

# Effects of Genotype, Latitude, and Weather Conditions on the Composition of Sugars, Sugar Alcohols, Fruit Acids, and Ascorbic Acid in Sea Buckthorn (*Hippophaë rhamnoides* ssp. *mongolica*) Berry Juice

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**S** Supporting Information

**ABSTRACT:** Sea buckthorn berries (*Hippophaë rhamnoides* ssp. *mongolica*) of nine varieties were collected from three growth locations in five inconsecutive years ( $n = 152$ ) to study the compositional differences of sugars, sugar alcohols, fruit acids, and ascorbic acid in berries of different genotypes. Fructose and glucose (major sugars) were highest in Chuiskaya and Vitaminaya among the varieties studied, respectively. Malic acid and quinic acid (major acids) were highest in Pertsik and Vitaminaya, respectively. Ascorbic acid was highest in Oranzhevaya and lowest in Vitaminaya. Berry samples of nine varieties collected from two growth locations in five years ( $n = 124$ ) were combined to study the effects of latitude and weather conditions on the composition of *H. rhamnoides* ssp. *mongolica*. Sea buckthorn berries grown at lower latitude had higher levels of total sugar and sugar/acid ratio and a lower level of total acid and were supposed to have better sensory properties than those grown at higher latitude. Glucose, quinic acid, and ascorbic acid were hardly influenced by weather conditions. The other components showed various correlations with temperature, radiation, precipitation, and humidity variables. In addition, fructose, sucrose, and *myo*-inositol correlated positively with each other and showed negative correlation with malic acid on the basis of all the samples studied ( $n = 152$ ).

**KEYWORDS:** sea buckthorn, *Hippophaë rhamnoides* ssp. *mongolica*, sugar, sugar alcohol, vitamin C, fruit acid, genotype, weather condition

## INTRODUCTION

Sea buckthorn (*Hippophaë rhamnoides* L.) berries are of high nutritive value and are widely consumed as food and used as raw materials for food and food supplements in Europe and Asia.<sup>1</sup> The contents of sugars and acids and the sugar/acid ratio are important factors affecting the flavor and consumer acceptance of the berry products.<sup>2,3</sup> Sea buckthorn berries are characterized as quite acidic and not very sweet in taste due to the abundance of the fruit acids and low levels of sugars compared to those of other edible fruits and berries.<sup>2–6</sup> Moreover, the berries have a typical bitter and astringent taste.

Fructose and glucose are the major sugars contributing to the sweetness of the berries. The major acid, malic acid, causes a sour and bitter taste.<sup>3,5</sup> These sensory components were thoroughly investigated and were reported to be affected by the genotype, harvesting time, and origin of sea buckthorn.<sup>5–9</sup> However, until now, little has been known about the effects of weather conditions on these components in sea buckthorn berries. The accumulation of sugars and acids in plants is a result of the complex metabolism of carbohydrates and is widely influenced by various weather conditions, such as temperature, light, and water supply.<sup>10</sup> Knowledge of the effects of weather conditions on the composition of sensory components is of great importance in view of berry breeding, cultivation, and raw material selection for industrial application.

Inositols and methylinositols, known as cyclitols, were recently identified in sea buckthorn berries by our research group.<sup>6,11,12</sup> Various beneficial effects of cyclitols on human health have been reported.<sup>13–16</sup> Cyclitols are cycloalkanes containing one hydroxyl group on three or more ring atoms and are normally formed in a plant as one of the compatible solutes in response to salt or water stress.<sup>10,17,18</sup> Clear influences of genotype, growth location, harvesting time, and weather conditions on the contents of inositols and methylinositols were detected.<sup>9,12</sup> Yang et al.<sup>12</sup> investigated the correlation between the contents of inositols and methylinositols in wild Chinese sea buckthorn (*H. rhamnoides* ssp. *sinensis*) berries and the parameters of temperature and precipitation. However, the influence of radiation and air humidity on the contents of inositols and methylinositols was not investigated in the study due to the lack of the corresponding weather data.

In the present study, nine varieties of sea buckthorn berries of *H. rhamnoides* ssp. *mongolica* were collected from three growth places in Finland and Canada in five inconsecutive

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Table 1. Harvesting Information of Sea Buckthorn (*H. rhamnoides* ssp. *mongolica*) Samples

variety	growth site	longitude	latitude	altitude (m)	harvest date
Avgustinka	Sammalmäki, Finland	22°09' E	60°23' N	1	Sept 5, 2003
	Kittilä, Finland	24°37' E	68°02' N	210	Sept 21, 2003
Botanicheskaya	Sammalmäki, Finland	22°09' E	60°23' N	1	Sept 5, 2003; Aug 28, 2008; Aug 26, 2010
	Kittilä, Finland	24°37' E	68°02' N	210	Sept 22, 2003
Trofimovskaya	Sammalmäki, Finland	22°09' E	60°23' N	1	Sept 5, 2003; Aug 28, 2008; Aug 31, 2009
	Kittilä, Finland	24°37' E	68°02' N	210	Sept 21, 2003
Pertsik	Sammalmäki, Finland	22°09' E	60°23' N	1	Sept 9, 2003
	Kittilä, Finland	24°37' E	68°02' N	210	Sept 22, 2003
Prevoshodnaya	Kittilä, Finland	24°37' E	68°02' N	210	Sept 8, 2003
	Québec, Canada	71°17' W	46°47' N	100	Aug 28, 2007; Sept 3, 2008; Aug 24, 2009; Aug 16, 2010
Prozharachnaya	Sammalmäki, Finland	22°09' E	60°23' N	1	Sept 4, 2003; Aug 28, 2008
	Kittilä, Finland	24°37' E	68°02' N	210	Sept 22, 2003
	Québec, Canada	71°17' W	46°47' N	100	Aug 28, 2007; Sept 3, 2008; Aug 24, 2009; Aug 23, 2010
Chuisakaya	Québec, Canada	71°17' W	46°47' N	100	Aug 28, 2007; Sept 3, 2008; Aug 24, 2009; Aug 16, 2010
Oranzhevaya	Québec, Canada	71°17' W	46°47' N	100	Aug 28, 2007; Sept 3, 2008; Aug 24, 2009; Aug 23, 2010
Vitaminaya	Québec, Canada	71°17' W	46°47' N	100	Aug 28, 2007; Sept 3, 2008; Aug 24, 2009; Aug 23, 2010

years. The collection of sea buckthorn samples of six varieties in Kittilä, northern Finland, was only available in 2003 probably because of the intolerance of the bushes to the extreme environmental stress in the far north after being transported from Sammalmäki, southern Finland, to Kittilä, northern Finland. The difference in compositional parameters between samples of different genetic backgrounds and the correlations between metabolites were studied on the basis of samples ( $n = 152$ ) collected from all three growth sites studied. However, the effects of latitude and weather conditions on the composition of sugars, sugar alcohols, fruit acids, and ascorbic acid were studied only in the samples ( $n = 124$ ) collected from Québec, Canada, and Sammalmäki, Finland. The sea buckthorn samples collected from Kittilä, Finland, in 2003 were excluded from the analysis because of the possible occurrence of undesirable influences caused by the transportation process of the bushes on the composition of sea buckthorn berries.

## MATERIALS AND METHODS

**Samples.** Sea buckthorn bushes of varieties Prevoshodnaya, Prozharachnaya, Chuisakaya, Oranzhevaya, and Vitaminaya were received bare root and planted in Québec, Canada (longitude 71°17' W, latitude 46°47' N, altitude 100 m) in June 2004. Sea buckthorn bushes of varieties Avgustinka, Botanicheskaya, Trofimovskaya, Pertsik, Prevoshodnaya, and Prozharachnaya were planted in Sammalmäki, Finland (22°09' E, 60°23' N, 1 m) in 1998–1999. To study the influence of growth sites and weather conditions on the composition of sea buckthorn berries, our research group transported some bushes of these six varieties from Sammalmäki, Finland, to Kittilä, Finland (24°37' E, 68°02' N, 210 m) in 2003. Whole bushes were dug out after pollination and transported with the roots and soil to minimize the stress to the plants. However, these bushes survived and produced berries only in 2003. This suggested that these six varieties were not good choices for sea buckthorn cultivation in such high latitude probably because of their intolerance to the extreme environmental stresses in the far north. During the study, no fertilization was applied on any of the bushes in these three growth locations. The harvesting information of sea buckthorn (*H. rhamnoides* ssp. *mongolica*) berries of the nine varieties from different growth locations and harvesting years in Finland and Canada is listed in Table 1. The berries were picked as soon as they were optimally ripe as defined by experienced horticulturists on the basis of the color, flavor, and structure of the berries. Due to the natural variation in these parameters in sea buckthorn, the optimal ripeness of the berries was determined by the local sea buckthorn experts, who have been investigating sea buckthorn for years and collected the berry samples for the study.

The berries from different growth sites were picked at the same stage of ripeness. The berries were loosely frozen immediately after picking and kept at  $-20\text{ }^{\circ}\text{C}$  before being analyzed within one year after the collection. An independent-sample  $t$  test showed that there was no significant difference ( $p > 0.05$ ) between the sea buckthorn samples analyzed after harvesting and the samples stored at  $-20\text{ }^{\circ}\text{C}$  for one year. Therefore, the compounds in sea buckthorn juice studied are stable at  $-20\text{ }^{\circ}\text{C}$  for one year.

**Reagents.** Reference compounds D-fructose, quinic acid, and ascorbic acid were purchased from Sigma Chemical Co. (St. Louis, MO). D-Glucose, myo-inositol, and the internal standard D-sorbitol were purchased from Fluka (Buchs, Switzerland). Malic acid and the internal standard tartaric acid were purchased from Merck (Darmstadt, Germany). Sucrose and citric acid were purchased from J. T. Baker (Deventer, Holland). L-Quebrachitol (1L-2-O-methyl-chiro-inositol) was purchased from Alexis Corp. (Läufelfingen, Switzerland).

**Analysis of Sugars, Sugar Alcohols, Fruit Acids, and Ascorbic Acid in Berry Juice.** Trimethylsilyl (TMS) derivatives of sugars, sugar alcohols, fruit acids, and ascorbic acid of sea buckthorn berry juice were prepared in quadruplicate with a method applied in our laboratory previously.<sup>9</sup> This analytical method, using sorbitol (for sugars and sugar alcohols) and tartaric acid (for acids and ascorbic acid) as internal standards, was developed by Tiitinen et al.<sup>5</sup> and showed good repeatability for quantification of sugars, sugar alcohols, fruit acids, and ascorbic acid in sea buckthorn berry juice. About 7 g of berries were weighed accurately in duplicate, thawed at room temperature for 15 min, and pressed manually 30 times with a potato masher. The slurry was centrifuged at 4360g for 10 min. The juice was separated, and the volume was determined. The TMS derivatives of the juice samples were then prepared in duplicate as described earlier.<sup>9</sup> The pH of the juice was determined with an Inolab pH level 1 meter (Wissenschaftlich Technische Werkstätten, Weilheim, Germany) and the content of soluble solids with a refractometer (0–32 °Brix, Atago, Tokyo, Japan). TMS derivatives of the dried juice samples were analyzed with a Hewlett-Packard 5890 series II gas chromatograph (Hewlett-Packard Co., Palo Alto, CA) equipped with a flame ionization detector (FID) and a Hewlett-Packard 7673 autosampler.<sup>9</sup> The analyses were carried out with a methyl silicone Supelco Simplicity-1 fused silica column (30 m × 0.25 mm i.d. × 0.25 μm  $d_f$ ) (Bellefonte, PA). A sample of 1 μL was injected automatically via a split/splitless injector. The internal standards sorbitol and tartaric acid were used for quantification. Ethyl β-D-glucopyranoside (henceforth ethyl glucose) was quantified as glucose and methyl-myoinositol as L-quebrachitol. The total sugar content was defined as the sum of sugars, sugar derivatives, and sugar alcohols.

**Information on Weather Conditions.** The data on weather conditions in Finland were provided by the Finnish Meteorological Institute (Helsinki, Finland). The data were recorded at the weather

Table 2. Weather Variables Investigated and Their Abbreviations

abbrev	weather variable	abbrev	weather variable
Dgs	growing season period (days)	Pjan, Pfeb, ..., Paug, Psep	precipitation in January, February, ..., August, September (mm)
SUMTgs	temp sum over 5 °C in growing season (°C)	Hgh	av humidity from start of growing season until day of harvest (%)
SUMTgh	temp sum over 5 °C from start of growing season until day of harvest (°C)	Hm	av humidity in last month before harvest (%)
SUMTm	temp sum over 5 °C in last month before harvest (°C)	Hw	av humidity in last week before harvest (%)
HDgh	no. of hot days (temp >25 °C) from start of growing season until day of harvest (days)	Hjan, Hfeb, ..., Haug, Hsep	av humidity in January, February, ..., August, September (%)
HDm	no. of hot days (temp >25 °C) in last month before harvest (days)	DH20to30gh	percentage of days with RH <sup>a</sup> = 20–30% from start of growing season until day of harvest (%)
Tm	av temp in last month before harvest (°C)	DH30to40gh	percentage of days with RH = 30–40% from start of growing season until day of harvest (%)
Tw	av temp in last week before harvest (°C)	DH40to50gh	percentage of days with RH = 40–50% from start of growing season until day of harvest (%)
TDm	mean daily temp difference in last month before harvest (°C)	DH50to60gh	percentage of days with RH = 50–60% from start of growing season until day of harvest (%)
LTm	av daily lowest temperature in last month before harvest (°C)	DH60to70gh	percentage of days with RH = 60–70% from start of growing season until day of harvest (%)
HTm	av daily highest temp in last month before harvest (°C)	DH70to80gh	percentage of days with RH = 70–80% from start of growing season until day of harvest (%)
Tjan, Tfeb, ..., Taug, Tsep	av temp in January, February, ..., August, September (°C)	DH80to90gh	percentage of days with RH = 80–90% from start of growing season until day of harvest (%)
Rgh	radiation from start of growing season until day of harvest (kJ/m <sup>2</sup> )	DH90to100gh	percentage of days with RH = 90–100% from start of growing season until day of harvest (%)
Rm	radiation during last month before harvest (kJ/m <sup>2</sup> )	DH50to60m	percentage of days with RH = 50–60% in last month before harvest (%)
Rw	radiation during last week before harvest (kJ/m <sup>2</sup> )	DH60to70m	percentage of days with RH = 60–70% in last month before harvest (%)
Rjan, Rfeb, ..., Raug, Rsep	radiation in January, February, ..., August, September (kJ/m <sup>2</sup> )	DH70to80m	percentage of days with RH = 70–80% in last month before harvest (%)
Pgh	precipitation from start of growing season until day of harvest (mm)	DH80to90m	percentage of days with RH = 80–90% in last month before harvest (%)
Pm	precipitation in last month before harvest (mm)	DH90to100m	percentage of days with RH = 90–100% in last month before harvest (%)
Pw	precipitation in last week before harvest (mm)		

<sup>a</sup>Relative humidity.

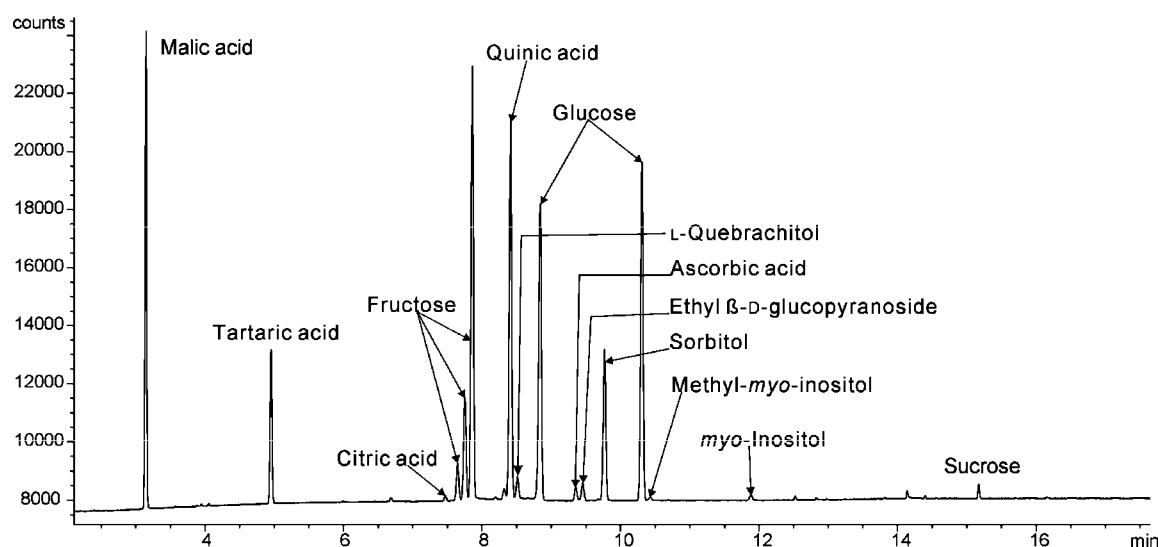


Figure 1. GC-FID chromatogram of sea buckthorn berries of variety Chuisakaya collected from Canada in 2008.

stations in Yltöinen Kaarina (longitude 22°33' E, latitude 60°23' N, altitude 6 m) and Artukainen Turku (22°10' E, 60°27' N, 8 m) in Finland for all the investigating years. The data recorded at the weather stations of the Jean Lesage International Airport (71°23' W, 46°47' N, 74 m) and Université Laval (71°17' W, 46°47' N, 100 m) in 2007–2010 were obtained from Environment Canada (Gatineau,

Québec, Canada) and the Centre de Recherche en Horticulture, Pavillon de l'Environnement, Université Laval (Hochelaga, Québec, Canada), respectively. The weather variables and their abbreviations used in the study are shown in Table 2.

**Statistical Analysis.** Statistical analyses were carried out with SPSS 16.0.1 (SPSS, Inc., Chicago, IL). Differences in the composition

between samples ( $n = 152$ ) of different varieties were analyzed with a one-way analysis of variance (ANOVA). The Student–Newman–Keuls (SNK) test for population with equal variances and Games–Howell test for population with unequal variances were performed for multiple comparisons. Differences reaching a confidence level of 95% were considered as being statistically significant. An independent-sample  $t$  test was used to investigate the difference between sea buckthorn berries grown in Sammalmäki, Finland, and Québec, Canada. Due to the transplanting process of the sea buckthorn bushes that happened in 2003 and the death of the bushes afterward, the samples collected from Kittilä, Finland, were not included in the latitudinal comparison of sea buckthorn berries. Pearson's correlation coefficients analysis and linear regression analysis were applied on sea buckthorn berries ( $n = 124$ ) collected from Sammalmäki, Finland, and Québec, Canada, to study the influence of weather conditions on the berry composition. Considering the transplanting practice of the bushes, the composition of sea buckthorn berries collected in Kittilä, Finland, in 2003 might have been influenced not only by the weather conditions but also by the stress caused by root cutting and the transportation process. In addition, the bushes were pollinated in Sammalmäki and fruits were produced in Kittilä in 2003, so the influences of weather conditions on the composition of the berries were complex effects of a combination of weather conditions in the two growth sites. Therefore, the sea buckthorn samples collected in Kittilä, Finland, in 2003 were excluded from Pearson's correlation coefficient analysis and linear regression analysis of the influence of weather conditions on the berry composition. Pearson's correlation coefficient analysis and linear regression analysis were applied on sea buckthorn samples ( $n = 152$ ) collected from all the growth sites (Sammalmäki and Kittilä, Finland, and Québec, Canada) in the investigating years to study the intercorrelations between the metabolites in sea buckthorn berries.

## RESULTS AND DISCUSSION

**Compositional Profile of Berry Juice.** Figure 1 presents the GC-FID chromatogram of the TMS-derivatized sea buckthorn juice. The contents of sugars and acids and the values of quality parameters of sea buckthorn berries of different varieties are shown in Tables 3 and 4. Glucose (62.5–74.8% of total sugar) was the most abundant sugar and fructose (11.7–30.7% of total sugar) the second most abundant sugar in all the varieties investigated except Chuiskaya. In the sea buckthorn berries of variety Chuiskaya, the contribution of fructose (49.9%) to total sugar was slightly higher than that of glucose (44.4%). Sucrose was absent in Avgustinka, Botanicheskaya, Trofimovskaya, Pertsik, and Oranzhevaya but present in Prevoshodnaya, Prozcharachnaya, Chuiskaya, and Vitaminaya at a level of 0.01–0.06 g/100 mL of juice. Ethyl glucose contributed 0.2–5.9% to the total sugar content in the berry juice.

L-Quebrachitol was a major sugar alcohol (2.9–8.4% of total sugar) in all the varieties of *H. rhamnoides* ssp. *mongolica* studied. It was reported as the major sugar alcohol in Chinese sea buckthorn (*H. rhamnoides* spp. *sinensis*) in our previous study as well.<sup>9</sup> The contents of methyl-myoinositol and myoinositol varied from 0.01 to 0.03 and from 0.01 to 0.04 g/100 mL of juice, respectively, in different varieties. Kallio et al.<sup>11</sup> reported a trace level of *chiro*-inositol in berries of wild Chinese sea buckthorn. However, no detectable *chiro*-inositol was found in any of the samples in this study.

Malic acid and quinic acid were the two major fruit acids in the sea buckthorn juice. For most of the varieties studied, malic acid (48.8–68.7% of total acid) appeared as the most abundant acid. However, in berries of the varieties Vitaminaya and Chuiskaya, quinic acid (69.2% and 50.2%, respectively, in the two varieties) was the most abundant acid, followed by malic

Table 3. Contents (g/100 mL) of Sugars, Sugar Alcohols, Fruit Acids, and Ascorbic Acid in Sea Buckthorn Berry Juice<sup>a</sup>

variety/ location	fructose	glucose	ethyl glucose	L- quebrachitol	methyl-myoinositol	myoinositol	sucrose	malic acid	citric acid	quinic acid	ascorbic acid	total sugar	total acid
Avgustinka	0.41 ± 0.13 ab	2.09 ± 0.19 a	0.12 ± 0.09 abcd	0.24 ± 0.09 a	0.02 ± 0.00 c	0.01 ± 0.01 abc	nd a	3.20 ± 0.37 de	0.05 ± 0.02 abc	1.30 ± 0.06 ab	0.11 ± 0.05 abc	2.89 ± 0.32 a	4.66 ± 0.26 d
Botanicheskaya	0.34 ± 0.13 a	2.14 ± 0.43 ab	0.14 ± 0.05 d	0.20 ± 0.05 a	0.03 ± 0.01 d	0.01 ± 0.01 b	nd a	2.89 ± 0.31 d	0.02 ± 0.01 ab	1.65 ± 0.23 c	0.09 ± 0.04 bc	2.86 ± 0.50 a	4.66 ± 0.32 d
Trofimovskaya	0.76 ± 0.17 c	2.90 ± 0.42 c	0.10 ± 0.05 d	0.23 ± 0.11 a	0.01 ± 0.01 abc	0.01 ± 0.00 a	nd a	2.82 ± 0.14 d	0.03 ± 0.02 ab	1.23 ± 0.16 a	0.14 ± 0.05 c	4.02 ± 0.64 b	4.22 ± 0.25 c
Pertsik	0.70 ± 0.36 abcd	3.13 ± 0.69 bcd	0.12 ± 0.05 cd	0.24 ± 0.09 a	0.03 ± 0.00 cd	0.01 ± 0.00 a	nd a	3.28 ± 0.05 e	0.03 ± 0.00 b	1.55 ± 0.40 abcd	0.13 ± 0.03 c	4.23 ± 1.01 abc	4.99 ± 0.42 d
Prevoshodnaya	1.44 ± 0.52 e	2.93 ± 0.35 c	0.05 ± 0.02 c	0.20 ± 0.10 a	0.02 ± 0.01 bc	0.02 ± 0.01 bc	0.03 ± 0.02 b	1.57 ± 0.88 bc	0.03 ± 0.01 b	1.51 ± 0.27 bc	0.10 ± 0.07 abc	4.68 ± 0.73 bc	3.22 ± 0.99 ab
Prozcharachnaya	0.46 ± 0.09 b	2.05 ± 0.51 a	0.17 ± 0.14 d	0.17 ± 0.06 a	0.02 ± 0.01 cd	0.01 ± 0.00 a	0.01 ± 0.01 a	2.90 ± 0.29 d	0.04 ± 0.01 b	1.39 ± 0.27 ab	0.08 ± 0.03 b	2.89 ± 0.67 a	4.40 ± 0.50 cd
Chuiskaya	2.62 ± 0.25 f	2.33 ± 0.43 ab	0.05 ± 0.02 c	0.15 ± 0.08 a	0.01 ± 0.00 ab	0.02 ± 0.00 c	0.06 ± 0.05 b	1.34 ± 0.19 b	0.03 ± 0.01 b	1.45 ± 0.25 abc	0.07 ± 0.02 ab	5.26 ± 0.64 c	2.90 ± 0.33 a
Oranzhevaya	0.75 ± 0.15 c	1.75 ± 0.56 a	0.02 ± 0.01 b	0.16 ± 0.07 a	0.03 ± 0.01 d	0.02 ± 0.01 bc	nd a	1.99 ± 0.13 c	0.05 ± 0.01 c	1.28 ± 0.12 a	0.20 ± 0.02 d	2.74 ± 0.57 a	3.52 ± 0.13 b
Vitaminaya	1.08 ± 0.18 de	3.50 ± 0.46 d	0.01 ± 0.00 a	0.18 ± 0.06 a	0.01 ± 0.00 a	0.04 ± 0.01 d	0.05 ± 0.04 b	0.84 ± 0.07 a	0.02 ± 0.00 a	2.05 ± 0.12 d	0.05 ± 0.01 a	4.86 ± 0.67 c	2.97 ± 0.14 a
Québec, Canada	1.30 ± 0.78 Y	2.56 ± 0.83 X	0.06 ± 0.10 X	0.16 ± 0.07 X	0.02 ± 0.01 X	0.02 ± 0.01 Y	0.03 ± 0.04 Y	1.58 ± 0.72 X	0.03 ± 0.01 X	1.54 ± 0.38 X	0.09 ± 0.06 X	4.15 ± 1.28 Y	3.25 ± 0.60 X
Sammalmäki, Finland	0.55 ± 0.28 X	2.45 ± 0.63 X	0.14 ± 0.06 Y	0.18 ± 0.05 X	0.02 ± 0.01 Y	0.01 ± 0.00 X	tr X	2.99 ± 0.34 Y	0.03 ± 0.02 X	1.45 ± 0.30 X	0.09 ± 0.03 X	3.36 ± 0.86 X	4.56 ± 0.38 Y

<sup>a</sup>Values in the same column followed by different letters (a–f and X and Y) are significantly different between varieties and latitudes, respectively ( $p < 0.05$ ). Key: tr, compound present at <0.005 g/100 mL of juice (trace); nd, not detected.

Table 4. Compositional Parameters in Sea Buckthorn Berry Juice<sup>a</sup>

variety	sugar/acid ratio	sugar/°Brix ratio	°Brix	pH	juice yield (mL/100 g)	berry weight (g/berry)
Avgustinka	0.62 ± 0.04 a	0.34 ± 0.04 a	8.4 ± 0.0 ab	2.66 ± 0.13 abcd	52.6 ± 0.4 a	0.42 ± 0.11 abc
Botanicheskaya	0.62 ± 0.13 ab	0.35 ± 0.05 a	8.1 ± 0.6 abc	2.57 ± 0.01 a	53.3 ± 5.5 ab	0.42 ± 0.10 a
Trofimovskaya	0.96 ± 0.16 c	0.43 ± 0.04 b	9.4 ± 0.8 abc	2.60 ± 0.01 b	51.7 ± 4.6 ab	0.48 ± 0.13 ab
Pertsik	0.87 ± 0.27 abc	0.45 ± 0.09 abcd	9.4 ± 0.5 abc	2.60 ± 0.01 ab	50.5 ± 1.1 a	0.37 ± 0.12 ab
Prevoshodnaya	1.61 ± 0.55 d	0.50 ± 0.07 c	9.3 ± 0.3 c	2.91 ± 0.20 abcd	55.2 ± 6.2 ab	0.55 ± 0.17 abc
Prozharachnaya	0.67 ± 0.20 ab	0.35 ± 0.07 a	8.1 ± 0.5 a	2.67 ± 0.07 ab	59.3 ± 2.5 b	0.60 ± 0.17 abc
Chuiskaya	1.83 ± 0.30 d	0.58 ± 0.06 d	9.0 ± 0.6 abc	2.97 ± 0.05 cd	57.7 ± 3.2 ab	0.74 ± 0.06 c
Oranzhevaya	0.78 ± 0.16 bc	0.37 ± 0.06 ab	7.4 ± 0.4 a	2.82 ± 0.05 c	59.4 ± 3.6 ab	0.57 ± 0.10 ab
Vitaminaya	1.64 ± 0.19 d	0.50 ± 0.04 c	9.6 ± 0.6 bc	3.05 ± 0.03 d	51.2 ± 5.1 ab	0.66 ± 0.10 bc
Canada	1.37 ± 0.56 Y	0.47 ± 0.11 Y	8.7 ± 1.0 X	2.91 ± 0.14 Y	56.8 ± 4.9 X	0.66 ± 0.12 Y
Sammalmäki	0.75 ± 0.22 X	0.39 ± 0.07 X	8.6 ± 0.8 X	2.61 ± 0.06 X	55.4 ± 4.1 X	0.51 ± 0.06 X

<sup>a</sup>Values in the same column followed by different letters (a–d and X and Y) are significantly different between varieties and latitudes, respectively ( $p < 0.05$ ).

acid (28.2% and 46.4%, respectively). The content of ascorbic acid varied from 0.05 to 0.20 g/100 mL of juice.

**Comparison between Varieties.** The multiple comparison study of compositional parameters of sea buckthorn berries showed considerable variation in juice composition among the varieties (Tables 3 and 4). Among all the varieties investigated, Chuiskaya and Vitaminaya showed the highest total sugar content and the lowest total acid content, hence the highest sugar/acid ratio ( $p < 0.05$ ). The berries of Chuiskaya and Vitaminaya were expected to be the sweetest and the most favored among all the berry samples.<sup>2,3</sup> In contrast, the berries of Avgustinka and Botanicheskaya were supposed to be the sourest due to the lowest total sugar content and sugar/acid ratio and the highest total acid content among the varieties studied ( $p < 0.05$ ). Therefore, in view of consumer acceptance and commercial purpose, the sea buckthorn varieties Chuiskaya and Vitaminaya would be better choices for sea buckthorn cultivation than the varieties Avgustinka and Botanicheskaya.

The berries of Botanicheskaya and Chuiskaya had, respectively, the lowest and the highest contents of fructose ( $p < 0.05$ ). The contents of quinic acid and glucose were the highest in the berries of Vitaminaya and the lowest in those of Oranzhevaya among all the varieties studied ( $p < 0.05$ ), whereas the contents of citric acid, ascorbic acid, and methyl-*myo*-inositol behaved vice versa. The content of the major acid, malic acid, appeared to be the highest in Pertsik and the lowest in Vitaminaya ( $p < 0.05$ ). Vitaminaya also showed the lowest content of ethyl glucose and the highest contents of *myo*-inositol and sucrose and pH. The highest levels of ethyl glucose were observed in Botanicheskaya, Trofimovskaya, and Prozharachnaya. There was no significant difference ( $p > 0.05$ ) between the contents of L-quebrachitol among berries of different varieties.

**Comparison between Growth Latitudes.** The sea buckthorn samples collected from two different growth sites, Québec, Canada, and Sammalmäki, Finland, were compared to study the effect of latitude on the composition of the berries (Tables 3 and 4). The altitudinal difference between Québec, Canada (100 m), and Sammalmäki, Finland (1 m), was ignored when compared to the high latitudinal difference between the two growth sites (46°47' N for Québec, Canada, vs 60°23' N for Sammalmäki, Finland). The independent-sample *t* test showed that the contents of fructose, *myo*-inositol, sucrose, and total sugar were significantly lower (by 57.9%, 44.7%, 92.4%, and 19.2%, respectively) in sea buckthorn berries grown at higher latitude (Sammalmäki, Finland) than those grown at

lower latitude (Québec, Canada). In contrast, the contents of ethyl glucose, methyl-*myo*-inositol, malic acid, and total acid were significantly higher (by 130.1%, 32.9%, 89.3%, and 40.6%, respectively) in berries grown in Finland than those grown in Canada. The sugar/acid ratio, sugar/°Brix ratio, pH, and berry weight were lower (by 45.4%, 17.3%, 10.4%, and 22.3%, respectively) in berries collected from Finland than those from Canada. As a result, the sea buckthorn berries grown at lower latitudes, with probably higher levels of total sugar and sugar/acid ratio and a lower level of total acid, may have better sensory properties than those grown at high latitudes.<sup>2,3</sup> This may give useful guidelines for the selection of growth areas for cultivation of sea buckthorn berries.

The effects of the latitudes of the growth places on the biosynthetic and the metabolic pathways in plants and therefore on the fruit composition are results of complex effects of growth environments such as soil conditions and weather conditions as well as the interaction between the genetic and the environmental factors. The only independent latitude-based variable is the day length. In Québec, Turku, and Kittilä, the day length is 12 h at both the vernal equinox (around March 30) and autumnal equinox (around September 23). Toward summer, the day lengths increase and reach the maximum level at the summer solstice (around June 21) as approximately 15.5 h in Québec and 19 h in Turku, with no sunset in Kittilä. Despite this, the total radiation during the growing season is the lowest in Kittilä and the highest in Québec due to the declination. Therefore, in view of plant growth and metabolism, radiation is considered to be a more important factor regulating the metabolic pathways of the plants compared to day length. The reducing effect of the low angle of sunshine on the total radiation in the north is even enhanced on cloudy days. Together with the other weather conditions, this causes a clear annual deviation in weather variables, such as length of the growing season. However, the growing season is always shortest in the north.

Clear differentiation between the effect of latitude and the effect of weather/climatic conditions cannot clearly be distinguished. Temperature and radiation often correlated negatively with latitude. The higher contents of fructose and sucrose in sea buckthorn berries grown at lower latitude compared to those grown at higher latitude in this study might be explained by the increased rate of photosynthesis with the higher temperature at lower latitude.<sup>19</sup> The biosynthesis of malic acid is known as a highly exothermic reaction,<sup>20</sup> and the inhibition of catabolism of malic acid caused by the exclusion of light has been reported

in grape.<sup>21,22</sup> The increased content of malic acid in sea buckthorn berries grown at higher latitude compared to those grown at lower latitude might be attributed to the decreased levels of temperature and radiation at higher latitude. However, a further investigation on the effects of weather conditions on the composition of sea buckthorn berries was of great importance and was required to validate the hypothesis.

#### Effects of Weather Conditions on Berry Composition.

The study focused on the effects of weather conditions on the composition of sea buckthorn berries. It is worth mentioning that since the sea buckthorn bushes were planted in different growth fields, differences in the soil quality exist to some extent. However, the influence of soil quality on the berry composition was not taken into account because of the unavailability of the soil information for the growth sites in Finland. Treatment of the bushes, such as fertilization and irrigation, was kept the same to minimize the variation of the soil quality. Pearson's correlation coefficient analysis was applied to investigate the correlations between the weather conditions and the composition of sea buckthorn berries of *H. rhamnoides* ssp. *mongolica* (Table 5). Linear regressions of L-quebrachitol and weather variables were taken as examples in Figure 2. The principal component analysis (PCA) applied in our previous studies<sup>23,24</sup> on the effects of weather conditions on the composition of currants was excluded in this study because it extracted a total of eight principal components (PCs) which gave too scattered and useless information for the study. Among all the components studied, the contents of glucose, quinic acid, ascorbic acid, total sugar, and soluble solids were hardly affected by the variation of weather conditions. Various correlations between the other components and weather parameters were found in this study.

**Temperature Parameters.** The temperature parameters had, more or less, positive correlations with the values of fructose, L-quebrachitol, myo-inositol, sucrose, sugar/acid ratio, sugar/°Brix ratio, and juice yield and negative correlations with the content of ethyl glucose ( $p < 0.05$ , Table 5). No correlation between citric acid and the temperature parameters was found. As described above, the positive correlations between the temperature and the contents of fructose and sucrose might provide a valid explanation for the negative correlation found between latitude and the concentrations of these two components in sea buckthorn berries. In our previous study on the wild Chinese sea buckthorn (*H. rhamnoides* ssp. *sinensis*), the content of L-quebrachitol was found to correlate negatively with the temperature parameters.<sup>12</sup> However, positive correlations between the temperature parameters and the contents of myo-inositol and L-quebrachitol were detected in the current study. Linear regression ( $p < 0.05$ ) of L-quebrachitol and the average temperature in March is shown in Figure 2. This is in disagreement with common knowledge and previous studies since the accumulation of inositols has always been in association with cold, salinity, and water stress.<sup>10,17,18</sup> The conflicting findings observed in this study might be caused by the collinearity of the weather parameters collected during the investigating years. Since the plants were naturally grown in the field without any control of the weather parameters, the collinearity of the parameters was thus unavoidable. In our study, some negative correlations were detected between temperature parameters and precipitation and humidity parameters during the investigating years ( $p < 0.05$ ). For example, the average temperature of January, February, and March all correlated negatively with the precipitation from the start of the growing season until the day of harvest ( $r = -0.54, -0.79, \text{ and } -0.92, p < 0.01$ ). Thus, the positive correlations between the temperature parameters and the

concentrations of myo-inositol and L-quebrachitol might be explained either by the metabolic pathways or by the collinear effects of the precipitation or humidity parameters on these components.

Moreover, it was observed that the levels of methyl-myoinositol, malic acid, and total acid, pH, and berry weight showed varying responses to the temperature parameters during different growth periods. Methyl-myoinositol correlated positively with the average temperature in July (T<sub>Jul</sub>) but negatively with the average temperature in the last week before harvest (T<sub>w</sub>) (Table 5). Malic acid and total acid contents correlated negatively with most of the temperature parameters except the monthly average temperature of January to March and of July. As an exception, the average temperature during January to March correlated positively with these two compositional parameters. In contrast, the pH and berry weight correlated negatively with the average temperature during January to March but positively with most of the other temperature parameters. In addition to the findings in this study, opposite responses of citrate accumulation to temperature during different growth periods were also detected by Lobit and colleagues in peach fruits.<sup>25</sup> An increase in temperature by 1 °C during the first part of the mesocarp growth before pit hardening led to an increase of citrate accumulation by 26–32%. In contrast, the accumulation of citrate decreased by 45–50% as the temperature increased by 1 °C during the second part of mesocarp growth after pit hardening. Richardson et al.<sup>26</sup> discovered that kiwifruit exposed to a higher temperature in early development showed an increase in the content of malate but the content of malate decreased when the kiwifruit was exposed to a higher temperature in later development. The different correlation trends observed in different growth periods in these studies, as well as in our study, suggested varying regulation of the biosynthesis of the compounds in response to certain weather parameters during different growth stages.

Moreover, it was an interesting finding of this study that the temperature parameters during the dormant season showed significant correlations ( $p < 0.05$ ) with the composition of sea buckthorn berries. This suggested that although the visible growth is suspended during the dormant season in winter, developmental changes may still occur, with an effect on the final composition of the fruits. The study conducted by Ruiz et al.<sup>27</sup> might support this speculation by showing that shading the whole tree during the dormant season had no effect on the fruit set, but reduced flower bud abscission and, as a result, increased the final fruit yield of apricot. The reduction of flower bud abscission was probably due to the decrease of gibberellins resulting from a reduction of temperature in the shaded trees.

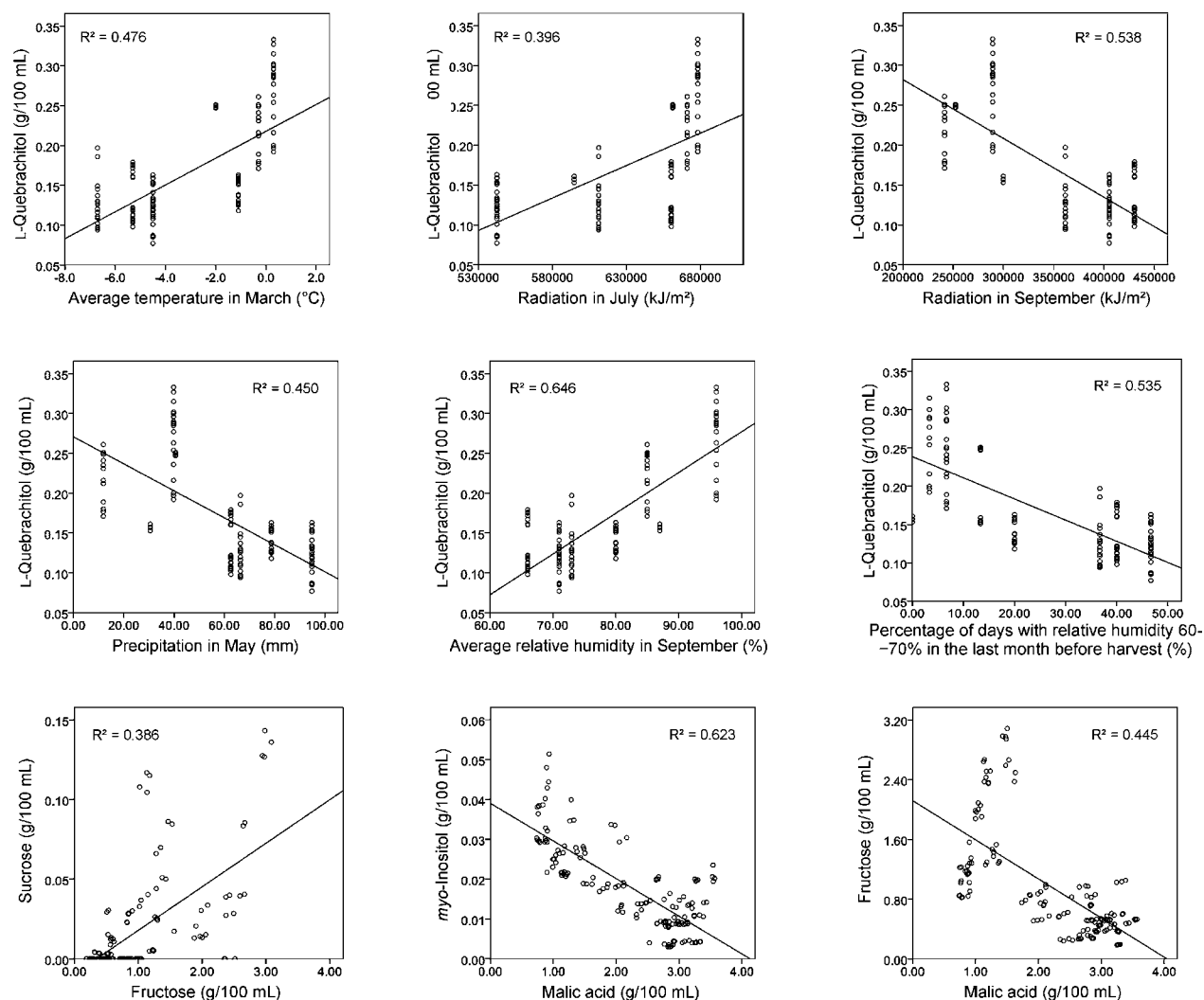
**Radiation Parameters.** The radiation parameters hardly influenced the values of ethyl glucose, methyl-myoinositol, and juice yield. Among the radiation variables, the radiation during January to March and August and that in the last month before harvest were the main factors affecting the composition of sea buckthorn berries. They correlated negatively with the contents of malic acid and total acid and positively with fructose, myo-inositol, citric acid, sugar/acid ratio, and pH. This is consistent with our previous findings of the positive correlation between latitude and malic acid content and the negative correlations between latitude and the contents of fructose and myo-inositol since the light intensity is commonly known to correlate negatively with latitude.

Exclusion of light has been reported to inhibit the degradation of malate and increase its content in grape.<sup>21,22</sup> This is in agreement with our study concerning the findings of

Table 5. Pearson's Correlation Coefficients between Compositional Parameters and Correlated Weather Parameters in Sea Buckthorn (*H. rhannonoides* ssp. *mongolica*)<sup>a</sup>

component	correlated weather variables (Pearson's correlation coefficient)	
	positive correlation	negative correlation
fructose	SUMTgs (0.40**), HDm (0.40**), Tjun (0.45**), Tsep (0.46**), Tw (0.45**), TDm (0.41**), HTm (0.42**), Rm (0.42**), Rjan (0.42**), Rfeb (0.42**), Rmar (0.43**), Raug (0.38**), DH30to40gh (0.40**), DH40to50gh (0.42**),	Hgh (-0.42**), Hjan (-0.48**), Hfeb (-0.47**), Hmar (-0.49**), Hapr (-0.42**), Hmay (-0.40**), DH90to100gh (-0.42**)
glucose		
ethyl glucose	Hgh (0.41**), Hjun (0.44**), DH80to90gh (0.44**)	SUMTgs (-0.40**), SUMTm (-0.41**), HDm (-0.43**), Tm (-0.41**), HTm (-0.42**), Pm (-0.41**), DH30to40gh (-0.49**), DH40to50gh (-0.43**),
l-quebrachitol	Dgs (0.52**), Tjan (0.50**), Tfeb (0.55**), Tmar (0.69**), Tapr (0.55**), Tmay (0.57**), Tjul (0.45**), Rgh (0.44**), Rmay (0.42**), Rjul (0.63**), Psep (0.53**), Hm (0.71**), Hw (0.78**), Haug (0.81**), Hsep (0.81**), DH80to90m (0.51**), DH90to100m (0.70**)	Rsep (-0.74**), Pgh (-0.54**), Papr (-0.55**), Pmay (-0.67**), Pjul (-0.66**), DH60to70m (-0.73**)
methyl-myoinositol	Tjul (0.43**), Hmay (0.48**), DH70to80gh (0.48**)	Tw (-0.43**), Pm (-0.55**), Pw (-0.42**), Pjul (-0.40**), DH30to40gh (-0.43**), DH40to50gh (-0.48**),
myo-inositol	Rm (0.52**), Rw (0.45**), Rjan (0.48**), Rfeb (0.46**), Rmar (0.61**), Raug (0.48**), Psep (0.54**), DH30to40gh (0.45**), DH40to50gh (0.43**), temp variables except Tjan, Tfeb, and Tmar	Hgh (-0.42**), Hjan (-0.54**), Hfeb (-0.50**), Hmar (-0.59**), Hapr (-0.52**), Hmay (-0.41**), DH80to90gh (-0.46**), DH90to100gh (-0.41**),
sucrose	HDm (0.41**), Tapr (0.44**), Taug (0.40**), Rw (0.55**), Rapr (0.42**), Psep (0.43**)	Hjan (-0.42**), Hfeb (-0.41**), Hmar (-0.44**), Hapr (-0.41**)
total sugar		
malic acid	Tjan (0.42**), Tfeb (0.44**), Tmar (0.45**), DH70to80gh (0.55**), DH80to90gh (0.55**), DH90to100gh (0.58**), DH70to80m (0.40**), av humidity variables	Rm (-0.52**), Rjan (-0.62**), Rfeb (-0.60**), Rmar (-0.62**), Raug (-0.53**), Rsep (-0.47**), DH30to40gh (-0.63**), DH40to50gh (-0.74**), DH50to60gh (-0.45**), DH60to70m (-0.46**), temp and precipitation variables except Tjan, Tfeb, Tmar, and Tjul
citric acid	Rm (0.60**), Rw (0.56**), Rjan (0.63**), Rfeb (0.61**), Rmar (0.69**), Rapr (0.46**), Raug (0.57**), Pmay (0.40**)	Hapr (-0.40**)
quinic acid		
ascorbic acid		
total acid	Tjan (0.43**), Tfeb (0.45**), Tmar (0.46**), DH70to80gh (0.52**), DH80to90gh (0.52**), DH90to100gh (0.59**), av humidity variables	Rm (-0.59**), Rjan (-0.75**), Rfeb (-0.73**), Rmar (-0.72**), Raug (-0.60**), Rsep (-0.52**), DH30to40gh (-0.64**), DH40to50gh (-0.75**), DH50to60gh (-0.41**), DH60to70m (-0.47**), temp and precipitation variables except Tjan, Tfeb, Tmar, and Tjul
sugar/acid ratio	Rm (0.42**), Rjan (0.46**), Rfeb (0.46**), Rmar (0.49**), Raug (0.39**), Pm (0.42**), Psep (0.41**), DH30to40gh (0.45**), DH40to50gh (0.47**), temp variables except Tjan, Tfeb, Tmar, and Tjul	Hgh (-0.47**), Hjan (-0.55**), Hfeb (-0.55**), Hmar (-0.57**), Hapr (-0.45**), Hmay (-0.47**), DH80to90gh (-0.41**), DH90to100gh (-0.42**),
sugar/Brix ratio	Tjun (0.40**), TDm (0.40**)	
<sup>o</sup> Brix		
pH	Pgh (0.57**), Pm (0.63**), Pmar (0.50**), Papr (0.52**), Pjun (0.47**), Pjul (0.50**), Psep (0.55**), DH30to40gh (0.66**), DH40to50gh (0.74**), DH50to60gh (0.40*), DH60to70m (0.41*), temp and radiation variables except Tjan, Tfeb, Tmar, Tjul, Rgh, Rmay, Rjun, and Rjul	Tjan (-0.45**), Tfeb (-0.42*), Tmar (-0.36*), Hgh (-0.65**), Hjan (-0.81**), Hfeb (-0.79**), Hmar (-0.82**), Hapr (-0.73**), Hmay (-0.65**), Hjun (-0.44*), DH70to80gh (-0.44*), DH80to90gh (-0.58**), DH90to100gh (-0.60**),
juice yield	SUMTm (0.45*), HDm (0.41*), Tapr (0.53**), Tm (0.45*), LTm (0.54**)	
berry weight	SUMTm (0.51**), HDm (0.40**), Tjun (0.41**), Taug (0.41**), Tsep (0.53**), Tm (0.50**), Tw (0.60**), LTm (0.48**), HTm (0.50**), Rjan (0.57**), Rfeb (0.56**), Rmar (0.34*), Rapr (0.42**), Raug (0.41**), Rsep (0.63**), DH40to50gh (0.62**), DH60to70m (0.74**), precipitation variables except Pw, Pjan, Paug, and Psep	Tjan (-0.59**), Tfeb (-0.55**), Tmar (-0.74**), Rmay (-0.43**), Rjul (-0.71**), DH90to100gh (-0.58**), DH70to80m (-0.40**), DH80to90m (-0.40**), DH90to100m (-0.66**), av humidity variables except Hjun and Hjul

<sup>a</sup>The abbreviations for the weather variables refer to Table 2. Key: \*,  $p < 0.05$ ; \*\*,  $p < 0.01$ .



**Figure 2.** Correlations ( $p < 0.05$ ) between the content of L-quebrachitol and weather conditions and between the different metabolites in sea buckthorn berries. Each data point in the figure presents a datum of a single replicate of the sample.

the negative correlations between the content of malic acid and the radiation parameters. The accumulation of malate in fruits depends on various biosynthetic reactions involved in glycolysis, photosynthesis, the tricarboxylic acid (TCA) cycle, and the glyoxylate cycle and on various catabolic reactions involved in gluconeogenesis, respiration, and fermentation in certain conditions.<sup>28</sup> Expression and activity of the enzymes that serve as regulatory points in the reactions, such as phosphoenolpyruvate carboxylase (PEPC) and malate dehydrogenase (MDH), which occur in glycolysis, and phosphoenolpyruvate carboxykinase (PEPCK) and pyruvate orthophosphate dikinase (PPDK), which occur in gluconeogenesis, should be investigated on the basis of the results of this study. It is worth exploring the reason for the changes in malate content in response to temperature and radiation in sea buckthorn berries.

myo-Inositol is synthesized *de novo* from D-glucose by the action of hexokinase, myo-inositol-1-phosphate synthase, and myo-inositol monophosphatase. The cyclization of D-glucose-6-phosphate to 1L-myo-inositol-1-phosphate (Ins(3)P<sub>1</sub>) by myo-inositol-1-phosphate synthase is the first committed and irreversible step in myo-inositol biosynthesis.<sup>29</sup> In addition to the commonly known drought and cold stresses, light was also found to have influences on expression of the genes encoding Ins(3)P<sub>1</sub>

synthase, which might give, to some extent, explanations for the observations in our study. A photoresponsive enhancement of chloroplast and cytosolic Ins(3)P<sub>1</sub> synthase activity was observed in salt-tolerant varieties of rice grown in a NaCl environment.<sup>30</sup> Keller et al.<sup>31</sup> even found that light greatly elevated the transcript levels of the Ins(3)P<sub>1</sub> synthase encoding gene (termed *STPS-1*) but drought stress had no effect.

Rosales and colleagues<sup>32</sup> reported an increase in sucrose synthase activity in cherry tomato in response to an increase in the temperature and solar radiation, coinciding with the reduction in sucrose content and the increase in the fructose and glucose contents. An increasing trend in the contents of fructose and glucose and a decreasing trend in the content of sucrose in response to high temperature were also found in passionfruit.<sup>33</sup> However, glucose and sucrose showed different varying trends in response to the variation of temperature and radiation in sea buckthorn berries, although fructose presented the same positive correlation with temperature and radiation as in the study of cherry tomato and passionfruit. Glucose had no correlation with the weather parameters, while sucrose showed positive correlation with some of the temperature and radiation parameters in sea buckthorn berries. The difference between these findings suggested a potential difference in the



biosynthetic and metabolic pathways in plants and fruits of different genotypes.

The content of L-quebrachitol correlated positively with the radiation from the start of the growing season until the day of harvest (Rgh) and the radiation in May (Rmay) and July (Rjul) but negatively with the radiation in September (Rsep) (Table 5). Figure 2 shows different associations between the content of L-quebrachitol and Rjul and Rsep. The berry weight correlated positively with the radiation during January to April and August to September but negatively with the radiation in May and July (Table 5). These opposite trends found in the content of L-quebrachitol and berry weight in response to the radiation in different growth periods suggested, again, the varying regulations of the metabolic pathways of the compounds at different growth stages in response to the weather conditions.

**Precipitation Parameters.** Pearson's correlation coefficients showed no correlation between juice yield and the precipitation parameters. Malic acid and total acid correlated negatively with all the precipitation parameters, whereas pH and berry weight correlated positively with most of the precipitation parameters. The positive correlation between the berry weight and the precipitation parameters indicated an increase in the size of the berries in response to an increase in precipitation. Therefore, irrigation could be a good choice for the commercial cultivation of the sea buckthorn berries grown in areas with low levels of precipitation. The other metabolites presented various correlations with individual precipitation parameters (Table 5). Citric acid correlated only positively with the precipitation in May (Pmay), while myo-inositol and sucrose correlated positively with the precipitation in September (Psep). Methyl-myoinositol and ethyl glucose both correlated negatively with the precipitation in the last month before harvest (Pm). In addition, methyl-myoinositol also showed negative correlations with the precipitation in the last week before harvest (Pw) and in July (Pjul). L-Quebrachitol correlated positively with Psep but negatively with the precipitation from the start of the growing season until the day of harvest (Pgh) and the precipitation in April, May, and July (Table 5 and Figure 2).

**Humidity Parameters.** Fructose, myo-inositol, sugar/acid ratio, pH, and berry weight all showed positive correlations with low humidity variables (relative humidity <70%) and negative correlations with high humidity variables (relative humidity >70%) from the start of the growing season until the day of harvest ( $p < 0.05$ , Table 5). However, opposite trends were found in the contents of ethyl glucose, methyl-myoinositol, malic acid, and total acid in response to the humidity variables ( $p < 0.05$ , Table 5). In accordance, the average humidity during January to May showed negative correlations with fructose, myo-inositol, sugar/acid ratio, pH, and berry weight and positive correlations with ethyl glucose, methyl-myoinositol, malic acid, and total acid although the correlations with ethyl glucose ( $r = 0.31-0.39$ ,  $p < 0.01$ ) and methyl-myoinositol ( $r = 0.26-0.48$ ,  $p < 0.01$ ) were weak. The content of L-quebrachitol was only affected by the humidity conditions in a short period before harvest. L-Quebrachitol correlated positively with the average humidity in August (Haug), September (Hsep), and the last month (Hm) and the last week (Hw) before harvest (Table 5 and Figure 2). The level of this compound also correlated positively with high humidity variables (relative humidity >70%) but negatively with low humidity variables (relative humidity <70%) in the last month before harvest (Table 5 and Figure 2).

**Correlation between Metabolites.** In the present study, a positive correlation between sucrose and fructose ( $r = 0.63$ ,  $p < 0.01$ , Figure 2) was detected. A weak positive correlation between sucrose and glucose was also observed ( $r = 0.32$ ,  $p < 0.01$ ). This could be well explained by the fact that fructose and glucose are the two substrates in the hexose phosphate pool and can be converted reversibly to sucrose.<sup>10,34</sup> Sucrose also correlated positively with myo-inositol ( $r = 0.52$ ,  $p < 0.01$ ) in berries of *H. rhamnoides* spp. *mongolica*. A weak positive correlation between myo-inositol and glucose ( $r = 0.41$ ,  $p < 0.01$ ), as well as between myo-inositol and fructose ( $r = 0.47$ ,  $p < 0.01$ ), was found in the current study. Among all the naturally occurring inositols and their methyl ethers in plants, only myo-inositol is synthesized de novo from D-glucose. Production of other isomeric (*scyllo*, *chiro*, *muco*, and *neo*) inositols and O-methylinositols (sequoyitol, bornesitol, quebrachitol, pinitol, ononitol, etc.) involves metabolic processing of myo-inositol under stressful environments.<sup>29</sup> The contents of myo-inositol, fructose, and sucrose showed negative correlation with the content of malic acid ( $r = -0.79$ ,  $-0.67$ , and  $-0.57$ , respectively,  $p < 0.01$ ). The linear regressions with 95% confidence intervals of myo-inositol and malic acid, and fructose and malic acid, are shown in Figure 2. Malate could be synthesized from and catabolized into glucose and fructose, and finally sucrose, via a series of reactions in glycolysis and gluconeogenesis in fruits.<sup>28</sup> In addition, a positive correlation between the content of L-quebrachitol and that of ascorbic acid ( $r = 0.51$ ,  $p < 0.01$ ) was detected as well.

In conclusion, among the nine varieties of sea buckthorn (*H. rhamnoides* spp. *mongolica*) berries studied, Chuisakaya and Vitaminaya were expected to be the sweetest and the most favored due to the highest total sugar content and sugar/acid ratio and the lowest total acid content. In contrast, berries of Avgustinka and Botanicheskaya were supposed to be the sourest. The sea buckthorn berries grown at higher latitude in this study had higher amounts of ethyl glucose, methyl-myoinositol, malic acid, and total acid but lower levels of fructose, myo-inositol, sucrose, and total sugar contents, sugar/acid ratio, pH, and berry weight than those grown at lower latitude. Among all the components studied, the contents of glucose, quinic acid, ascorbic acid, total sugar, and soluble solids were hardly influenced by the weather conditions, whereas the other components correlated significantly with various weather variables. Moreover, it was found that some of the compositional parameters responded to the weather parameters differently in different growth stages. Correlations between metabolites of sea buckthorn berries were detected in the current study. The contents of fructose, sucrose, and myo-inositol, which correlated positively with each other, were found to correlate negatively with the content of malic acid.

## ■ ASSOCIATED CONTENT

### 📄 Supporting Information

Tables of compositional information of sea buckthorn berry juice collected at different growth sites and in different harvesting years and Pearson's correlation coefficients between weather conditions and compositional parameters in sea buckthorn berry juice. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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## Notes

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