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Secoisolariciresinol and Matairesinol of Sea Buckthorn (*Hippophaë rhamnoides* L.) Berries of Different Subspecies and Harvesting Times

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Sea buckthorn (*Hippophaë rhamnoides*) seeds, berries, and berry fractions are often used as sources of bioactive ingredients for health products. The aim of the present study was to analyze lignans in these fractions of sea buckthorn. Secoisolariciresinol and matairesinol in seeds, fruit pulp/peel, and whole berries of sea buckthorn of three subspecies were analyzed by isotope dilution gas chromatography−mass spectrometry. The total content of the two lignans secoisolariciresinol and matairesinol varied widely from 8 to 139 μg/100 g in fresh berries and from 51 to 319 μg/100 g in dry berries. The content of secoisolariciresinol varied in the range of 34−313 μg/100 g of dry mass in the fruit pulp/peel and 93−355 μg/100 g in dry seeds. The content of matairesinol fell within the range of 3−25 μg/100 g in dry pulp/peel and 1−13 μg/kg in dry seeds. Wild *H. rhamnoides* ssp. *sinensis* contained a significantly higher total level of secoisolariciresinol and matairesinol in dry seeds, dry berries, and fresh berries compared with wild ssp. *rhamnoides* (253 vs 135 μg/100 g, $P < 0.01$, in seeds; 224 vs 153 μg/100 g, $P < 0.05$, in dry berries; 71 vs 29 g/100 g, $P < 0.01$, in fresh berries) and the cultivar of ssp. *mongolica* (253 vs 112 μg/100 g in seeds, 71 vs 9 μg/100 g in fresh berries). Harvesting dates had a significant influence on the content of the two lignans in seeds, fruit pulp/peel, and whole berries. This is the first report of lignans in sea buckthorn.

KEYWORDS: Sea buckthorn; *Hippophaë rhamnoides*; seeds; berries; fruit pulp/peel; lignans; secoisolariciresinol; matairesinol

INTRODUCTION

Vegetables and fruits are irreplaceable constituents of a healthy diet. In addition to vitamins, antioxidants, and dietary fibers, a group of phenolic compounds known as phytoestrogens are increasingly recognized as important contributors to the beneficial effects of vegetables and fruits on human health.

Phytoestrogens are weakly estrogenic compounds found in plants and consist of isoflavones, coumestans, and lignans. The dietary lignans are converted in the colon to weakly estrogenic compounds called mammalian lignans or enterolignans. Phytoestrogens have been shown to bind estrogen receptors and to behave as either agonists or antagonists of estrogens in both animals and humans depending on the level of endogenous estrogens (*1*). Evidence from epidemiological, experimental, and intervention studies suggests health-beneficial properties of phytoestrogens extending from lowering the incidence of hormone-dependent breast and prostate cancers and osteoporosis to reducing the risk of inflammation and cardiovascular disease and improving cognitive and immune functions (*2*−*10*).

The suggested mechanisms of the health effects of phytoestrogens are many and highly diversified, such as interference with the release of gonadotrophins, competition with endogenous estrogens for estrogen receptor binding, inhibition of the biosynthesis of estradiol, influence on early mammary gland maturation, inhibition of 5α-reductase activity, reduction of the plasma cholesterol level, enhancement of LDL clearance, and inhibition of LDL oxidation (*11*−*13*).

Lignans occur widely in the plant kingdom both in free form and as glycosides. Over 500 lignans have been reported in 150 plant species (*11*). The lignans often studied in plant materials are secoisolariciresinol, matairesinol, lariciresinol, isolariciresinol, arctigenin, pinoresinol, and syringaresinol, the first two being the most frequently found plant lignans (*11*). **Figure 1** presents the structures of the two major plant lignans, secoisolariciresinol and matairesinol.

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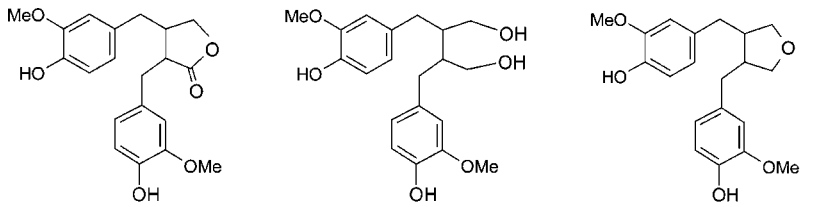

Figure 1. Chemical structures of two major plant lignans (matairesinol and secoisolariciresinol) and anhydrosecoisolariciresinol (acid hydrolysis product of secoisolariciresinol).

Table 1. Content of Lignans in Dry Seeds and Fruit Pulp/Peel and Fresh and Dry Berries of Sea Buckthorn of Different Origins[a]

sample			dry pulp/peel			dry seeds			SECO + MAT in berries	
growth location	ssp.	harvesting date[b]	SECO	MAT	SECO + MAT	SECO	MAT	SECO + MAT	fresh berry	dry berry
Wenshui, Shanxi	*sinensis*	Oct 4	197.1	24.8	221.8	354.7	12.9	367.6	71.3	278.3
Fuxian, Shanxi	*sinensis*	Oct 5	312.7	20.0	332.7	292.1	8.8	300.9	114.8	319.3
Wangtao, Shanxi	*sinensis*	Oct 27	261.9	19.1	281.0	293.7	9.2	302.9	83.1	288.9
Kelan, Shanxi	*sinensis*	Oct 29	141.9	10.8	152.7	265.9	5.4	271.2	59.2	199.5
Youyu, Shanxi	*sinensis*	Nov 7	132.5	8.7	141.3	233.2	3.8	236.9	53.8	182.0
Xixian, Shanxi	*sinensis*	Oct17	187.3	7.1	194.4	233.4	2.6	236.0	65.1	212.4
Heshun, Shanxi	*sinensis*	Nov 5	226.0	10.2	236.2	258.5	4.4	263.0	71.5	247.8
Xunyi, Shanxi	*sinensis*	Nov 3	305.1	3.7	308.8	243.0	3.7	246.7	93.3	279.1
Wuzai, Shanxi	*sinensis*	Nov 26	132.0	6.4	138.4	194.9	1.5	196.3	43.5	159.4
Youyu, Niuxin, Shanxi	*sinensis*	Nov 23	152.1	7.6	159.8	182.0	2.1	184.1	47.3	169.5
Wutai, Shanxi	*sinensis*	Nov 21	181.7	14.3	196.0	140.2	2.2	142.4	40.9	174.9
Yongshou, Shaanxi	*sinensis*	Nov 21	231.2	17.9	249.1	189.8	3.5	193.3	72.9	226.2
Ningwu , Shanxi	*sinensis*	Nov 5	84.1	8.6	92.7	304.4	2.4	306.8	32.4	156.4
Datong, Qinghai	*sinensis*	Nov 7	131.4	17.5	149.0	258.3	2.5	260.8	67.1	194.8
Longde, Ningxia	*sinensis*	Nov 20	246.0	15.0	261.0	311.1	2.7	313.8	138.8	281.4
Dingxi, Gansu	*sinensis*	Nov 9	201.9	15.3	217.2	213.4	3.5	216.9	76.1	217.1
Siikajoki, Finland	*rhamnoides*	Sept 10	209.6	8.6	218.2	163.5	3.1	166.6	31.3	195.1
Vaasa, Finland	*rhamnoides*	Sept 10	106.1	4.4	110.5	133.4	2.6	135.9	23.0	120.0
Pyhämaa, Finland	*rhamnoides*	Sept 9	160.8	5.2	166.0	119.2	3.0	122.3	32.3	147.7
Pyhämaa, Finland	*rhamnoides*	Sept 9	159.7	6.7	166.4	113.5	3.0	116.5	28.7	148.1
mean ± st dev	*sinensis*		195.3 ± 65.6	13.0 ± 5.9[c]	208.3 ± 62.9	248.0 ± 56.0[d]	4.4 ± 3.2	252.5 ± 58.1[d]	70.7 ± 27.6[d]	224.2 ± 52.1[c]
mean ± st dev	*rhamnoides*		159.1 ± 42.3	6.2 ± 1.8	165.3 ± 29.6	132.4 ± 22.3	2.9 ± 0.2	135.3 ± 22.4	28.8 ± 4.2	152.7 ± 31.1
Satava, Finland	*mongolica*	Aug 25, Sept 8 and 22	36.3, 33.9, 48.0	2.6, 2.5, 3.2	38.9, 36.3, 51.2	93.0, 128.2, 112.2	0.6, 0.7, 0.8	93.7, 128.8, 113.0	8.3, 9.0, 8.4	51.4, 56.7, 58.6

[a] SECO = secoisolariciresinol, and MAT = matairesinol. The contents of the lignans are expressed as μg/100 g. [b] The berries of ssp. *sinensis* were collected in China in 1997 and the berries of ssp. *rhamnoides* and *mongolica* in Finland in 1999. [c] $P < 0.05$, compared with ssp. *rhamnoides*. [d] $P < 0.01$, compared with ssp. *rhamnoides*.

The content of lignans varies greatly among different food materials of plant origin (*14*−*17*). Linseed contains lignans up to 400 000 μg/100 g of the dry mass, being the richest natural source of the compounds. Cereal grains contain lignans at levels of 10−100 μg/100 g of dry mass. In tea and some vegetables such as pumpkin, asparagus, and leek, the levels of lignans are typically 1000−4000 μg/100 g of dry mass. The lignan contents in berries have been reported with large variation among different species (*16*, *17*). Cranberry, lingonberry, and strawberry contain lignans at 1000−1500 μg/100 g of dry mass. The levels in raspberry, blackcurrant, and redcurrant are typically 100−400 μg/100 g of dry mass (*16*, *17*).

A wide range of beneficial effects have been demonstrated for sea buckthorn (*Hippophaë rhamnoides*) berries on human health (*18*−*22*). The berries are increasingly popular as a source of nutraceuticals and cosmeceuticals as well as potential raw material for functional foods. It is generally believed that the health effects of sea buckthorn are often the results of the synergy among multiple lipophilic and hydrophilic bioactive compounds in the seeds and berries. While fatty acids, flavonoids, vitamin C, carotenoids, sterols, tocopherols, and tocotrienols in sea buckthorn have been well documented (*22*−*27*), the content of lignans in the species has not been reported.

The aim of the present study was to characterize and quantify two key lignans, secoisolariciresinol and matairesinol, in the seeds and fruit pulp/peel of sea buckthorn. The differences among subspecies as well as the effect of harvesting time on the content of the two compounds were also investigated.

MATERIALS AND METHODS

Sample Collection. For the analysis of the selected lignans in sea buckthorn and for comparison of different subspecies, berry samples were collected from China from early October to late November 1997 and Finland in early September 1999 (**Table 1**). Chinese wild berries of sea buckthorn of subspecies *H. rhamnoides* ssp. *sinensis* were collected from 16 natural growth sites in Shanxi, Shaanxi, Ningxia, Gansu, and Qinghai provinces. The Finnish wild berries belonging to *H. rhamnoides* ssp. *rhamnoides* were picked from four natural populations on the southwestern coast of Finland. One sample was collected from each growth site. The collecting dates (**Table 1**) represented the common local harvesting times for properly ripened berries.

To follow the changes in the content of secoisolariciresinol and matairesinol during the harvesting period, berry samples were collected from a natural growth site in Wenshui, Shanxi, China, at seven different dates during the harvesting period from the end of August to the end of November 1998. In Satava, Turku, Finland, berries of a commercial cultivar of *H. rhamnoides* ssp. *mongolica* were picked on three different dates, Aug 25, Sept 8, and Sept 22, 1999.

For each sample, 20 kg of fresh berries were collected and loosely frozen immediately after picking and stored at −20 °C until they were analyzed in 2000.

Preparation of Berry Samples. For each of the samples, frozen berries of 1 kg were pulled from the 20 kg lot using a multiple-site sampling procedure, from which a smaller sample of frozen berries (about 20 g) was then taken for analysis. The dry matter content of the freshly thawed berries was determined using a Moisture Analyser 40 (Sartorius AG, Göttingen, Germany).

Freshly thawed berries were manually separated into two fractions, the seeds and the fruit pulp/peel, which were freeze-dried immediately after the separation. The freeze-dried samples were homogenized by milling. For each analysis, a powdered sample of 50 mg was weighed, 500 μL of distilled water was added, and the sample was kept at room temperature overnight for rehydration.

Isolation of Lignans. Lignans were isolated from rehydrated samples using a modification of the procedures described by Mazur et al. (*28*). Briefly, enzymatic hydrolysis with purified *Helix pomatia* (Biosepra IBF/Sepracor, Villeneuvela Lagarenne, France) juice was carried out to hydrolyze the glycosides of isoflavonoids possibly present in the samples (*28*). A hydrolysis reagent was prepared by addition of Milli-Q water (1:1) and ascorbic acid (5 mg/mL) to the purified enzyme. After the enzymatic reaction, the isoflavonoids and aglycons of lignans were extracted from the samples with diethyl ether (2 × 5 mL). The water and solid phases containing lignan glycosides were saved for acid hydrolysis. Isoflavonoid analyses are not reported here.

The lignan glycosides in the water and solid phases from the enzymatic hydrolysis step were hydrolyzed in the presence of 2 M HCl (by addition of approximately 500 μL of 6 M HCl) at 100 °C for 2.5 h. After hydrolysis, the sample was cooled to room temperature and the pH was adjusted to 3−5 with 10 M NaOH solution. After acid hydrolysis, deuterated lignans (d_6-secoisolariciresinol, d_8-anhydrosecoisolariciresinol, and d_6-matairesinol, 85.5 ng of each) (synthesized at the Department of Chemistry, University of Helsinki, Finland) were added as internal standards. The sample was then extracted with 2 × 6.5 mL of diethyl ether. The ether phases were combined and evaporated to dryness under nitrogen. The samples were redissolved in methanol/chloroform/water (8:2:2, v/v/v) and transferred to a 5 × 0.5 cm Lipidex-5000 column (Packard Instrument Co. Inc./Canberra, Meriden, CT). The lignans were eluted with 4 mL of the same solvent and further purified using successive ion exchange chromatographic processes on a DEAE-OH-Sephadex A-25 (Pharmacia Biotech AB, Uppsala, Sweden) column and a QAE-Sephadex A-25 (Pharmacia Biotech AB) column (*28*).

Analysis of Lignans. Secoisolariciresinol and matairesinol in the samples were analyzed by an earlier described method of gas chromatography−mass spectrometry (GC−MS) (*28*) after slight modification. After complete evaporation of the solvents, the purified lignan fraction was silylated by addition of 100 μL of silylating reagent (Silyl 8, pyridine/hexamethyldisilizane/trimethylchlorosilane, 9:3:1, v/v/v) (Pierce Chemical Co., Rockford, IL) followed by 30 min of incubation at room temperature. After that, the solvents were evaporated, and the sample was dissolved in 208 μL of hexane/Silyl 8 (200:8, v/v). The silylated samples were analyzed in duplicates with an Autoinjector 7673A gas chromatograph combined with an HP 5995 quadruple mass spectrometer and an HP 59970C MS Chem Station data system (Hewlett-Packard Oy, Espoo, Finland). A 12.5 m × 0.22 mm i.d., 0.25 μm d_f bonded phase BP-1 vitreous silica column (SGE International Pty. Ltd., Ringwood, Australia) directly connected to the ionization chamber was used. The temperature of the ion source was 240 °C. The oven temperature was programmed as follows: 150 °C for 1 min, increased to 240 °C at a rate of 40 °C/min, further increased to 280 °C at a rate of 3 °C/min, held at 280 °C for 1 min, increased to 290 °C at a rate of 10 °C/min, held at 290 °C for 5 min.

The lignans were identified by comparing the retention times and the mass spectra with those of reference compounds. The intensities of the molecular ions of trimethylsilyl (TMS) ethers of lignans were used for quantification (*28*). The ions monitored were *m*/*z* 566.5 for d_6-secoisolariciresinol, *m*/*z* 560.5 for secoisolariciresinol, *m*/*z* 508.4 for d_6-matairesinol, *m*/*z* 502.4 for matairesinol, *m*/*z* 488.5 for anhydrosecoisolariciresinol, and *m*/*z* 496.5 for d_8-anhydrosecoisolariciresinol. The lignans were quantified by relating the ratio of the peak area of the sample and deuterated internal standard to the respective ratios of the peak areas of standard compounds of known concentrations. Anhydrosecoisolariciresinol was formed from secoisolariciresinol during acid hydrolysis. The sum of secoisolariciresinol and anhydrosecoisolariciresinol was calculated and used to represent the total amount of secoisolariciresinol in the samples. The averages of the duplicate analyses were calculated and used in comparison of the samples and in the further analysis of the results.

Statistical Analysis. The data analysis was carried out using the statistical program SPSS 12.0 for Windows. Independent sample *t* tests were used to compare the content of lignans in different subspecies of sea buckthorn. Differences reaching a confidence level of 95% ($P < 0.05$) were considered as statistically significant.

RESULTS AND DISCUSSION

Two lignans, secoisolariciresinol and matairesinol (**Figure 1**), were quantified in sea buckthorn samples. Anhydrosecoisolariciresinol was formed from secoisolariciresinol during hydrolysis. Examples of single-ion monitoring (SIM) chromatograms of TMS derivatives of lignans of the sea buckthorn pulp/peel fraction and the seed fraction are presented in parts **A** and **B** of **Figure 2**, respectively. The total ion chromatograms (TICs) are also displayed. The chromatograms, especially the TICs clearly show the presence of unidentified compounds, which may be related to lignans and their metabolites. The amounts of anhydrosecoisolariciresinol and secoisolariciresinol were summed for the original secoisolariciresinol in the samples. The contents of secoisolariciresinol and matairesinol in dry seeds, dry fruit pulp/peel, and fresh and dry berries of sea buckthorn of different origins are summarized in **Table 1**.

Secoisolariciresinol was a major lignan in sea buckthorn representing 34−313 μg/100 g of dry fruit pulp/peel and 93−355 μg/100 g of dry seeds. Matairesinol was found at levels of 3−25 μg/100 g in dry pulp/peel and 1−13 μg/100 g in dry seeds. The sum of the two compounds ranged from 36 to 333 μg/100 g in dry pulp peel, from 94 to 368 μg/100 g in dry seeds, and from 51 to 319 μg/100 g in dry berries.

The level of secoisolariciresinol in the seeds of ssp. *sinensis* was significantly higher than that in the seeds of ssp. *rhamnoides* (248 vs 132 μg/100 g, $P < 0.01$). The content of matairesinol in the pulp/peel fraction was higher in ssp. *sinensis* than in ssp. *rhamnoides* (13 vs 6 μg/100 g, $P < 0.05$). The pulp/peel fraction of ssp. *sinensis* had a slightly higher average content of secoisolariciresinol and total content of the two lignans than that of ssp. *rhamnoides*, but the difference was not of statistical significance due to the relatively high variation. The seeds and pulp/peel of berries of one commercial cultivar of ssp. *mongolica* harvested on three different dates were also analyzed. The total content of secoisolariciresinol and matairesinol in dry seeds and dry fruit pulp/peel of this cultivar was clearly lower compared with the corresponding values found in the other two subspecies. In addition to different subspecies, the different growth conditions (such as soil and climate), variation among individual bushes within each growth site, and some differences in the maturity stages of the collected samples may also have contributed the the differences observed.

The total content of secoisolariciresinol and matairesinol varied widely in fresh (from 8 to 139 μg/100 g) and dry (from 51 to 319 μg/100 g) berries, the average level in the Chinese berries being significantly higher compared with those in the other two subspecies (*sinensis* vs *rhamnoides*, $P < 0.01$ in fresh berries, $P < 0.05$ in dry berries). Berries of the cultivar of ssp. *mongolica* were the poorest source of these lignans among the three subspecies analyzed, the content in fresh berries being

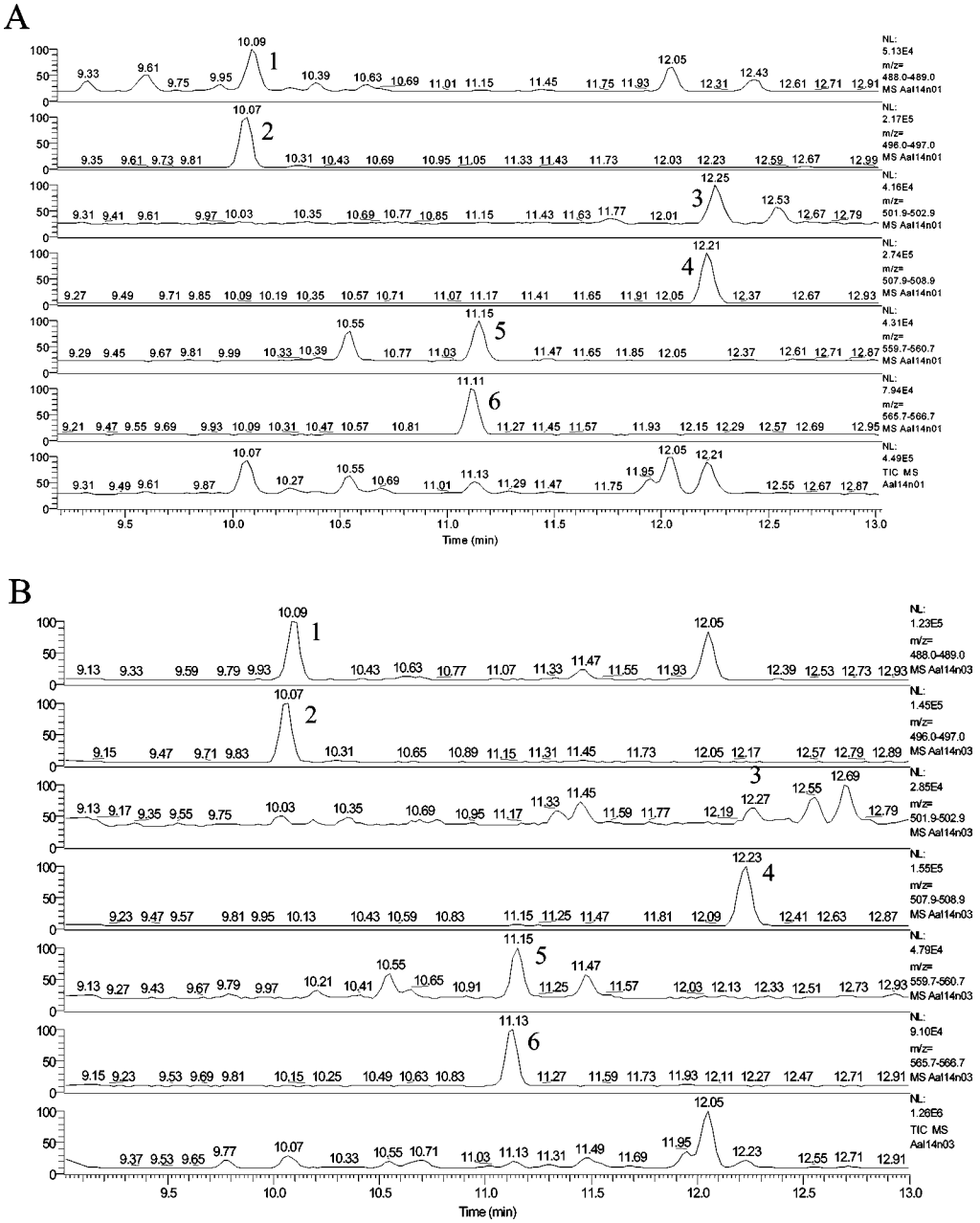

Figure 2. SIM chromatograms and TICs of trimethylsilyl ether derivatives of anhydrosecoisolariciresinol (**1**) (*m*/*z* 488.0–489.0), d_8-anhydrosecoisolariciresinol (**2**) (*m*/*z* 496.0–497.0), matairesinol (**3**) (*m*/*z* 501.9–502.9), d_6-matairesinol (**4**) (*m*/*z* 507.9–508.9), secoisolariciresinol (**5**) (*m*/*z* 559.7–560.7), and d_6-secoisolariciresinol (**6**) (*m*/*z* 565.7–566.7) of sea buckthorn: **A**, pulp/peel, **B**, seeds.

about one-eighth of the average level in fresh berries of ssp. *sinensis* and one-third of that of ssp. *rhamnoides*. In most of the samples of ssp. *sinensis*, the seeds contain more lignans than the fruit pulp/peel. The fresh berries of this subspecies were also smaller with a higher proportion of seeds compared with berries of the other two subspecies. This partially explains the higher lignan content in fresh berries of ssp. *sinensis*. It is not clear whether the breeding and selection process has played a role in the extremely low lignan content of the commercial cultivars of ssp. *mongolica* since no information is available on the lignan content in wild berries of this subspecies.

Figure 3 presents the changes in the content of secoisolariciresinol and matairesinol analyzed in dry pulp/peel, dry seeds, and dry berries of wild ssp. *sinensis* in Wenshui County, Shanxi Province, China, during the harvesting period in 1998. The highest content of secoisolariciresinol in the pulp/peel fraction was found for berries collected in mid-September (249 μg/100 g of dry mass), which was followed by a sharp decrease by almost two-thirds till mid-November to a level of 99 μg/100 g of dry mass. The level of secoisolariciresinol in the dry seeds increased from 210 μg/100 g at the end of August to a maximum of 274 μg/100 g in mid-October and decreased thereafter to a level of 175 μg/100 g by the end of November. The content of matairesinol in the pulp/peel fraction reached its maximum at the end of September, whereas the level in seeds showed an overall increasing trend throughout the period followed. The total content of secoisolariciresinol and matairesinol in the berry pulp/peel and the seeds followed the same changing pattern as that of secoisolariciresinol due to the clear dominance of this compound. After mid-October, the total content of secoisolariciresinol and matairesinol decreased considerably in dry seeds, dry pulp/peel, and dry berries.

In the dry pulp peel of the commercial cultivar of ssp. *mongolica*, the content of the two lignans increased from late

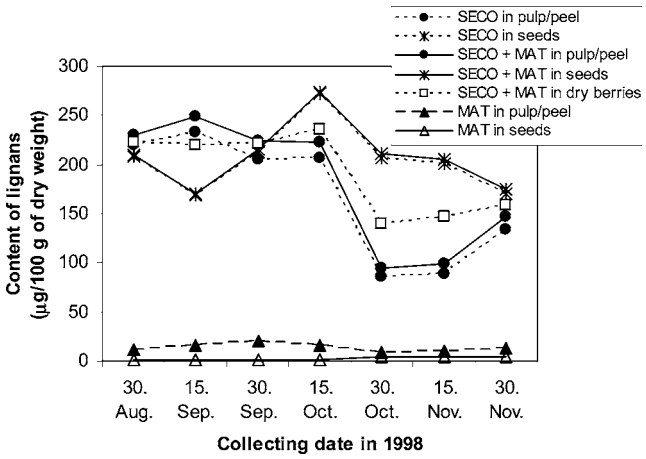

Figure 3. Changes in the content of secioisolariciresinol (SECO) and matairesinol (MAT) in dry fruit pulp/peel, dry seeds, and dry berries of sea buckthorn during the harvesting period in 1998. The samples were collected in Wenshui, Shanxi Province, China.

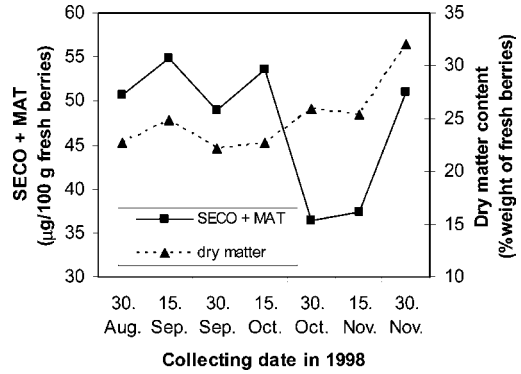

Figure 4. Changes in the total content of secoisolariciresinol (SECO) and matairesinol (MAT) as well as dry matters in fresh berries of sea buckthorn during the harvesting period in 1998. The samples were collected in Wenshui, Shanxi Province, China.

August to late September 1999. In the seeds, the level of secoisolariciresinol was highest in the sample collected on Sept 8, 1999 (**Table 1**).

In fresh wild berries of ssp. *sinensis*, the total content of secoisolariciresinol and matairesinol dropped sharply from 54 μg/100 g in mid-October to 36 μg/kg at the end of October (**Figure 4**). By the end of November, the total content of the two lignans in fresh berries returned to a level close to that found at the end of August, mostly due to the loss of water from the berries (the dry matter content increased by close to 40%). From Aug 30 to Oct 15, 1998, the changes found in the lignan content of fresh berries corresponded to the changes in the dry matter content of the berries. However, the sharp drop in the lignan content from late October to mid-November could not be explained by the dry matter content. Thus, the results suggest that the different ripening stages had a major impact on the content of the two lignans in the berries. Since only one sample was collected on each harvesting date, the variation among sampling within each growth site may also have contributed to the observed changes.

On the basis of the information presented in **Figures 3** and **4**, it is clear that mid-October was the best time for harvesting the Chinese berries to obtain a high content of secoisolariciresinol and matairesinol in both seeds and the berry pulp/peel. This time point falls in the normal harvesting time of wild sea buckthorn berries in China. In the fresh berries of ssp. *mongolica*, the lignan content was quite constant among the three harvesting dates (**Table 1**).

This paper is the first report of lignans of sea buckthorn berries. The total content of secoisolariciresinol and matairesinol found in dry berries and fruit pulp/peel of sea buckthorn was at levels similar to those reported for cloudberry (203 μg/100 g) and raspberry (139 μg/100 g) of the genus *Rubus* and black- and redcurrant of the genus *Ribes* (*17*). The highest content of lignans in berries (3740 μg/100 g of dry mass) has been reported for blackberries (*17*). Strawberry (1504 μg/100 g) and berries of the genus *Vaccinium* are also rich sources of secoisolariciresinol (1510 μg/100 g of dry mass in lingonberry, 835 μg/100 g in blueberry, 1054 μg/100 g in cranberry) (*17*).

In our earlier studies, significant compositional differences were found among berries of the three major subspecies of sea buckthorn, *H. rhamnoides* ssp. *sinensis*, ssp. *rhamnoides*, and ssp. *mongolica* (*22*−*27*). For example, the fruit pulp oil of ssp. *mongolica* has the highest proportion of palmitoleic acid (16:1*n*−7) (*26*), and the three subspecies clearly differ in the content of tocopherols in seeds and berries (*26*, *27*). Berries of ssp. *sinensis* contain vitamin C at levels around 20-fold higher compared with the berries of ssp. *mongolica* (*27*). The result of the present study provides more information for characterization of compositional differences among sea buckthorn berries of different subspecies.

The content and composition of the bioactive compounds, especially the hydrophilic components, in sea buckthorn berries vary considerably with the stages of maturity (*22*, *23*, *27*). The results of the present study suggest that lignan contents in berries of sea buckthorn are sensitive to the influence of harvesting dates.

At the present state of the art, processing of sea buckthorn berries on industrial scales involves mainly juice pressing from fresh berries and oil isolation from seeds and berries. The press residues from juice production and the residues after oil extraction are often discarded. Further information about the composition of the bioactive components in these fractions provides important guidance for more efficient utilization of the highly valuable berries. A major fraction of the lignans and many other phenolic compounds in sea buckthorn berries remain in the press cake and in the extraction residues after the juice and oil production processes. It is possible to give a rough estimation of the lignan content in these fractions based on the data presented by this paper. However, a thorough investigation of abundant lignans and phenolic compounds of sea buckthorn other than secoisolariciresinol and matairecinol would be worthwhile.

ABBREVIATIONS USED

GC−MS, gas chromatography−mass spectrometry; LDL, low-density lipoprotein; SIM, single-ion monitoring; TIC, total ion chromatogram; TMS, trimethylsilyl ether.

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