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Carotenoids in Sea Buckthorn (*Hippophae rhamnoides* L.) Berries during Ripening and Use of Pheophytin *a* as a Maturity Marker

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Four cultivars of sea buckthorn berries were analyzed for their carotenoid and chlorophyll contents during ripening in three consecutive years. The different carotenoids generally increased in concentration during ripening and comprised from 120 to 1425 μg/g of DW of total carotenoids (1.5−18.5 mg/100 g of FW) depending on cultivar, harvest time, and year. GLM analyses revealed the effect of cultivar to be considerably larger than that of year and harvest time. The content of pheophytin *a*, a chlorophyll *a* derivate, steadily decreased during berry ripening. Pheophytin *a* therefore acted as a marker of the degree of ripening of sea buckthorn berries and was used here to convert harvest date into an estimated ripening time.

KEYWORDS: Antioxidant; berries; carotenoids; chlorophyll; cultivar; esterified carotenoids; fruit; harvest; *Hippophae rhamnoides*; lycopene; maturity; pheophytin; ripening; sea buckthorn; irradiation; temperature; variation between years; xanthophyll; zeaxanthin

INTRODUCTION

Sea buckthorn (*Hippophae rhamnoides* L.) berries are considered to be beneficial to health (*1*). Different applications for the berries include food, cosmetics, and pharmaceutical products (*2*−*4*). The berries generally contain high amounts of phytochemicals, for example, ascorbic acid, carotenoids, healthy fatty acids, phenolic compounds, and tocopherols (*3*, *5*−*9*). Some products of sea buckthorn berries are used for treatment of the skin, mucosa, and cardiovascular and immune systems (*1*, *4*).

For the manufacturing of many products, for example, nutritional and health products, it is important to have access to raw material with a high content of bioactive compounds such as carotenoids (*10*, *11*). Previous investigations on sea buckthorn berries have shown differences in composition and content of carotenoids (*1*, *2*), which may be due to genetic variation, berry parts analyzed, climate and growing conditions, variation between years, degree of ripening when harvested, storage conditions, and methods of analysis. Forty-one different carotenoids have been reported in various cultivars of sea buckthorn berries (*12*), the major types being zeaxanthin, β-cryptoxanthin, and β-carotene (*1*, *4*, *13*, *14*). Raffo et al. (*4*) showed increasing contents of zeaxanthin, β-cryptoxanthin, and β-carotene during ripening from early July to mid-September in two of three cultivars. Studies on the total content of carotenoids have also been reported (*15*−*17*). One of these investigations, performed during a period of 19 days in August, showed a consistent increase in total carotenoids in three different cultivars (*17*). To our knowledge, no previous study has compared carotenoids during ripening in various cultivars of sea buckthorn berries in different years.

When fruits and berries are analyzed, an objective reference scale for the stage of ripening is very useful. Without such a scale, it is, for example, not possible to reliably compare studies of different cultivars/genotypes in different years and at different ripening stages. To date, no such scale has been available for sea buckthorn berries.

The main aim of this study was therefore to determine the variation in composition and levels of major carotenoids in the soft parts of sea buckthorn berries during ripening in different years. A further aim was to study the possibility of using chlorophyll and its derivates as markers for sea buckthorn berry maturity.

MATERIALS AND METHODS

Plant Material. The plant material used in the present investigation was identical to that used for tocopherol and tocotrienol determination

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Table 1. Harvest Date for Sampling of Sea Buckthorn Berries in Different Years and Average Harvest Dates (ahd) 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	July 28	Aug 4	Aug 10	Aug 17	Aug 24	Sept 1	Sept 7	Sept 15

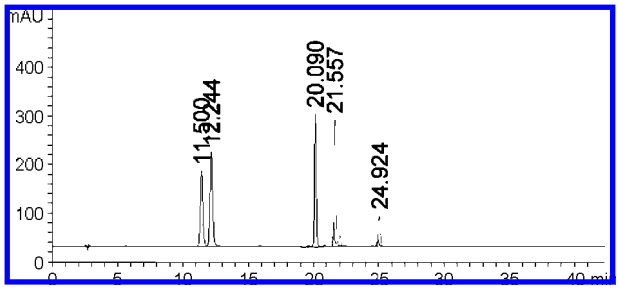

Figure 1. Total profile at 458 nm for standards of carotenoids with respective retention times: lutein, 11.500 min; zeaxanthin, 12.244 min; β-cryptoxanthin, 20.090 min; lycopene, 21.557 min; β-carotene, 24.924 min.

in Andersson et al. (*5*). The experimental site was Balsgård (56° 06′ N, 14° 10′ E), Swedish University of Agricultural Sciences, Sweden, and the experiment was carried out in 2004−2006. Berry harvesting was carried out weekly during the ripening period (**Table 1**) of one sea buckthorn cultivar (cv. Ljublitelskaja) and three advanced selections (BHi 72587, BHi 72588, and BHi 727102, hereafter referred to as cultivars), which were planted in the field year 2000 at the age of 2 years. Berries of five ramet plants from each cultivar were pooled on every harvesting occasion. The sampling period was from onset of the change to characteristic ripening color (orange) of berries until over-ripening was apparent (rancid aroma, desiccation). Berries were harvested by cutting complete branches (with berries attached) from the plants. The branches were then frozen as quickly as possible at −20 °C, and the berries were thereafter shaken off while still frozen. When all plants were harvested, the berries were transferred to −80 °C for storage until analysis.

Analysis of Carotenoids and Chlorophylls. About 40−80 g of fresh berries was lyophilized for 4 days, and the seeds were removed. The lyophilized berry soft parts [equivalent to 5−10 g of dry weight (DW)] were ground for 5 s/20000 rpm in a laboratory mill (Yellow line, A10, IKA-Werke, Staufen, Germany), and 1 g of each sample was homogenized in 20 mL of ethanol (99.7%)/*n*-hexane, in proportions 4:3 and including 0.01% butylated hydroxytoluene (BHT), using an Ultraturrax (T8, IKA-Werke, Staufen, Germany). Samples were sealed and placed in an orbital shaker (Forma Scientific Inc., Marietta, OH) in darkness at 4 °C for 20 h for further extraction and thereafter centrifuged at 10000*g* for 10 min. The samples were analyzed by an Agilent 1100 HPLC system (Agilent Technology) equipped with a diode array detector according to the methods of Khachik et al. (*18*) with modifications. The eluent consisted of solvent A [80% acetonitrile, 15% MeOH, and 5% dichlormethane (v/v)] and solvent B [30% acetonitrile, 20% MeOH, and 50% dichlormethane (v/v)]. The binary gradient was as follows: 0% B (0−2 min), 0−25% B (2−15 min), 25−60% B (15−17 min), 60−90% B (17−29 min), 90% B (29−39 min), 90−0% B (39−41 min), and 0% B (41−47 min). A Phenomenex Synergi 4 μ Hydro-RP 80A, 250 × 4.60 mm column and a Security Guard C18 precolumn were used, and detection was carried out at 458 and 665 nm. Data were evaluated by Chemstation A09.03 software (Agilent Technology). For quantification of all samples, standard curves of *all-trans*-β-carotene (Sigma-Aldrich) were used, which was prequantified by a spectrophotometric method according to the method given in ref *19*. Identification was done by use of spectral data for external standards (*all-trans* forms of lutein, zeaxanthin, β-cryptoxanthin, lycopene; Extrasynthese, Genay, France) (**Figure 1**) and from literature data (*20, 21*), which were compared with spectra in the samples. The stereochemical identification of the carotenoids was made by comparing literature data (*21*−*24*), but not quantified. Esterified carotenoids were separated from carotenes and xanthophylls by saponification of trial samples (*25*), but not quantified as single compounds. Chlorophyll *a* and its derivates were identified by comparing literature data (*26*) with spectra in the samples. The content of pheophytin *a* in sea buckthorn berries was approximated from a standard curve constructed on HPLC by use of extractions of chlorophyll *a* and its derivates (pheophytin *a* and pheophorbide *a*) from unripe fruits with high chlorophyll content (*Rosa rubiginosa*), the content of which was quantified using spectrophotometric methods (*27*). The extraction efficiency for chlorophylls with ethanol/*n*-hexane 4:3 was 1.16 times higher than with 80% acetone.

Statistics. All HPLC results are based on three replicate samples for each cultivar and harvest date. General linear model (GLM) analyses of variance were carried out using SAS software (SAS Institute Inc., Cary, NC). The GLM analyses of means were followed by the *t* test for comparisons for differences of means for each parameter, harvest date, year, and cultivar, and significant levels were determined by LSD < 0.05. The statistics were calculated both before and after adjustment to ripening time. Total minor unidentified peaks of carotenes were not evaluated as a single group in the GLM analyses due to varying individual carotenoids in this group.

A conversion of harvest date into ripening time was made: the different cultivars were correlated against each other in each year with regard to their contents of carotenoids and pheophytin *a* in order to investigate any relationship. For this purpose the mean values of three replicates at each comparable harvest date were used. A strong correlation was found for pheophytin *a*, which consistently decreased during the early stages of ripening, and therefore it was proposed to be useful as a measurable marker for berry ripening. For each cultivar the harvest date was then converted into an estimated ripening time for each year, by fitting the cultivars by the whole sequence of weekly harvest dates to the content of pheophytin *a*. The best fit was obtained by comparing the coefficient of variation for differently shifted ripening times for all cultivars and years, using the smallest value of coefficient of variation as the selection criterion. The ripening time was then plotted against the mean contents of pheophytin *a*, a trend line was added, and an equation for pheophytin *a* was derived (Excel 2003, Microsoft).

Pearson correlation coefficients were calculated for climate and biochemical data to investigate any relationships using Minitab 15 (Minitab Inc.). For every calculation, mean values (of total carotenoids, pheophytin *a*, totals of different groups of carotenoids) of single cultivars and of four cultivars per year and harvest date were used. Minor unidentified peaks were not included in the correlation analyses. Climate data were obtained from the Swedish Meteorological and Hydrological Institute (SMHI), Kristianstad airport, station 1651 (55° 55′ N, 14° 05′ E). Data included were temperature (daily mean, 2 m above ground level) and irradiation (cumulative daily total, MJ/m^2) on day of harvest, 1−7 days before harvest, weekly mean value before harvest, and cumulative temperature at harvest calculated from meteorological spring (daily mean temperature when increasing between 0−10 °C).

RESULTS

Conversion of Harvest Date into Ripening Time. Of the chlorophylls and chlorophyll derivates, only pheophytin *a* was present in detectable amounts in the berries during the sampling period. The correlation analysis among different cultivars in each year for amounts of carotenoids and pheophytin *a* revealed a strong relationship, mainly for pheophytin *a* (**Table 2**). To investigate whether the content of pheophytin *a* could be used as a marker for maturity, the harvest date was converted into a ripening time (**Figures 2** and **3**). The use of ripening time instead of harvest date (1−8) (**Tables 3** and **4**) resulted in a lower coefficient of variation for 71% of the comparisons performed (60 of 84, results not shown). The relationship between the ripening time (adjusted harvest date) and mean pheophytin *a*

Table 2. Pearson Correlation Coefficient for Pheophytin *a* Content at Comparable Harvest Dates in the Respective Year in Berries of Different Cultivars of Sea Buckthorn in Three Years[a]

		correlated cultivar for respective year		
year	cultivar	BHi 72587	BHi 72588	BHi 727102
2004	Ljublitelskaja	0.908	0.994	0.986
	BHi 72587		0.923	0.922
	BHi 72588			0.971
2005	Ljublitelskaja	0.997 (0.052)	0.996	0.973
	BHi 72587		0.966 (0.166)	1.000
	BHi 72588			0.992
2006	Ljublitelskaja	0.991	0.950	0.999
	BHi 72587		0.968	0.989
	BHi 72588			0.936

[a] All values are significant ($P < 0.05$) except for values with P value in parentheses.

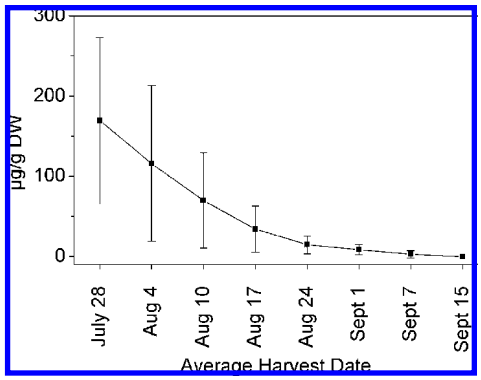

Figure 2. Mean pheophytin *a* content ± standard deviation (μg/g of DW) in berries of four cultivars of sea buckthorn on various harvest dates in three years.

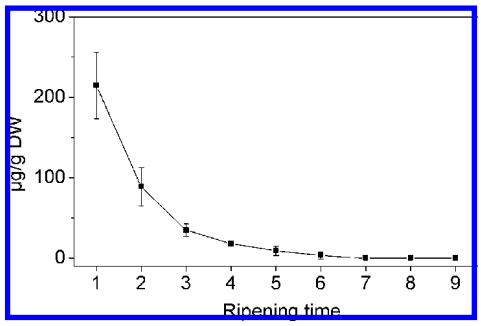

Figure 3. Mean pheophytin *a* content ± standard deviation (μg/g of DW) in berries of four cultivars of sea buckthorn at various ripening times in three years.

content of the four cultivars (for three years) had an exponential trend (presented in a logarithmic scale, **Figure 4**), with the equation

$$y = 449.23\ e^{-0.8042x} \quad (1)$$

The trend line for the relationship had an almost perfect fit ($R^2 = 0.9958$) and eq 1 could therefore be used to predict ripening time in a sample with a known amount of pheophytin *a*.

GLM Analysis. The GLM analysis of variance of carotenoids and pheophytin *a* resulted in significant results for all factors included in the model: harvest date, year, and cultivar, as well as their interactions in all possible combinations (**Table 5**).

For all single and total carotenoids studied, cultivar had the largest influence. For the xanthophylls (except β-cryptoxanthin),

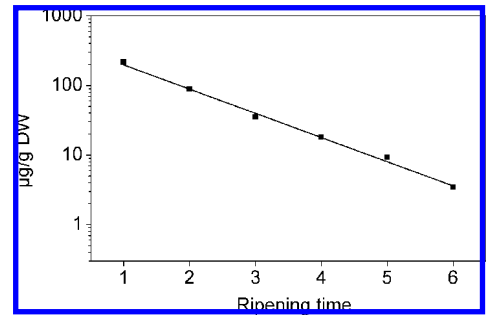

Figure 4. Mean pheophytin *a* content (μg/g of DW) in berries of four cultivars of sea buckthorn at various ripening times in three years with trend line (ripening times 7−9 are below measureable limits).

harvest date had a higher influence than year, but for the carotenes and β-cryptoxanthin, year had a larger influence than harvest date. For pheophytin *a* the influence of the different factors decreased in the order harvest date > cultivar > year. The influences of the interactions were generally of lower magnitude than the influence of single factors. Of the interactions, year × cultivar had the highest influence for zeaxanthin, β-cryptoxanthin and total xanthophylls, lycopene, γ- and β-carotenes, total carotenes, esterified carotenoids, and total carotenoids. For lutein the highest interaction was harvest date × year × cultivar, and for pheophytin *a* it was harvest date × cultivar.

After conversion of harvest date to ripening time, GLM analysis of variance showed similar results as for harvest date (**Table 6**). Cultivar was the factor of highest magnitude for all carotenoids and ripening time for pheophytin *a*. The only difference at ripening time compared to harvest date for single estimates was for lutein, for which year was of higher magnitude than ripening time. For the interactions, the major differences at ripening time compared to harvest date were that ripening time × year × cultivar was more important for β-cryptoxanthin, ripening time × year was more important for lutein, and year × cultivar was more important for pheophytin *a*.

Variation in Content of Different Carotenoids and Pheophytin *a*. In the sea buckthorn berries investigated, the major identified peaks of carotenoids were xanthophylls [lutein (*all-trans*); zeaxanthin (*all-trans*, 9/9′-*cis*); β-cryptoxanthin (*all-trans*)] and carotenes [lycopene (*all-trans*, 9/9′-*cis*, 13/13′-*cis*); γ-carotene (*all-trans*); β-carotene (*all-trans*, 9/9′-*cis*, 13/13′-*cis*), and esterified carotenoids (**Figure 5**)]. The major part of the carotenoids was identified as *all-trans*. The total amount of all carotenoids varied from 119.9 to 1424.9 μg/g of DW, with a mean value of 834.8 μg/g of DW (four cultivars, three years, and all harvest occasions) (**Figure 6**). On average, the xanthophylls corresponded to 9.1%, the carotenes to 35.5%, and the esterified carotenoids to 55.4% of total carotenoids.

The total xanthophyll content varied from 35.5 to 157.0 μg/g of DW, with a mean value of 75.9 μg/g of DW; the carotene content varied from 65.3 to 662.9 μg/g of DW, with a mean value of 296.6 μg/g of DW; and the esterified carotenoid content varied from 10.9 to 827.9 μg/g of DW, with a mean value of 462.3 μg/g of DW.

Among the xanthophylls, lutein varied from trace amounts to 28.7 μg/g of DW, with a mean value of 8.7 μg/g of DW, and corresponded to 11.5% of the xanthophylls. Zeaxanthin varied from 17.2 to 137.2 μg/g of DW, with a mean value of 65.2 μg/g of DW, and corresponded to 85.9% of the xanthophylls, whereas β-cryptoxanthin varied from trace to 9.8 μg/g of DW, with a mean value of 2.2 μg/g of DW, and corresponded to 2.9% of the xanthophylls.

Table 3. Harvest Dates for Mean Major Carotenoid Content and Pheophytin *a* Content (Micrograms per Gram of DW) in Berries of Four Cultivars of Sea Buckthorn Plants in Three Years[a]

	xanthophylls				carotenes							
ahd[c]	lutein	zeaxanthin	β-cryptoxanthin	total	lycopene	γ	β	minor[b]	total	esterified carotenoids	total carotenoids	pheophytin *a*
July 28	17.5 h	43.9 a	0.0 a	61.4 a	41.3 a	14.9 a	46.7 a	58.6	161.5 a	100.5 a	323.4 a	169.4 h
Aug 4	14.4 g	51.3 b	1.7 d	67.4 b	48.8 b	18.6 b	60.4 b	61.4	189.2 b	203.7 b	460.3 b	115.9 g
Aug 10	10.7 f	56.9 c	0.5 b	68.1 b	61.5 c	26.4 c	103.2 c	76.9	268.0 c	346.5 c	682.6 c	69.9 f
Aug 17	8.1 d	65.6 d	1.0 c	74.7 c	67.3 e	28.4 d	115.2 d	85.4	296.3 e	419.9 d	790.9 d	34.3 e
Aug 24	7.8 c	71.5 f	2.4 e	81.7 e	74.7 f	33.1 e	128.1 e	92.4	328.3 g	510.4 e	920.4 e	14.6 d
Sept 1	8.6 e	70.9 f	3.0 f	82.5 e	73.9 f	35.1 f	138.3 g	91.2	338.5 h	545.9 f	966.9 f	8.6 c
Sept 7	7.2 b	66.7 e	3.0 f	76.9 d	65.4 d	34.5 f	133.0 f	84.0	316.9 f	567.5 g	961.3 f	2.9 b
Sept 15	4.5 a	71.8 f	6.3 g	82.6 e	48.2 b	35.3 f	137.5 g	62.6	283.6 d	648.7 h	1014.9 g	0.0 a

[a] Means based on three replicates for each sample. Different letters within columns indicate significant differences between harvest dates ($P < 0.05$). [b] Minor = total of minor unidentified peaks. [c] ahd = average harvest date.

Table 4. Ripening Times for Mean Major Carotenoid Content and Pheophytin *a* Content (Micrograms per Gram of DW) in Berries of Four Cultivars of Sea Buckthorn Plants in Three Years[a]

	xanthophylls				carotenes							
ripening time	lutein	zeaxanthin	β-cryptoxanthin	total	lycopene	γ	β	minor[b]	total	esterified carotenoids	total carotenoids	pheophytin *a*
1	15.8 h	25.3 a	0.0 a	41.1 a	19.4 a	8.8 a	46.0 a	25.8	100.0 a	91.4 a	232.5 a	214.7 g
2	10.4 f	45.4 b	0.0 a	55.8 b	45.6 b	14.4 b	75.7 b	60.5	196.2 b	221.0 b	473.0 b	88.9 f
3	10.4 f	64.8 c	0.5 b	75.7 c	71.1 e	26.5 c	106.1 c	89.5	293.2 d	403.9 c	772.8 c	34.9 e
4	9.1 e	66.2 d	0.9 c	76.2 c	75.1 f	32.5 d	122.3 d	92.5	322.4 e	475.6 d	874.2 d	18.1 d
5	8.0 d	73.8 f	3.0 e	84.8 e	80.8 g	36.4 e	133.0 e	101.9	352.1 f	533.9 e	970.8 e	9.3 c
6	5.8 b	71.4 e	2.3 d	79.5 d	66.9 d	36.3 e	136.4 f	85.4	325.0 e	571.5 f	976.0 e	3.4 b
7	6.4 c	78.2 g	5.9 f	90.5 f	62.9 c	37.9 f	146.8 g	76.8	324.4 e	605.9 g	1020.8 f	0.0 a
8	5.2 a	71.9 e	7.5 g	84.6 e	44.5 b	32.8 d	135.7 f	55.9	268.9 c	651.8 h	1005.3 f	0.0 a
9[c]	11.8 g	100.2 h	9.0 h	121.0 g	93.0 h	74.9 g	246.4 h	120.5	534.8 g	690.4 i	1346.2 g	0.0 a

[a] Means based on three replicates for each sample. Different letters within columns indicate significant differences between ripening times ($P < 0.05$). [b] Minor = total of minor unidentified peaks. [c] Amount based on one cultivar one year.

Table 5. Mean Squares from GLM Analyses of Major Carotenoid Content and Pheophytin *a* Content in Berries of Four Cultivars of Sea Buckthorn Plants Sampled at Different Harvest Dates in Three Years[a]

		xanthophylls				carotenes						
source	df	lutein	zeaxanthin	β-cryptoxanthin	total	lycopene	γ	β	total	esterified carotenoids	total carotenoids	pheophytin *a*
harvest date	7	264.39	1930.18	64.52	1697.39	1333.11	662.05	7429.81	35215.48	343820.52	647739.26	11282.70
year	2	218.65	536.07	101.78	860.20	9815.60	2157.52	56701.01	135935.07	672184.93	952588.28	3762.22
cultivar	3	742.76	41685.43	156.54	56168.25	99397.14	21492.25	192975.55	1682551.23	770913.91	5526449.44	6772.40
harvest × year	9	60.00	177.60	7.21	203.93	350.42	107.08	311.96	3464.43	9842.56	18864.02	158.31
harvest × cultivar	18	68.97	327.89	21.41	462.46	1572.76	267.24	1031.50	16593.95	20179.36	69543.78	1876.93
year × cultivar	6	57.18	875.43	24.35	771.94	1758.68	784.43	15492.43	34488.42	39254.01	87825.67	1034.31
harvest × year × cultivar	20	139.70	428.21	7.71	311.03	360.24	58.47	326.96	4001.27	5502.01	17254.67	28.34
error	132	0.39	2.91	0.03	7.17	2.94	2.54	6.25	35.80	213.50	385.55	3.92

[a] All values are significant ($P < 0.05$).

Among the carotenes, lycopene varied from 4.2 to 155.6 μg/g of DW, with a mean value of 65.2 μg/g of DW, and corresponded to 22.0% of the carotenes, whereas γ-carotene varied from 5.9 to 74.9 μg/g of DW, with a mean value of 30.5 μg/g of DW, and corresponded to 10.3% of the carotenes. β-Carotene varied from 33.3 to 248.9 μg/g of DW, with a mean value of 118.7 μg/g of DW, and corresponded to 40.0% of the carotenes. The only chlorophyll *a* metabolite found was pheophytin *a*, which varied from trace to 264.2 μg/g of DW, with a mean value of 33.7 μg/g of DW (**Figure 7**). There was a total of up to seven minor unidentified/mixed peaks of carotenes, depending on cultivar and ripeness, and these peaks varied from trace to 179.2 μg/g of DW, with a mean value of 82.2 μg/g of DW.

Variation between Cultivars. The cultivars differed in their contents of total xanthophylls, lutein, β-cryptoxanthin, total carotenes, lycopene, γ- and β-carotenes, total carotenoids, and pheophytin *a* (**Table 7**). Cultivars Ljublitelskaja and BHi 727102 did not differ in their contents of zeaxanthin and esterified carotenoids. Cultivar BHi 72587 had the highest amount of total xanthophylls, including lutein and zeaxanthin, esterified carotenoids, and total carotenoids. Cultivar BHi 72588 had the highest content of all carotenes and β-cryptoxanthin, whereas cultivar BHi 727102 had the highest content of pheophytin *a*. In cv. BHi 727102 no β-cryptoxanthin was detected. The major unidentified peaks varied from trace to three minor unidentified peaks in cv. Ljublitelskaja, whereas in each of the other cultivars they varied from trace to seven peaks.

Variation during the Ripening Period. The content of carotenoids and pheophytin *a* varied depending on date of harvest (**Table 3**). The esterified carotenoids and total carotenoids increased from the first to the last date of harvest. Total xanthophylls and zeaxanthin also increased from the first date of harvest but stayed almost constant from August 24 to the last date of harvest. Lutein showed a general decrease from the first to the last date of harvest, whereas β-cryptoxanthin generally increased over the same period. The content of

Table 6. Mean Squares from GLM Analyses of Major Carotenoid Content and Pheophytin *a* Content in Berries of Four Cultivars of Sea Buckthorn Plants at Different Ripening Times in Three Years[a]

		xanthophylls				carotenes						
source	df	lutein	zeaxanthin	β-cryptoxanthin	total	lycopene	γ	β	total	esterified carotenoids	total carotenoids	pheophytin *a*
ripening time	8	188.22	12240.34	75.53	1236.50	2976.70	802.92	6676.46	42184.08	329870.27	637272.12	55523.69
year	2	218.65	536.07	101.78	860.20	9815.60	2157.52	56701.10	135935.07	672184.93	952588.28	14737.88
cultivar	3	742.76	41685.43	156.54	56168.25	99397.14	21492.25	192975.55	1682551.23	770913.91	5526449.44	26529.76
ripening time × year	13	199.15	196.59	7.46	91.10	306.59	81.37	266.63	2981.18	12277.54	21148.53	542.41
ripening time × cultivar	17	67.70	508.47	12.00	626.49	934.64	193.99	887.09	11171.43	7091.76	31093.02	776.47
year × cultivar	6	37.76	1092.52	12.49	1031.78	1966.26	806.37	16286.4	43936.75	40843.29	129909.46	1455.28
ripening × year × cultivar	16	81.07	302.09	13.88	311.02	191.64	41.69	200.15	2098.26	2495.60	9165.03	41.97
error	132	0.39	3.09	0.03	7.47	3.07	2.61	6.79	38.49	263.33	474.05	3.92

[a] All values are significant ($P < 0.05$).

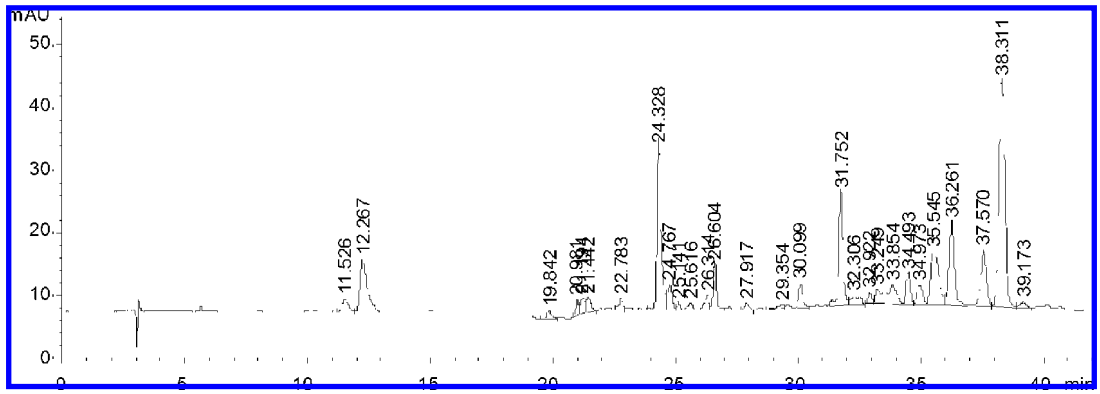

Figure 5. Total profile at 458 nm for the sea buckthorn berry, cultivar Ljublitelskaja harvested August 11, 2004. Major carotenoids with respective retention times: lutein, 11.526 min; zeaxanthin, 12.267 min; β-cryptoxanthin, 19.842 min; lycopene, 20.981 min; lycopene, 21.194 min; mixed peak, 21.442 min; γ-carotene, 22.783 min; β-carotene, 24.328 min; β-carotene, 24.767 min; esterified carotenoids, 25.141−39.173 min.

lycopene reached a maximum on August 24 and September 1. γ- and β-carotenes increased from the first day of harvest to September 1 and then remained fairly constant. Pheophytin *a* decreased from the first to the last date of harvest.

After the harvest date was converted to the ripening time on the basis of the content of pheophytin *a*, all carotenoids except lutein increased from the first to the last ripening time, with the highest content at the last ripening time. However, the increases in carotenoid concentrations were not observed in all ripening times. The lutein decreased from the first to the second to last ripening time (ripening time 8). Pheophytin *a* decreased from the first to the three last ripening times, when no content was detectable (**Table 4**).

Variation between Years. The content of carotenoids varied depending on compound and year (**Table 8**). The highest contents of β-cryptoxanthin, γ- and β-carotenes, esterified carotenoids, and total carotenoids were found in 2004. The contents of lutein, zeaxanthin, total xanthophylls, lycopene, and total carotenes were higher in 2006 than in 2004 and 2005. The lowest contents of all carotenoids except β-cryptoxanthin and minor carotenes were found in 2005. Over the three years, the content of pheophytin *a* was highest in 2005 and lowest in 2004.

Climate Data. The year 2006 had a higher cumulative temperature than either of the other years when compared at corresponding harvest dates, 103−124 °C higher than 2004 and 141−159 °C higher than 2005. The year 2004 in turn had a 26−45 °C higher cumulative temperature than 2005 (**Table 9**). However, the increase in temperature between harvest dates within years did not show any consistent trends when the different years were compared. In 2005 cumulative irradiation was 26.6−36.7 MJ/m^2 higher than in 2004 and 8.0−39.9 MJ/m^2 higher than in 2006. In 2006 cumulative irradiation was 3.2−24.3 MJ/m^2 higher than in 2004 on four of five corresponding dates. However, the increase in cumulative irradiation between each harvest date within years did not show any consistent trends when the different years were compared.

Correlation with Climate Data. Of the correlations between different climate data and the mean values of the different carotenoids or pheophytin *a*, the relationships between cumulative temperature and irradiation on the one hand and esterified carotenoids and pheophytin *a* on the other were the most consistent over years (**Table 10**). No other consistent correlations were revealed (results not shown). Negative correlations were found between pheophytin *a* and cumulative temperature and irradiation, whereas the corresponding correlations with esterified carotenoids were positive.

DISCUSSION

The total amount of carotenoids found in sea buckthorn berries in this investigation was of the same magnitude as in other studies (*2*, *4*, *17*). The major carotenoids detected were zeaxanthin, β-carotene, β-cryptoxanthin, lutein, lycopene, and γ-carotene. This is in agreement with earlier studies, which also reported zeaxanthin and β-carotene to be the dominant carotenoids in sea buckthorn berries (*1*, *4*, *13*, *14*). However, after prior saponification, the amounts of zeaxanthin have been found to increase relative to the total amounts of carotenoids, for example, to 49−91% of the total main carotenoids depending on cultivar (*4*), or 31−39% of total carotenoids (*28*). The relatively low values of zeaxanthin and β-cryptoxanthin in the present study are probably due to the fact that esterified carotenoids consist of zeaxanthin and β-cryptoxanthin to a high

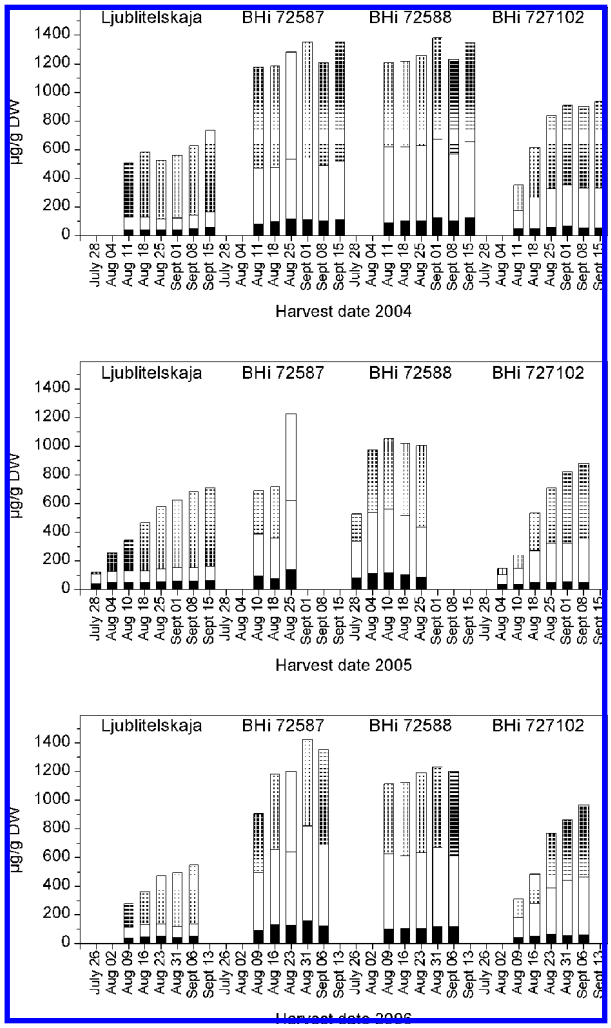

Figure 6. Total carotenoids, xanthophylls (black bars), carotenes (white bars), and esterified carotenoids (shaded bars) (μg/g of DW) in berries of four cultivars of sea buckthorn at various ripening times in three years.

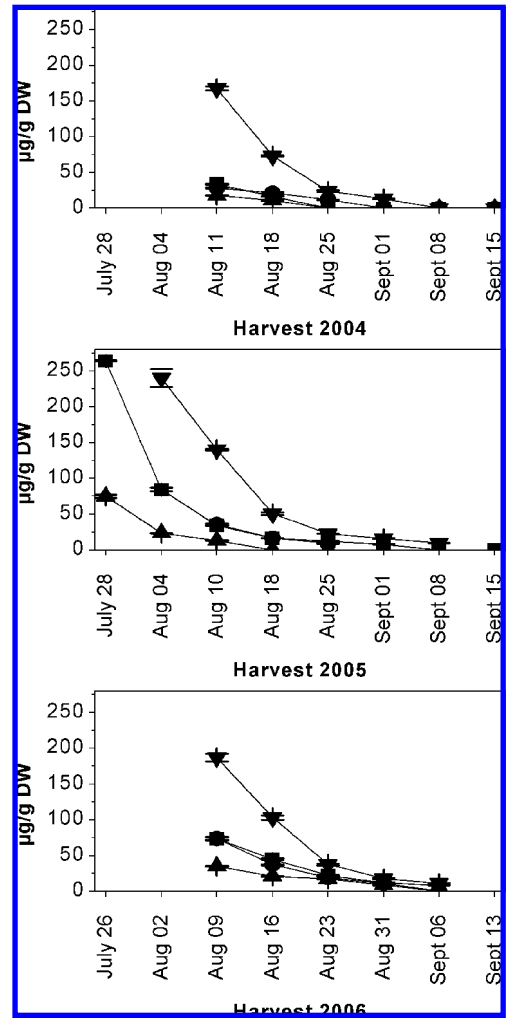

Figure 7. Pheophytin *a* ± standard deviation (μg/g of DW) in berries of four cultivars of sea buckthorn sampled at various harvest dates over three years. Cultivars: Ljublitelskaja (■); BHi 72587 (●); BHi 72588 (▲); BHi 727102 (▼).

degree (*14*). In the present study esterified carotenoids represented 55.4% of total carotenoids. Differences in amounts of various compounds may also be due to differences in cultivars, ripening, year, climate factors, and methods of analysis.

Precise criteria for ripening and preferably objective measurements of different stages of ripening/maturity are highly necessary when the influence of the ripening process on the content of bioactive compounds is studied. The results from the present study regarding degradation and content of chlorophyll *a* and its derivates during the ripening process, together with the correlations between the different cultivars in amount of pheophytin *a*, make pheophytin *a* a good candidate as a measurable marker associated with the degree of ripening in sea buckthorn berries. However, when pheophytin *a* has decreased below detectable limits, it is not possible to distinguish between the ripening times (ripening times 7−9). For a compound to be generally applicable as an objective marker for ripening, any change in amount of the compound must be similar in different cultivars/genotypes. The GLM analyses of variance showed that date of harvest influenced pheophytin *a* content in sea buckthorn berries to a considerably larger extent than cultivar and year. Due to the breakdown of chlorophyll during ripening/senescence (*29*), chlorophyll compounds could be associated with, and useful for, determining the stage of ripeness in many fruits and berries. A change from green to a characteristic ripe color during the ripening process is associated with maturity in many other fruits, and chlorophyll is used to monitor the ripening process of fruits such as apple, banana, and mango (*30*−*32*). The color of the sea buckthorn berries derives from their high content of carotenoids, which are, during the early stages of maturation, masked by the content of chlorophylls. Chlorophyll *a* and its derivates can be measured using simple methods such as spectrophotometry, colorimetry, or fluorometry, which renders these compounds very useful for the estimation of ripening stages by use of eq 1 when plant materials are being compared.

By the use of GLM analyses, the factor cultivar was found to have the strongest impact on all compounds except pheophytin *a*. Cultivars BHi 72587 and BHi 72588 had almost 2-fold higher contents of carotenoids than cvs. Ljublitelskaja and BHi 727102 and 2-fold lower contents of pheophytin *a*. The lower amounts of carotenoids in cvs. Ljublitelskaja and BHi 727102 can partly depend on a higher degree of unripe berries. However, as harvesting time was of less magnitude than cultivar, cultivar differences have to be the main reason for differences in carotenoid content.

In 2004 and 2006 the amounts of carotenoids were higher than in 2005, except for β-cryptoxanthin, and the pheophytin *a* content was lower in 2004 and 2006 than in 2005. The variation between years in amounts of carotenoids and pheophytin *a* might

Table 7. Cultivar Differences of Mean Major Carotenoid Content and Pheophytin *a* Content (Micrograms per Gram of DW) in Berries of Four Cultivars of Sea Buckthorn Sampled at Various Harvest Dates in Three Years[a]

	xanthophylls				carotenes							
cultivar	lutein	zeaxanthin	β-cryptoxanthin	total	lycopene	γ	β	minor[b]	total	esterified carotenoids	total carotenoids	pheophytin *a*
Ljublitelskaja	4.0 a	42.3 a	1.7 b	48.0 a	11.3 a	8.5 a	54.4 a	12.1	86.3 a	364.7 a	499.0 a	33.1 c
BHi 72587	13.2 d	95.0 c	3.5 c	111.7 d	92.4 c	44.3 c	175.2 c	125.0	436.9 c	612.9 c	1161.5 d	18.8 b
BHi 72588	10.4 c	90.7 b	3.9 d	105.0 c	110.9 d	53.4 d	177.1 d	136.0	477.4 d	547.9 b	1130.3 c	13.9 a
BHi 727102	8.8 b	42.3 a	0.0 a	51.1 b	60.1 b	22.1 b	88.8 b	74.8	245.8 b	366.8 a	663.7 b	65.4 d

[a] Means based on three replicates for each sample. Different letters within columns indicate significant differences between cultivars ($P < 0.05$). [b] Minor = total of minor unidentified peaks.

Table 8. Yearly Differences of Mean Major Carotenoid Content and Pheophytin *a* Content (Micrograms per Gram of DW) in Berries of Four Cultivars of Sea Buckthorn Sampled at Various Harvest Dates in Three Years[a]

	xanthophylls				carotenes							
year	lutein	zeaxanthin	β-cryptoxanthin	total	lycopene	γ	β	minor[b]	total	esterified carotenoids	total carotenoids	pheophytin *a*
2004	7.8 b	65.7 b	3.5 c	77.0 b	60.2 b	37.3 c	144.1 c	17.5	313.2 b	579.5 c	969.7 c	17.5 a
2005	7.5 a	59.4 a	1.8 b	68.7 a	54.0 a	21.4 a	75.1 a	48.5	221.8 a	361.6 a	652.1 a	48.5 c
2006	11.3 c	71.1 c	1.0 a	83.4 c	83.5 c	32.3 b	136.2 b	107.0	359.0 c	432.4 b	874.8 b	37.0 b

[a] Means based on three replicates for each sample. Different letters within columns indicate significant differences between years ($P < 0.05$). [b] Minor = total of minor unidentified peaks.

Table 9. Climate Data at Different Harvest Dates in the Three Years[a]

		harvest dates							
	year	July 28	Aug 4	Aug 10	Aug 17	Aug 24	Sept 1	Sept 7	Sept 15
cumulative temperature (°C)	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	
cumulative irradiation (MJ/m^2)	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 were collected by SMHI at Kristianstad airport, station 1651, (55° 55′ N, 14° 05′ E).

Table 10. Pearson Correlation Coefficient between Pheophytin *a* Content and Esterified Carotenoid Content in Berries of Different Cultivars of Sea Buckthorn and Weather Data during the Ripening Period in Three Years[a]

		pheophytin *a*		esterified carotenoids	
year	cultivar	cumulative temperature	cumulative irradiation	cumulative temperature sum	cumulative irradiation
2004	Ljublitelskaja	−0.852	−0.856	0.858	0.850
	BHi 72587	−0.947	−0.949	0.672 (0.144)	0.688 (0.147)
	BHi 72588	−0.862	−0.867	0.848	0.849
	BHi 727102	−0.894	−0.895	0.937	0.939
2005	Ljublitelskaja	−0.772	−0.781	0.980	0.984
	BHi 72587	−0.959 (0.184)	−0.964 (0.170)	0.947 (0.208)	0.940 (0.222)
	BHi 72588	−0.884	−0.878	0.896	0.888
	BHi 727102	−0.901	−0.914	0.988	0.988
2006	Ljublitelskaja	−0.952	−0.958	0.983	0.986
	BHi 72587	−0.954	−0.958	0.973	0.973
	BHi 72588	−0.986	−0.986	0.979	0.979
	BHi 727102	−0.939	−0.945	0.983	0.984

[a] Carotenoid and pheophytin a values are based on the mean values of triplicates for the corresponding harvest dates for each year. All values are significant ($P < 0.05$) except for values with *P* value in parentheses.

partly depend on variation in earliness of ripening. Cumulative temperature was also higher in 2004 and 2006 than in 2005, whereas cumulative irradiation was higher in 2005. Thus, in addition to cultivar, carotenoid and pheophytin *a* contents are influenced by the environment, with high cumulative temperature and low cumulative irradiation leading to higher amounts of carotenoids and lower amounts of pheophytin *a*. There was a general increase in all carotenoids except for lutein during ripening of the berries. The biosynthesis of many carotenoids has been studied in different plants, and temperature and irradiation influence the accumulation/degradation of different carotenoids, but light is not essential for carotenogenesis (*33*). The biosynthesis of β-carotene, lutein, and zeaxanthin has been shown to increase in sun-exposed leaves compared with shaded leaves (*34*). However, investigations on *Capsicum annuum* showed that capsanthin was inhibited by shade, whereas capsorubin, which is responsible for the ripe color, was enhanced in shade (*35*). It has also been concluded that the optimum temperature for the synthesis of lycopene in tomatoes is 16−21 °C and that temperatures higher than 30 °C inhibit the synthesis

of lycopene but not that of β-carotene (*33*). Warm and sunny weather with moderate precipitation has been suggested to favor accumulation of carotenoids in sea buckthorn berries, and the carotenoid content varies widely depending on the climate during the vegetative period (*12*). However, this study showed that the influence of cultivar was more significant than climate variations between years in all three years. The decrease in lutein during the ripening process might be due to esterification. The present investigation showed that the content of total xanthophylls was influenced to a larger extent by harvest/ripening time than by year, whereas the converse was true for the carotene content. This converse relationship might be partly due to esterification of the carotenoids. The conversion of harvest date to ripening time led to an adjustment of cv. BHi 727102 toward a lower degree of ripeness during all years (total minus 5 weeks for all years). Cultivar BHi 727102 generally had lower amounts of carotenoids than cvs. BHi 72587 and BHi 72588. Furthermore, no β-cryptoxanthin was detected in cv. BHi 727102, whereas it was found in the other cultivars investigated. After conversion to ripening time, β-cryptoxanthin was not detected during the first two ripening times and increased in small amounts thereafter.

The content of esterified carotenoids continuously increased with ripening time. It has been suggested that esterification of the carotenoids makes them more liposoluble and eases their integration into membranes and plastoglobules; also, esterified carotenoids have been shown to be more stable than nonesterified forms (*36*). The suggested reason for esterification of carotenoids in fruits and flowers is to enhance the color by overaccumulation of carotenoids in the chromoplast organelles, that is, the plastoglobules (*37*). Most esterification takes place when the chloroplasts degenerate and carotenoids leak out into the less soluble stroma (*37*), which is also apparent in the decrease in pheophytin *a* and the increase in esterified carotenoids. It has been shown that the esterified and nonesterified capsanthins in *C. annuum* also suppress oxidation of methyl linoleate with the same ability, which suggests that the esterified capsanthins serve as an antioxidant in the plastoglobules (*38*). The xanthophylls detected here in sea buckthorn berries (zeaxanthin, lutein, and β-cryptoxanthin) have good antioxidative capacity (*39*). It has also been shown that esterified capsanthins are more stable toward lipoxygenase and that esterification does not affect their photostability (*38, 40*). The esterified carotenoids have previously been proposed as useful ripening markers for *C. annuum* (*37*); however, the variation in content between cultivars and years makes esterified carotenoids less suitable as a maturity marker in sea buckthorn berries.

In an earlier investigation (*5*), tocopherol and tocotrienol contents were reported using the same sea buckthorn berry material as in the present study. When these data were reanalyzed following conversion of harvest date to ripening time by use of pheophytin *a* content, the coefficient of variance was lower in 50% of the comparable harvest/ripening times for the tocopherols and tocotrienols. This result indicates the usefulness of ripening time instead of harvest date for these compounds, too. In these two ripening investigations the differences observed in carotenoids, tocopherol and tocotrienol content between first and last harvest date were significant, and if berries are harvested only once, it is very important to know their degree of maturity. However, commercial harvest is possible during the whole investigated time period, with few exceptions on the first and last harvest dates due to immaturity or over-ripeness, which have been shown to differ in the content of different bioactive compounds. Therefore, the optimal ripening stage should be decided by the demands of consumers and technical producers for the final product, whose preferences may be different in taste, content of oil or bioactive compounds, etc.

The content of bioactive compounds was most influenced by cultivar and often also by environmental factors within different years. To maximize the content of carotenoids in sea buckthorn berries, delaying harvest of selected cultivars to as late as possible is recommended.

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LITERATURE CITED

(1) Yang, B.; Kallio, H. Composition and physiological effects of sea buckthorn (Hippophae) lipids. *Trends Food Sci. Technol.* **2002**, *13*, 160–167.

(2) Beveridge, T.; Li, T. S. C.; Oomah, B. D.; Smith, A. Sea buckthorn products: manufacture and composition. *J. Agric. Food Chem.* **1999**, *47*, 3480–3488.

(3) Zadernowski, R.; Nowak-Polakowska, H.; Lossow, B.; Nesterowicz, J. Sea-buckthorn lipids. *J. Food Lipids* **1997**, *4*, 165–172.

(4) Raffo, A.; Paoletti, F.; Antonelli, M. Changes in sugar, organic acid, flavonol and carotenoid composition during ripening of berries of three seabuckthorn (*Hippophae rhamnoides* L.) cultivars. *Eur. Food Res. Technol.* **2004**, *219*, 360–368.

(5) Andersson, S. C.; Rumpunen, K.; Johansson, E.; Olsson, M. E. Tocopherols and tocotrienols in sea buckthorn (*Hippophae rhamnoides* L.) berries during ripening. *J. Agric. Food Chem.* **2008**, *56*, 6701–6706.

(6) Olsson, M. E.; Gustavsson, K.-E.; Andersson, S.; Nilsson, Å.; Duan, R.-D. Inhibition of cancer cell proliferation in vitro by fruit and berry extracts and correlations with antioxidant levels. *J. Agric. Food Chem.* **2004**, *52*, 7264–7271.

(7) Suomela, J.-P.; Ahotupa, M.; Yang, B.; Vasankari, T.; Kallio, H. Absorption of flavonols derived from sea buckthorn (*Hippophae rhamnoides* L.) and their effect on emerging risk factors for cardiovascular disease in humans. *J. Agric. Food Chem.* **2006**, *54*, 7364–7369.

(8) Rösch, D.; Bergmann, M.; Knorr, D.; Kroh, L. W. Structure−antioxidant efficiency relationships of phenolic compounds and their contribution to the antioxidant activity of sea buckthorn juice. *J. Agric. Food Chem.* **2003**, *51*, 4233–4239.

(9) Zadernowski, R.; Naczk, M.; Czaplicki, S.; Rubinskiene, M.; Szalkiewicz, M. Composition of phenolic acids in sea buckthorn (*Hippophae rhamnoides* L.) berries. *J. Am. Oil Chem. Soc.* **2005**, *82*, 175–179.

(10) Deli, J.; Molnar, P. Paprika carotenoids: analysis, isolation, structure elucidation. *Curr. Org. Chem.* **2002**, *6*, 1197–1219.

(11) Maoka, T.; Mochida, K.; Kozuka, M.; Ito, Y.; Fujiwara, Y.; Hashimoto, K.; Enjo, F.; Ogata, M.; Nobukuni, Y.; Tokuda, H.; Nishino, H. Cancer chemopreventive activity of carotenoids in the fruits of red paprika *Capsicum annuum* L. *Cancer Lett.* **2001**, *172*, 103–109.

(12) Bekker, N. P.; Glushenkova, A. I. Components of certain species of the Elaeagnaceae family. *Chem. Nat. Compd.* **2001**, *37*, 97–116.

(13) Kudritskaya, S. E.; Zagorodskaya, L. M.; Shishkina, E. E. Carotenoids of the sea buckthorn, variety Obil'naya. *Chem. Nat. Compd.* **1990**, *25*, 724–725.

(14) Pintea, A.; Varga, A.; Stepnowski, P.; Socaciu, C.; Culea, M.; Diehl, H. A. Chromatographic analysis of carotenol fatty acid esters in *Physalis alkekengi* and *Hippophae rhamnoides*. *Phytochem. Anal.* **2005**, *16*, 188–195.

(15) Ranjith, A.; Kumar, K. S.; Venugopalan, V. V.; Arumughan, C.; Sawhney, R. C.; Singh, V. Fatty acids, tocols, and carotenoids in pulp oil of three sea buckthorn species (*Hippophae rhamnoides*, *H. salicifolia*, and *H. tibetana*) grown in the Indian Himalayas. *J. Am. Oil Chem. Soc*. **2006**, *83*, 359–364.
(16) Ul'chenko, N. T.; Zhmyrko, T. G.; Glushenkova, A. I.; Murdakhaev, Y. M. Lipids of *Hippophae rhamnoides* pericarp. *Chem. Nat. Compd*. **1995**, *31*, 565–567.
(17) Gao, X. Q.; Ohlander, M.; Jeppsson, N.; Bjork, L.; Trajkovski, V. Changes in antioxidant effects and their relationship to phytonutrients in fruits of sea buckthorn (*Hippophae rhamnoides* L.) during maturation. *J. Agric. Food Chem*. **2000**, *48*, 1485–1490.
(18) Khachik, F.; Beecher, G. R.; Goli, M. B. Separation, identification, and quantification of carotenoids in fruits, vegetables and human plasma by high performance liquid chromatography. *J. Agric. Food Chem*. **1991**, *1*, 71–80.
(19) Chen, B. H.; Yang, S. H. An improved analytical method for the determination of carotenes and xanthophylls in dried plant materials and mixed feeds. *Food Chem*. **1992**, *44*, 61–66.
(20) Britton, G.; Liaaen-Jensen, S.; Pfander, H. E. *Carotenoids 1A, 1B and Key to Carotenoids*; Birkhauser Verlag: Basel, Switzerland, 1995.
(21) Rodriguez-Amaya, D. B. *A Guide to Carotenoid Analysis in Foods*; ILSI Press: Washington, DC, 2001.
(22) Khachik, F.; Beecher, G. R.; Lusby, W. R. Separation, identification, and quantification of the major carotenoids in extracts of apricots, peaches, cantaloupe, and pink grapefruit by liquid chromatography. *J. Agric. Food Chem*. **1989**, *37*, 1465–1473.
(23) Yeum, K.-J.; Booth, S. L.; Sadowski, J. A.; Liu, C.; Tang, G.; Krinsky, N. I.; Russell, R. M. Human plasma carotenoid response to the ingestion of controlled diets high in fruits and vegetables. *Am. J. Clin. Nutr*. **1996**, *64*, 594–602.
(24) Khachik, F.; Beecher, G. R.; Whittaker, N. F. Separation, identification, and quantification of the major carotenoid and chlorophyll constituents in extracts of several green vegetables by liquid chromatography. *J. Agric. Food Chem*. **1986**, *34*, 603–616.
(25) Granado, F.; Olmedilla, B.; Gil-Martinez, E.; Blanco, I. A fast, reliable and low-cost saponification protocol for analysis of carotenoids in vegetables. *J. Food Compos. Anal*. **2001**, *14*, 479–489.
(26) Johnson-Flanagan, A. M.; Thiagarajah, M. R. Degreening in canola (*Brassica napus*, cv. Westar) embryos under optimum conditions. *J. Plant Physiol*. **1990**, *136*, 180–186.
(27) Hendry, G. A. F.; Grime, J. P. E. *Methods in Comparative Plant Ecology*; Chapman and Hall: London, U.K., 1993; pp 150−151.
(28) Weller, P.; Breithaupt, D. E. Identification and quantification of zeaxanthin esters in plants using liquid chromatography−mass spectrometry. *J. Agric. Food Chem*. **2005**, *51*, 7044–7049.
(29) Amir-Shapira, D.; Goldschmidt, E. E.; Altman, A. Chlorophyll catabolism in senescing plant tissues: in vivo breakdown intermediates suggest different degradative pathways for citrus fruit and parsley leaves. *Proc. Natl. Acad. Sci. U.S.A*. **1987**, *84*, 1901–1905.
(30) Knee, M. Methods of measuring green colour and chlorophyll content of apple fruit. *J. Food Technol*. **1980**, *15*, 493–500.
(31) Bron, I. U.; Ribeiro, R. V.; Azzolini, M.; Jacomino, A. P.; Machadoc, E. C. Chlorophyll fluorescence as a tool to evaluate the ripening of 'Golden' papaya fruit. *Postharvest Biol. Technol*. **2004**, *33*, 163–173.
(32) Li, M.; Slaughter, D. C.; Thompson, J. F. Optical chlorophyll sensing system for banana ripening. *Postharvest Biol. Technol*. **1997**, *12*, 273–283.
(33) Goodwin, T. W. *The Biochemistry of the Carotenoids*, 2nd ed.; Chapman and Hall: New York, 1980; Vol. 1.
(34) Demmig-Adams, B.; Gilmore, A. M.; Adams, W. W. In vivo functions of carotenoids in higher plants. *FASEB J*. **1996**, *10*, 403–412.
(35) Lopez, M.; Candela, M.; Sabater, F. Carotenoids from Capsicum annuum fruits: Influence of spectral quality of radiation. *Biol. Planta*. **1986**, *28*, 100–104.
(36) Minguez-Mosquera, M. I.; Hornero-Mendez, D. Changes in carotenoid esterification during the fruit ripening of *Capsicum annuum* Cv. Bola. *J. Agric. Food Chem*. **1994**, *42*, 640–644.
(37) Hornero-Mendez, D.; Minguez-Mosquera, M. I. Xanthophyll esterification accompanying carotenoid overaccumulation in chromoplast of *Capsicum annuum* ripening fruits is a constitutive process and useful for ripeness index. *J. Agric. Food Chem*. **2000**, *48*, 1617–1622.
(38) Matsufuji, H.; Nakamura, H.; Chino, M.; Takeda, M. Antioxidant activity of capsanthin and the fatty acid esters in paprika (*Capsicum annuum*). *J. Agric. Food Chem*. **1998**, *46*, 3468–3472.
(39) Krinsky, N. I.; Johnson, E. J. Carotenoid action and their relation to health and disease. *Mol. Aspects Med*. **2005**, *26*, 459–516.
(40) Biacs, P. A.; Daood, H. G.; Pavisa, A.; Hajdu, F. Studies on the carotenoid pigments of paprika (*Capsicum annuum* L. var. Sz-20). *J. Agric. Food Chem*. **1989**, *37*, 350–353.

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