Therefore, the leaves are clearly poorer than the fruits as sources of anthocyanins for pharmaceutical or nutraceutical use.

With respect to the proanthocyanidins, blueberry fruit pulp contains a fairly high concentration of procyanidins, in accordance with previous publications (Gu et al., 2004). However, both green and red leaves of bilberry and blueberry are the best potential sources of procyanidins (Fraisse, Carnat, & Lamaison, 1996; Jaakola et al., 2004). Even though the results in Table 1 are analytically reliable and adequate for the comparison of plant parts as sources of proanthocyanidins, they do underestimate the contents (0.07–0.09% of fresh weight), since the yield of acid released anthocyanidins of proanthocyanins is low (Rohr, Meier, & Sticher, 2000). The content of procyanidins in bilberry leaves is about 7% of dry weight as measured spectrophotometrically (Fraisse et al., 1996). The leaves are the main waste products from the cleaning process of wild bilberries in the fruit industry. This waste should be viewed as an excellent source for proanthocyanidin-containing products. Bilberry leaf extract could be used in cosmetics and pharmaceuticals similarly to the phenolic compounds of green tea (Hsu, 2005), since proanthocyanidins are known to possess both antimicrobial and antioxidant activities (Heinonen, 2007).

Quercetin, myricetin, kaempferol and isorhamnetin are the most abundant flavonols in plant-based foods (Markham, 1989). The main sources of flavonols in an average diet are onions, tea, berries and apples (Hollman & Arts, 2000). In the fruits of bilberry and blueberry, quercetin is the predominant flavonol. Myricetin is the most abundant flavonol in the peels of both fruits. A similar observation was made with black currant peels and pulps (Vuorinen, Määttä, & Törrönen, 2000). Myricetin is synthesized during the late stages of bilberry ripening together with other phenolic compounds, since their biosynthesis is most active during this growth stage (Jaakola et al., 2004). Flavonols are clearly concentrated in blueberry peel and are present at higher concentrations in the peel and at the flower stage compared to the respective plant parts in bilberry. However, when the whole bilberry and blueberry fruits are analysed, the fruits are comparable sources of flavonols in the human diet (Määttä-Riihinen et al., 2004; Taruscio et al., 2004). While quercetin was found to be the main flavonol in the leaves, kaempferol is also detected, in agreement with other studies (Fraisse et al., 1996; Harris et al., 2007; Jaakola et al., 2004). According to our previous results, solar radiation increases the contents of both quercetin and kaempferol in bilberry leaves, which suggests that these compounds have a role in photo-protection (Jaakola et al., 2004). This explains the higher content of quercetin and kaempferol in the red leaves of both bilberry and blueberry compared to the respective green leaves. Bilberry leaves may be considered as excellent sources of flavonols for cosmetic products since the contents are as high as 1% of fresh weight.

At least one of the common hydroxycinnamic acids, i.e., p-coumaric acid, caffeic acid, ferulic acid or sinapic acid, are present in nearly all plants (Ibrahim & Barron, 1989). These acids are found in insoluble form as structural components and in soluble form as simple conjugates. While hydroxycinnamic acids is found in all parts of bilberry and blueberry, the highest contents are present in the leaves, and represent the major phenolic compounds in the rhizome. Hydroxycinnamic acids are the precursors of cellular support material lignin in the plants, but they also provide defense against plant pathogens (Dixon & Paiva, 1995) and other stress factors such as wounding (Housti, Andary, Gargadennec, & Amssa, 2002), intense solar radiation (Jaakola et al., 2004), and low temperature (Solecka & Kacperska, 2002).

In conclusion, anthocyanins and flavonols are the predominant flavonoids in the fruits of bilberry and blueberry but, their content in bilberry fruits is over three-fold compared to that in blueberry. However, blueberry fruits contain higher levels of hydroxycinnamic acids and flavonols. The most complex profile and highest content of diverse flavonoids and hydroxycinnamic acids are present in the leaves, whereas only hydroxycinnamic acids and procyanidins are found in the rhizomes. All of the studied plant parts could serve as potential sources of phenolic compounds for nutraceutical or pharmaceutical industry.

Acknowledgements

This work was supported by the Emil Aaltonen Foundation. We thank Mrs. Eeva-Liisa Palkispää for technical assistance in the analysis of flavonoids and hydroxycinnamic acids.

References

Baliga, M. S., & Katiyar, S. K. (2006). Chemoprevention of photocarcinogenesis by selected dietary botanicals. *Photochemical & Photobiological Sciences, 5*(2), 243–253.

Canter, P. H., & Ernst, E. (2004). Anthocyanosides of *Vaccinium myrtillus* (bilberry) for night vision – a systematic review of placebo-controlled trials. *Survey of Ophthalmology, 49*(1), 38–50.

Dixon, R. A., & Paiva, N. L. (1995). Stress-induced phenylpropanoid metabolism. *Plant Cell, 7*(7), 1085–1097.

Fraisse, D., Carnat, A., & Lamaison, J.-L. (1996). Composition polyphénolique de la feuille de myrtille. *Annual Pharmacy of France, 54*, 280–283.

Ghosh, D., & Konishi, T. (2007). Anthocyanins and anthocyanin-rich extracts: role in diabetes and eye function. *Asia Pacific Journal of Clinical Nutrition, 16*(2), 200–208.

Gu, L., Kelm, M. A., Hammerstone, J. F., Beecher, G., Holden, J., Haytowitz, D., et al. (2004). Concentrations of proanthocyanidins in common foods and estimations of normal consumption. *Journal of Nutrition, 134*(3), 613–617.

Harris, C. S., Burt, A. J., Saleem, A., Le, P. M., Martineau, L. C., Haddad, P. S., et al. (2007). A single HPLC-PAD-APCI/MS method for the quantitative comparison of phenolic compounds found in leaf, stem, root and fruit extracts of *Vaccinium angustifolium*. *Phytochemical Analysis, 18*(2), 161–169.

Heinonen, M. (2007). Antioxidant activity and antimicrobial effect of berry phenolics – a finnish perspective. *Molecular Nutrition & Food Research, 51*(6), 684–691.

Hollman, P. C. H., & Arts, I. C. W. (2000). Flavonols, flavones and flavanols – nature, occurrence and dietary burden. *Journal of the Science of Food and Agriculture, 80*(7), 1081–1093.

Housti, F., Andary, C., Gargadennec, A., & Amssa, M. (2002). Effects of wounding and salicylic acids on hydroxycinnamoylmalic acids in Thunbergia alata. *Plant Physiology and Biochemistry, 40*, 761–769.

Hsu, S. (2005). Green tea and the skin. *Journal of the American Academy of Dermatology, 52*(6), 1049–1059.

Häkkinen, S. H., Kärenlampi, S. O., Heinonen, I. M., Mykkänen, H. M., & Törrönen, A. R. (1999). Content of the flavonols quercetin, myricetin, and kaempferol in 25 edible berries. *Journal of Agricultural and Food Chemistry, 47*(6), 2274–2279.

Ibrahim, R., & Barron, D. (1989). In P. M. Dey & J. B. Harborne (Eds.), *Phenylpropanoids. Methods in plant biochemistry* (pp. 75–112). London, UK: Academic Press.

Jaakola, L., Määttä, K., Pirttilä, A. M., Törrönen, R., Kärenlampi, S., & Hohtola, A. (2002). Expression of genes involved in anthocyanin biosynthesis in relation to anthocyanin, proanthocyanidin, and flavonol levels during bilberry fruit development. *Plant Physiology, 130*(2), 729–739.

Jaakola, L., Määttä-Riihinen, K., Kärenlampi, S., & Hohtola, A. (2004). Activation of flavonoid biosynthesis by solar radiation in bilberry (*Vaccinium myrtillus* L.) leaves. *Planta, 218*, 721–728.

Markham, K. R. (1989). In P. M. Dey & J. B. Harborne (Eds.), *Flavones, flavonols and their glycosides. Methods in plant biochemistry* (pp. 197–236). London, UK: Academic Press.

Morton, L. W., Abu-Amsha Caccetta, R., Puddey, I. B., & Croft, K. D. (2000). Chemistry and biological effects of dietary phenolic compounds: Relevance to cardiovascular disease. *Clinical and Experimental Pharmacology, 27*(3), 152–159.

Määttä, K., Kamal-Eldin, A., & Törrönen, R. (2001). Phenolic compounds in berries of black, red, green, and white currants (*Ribes* sp.). *Antioxidant & Redox Signaling, 3*(6), 981–993.

Määttä, K. R., Kamal-Eldin, A., & Törrönen, A. R. (2003). High-performance liquid chromatography (HPLC) analysis of phenolic compounds in berries with diode array and electrospray ionization mass spectrometric (MS) detection: Ribes species. *Journal of Agricultural and Food Chemistry, 51*(23), 6736–6744.

Määttä-Riihinen, K. R., Kamal-Eldin, A., Mattila, P. H., González-Paramás, A. M., & Törrönen, A. R. (2004). Distribution and contents of phenolic compounds in eighteen Scandinavian berry species. *Journal of Agricultural and Food Chemistry, 52*(14), 4477–4486.

Rohr, G. E., Meier, B., & Sticher, O. (2000). In Atta-ur-Rahman (Ed.), *Analysis of procyanidins. Studies in natural products chemistry* (pp. 497–569). Elsevier Science BV.

Solecka, D., & Kacperska, A. (2002). Phenylpropanoid deficiency affects the course of plant acclimation to cold. *Physiologia Plantarum, 119*, 253–262.

Tapiero, H., Tew, K. D., Ba, G. N., & Mathe, G. (2002). Polyphenols: Do they play a role in the prevention of human pathologies?. *Biomedicine & Pharmacotherapy 56*(4), 200–207.

Taruscio, T. G., Barney, D. L., & Exon, J. (2004). Content and profile of flavanoid and phenolic acid compounds in conjunction with the antioxidant capacity for a variety of northwest *Vaccinium* berries. *Journal of Agricultural and Food Chemistry, 52*(10), 3169–3176.

Treutter, D. (2005). Significance of flavonoids in plant resistance and enhancement of their biosynthesis. *Plant Biology (Stuttg), 7*(6), 581–591.

Vuorinen, H., Määttä, K., & Törrönen, R. (2000). Content of the flavonols myricetin, quercetin, and kaempferol in Finnish berry wines. *Journal of Agricultural and Food Chemistry, 48*(7), 2675–2680.