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Tocopherols and Tocotrienols in Sea Buckthorn (*Hippophae rhamnoides* L.) Berries during Ripening

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Sea buckthorn berries (*Hippophae rhamnoides* L.) are used in foods, cosmetics, and pharmaceutical products. They are of particular interest for their high content of healthy phytochemicals, including vitamin E-related compounds (tocopherols and tocotrienols). This study investigated the content of tocopherols and tocotrienols during ripening in berries from four cultivars of sea buckthorn over a three-year period. The results showed large variations in tocopherols and tocotrienols depending on harvest date, cultivar, and year. Levels of α-tocopherol were higher early in the ripening period, while at later dates, δ-tocopherol levels increased. Great differences in amounts and composition of tocopherols and tocotrienols were observed between cultivars. Tocopherol levels were positively correlated with daily temperature, but this trend varied between years. Variations in tocopherols and tocotrienol levels in sea buckthorn berries due to cultivar, year, and ripening stage should therefore be considered in the production of nutritional products.

KEYWORDS: Antioxidant; berries; cultivar; fruit; harvest; *Hippophae rhamnoides*; ripening; sea buckthorn; sun irradiation; temperature; tocopherols; tocotrienols; yearly variation

INTRODUCTION

Sea buckthorn (*Hippophae rhamnoides* L.) is a plant native to the temperate areas of Europe and Asia that is now being cultivated in other parts of the world. Sea buckthorn berries when ripe are yellow−orange−red in color depending on the cultivar and normally have a shiny appearance. The round−oblong berries develop on shoots from the previous year and ripen uniformly. The berries of different cultivars usually have a weight within the range 70−80 g/100 berries (*1*). The berries grow on 1−2 mm long stems and lack an abscission layer. The aroma is characterized by several aliphatic esters (*2*), and sea buckthorn juice has been characterized and compared with aromas such as strawberry, peach, mango, apricot, papaya, and citrus. However, sourness is the dominant taste (*3, 4*). Sea buckthorn berries are used raw or processed in foods, cosmetics, and pharmaceutical products (*5*), and are considered to possess health-related potential (*6*). The berries are rich in different phytochemicals such as ascorbic acid, phenolic compounds, carotenoids, tocopherols, and healthy fatty acids (*5, 7–11*), and have a comparatively strong antioxidant activity (*12*). Sea buckthorn berry products have been used for the treatment of different conditions and diseases such as burns, external wounds and skin inflammations, and gastric ulcers (*5, 13*). It has also been reported that sea buckthorn berries reduce plasma cholesterol levels and inhibit platelet aggregation (*8, 14*).

Previous studies of sea buckthorn berries reported differences in tocopherol and tocotrienol composition (*9, 15–17*), which may be due to different causes, e.g., methods of extraction and analysis used, berry parts analyzed, genetic variation, climate, growing conditions, yearly variations, degree of ripening when harvested, storage conditions, and harvesting method.

A few one-year studies on ripening in sea buckthorn berries and their composition of tocopherols and tocotrienols have been performed. For example, Zadernowski et al. studied tocopherol composition of berry oil from one cultivar during ripening and observed the highest levels of α- and δ-tocopherol at change in color and two to three months later (*17*). In soft parts from pooled samples of different wild bushes of *H. rhamnoides* ssp. *sinensis* grown in two different locations in China, maximum levels of total tocopherols and tocotrienols were found in ripe berries in early to mid-September and in desiccated berries in mid-October to late November (study period late August−late November) (*18*). To our knowledge, no previous studies comparing tocopherols and tocotrienols during ripening in various cultivars of sea buckthorn berries in consecutive years have been performed.

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Table 1. Harvest Dates for the Sampling of Sea Buckthorn in Different Years and Average Harvest Dates for All Years

	harvest date							
2004			Aug 11	Aug 18	Aug 25	Sept 1	Sept 8	Sept 15
2005	July 28	Aug 4	Aug 10	Aug 18	Aug 25	Sept 1	Sept 8	Sept 15
2006			Aug 9	Aug 16	Aug 23	Aug 31	Sept 6	
ahd[a]	July 28	Aug 4	Aug 10	Aug 17	Aug 24	Sept 1	Sept 7	Sept 15

[a] ahd = average harvest date

Vitamin E is represented by α-, β-, γ-, and δ-tocopherol, and α-, β-, γ-, and δ-tocotrienol, all of which occur in nature, and 14 vitamers are theoretically possible (*19*). All tocopherols are considered to have a vitamin E effect. However, α-tocopherol is regarded as the most active and predominant form (*20*). Of the tocotrienols, only α-tocotrienol has been claimed to have a significant vitamin E effect (*21*). Tocotrienols have been suggested to suppress the effects of reactive oxygen species more effectively than tocopherols, and different *in vitro* and *in vivo* studies indicate that tocotrienols may lower cholesterol levels and suppress tumor growth (*22*). Furthermore, a combination of tocopherols and tocotrienols, preferably from natural sources, has been suggested to be an essential part of a diet preventing Alzheimer's disease (*23*).

The aim of this study was to determine the variation in composition and levels of tocopherols and tocotrienols in soft parts of sea buckthorn berries during ripening in three consecutive years.

MATERIALS AND METHODS

Plant Material. The experiment was carried out during the period 2004−2006 at Balsgård (56°06′N, 14°10′E), Swedish University of Agricultural Sciences, Sweden. Berries from one cultivar and three advanced selections (below referred to as cultivars) of sea buckthorn (Ljublitelskaja, BHi 72587, BHi 72588, and BHi 727102) were harvested weekly during the ripening period. Each of the four cultivars was represented by five ramet plants, the berries of which were pooled on every harvest occasion. The berries were collected during three to eight weeks in July−September, depending on the availability of berries and the degree of ripeness in different years (**Table 1**). Sampling started when the berries were changing to their characteristic ripe color and ended when over-ripeness was apparent (rancid aroma, desiccation). All berries from a branch were harvested by cutting complete branches from the plants in the field. The branches were then frozen as quickly as possible at −20 °C, and the berries were shaken off the branches while still frozen. The berries were stored at −20 °C until all berries of the year had been sampled and thereafter transferred to −80 °C for long-term storage. Before analysis, the berries were lyophilized for four days and the seeds removed.

Analysis of Tocopherols and Tocotrienols. For the analysis of tocopherols and tocotrienols, about 5−10 g of lyophilized berries (equivalent to 40−80 g fresh weight (FW)) were ground in a laboratory mill (Yellow line, A10, IKA-Werke, Staufen, Germany), and for each sample, three 1 g replicates were homogenized in 20 mL of ethanol (99.7%)/*n*-hexane 4:3 with 0.01% (w/v) BHT (butylated hydroxytoluene) using an Ultraturrax (T8, IKA-Werke, Staufen, Germany). The extraction efficiency was 1.17 times higher than extraction in *n*-hexane/ethyl acetate 9:1, and the exchange rate was close to 100% (estimated with α-tocopherol). The vials were thereafter sealed and placed in an orbital shaker (Forma Scientific Inc., Merietta, Ohio, USA) in darkness at 4 °C for 20 h for further extraction. The extracts were then centrifuged at 10,000*g* for 10 min and analyzed isocratically on a D-6000 HPLC-system (Merck-Hitachi) with a fluorescence detector, according to methods described by Panfili et al. (*24*) with modifications. The eluent consisted of a solvent mixture (97.3% (v/v) *n*-hexane, 1.8% (v/v) ethyl acetate, and 0.9% (v/v) acetic acid). A Phenomenex Luna 5 μm silica (2) 100A, 250 × 4.60 mm column with a Security Guard silica 4 mm L × 3.0 mm precolumn was used, with an injection volume of 20 μL. Detection was carried out at 290 nm excitation and 330 nm emission. Evaluation of data was performed by D-7000 HSM 4.1 software (Hitachi Ltd.), using retention times compared against α-, β-, γ-, and δ-tocopherol standards (Calbiochem, Merck, Darmstadt, Germany), and tocotrienol data from the literature (*24–26*). DL-α-tocopherol 97% (Alfa Aesar, Karlsruhe, Germany) was used for the quantification of all tocopherols and tocotrienols. The quantification limit was estimated to 14 ng and the detection limit to 3 ng. The content of biologically active vitamin E (C_E) was calculated using the formula described by Bramley et al. (*19*):

$$C_E = C_\alpha + C_\beta \times 0.5 + C_\gamma \times 0.1 + C_\delta \times 0.03 + C_{\alpha 3} \times 0.3 \quad (1)$$

where C_α = α-tocopherol content; C_β = β-tocopherol content; C_γ = γ-tocopherol; C_δ = δ-tocopherol; and $C_{\alpha 3}$ = α-tocotrienol content.

Statistics. Samples were analyzed by HPLC in triplicate for each cultivar and harvest date. Berries were picked from plants in a germplasm collection in which plants had been placed in the same field, at maximum 30 m spacings. Each sample consisted of pooled berries from five ramet plants. To estimate the differences in cultivars, harvest time, and year, and their interactions, a general linear model (GLM) was applied using SAS (SAS Inst. Inc., Cary, North Carolina). The first calculation was followed by GLM of means for each parameter per harvest date, year, and cultivar, and significance levels by LSD < 0.05.

Correlation with Climate Data. Pearson correlations were made for climate data and amounts of tocopherols and tocotrienols in order to reveal any relationships. Mean values of single cultivars and of four cultivars per year and harvest date were used for α-, γ-, and δ-tocopherol, α-, and δ-tocotrienol, total tocopherols, total tocotrienol, total tocopherols plus tocotrienols, and dry matter content. The climate data included temperature (daily mean, 2 m above ground) and irradiation (daily sum, MJ/m^2) at day of harvest, one to seven days before harvest, mean value of week before harvest, and sum at harvest calculated from the meteorological spring (daily mean temperature increasing between 0−10 °C). Climate data for 2004, 2005, and 2006 were obtained from the Swedish Meteorological and Hydrological Institute (SMHI) at Kristianstad airport, station no. 1651, (55°55′N, 14°05′E).

RESULTS

The GLM analysis showed significant relationships between all tocopherol and tocotrienol compounds analyzed, harvest date, cultivar, and year, as well as their interactions. The cultivar had the highest influence on α- and δ-tocopherol, total tocopherols, total tocopherols plus tocotrienols, and vitamin E activity. Yearly variation had the highest influence on γ-tocopherol, α- and δ-tocotrienol, and total tocotrienols. Harvest date always had the lowest influence on all compounds except for δ-tocotrienol, for which cultivar had the lowest influence.

Variation in Different Tocopherols and Tocotrienols. In the sea buckthorn cultivars investigated, the tocopherols identified were α-, γ-, and δ-tocopherol, while β-tocopherol was found only as trace. The tocotrienols found were α-, γ-, and δ-tocotrienol. The total amount of tocopherols plus tocotrienols varied from 316.6 to 1250.9 μg/g DW, with a mean value of 561.5 μg/g DW (average of four cultivars, three years, and all harvest occasions). On average, the tocopherols corresponded to 86.4% and the tocotrienols to 13.6% of the total amount of tocopherols plus tocotrienols.

Total tocopherol content varied from 260.4 to 1173.8 μg/g DW, with a mean value of 485.1 μg/g DW. The predominant tocopherol was α-tocopherol, with contents varying from 260.4 to 540.1 μg/g DW and a mean value of 363.6 μg/g DW (75.0% of total tocopherols). γ-Tocopherol was found in smaller amounts from trace to 35.6 μg/g DW, with a mean value of 19.1 μg/g DW (3.9% of total tocopherols). δ-Tocopherol was found in large amounts from trace to 702.8 μg/g, with a mean

Table 2. Means of Tocopherols and Tocotrienols in Berries of Different Cultivars of Sea Buckthorn Sampled at Various Harvest Dates over Three Years (μg/g DW)[a]

	tocopherols				tocotrienols			total	
cultivar	α	γ	δ	total	α	δ	total	tocols	vitamin E
Ljublitelskaja	432 c	23 b	261 c	717 d	34 b	50 b	84 b	801 d	452 c
BHi 72587	385 b	21 b	60 b	465 c	29 a	58 b	87 b	553 c	398 b
BHi 72588	317 a	10 a	57 b	385 b	28 a	38 a	66 a	451 b	329 a
BHi 727102	313 a	22 b	0 a	335 a	29 a	40 a	69 a	406 a	324 a

[a] The results are presented as means, based on three replicates for each sample. The same letter indicates no significant differences between the cultivars within the same column (LSD <0.05). Total tocols = total tocopherols plus tocotrienols.

value of 102.4 μg/g DW (21.0% of total tocopherols). The δ-tocopherol content occasionally had a large impact on the content of total tocopherols. Cultivar Ljublitelskaja had an extremely high content of δ-tocopherol, especially in 2004, while in cv. BHi 717202, only traces were found (**Table 2**).

The total tocotrienol content varied from 30.5 to 197.4 μg/g DW, with a mean value of 76.4 μg/g DW. The content of α-tocotrienol varied from 22.2 to 38.2 μg/g DW, with a mean value of 30.2 μg/g DW (39.6% of total tocotrienols). γ-Tocotrienol was only found as trace. δ-Tocotrienol content varied from trace to 161.9 μg/g DW, with a mean value of 46.2 μg/g DW (60.4% of total tocotrienols).

Vitamin E activity varied from 270.7 to 559.8 μg/g DW (**Figure 1**), with a mean value of 379.1 μg/g DW, of which about 96% was α-tocopherol.

Variation between Cultivars. The contents of tocopherols, tocotrienols, and vitamin E differed significantly between cultivars. Cultivar Ljublitelskaja had the highest levels of α- and δ-tocopherol, α-tocotrienol, total tocopherols, and total tocopherols plus tocotrienols, and it also had the highest vitamin E activity compared with that of the other cultivars (**Table 2**). The content of γ-tocopherol did not differ in cv. Ljublitelskaja compared with that in cv. BHi 72587 and cv. BHi 727102. Cultivar Ljublitelskaja and cv. BHi 72587 had the highest δ-tocotrienol level of the cultivars investigated. The highest levels of total tocotrienols were found in cv. Ljublitelskaja and cv. BHi 72587. In cv. BHi 727102, only traces of δ-tocopherol were found.

Variation during the Ripening Period. The amounts of all types of tocopherols and tocotrienols and of vitamin E were found to vary depending on ripening. The contents of α- and γ-tocopherol, α-tocotrienol, and vitamin E generally decreased from the first to the last harvest date, while the δ-tocopherol content generally increased during the same period (**Table 3**). δ-Tocotrienol reached its highest content in the middle of the harvest period. Total tocotrienols reached their highest content on average on harvest occasions August 17th and 24th.

Variation between Years. The GLM analyses showed an influence of year on amounts of all types of tocotrienols and tocotrienols, and on vitamin E. For all compounds except δ-tocopherol in 2005 and 2006, the amounts varied significantly between years (**Table 4**). The levels of δ-tocopherol, total tocopherols, and total tocopherols plus tocotrienols were higher in 2004 than in 2005 and 2006. In 2005, the levels of α-tocotrienol were higher than those in 2004 and 2006. Most of the other compounds occurred in lowest amounts in 2005. Levels of α- and γ-tocopherol, δ-tocotrienols, and total tocotrienols and vitamin E activity were highest in 2006.

Correlation with Climate Data. Among the most consistent relationships revealed were correlations of mean values of α-, γ-, and δ-tocopherol with temperature on the day of harvest

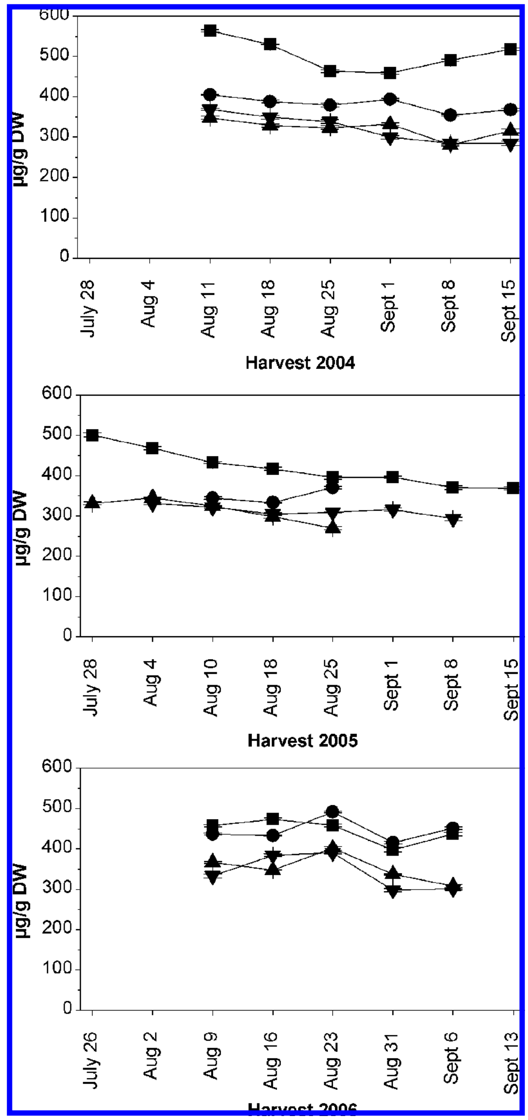

Figure 1. Vitamin E activity ± standard deviation in berries of four cultivars of sea buckthorn sampled at various harvest dates over three years (μg/g DW). Cultivar: Ljublitelskaja (■); BHi 72587 (●); BHi 72588 (▲); BHi 727102 (▼).

Table 3. Means of Tocopherols and Tocotrienols in Berries of Four Cultivars of Sea Buckthorn Sampled at Various Harvest Dates during Three Years, μg/g DW[a]

average harvest	tocopherols				tocotrienols			total	
date	α	γ	δ	total	α	δ	total	tocols	vitamin E
July 28	403 e	24 c	20 a	447 a	34 cd	13 a	46 a	493 ab	416 e
Aug 4	368 bcd	24 c	30 a	422 a	34 d	23 ab	57 ab	479 a	382 bcd
Aug 10	379 de	23 c	76 a	477 a	29 ab	31 b	60 a	537 ab	392 de
Aug 17	368 cd	22 c	86 ab	476 a	31 bc	58 ef	89 de	565 bc	382 cd
Aug 24	368 cd	20 bc	94 ab	482 a	32 cd	66 f	98 e	580 bc	383 cd
Sept 1	350 ab	18 b	125 bc	493 ab	28 a	51 de	79 cd	572 bc	364 ab
Sept 7	343 a	13 a	136 cd	493 ab	30 ab	43 cd	72 bc	565 bc	357 a
Sept 15	356 abc	11 a	189 d	555 b	29 ab	32 abc	61 ab	616 c	371 abc

[a] The results are presented as means, based on three replicates for each sample. The same letter indicates no significant differences between the harvest times in the same column (LSD <0.05). Total tocols = total tocopherols plus tocotrienols.

and temperature and irradiation sums in 2004 (**Table 5**). Correlations between tocopherols/tocotrienols and climate data one to seven days before harvest showed less significance (results not shown). The amounts of α- and γ-tocopherol were positively correlated with the temperature on the day of harvest

Table 4. Means of Tocopherols and Tocotrienols in Berries of Four Cultivars of Sea Buckthorn Sampled at Various Harvest Dates over Three Years (μg/g DW)[a]

	tocopherols				tocotrienols			total	
year	α	γ	δ	total	α	δ	total	tocols	vitamin E
2004	367 b	14 a	188 b	569 c	28 a	39 b	67 a	636 c	382 b
2005	343 a	18 b	43 a	405 a	33 c	28 a	62 a	466 a	356 a
2006	382 c	26 c	64 a	473 b	30 b	74 c	104 b	577 b	396 c

[a] The results are presented as means, based on three replicates for each sample. The same letter indicates no significant differences between the years within the same column (LSD <0.05). Total tocols = total tocopherols plus tocotrienols.

Table 5. Pearson Correlation Coefficient among Amounts of Different Tocopherols in Sea Buckthorn Berries and Weather Data during the Ripening Period in Three Years[a]

		temperature at d. h.		temperature sum		irradiation sum	
	year	corr. coef.	P-value	corr. coef.	P-value	corr. coef.	P-value
α-tocopherol	2004[4]	0.919	0.009(2)	−0.885	0.019(1)	−0.890	0.017(1)
	2005[4]	0.630	0.566(0)	−0.901	0.285(3)	−0.910	0.272(3)
	2006[4]	0.749	0.145(0)	−0.537	0.351(0)	−0.541	0.346(0)
γ-tocopherol	2004[3]	0.931	0.007(3)	−0.742	0.091(2)	−0.747	0.088(2)
	2005[4]	0.925	0.248(1)	−0.999	0.033(2)	−0.997	0.047(2)
	2006[4]	0.634	0.250(0)	−0.436	0.463(0)	−0.434	0.465(0)
δ-tocopherol	2004[3]	−0.984	0.000(1)	0.875	0.023(2)	0.881	0.021(2)
	2005[2]	−0.985	0.109(1)	0.964	0.171(1)	0.985	0.185(1)
	2006[3]	−0.735	0.157(1)	0.599	0.286(0)	0.603	0.282(0)

[a] Tocopherol values are based on the mean values of four cultivars per year for the corresponding date of harvest. Values in parenthesis are the number of significant ($P < 0.05$) single cultivar estimates (mean value of triplicates). Superscripts indicate the number of cultivars with quantified amounts of respective tocopherol. corr. coef. = correlation coefficient; d. h. = day of harvest.

in 2004, but this relationship was not significant in 2005 and 2006. The correlation between the amount of δ-tocopherol and temperature on the day of harvest was found to be negative in 2004.

Negative correlations were found between α-tocopherol and γ-tocopherol, and temperature and irradiation sums in 2004 and 2005, respectively, while there was a positive correlation between the amount of δ-tocopherol and temperature and irradiation sums in 2004.

Single cultivars showed some correlations with temperature on the day of harvest and temperature and irradiation sums (number of cultivars in brackets; **Table 5**), although the mean of all cultivars did not show a significant relationship with climate data.

Vitamin E activity, mostly determined by α-tocopherol amount, showed similar correlations to climate data as did α-tocopherol (results not shown). For the rest of the weather parameters and amounts of compounds investigated, only few correlations were found (results not shown).

The temperature on the day of harvest was 0.8−1.8 °C lower at harvest times from August 10th to 24th in 2005 compared with that in 2004 and 2006 (**Table 6**). In 2005, the maximum temperature on the day of harvest was recorded for September 7th, while in 2004 and 2006, the temperature on the day of harvest continually declined.

Dry Weight Determination. Mean dry weight (DW) of the soft parts of the berries analyzed was 12.8% of FW. When seeds were included, DW was 15.4% of FW. DW generally decreased by 0.5−4% during the sampling period, and the decrease was partly dependent on the length of the harvest period. The mean percentage DW ± standard deviation of the soft berry parts (excluding seeds) during the three years was 11.7 ± 0.9 for cv. Ljublitelskaja, 13.0 ± 0.7 for cv. BHi 72587; 12.2 ± 0.8 for cv. BHi 72588; and 14.2 ± 0.7 for cv. BHi 727102.

DISCUSSION

Similar to the results of the present study, Zadernowski et al. (*17*) reported the presence of α-, γ-, and δ-tocopherols in berry oil of sea buckthorn berries. Other studies have also shown contents of β-tocopherol in, e.g., pulp oil (*15, 16*) and in the pulp and peel of berries (*9, 18*).

For tocotrienol content, the results from the present study confirmed results from, e.g., Ranjith et al. (*15*) showing the presence of α-, γ-, and δ-tocotrienols. Other studies have shown the presence of only δ-tocotrienols in the pulp oil (*16*) or α-, β-, and γ-tocotrienols in the soft berry parts (*18*).

One reason for the differences cited in the literature concerning the types of tocopherols and tocotrienols present in sea buckthorn berries might be that different parts of the berries, e.g., pulp oil (*15, 16*) and soft berry parts (*18*), were analyzed. The use of different cultivars and/or yearly variations or differences in time of harvest might also have influenced the results.

A problem with ripening studies on sea buckthorn berries is that there are no well-recognized objective measurements to precisely determine the stage of maturity for different cultivars. Various methods investigated, e.g., length, weight, acidity, sugar, and color, have resulted in different estimates in pilot studies and appear to be inadequate parameters. In the present study, berries were harvested at different stages of maturity spanning the optimum stage of ripeness.

In this study, genetic differences in the content of tocopherols and tocotrienols were prominent. Cultivar Ljublitelskaja generally had a higher content of most tocopherols and tocotrienols than the other cultivars. The total amounts of tocopherols plus tocotrienols were 2-fold higher in cv. Ljublitelskaja compared with cv. BHi 727102. The most extreme difference between the cultivars was for δ-tocopherol, with the highest amounts in cv. Ljublitelskaja and only trace amounts in cv. BHi 727102.

The yearly variation in the content of tocopherols and tocotrienols was of similar magnitude to the variation between cultivars. The largest variation was found for δ-tocopherol, with more than 4-fold higher content in 2004 than in 2005. The other tocopherols and tocotrienols also showed yearly variations, resulting in different total amounts of tocopherols plus tocotrienols between years. Different stress factors, such as drought, low temperature, and high light intensity, have been shown to influence the formation of tocopherols (*27–30*). Tocopherols and tocotrienols have antioxidant properties and have been suggested to prevent lipid peroxidation in plant membranes (*31*). Both formation and degradation of tocopherols and tocotrienols might be affected by the degree of stress; therefore, a high stress level might overwhelm the capacity of the antioxidant system and result in lower content of these antioxidants, whereas a lower degree of stress might induce higher contents (*31*). Investigations of yearly variations in tocopherols or tocotrienols are scarce, but significant yearly variations in tocopherols have been found in olive oil (*32*). Seasonal variations were found in another investigation in drought-stressed leaves of *Salvia officinalis* in March, May, and July, and the highest levels of α-tocopherol were found in March, the period with the lowest degree of drought stress, which progressively increased from March to July (*33*). Therefore, the yearly variation shown in this investigation may be the result of different complementary or

Table 6. Temperature Data at Different Harvest Dates over Three Years[a]

		average harvest date							
	year	July 28	Aug 4	Aug 10	Aug 17	Aug 24	Sept 1	Sept 7	Sept 15
temperature at day of harvest (°C)	2004			18.2	17.7	16.3	13.7	11.2	13.5
	2005	16.0	15.2	16.6	16.5	15.0	15.8	19.9	13.1
	2006			17.6	17.3	16.8	15.2	15.4	
temperature sum	2004			1706.3	1828.8	1941.0	2041.9	2144.4	2242.2
	2005	1454.7	1573.2	1677.8	1785.8	1904.2	1996.6	2112.3	2215.8
	2006			1818.9	1932.2	2048.3	2155.6	2268.8	
irradiation sum	2004			2396.3	2501.3	2610.3	2698.5	2793.1	2875.3
	2005	2221.8	2322.4	2428.6	2531.9	2636.9	2728.0	2829.8	2911.8
	2006			2420.6	2516.8	2613.5	2707.8	2789.9	

[a] Climate data was collected by SMHI at Kristianstad airport, station no. 1651, (55°55′N, 14°05′E).

conflicting environmental factors. Pruning activities, with lower cutting frequency in the years preceding 2004 and cutting each year thereafter, might also have affected the results.

Contents of α-tocopherol and γ-tocopherol decreased during the ripening period, whereas δ-tocopherol increased. This is in accordance with earlier findings showing decreasing levels of α-tocopherol and increasing levels of δ-tocopherol in sea buckthorn during ripening (*17*). δ-Tocopherol and γ-tocopherol are precursors to α-tocopherol and β-tocopherol, respectively (*34*), but the factors that modulate transformation from one form to the other are not clear. The negative correlation between α- and γ-tocopherol and temperature irradiation sum and its positive correlation with δ-tocopherol suggest that α- and γ-tocopherol decrease in response to oxidative stress caused by heat, irradiation, and/or senescence among other possible factors, whereas δ-tocopherol might be a precursor to replenish the tocopherol pool.

The positive correlations between contents of α-tocopherol and γ-tocopherol and temperature on the day of harvest, as well as the negative correlation between δ-tocopherol and temperature on day of harvest, indicate that the daily temperature might have an influence on the content of these tocopherols, and thus, there may be fairly rapid fluctuations in the levels. The content of α-tocopherol has previously been found to follow a weak diurnal rhythm in leaves of a high alpine plant species, though large fluctuations were found, possibly due to rapid turnover of the compound (*35, 36*). A maximum content was found at midday and a minimum during night. However, in another investigation in drought-stressed leaves of *Salvia officinalis*, the lowest levels of α-tocopherol were found at midday (night levels were not measured) (*33*). Diurnal fluctuations have also been found in other antioxidants (*35*). Although these diurnal fluctuations have been found in photosynthetic tissues, similar changes may be found in nonphotosynthetic tissues such as orange sea buckthorn berries. Previous investigations have found that tocopherol-deficient mutants subjected to high light stress showed only subtle responses to stress tolerance (*37*), indicating that tocopherols may have a more common antioxidant function, interchangeable with other antioxidants, and not specifically linked to some photosynthetic function. The results found in the present investigation indicate that the daily temperature might be important for the levels of tocopherols, but the influence may vary within years, as other environmental factors may have greater importance in some years.

In this study, cv. Ljublitelskaja was the most promising of the cultivars as a source of high vitamin E sea buckthorn berries as it had significantly higher levels of α-tocopherol in 2004 and 2005, and significantly higher levels of δ-tocopherol in all years investigated compared with those of the other cultivars.

The optimum time of harvest depends on the desired contents of different compounds. If high levels of vitamin E activity are mostly desired, early harvest on a warm day when berries have changed to the characteristic orange color is recommended. However, it is important to note that a relationship was observed between cultivar × harvest interactions and content of the compounds investigated, making precise predictions of optimum harvest date difficult. The decrease in vitamin E activity during ripening is mostly dependent on the decrease in the content of α-tocopherol. Premature harvest (before the change to the characteristic ripe color) has previously been shown to result in lower levels of α- and δ-tocopherol (*17*). The differences between cultivars in the content of bioactive compounds revealed in this study show that it is possible to increase the levels of healthy compounds by the appropriate choice of cultivars. Advanced plant breeding could further improve the plant material and produce superior cultivars for use, e.g., in the fortification of various foods.

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