



## 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 quercetin-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.

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### 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 111°41'E, latitude 37°32'N, altitude 1600 m) and Xixian (longitude 111°02'E, latitude 36°48'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, Casascelli, 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

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