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Quality Components of Sea Buckthorn (*Hippophae rhamnoides*) Varieties

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The sensory quality and chemical constituents of juices from seven sea buckthorn (*Hippophaë rhamnoides* L.) varieties were studied in two consecutive seasons. The juices were generally described as sour and astringent, with low sweetness and fruity flavor. The differences in sensory quality as well as in chemical composition between samples and years were significant (p < 0.05) in most parameters studied. The Chuiskaya variety was described as the sweetest, with the strongest fruity flavor, whereas the varieties Avgustinka, Botanicheskaya, Trofimovskaya, and Raisa were the sourest and most astringent. Total sugar (fructose and glucose) varied from 1.9 to 7.1 g/100 mL in juice, total acid (malic and quinic acids) from 3.1 to 5.1 g/100 mL, vitamin C from 29 to 176 mg/100 mL, and pulp oil from 0.7 to 3.6%. The soluble solids were between 7.4 and 12.6, the pH between 2.7 and 2.9, and the titrable acidity between 2.0 and 3.7. The redness was highest on Avgustinka and Raisa, but there were no differences in yellowness. Total sugar and the sugar/acid ratio correlated positively with sourness and astringency, whereas total acid and titrable acidity correlated positively with sourness and astringency and negatively with sweetness.

KEYWORDS: *Hippophae rhamnoides*; varieties; sensory quality; sugars; organic acids; vitamin C; pulp oil; color

INTRODUCTION

Sea buckthorn (*Hippophaë rhamnoides*) berries have a unique, strong flavor. They are known to be quite acidic and not very sweet with a mild but characteristic aroma. The nutritional value of the berries is high. They are rich in flavonoids and vitamin C, and their seed oil contains α -linolenic acid in abundance. The fruit has high contents of pulp oil and oil soluble bioactive compounds, such as tocopherols, tocotrienols, carotenoids, and plant sterols (1–5). Many health claims are associated with sea buckthorn. Vitamin C in sea buckthorn, together with tocopherols and tocotrienols, has a strong antioxidative effect (1). The berries seem to have preventive effects against cardiovascular diseases, mucosa injuries, and skin problems, evidently through the enhancement of cell membrane regeneration (2, 6).

Besides the nutritional value, the sensory quality of berries is important from the consumers' point of view. However, the literature concerning the sensory quality of sea buckthorn focuses on a limited number of issues. Tang et al. (7) found sourness, astringency, and bitterness to be the sensory attributes that characterize sea buckthorn flavor. Beveridge et al. (8) also reported on the sourness in sea buckthorn juice. The titrable acidity in the berry correlates positively with sourness and astringency but negatively with sweetness (7). In sea buckthorn, sourness arises from organic acids, primarily malic acid (2, 9). Sweetness has a strong positive effect on the overall pleasantness of the berry, whereas sourness, astringency, and bitterness correlate negatively with pleasantness in sea buckthorn (7). To better understand the flavor of sea buckthorn, the sensory quality and related chemical composition of the berry need to be studied more intensively.

The aim of our study was to examine the sensory properties (odor and taste) of several sea buckthorn varieties. The paper focuses on the correlation between the sensory quality and chemical compositions, such as sugars and organic acids, vitamin C, pulp oil content, and color as Hunter *L*, *a*, *b* values in sea buckthorn berries.

MATERIALS AND METHODS

Samples. The sea buckthorn varieties to be investigated were chosen by their availability and familiarity among farmers and specialists in Finland. The sea buckthorn varieties Avgustinka, Botanicheskaya, and Trophimovskaya were grown by a sea buckthorn society in southwestern Finland in Turku. A professional farmer in southern Finland, in Riihimäki, cultivated berries of cv. Prevoshodnaya, Oranzhevaya, Chuiskaya, and Raisa. (The names of varieties are transliterated from Russian to English according to the general transliterating rules.) Both berry producers were known to be reliable and dedicated to the matter. All varieties were of the Russian ssp. *mongolica* origin except Raisa, which is a Finnish product of the ssp. *rhamnoides* origin. The berries were handpicked fully ripe during the seasons of 2002 and 2003, immediately frozen at -20 °C, pooled after freezing, and stored for analyses.

Sample Preparation. Sensory Evaluation. Sea buckthorn berries (100-200 g) were thawed in a microwave oven (AEG Micromat,

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Table 1.	Sensory	Attributes,	Descriptions	with	References,	and	Intensities	Used	in [Descriptive	Profiling
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attribute	description	intensity level	reference compound
strength of odor	strength of odor typical of sea buckthorn		
fruity flavor	fruity flavor of tropical fruits and strong-tasting berries,	7	fruit nectar ^a /cranberry juice
	typical of sea buckthorn		concentrate ^b /water 6:1:5 +
			3 g of sucrose/100 mL
			reference mixture
sweetness	taste caused by sugars and other sweeteners	2	1% glucose solution
		7	2% glucose solution
sourness	taste caused by organic acids	2	0.1% citric acid solution
		6	0.5% citric acid solution
astringency	tingling, drying mouth feeling	2	0.1% AISO ₄
		7	0.2% AISO4
off-odors	odors that are not innate for sea buckthorn, usually		
	refer to storage, mishandling, contaminations		
off-flavors	flavors that are not innate for sea buckthorn (see off-odors)		

^a Granini, Eckes-Granini GmbH & Co. KG, Germany. ^b Meritalo cranberry juice, Finland.

Nürnberg, Germany) with 30% power for $2 \times 20-25$ s. The thawed berries were homogenized for 30 s by a bamix blender. The mash was pressed for juice with a hydraulic press (Hafico) at a pressure of 19.6 MPa to ensure that the seeds were not broken, and the juice was stored at 4 °C overnight. In the morning of the analysis, the juice was kept at room temperature for 45–60 min, mixed and diluted 1:1 (v/v) in carbon-filtered water, and kept at room temperature for another 30 min to reach the final temperature of 20 ± 2 °C. The samples were mixed and divided in 50-mL beakers, covered with lids, and kept at room temperature for an additional 30 min to stabilize the headspace. Sample serving beakers were randomly coded with three-digit numbers.

Chemical Analysis. Sea buckthorn berries (50 g) were thawed in the microwave oven with 30% power for 2×15 s and homogenized as previously described. For the analysis of pulp oil content and color, the juice was prepared as for the sensory analysis. For the analysis of pH, soluble solids, titrable acidity, sugars, and acids, as well as vitamin C, the juice of homogenized berries was filtered through cheesecloth under vacuum.

Sensory Evaluation. Sensory Panel. The panel for descriptive profiling consisted of volunteer students and staff of the university. Of the 11 assessors in 2002, 8 were women and 3 were men (21-50 years of age), whereas in 2003, 9 were women and 2 were men (21-38 years of age). Six of the assessors evaluated samples in both years. The assessors were chosen on the basis of their willingness, availability, and good health (self-reported), together with their ability to recognize selected odors, basic tastes, and flavor differences in samples.

Creating the Descriptors and Training of the Panel. The descriptors were created in 2002. The assessors were asked to define the odor and flavor of two samples during the first 1-h session. Odor was most commonly described as fruity, berrylike, citrus fruit, sweet, exotic soft fruit, tingling, and fermented and flavor as sour, astringent/drying, citrus fruit/orange, sweet, fermented, full, and bitter/acrid. On the basis of preprofiling attributes, "strength of odor", "fruity flavor", "sweetness", "sourness", and "astringency" were chosen for evaluation (**Table 1**.). Three training sessions were arranged each year to introduce the attributes, the intensity scale, and the use of Compusense *five* data collection software (version 2.4. in 2002 and version 4.1 in 2003, Compusense, Guelph, Canada).

Sample Evaluation. During three separate sessions, each assessor evaluated all of the samples in triplicate by their intensity on the line scale of 0-10 (0 = none, 10 = very strong) with the help of references (**Table 1**). The sample presentation order was randomized between and within assessors. The sensory analyses were performed in the sensory laboratory in accordance with ISO 8589-1988 standard. The assessors were forced to take a 30-s break between samples, and they were asked to clean their mouths with water biscuits (Carr's Table Water Biscuits, Carr's of Carlisle Ltd., Carlisle, U.K.) and carbon-filtered water.

Chemical Analysis. Soluble Solids, pH, and Titrable Acidity. Soluble solids, pH, and titrable acidity were analyzed as triplicate juice subsamples, each subsample in three measurements. The soluble solids were determined by 0-32 °Brix refractometer (Atago, Tokyo, Japan),

and pH was determined with a PHM80 portable pH-meter (Radiometer, Copenhagen, Denmark) at room temperature. The juice was titrated with 0.1 M NaOH to the end point of pH 8.1, the third pK value of citric acid (AOAC 22.058), and the total acidity was calculated as citric acid.

Sugars and Acids. Sugars and acids were analyzed in triplicate for each sample according to the method applied by Kallio et al. (2, 10). The juice was diluted 1:20 in water, and the internal standards sorbitol (0.1 g/100 mL in dilution) and tartaric acid (0.05 g/100 mL) (Merck, Darmstadt, Germany) and 6 mL 0.1 N NaOH were added in the total volume of 20 mL. One milliliter of the dilution was fractionated by dual solid-phase extraction. The anthocyanin colors were absorbed in the upper nonpolar cyclohexyl Isolute CH (EC) column (100 mg/mL) (International Sorbent Technology, Hengoed, U.K.), and the acids were trapped for a second lower anion exchanger Isolute SAX column in the formate form (200 mg/3 mL) (International Sorbent Technology). The columns were activated separately as described by the manufacturer, and they were connected in a Baker-10 extraction system (J. T. Baker, Phillipsburg, NJ) prior to fractionation. Sugars were eluted from the SAX column by 2 mL of water, and after the removal of the CH column, organic acids were eluted from the SAX column by 1 mL of 15 N formic acid. Sugar and acid fractions were diluted to the final volumes of 3 mL and shaken, whereafter 1 mL of sample was evaporated to dryness under nitrogen stream at 40 °C and dried in a desiccator on P₂O₅ not less than overnight. Trimethylsilyl (TMS) derivatives of sugars and acids were prepared by adding 200 μ L of Tri-Sil (Pierce, Rockford, IL) reagent for each fraction, and vials were closed with butyl Teflon septa, shaken vigorously by a vortex (Vortex-Genie, Springfield, MA) for 5 min, and incubated at 60 °C for 30 min and at room temperature overnight. The TMS derivatives of sugars and acids were analyzed with a Varian 3300 gas chromatograph (Varian, Palo Alto, CA) equipped with a flame ionization detector (Limerick, Ireland) directed by Perkin-Elmer Turbochrom Navigator 4.1 software (Perkin-Elmer, San Jose, CA). The chromatograph and the workstation were connected with a PE Nelson 900 series interface. The analyses were carried out with a methyl silicone Supelco Simplicity-1 fused silica column (30 m, i.d. = 0.25 mm, film thickness = 0.25 μ m) (Bellefonte, PA). One microliter of sample was injected manually into the split injector (1:20). The temperature of the injector was 210 °C and that of the detector, 290 °C. The column temperature was programmed as 2 min at 90 °C, raised to 275 °C at a rate of 4 °C/min, and held at 275 °C for 10 min.

Vitamin C. Vitamin C was analyzed with HPLC in triplicate as ascorbic acid after the conversion of dehydroascorbic acid to ascorbic acid form with dithiothreitol (DTT; Promega Co., Madison, WI). The berry juice was diluted 1:40 with DTT in water solution (final volume = 80 mg/1 mL juice), each juice in two measurements. The dilution was kept in the dark for 2 h at room temperature and filtered (0.45 μ m) for HPLC analysis. A sample of 20 μ L was injected into a Shimadzu SLC-6A system equipped with a Shimadzu SPD-6AV UV detector and an HP 3393A integrator (Shimadzu, Kyoto, Japan). The

column used was a LiChrochart 250-4-LiChrosphar RP-18, 5 μ m (Merck, Darmstadt, Germany), and 0.5% KH₂PO₄ buffer and 0.1% DTT with a flow rate of 0.4 mL/min were used as mobile phase. DTT was added to the mobile phase to prevent the oxidation of ascorbic acid during the chromatographic analysis.

A stock solution in water, consisting of 5 mg/mL of ascorbic acid (AA) and 1 mg/mL of DTT, was prepared daily. The stock solution was diluted with water to final concentrations of AA of 25, 50, 150, and 300 μ g/mL. Each dilution was analyzed in HPLC in two measurements, and quantification of unknown samples was measured with an external standard method (*1*, *2*, *10*, *11*).

Pulp Oil Content. The pulp oil from triplicate juice samples was extracted by chloroform/methanol, as described by Christie (12). A juice sample of 1 mL was weighed and homogenized with an Ultra-Turrax (Janke & Kunkel GmbH & Co. KG, Staufen, Germany) in methanol (10 mL) for 1 min in a blender, and chloroform (20 mL) was added. The mixture was, further, homogenized and filtered. The filter paper (Whatman no. 1, Whatman Laboratory Division, Maidstone, U.K.) was washed with 60 mL of chloroform/methanol (2:1 v/v). The filtrate was transferred into a measuring cylinder, one-fourth of the total volume of 0.88% KCl in water was added, and the mixture was shaken thoroughly before being allowed to settle. The lower layer was removed and washed with one-fourth of its volume of methanol/water (1:1, v/v). The fraction containing lipid was rewashed, and the solvent was evaporated to dryness with a rotary film evaporator (Heidolph VV2000, Heidolph Eletkro GmbH & Co. KG, Kelheim, Germany) and nitrogen stream at 40 °C. The lipids were weighed, and the oil content of juice was calculated (4).

Color. The tristimulus color properties, Hunter a^* (green-red), b^* (blue-yellow), and *L* (lightness), of each juice sample were measured in three replicates and three measurements using a Minolta Chroma meter CR 200 (Minolta Camera Co., Ltd., Osaka, Japan). The instrument used a D₆₅ illuminant, diffuse illumination, and a 0° viewing angle. Hue (H°), expressed as angular measurement, and chroma (C^*) were calculated as follows (CIE, 1976): $H^\circ = \arctan(b^*/a^*)$ and $C^* = (a^{*2} + b^{*2})^{1/2}$.

Statistical Analysis. The statistical analyses were performed using SPSS (SPSS 12.0.1, SPSS Inc. H, Chicago, IL) and Unscrambler 7.8 (Camo Process AS, Oslo, Norway). The effects of samples, assessors, and replications on sensory intensities were analyzed using a threeway analysis of variance (ANOVA). The differences in sensory intensities and chemical properties among samples were analyzed with a one-way ANOVA and with the nonparametric Kruskal-Wallis test and Mann-Whitney U-test when the population was not normally distributed. Tukey's HSD test for populations with equal variances and Tamhane's test for those with unequal variances were used for the multiple comparison of the mean intensities of attributes at p < 0.05. The sample \times year interaction was also tested. For interpreting the sensory data, principal component analysis (PCA) was used. The relationship between the sensory and chemical data matrices was analyzed with the partial least-squares regression (PLS) method using jack-knifing (13).

RESULTS AND DISCUSSION

Sensory Evaluation. The mean intensities (n = 3 sessions \times 11 assessors = 33) for the odor and flavor properties of each sample in two years, including the multiple comparison between samples (years and sample \times year interaction), are given in **Table 2**. Statistical analysis showed significant differences (p < 0.05) between samples in odor and flavor descriptors in both years (**Table 2**). The differences were strongest in sourness (F = 8.7, p < 0.001) as well as in astringency (F = 5.7, p < 0.001) and sweetness (F = 5.4, p < 0.001). In general, Chuiskaya was described as the sweetest and least sour or astringent among the varieties, whereas Raisa was regarded as sour, strongly astringent, and the least sweet. Likewise, Avgustinka, Botanicheskaya, and Trofimovskaya were high in sourness and astringency, whereas Botanicheskaya was also low in sweetness

Table 2. Mean Intensities (n = 33) for Sensory Attributes in Sea Buckthorn Varieties in 2002 and 2003^a

sample	year	total odor	fruity flavor	sweet- ness	sour- ness	astringency
Avgustinka	2002	4.6ab	3.2a	2.9bc	6.1c	5.4b
Botanicheskaya	2002	5.2b	3.6a	2.4b	5.7bc	5.4b
Oranzhevaya	2002	4.0a	3.5a	2.9bc	6.0bc	5.3ab
Prevoshodnaya	2002	4.8ab	3.8a	3.4cd	5.7bc	4.5a
Raisa	2002	4.9ab	3.2a	1.8a	7.3d	6.9c
Trofimovskava	2002	3.9a	3.1a	3.0bc	5.6ab	4.9ab
Chuiskaya	2002	5.0ab	4.0a	3.7a	4.6a	4.4ab
Avgustinka	2003	3.9E	2.8EF	2.3F	6.5GH	6.6G
Botanicheskaya	2003	4.3E	3.2FG	2.2F	7.2HI	6.5G
Oranzhevaya	2003	3.8E	3.9GHI	3.7G	5.9FG	5.3F
Prevoshodnaya	2003	4.4E	4.21	4.1G	5.6EF	5.3EF
Raisa	2003	4.4E	2.1E	1.3E	6.2FG	6.8G
Trofimovskaya	2003	4.1E	3.2FH	2.2F	7.11	6.3G
Chuiskaya	2003	4.3E	4.11	4.3G	5.2E	4.3E
sample ^a		*	***	***	***	***
vear ^a		**	ns	ns	**	***
sample × yea	ar ^a	ns	ns	***	***	ns

^a Significant differences between samples based on Tukey's HSD test or Mann–Whitney U-test are marked a–d in 2002 and E–I in 2003: ns, not significant; *, p < 0.05; **, p < 0.01; ***, p < 0.001.

in both years. The differences in the strength of odor and fruity flavor were either small or not significant. Chuiskaya, together with Prevoshodnaya, was described as having the most fruity flavor, and the odor was strongest in Prevoshodnaya and Raisa. Tang et al. (7) also found differences in sourness, astringency, and sweetness as well as in the pleasantness of sea buckthorn juices from different origins. Sweetness is known to correlate closely with the overall liking of soft fruit juice (14).

The biplot of PCA between all sensory attributes and samples is shown in **Figure 1**. The first two PCs explained 87% of the variance of the data. The closer the samples lay in the PCA plot, the more similar they are in view of sensory attributes. The first PC separates samples with fruity flavor and sweetness from samples with sourness and astringency, whereas the second PC seems to separate total odor from the flavor attributes. The second PC also seems to separate samples between years. The third PC explains 11% of the variance of data and also separates astringency and sourness as well as sweetness and fruity flavor. The differences between years were significant (p < 0.05) in the strength of odor, sourness, and astringency. In general, the odor was stronger in 2002, whereas sourness and astringency increased in 2003.

Chemical Analysis. The content of sugars and soluble solids and their standard deviations in samples in 2002 and 2003 are given in Table 3. Fructose (37.3-50.4% of total sugar) and glucose (49.5-62.1% of total sugar) are the main sugars in sea buckthorn juice (9). In our study, the amount of fructose and glucose varied from 0.2 to 3.8 g/100 mL and from 1.6 to 4.3 g/100 mL, respectively. This is in good accordance with the previous studies reported (2, 7, 15). The statistical differences (p < 0.05) between the samples are shown in **Table 6**. Botanicheskaya was the lowest in both fructose and glucose, and Chuiskaya was the highest in fructose, whereas Prevoshodnaya and Trofimovskaya were the highest in glucose. The range of total sugar (sum of fructose and glucose) was between 1.9 and 7.1 g/100 mL, Botanicheskaya being the lowest and Chuiskaya and Prevoshodnaya the highest. Jalakas et al. (16) reported that Trofimovskaya had higher levels of total sugar than Avgustinka or Botanicheskaya, which corresponds with our results. In the sugar fraction we also found some trace



Figure 1. Differences in PCA between sea buckthorn varieties based on their sensory attributes. AUG, Avgustinka; BOT, Botanicheskaya; ORA, Oranzhevaya; PRE, Prevoshodnaya; RAI, Raisa; TRO, Trofimovskaya; TSU, Chuiskaya.

Table 3. Sugar Main Values and Standard Deviations (SD) Analyzed by GC as TMS Derivatives, Soluble Solids (°Brix), and Sugar/Acid Ratio of Sea Buckthorn Varieties from 2002 and 2003

sample	year	fructose (g/100 mL \pm SD)	glucose (g/100 mL \pm SD)	total sugar ^a (g/100 mL \pm SD)	°Brix	sugar/acid
Avgustinka	2002	0.6±0.1	2.4 ± 0.1	3.1 ± 0.1	8.7 ± 0.1	0.8
0	2003	0.7 ± 0.1	2.2 ± 0.1	2.9 ± 0.1	8.5 ± 0.2	0.6
Botanicheskaya	2002	0.2 ± 0.1	2.3 ± 0.1	2.5 ± 0.1	7.4 ± 0.3	0.8
	2003	0.3 ± 0.1	1.6 ± 0.3	1.9 ± 0.4	7.5 ± 0.1	0.4
Oranzhevaya	2002	1.5 ± 0.1	2.1 ± 0.1	3.6 ± 0.2	8.1 ± 0.2	1.0
	2003	3.1 ± 0.4	2.9 ± 0.2	6.0 ± 0.5	10.9 ± 0.3	1.2
Prevoshodnaya	2002	1.6 ± 0.1	3.6 ± 0.1	5.2 ± 0.2	10.3 ± 0.3	1.5
	2003	3.0 ± 0.2	3.9 ± 0.2	6.9 ± 0.2	12.0 ± 0.2	1.7
Raisa	2002	1.7 ± 0.2	3.9 ± 0.3	5.6 ± 0.4	10.3 ± 0.4	1.7
	2003	0.8 ± 0.1	1.9 ± 0.2	2.6 ± 0.2	12.6 ± 0.1	0.5
Trofimovskaya	2002	0.7 ± 0.1	4.3 ± 0.2	4.9 ± 0.2	9.7 ± 0.2	1.5
	2003	1.0 ± 0.2	2.4 ± 0.3	3.4 ± 0.3	8.9 ± 0.3	0.7
Chuiskaya	2002	2.1 ± 0.2	2.5 ± 0.1	4.6 ± 0.2	9.1 ± 0.4	1.4
	2003	$\textbf{3.8}\pm\textbf{0.4}$	2.9 ± 0.5	7.1 ± 0.2	11.4 ± 0.1	1.9

^a Total sugar is the sum of fructose and glucose.

amounts of an unknown compound. It remained unidentified, because its chromatographic behavior did not resemble that of any common sugar tested. In particular, Raisa contained noticeable amounts of the compound. The existence of an unknown sugar has also been reported by Beveridge et al. (15). Soluble solids varied from 7.4 to 12.6 between samples and

years. The differences between years in fructose, glucose, total sugar, and soluble solids were significant (p < 0.05). The amount of fructose was higher in 2003 than in 2002 in all varieties but Raisa. The direction of changes in glucose as well as in total sugar and in soluble solids was variety dependent between the two years studied. The sugar/acid ratio varied from

Table 4. Mean Values and Standard Deviations (SD) of Organic Acids Analyzed by GC as TMS Derivatives, pH, Titrable Acidity, and Vitamin C in Sea Buckthorn Varieties in 2002 and 2003

sample	year	malic acid (g/100 mL \pm SD)	quinic acid (g/100 mL \pm SD)	total acid ^a (g/100 mL \pm SD)	рН	titrable acidity	vitamin C (mg/100 mL \pm SD)
Avgustinka	2002	2.8 ± 0.1	1.0 ± 0.2	3.7 ± 0.1	2.9 ± 0.1	2.8 ± 0.2	68 ± 5
	2003	3.6 ± 0.2	1.1 ± 0.2	4.8 ± 0.1	2.9 ± 0.1	3.3 ± 0.1	67 ± 5
Botanicheskaya	2002	1.8 ± 0.1	1.4 ± 0.2	3.1 ± 0.2	2.9 ± 0.1	2.1 ± 0.2	74 ± 5
	2003	3.5 ± 0.2	1.7 ± 0.2	5.1 ± 0.2	2.6 ± 0.1	3.7 ± 0.2	54 ± 1
Oranzhevaya	2002	2.5 ± 0.1	1.2 ± 0.1	3.7 ± 0.1	2.8 ± 0.1	2.9 ± 0.1	176 ± 8
,	2003	3.0 ± 0.3	1.8 ± 0.2	4.8 ± 0.3	2.8 ± 0.2	2.2 ± 0.1	128 ± 9
Prevoshodnaya	2002	1.6 ± 0.2	1.8 ± 0.1	3.4 ± 0.3	2.8 ± 0.1	2.1 ± 0.1	159 ± 5
,	2003	2.2 ± 0.2	1.8 ± 0.3	4.0 ± 0.5	2.8 ± 0.1	2.5 ± 0.1	87 ± 7
Raisa	2002	1.6 ± 0.1	1.6 ± 0.2	3.2 ± 0.3	2.9 ± 0.1	3.1 ± 0.2	46 ± 4
	2003	2.9 ± 0.2	2.6 ± 0.4	5.4 ± 0.3	2.7 ± 0.1	3.2 ± 0.1	29 ± 2
Trofimovskava	2002	2.1 ± 0.2	1.1 ± 0.2	3.2 ± 0.3	2.8 ± 0.1	2.0 ± 0.1	107 ± 8
	2003	3.5 ± 0.3	1.2 ± 0.1	4.7 ± 0.3	2.7 ± 0.2	3.4 ± 0.2	120 ± 4
Chuiskava	2002	1.7 ± 0.1	1.6 ± 0.2	3.3 ± 0.2	2.9 ± 0.1	2.0 ± 0.2	96 ± 3
J~	2003	2.0 ± 0.1	1.8 ± 0.2	3.8 ± 0.2	2.8 ± 0.1	2.2 ± 0.1	68 ± 6

^a Total acid is the sum of malic and quinic acids.

Table 5. Pulp Oil Content and Tristimulus Color Coordinates (Hunter *L*, a^* , and b^*) and Hue and Chroma Color Dimensions and Standard Deviations (SD) in Sea Buckthorn Juice in 2002 and 2003

sample	year	pulp oil (% w/w ± SD)	Hunter $L \pm SD$	Hunter $a^* \pm SD$	Hunter $b^* \pm SD$	hue deg \pm SD	$chroma \pm SD$
Avgustinka	2002	2.4 ± 0.3	52.6 ± 0.9	17.6 ± 0.8	42.5 ± 0.7	1.18 ± 0.01	46.0 ± 0.9
	2003	2.1 ± 0.3	40.5 ± 0.3	17.9 ± 0.5	38.9 ± 1.1	1.14 ± 0.01	42.8 ± 1.2
Botanicheskaya	2002	3.2 ± 0.2	55.8 ± 0.8	14.2 ± 0.8	41.4 ± 1.3	1.24 ± 0.01	2.5 ± 0.1
	2003	2.9 ± 0.5	44.4 ± 0.3	10.9 ± 0.1	39.9 ± 1.0	1.30 ± 0.01	1.9 ± 0.4
Oranzhevaya	2002	0.7 ± 0.1	41.0 ± 2.5	4.0 ± 2.7	22.8 ± 5.6	1.41 ± 0.08	3.6 ± 0.2
	2003	0.9 ± 0.1	33.6 ± 1.1	5.6 ± 1.4	26.5 ± 3.2	1.36 ± 0.03	6.0 ± 0.5
Prevoshodnaya	2002	1.3 ± 0.2	53.6 ± 0.9	11.9 ± 1.4	43.2 ± 2.7	1.30 ± 0.02	5.2±0.2
	2003	2.1 ± 0.2	40.0 ± 0.8	11.2 ± 0.6	37.3 ± 0.4	1.28 ± 0.02	6.9±0.2
Raisa	2002	3.3 ± 0.2	57.8 ± 3.2	17.3 ± 0.4	45.5 ± 1.5	1.21 ± 0.02	5.6 ± 0.4
	2003	2.9 ± 0.3	39.9 ± 0.1	15.9 ± 0.4	37.9 ± 0.7	1.17 ± 0.02	2.6±0.2
Trofimovskaya	2002	3.6 ± 0.3	55.4 ± 2.2	18.5 ± 1.0	45.7 ± 3.6	1.18 ± 0.01	4.9 ± 0.2
	2003	3.0 ± 0.1	42.9 ± 0.5	13.6 ± 0.5	40.6 ± 1.3	1.25 ± 0.01	3.4 ± 0.3
Chuiskaya	2002	1.7 ± 0.3	52.4 ± 2.7	13.4 ± 0.5	41.0 ± 1.7	1.25 ± 0.01	4.6 ± 0.2
	2003	2.9 ± 0.2	40.2 ± 0.4	12.7 ± 0.4	39.1 ± 1.0	1.26 ± 0.01	7.1 ± 0.2

Table 6.	Significant	Differences	(p	< 0.05)	in	Chemical	Data in	Sea	Buckthorn	Samples	s in	2002	and	2003	1
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	fructose		glucose		Σ	ΣS		malic acid		quinic acid		ΣΑ		°Brix		рН	
	2002	2003	2002	2003	2002	2003	2002	2003	2002	2003	2002	2003	2002	2003	2002	2003	
Avgustinka	bc	EFG	b	FG	b	EG	d	Н	а	Е	С	G	ab	Е	а	EF	
Botanicheskaya	а	E	ab	E	а	E	а	GH	ab	EFG	а	G	а	E	а	EF	
Oranzhevaya	d	FG	а	HI	abc	FGH	С	FG	ab	G	bc	G	а	G	а	EF	
Prevoshodnaya	cd	Н	С	J	d	Н	а	E	С	G	abc	EF	bc	G	а	EF	
Raisa	cd	EFG	С	EF	abd	EF	а	F	bc	Н	а	G	bc	G	а	Е	
Trofimovskaya	ab	F	d	GH	d	E	b	GH	а	EF	ab	F	С	EF	а	EF	
Chuiskaya	d	GH	b	Ι	cd	Н	а	Е	bc	FG	abc	E	abc	FG	а	F	
	titr		vitamin C		pulp oil %		color L		color a*		color b*		hue		chroma		
	2002	2003	2002	2003	2002	2003	2002	2003	2002	2003	2002	2003	2002	203	2002	2003	
Avgustinka	b	F	ab	EG	abd	EFG	ab	EFG	b	G	а	E	а	E	а	E	
Botanicheskaya	а	G	abc	GH	d	EFG	ab	Н	ab	E	а	Е	b	Н	а	Е	
Oranzhevaya	b	E	d	HI	ab	E	а	E	ab	EF	а	E	abc	EFGH	а	Е	
Prevoshodnaya	а	E	d	EGHIJ	ac	F	ab	FGH	ab	E	а	E	С	GH	а	E	
Raisa	b	F	а	EF	d	FG	ab	EFG	b	FG	а	E	ab	EF	а	E	
Trofimovskaya	а	FG	bc	J	d	G	b	FH	ab	EF	а	Е	а	FGH	а	Е	
Chuiskaya	а	Е	С	EG	bc	FG	ab	EGH	а	Е	а	Е	bc	FG	а	Е	

^a Significant differences between samples based on Tukey's HSD test, Tamhane's test, or Mann–Whitney U-test are marked as a–d in 2002 and E–J in 2003. ΣS, total sugar; ΣA, total acid; °Brix, soluble solids; titr, titrable acidity.

0.4 to 1.9 between varieties and years, being the lowest in Avgustinka and Botanicheskaya and the highest in Chuiskaya and Prevoshodnaya. In comparison, Jalakas et al. (*16*) reported low sugar/acid ratios for Avgustinka and Botanicheskaya and a higher ratio for Trofimovskaya.

Malic and quinic acids were the most abundant organic acids; small amounts of citric acid were also found as reported previously (2, 9). Organic acids, titrable acidity, and pH values of sea buckthorn varieties are shown in **Table 4**. The content of malic acid was between 1.6 and 3.6 g/100 mL and that of



Figure 2. PLS2 plot regarding the relationships between sensory properties and chemical variables. TA, titrable acidity; Fru, fructose; Glu, glucose; Tot.Sug, sum of fructose and glucose; Mal, malic acid; Quin, quinic acid; Tot.Acid, sum of malic and quinic acids; S:A, sugar/acid ratio; Vit.C, vitamin C.

Table 7. Pearson's Correlation Coefficients between Sensory Attributes and Chemical Parameters^a

	pН	soluble solids	titrable acidity	total sugar	total acid	sugar/acid	vitamin C	pulp oil
odor	0.13	-0.09	-0.29	-0.04	-0.47**	0.17	-0.22	0.16
fruity	0.22	0.01	0.58**	0.60**	0.35*	0.53**	0.41**	0.53**
sweetness	0.39*	0.23	-0.64**	0.69**	-0.30	0.59**	0.38*	-0.41**
sourness	-0.48**	-0.20	0.77**	-0.39*	0.41**	-0.43**	-0.23	0.28
astringency	-0.39*	-0.07	0.76**	-0.46**	0.49**	-0.49**	-0.47**	0.29

^{*a*}*, *p* < 0.05; **, *p* < 0.01.

quinic acid between 1.0 and 2.6 g/100 mL, depending on variety and year. Significant differences (p < 0.05) between samples were found (**Table 6**). Avgustinka was the highest in malic acid and Prevoshodnaya and Chuiskaya were the lowest, but quinic acid was the lowest in Avgustinka and the highest in Prevoshodnaya and Raisa. Even though the amount of acids was higher in 2003, which was noted as an increase in titrable acidity (2.0–3.7 g/100 mL between samples and years), the pH values altered only slightly between years. Trofimovskaya has been reported to have lower titrable acidity than Avgustinka or Botanicheskaya (*16*).

Soluble solids is a commonly used variable to describe the sugar content in fruit and berries. Kallio et al. (10) reported correlation (0.90) between soluble solids and total sugar in strawberry. We found the Pearson's correlation of 0.635 (p < 0.01) between soluble solids and total sugar, whereas the Pearson's correlation between soluble solids and total sugar and total acid together was 0.762 (p < 0.01) in sea buckthorn. This indicates that, in sea buckthorn, soluble solids is not good for the estimation of sugars but there are also other dissolved components affecting the °Brix value.

The vitamin C content of varieties is summarized in **Table 4**, and differences (p < 0.05) between samples are given in **Table 6**. Sea buckthorn juice is high in vitamin C, but there is a great variation depending on the stage of ripeness (*18, 19*) and the origin of the subspecies (*1, 17*). In sea buckthorn juice, vitamin C varied from 29 to 176 mg/100 mL depending on the variety and the year. Oranzhevaya and Prevoshodnaya were the highest in vitamin C, but the Finnish agricultural product Raisa was noticeably lower than the other varieties. The vitamin C content was significantly (p < 0.05) lower in 2003 than in 2002. Previous studies have reported typical variation of 30–500 mg/

100 g of ascorbic acid content in sea buckthorn, depending on the origin (1, 17, 18), stage of ripeness (1, 17-19), and variety (16, 19-21).

The pulp oil content was between 0.7 and 3.6%, varying between varieties and years (**Tables 5** and **6**). Trofimovskaya had the highest amounts of oil, whereas Oranzhevaya had the least oil. There was some variation between years, but no clear evidence of the direction of change. Ma et al. (22) reported pulp oil content of 2.0-2.4% in Chinese sea buckthorn, which is in good accordance with our results.

The mean values and standard deviations for tristimulus color coordinates (Hunter *L*, a^* , and b^*) and hue and chroma color dimensions are reported in **Table 5**, and the differences (p < 0.05) are marked in **Table 6**. The lightness (*L* value) differed between samples, and there was a significant (p < 0.001) decrease from the year 2002 to 2003. Redness (a^* value) was the highest on Avgustinka and Raisa, but there were no differences in yellowness (b^* value). The ratio of yellowness and redness (hue degree) was the highest on Oranzhevaya and Avgustinka. Chroma, which expresses the colorfulness (related to the brightness of white), was not different between samples or years.

Relationship between Sensory and Chemical Data. The PLS method was used to relate the sensory and chemical data matrices (23). The predicted *Y* values (sensory properties, n = 5) were computed by applying the model equation to the observed *X* variables (chemical data, n = 18). The model was applied on all sensory properties and all chemical variables (PLS2) with all samples (n = 7) (**Figure 2**). Sixty-five percent of chemical variables explained 56% of the sensory properties with the first two components.

Poll (24) suggested that the sugar/acid ratio was an important factor in determining the sweetness or sourness of apple juice. Tang et al. (7) also reported that the sugar/acid ratio responded better than the total sugar with sweetness. We also noted that the total sugar as well as the sugar/acid ratio (Table 7) can be used to explain sweetness in sea buckthorn. Sweetness also correlated negatively with titrable acidity and, to some extent, with the pulp oil content. The fruity flavor in sea buckthorn has a positive correlation with both total sugar and sugar/acid ratio as well as with titrable acidity. The positive correlation between fruity flavor and pulp oil content may be interesting for future studies on sea buckthorn flavor. Both sourness and astringency were found to correlate positively with titrable acidity and also with the total acid but negatively with the total sugar and with the sugar/acid ratio. Astringency is the tactile stimulus sensed as dryness, puckering, and rough mouth feel (25, 26). It is an important sensory character in many fruit juices as well as in wine. It is caused by the presence of acids and phenolic compounds (26, 27). Organic acids have been reported to increase not only sourness but also astringency (27, 28), whereas the addition of sucrose has been found to lower astringency (29). We also found significant (p < 0.01) correlation (Pearson's correlation = 0.774) between sourness and astringency, whereas the total sugar correlated negatively with astringency (Table 7). Astringency elicited by acids has been reported to be a function of pH rather than the concentration of acid (30, 31). No effect of quinic acid on astringency has been found in cranberry juice (31).

Our study shows that there are obvious differences among sea buckthorn varieties in the sensory quality and the chemical composition. The chemical composition of sea buckthorn can be used to explain to some extent the sensory quality of the berry. The berry flavor is generally described as sour and astringent, which is caused by the high levels of organic acids in berries. The sweetness, as well as the sugar/acid ratio in sea buckthorn, is low compared to that of other berries. This may also reduce the overall liking of the berry among consumers. However, the great differences between varieties show the potential for selecting the raw material for different purposes, such as table dessert berries or processing for juice, jams, or jellies.

ABBREVIATIONS USED

AA, ascorbic acid; ANOVA, analysis of variance; C^* , chroma; DTT, dithiotreitol; GC, gas chromatography; H° , hue angle; HPLC, high-performance liquid chromatography; ssp., subspecies; M, molarity; MPa, megapascal; PC, principal component; PCA, principal component analysis; PLS, partial least-squares regression; TMS, trimethylsilyl; v/v, volume/ volume.

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LITERATURE CITED

 Kallio, H.; Yang, B. R.; Peippo, P. Effects of different origins and harvesting time on vitamin C, tocopherols, and tocotrienols in sea buckthorn (*Hippophaë rhamnoides*) berries. J. Agric. Food Chem. 2002, 50, 6136–6142.

- (2) Kallio, H.; Yang, B.; Tahvonen, R.; Hakala, M. Composition of sea buckthorn berries of various origins. *Proceedings of International Workshop on Sea Buckthorn IWS-99*, Beijing, China, Aug 29–Sept 2, 1999; International Centre for Research and Training on Sea Buckthorn: Beijing, China, 1999; pp 17–23.
- (3) Rösch, D.; Bergman, 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.
- (4) Yang, B. Lipophilic components of sea buckthorn (*Hippophaë rhamnoides*) seeds and berries. Ph.D. Thesis, Department of Biochemistry and Food Chemistry, University of Turku, Turku, Finland, 2001.
- (5) Yang, B.; Kallio, H. Effects of harvesting time on triacylglycerols and glycerophospholipids of sea buckthorn (*Hippophaë rham-noides* L.) berries of different origins. J. Food Compos. Anal. 2002, 15, 143–157.
- (6) Yang, B.; Kallio, H.; Kalimo, K.; Mattila, L.; Kallio, S.; Tahvonen, R.; Katajisto, J. Effect of dietary supplementation of sea buckthorn (*Hippophaë rhamnoides*) oils on fatty acid composition of skin glycerophospholipids of patients with atopic dermatitis. J. Nutr. Biochem. 2000, 11, 339–340.
- (7) Tang, X.; Kälviäinen, N.; Tuorila, H. Sensory and Hedonic Characteristics of Juice of Sea Buckthorn (*Hippophaë rham-noides* L.) Origins and Hybrids. *Lebensm. Wiss. Technol.* 2001, 34, 102–110.
- (8) Beveridge, T. H. J.; Cliff, M.; Sigmund, P. Supercritical carbon dioxide percolation of sea buckthorn press juice. *J. Food Qual.* 2003, 27, 41–54.
- (9) Beveridge, T.; Li, T.; Oomah, B.; Smith, A. Sea Bucthorn Products: Manufacture and Composition. J. Agric. Food Chem. 1999, 47, 3480–3488.
- (10) Kallio, H.; Hakala, M.; Pelkkikangas, A.-M.; Lapveteläinen, A. Sugars and acids of strawberry varieties. *Eur. Food Res. Technol.* 2000, 212, 81–85.
- (11) Gökmen, V.; Acar, J. A simple HPLC method for the determination of total vitamin C in fruit juices and drinks. *Fruit Processing* **1996**, *5*, 198–201.
- (12) Christie, W. W. Fatty acids and lipids: Structures, extraction and fractionation into classes. In *Gas Chromatography and Lipids*; Christie, W., Ed.; The Oily Press: Glasgow, U.K., 1989.
- (13) Martens, H.; Martens, M. Modified Jack-knife estimation of parameters uncertainty in bilinear modeling by partial least squares regression (PLSR). *Food Qual. Pref.* 2000, 11, 5–16.
- (14) Tuorila-Ollikainen, H.; Mahlamäki-Kultanen, S.; Kurkela, L. Relative importance of color, fruity flavor and sweetness in overall liking of soft drinks. *J. Food Sci.* **1984**, *49*, 1598–1603.
- (15) Beveridge, T.; Harrison, J.; Drover, J. Processing effects on the composition of sea buckthorn juice from *Hippophaë rhamnoides* L. Cv. Indian Summer. *J. Agric. Food Chem.* 2002, *50*, 113–116.
- (16) Jalakas, M.; Kelt, K.; Karp, K. The yield and fruit quality of sea buckthorn (*Hippophaë rhamnoides* L.) after rejuvenation cutting. *Agron. Res.* **2003**, *1*, 31–36.
- (17) Yao, Y. M.; Tigerstedt, P. M. A.; Joy, P. Variation of Vitamin-C Concentration and Character Correlation between and within Natural Sea Buckthorn (*Hippophae rhamnoides* L) Populations. *Acta Agric. Scand. Sect. B: Soil Plant Sci.* **1992**, *42*, 12–17.
- (18) Rousi, A.; Aulin, H. Ascorbic acid content in relation to ripeness in fruits of six *Hippophaë rhamnoides* clones from Pyhäranta, SW Finland. *Ann. Agr. Fenn.* **1977**, *16*, 80–87.
- (19) Jeppsson, N.; Gao, X. Q. Changes in the contents of kaempherol, quercetin and L-ascorbic acid in sea buckthorn berries during maturation. *Agric. Food Sci. Finland* **2000**, *9*, 17–22.
- (20) Gao, X.; 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.

- (22) Ma, Z.; Cui, Y.; Feng, G. Studies on the fruit character and biochemical compositions of some forms within Chinese sea buckthon (*Hippophaë rhamnoides* ssp. *sinensis*) in Shanxi, China. *Proceedings of International Symposium on Sea Buckthorn*, Xian, China, Oct 19–23, 1989; Secretariat of International Symposium on Sea Buckthorn: Xian, China, 1989; pp 106–113.
- (23) Wold, S.; Sjöström, M.; Eriksson, L. Partial Least Squares Projections to Latent Structures (PLS) in Chemistry. In *Encyclopedia of Computational Chemistry*; Wiley: New York, 1999; pp 2006–2022.
- (24) Poll, L. Evaluation of 18 Apple Varieties for their Suitability for Juice Production. J. Sci. Food Agric. 1981, 32, 1081–1090.
- (25) Ishikawa, T.; Noble, A. C. Temporal Perception of Astringency and Sweetness in Red Wine. *Food Qual. Pref.* **1995**, *6*, 27–33.
- (26) Noble, A. C. Astringency and bitterness of flavonoid phenols. *Chemistry of Taste: Mechanisms, Behaviors, and Mimics*; ACS Symposium Series 825; American Chemical Society: Washington, DC, 2002; pp 192–201.

- (27) Peleg, H.; Bodine, K. K.; Noble, A. C. The influence of acid on astringency of alum and phenolic compounds. *Chem. Senses* **1998**, *23*, 371–378.
- (28) Thomas, C. J. C.; Lawless, H. T. Astringent subqualities in Acids. *Chem. Senses* 1995, 20, 593–600.
- (29) Courregelongue, S.; Schlich, P.; Noble, A. C. Using repeated ingestion to determine the effect of sweetness, viscosity and oiliness on temporal perception of soymilk astringency. *Food Qual Pref.* **1999**, *10*, 273–279.
- (30) Sowalsky, R. A.; Noble, A. C. Comparison of the effects of concentration, pH and anion species on astringency and sourness of organic acids. *Chem. Senses* **1998**, *23*, 343–349.
- (31) Peleg, H.; Noble, A. C. Effect of viscosity, temperature and pH on astringency in cranberry juice. *Food Qual. Pref.* **1999**, *10*, 343–347.

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