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Trace element concentration and stable isotope ratio analysis in blueberries and bilberries: a tool for quality and authenticity control

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Abstract. *Vaccinium* genus berries - wild bilberries (*Vaccinium myrtillus* L.) and cultivated highbush blueberries (*Vaccinium corymbosum* L.) are consumed worldwide and their consumption have a trend of stable increase. Thus, of a special importance are studies of the composition of these berries considering their wide use in ethnomedicine, for juice and jam production, functional food as well as the use in preparations of extracts that can have applications in pharmaceutical and cosmetics industries. The aim of this study was to characterise elemental and isotopic composition, variation in element concentration as well as to develop tools for berry authenticity and quality control of bilberries gathered in Northern Europe and commercially available blueberry samples from across the World. The elemental composition of berries was analysed using inductively coupled plasma with optical emission detection, while isotope ratio mass spectrometry (IRMS) was used for determination of isotope ratio values. The results demonstrated detectable differences between bilberry macro- and microelement values. IRMS analysis of blueberries revealed significant differences in isotope ratios based on the place of origin, which indicates the possibility to use this analytical method for authenticity testing. In none of the samples, pollution was detected, even though there are indications of different growth conditions and geochemical differences affecting bilberry composition.

Keywords: blueberries 1; trace elements 2; heavy metals 3; light stable isotope ratio 4; pollution 5; authenticity 6; bilberries 7

1. Introduction

Consumption of berries is becoming increasingly popular worldwide due to health benefits and excellent taste properties. Growing popularity increases production and consumption of two *Vaccinium* genus (Ericacae family) berries - wild bilberries (*Vaccinium myrtillus*) and cultivated blueberries (*Vaccinium corymbosum*). Consumers are becoming more health-conscious, and fresh berries with attractive appearance, balanced sweet-sour taste and high nutritional and health beneficial value are appreciated [1]. Commercial production of blueberries has reached 682,790 tons (80.2 % in Americas, 18.2 % in Europe) [2], and picking of wild bilberries in Northern Europe and Russia reach several hundreds of tons yearly with stable increasing trend. Widely recognised are the various health-promoting properties of blueberries and bilberries as their consumption can reduce risk of many infectious and degenerative disease [3,4]. Blueberry and bilberry fruits have been used fresh, dried and as juice for alleviation for gastrointestinal tract and diabetes, but herbal supplements containing these berries are available in the market as aids to improve vision, to treat diarrhoea, considering their antimicrobial, anti-inflammatory and antioxidant properties [5-8]. Biological and pharmacological activity of blueberries and bilberries are associated with high concentrations in their composition of polyphenolics, especially anthocyanins and their capacity to scavenge oxygen and other radical species and thus reduce the oxidative stress [9]. Additionally, berry lipids, such as terpenes, sterols, unsaturated fatty acids, and waxes are contributing to the biological activities of these berries and their extracts [10].

Another group of valuable components of blueberries and bilberries are mineral substances (K, Ca, Mg, P, Fe, Mn, Zn and others) which are essential for sustaining various functions of the human body and are important components of the diet. At the same time many inorganic elements (Cd, Pb, As and others) are non-essential and might indicate presence of anthropogenic pollution [11]. Thus, of a special importance are studies of the mineral composition of the berries as well as analysis of the presence of toxic trace elements. The environmental pollution sources on the presence of toxic trace elements has been studied to identify the adverse impacts of known pollution sources, such as metal processing industries. For example, significantly elevated concentrations of trace elements in comparison with the background pollution sites were found in berries sampled in vicinity of ferrochrome and stainless-steel factories in Northern Finland [12, 13]. As a result of mining and metal processing industries, high concentrations of Ag, As, Be, Bi, Br, Cd, Hg, I, Ni, Pb, Sb, and Tl were found in berries from mining areas in Northern Sweden [14]. Recently, the impact of wood ash applications in forests on the elemental composition in berries has been studied and risks related to the increasing concentrations of trace elements has been found [15]. Another aspect on the berry quality studies offers evaluation of element concentrations in edible products available on the market [16]. However, in this case it is nearly impossible to relate the found concentrations with the origin of the samples as possible adulteration of products may exist, thus requiring development of berry origin authentication methods.

As the growth conditions as well as metal accumulation patterns for different plant species vary, it is important to study contamination levels in species which are of importance for human consumption, such as blueberries and bilberries. Only few studies with only few studied elements are dedicated to elemental composition of trace elements in blueberries and bilberries [15,17,18]. Another important aspect is related to possibilities to identify the origin and cultivation practices based on the compositional analysis of berries. The aim of this study was to characterise elemental and isotopic composition, concentration variability of elements as well as develop and suggest tools for berry authenticity and quality control of bilberries gathered in Northern Europe and commercially available blueberry samples from across the World.

2. Materials and Methods

2.1. Sampling

The fruits of wild bilberries (*Vaccinium myrtillus* L.) were collected during 2018 – 2020 vegetation seasons in 26 sampling sites in the territory of Latvia as well as in three sampling sites in Norway, Finland, and Lithuania. Samples of different varieties of highbush blueberries (*Vaccinium corymbosum* L.) were sampled in 2018 in farm "Strelnieki" (Latvia). Once the samples were collected, they were frozen and stored in freezer at -20 °C. Commercial samples of blueberries were obtained from supermarkets during 2018 – 2020 and the country of origin was indicated on the labels being Peru, Argentina, Uruguay, Chile, Morocco, Spain, Germany, Poland, and Latvia. For analysis, the samples were lyophilised, and homogenised using pestle and mortar. Agate pestle and mortar was used for homogenising of samples for IRMS analysis.

2.2. Analysis of trace elements

Dried berry samples (1.00 g) were weighed into Teflon tubes followed by addition of 8 mL 65% HNO_3 (Sigma Aldrich, ≥65%) and 2 mL 30% H_2O_2 (Enola, Latvia). The tubes were closed (to provide high pressure) prior to sample digestion using a microwave system (Milestone Advanced Microwave digestion system, Ethos Easy) at 200 °C for 30 min. The resulting samples were diluted to 50 mL with deionised water (Millipore, 7,4 µS/cm). The concentrations of inorganic elements were determined by inductively coupled plasma spectrometry with optical emission detection (Thermo Scientific iCAP 700 series ICP spectrometer). The elements determined were Al, As, B, Ba, Ca, Co, Cr, Cu, Fe, K, Li, Mg, Mn, Mo, Na, Ni, Pb, Rb, Sb, Se, Sn, Sr, Ti, Tl, V, and Zn. The detection limit was 1–10 ppb for Al, Ca, and Fe, 0.1–1 ppb for Mg, K, and Na, and 0.1 ppb for all other elements. The concentrations were expressed per dry weight of berries. The accuracy of the analysis of berry samples was checked by the following certified reference materials: SRM 3287 - Blueberry (Fruit) (National Institute of Standards and Technology (NIST) and the National Institutes of Health Office of Dietary Supplements). The difference between the data of berry samples analysed and the reference materials was generally lower than 15% for all elements.

2.3. Light stable isotope ratio analysis ($\delta^{13}C$, $\delta^{15}N$, $\delta^{18}O$)

For determination of the stable isotope ratio, dried berry samples (5.00 mg) were weighed into tin capsules (EuroVector) for C and N analyses and 1.00 mg into silver capsules (EuroVector) for O analysis, and the capsules were carefully folded. All samples were prepared in triplicate. Glutamic acid (C and N analysis) and sucrose (O analysis) laboratory standards (Sigma Aldrich) were used for calibration (0.2, 0.5, 0.8, 1.0 and, 1.5 mg). To monitor the stability of the obtained values, one glutamic acid or sucrose control sample (1.0 mg) was analysed after every 10 samples. To verify trueness of the obtained results, glutamic acid (USGS-40, d^{13}C -26.39 ± 0.04 ‰ VPDB, d^{15}N -4.52 ± 0.06 ‰ AIR, wC = 40,8%, wN = 9,52%) and benzoic acid (IAEA-601, d^{18}O 23.14 ± 0.19 ‰ VSMOW, wO = 26,2%) reference materials were used. The ratio of C, N, and O isotopes in samples was measured on the isotope ratio mass spectrometer (Nu Horizon, acceleration voltage: 5kV, mass range: 2–100 Da, mass dispersion: > 30 cm) using an element analyser (EuroVector Euro EA3000) with quartz combustion column filled with chromium (III) oxide and silvered cobaltous oxide (1030°C) and a quartz reduction tube filled with copper shards (650 °C) for the determination of C and N isotope ratio. For the determination of the O isotope ratio, high-temperature element analyser unit was used (EuroVector HTEA PyrOH) with outer ceramic tube and inner glassy carbon tube filled with glassy carbon chips and nickelled carbon (1420°C). The results were processed by the Nu Stable Control Software v1.69.

2.4. Statistical analysis of results

The Kruskal–Wallis nonparametric test was used to detect differences among metal concentrations in samples with different origin or variety. Statistical data analysis, including Principal Component Analysis (PCA) of metal concentration and stable isotope ratios were done using statistical data discovery software SAS JMP®, Version 14 (SAS Institute Inc., Cary, NC, USA).

3. Results and Discussion

In blueberry and bilberry wet digested samples, total element concentrations were determined by ICP OES method. Sinve highbush blueberries are cultivated, many varieties are available differing in berry ripening time, size, taste, and other properties [19]. Differences in element concentrations in eight varieties of blueberries growing on peat soil in one location (biological farm in Latvia) were compared (Table 1). Our results show that blueberries are a rich source of mineral elements, especially K, Ca, Mg, P, and S. Blueberries as a source for K, Ca, Mg, and Mn is shown also in other studies [17,18]. However, the concentrations of toxic trace elements (Co, Se, Pb, Ni, Cr, Co, As) have been shown to be lower than in berries growing on soils with elevated metal concentrations [20]. For nearly all elements, differing element concentrations in berries were found in studied cultivars, thus, reflecting impact of berry cultivars.

As blueberries are cultivated worldwide it was of interest to compare their elemental composition also in commercially available samples. Elemental composition was analysed in samples obtained from nine countries for the period 2018 – 2020 and altogether 24 elements were analysed (Table 2). In all studied samples, the highest concentrations were found for the elements important for human consumption, namely K, Ca, Mg, Fe, P, S, and Mn. However, the variability was high and, for example, the K concentration in berries from South America or North Africa is twice as high than in samples from North Europe, where the cultivation usually takes place in mineral poor peat soils. In commercially available cultivated berry samples, values of trace elements were found to be low, but comparable with element concentration values, found in other studies [17,18]. However, variability of some of trace elements in some of berry samples was significantly higher than in others For example, in samples from Europe, V concentration was 0.03±0.01, when in samples for other countries, the concentration was nearly five times higher. Similar differences in concentrations were found also for elements, such as Mo, Se, Pb, and Ni. Considering the amounts of blueberries that are cultivated and consumed, and the toxicity values of the studied elements [21], it can be concluded that the trace element concentrations found in the commercially available berry samples do not pose risk to human health. However, considering the high volumes of blueberries sold on the markets worldwide, it would be important to establish quality criteria of elemental composition of trace elements in their composition.

Values of element concentrations in bilberries in territory of Latvia (64 000 km^2) also demonstrate variability and are shown in Figure 1. Maximal values of the studied elements are associated with the known local and regional environmental pollution sites. For example, elevated concentrations in western part of Latvia indicates presence of industrial pollution of long-time functioning cement production as well as metallurgical factories, as it has been found previously [25]. Another major factor influencing bilberry elemental composition seems to be geochemical differences in soils of Latvia, for example differences in Ca/Mg ratio for different parts of the country [26]. Geochemical characteristics of soil is one of the major factors affecting element composition of bilberries, as it has been stated in earlier studies [13,16,22]. Nevertheless, the variability of concentrations of trace elements in wild bilberries reflect specific pattern of metal and other trace element concentrations in soils and thus can be considered as specific for the territory of Latvia. At the same time, the low concentrations of toxic elements and heavy metals found in our study in all locations can be considered low, especially if compared with concentrations found in other studies [7,14,18,22], and Latvian bilberries can be considered as valuable source of mineral substances and essential elements.

Table 1. Concentrations (mg/kg) of elements in blueberry cultivar samples from Latvia.

Element	‘Patriot’	‘Polaris’	‘Bluecrop’	‘Northblue’	‘Chandler’	‘Duke’	‘Chippewa'	‘Blueray 17’
K***	4080±136	4823±118	5509±249	5247±190	5626±249	4955±126	6208±94	4706±284
Ca***	302±27	388±17	386±13	239±53	382±14	432±29	411±37	427±58
Mg***	265±6	322±14	341±15	286±16	326±8	361±12	291±18	38±22
Na***	89±5	123±7	57±9	65±9	112±10	208±19	97±14	83±6
P***	779±22	755±19	713±41	811±31	671±4	658±54	713±27	865±10
S***	669±15	665±18	601±41	764±29	532±11	546±33	592±26	583±20
Al***	6.0±1.0	7.3±0.0	7.7±0.5	7.5±0.3	6.2±0.5	5.8±0.4	5.4±0.4	9.2±0.4
B***	2.17±0.28	3.09±0.25	3.59±0.29	1.41±0.11	2.44±0.17	3.20±0.14	2.12±0.15	2.98±0.29
Ba***	1.02±0.16	1.09±0.12	1.07±0.06	0.64±0.26	0.75±0.07	1.19±0.32	0.93±0.11	1.46±0.37
Cu***	2.44±0.29	2.45±0.68	3.51±0.33	1.65±0.20	4.28±0.38	1.73±0.36	2.62±0.61	2.81±0.43
Fe***	17.4±2.5	20.6±1.6	17.1±0.7	16.1±1.3	20.6±2.0	20.3±2.9	21.7±2.2	18.6±1.4
Mn***	10.6±0.4	19.1±1.4	15.5±0.9	12.7±1.4	11.0±1.1	12.5±0.5	10.4±0.9	13.5±3.4
Si***	30.7±5.0	26.1±3.5	41.2±4.1	35.6±2.5	32.8±2.4	23.9±3.4	35.6±4.9	52.2±11.4
Sr***	0.88±0.15	0.95±0.07	0.87±0.05	0.49±0.11	0.84±0.08	1.37±0.11	0.97±0.07	1.31±0.07
Zn***	3.7±0.1	3.8±0.3	4.8±0.4	3.9±0.2	3.7±0.1	4.0±0.2	3.7±0.3	4.0±0.0
As***	0.16±0.05	0.12±0.04	0.18±0.01	0.19±0.01	0.12±0.01	0.31±0.01	0.17±0.02	0.31±0.02
Cd***	0.030±0.001	0.059±0.001	0.057±0.001	<LOD	0.013±0.001	0.036±0.001	0.015±0.009	0.021±0.002
Co***	0.07±0.01	0.02±0.01	0.05±0.01	0.05±0.01	0.05±0.01	0.06±0.01	0.03±0.01	0.05±0.01
Cr***	0.03±0.01	0.06±0.01	0.01±0.01	<LOD	<LOD	0.08±0.01	0.06±0.01	<LOD
Mo***	0.03±0.01	0.03±0.01	0.03±0.01	0.04±0.01	0.05±0.01	0.05±0.01	0.05±0.01	0.08±0.01
Ni***	0.16±0.01	0.18±0.01	0.15±0.01	0.08±0.01	0.06±0.01	0.12±0.01	0.18±0.01	0.03±0.01
Pb***	0.09±0.01	0.07±0.01	0.11±0.01	0.03±0.01	0.07±0.01	0.11±0.01	0.08±0.01	0.15±0.01
Se***	0.30±0.01	0.28±0.01	0.11±0.01	<LOD	0.11±0.01	0.07±0.01	0.12±0.01	0.23±0.01
V***	0.024±0.001	0.067±0.001	0.018±0.005	<LOD	<LOD	0.018±0.001	0.061±0.004	<LOD

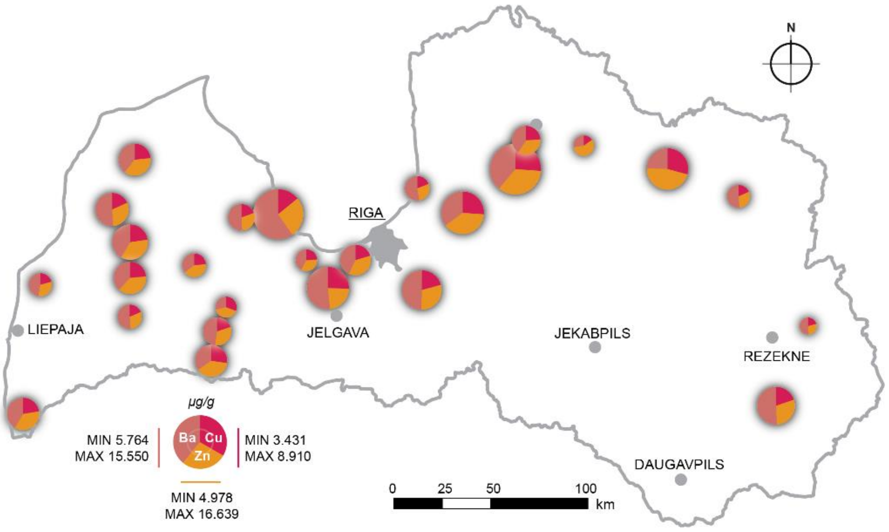
A nonparametric multiple test (Kruskal–Wallis) was applied with p-values: ns not significant; *0.05; **0.01; ***0.001.

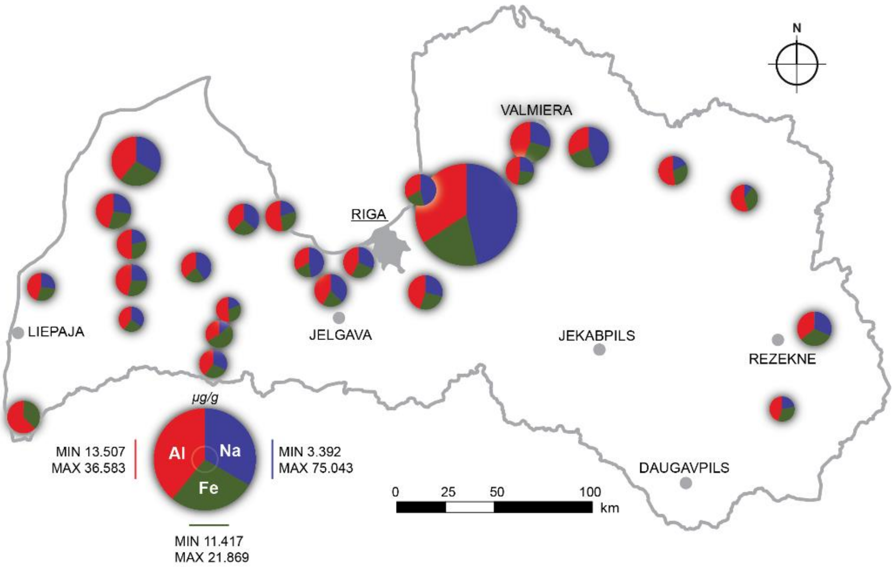
Table 2. Concentrations (mg/kg) of elements in blueberry samples from the different countries.

Element	Peru	Chile	Urugvay	Argentina	Morocco	Spain	Germany	Poland	Latvia
K***	6592±59	7126±66	5583±72	6505±26	7058±94	5372±63	4443±31	6092±62	4144±280
Ca***	1293±32	520±75	508±40	1028±68	834±88	477±30	550±58	360±16	371±31
Mg***	459±34	423±44	370±40	438±34	475±40	388±14	342±20	275±1	322±14
Na***	90±12	38±7	20±5	41±9	134±8	25±3	22±2	45±4	34±4
P***	586±14	804±14	767±11	882±27	918±53	907±24	533±26	875±50	746±26
S***	531±46	655±64	578±32	620±41	587±50	658±12	363±17	576±12	619±24
Al***	33.2±6.8	14.9±2.8	14.6±1.9	33.4±3.2	9.7±8.1	5.9±0.4	7.1±0.3	10.7±0.9	6.9±0.4
B***	3.77±0.46	3.83±0.73	1.82±0.60	3.48±0.34	3.92±0.20	2.22±0.11	2.79±0.23	2.97±0.11	2.62±0.21
Ba***	2.74±0.08	2.82±0.09	2.73±0.81	8.54±0.71	0.45±0.08	0.54±0.05	2.96±0.43	0.68±0.04	1.02±0.18
Cu***	1.90±0.11	7.91±0.33	3.15±0.46	3.90±0.39	2.83±0.63	1.54±0.16	2.03±0.17	1.58±0.08	2.69±0.41
Fe***	31.7±4.1	13.3±2.8	18.4±2.3	26.0±2.9	7.5±3.8	14.9±0.5	14.4±1.4	16.0±0.5	19.0±1.8
Mn***	27.5±3.4	21.5±4.9	21.9±8.0	117.2±6.9	18.3±5.4	15.6±1.1	7.2±0.5	36.1±1.8	13.2±1.3
Si***	88.3±7.1	69.3±6.9	85.6±4.7	144.3±11.4	81.8±9.4	16.0±2.4	27.5±1.0	46.7±2.8	34.8±4.7
Sr***	3.77±0.34	1.76±0.18	1.58±0.40	3.70±0.62	0.39±0.08	0.36±0.03	0.80±0.06	0.31±0.06	0.96±0.09
Zn***	3.5±0.4	6.5±1.3	4.0±0.4	5.2±0.1	6.1±0.5	5.1±0.1	3.7±0.5	2.8±0.1	3.97±0.20
As***	0.15±0.01	0.03±0.01	0.13±0.02	0.14±0.02	0.18±0.06	0.01±0.01	0.30±0.03	0.04±0.01	0.08±0.01
Cd***	0.030±0.009	<LOD	0.016±0.006	0.073±0.006	0.012±0.001	0.005±0.001	0.030±0.002	0.022±0.006	0.02±0.002
Co***	0.03±0.01	0.02±0.01	0.10±0.02	0.02±0.001	0.08±0.005	0.02±0.001	0.01±0.005	0.02±0.008	0.02±0.01
Cr***	0.10±0.008	0.29±0.005	<LOD	0.06±0.008	<LOD	0.01±0.006	<LOD	0.04±0.007	0.02±0.001
Mo***	0.50±0.002	<LOD	<LOD	0.07±0.02	0.20±0.02	0.07±0.02	0.03±0.02	0.06±0.02	0.04±0.02
Ni***	0.42±0.015	0.60±0.144	0.44±0.065	0.26±0.063	0.20±0.05	0.04±0.001	0.12±0.037	0.22±0.010	0.12±0.03
Pb***	0.12±0.01	<LOD	0.11±0.01	0.16±0.031	0.13±0.02	<LOD	0.13±0.044	0.06±0.01	0.05±0.01
Se***	0.24±0.20	<LOD	0.37±0.02	0.08±0.01	0.30±0.01	<LOD	0.08±0.01	0.21±0.01	0.13±0.01
V***	0.130±0.02	<LOD	0.111±0.01	0.025±0.01	0.105±0.01	0.04±0.01	0.028±0.01	0.03±0.01	0.02±0.01

A nonparametric multiple test (Kruskal–Wallis) was applied with p values: ns not significant; *0.05; **0.01; ***0.001. Values are expressed in mg/kg

Comparison of elemental composition of bilberries sampled from randomly selected natural forest stands in the Baltic Sea region countries (Latvia, Lithuania, Finland) and Norway demonstrates more variability than nationwide sampling in Latvia (Table 3). However, elemental composition of bilberries from Northern Europe shows differences in element concentrations in berries sampled in Latvia, Lithuania and Finland on one hand and in Norway on the other hand (Table 3). In bilberries from Norway, higher concentrations of major elements (K, Na, Ca, Mg, Mn, Fe, P) was found compared to berries sampled in the studied Baltic Sea region countries





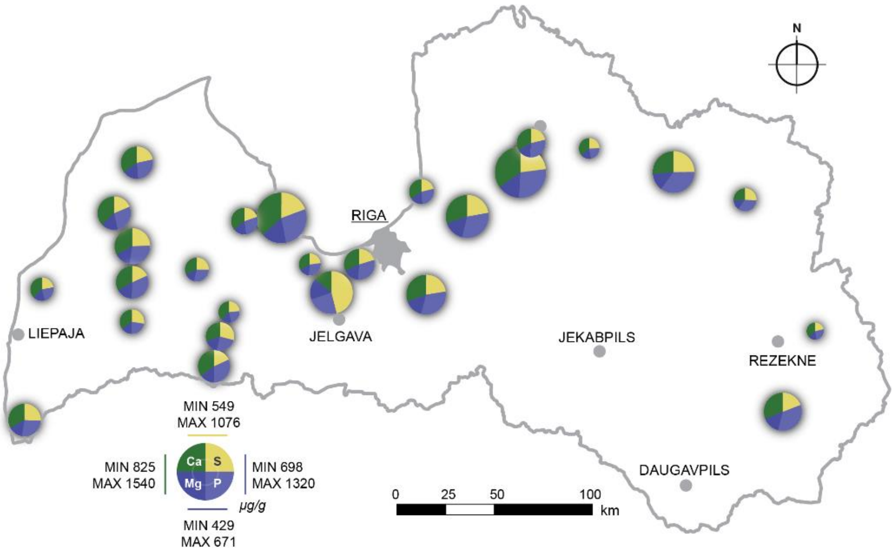


Figure 1. Elemental composition of bilberries in Latvia.

Table 3. Concentrations (mg/kg) of elements in bilberry samples from Northern Europe.

Element	Latvia	Lithuania	Finland	Norway
Ca***	971±32	1020±46	1134±72	1161±56
K***	5662±66	5755±116	5083±72	5339±54
Mg***	436±34	439±43	471±14	532±28
Na***	15±4	9±3	9±3	42±2
P***	868±14	1021±12	1074±18	1234±15
S***	791±18	741±26	617±21	815±21
Al***	27.8±6.8	20.7±3,2	15.9±7.1	16.8±1.8
B***	4.52±0.4	4.84±0.73	5.17±0.53	6.28±0.34
Ba***	9.71±0.08	10.16±0.09	10.34±0.07	12.73±0.08
Cu***	4.22±0.39	5.35±0.32	3.57±0.29	3.42±0.42
Fe***	16.5±2.3	81.8±3.2	10.9±2.8	18.1±2.9
Mn***	32.7±1.1	243.3±11.8	154.8±21.2	216.6±18.2
Si***	12.7±4.7	39.6±5.9	26.7±3.2	20.9±3.9
Sr***	1.14±0.40	2.36±0.62	2.71±0.28	2.14±0.48
Zn***	6.5±0.4	7.8±0.4	5.5±0.5	7.6±0.3
As***	0.12±0.01	0.16±0.01	0.03±0.01	0.12±0.02
Cd***	0.049±0.001	0.033±0.002	0.021±0.006	0.033±0.004
Co***	0.070±0.01	0.067±0.03	0.102±0.03	0.049±0.02
Cr***	0.213±0.008	0.177±0.008	0.149±0.007	0.297±0.009
Mo***	0.073±0.002	0.091±0.002	0.281±0.002	0.096±0.002
Ni***	0.428±0.019	0.455±0.015	0.322±0.013	0.089±0.016
Pb***	0.147±0.012	0.456±0.023	0.213±0.016	0.247±0.037
Se***	0.427±0.027	0.347±0.022	0.646±0.025	0.619±0.023
V***	0.067±0.014	0.157±0.021	0.050±0.018	0.071±0.014

A nonparametric multiple test (Kruskal–Wallis) was applied with p values: ns not significant; *0.05; **0.01; ***0.001. Values are expressed in mg/kg.

Considering the significant effect of origin on the composition of blueberries and bilberries, development of origin authentication methods would be of importance to exclude the forgery. As a prospective approach can be suggested the use of elemental composition analyses as well as light stable element isotope ($\delta^{13}C$, $\delta^{15}N$, $\delta^{18}O$) ratio analyses, used for authentication of wine and table grape origin [27], origin of goji berries [28] and other berries [29]. Figure 2 shows light stable element isotope ($\delta^{13}C$, $\delta^{15}N$, $\delta^{18}O$) ratio values in commercial blueberries samples from different countries. The most significant changes appear in $\delta^{15}N$ values (-12,9 – 3,7 ‰). Less but still significant changes are seen in $\delta^{18}O$ values (25,7 – 38,3 ‰) and the lowest changes are in $\delta^{13}C$ values (-23,9 – -28,6 ‰). $\delta^{15}N$ most commonly is used as nitrogen cycle indicator in different plants [30]. $\delta^{15}N$ value is dependent on many factors, for example on the availability of nitrogen in soil or nitrogen source in plants, atmosphere, soil, fertilizer as well as nitrogen uptake processes in plant itself [31]. However, for plants it indicates fertilization practices. $\delta^{15}N$ values are not characteristic for large geographical areas but is more specific to each location (farm). The comparison of $\delta^{15}N$ values in commercially available blueberries on our study (Figure 2) demonstrates differing patterns of blueberry fertilization. In some samples, $\delta^{15}N$ values varies from -6 to – 12 ‰, while in cases of organic farming with minimal fertilizer application, $\delta^{15}N$ values are in range from –2 to 5 ‰ as it was found in case of different blueberry cultivars from one farm in Latvia (Figure 3). Also, in wild bilberries from different places in Latvia (sampled in forests), $\delta^{15}N$ values are in range from –2 to 5 ‰. Thus, this interval of $\delta^{15}N$ values can be considered as indicative for natural berry cultivation (growth) conditions.

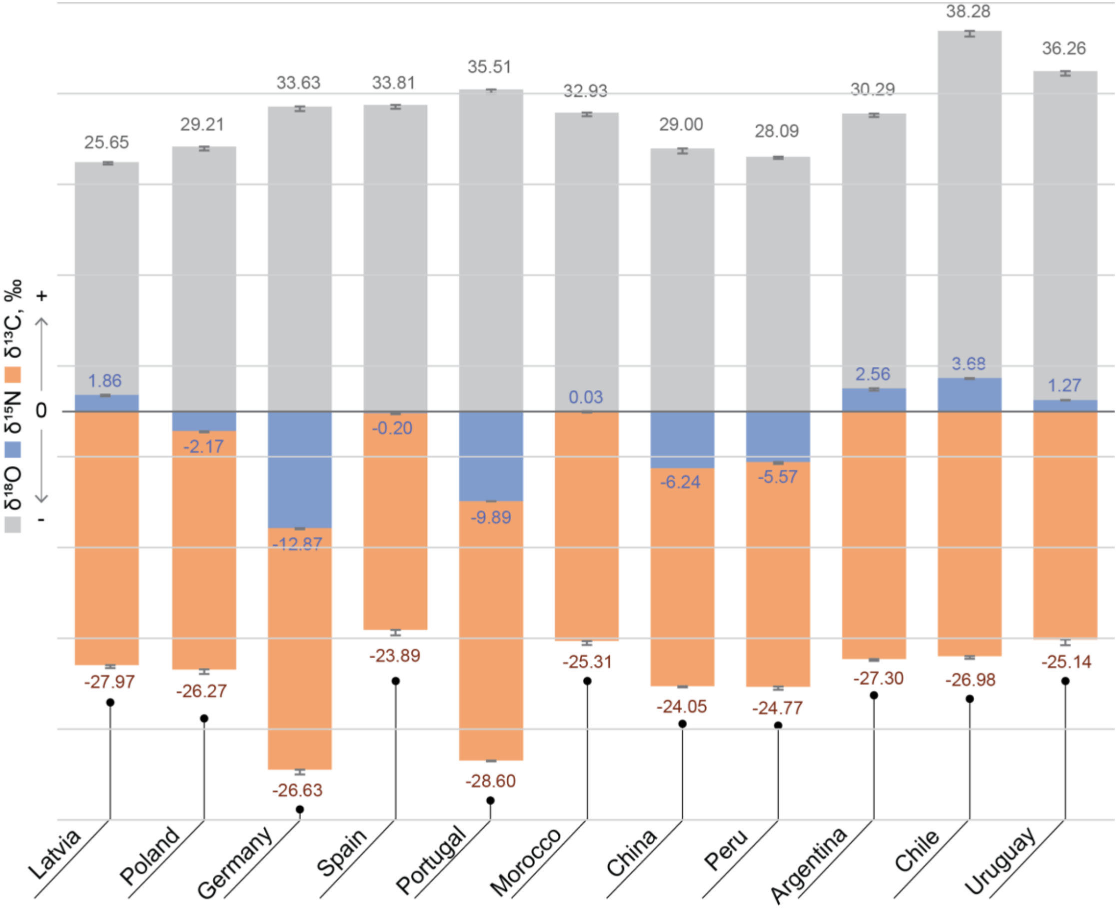


Figure 2. Light stable element isotope ($\delta^{13}C$, $\delta^{15}N$, $\delta^{18}O$) ratio values in blueberry from different countries.

δ^{18}O values are more characteristic to the geographical location and usually are associated with the climatic conditions, such as temperature [32]. From δ^{18}O values in blueberries from different countries, somewhat similar pattern as in the case of δ^{18}O in water can be seen. Because of natural water cycle and precipitations heavy oxygen isotope (^{18}O) tends to concentrate more in oceans [33], the lower δ^{18}O values indicate distance from the ocean. Similar pattern can be seen in Figure 4; countries with distance from ocean (Latvia, Poland, continental China) have lower d^{18}O values (25 – 29 ‰) while countries closer to oceans and warmer climate, the d^{18}O value is larger than 30‰.

δ^{13}C values are associated with the photosynthetic cycle of plants [34]. As all the studied blueberry varieties are considered as C3 plants [29] the changes in these values are least significant. Of course, there are some differences which are most likely related to climatic conditions in which they are growing, such as annual mean temperature and availability of sunlight. It is evident that light stable isotope ratio analysis can help to identify the place of origin of studied berries as well as can tell significant amount of information from their growth conditions.

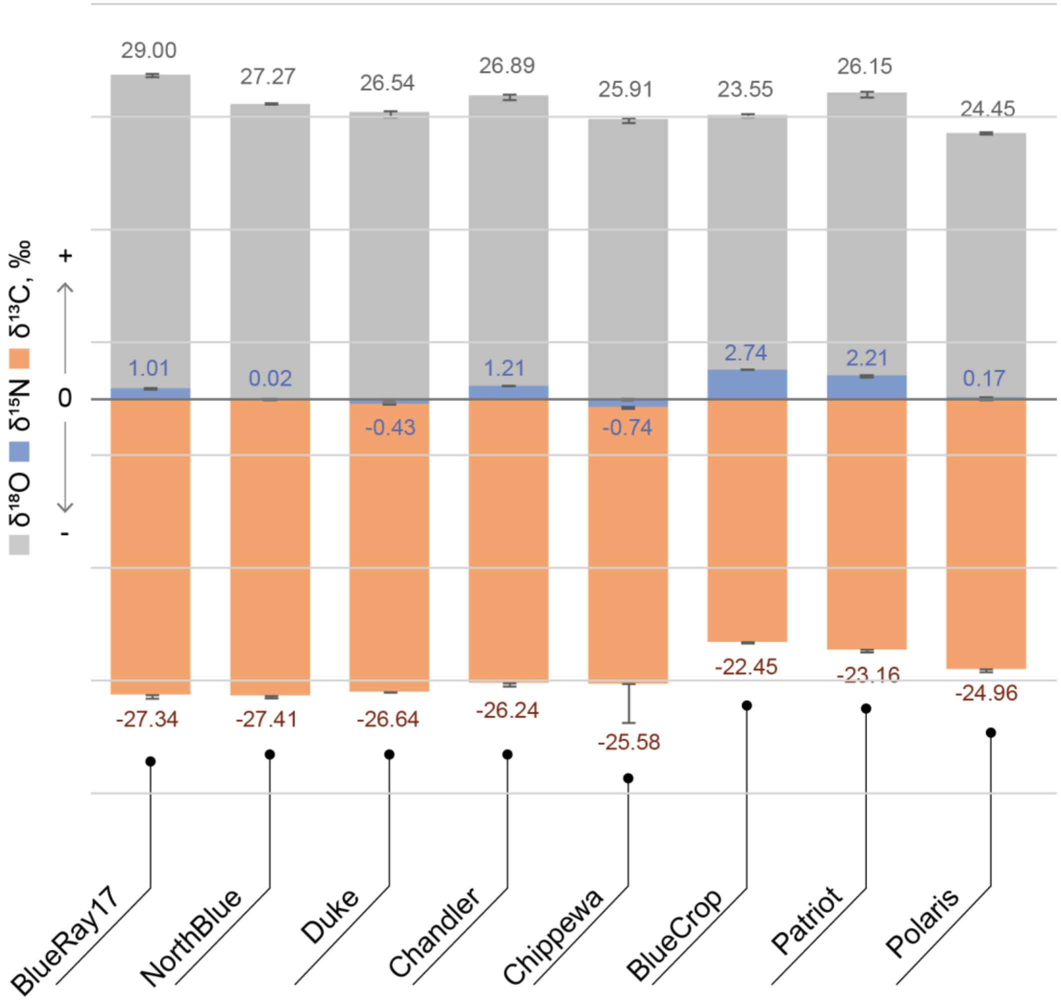


Figure 3. Light stable element isotope (δ^{13}C, δ^{15}N, δ^{18}O) ratio values in different varieties of blueberry from one location in Latvia.

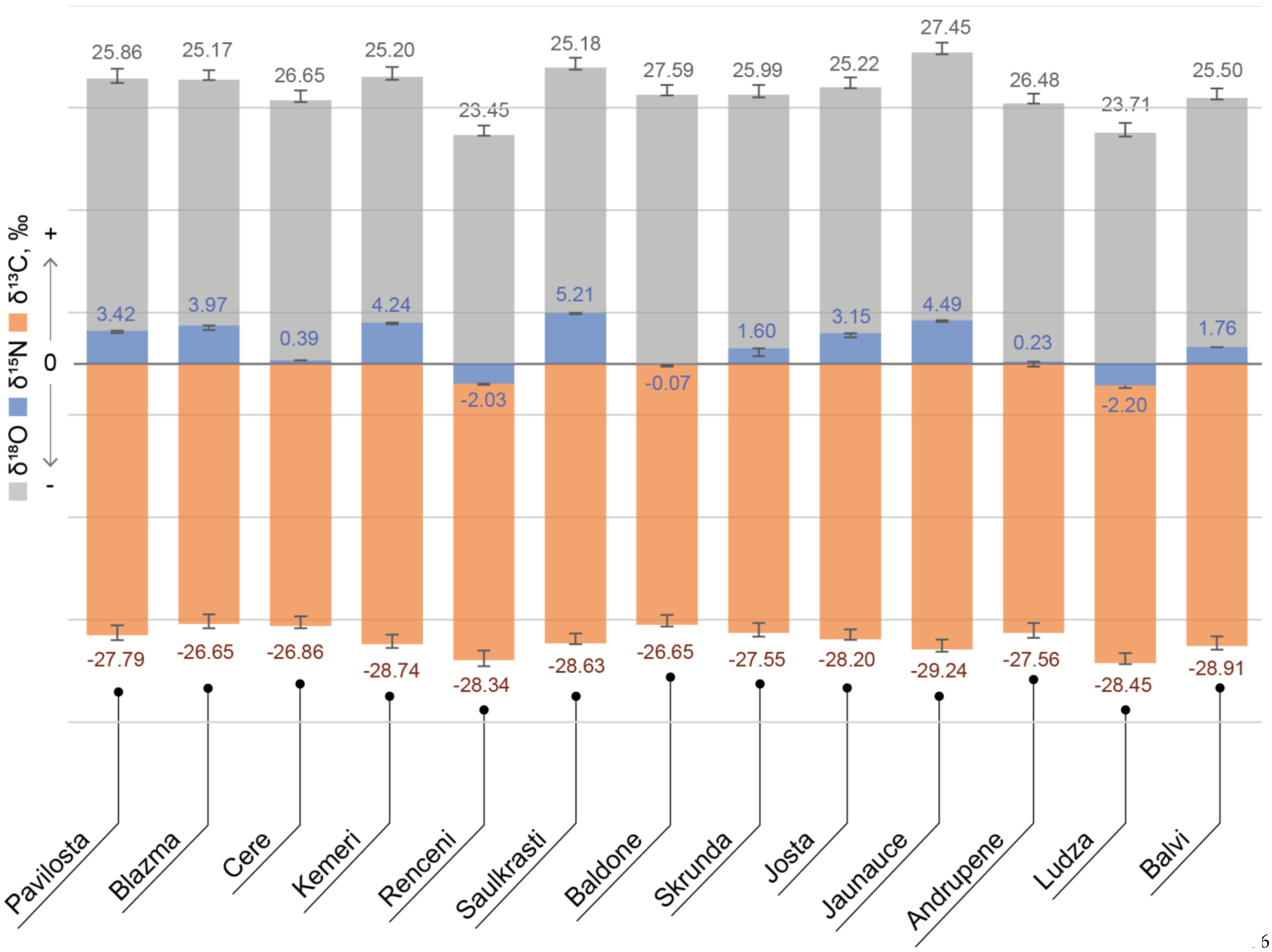


Figure 4. Light stable element isotope ($\delta^{13}C$, $\delta^{15}N$, $\delta^{18}O$) ratio values in bilberries from different places in Latvia.

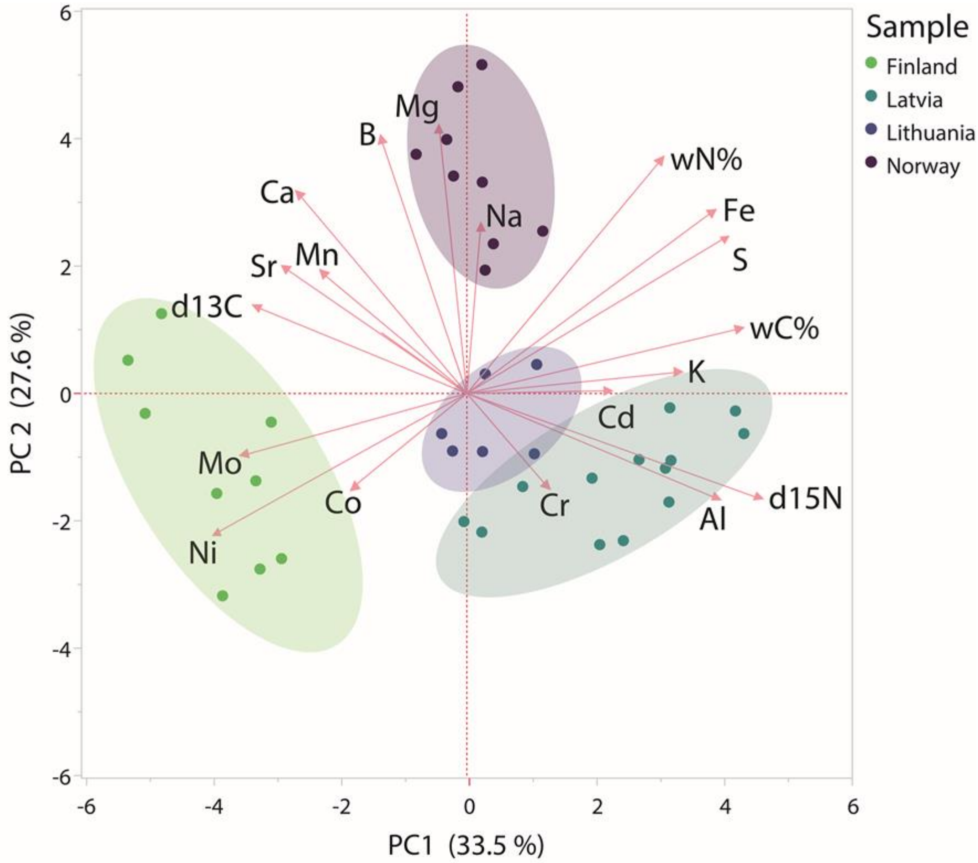


Figure 5. Principal component analysis of metal and light stable isotope contents in bilberries harvested in the Baltic Sea region (*n*=*9*). Ellipses represent 95% confidence intervals.

The obtained data from metal and stable light isotope analysis in bilberries, collected from Finland, Latvia, Lithuania, and Norway, was analysed using principal components analysis to visualize the possible differences among the regions of berry harvest. Samples gathered in their respective countries clustered together within the matrix, and the analysed data explained 61.1% of the data variability (Figure 5). The loadings plot indicates presence of certain metals in higher or lower concentration in the respective countries, which could be explained by the differences in the geochemical composition. Clusters of Lithuania and Latvia overlap (Figure 5) and these neighbouring countries (Baltic States) are known to share closely related types of soils [26]. The use of multivariate methods for distinguishing among groups of parameters and their variables could be successfully used to identify specific regions of berry product origin, however, to identify specific countries, even more detailed analysis would be necessary. IRMS data has been previously successfully used to identify the origin of meat [34], extra virgin olive oil [35], fruits and vegetables [36, 37]. However, an approach where in addition to stable isotope ratios, also metal analysis are used, could provide more specific method to determine the place of origin in greater detail. Additionally, similarly to previous studies where IRMS has been used to determine adulteration in fruit juices [38], essential oils [39], instant coffee [40], such approach could be used for bilberry products, which are often diluted with much cheaper, cultivated blueberries, or even other fruits.

Author Contributions: L.K. contributed to the conception of the study, data interpretation and drafted the manuscript. I.M. performed the formal analysis and investigation. M.B. contributed to the investigation of the plant material. K.S. contributed to the visualization of the data. M.K. critically revised the manuscript and contributed to the data interpretation and discussion, original draft preparation. All authors have read and agreed to the published version of the manuscript.

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