Tocopherols in Sea Buckthorn (*Hippophaë rhamnoides* L.) Berry Oil

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ABSTRACT: The free tocopherol content in whole berries of six sea buckthorn cultivars grown in northeastern Poland and Belorussia was determined with HPLC. The total free tocopherol content in oil from whole berries was 101.4–128.3 mg/100 g of oil. α-Tocopherol was the predominant tocopherol of sea buckthorn berries, and only traces of γ-tocopherol were detected in the oil. α- and δ-Tocopherols constituted 62.5–67.9% and 32.1–37.5% of total tocopherol, respectively. The total free tocopherol content in oil of sea buckthorn cv. Nadbaltycka increased during maturation from 40.4 to 109.8 mg/100 g of oil. Green berries contained a marked amount of γ-tocopherol, but its content rapidly declined to traces when the color of berries turned from green to olive-yellow.

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Sea buckthorn (*Hippophaë rhamnoides* L.), a hardy bush that grows wild in temperate areas of Central Asia and Europe, produces nutritious and delicious berries (1). It is currently domesticated in some parts of the world (2). Sea buckthorn berries can be processed to jams, juices, yellow pigments, and seed oils (2,3). The chemical composition of the berries is affected by growing conditions and maturity (2,4). The soft tissue of the berries contains 3–5% of oil, whereas the oil content of the seeds is 12–13% (5,6). Sea buckthorn berries are an excellent source of phytochemicals such as ascorbic acid, tocopherols, unsaturated FA, and carotenoids (2,3,5,6). Berries have been used for the treatment of radiation damage, burns, oral inflammation, and gastric ulcers (7). Other claimed positive health effects included reduction in plasma cholesterol level, inhibition of platelet aggregation, and regulation of immune function (8). Accordingly, there is a growing interest in the use of sea buckthorn berries for medicinal and cosmetic applications as well as in functional foods. (2,4). Many components of the sea buckthorn berries such as vitamin C, organic acids, unsaturated FA, carotenoids, minerals, and phytosterols have been extensively studied (2,5,6,8). Linoleic and α-linolenic acids constitute approximately 70% of the seed oil FA, whereas palmitoleic acid is the predominant FA in soft-tissue oil (5). Sitosterol is the predominant phytosterol of sea buckthorn, constituting 57–76% and 61–83% of seed and soft-tissue sterols, respectively (8). Published data on tocopherols, however, are still diverse and fragmentary. For example, the concentrations of vitamin E in Chinese cultivars of sea buckthorn range from 40.1 to 103.0 mg/100 g in whole berries (2,9), from 61 to 113 mg/100 g in seed oil, from 162 to 255 mg/100 g in juice oil and from 390 to 540 mg/100 g in the residue (2,4). Fu *et al.* (10) reported that sea buckthorn berries contained from 1 to 10 mg/100 g of tocopherols. Recently, Kallio *et al.* (11) reported that the total content of tocopherols and tocotrienols in sea buckthorn berries (ssp. *sinensis* and *mongolica*) ranged from 56 to 140 mg/kg of whole berries, and the total content of tocotrienols varied from 1.5 to 8.1 mg/kg of whole berries. α-Tocopherol was the predominant tocopherol found in sea buckthorn berries, where it constituted 49% of total tocopherols in seed buckthorn seed oil (2) and 76–89% of total tocopherols in sea buckthorn berries (*H. rhamnoides* ssp. *sinensis* and *mongolica*) (11). Moreover, research is still needed to determine the composition of tocopherols in sea buckthorn berries of different origins.

The aim of this study was to determine (i) the composition of tocopherols in whole berries of selected sea buckthorn varieties grown in eastern Europe and (ii) the effect of the maturity of sea buckthorn berries on the composition of tocopherols.

MATERIALS AND METHODS

Berries of six sea buckthorn (*H. rhamnoides* L.) cultivars—Nadbaltycka, Nevlejena, Otradnaja, Podarok Sadu, Trofimovskaja, and 29-88 hybrid—were used in this study. The oil content in whole berries ranged from 2.3 to 3.0% of berry weight (Table 1). Berries of Nadbaltycka cv. were collected near Olsztyn, Poland, while all of the other cultivars were obtained from the Belorussian Horticulture Research Institute in Samochwalowicze (Belorussia). Mature fruits were harvested by hand in the fall of 1999 and 2000 at the stage of commercial maturity as judged by hand manipulation and juiciness. In addition, berries of Nadbaltycka cv. also were collected at different stages of maturity between July and December of 2000. The berries were cleaned to remove diseased or pest-infested fruits, stems, and leaves and then stored in polyethylene bags at −18°C until analysis.

Lipids were isolated from whole berries using a chloroform/methanol extraction procedure (6,12). The crushed whole

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TABLE 1
Oil Content of Whole Sea Buckthorn Berries[a]

Cultivar	% of fresh berries	% of dry matter
Hybrid 29-88	2.45 ± 0.25[a]	19.1 ± 2.00[a]
Nadbaltycka	2.33 ± 0.31[a,b]	19.4 ± 2.57[a,b]
Nevlejena	2.50 ± 0.20[a–c]	18.00 ± 3.86[a–c]
Otradnaja	2,32 ± 0.30[a–d]	20.00 ± 3.09[a–d]
Podarok Sadu	3.00 ± 0.34[e]	20.90 ± 4.35[a–e]
Trofimovskaja	2.32 ± 0.15[a–d]	17.20 ± 2.10[a–e]

[a]Results are mean values of six determinations. Values within the same column followed by the same superscript are not significantly different ($P > 0.05$).

berries (100 g) were macerated with 200 mL of methanol/chloroform (1:2, vol/vol) for 20 min at room temperature. The mixture was then filtered through Whatman #1 filter paper and the residue extracted with methanol/chloroform (1:2, vol/vol) five more times. The methanol/chloroform extracts were combined and transferred into a separatory funnel, and then the chloroform/methanol/water ratio was adjusted to 1:1:0.9 (by vol) by the addition of methanol and water, after which the chloroform layer was separated, dried over anhydrous sodium sulfate, filtered, and divided into two equal portions. One portion of the chloroform extract was evaporated to dryness under vacuum at ≤40°C to determine the oil content of the berries. The other portion of the extract was concentrated under vacuum and nitrogen at ≤40°C, and the chloroform solution of the oil was then stored at −18°C until analyzed for tocopherols.

The tocopherols in sea buckthorn oils were determined as described by Thompson and Hatine (13). The oil was dissolved in the elution solvent (hexane/diethyl ether, 95:5, vol/vol) to a final volume of 1 g/10 mL. The solution was then filtered through a 0.45 μm Gelman Acrodisc filter (VWR International, Missisauga, Ontario, Canada). Care was taken to preclude exposure of the samples to sunlight throughout the analytical procedure. Samples (20 μL) were analyzed using a Shimadzu HPLC system (Kyoto, Japan) consisting of a pump (Model LC 10AD), a system controller (Model SCTL 10A), a column oven (Model CTO 10 AS), and a Shimadzu diode array detector (Model SPD M10A) interfaced with a personal computer. The HPLC system was equipped with a Luna silica column (5 μm, 4.6 × 250 mm) Phenomenex, Torrance, CA). The separation was carried out at 25°C using hexane/diethyl ether (95:5, vol/vol) as the mobile phase. The system was operated isocratically at a flow rate of 2 mL per min, and tocopherols were detected at 295 nm. A 10-min equilibration period was used between sample injections. The identification of α-, β-, γ-, and δ-tocopherol was made by comparison of retention times of unknown peaks to those of reference authentic standards (Sigma Chemical Co., St. Louis, MO) and confirmed by comparison of DAD-UV (diode array detector-UV) spectra. Quantification of tocopherols was based on the external standard method. Calibration curves for each standard were established by plotting peak areas at four different concentrations for each standard. The correlation coefficients R were between 0.98 and 0.99. Retention times for α-, β-, δ-, and γ-tocopherols were 7.48, 11.24, 20.05, and 13.16 min, respectively.

The content of biologically active vitamin E (C_E) was calculated using the following formula:

$$C_E = C_1 + 0.1 \cdot C_2 + 0.03 \cdot C_3 \quad [1]$$

proposed by Eittenmiller *et al.* (14), where C_1 = α-tocopherol content, C_2 = γ-tocopherol content, and C_3 = δ-tocopherol content.

The results listed in the tables are mean values of duplicate experiments with three to six replicates per experiment. Statistical analysis of data (ANOVA and *t*-test) was carried out using the SigmaStat v.2.03 (SSPS, Chicago, IL) software package. No statistically significant difference (*t*-test; $P > 0.05$) was found among the experiments. In the tables, means within the same column followed by the same superscript are not significantly different (*t*-test, $P > 0.05$).

RESULTS AND DISCUSSION

Table 2 summarizes the tocopherol content in six sea buckthorn cultivars. In this study, we measured the content of tocopherols directly in sea buckthorn oil as recommended by the current standard analytical protocol used for determination of tocopherols and tocotrienols in oils (15). This methodology measures only free tocopherols and tocotrienols. Only α- and δ-tocopherols were detected in the oil of matured sea buckthorn berries in significant quantities. The total tocopherol content in the whole berries was between 101.4 and 128.3 mg/100 g of oil.

TABLE 2
Tocopherol and Vitamin E Contents in Berries of Sea Buckthorn Cultivars[a]

	Tocopherols (mg/100 g of oil)				
Cultivar	α	γ	δ	Total	Vitamin E
Nadbaltycka	70.1 ± 7.6[a]	Traces	36.6 ± 5.0[a]	106.7 ± 9.3[a]	71.2
Nevlejena	72.2 ± 5.2[a,b,d]	Traces	40.5 ± 3.3[b]	112.5 ± 7.6[a,b]	73.4
Otradnaja	83.3 ± 9.2	Traces	45.0 ± 6.9	128.3 ± 10.6	84.6
Podarok Sadu	72.0 ± 3.6	0.25 ± 0.1	38.0 ± 3.9[a,b]	110.3 ± 8.4[a,b]	73.2
Trofimovskaja	63.4 ± 1.8	Traces	38.0 ± 1.0[a,b,c]	101.4 ± 2.4[c]	64.5
Hybrid 29-88	70.5 ± 3.3[a,b,d]	0.3 ± 0.3	33.0 ± 3.0[a]	103.8 ± 4.5[a,c]	71.5

[a]Results are mean values of 12 determinations. Values within the same column followed by the same superscript are not significantly different ($P > 0.05$). Biologically active vitamin E was calculated as described by Eittenmiller *et al.* (14).

TABLE 3
Effect of Harvesting Time on the Composition[a] of Tocopherols in Berries of Sea Buckthorn cv. Nadbaltycka[b]

Harvesting time	Tocopherols (mg/100 g oil)					Diameter of berry (mm)	Color of berry
	α	γ	δ	Total	Vitamin E		
July 10	30.1 ± 1.6[a]	10.1 ± 1.4[a]	10.2 ± 2.5	40.4 ± 2.7	31.4	4	Green
July 28	39.3 ± 2.0	11.0 ± 0.6[a]	30.6 ± 3.1[b]	80.9 ± 2.0	41.3	4.5	Green
August 16	31.7 ± 1.0[a]	6.0 ± 0.8	31.0 ± 2.1[b]	68.7 ± 0.5	33.2	5	Olive
September 13	74.9 ± 0.8[c]	Traces	34.9 ± 2.6[c]	109.8 ± 2.5[c]	75.9	5.5	Olive-yellow
October 3	67.9 ± 1.7[d]	Traces	35.7 ± 1.9[c]	103.6 ± 1.9[d]	69.0	6	Light orange
October 14	65.4 ± 3.0[d]	Traces	36.1 ± 2.2[c–e]	101.5 ± 2.1[d]	66.5	7	Orange
November 15	74.0 ± 2.1[c]	Traces	36.3 ± 1.2[c–f]	110.3 ± 3.8[c,f]	75.1	10	Orange
December 1	71.4 ± 2.8[g]	Traces	36.6 ± 1.1[c–g]	108.0 ± 3.0[c,f,g]	72.5	10	Orange
December 29	71.1 ± 1.6[g]	Traces	38.6 ± 2.3[e–g]	109.7 ± 1.8[c,f,g]	72.3	10	Orange

[a]Results are mean values of six determinations. Values within the same column followed by the same superscript are not significantly different ($P > 0.05$). The biologically active vitamin E was calculated as described by Eittenmiller *et al.* (14).
[b]Berries collected near Olsztyn, Poland, in 2000.

These values were in the upper range of values reported for Chinese sea buckthorn cultivars (2, 9). α-Tocopherol was the predominant tocopherol found in the oil, and it constituted 62.5–67.9% of the total tocopherols. On the other hand, α-tocopherol constituted 49% of total tocopherols in seed buckthorn seed oil (2) and 76–89% of total tocopherols in sea buckthorn berries (*H. rhamnoides* ssp. *sinensis* and *mongolica*) (11). In our study we detected only α-, δ-, and γ-tocopherols in sea buckthorn berry oil, but both Beveredge *et al.* (2) and Kallio *et al.* (11) found small quantities of β-tocopherol as well. This discrepancy may be due to differences in cultivars as well as in the HPLC methodologies employed for the analysis of tocopherols. The distribution of tocopherols in sea buckthorn oil differs from those reported for poppy seed (16), flaxseed (17), and olive oil (18). The content of biologically active vitamin E ranged from 64.5 to 84.6 mg/100 g of oil for the cultivars analyzed in this study. We also noted small peaks on chromatograms that may indicate the presence of tocotrienols in sea buckthorn oil. However, in this study we did not identify and quantify tocotrienols owing to the lack of appropriate tocotrienol standards.

The effect of maturation on the color and size of berries and the content of tocopherols in sea buckthorn cv. Nadbaltycka berry oil is summarized in Table 3. The berries of this cultivar, grown in northeastern Poland, reach commercial maturity in September/October. As the berries matured, the total tocopherol content increased from 40.4 to 109.8 mg/100 g of oil. The maximum level of total tocopherols was found in berries collected in September. α-, γ-, and δ-Tocopherols were detected at the early stages of berry development. The green berries (July harvest) contained about 10 mg γ-tocopherol/100 g of oil, but their content rapidly declined to traces when the berry color turned from green to olive-yellow (September harvest). According to Demurin (19) and Hashim *et al.* (20), accumulation of γ-tocopherol in seeds is probably the result of α-tocopherol demethylation, and it is responsible for seed stability. It is possible that γ-tocopherol may play a similar role at the early stages of development of sea buckthorn berries.

α-Tocopherol was the predominant tocopherol found in maturing berries, where it constituted 48.5–74% of total tocopherol content. The α-tocopherol content increased from 30.1 to 74 mg/100 g oil, reaching the maximal level in berries collected in November (Table 3). The content of α-tocopherol in sea buckthorn oil was two- to sevenfold higher than that found in Greek virgin olive oils (18). The high level of α-tocopherol may contribute to the stability of berries at later stages of development, as it is the most potent tocopherol in quenching singlet oxygen (21). In addition, as the berries matured, the δ-tocopherol content also increased from 10.2 to 38.6 mg/100 g oil, reaching its maximal level in berries harvested in October.

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