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Flavonol glycosides in wild and cultivated berries of three major subspecies of *Hippophaë rhamnoides* and changes during harvesting period

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ABSTRACT

Flavonol glycosides are an important group of bioactive components of sea buckthorn (*Hippophaë rhamnoides*). The content and profile of flavonol glycosides of some major subspecies and most cultivars as well as the variation amongst the harvesting years and dates are largely unknown. This study investigated flavonol glycosides in wild berries of two major subspecies *H. rhamnoides* ssp. *rhamnoides* and ssp. *sinensis* and berries of eight cultivars of ssp. *rhamnoides* and *mongolica* by reverse phase high performance liquid chromatography combined with diode array detection. The major flavonol glycosides were isorhamnetin-3-O-glucoside-7-O-rhamnoside, isorhamnetin-3-O-rutinoside, isorhamnetin-3-O-glucoside, isorhamnetin-3-O-sophoroside-7-O-rhamnoside, quercetin-3-O-rutinoside, quercetin-3-O-glucoside and quecertin-3-O-sophoroside-7-O-rhamnoside. The total content of flavonol glycosides fell in the range of 27–130 mg per 100 g fresh berries with considerable variation amongst the origins and the harvesting years. Compared with the berries of ssp. *sinensis* and ssp. *mongolica*, the berries of ssp. *rhamnoides* contained high levels of isorhamnetin-3-O-glucoside-7-O-rhamnoside and isorhamnetin-3-O-glucoside and lower levels of quercetin-3-O-rutinoside and quercetin-3-O-glucoside. In the wild berries of ssp. *sinensis*, the contents of flavonol glycosides reached maxima around late September to early October and decreased thereafter, whereas a general decreasing trend was seen in the cultivated berries of ssp. *rhamnoides* from the end of August to the end of October.

1. Introduction

Flavonoids form the largest group of antioxidants in nature. A wide spectrum of biological activities have been reported of flavonoids, often flavonols, from different sources such as inhibiting the formation of reactive radicals, scavenging of different radical species, enhancing the activity of antioxidative enzymes, inhibiting platelet aggregation, improving blood circulation, reducing inflammation, and inhibiting the growth and speeding up the apoptosis of cancer cells (Cao et al., 2003; Cheng et al., 2007; Cos et al., 1998; da Silva, Tsushida, & Terao, 1998; Hertog et al., 1995; Nichenametla, Taruscio, Barney, & Exon, 2006; Bestwick, Milne, & Duthie, 2007). Along with the widely shown health benefits, controversial results have been reported from both epidemiological studies and intervention trials on the effects of flavonoids in humans (Hertog, Feskens, Hollman, Katan, & Kromhout, 1994; Hertog, Sweetnam, Fehily, Elwood, & Kromhout, 1997; Knekt et al., 1997, 2002; Rimm, Katan, Ascherio, Stampfer, & Willet, 1998). In addition to differences in study protocols and other dietary factors, variations in the biological activity and bioavailability amongst flavonols from different sources may have played an important role in the discrepancy of the results from different studies. Therefore, information on both the content and the composition of flavonoids such as flavonols and flavonol glycosides in different sources is important for clearing up the discrepancies observed amongst the studies and for evaluating the potential health benefits of food materials.

Despite some inconsistent lines of evidence, several structure-activity relationships of flavonoids have been well established *in vitro*. The presence of multiple hydroxy groups in the benzene rings confer upon the molecule substantial antioxidant, chelating and prooxidant activities. Substitution of the hydroxy groups with methoxy groups introduces unfavourable steric effects and increases lipophilicity and membrane partitioning. Double bonds and carbonyl groups in the heterocyclic ring increase the antioxidative activities of the molecule by stabilizing flavonoid radical formation through conjugation and electron delocalization. Flavonoids differing in the pattern of substitution and conjugation in the phenolic and heterocyclic rings are likely to have different biological activities (Heim, Tagliaferro, & Bobilya, 2002).

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Flavonols are a major group of flavonoids, which occur mainly in the form of glycosides in plants. The most common aglycons are quercetin, myricetin and kaempferol. The common sugar residues are glucose and galactose, but rutinose, xylose, arabinose and rhamnose are also found. The bioavailability of flavonols is influenced by both the presence and the type of sugar moiety attached to the aglycons. When flavonols are present in the diet as aglycons, they are partially absorbed in the stomach, where the glycosidic forms of these flavonols are not (Crespy et al., 2002). The speed and efficiency of absorption of flavonol glycosides depends largely on species and location of the sugar moieties of the molecules (Chang, Zuo, Chow, & Ho, 2005; Hollman et al., 1999). The peak concentration of quercetin in plasma was much higher and was reached much faster after intake of quercetin-3-O-glucoside than after intake of equal amount of quercetin-3-O-rutinoside by man. Quercetin glucoside was likely to be actively absorbed from the small intestine, whereas quercetin rutinoside was absorbed only from the colon after deglycosylation. (Hollman et al., 1999). There is also evidence indicating that quercetin-3-O-glucoside may be more readily absorbed than quercetin-3-O-galactoside (Chang et al., 2005).

Sea buckthorn (*Hippophaë* sp.) berries are increasingly recognised as food material having multiple health benefits in man. Flavonoids (mainly flavonol glycosides and proanthocyanidins accompanied by minor components such as flavanols and phenolic acids) are an important group of bioactive compounds in the berries. Human intervention studies and investigations using experimental models have shown great potential of flavonols (mainly as aglycons) isolated from sea buckthorn in supporting the health of the heart and the vascular system (Bao & Lou, 2006; Cao et al., 2003; Cheng et al., 2007; Wang, Feng, Yu, Zhang, & Zhu, 1993; Wang, Zhang, Xu, Zhang, & Cheng, 1985; Wu, Yu, Li, & Liu, 1994; Yu, Wu, Zang, Liu, & Chen, 1992).

Being widely distributed in Europe and Asia, naturally growing sea buckthorn covers several species and subspecies of *Hippophaë*. In addition, varieties and cultivars with different characteristics are commercially available. The content and profile of flavonols and flavonol glycosides are important compositional characteristics influencing the quality and health effects of the berries. At the moment, no systematic information is available on the flavonol glycosides in berries of the species, subspecies, varieties and cultivars. The Chinese subspecies *H. rhamnoides* ssp. *sinensis* is the commercially most important subspecies. *H. rhamnoides* ssp. *rhamnoides* and ssp. *mongolica* are the major subspecies in Europe and Russia, respectively. Cultivars of ssp. *mongolica* from the Altai region are widely cultivated in Asia, Europe and North America. The Finnish cultivars are amongst the major cultivars in Finland, the Baltic countries, Canada and the US.

In the present study the profile and content of flavonol glycosides were studied in wild and cultivated berries of *H. rhamnoides* ssp. *sinensis*, *rhamnoides* and *mongolica* from China, Finland and Russia. The changes during harvesting period were studied in cultivated berries of *H. rhamnoides* ssp. *rhamnoides* from Finland and wild berries of *H. rhamnoides* ssp. *sinensis* from China. To our best knowledge, this is the first report on the flavonol glycosides in sea buckthorn berries of these origins and the influence of harvesting date on flavonol glycosides in sea buckthorn berries.

2. Materials and methods

2.1. Berry samples

In order to investigate the flavonol glycosides in different subspecies and cultivars, wild sea buckthorn berries were collected in China (*H. rhamnoides* ssp. *sinensis*) and Finland (*H. rhamnoides* ssp. *rhamnoides*), cultivated berries in Finland (*H. rhamnoides* ssp. *rhamnoides* and ssp. *mongolica*) and Russia (*H. rhamnoides* ssp. *mongolica*) during the years 1996–1999 (Table 2). Wild and cultivated berries of ssp. *rhamnoides* and two cultivars of ssp. *mongolica*, Tsuiskaya and Oranzevaya, were collected in southwest Finland (longitudes 2104′E–2424′E, latitudes 6045′N–6447′N, altitudes 0–50 m). Berries of cultivar Vitaminaya (*H. rhamnoides* ssp. *mongolica*) were picked from the Institute of Horticulture, Siberian Branch of the Russian Academy of Science, Novosibirsk (longitude 82°55′E, latitude 55°02′N, altitude 200 m), Russia. Wild Chinese berries were picked at two natural growth sites, Wenshui (longitude 11141′E, latitude 3732′N, altitude 1600 m) and Xixian (longitude 11102′E, latitude 3648′N, altitude 1500 m), in Shanxi Province, China. The berry-picking time was mostly from late August to mid September in Finland, around mid August in Russia, and from mid September to mid October in China, representing, respectively, the common time period for harvesting optimally ripe berries in these regions.

In order to study the influence of harvesting date on the content and profile of flavonol glycosides, wild berries were picked from two natural growth sites of ssp. *sinensis* in China. Berries from two Finnish cultivars of ssp. *rhamnoides* were picked in Finland. These berries were harvested at different dates from the end of August to the end of November in the years 1998 and 1999.

Each sample consisted of berries picked from 20 different bushes from five different locations at each natural growth site or the cultivation field of each cultivar, in order to obtain samples representing the population or cultivar. The berries were loosely frozen within one day after picking until analysed within half year after harvesting.

2.2. Reagents and reference compounds

All reagents used were of HPLC grade. The reference compounds isorhamnetin-3-O-rutinoside, isorhamnetin-3-O-glucoside, quercetin-3-O-rutinoside and quercetin-3-O-glucoside were from Extrasynthèse S.A. (Genay, France) and the internal standard phlorizin from Sigma–Aldrich (Steinheim, Germany). Two sets of reference compounds isorhamnetin-3-O-glucoside-7-O-rhamnoside, isorhamnetin-3-O-sophoroside-7-O-rhamnoside, and quercetin-3-O-sophoroside-7-O-rhamnoside isolated from sea buckthorn berries were kindly donated by Professor Zhang Hao (West China School of Pharmacy, Sichuan University, Chengdu, China) and Professor Lothar W. Kroh and Dr Daniel Rösch (Institut für Lebensmitteltechnologie und Lebensmittelchemie, Technische Universität Berlin, Berlin, Germany), who have carried out the isolation and the structure determination independently from each other.

2.3. Extraction of flavonol glycosides

The extraction of flavonol glycosides was carried out using a previously reported procedure after slight modification (Price, Casuscelli, Colquhoun, & Rhodes, 1998; Price, Colquhoun, Barnes, & Rhodes, 1998; Price & Rhodes, 1997). A frozen berry sample of 20 g was taken from a 5 kg lot using a sample partitioning procedure. The berries were thawed in a microwave oven. The berries were homogenised and extracted three times in 50 ml solvent mixture consisting of methanol:water:acetic acid (70:30:5), taking the

Table 1
Program for mobile phase composition during the HPLC analysis. A, water – tetrahydrofuran – trifluoroacetic acid (98:2:0.1); B, acetonitril.

Time (min)	0	2	14	19	24	28	30	35	40	50
A (v/v,%)	85	85	75	75	40	40	10	10	85	85
B (v/v,%)	15	15	25	25	60	60	90	90	15	15

Table 2
Flavonol glycosides[b] in wild and cultivated sea buckthorn of different origins (mg/100 g fresh berries).

Origin	Samples	Subsp[a]	Collecting date	Q-3-S-7-Rh	I-3-S-7-Rh	I-3-G-7-Rh	Q-3-R	Q-3-G	I-3-R	I-3-G	Total
Finnish	74006003	*R*	Sep. 4, 96 (SAT)[c]	3.7	8.9	18.0	1.5	0.5	9.4	7.1	49.2
Cultivar		*R*	Aug. 25, 97 (TUO)[d]	3.4	6.6	21.3	2.0	1.2	16.5	22.4	73.3
		R	Sep. 17, 99 (SAT)	3.8	10.0	17.6	1.9	1.1	11.6	16.9	62.8
	74006005	*R*	Sep. 4, 96 (SAT)	2.9	6.4	16.3	1.9	0.5	15.1	5.9	49.0
		R	Aug. 25, 97 (TUO)	2.3	3.2	19.8	2.8	1.6	37.3	25.9	93.0
		R	Sep. 17, 99 (SAT)	3.2	7.1	15.0	3.3	1.4	17.7	12.3	59.9
	s3006	*R*	Sep. 10, 96 (SAT)	2.3	6.9	34.0	1.3	1.2	18.0	15.5	79.2
		R	Aug. 28, 97 (SAT)	2.8	6.2	18.7	1.9	1.0	21.2	17.4	69.3
		R	Sep. 17, 99 (SAT)	2.8	7.8	23.8	3.0	2.0	25.9	23.3	88.5
	s3003	*R*	Sep. 10, 96 (SAT)	3.0	7.3	25.7	1.8	1.1	8.3	9.7	56.9
		R	Aug. 28, 97 (SAT)	2.8	5.4	17.6	1.9	1.6	9.4	15.8	54.6
		R	Sep. 17, 99 (SAT)	3.5	9.4	27.3	2.7	3.0	14.0	24.7	84.6
	Raisa	*R x C*[f]	Sep. 1, 96 (SAT)	2.1	2.2	44.7	6.5	2.4	51.6	20.2	129.6
			Sep. 17, 99 (PTL)[e]	2.1	6.2	26.3	7.4	3.3	52.0	25.4	122.8
	Median, range			2.9, 1.7	6.8, 7.8 x	20.5, 29.7 x	2.0, 6.2 x	1.3, 2.8 x	17.1, 43.7	17.2, 20.1	
Finnish	Pyhämaa	*R*	Aug. 30, 99	3.2	13.6	15.6	5.3	2.7	35.3	25.2	100.9
Wild	Siikajoki	*R*	Sep. 3, 99	3.6	9.5	27.9	4.9	2.5	18.5	7.0	74.0
	Vaasa	*R*	Sep. 3, 99	4.4	12.8	24.5	9.8	3.0	33.3	12.6	100.4
	Vaasa	*R*	Sep. 14, 2000	1.8	4.8	25.3	3.9	3.3	17.0	5.4	81.4
	Pyhämaa	*R*	Sep. 8, 99	3.4	9.0	22.9	6.1	6.5	26.1	25.8	99.7
	Pyhämaa	*R*	Sep. 8, 99	2.0	6.6	22.7	4.8	4.6	17.4	14.2	72.3
	Median, range			3.3, 2.6	9.2, 8.8 x	23.7, 12.3 x	5.1, 5.9 x	3.2, 4.1 y	22.3, 18.3	13.4, 20.4	
Russian	Vitaminaya	M	Aug. 23, 97	5.2	4.2	1.7	4.8	2.4	7.1	1.8	27.2
Cultivars	Tsuiskaya	M	Aug. 23, 99	3.3	7.3	8.7	10.9	6.6	26.0	11.9	74.8
	Oranzevaja	M	Aug. 23, 99	3.3	6.7	9.1	12.1	6.5	30.5	12.5	80.6
	Median, range			3.3, 1.9	6.7, 3.1	8.7, 7.4	10.9, 7.3	6.5, 4.2	26.0, 23.4	11.9, 10.7	
Chinese	Wenshui	*S*	Oct. 23, 96	2.1	1.1	7.0	7.3	3.0	10.3	4.8	35.6
Wild		*S*	Oct. 15, 98	4.1	5.3	11.3	19.2	9.2	27.3	16.4	92.7
		S	Oct. 15, 99	2.0	4.9	21.6	16.5	10.0	33.2	18.9	107.0
	Xixian	*S*	Oct. 12, 96	3.8	0.0	6.7	9.7	4.2	19.1	5.3	48.8
		S	Oct. 15, 98	4.6	7.0	18.5	6.7	4.0	26.1	11.1	77.9
		S	Sep. 15, 99	5.3	3.6	13.2	16.4	6.8	47.2	11.5	104.0
	Median, range			3.9, 3.4	4.2, 7.0 y	12.3, 14.8 y	13.1, 12.5 y	5.5, 7.1 z	26.7, 36.9	11.3, 14.2	

[a] R, ssp. *rhamnoides*; M, ssp. *mongolica*; S, ssp. *sinensis*.
[b] Q-3-S-7-Rh, quercetin-3-O-sophoroside-7-O-rhamnoside; I-3-S-7-Rh, isorhamnetin-3-O-sophoroside-7-O-rhamnoside; I-3-G-7-Rh, isorhamnetin-3-O-glucoside-7-O-rhamnoside; Q-3-R, quercetin-3-O-rutinoside; Q-3-G, quercetin-3-O-glucoside; I-3-R, isorhamnetin-3-O-rutinoside; I-3-G, isorhamnetin-3-O-glucoside.
[c] Agrifood Research Finland, Research Station at Satakunta (Kokemäki, Finland).
[d] Agrifood Research Finland, Research Station at Tuorla (Tuorla, Piikkiö, Finland).
[e] Agrifood Research Finland, Research Station at Piikkiö (Piikkiö, Finland).
[f] *H. rhamnoides* ssp. *rhamnoides* (♂) X *H. rhamnoides* ssp. *caucasica* (♀). Different letters (x, y, z) following the values in the same column indicate statistically significant difference ($P < 0.05$) between the values.

water content in the berries into account at the first extraction. After each extraction, the sample was filtered. The filtrates from three extractions were combined, and the solvents were evaporated in a rotary evaporator. After addition of 5.0 ml of phloridzin (1.0 mg/ml in MeOH) solution as internal standard, the sample was dissolved in methanol to a final volume of 25 ml. A volume of 10 ml of the methanol solution was taken, evaporated to dryness and re-dissolved in 10 ml water. Thereafter, the sample was purified on a polyamide column (1.0 g Polyamide CC 6, particle size 50–160 μm; Fluka, Milwaukee, WI, USA) pre-activated with methanol (20 ml) and water (60 ml). The sample was applied on the column, and the strongly polar compounds such as fruit sugars and acids were eluted by 3 ml of water and discarded after elution. Flavonol glycosides were eluted with 40 ml of methanol, evaporated to dryness and re-dissolved in 3 ml methanol for HPLC analysis. Each berry sample was extracted and purified in duplicates. Two HPLC runs were carried out for each purified sample.

2.4. HPLC-DAD analysis of flavonol glycosides

The HPLC instrument consisted of a Shimadzu SIL-10A auto sampler, a sample cooler, two CTO-10A pumps, a CTO-10A column oven, a SPD-M10AVP diode array detector and a SCL 10AVP central unit (Shimadzu Corporation, Kyoto, Japan). The samples were analysed using a Phenomex Prodigy ODS 5 μ (3) column (250 × 4.60 mm, particle size 5 μm). The mobile phase consisted of a binary gradient eluting system. Solvent A was a mixture of water:tetrahydrofuran:trifluoroacetic acid (98:2:0.1); solvent B was acetonitrile. The eluting gradient program used in flavonol glycoside analysis is presented in Table 1. The flow rate of the mobile phase was 1 ml/min, the volume of injection 10 μl, and the column temperature 30 °C. The detection wave length was 270 nm. UV absorption spectra were scanned in the range of 200–450 nm and stored during the analysis. The peaks were identified by co-injections with reference compounds and comparisons of the UV absorption spectra to those of the reference compounds. The quantification of flavonol glycosides was carried out using the internal standard phlorizin. Correction factors for four flavonol glycosides were determined by HPLC analyses of mixtures of reference compounds (flavonol glycosides and phloridzin) before and after the sample preparation process, and the results showed that the sample preparation procedure did not lead to discrimination between the compounds. The correction factors used, 0.782 for isorhamnetin-3-O-rutinoside, 1.025 for isorhamnetin-3-O-glucoside, 0.944 for quercetin-3-O-rutinoside and 0.726 for quercetin-3-O-glucoside. For other compounds, correction factor 1 was applied due to the lack of pure reference compounds for an accurate determination of the correction factors. The compounds were quantified as flavonol glycosides without conversion into the quantity of the aglycones.

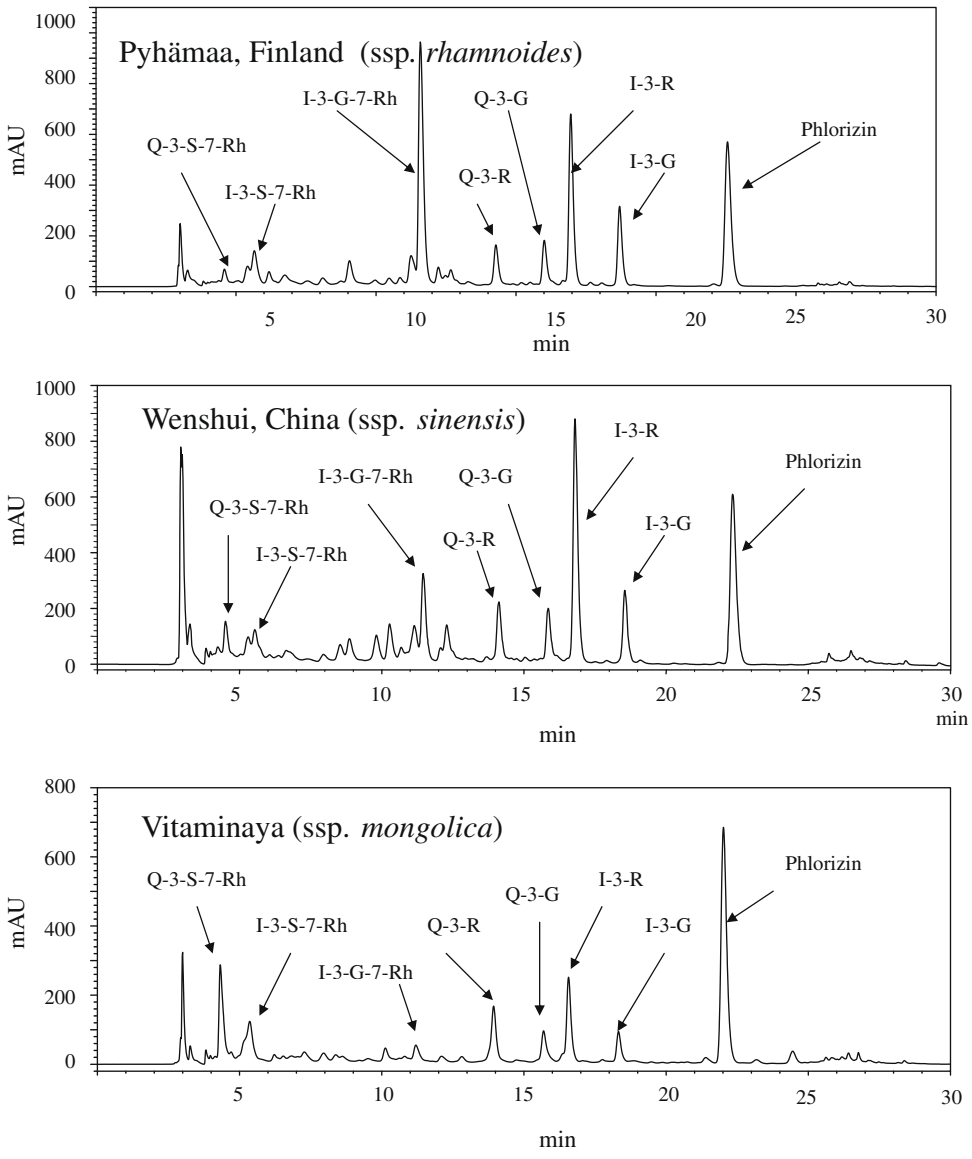
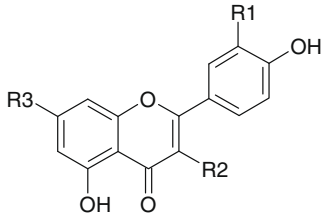


Fig. 1. HPLC chromatograms of flavonol glycosides from berries of three different subspecies of sea buckthorn. Q-3-S-7-Rh, quercetin-3-O-sophoroside-7-rhamnoside; I-3-S-7-Rh, isorhamnetin-3-O-sophoroside-7-O-rhamnoside; I-3-G-7-Rh, isorhamnetin-3-O-glucoside-7-O-rhamnoside; Q-3-R, quercetin-3-O-rutinoside; Q-3-G, quercetin-3-O-glucoside; I-3-R, isorhamnetin-3-O-rutinoside; I-3-G, isorhamnetin-3-O-glucoside. The first major peak (retention time ca. 3 min) in the chromatogram is a solvent peak.



Flavonol glycoside	R1	R2	R3
Isorhamnetin-3-O-sophoroside-7-rhamnoside	OCH_3	O-sophorosyl	O-rhamnosyl
Isorhamnetin-3-O-glucoside-7-rhamnoside	OCH_3	O-glucosyl	O-rhamnosyl
Isorhamnetin-3-O-rutinoside	OCH_3	O-rutinosyl	OH
Isorhamnetin-3-O-glucoside	OCH_3	O-glucosyl	OH
Quercetin-3-O-sophoroside-7-rhamnoside	OH	O-sophorosyl	O-rhamnosyl
Quercetin-3-O-rutinoside	OH	O-rutinosyl	OH
Quercetin-3-O-glucoside	OH	O-glucosyl	OH

Fig. 2. Structures of the flavonol glycosides identified in the sea buckthorn samples analysed in the present study.

2.5. Statistical analysis

Statistical analysis was carried out using the statistical software SPSS for Windows version 16.0 (SPSS Inc., Chicago, IL). Mann-Whitney *U*-Test was used for the comparison amongst wild subspecies ssp. *sinensis*, wild ssp. *rhamnoides* and the Finnish cultivars. Differences reaching a minimal confidence level of 95% were considered as being statistically significant. Due to the small number of samples analysed, the Russian cultivars were not compared statistically with the samples of the other origins.

3. Results and discussion

Fig. 1 shows chromatograms of the flavonol glycoside fraction from the berry samples of *H. rhamnoides* ssp. *rhamnoides*; ssp. *sinensis*, and ssp. *mongolica*. Seven major flavonol glycosides were identified and quantified. The structures of these compounds are presented in Fig. 2. Isorhamnetin and quercetin are known to be the major flavonols in sea buckthorn (Chen, Zhang, Xiao, Yong, & Bai, 2007; Lachman, Pivec, Hubácek, & Reháková, 1985; Rösch, Bergmann, Knorr, & Kroh, 2003; Rösch, Krumbein, Mügge, & Kroh, 2004). Rösch and colleagues reported major flavonol glycosides in the berries of a German cultivar, *H. rhamnoides* ssp. *rhamnoides* cv. Hergo (Rösch et al., 2004). Chen and colleagues studied the flavonol glycosdies in the berries of wild sea buckthorn from the natural growth sites in Sichuan province, China (Chen et al., 2007). The identification of the major flavonol glycosides in the present study were in agreement with the compounds reported by these two previous studies (Chen et al., 2007; Rösch et al., 2004). Thus it was considered as sufficient that the identification was confirmed by the co-injection of the reference compounds; especially, the identities of three less common flavonol glycosides were confirmed by co-injections with two sets of reference compounds obtained from two different sources.

Contents of the major flavonol glycosides in the samples are summarised in Table 2. The data in Table 2 shows sea buckthorn berries in general as a rich source of flavonols. The total content of flavonol glycosides was in the range of 27–130 mg per 100 g fresh berries with considerable variation amongst samples of different origins (Table 2). The contents were comparable to the levels reported in fresh onions (15–200 mg per 100 g) and fresh broccoli florets (38 mg per 100 g) but about 10–100 times higher than the levels reported in fresh green beans (1–2 mg aglycons per 100 g) (Price & Rhodes, 1997; Price, Casuscelli, et al., 1998; Price, Colquhoun, et al., 1998). The Finnish cultivar Raisa contained the highest level of flavonol glycosides (up to 130 mg per 100 g fresh berries) amongst the berry samples analysed. In addition, high contents of flavonol glycosides were seen in some wild berries from Finland and China. The Russian cultivar Vitaminaya contained the lowest level of flavonol glycosides (27 mg per 100 g fresh berries).

Typical intake of flavonoids from foodstuffs in industrial countries varies between 20 and 50 mg/day (Arai et al., 2000; Hertog, Hollman, Katan, & Kromhout, 1993; Justesen, Knuthsen, & Leth, 1997). Quercetin is the most common flavonol in the diet, abundant in tea, apples, onion, broccoli and many other vegetables. Some red wines also have high contents of flavonols (Frankel, Waterhouse, & Teissedre, 1995). With the high content of flavonols in sea buckthorn berries, it is possible to significantly increase the daily intake of flavonoids through a reasonable addition of the berries to the diet. Some clinical evidence suggests that dietary supplementation with as little as 10 mg sea buckthorn flavonols may have some positive effects on the health and function of the heart and the vascular system (Wang et al., 1993).

Overall, isorhamnetin-3-O-glucoside-7-O-rhamnoside and isorhamnetin-3-O-rutinoside, and isorhamnetin-3-O-glucoside were the most abundant flavonol glycosides in the wild and cultivated berries of ssp. *rhamnoides*. In the berries of wild ssp. *sinensis* from Wenshui and Xixian, China and the Russian cultivars,

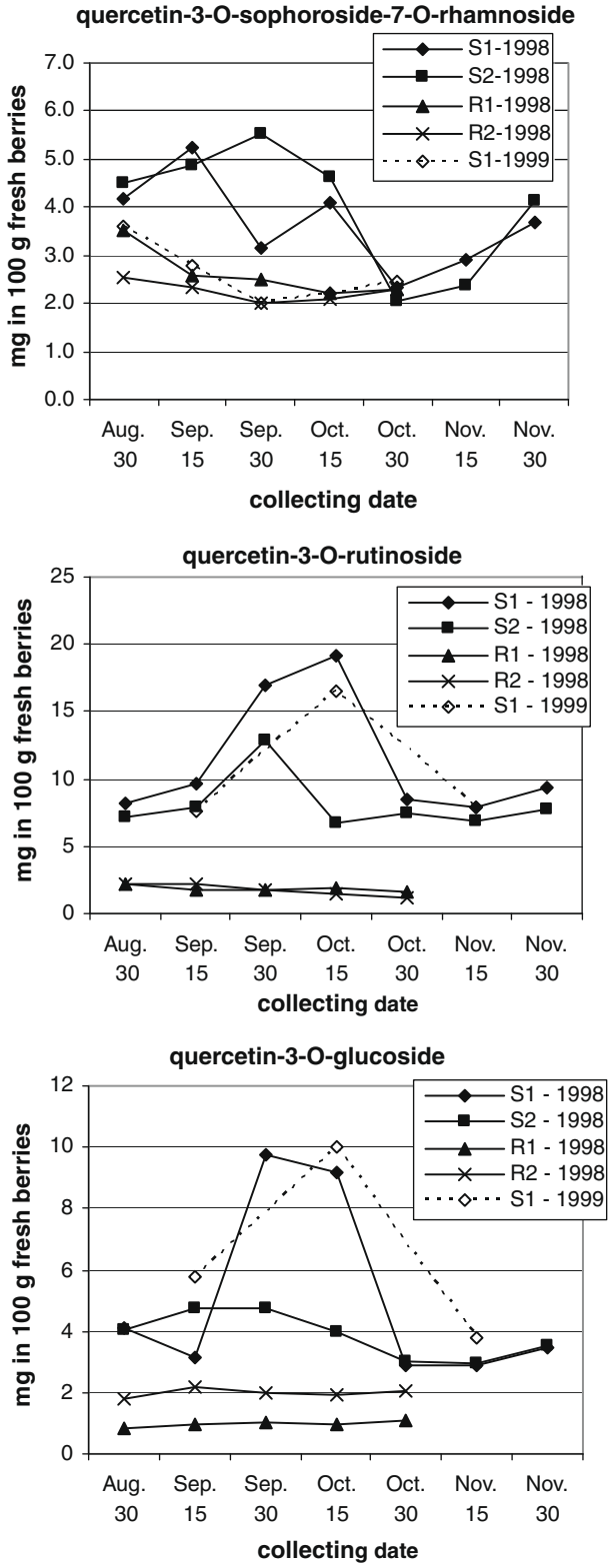


Fig. 3. Changes in the content of quercetin glycosides in the berries of ssp. *sinensis* and ssp. *rhamnoides* during the harvesting period in 1998 and 1999. S1, wild berries of ssp. *sinensis* from Wenshui, Shanxi, China; S2, wild berries of ssp. *sinensis* from Xixian, Shanxi, China. R1 and R2 are cultivars selected from wild ssp. *rhamnoides*, R1, 74006003; R2, S3003.

isorhamnetin-3-O-rutinoside was clearly the most abundant flavonol glycoside, followed by isorhamnetin-3-O-glucoside-7-O-rhamnoside, isorhamnetin-3-O-glucoside and quercetin-3-O-rutinoside (Fig. 1 and Table 2). The Finnish wild and cultivated berries of ssp. *rhamnoides* contained higher levels of isorhamnetin-3-O-glucoside-7-O-rhamnoside and isorhamnetin-3-O-sophoroside-7-O-rhamnoside and lower levels of quercetin-3-O-rutinoside compared with the Chinese berries of ssp. *sinensis*, the difference reaching a statistically significant level ($P < 0.05$, Table 2).

Chen and colleagues reported significant difference in the content and profile of flavonol glycosides amongst the berries *H. rhamnoides* ssp *sinensis* and *H. neurocapa* ssp. *stellatopilosa* and *H. tibetana*. The profiles of flavonol glycosides were found to be similar in the berries of *H. rhamnoides* ssp. *sinensis* and *H. rhamnoindes* ssp. *yunnanensis*. The authors suggested that the profile of flavonol glycosdies in berries may be a useful chemotaxonomic feature distinguishing different species of sea buckthorn (Chen et al., 2007).

The results of the present study indicate compositional difference in flavonol glycosides between the wild berries of two major subspecies as well as amongst the Russian and Finnish cultivars. However, the difference shall be verified by future studies involving larger number of samples of especially ssp. *mongolica*.

The content of flavonol glycosides, the relative abundance of different aglycon species, as well as the structure and position of the sugar moieties attached to flavonols, all contribute to the biological effects and efficacies of the berries and the berry products. Quercetin is known to be a stronger antioxidant than isorhamnetin *in vitro*. On the other hand, the 3′ 4′-catechol B-ring structure of quercetin also presents higher potential of prooxidantive activities compared with isorhamnetin. Dietary flavonol glucosides e.g. quercetin-3-O-glucoside and kaempferol-3-O-glucoside have been shown to have better bioavailability than the corresponding 3-O-rutinosides. The glucosides are actively absorbed in the small intestine, whereas the rutinosides are only absorbed in the colon after hydrolysis by the activities of colonic microflora. In addition to influencing the effectiveness of absorption, the different locations of uptake probably lead to different metabolic fates of these glycosides (Chang et al., 2005; Crespy et al., 2002; Heim et al., 2002; Hollman et al., 1999; Rösch et al., 2003). Based on the data provided by the present study, it is possible to select sea buckthorn berries amongst the cultivars and subspecies to obtain specific content and composition of flavonol glycosides for targeted applications.

Considerable variation was also recognised amongst the samples harvested in different years. The variation may have resulted from different climate conditions in these years. It is also important to notice that the optimal harvesting dates (for optimally ripe fruits) of sea buckthorn do vary slightly amongst different years. This factor may have contributed to the differences observed in samples of the same origin but collected in different years.

The influence of harvesting dates on the content flavonol glycosides was studied in wild berries of ssp. *sinensis* collected from two

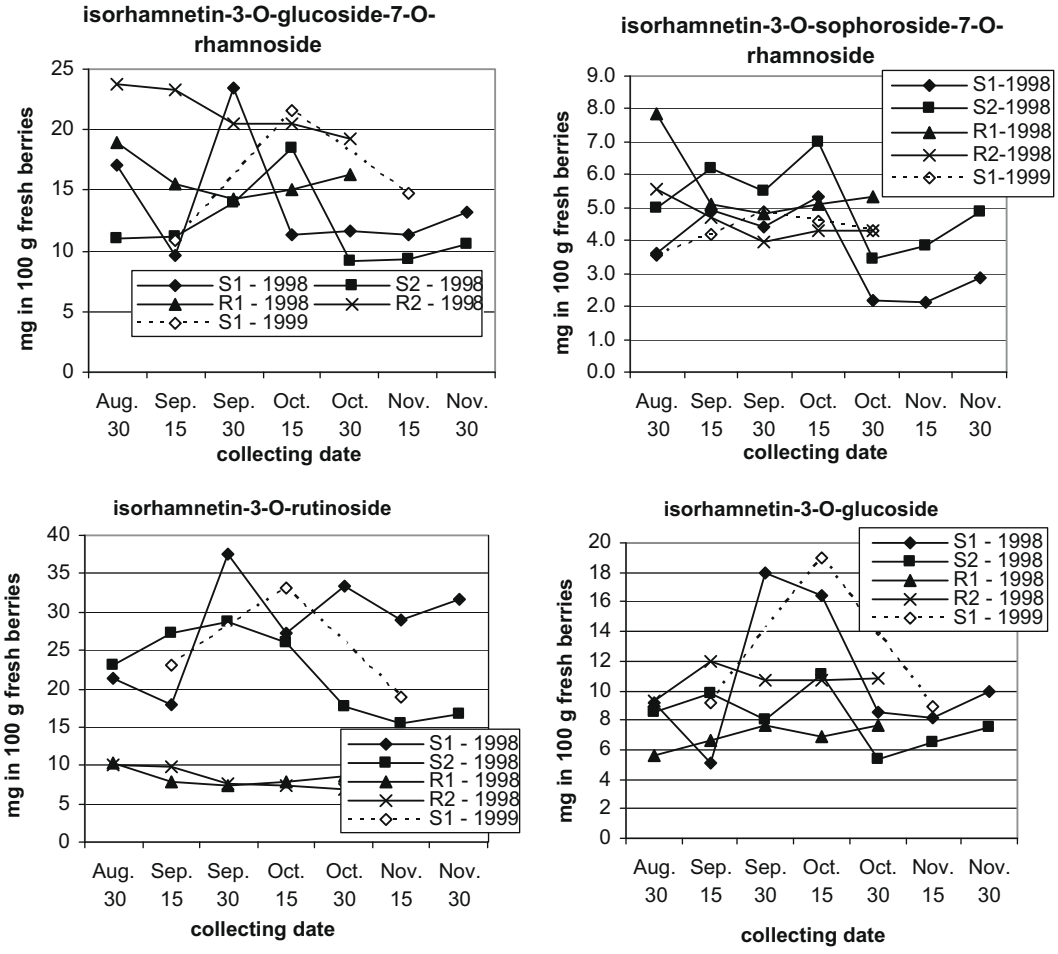


Fig. 4. Changes in the content of isorhamnetin glycosides in the berries of ssp. *sinensis* and ssp. *rhamnoides* during the harvesting period in 1998 and 1999. S1, wild berries of ssp. *sinensis* from Wenshui, Shanxi, China; S2, wild berries of ssp. *sinensis* from Xixian, Shanxi, China. R1 and R2 are cultivars selected from wild ssp. *rhamnoides*, R1, 74006003; R2, S3003.

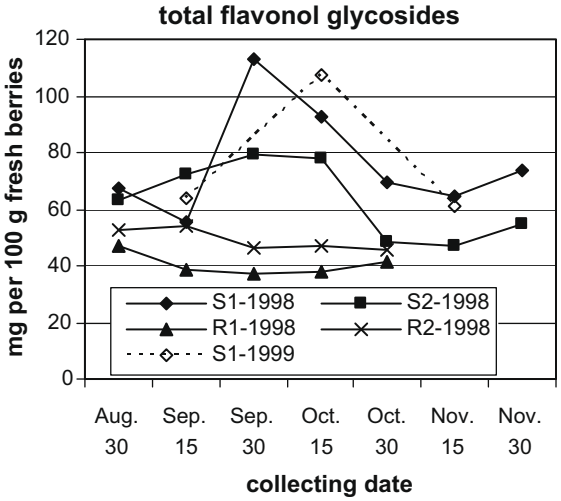


Fig. 5. Changes in the total content of flavonol glycosides in the berries of ssp. *sinensis* and ssp. *rhamnoides* during the harvesting period in 1998 and 1999. S1, wild berries of ssp. *sinensis* from Wenshui, Shanxi, China; S2, wild berries of ssp. *sinensis* from Xixian, Shanxi, China. R1 and R2 are cultivars selected from wild ssp. *rhamnoides*, R1, 740,06,003; R2, S3003.

natural growth sites, Xixian and Wenshui in Shanxi Province, China and cultivated berries of ssp. *rhamnoides* from Kokemäki, Finland from late August to late November 1998. In 1999, flavonol glycosides were studied in berries collected from Wenshui County at three different time points. The changes in the content of flavonol glycosides in these berry samples during the harvesting period are shown in Figs. 3–5. In the Chinese berries, the content of major flavonol glycosides increased from mid September to mid October, reached the maxima around the period from late September to early October and decreased thereafter. In contrast to the Chinese berries, the level of flavonol glycosides in the two Finnish cultivars showed a decreasing trend during the period studied.

Raffo and colleagues (Raffo, Paoletti, & Antonelli, 2004) studied the changes in the content of flavonols in three German cultivars Askola, Hergo and Leikora during the ripening period from early July to late September. The content of isorhamnetin and quercetin decreased all the way through the period in the berries of Hergo whilst remaining relatively constant in the berries of Askola and Leikora. The difference in the peaking dates of flavonol glycosides in Finnish and Chinese berries as well as the different changing patterns reported in the flavonol content in German cultivars reflected differences in ripening rate and the metabolism pathways of the secondary metabolites as a result of multiple interactions amongst genetic, geographical and climate factors.

In Finland the berries of most cultivated and wild sea buckthorn are harvested around late August to mid September, whereas the harvesting period of wild berries of ssp. *sinensis* in northern China starts at early October and extends until January of the following year in some mountainous areas. The results of the present and previous studies suggest that the harvesting date should be carefully selected according to the genetic background and the growth place of sea buckthorn in order to obtain berries with the highest content of flavonols.

Previously, the authors reported the decreasing trend of vitamin C content in four Finnish cultivars (74006003, 74006005, S3003, S3006) from the end of August to the end of November 1998 (Kallio, Yang, & Peippo, 2002). The changing patterns in the content of vitamin C are quite identical to those of flavonol glycosides observed in the present study in the berries of two cultivars, 74006003 and S3003. In the wild Chinese berries, the changes in the content of flavonol glycosides seemed to parallel the changes of lignans during the same harvesting period (Yang, Linko, Adlercreuts, & Kallio, 2006). The influence of harvesting date on the content of multiple bioactive compounds may result in great variation in the biological activities and health benefits of sea buckthorn berries and berry product (Gao, Ohlander, Jeppsson, Bjork, & Trajkovski, 2000). In addition to harvesting date, attention should be paid to the handling and storage of the berries in order to minimise post-harvesting loss of flavonols and their glycosides.

In conclusion, the content and profile of flavonol glycosides in sea buckthorn berries are highly dependent on the origins, subspecies and cultivars and strongly influenced by the harvesting dates and annual variations. The present study provided valuable information on the flavonol glycosides in wild and cultivated sea buckthorn from Finland, China and Russia as well as the optimal harvesting dates for Chinese and Finnish sea buckthorn. These together with the existing information published on other bioactive components of these berries are useful guidance for plant breeding and industrial utilisation of sea buckthorn.

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