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Effects of Different Origins and Harvesting Time on Vitamin C, Tocopherols, and Tocotrienols in Sea Buckthorn (*Hippophaë rhamnoides*) Berries

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Vitamin C, tocopherols, and tocotrienols in berries of wild and cultivated sea buckthorn (*Hippophaë rhamnoides* L.) of different origins and harvesting dates were determined with HPLC. Wild berries of subsp. *sinensis*, native to China, contained 5−10 times more vitamin C in the juice fraction than the berries of subsp. *rhamnoides* from Europe and subsp. *mongolica* from Russia (4−13 vs 0.02−2 g/L juice). Genetic background and berry-harvesting date were two primary factors determining the vitamin C content in the berries. Crossing different subspecies influenced the vitamin C content to some extent. For bushes cultivated in southwest Finland, the best berry-harvesting date for high vitamin C content was the end of August. The seeds of subsp. *sinensis* contained less tocopherols and tocotrienols (average 130 mg/kg) compared with seeds of subsp. *rhamnoides* (average 290 mg/kg) and *mongolica* (average 250 mg/kg). The fruit flesh of *sinensis* berries had contents of tocopherols and tocotrienols 2−3 times higher than those found in the other two subspecies (120 mg/kg vs 40 mg/kg in *rhamnoides* and 50 mg/kg in *mongolica*). The fresh whole berries of subsp. *sinensis* were clearly the best source of total tocopherols and tocotrienols. The total content of tocopherols and tocotrienols in the soft parts of the berries reached the maximum level around early- to mid-September, whereas the content in seeds continued to increase until the end of November. The excellent combination of the highest content of vitamin C and tocopherols and tocotrienols makes the berries of subsp. *sinensis* an optimal raw material for nutritional investigation as a candidate for functional foods with special antioxidative properties.

KEYWORDS: Sea buckthorn; *Hippophaë rhamnoides*; vitamin C; tocopherols and tocotrienols; seeds; fruit flesh; origins; harvesting time

INTRODUCTION

Sea buckthorn (*Hippophaë rhamnoides* L.) is a berry-bearing bush naturally distributed over Asia and Europe (*1*). Used traditionally as raw material for foods and lately as a candidate for functional food ingredient, the berries are especially rich in vitamin C (up to 20 g/kg fresh berries from some sources) and flavonoids in the juice fraction (*2*−*7*). Both the soft parts (pulp and peel) and the seeds contain oil and high levels of tocopherols and plant sterols (*8*−*11*). Oral administration of sea buckthorn berry juice decreased the susceptibility of LDL to oxidation in humans (*11*). The addition of sea buckthorn juice (*12*) and oils (*13*−*15*) to feed decreased lipid peroxidation in plasma, liver, and erythrocyte membranes in rats. Sea buckthorn seed oil inhibited cell-mediated and Cu^{2+}-catalyzed LDL oxidation in vitro (*16*). Vitamin C and tocopherols and tocotrienols (the latter two also referred to as vitamin E) are among the major antioxidants in sea buckthorn berries. Vitamin C plays an important role in human physiology as a cofactor or cosubstrate of several enzymes and as an intra- and extracellular antioxidant. Vitamin E is the major antioxidant in lipoproteins and cell membranes. An effect of synergy has been suggested between vitamins C and E (*17*, *18*). Extracelluler vitamin C may reduce tocopherol radicals in lipoproteins and cell membranes (*18*). Long-term combined supplementation of vitamin C and vitamin E reduced lipid peroxidation in vitro and in vivo (*19*). Therefore, the content of these components as vitamins and natural antioxidants is an important parameter defining the quality of the berries. Data reported on the vitamin C and tocopherols in the berries showed considerable variation, particularly in the case of vitamin C content, and extreme variation has been reported both within and among natural populations (*2*−*4*, *11*). The variation has been attributed to genetic factors, weather conditions, and different harvesting dates (*2*−*4*, *11*). Data have been published dealing with the content of α-tocopherol and the total content of vitamin E in sea buckthorn oils isolated from different raw materials using different methods. The content and composition of the tocopherol and tocotrienol isomers in berries of different origins and subspecies have not yet been thoroughly investigated. In our previous study, we

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Table 1. Berry Samples of Different Origins

code	cultivar/clone	growth location	subsp.[a]	date of collection
S1[b]	wild[c]	Wenshui, Shanxi, China	S	Oct 17, 1997
S2	wild[c]	Fuxian, Shanxi, China	S	Nov 23, 1997
S3	wild[c]	Wangtao, Shanxi, China	S	Nov 3, 1997
S4	wild[c]	Kelan, Shanxi, China	S	Oct 29, 1997
S5	wild[c]	Youyu, Shanxi, China	S	Oct 25, 1997
S6[b]	wild[c]	Xixian, Shanxi, China	S	Nov 26, 1997
S7	wild[c]	Heshun, Shanxi, China	S	Nov 5, 1997
S8	wild[c]	Xunyi, Shaanxi, China	S	Nov 21, 1997
S9	wild[c]	Youyu, Shanxi, China	S	Oct 24, 1997
S10	wild[c]	Wutai, Shanxi, China	S	Nov 7, 1997
S11	wild[c]	Yongshou, Shaanxi, China	S	Nov 21, 1997
S12	wild[c]	Ningwu, Shanxi, China	S	Oct 27, 1997
S13	wild[c]	Datong, Qinghai, China	S	Nov 17, 1997
S14	wild[c]	Dingxi, Gansu, China	S	Nov 19, 1997
S15	clone 21V, Chinese	Viikki, Helsinki	S	Sept 15, 1999
R1[b,d]	S3003	Satakunta, Finland	R	Aug 30–Sept 15, 1997–99
R2[b,d]	S3006	Satakunta, Finland	R	Aug 30–Sept 15, 1997–99
R3[b,d]	74006003	Satakunta, Finland	R	Aug 30–Sept 15, 1997–99
R4[b,d]	74006005	Satakunta, Finland	R	Aug 30–Sept 15, 1997–99
R5	wild[c]	Pyhämaa, Finland	R	Sept 10, 1999
R6	wild[c]	Hirsilahti, Pyhäranta, Finland	R	Sept 10, 1999
R7	wild[c]	Siikajoki, Finland	R	Sept 12, 1999
R8	wild[c]	Vaasa, Finalnd	R	Sept 13, 1999
R9	wild[c]	Pyhämaa, Finland	R	Sept 10,1999
R10	wild[c]	Pyhämaa, Finland	R	Sept 10, 1999
R11	wild[c]	Romania	R	Sept 1999
R12	clone 8, Finnish	Viikki, Helsinki, Finland	R	Sept 15, 1999
R13	clone 99, Finnish	Viikki, Helsinki, Finland	R	Sept 15, 1999
R14	clone 94, Danish	Viikki, Helsinki, Finland	R	Sept 15, 1999
R15	clone 18, Danish × Finnish	Viikki, Helsinki, Finland	R	Sept 15, 1999
R16	clone 133, Danish × Finnish	Viikki, Helsinki, Finland	R	Sept 15, 1999
R17	clone 134, Danish × Finnish	Viikki, Helsinki, Finland	R	Sept 15, 1999
R × S	clone 92, Finnish × Chinese	Viikki, Helsinki, Finland	R × S	Sept 15, 1999
R × M	clone 87, Finnish × Siberian	Viikki, Helsinki, Finland	R × M	Sept 15, 1999
R × M	clone 131, Finnish × Siberian	Viikki, Helsinki, Finland	R × M	Sept 15, 1999
M1	Tsuiskaya[c]	Riihimäki, Finland	M	Aug 23, 1999
M2	Oranzevaya[c]	Riihimäki, Finland	M	Aug 23, 1999
M3	Ruet[c]	Novosibirsk, Russia	M	early Sept 1997
M4	Dar Katuni[c]	Novosibirsk, Russia	M	early Sept 1997
M5	Luchezarnaya[c]	Novosibirsk, Russia	M	early Sept 1997
M6	Vitaminaya[c]	Novosibirsk, Russia	M	early Sept 1997
M7	Maslichnaya[c]	Novosibirsk, Russia	M	early Sept 1997
M8	cultivated	Kakskerta, Turku, Finland	M	Aug 25–Sept 27
M9	cultivated	Kakskerta, Turku, Finland	M	1999, 2000

[a] S, sinensis; R, rhamnoides, M, mongolica. [b] Berries samples were also collected at different time points from the end of August to the end of November, 1998. [c] Pooled from 10 to 20 bushes. [d] R1–R4 are four cultivated clones from the Finnish Agricultural Research Center, Horticulture Piikkiö, and Satakunta.

compared tocopherols and tocotrienols in seeds and berries of wild subsp. *sinensis* from China, and five cultivars of subsp. *mongolica* from Russia (*11*). The aim of the present study was to compare the content of vitamin C and tocopherols and tocotrienols in wild and cultivated sea buckthorn berries of three different subspecies and of crosses between subspecies, and to follow the changes among different harvesting years and during the harvesting period. The stability of vitamin C during the storage of berries and juice was also investigated. The results of the present study provide important guidance for breeding and large-scale planting of sea buckthorn and for the selection of the optimal harvesting date for berries with high content of these vitamins.

MATERIALS AND METHODS

Berries. Wild berries of *Hippophaë rhamnoides* subsp. *sinensis* were collected from fourteen different locations at natural growth sites in China from mid-October to late November, 1997. The areas range between longitudes 108°04′ E and 113°40′ E, latitudes 34°56′ N and 40°03′ N, and altitudes 1020 and 2800 m. Wild and cultivated berries belonging to the subsp. *mongolica* (four samples) and subsp. *rhamnoides* (sixteen samples), were collected from the coastal area in southwest Finland (longitudes 21°04′ E − 24°24′ E, latitudes 60°45′ N − 64°47′ N, and altitudes 0−50 m) around late August and early September 1996−1999. Of these *rhamnoides* clones, six were cultivated at the Viikki experimental field of the Department of Applied Biology, University of Helsinki, and four were cultivated at the test field of the Finnish Agricultural Research Centre, Satakunta. Samples of five *mongolica* cultivars originating from the natural population of the Altai region were picked from Novosibirsk, Russia, in early September 1997. One wild *rhamnoides* sample from Romania, and one *sinensis* and three crossing samples grown in Viikki were also analyzed.

To follow the changes in vitamin C content (*sinensis* and *rhamnoides*) and tocopherols and tocotrienols (*sinensis*) during the elongated harvesting period, berries were collected at different time points from the end of August to the end of November 1998. The *rhamnoides* berries were picked from four separate bushes (not pooled), whereas the two wild *sinensis* samples were both pooled collections from over twenty bushes of the same area. All the samples were stored at −20 °C and analyzed typically within some months, in some cases within a year. For the analyses, two sub-samples of each sample coded in **Table 1** were prepared and analyzed in duplicate.

The vitamin C stability test was carried out with berries picked in autumn 1999 and autumn 2000 from four cultivars of subsp. *mongolica* grown in two areas near Turku, Finland. Each sample originated from individual bushes. **Table 1** presents detailed information on the samples analyzed in the present study. The berries were hand-picked at the time periods when they are typically harvested for commercial purposes. Exceptions were the late-harvested samples of the S1, S6, R1, R2, R3, and R4 berries. Immediately after the berries were picked, they were loosely frozen in sealed plastic bags at −20 °C in order to avoid desiccation and external moisture condensation.

Analysis of Vitamin C. Frozen berries were melted in a microwave oven to the temperature c.a. 10 °C. Juice was pressed manually and diluted 1 to 25 with Milli-Q purified water. To determine the total vitamin C content, dithiothreitol (DTT) was added to the solution as a reducing agent at a final concentration of 1 mg/mL, and the mixture was allowed to react at room temperature for 2 h. The sample solution was filtered and analyzed with a Shimadzu LC-10ATvp high-performance liquid chromatograph equipped with a SPD-M10AVP diode array detector (Shimadzu Corp., Kyoto, Japan) and a LiChrospher 100 RP-18 column (250 × 4 mm, 5 μm) (Merck KGaA, Darmstadt, Germany). The mobile phase was 0.5% KH_2PO_4 deionized water solution containing 0.1% DTT. The vitamin C concentration in the sample solution, and, thus, in the juice was determined according to the absorption peak area at 254 nm, using an external standard method (vitamin C, J. T. Baker Chemicals B. V., Deventer, The Netherlands) (*20*). Four parallel analyses were carried out, and an average value of vitamin C content was calculated for each sample.

Stability Test of Vitamin C in Berries and Juice. Fresh berries, and juice manually pressed from fresh berries, were stored at 5 °C, and the vitamin C content was determined after 0, 3, 6, and 14 days of storage of berries and juice. The storage test was carried out with berries from four different cultivars/bushes in 1999, and from two different bushes collected at two different time points in 2000. The long-term (half year and one year) storage test of vitamin C content in frozen berries (at −20 °C) was carried out with berries from the four cultivars/bushes collected in 1999.

Lipid Extraction. Samples (1 g) of seeds and the soft parts (pulp and peel) isolated from freeze-dried berries were crushed separately in a mortar in liquid nitrogen, and the lipids were isolated using a methanol−chloroform extraction procedure (*8*, *10*). The sample was homogenized in methanol (10 mL) for 1 min in a blender, chloroform (20 mL) was added, and homogenization continued for a further 2 min. The mixture was filtered and the solid residue was re-suspended in chloroform/methanol (2:1, v/v, 30 mL) and homogenized for three minutes. The mixture was filtered again and washed with fresh solvent (chloroform/methanol, 2:1, v/v, 30 mL). The combined filtrates were transferred into a measuring cylinder, one-fourth of the total volume of 0.88% potassium chloride water solution was added, and the mixture was shaken thoroughly before being allowed to settle. The lower layer was removed and washed with one-fourth of its volume of methanol/water (1:1, v/v). The washing procedure was repeated, and the bottom layer containing the purified lipids was filtered before the solvent was removed on a rotary film evaporator. Lipids were stored in chloroform at −20 °C for a short period until the analysis of tocopherols and tocotrienols.

Analysis of Tocopherols and Tocotrienols. The extracted lipids were dissolved in 3 mL of hexane, and D,L-tocol was added as internal standard (Roche, Basel, Switzerland; concentration in the final solution, 10 μg/mL). Tocopherols and tocotrienols were analyzed with a normal-phase HPLC method. The instrumentation was a Shimadzu LC-10ATvp equipped with a Shimadzu SIL-10A autoinjector, a CTO-10A oven, and a RF-530 fluorescence detector (Shimadzu Corp., Kyoto, Japan). The excitation wavelength was 295 nm, and the emission wavelength was 330 nm. The column was a Merck LiChroCART 250-4, Superspher Si 60 connected to a guard column Merck LiChroCART 4-4, LiChrospher Si 60. The sample injection volume was 20 μL. The tocopherols and tocotrienols were eluted at 30 °C with the eluting solvents programmed as follows: 0−5 min, 92% hexane/8% diisopropyl ether; 5−30 min, programmed change in eluting solvent from 92% hexane/8% diisopropyl ether to 83% hexane/17% diisopropyl ether; 30−35 min, 83% hexane/17% diisopropyl ether. The identification of individual peaks was carried out by co-injection with standard compounds. The quantification was carried out with internal standard tocol and corrected with specific correction factors determined by analysis of standard compounds.

Statistical Analysis. Data analysis was carried out with SPSS 10.0 for Windows. Independent−Samples *t* test was used to compare the differences between the subspecies. Difference levels at $p < 0.05$ were taken as statistically significant.

Table 2. Vitamin C Content in Juice (g/L) of Sea Buckthorn Berries of Different Origins[a]

subspecies										cross between subsp.	
sinensis	97	rhamnoides	96	97	98[b]	99	mongolica				
S1	13.2	R1	1.8	2.0	2.0	1.8	M1	1.2		R[c] × S[c]	3.0
S2	8.7	R2	1.4	1.7	1.9	1.5	M2	1.1		R × M[c] 87	1.1
S3	5.6	R3	1.3	0.9	1.6	1.3	M3	< 0.1		R × M 131	0.4
S4	8.6	R4	1.1	0.7	1.3	1.0	M4	< 0.1			
S5	4.2	R5				2.0	M5	0.3			
S6	9.5	R6				0.9	M6	0.5			
S7	6.2	R7				2.9	M7	0.5			
S8	7.0	R8				2.2	M8	0.9			
S9	8.3	R9				1.1	M9	0.9			
S10	8.6	R10				2.2					
S11	10.1	R11				1.2					
S12	7.8	R12				1.2					
S13	9.9	R13				2.0					
S14	12.3	R14				1.6					
S15	9.0	R15				1.2					
		R16				2.1					
		R17				1.6					
mean[d]	8.6	mean[d]		1.6			mean[d]	0.6			
SD	2.3	SD		0.5			SD	0.5			

[a] Each value represents the average of results of analyses of two subsamples. [b] Value 1 from Aug. 30, 98. [c] R, S, and M represent subspecies *rhamnoides*, *sinensis*, and *mongolica*, respectively. [d] S > R, S > M, $p < 0.001$; R > M, $p < 0.01$.

RESULTS AND DISCUSSION

Vitamin C Content in Berries of Different Origins and Harvesting Dates. The vitamin C content in berries of different origins is summarized in **Table 2**. The level measured as g/L juice varied in a wide range from the <0.1 g/L juice of berries from two Russian cultivars (Ruet and Dar Katuni) to the 13.2 g/L juice from Chinese berries collected from Wenshui, Shanxi Province. The berries of subsp. *sinensis* are clearly the best source of vitamin C among the three subspecies studied (8.6 ± 2.4 in subsp. *sinensis* vs 1.7 ± 0.5 in subsp. *rhamnoides* and 0.5 ± 0.5 g/L in subsp. *mongolica*) ($P < 0.001$). Among the berries of different origins analyzed, the wild Chinese berries have the smallest size (long diameter 4−8 mm, short diameter 3−5 mm) with the lowest yield of juice (∼50−60%), whereas the corresponding values were the highest in the berries from the Russian cultivars of subsp. *mongolica* (long diameter 13−16 mm, short diameter 6−10 mm, juice yield ∼70%). The vitamin C content measured as weight percentage in fresh berries was reported to correlate negatively with the size of the berries among natural populations of sea buckthorn growing in the southwest coastal area in Finland (*1*). The vitamin C content in berries from a single bush did not correlate with the size of the berries (*3*). The results of the present study showing the highest content of vitamin C in the small Chinese berries of subsp. *sinensis* supported and extended the findings of the previous investigations. Berries from one bush of Chinese origin (S15) cultivated in Finland contained a clearly higher level of vitamin C (9.0 g/L) than the bushes of other subspecies grown in the same field (0.4−2.1 g/L), suggesting that genetic background is the most important factor determining the vitamin C content of the berries. Comparison of the values of the crosses and the different subspecies shown in **Table 2** indicated that modification of the genetic background by crossing different subspecies probably influenced the vitamin C content in the berries to some extent.

Comparing the berries of four cultivated clones of subsp. *rhamnoides* (R1−R4) collected in four different years (1996−1999), the vitamin C content was not significantly different ($p > 0.05$). **Figure 1** presents the vitamin C content in berries harvested at different time points in autumn 1998. The berries

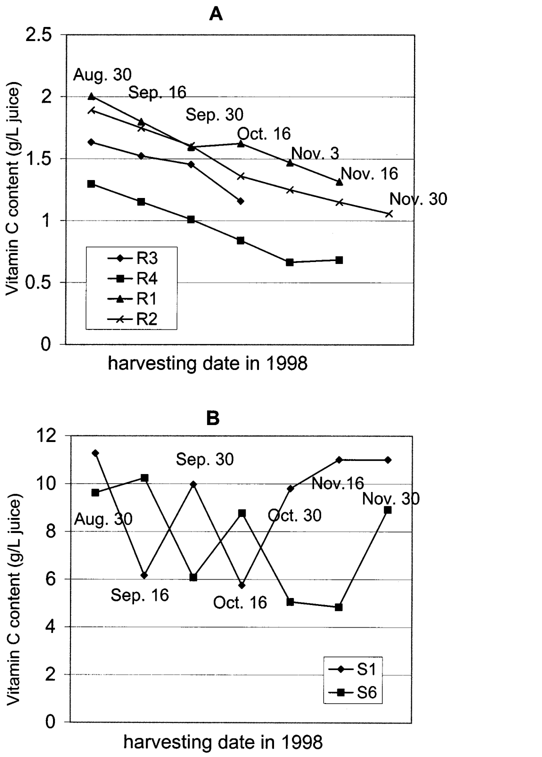

Figure 1. Changes in vitamin C content in the juice fraction from berries of four cultivated clones of subsp. *rhamnoides* from Finland (A), and wild subsp. *sinensis* from two different locations at natural growth sites in China (B), harvested from the end of August to the end of November, 1998. The Finnish berries were collected from the same bushes, whereas Chinese berries from different bushes were included at different time points.

of subsp. *rhamnoides* were collected from the same bushes of four different cultivated clones. There is a clear decreasing trend in the vitamin C content in berry juice from the beginning of September to the end of November (**Figure 1A**). Rousi and Aulin (*2*) reported a decreasing trend in vitamin C content, accompanied by a steady increase in the fresh weight of the berries, from six bushes growing naturally in Pyhäranta in southwest Finland from the August 17 to September 21. In berries from three cultivars (Botanitjetskaja, Trofimovskaja, and Aromatnaja) grown in Kristianstad, Sweden, the vitamin C content decreased from August 6 to August 25, 1997 (*21*). The change in vitamin C content correlated positively with the decrease in the free-radical scavenging capacity of the corresponding fraction (*21*). It has been suggested that the effective temperature (>5 °C) sum (degree-days) is a better predictor than the calender date of the optimum harvesting date for berries with the highest vitamin C content (*4*). For the berries of seven of the eight bushes investigated from an experimental field near Helsinki harbor, the vitamin C content reached maximal levels when the temperature sum reached 1299 degree-days, corresponding to August 31 in 1990, and then decreased during the rest of the period followed in the study (*4*). **Figure 1B** presents the vitamin C content in the juice fraction of berries of subsp. *sinensis* collected at different time points in 1998. Instead of a clear changing trend, the figure shows considerable variation among the different time points during the period investigated. Yao et al. (*3*) determined the vitamin C content of 701 berries from 71 bushes representing 10 Finnish populations of *H. rhamnoides* subsp. *rhamnoides*. Significant variation has been found among different bushes both within and among populations, suggesting genotypes are the most potent factors influencing the vitamin C content in the berries (*3*). The collection of

Table 3. Storage Test of Vitamin C Content (g/L juice) in Sea Buckthorn Juice and Berries[a]

sample	storage form	storage at 5 °C				storage at –20 °C	
		0 days	3 days	6 days	14 days	6 months	13 months
Picked on August 23, 1999							
M1 (Tsuiskaya)	berries	1.2	1.3	1.3	1.3	1.3	
	juice	1.2	1.3	1.3	1.5		
M2 (Oranzevaya)	berries	1.1	1.1	1.1	1.4	1.3	
	juice	1.1	1.2	1.1	1.4		
Picked on August 25, 1999							
M8	berries	0.9	0.9	0.9	1.1	0.9	1.0
	juice	0.9	0.9	0.9	0.9		
M9	berries	0.9	0.9	0.8	0.9	0.9	0.9
	juice	0.9	0.9	0.9	0.9		
Picked on September 5, 2000							
M8	berries	0.6	0.9	0.9	1.0		
	juice	0.6	0.8	0.8	0.7		
M9	berries	0.9	0.9	0.8	0.8		
	juice	0.9	0.8	0.9	0.7		
Picked on September 27, 2000							
M8	berries	0.6	0.8	0.7	0.8		
	juice	0.6	0.6	0.5	0.6		
M9	berries	0.5	0.6	0.5	0.6		
	juice	0.5	0.6	0.4	0.5		

[a] Each value represents the average of analysis results of two subsamples, each analyzed in duplicate.

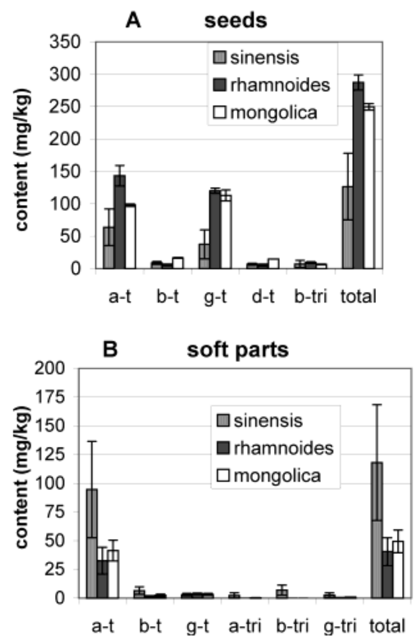

Figure 2. Content (mean ± SD, mg/kg fresh weight) of isomers of tocopherols and tocotrienols in seeds (A) and soft parts (B) of berries of different subspecies: a-t, α-tocopherol; b-t, β-tocopherol; g-t, γ-tocopherol; d-t, δ-tocopherol; a-tri, α-tocotrienol; b-tri, β-tocotrienol; g-tri, γ-tocotrienol; s, ssp. *sinensis*; m, ssp. *mongolica*.

the Chinese berries shown in **Figure 1B** was not confined to a single bush at different dates. Therefore, the less clear changing trend during the harvesting period in these berries was probably a result of the variation among the bushes.

Stability of Vitamin C in Berries and Juice. **Table 3** summarizes the results of stability tests in berries and juice stored at 5 °C, and in berries frozen at −20 °C. No clear changes were found in the content of vitamin C during the first 6 days of storage in either berries or juice. In most of the samples investigated, the vitamin C level remained unchanged during the first 14 days of storage. Berries and juice of the two cultivars (Tsuiskaya and Oranzevaya) from Riihimäki became mouldy on the 14th day, so the vitamin C content was not determined

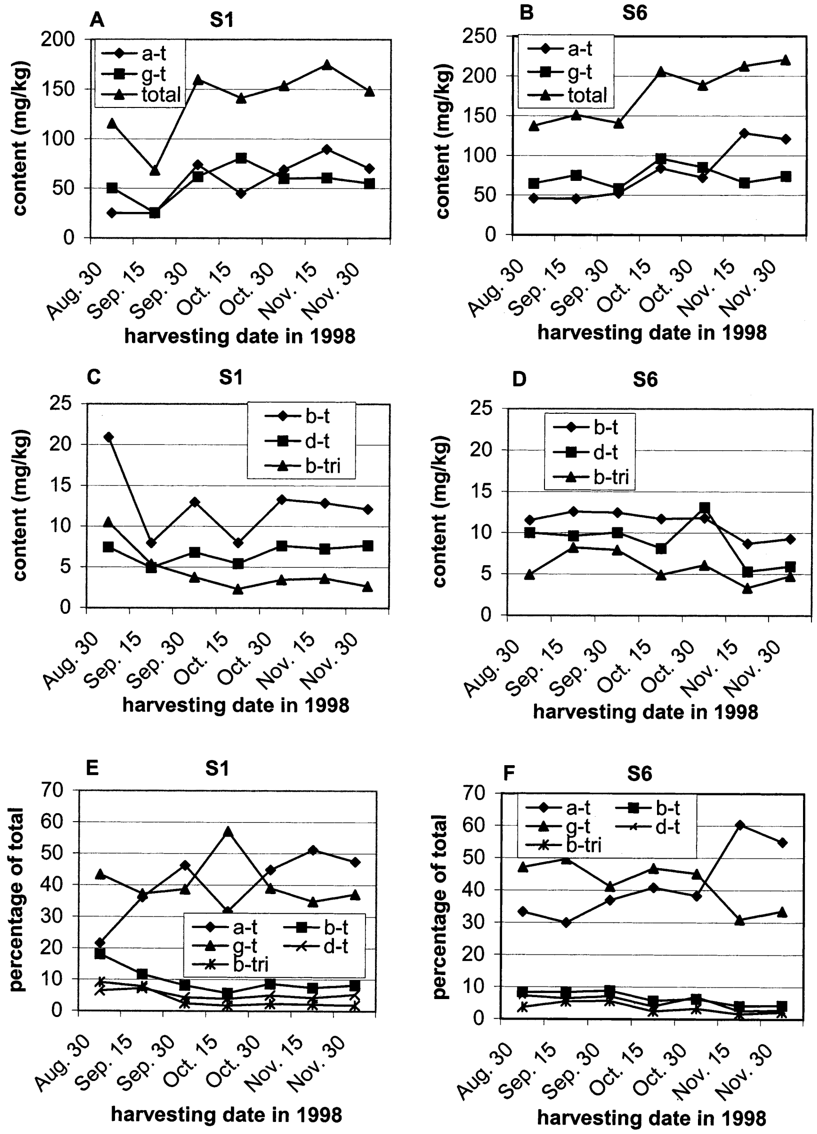

Figure 3. Changes in content (mg/kg fresh weight) and proportion of tocopherols and tocotrienols in seeds of sea buckthorn berries of subsp. *sinensis* collected at the two locations (Wenshui, S1; Xixian, S6) in China in 1998: a-t, α-tocopherol; b-t, β-tocopherol; g-t, γ-tocopherol; d-t, δ-tocopherol; b-tri, β-tocotrienol.

from these samples. After one-half year and one year of storage as frozen berries, the total vitamin C content also did not change.

Tocopherols and Tocotrienols in Seeds. All the four tocopherol isomers and β-tocotrienol were found in seeds. α- and γ-Tocopherols were the two major isomers, representing typically 40−50% and 20−40%, respectively, of the total tocopherols and tocotrienols. The proportions of each of the other isomers were typically 5−10%. The total content of tocopherols and tocotrienols in seeds varied from 40 to 220 mg/kg in subsp. *sinensis*, from 280 to 300 mg/kg in subsp. *rhamnoides*, and was ∼250 mg/kg in the two samples of subsp. *mongolica*. Seeds of the latter two subspecies were clearly richer in tocopherols and tocotrienols than those of subsp. *sinensis* (287.4 ± 11.7 mg/kg in subsp. *rhamnoides* and 249.8 ± 5.1 mg/kg in subsp. *mongolica* vs 126.7 ± 59.8 mg/kg in *sinensis*, $p < 0.01$) (**Figure 2A**). The result is in agreement with our previous investigation (*11*) showing that the seeds of subsp. *mongolica* were a better source of tocopherols than those of subsp. *sinensis*.

In the extracted seed oil of different samples, the total content of tocopherols and tocotrienols was typically 100−300 mg/100 g.

During the harvesting period from the end of August to the end of November 1998, the total content of tocopherols and tocotrienols and the two major tocopherols α- and γ-isomers seemed to increase in seeds from both of the two locations in China (**Figure 3A**,**B**). The level of β-tocopherol and β-tocotrienol showed a slight decreasing trend during the same period (**Figure 3C**,**D**). These changes resulted in a further increase in the proportion of the major isomer α-tocopherol, and a decrease in the other isomers in the total tocopherols and tocotrienols in seeds (**Figure 3E**,**F**). The trends were surprisingly clear, when taking into account that the berries collected at different harvesting times were not from the same individual bushes.

Liu et al. (*22*) followed the changes in vitamin E content in seeds of sea buckthorn (*H. tibetana* and *H. rhamnoides* L. subsp. *sinensis*) growing naturally in Gansu Province (latitude 35°15′5″ N, longitude 102° 54′ 20″ E, altitude 2865−2895 m, China) from the 30th to the 150th day after flowering (DAF) (corresponding to the period from mid-May to mid-October). The total content of vitamin E in seeds of subsp. *sinensis* increased sharply after the 107th DAF, reached a maximum level (800 mg/kg fresh weight) on the 113rd DAF (early to mid September) and decreased during the following period. For the seeds of *H.*

tibetana the total vitamin E content increased steadily from the 107th to the 139th (over 800 mg/kg) DAF (early October) and decreased during the following period. In the samples of subsp. *sinensis* analyzed in the present study, the total content of tocopherols and tocotrienols showed a continuously increasing trend from the end of August to the end of November, 1998. This discrepancy may be explained by the different geographical and climatic conditions of the growth areas, which may have influenced the process of maturity of the berries and seeds. According to the results of the present and previous investigations (*22*), the highest level of total tocopherols and tocotrienols could only be obtained in seeds from the berries normally considered as being over-ripe, the exact optimal harvesting date depending on origins and growth conditions.

Tocopherols and Tocotrienols in Soft Parts of Berries. α-Tocopherol typically represented 70−80% of total tocopherols and tocotrienols in the soft parts of the berries (**Figure 2B**). The proportion of β- and γ-tocopherols and γ-tocotrienol was 4−9%, 2−6%, and 2−4%, respectively. The berries of subsp. *sinensis* also contained α- and β-tocotrienols at levels of 1−2% and 4−9%, respectively, whereas the two isomers were almost undetectable in the samples of the other two subspecies. The total content of tocopherols and tocotrienols varied from 30 to 210 mg/kg based on fresh weight in the samples analyzed. The soft parts of the berries of subsp. *sinensis* were the best source of tocopherols and tocotrienols among the three subspecies (118 ± 50 vs 40 ± 12 in subsp. *rhamnoides* and 50 ± 9.0 in subsp. *mongolica*, mg/kg, **Figure 2B**). In the soft parts of berries of subsp. *sinensis*, the dry matter content (20−30%, according to the weight % of freeze-dried material in fresh soft parts) was higher than the corresponding values in subsp. *rhamnoides* and *mongolica* (11−14%). This, together with the high content of vitamin C, vitamin E, sugars, and acids (*10*), suggested that the nutrients in the seedless fraction were more concentrated in the small berries of subsp. *sinensis* than in the large berries of subsp. *mongolica*.

In the extracted soft part oil, the total content of tocopherols and tocotrienols was typically 400−700 mg/100 g in subsp. *sinensis*, significantly higher than the corresponding values in the other two subspecies (100−200 mg/100 g oil) ($p < 0.001$).

Figure 4 presents the changes in content of tocopherols and tocotrienols in the soft parts of the berries of subsp. *sinensis* collected from two different locations in China, showing greater deviation than that in the seeds. This may be partly the result of changes in the water content in the soft parts during the harvesting period. In berries from Wenshui (S1), the total contents of tocopherols and tocotrienols at the end of September (total content 68 mg/kg, α-tocopherol 52 mg/kg) and the middle of October (total level 72 mg/kg, α-tocopherol 60 mg/kg) were lower than the corresponding levels at the other three time points from September to November (total content 80−97 mg/kg, α-tocopherol 75−83 mg/kg) (**Figure 4A**). In berries from Xixian (S6), these levels were at their lowest (∼60 mg/kg for total and 50 for α-tocopherol) around the end of September and mid-October, whereas the corresponding values at the other time points were around 80−90 mg/kg for the total and 60−78 mg/kg for α-tocopherol (**Figure 4B**).

Among the minor isomers, the levels of β-tocotrienol and γ-tocotrienol decreased in the berries from both locations during the harvesting period (**Figure 4C**,**D**). The levels of δ-tocopherol (<1 mg/kg) and α-tocotrienol (<2 mg/kg) remained constantly low. Nor was a clear changing trend found in the other isomers (**Figure 4C**,**D**). When the proportion of different isomers was studied, the proportion of α-tocopherol increased slightly (from

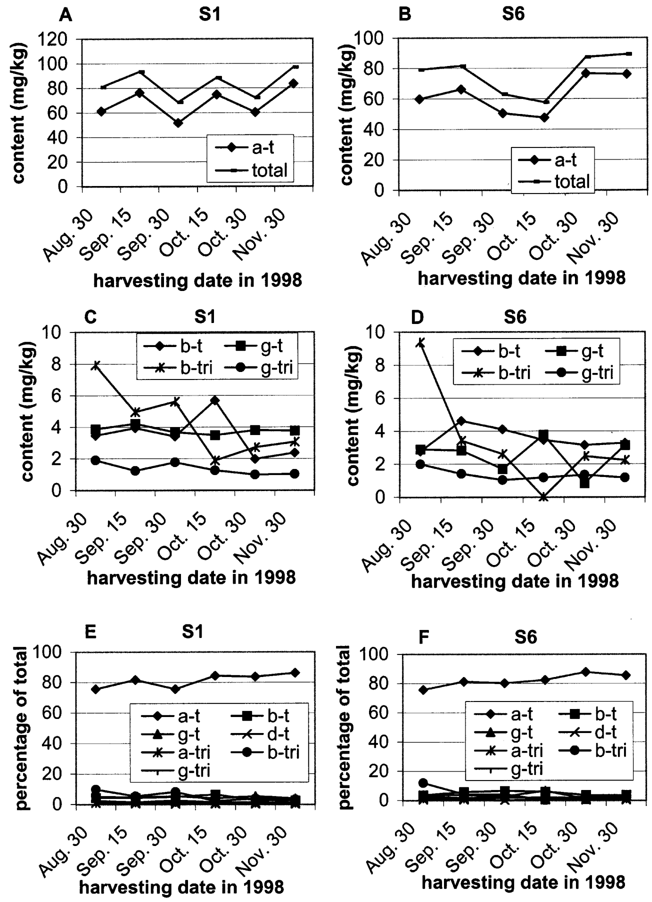

Figure 4. Changes in content (mg/kg fresh weight) and proportion of tocopherols and tocotrienols in the soft parts of sea buckthorn berries of subsp. *sinensis* collected at the two locations (Wenshui, S1; Xixian, S6) in China in 1998: a-t, α-tocopherol; b-t, β-tocopherol; g-t, γ-tocopherol; d-t, δ-tocopherol; a-tri, α-tocotrienol; b-tri, β-tocotrienol; g-tri, γ-tocotrienol.

75 to 85%) and that of the others decreased slightly during this period (**Figure 4E**,**F**).

The total vitamin E content (method of calculation not defined) in fresh soft parts of berries was reported to increase from the 107th to the 113rd DAF (early to mid-September), reaching a maximum level of ∼20 mg/kg (fresh weight), followed by a decrease from the 114th to the 125th DAF in subsp. *sinensis* (*22*). In the present study, the total content of tocopherols and tocotrienols was highest in mid-September among all the levels measured before mid-October. The increase seen in these levels during the period from mid-October to late November may have resulted from the desiccation of the berries while still on the bushes.

In additon, the vitamin E level in fresh soft parts reported by Liu et al. (*22*) was considerably lower than the total content of tocopherols and tocotrienols in the present study. The difference may have been due to the different origin of the berries or the possibly different methods of anaysis, as the methods were not described in detail in the reference (*22*).

The results of the present study and the previous investigation (*22*) suggested that, unlike the situation for seeds, mid-September was the best harvesting date for berries, with both the highest total content of tocopherols and tocotrienols and other desired properties (properly ripened) in the natural growth sites in northwest China.

Tocopherols and Tocotrienols in Whole Berries. Figure 5 presents an overview of the total content of tocopherols and tocotrienols in fresh berries, and the overall shares of the seeds

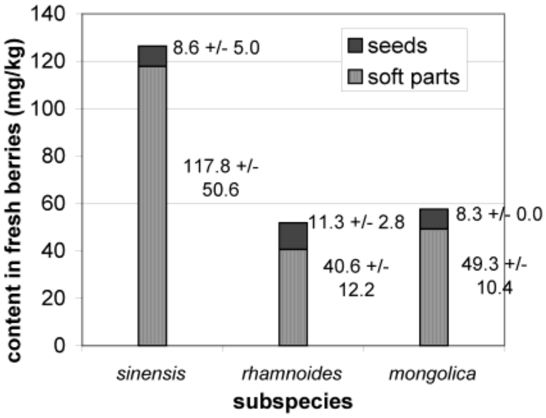

Figure 5. Total content of tocopherols and tocotrienols in whole berries (mg/kg fresh weight) of different origins, showing the shares of seeds and soft parts.

and the soft parts in berries of different origins. Depending on the origin, the total content of tocopherols and tocotrienols in fresh whole berries varied from 40 to 220 mg/kg, of which 70−80% was α-tocopherol. The share of the soft parts and the seeds also varied from 77 to 97% and from 3 to 13%, respectively. The total content of tocopherols and tocotrienols was higher in the fresh whole berries of subsp. *sinensis* than in the berries of the other two subspecies (average 130 vs 50 and 60 mg/kg).

A significant synergy has been reported in the protective effects of long-term combined supplementation of vitamin C and vitamin E against oxidation of lipoproteins and other lipids in man (*19*). The excellent combination of the highest content of vitamin C and tocopherols and tocotrienols makes the berries of subsp. *sinensis* a special food raw material with antioxidative properties.

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