



# 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 16 blueberries (Vaccinium corymbosum L.) are consumed worldwide and their consumption have a 17 trend of stable increase. Thus, of a special importance are studies of the composition of these berries 18 considering their wide use in ethnomedicine, for juice and jam production, functional food as well 19 as the use in preparations of extracts that can have applications in pharmaceutical and cosmetics 20 industries. The aim of this study was to characterise elemental and isotopic composition, variation 21 in element concentration as well as to develop tools for berry authenticity and quality control of 22 bilberries gathered in Northern Europe and commercially available blueberry samples from across 23 the World. The elemental composition of berries was analysed using inductively coupled plasma 24 with optical emission detection, while isotope ratio mass spectrometry (IRMS) was used for deter-25 mination of isotope ratio values. The results demonstrated detectable differences between bilberry 26 macro- and microelement values. IRMS analysis of blueberries revealed significant differences in 27 isotope ratios based on the place of origin, which indicates the possibility to use this analytical 28 method for authenticity testing. In none of the samples, pollution was detected, even though there 29 are indications of different growth conditions and geochemical differences affecting bilberry com-30 position. 31

Keywords: blueberries 1; trace elements 2; heavy metals 3; light stable isotope ratio 4; pollution 5;32authenticity 6; bilberries 733

## 1. Introduction

Consumption of berries is becoming increasingly popular worldwide due to health 36 benefits and excellent taste properties. Growing popularity increases production and con-37 sumption of two Vaccinium genus (Ericacae family) berries - wild bilberries (Vaccinium 38 myrtillus) and cultivated blueberries (Vaccinium corymbosum). Consumers are becoming 39 more health-conscious, and fresh berries with attractive appearance, balanced sweet-sour 40 taste and high nutritional and health beneficial value are appreciated [1]. Commercial pro-41 duction of blueberries has reached 682,790 tons (80.2 % in Americas, 18.2 % in Europe) [2], 42 and picking of wild bilberries in Northern Europe and Russia reach several hundreds of 43 tons yearly with stable increasing trend. Widely recognised are the various health-44

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promoting properties of blueberries and bilberries as their consumption can reduce risk 45 of many infectious and degenerative disease [3,4]. Blueberry and bilberry fruits have been 46 used fresh, dried and as juice for alleviation for gastrointestinal tract and diabetes, but 47 herbal supplements containing these berries are available in the market as aids to improve 48 vision, to treat diarrhoea, considering their antimicrobial, anti-inflammatory and antioxi-49 dant properties [5-8]. Biological and pharmacological activity of blueberries and bilberries 50 are associated with high concentrations in their composition of polyphenolics, especially 51 anthocyanins and their capacity to scavenge oxygen and other radical species and thus 52 reduce the oxidative stress [9]. Additionally, berry lipids, such as terpenes, sterols, un-53 saturated fatty acids, and waxes are contributing to the biological activities of these berries 54 and their extracts [10]. 55

Another group of valuable components of blueberries and bilberries are mineral sub-56 stances (K, Ca, Mg, P, Fe, Mn, Zn and others) which are essential for sustaining various 57 functions of the human body and are important components of the diet. At the same time 58 many inorganic elements (Cd, Pb, As and others) are non-essential and might indicate 59 presence of anthropogenic pollution [11]. Thus, of a special importance are studies of the 60 mineral composition of the berries as well as analysis of the presence of toxic trace ele-61 ments. The environmental pollution sources on the presence of toxic trace elements has 62 been studied to identify the adverse impacts of known pollution sources, such as metal 63 processing industries. For example, significantly elevated concentrations of trace elements 64 in comparison with the background pollution sites were found in berries sampled in vi-65 cinity of ferrochrome and stainless-steel factories in Northern Finland [12, 13]. As a result 66 of mining and metal processing industries, high concentrations of Ag, As, Be, Bi, Br, Cd, 67 Hg, I, Ni, Pb, Sb, and Tl were found in berries from mining areas in Northern Sweden [14]. 68 Recently, the impact of wood ash applications in forests on the elemental composition in 69 berries has been studied and risks related to the increasing concentrations of trace ele-70 ments has been found [15]. Another aspect on the berry quality studies offers evaluation 71 of element concentrations in edible products available on the market [16]. However, in 72 this case it is nearly impossible to relate the found concentrations with the origin of the 73 samples as possible adulteration of products may exist, thus requiring development of 74 berry origin authentication methods. 75

As the growth conditions as well as metal accumulation patterns for different plant 76 species vary, it is important to study contamination levels in species which are of im-77 portance for human consumption, such as blueberries and bilberries. Only few studies 78 with only few studied elements are dedicated to elemental composition of trace elements 79 in blueberries and bilberries [15,17,18]. Another important aspect is related to possibilities 80 to identify the origin and cultivation practices based on the compositional analysis of ber-81 ries. The aim of this study was to characterise elemental and isotopic composition, con-82 centration variability of elements as well as develop and suggest tools for berry authen-83 ticity and quality control of bilberries gathered in Northern Europe and commercially 84 available blueberry samples from across the World. 85

#### 2. Materials and Methods

#### 2.1. Sampling

The fruits of wild bilberries (Vaccinium myrtillus L.) were collected during 2018 – 2020 88 vegetation seasons in 26 sampling sites in the territory of Latvia as well as in three sam-89 pling sites in Norway, Finland, and Lithuania. Samples of different varieties of highbush 90 blueberries (Vaccinium corymbosum L.) were sampled in 2018 in farm "Strelnieki" (Latvia). 91 Once the samples were collected, they were frozen and stored in freezer at -20 °C. Com-92 mercial samples of blueberries were obtained from supermarkets during 2018 - 2020 and 93 the country of origin was indicated on the labels being Peru, Argentina, Uruguay, Chile, 94 Morocco, Spain, Germany, Poland, and Latvia. For analysis, the samples were lyophilised, 95

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and homogenised using pestle and mortar. Agate pestle and mortar was used for homog-96 enising 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 99 8 mL 65% HNO<sub>3</sub> (Sigma Aldrich, ≥65%) and 2 mL 30% H<sub>2</sub>O<sub>2</sub> (Enola, Latvia). The tubes 100 were closed (to provide high pressure) prior to sample digestion using a microwave sys-101 tem (Milestone Advanced Microwave digestion system, Ethos Easy) at 200 °C for 30 min. 102 The resulting samples were diluted to 50 mL with deionised water (Millipore, 7,4  $\mu$ S/cm). 103 The concentrations of inorganic elements were determined by inductively coupled plasma 104 spectrometry with optical emission detection (Thermo Scientific iCAP 700 series ICP spec-105 trometer). The elements determined were Al, As, B, Ba, Ca, Co, Cr, Cu, Fe, K, Li, Mg, Mn, 106 Mo, Na, Ni, Pb, Rb, Sb, Se, Sn, Sr, Ti, Tl, V, and Zn. The detection limit was 1–10 ppb for 107 Al, Ca, and Fe, 0.1–1 ppb for Mg, K, and Na, and 0.1 ppb for all other elements. The con-108 centrations were expressed per dry weight of berries. The accuracy of the analysis of berry 109 samples was checked by the following certified reference materials: SRM 3287 - Blueberry 110 (Fruit) (National Institute of Standards and Technology (NIST) and the National Institutes 111 of Health Office of Dietary Supplements). The difference between the data of berry sam-112 ples analysed and the reference materials was generally lower than 15% for all elements. 113

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 115 weighed into tin capsules (EuroVector) for C and N analyses and 1.00 mg into silver cap-116 sules (EuroVector) for O analysis, and the capsules were carefully folded. All samples 117 were prepared in triplicate. Glutamic acid (C and N analysis) and sucrose (O analysis) 118 laboratory standards (Sigma Aldrich) were used for calibration (0.2, 0.5, 0.8, 1.0 and, 1.5 119 mg). To monitor the stability of the obtained values, one glutamic acid or sucrose control 120 sample (1.0 mg) was analysed after every 10 samples. To verify trueness of the obtained 121 results, glutamic acid (USGS-40, d<sup>13</sup>C -26.39 ± 0.04 ‰ VPDB, d<sup>15</sup>N -4.52 ± 0.06 ‰ AIR, wC 122 = 40,8%, wN = 9,52%) and benzoic acid (IAEA-601, d<sup>18</sup>O 23.14 ± 0.19 ‰ VSMOW, wO = 123 26,2%) reference materials were used. The ratio of C, N, and O isotopes in samples was 124 measured on the isotope ratio mass spectrometer (Nu Horizon, acceleration voltage: 5kV, 125 mass range: 2–100 Da, mass dispersion: > 30 cm) using an element analyser (EuroVector 126 Euro EA3000) with quartz combustion column filled with chromium (III) oxide and sil-127 vered cobaltous oxide (1030°C) and a quartz reduction tube filled with copper shards (650 128 °C) for the determination of C and N isotope ratio. For the determination of the O isotope 129 ratio, high-temperature element analyser unit was used (EuroVector HTEA PyrOH) with 130 outer ceramic tube and inner glassy carbon tube filled with glassy carbon chips and nick-131 elled carbon (1420°C). The results were processed by the Nu Stable Control Software 132 v1.69. 133

#### 2.4. Statistical analysis of results

The Kruskal-Wallis nonparametric test was used to detect differences among metal 135 concentrations in samples with different origin or variety. Statistical data analysis, includ-136 ing Principal Component Analysis (PCA) of metal concentration and stable isotope ratios 137 were done using statistical data discovery software SAS JMP®, Version 14 (SAS Institute 138 Inc., Cary, NC, USA).

### 3. Results and Discussion

In blueberry and bilberry wet digested samples, total element concentrations were 141 determined by ICP OES method. Sinve highbush blueberries are cultivated, many 142

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varieties are available differing in berry ripening time, size, taste, and other properties 143 [19]. Differences in element concentrations in eight varieties of blueberries growing on 144 peat soil in one location (biological farm in Latvia) were compared (Table 1). Our results 145 show that blueberries are a rich source of mineral elements, especially K, Ca, Mg, P, and 146 S. Blueberries as a source for K, Ca, Mg, and Mn is shown also in other studies [17,18]. 147 However, the concentrations of toxic trace elements (Co, Se, Pb, Ni, Cr, Co, As) have been 148 shown to be lower than in berries growing on soils with elevated metal concentrations 149 [20]. For nearly all elements, differing element concentrations in berries were found in 150 studied cultivars, thus, reflecting impact of berry cultivars. 151

As blueberries are cultivated worldwide it was of interest to compare their elemental 152 composition also in commercially available samples. Elemental composition was analysed 153 in samples obtained from nine countries for the period 2018 - 2020 and altogether 24 ele-154 ments were analysed (Table 2). In all studied samples, the highest concentrations were 155 found for the elements important for human consumption, namely K, Ca, Mg, Fe, P, S, 156 and Mn. However, the variability was high and, for example, the K concentration in ber-157 ries from South America or North Africa is twice as high than in samples from North 158 Europe, where the cultivation usually takes place in mineral poor peat soils. In commer-159 cially available cultivated berry samples, values of trace elements were found to be low, 160 but comparable with element concentration values, found in other studies [17,18]. How-161 ever, variability of some of trace elements in some of berry samples was significantly 162 higher than in others For example, in samples from Europe, V concentration was 163  $0.03\pm0.01$ , when in samples for other countries, the concentration was nearly five times 164 higher. Similar differences in concentrations were found also for elements, such as Mo, Se, 165 Pb, and Ni. Considering the amounts of blueberries that are cultivated and consumed, 166 and the toxicity values of the studied elements [21], it can be concluded that the trace 167 element concentrations found in the commercially available berry samples do not pose 168 risk to human health. However, considering the high volumes of blueberries sold on the 169 markets worldwide, it would be important to establish quality criteria of elemental com-170 position of trace elements in their composition. 171

Values of element concentrations in bilberries in territory of Latvia (64 000 km<sup>2</sup>) also 172 demonstrate variability and are shown in Figure 1. Maximal values of the studied ele-173 ments are associated with the known local and regional environmental pollution sites. For 174example, elevated concentrations in western part of Latvia indicates presence of industrial 175 pollution of long-time functioning cement production as well as metallurgical factories, 176 as it has been found previously [25]. Another major factor influencing bilberry elemental 177 composition seems to be geochemical differences in soils of Latvia, for example differ-178 ences in Ca/Mg ratio for different parts of the country [26]. Geochemical characteristics of 179 soil is one of the major factors affecting element composition of bilberries, as it has been 180 stated in earlier studies [13,16,22]. Nevertheless, the variability of concentrations of trace 181 elements in wild bilberries reflect specific pattern of metal and other trace element con-182 centrations in soils and thus can be considered as specific for the territory of Latvia. At the 183 same time, the low concentrations of toxic elements and heavy metals found in our study 184 in all locations can be considered low, especially if compared with concentrations found 185 in other studies [7,14,18,22], and Latvian bilberries can be considered as valuable source 186 of mineral substances and essential elements. 187





Elemen	'Patriot'	'Polaris'	'Bluecrop'	'Northblue'	'Chandler'	'Duke'	'Chippewa'	'Blueray 17'
t 1⁄***	4080+126	4822+118	5509+249	5247+190	5626+249	4955+126	6208+94	4706+284
<u>к</u> Са***	4080±130	4023±110	3309±249	229±52	382±14	4933±120	0200±94	4700±204
Ca Ma***	265+6	300±17	341±15	239±33 286±16	376+8	432±29	411±37 201±18	427±38
No***	203±0	122±14	57+0	200±10	112+10	208+10	291±16	<u> </u>
D***	770+22	755+10	57±9	00±9	671+4	200±19	97±14 712+07	05±0
I	779±22	755±19	/15±41	011±31	671±4	636±34	713±27	865±10
5***	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	$5.8 \pm 0.4$	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< td=""><td>0.013±0.001</td><td>0.036±0.001</td><td>0.015±0.009</td><td>0.021±0.002</td></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< td=""><td><lod< td=""><td>0.08±0.01</td><td>0.06±0.01</td><td><lod< td=""></lod<></td></lod<></td></lod<>	<lod< td=""><td>0.08±0.01</td><td>0.06±0.01</td><td><lod< td=""></lod<></td></lod<>	0.08±0.01	0.06±0.01	<lod< td=""></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< td=""><td>0.11±0.01</td><td>0.07±0.01</td><td>0.12±0.01</td><td>0.23±0.01</td></lod<>	0.11±0.01	0.07±0.01	0.12±0.01	0.23±0.01

<LOD

<LOD

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

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

 $0.018 \pm 0.005$ 

0.024±0.001

0.067±0.001

V\*\*\*

 $0.018 \pm 0.001$ 

0.061±0.004

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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	$1.58 \pm 0.40$	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	$4.0\pm0.4$	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< td=""><td>0.016±0.006</td><td>0.073±0.006</td><td>0.012±0.001</td><td>0.005±0.001</td><td>0.030±0.002</td><td>0.022±0.006</td><td>0.02±0.002</td></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< td=""><td>0.06±0.008</td><td><lod< td=""><td>0.01±0.006</td><td><lod< td=""><td>0.04±0.007</td><td>0.02±0.001</td></lod<></td></lod<></td></lod<>	0.06±0.008	<lod< td=""><td>0.01±0.006</td><td><lod< td=""><td>0.04±0.007</td><td>0.02±0.001</td></lod<></td></lod<>	0.01±0.006	<lod< td=""><td>0.04±0.007</td><td>0.02±0.001</td></lod<>	0.04±0.007	0.02±0.001
Mo***	0.50±0.002	<lod< td=""><td><lod< td=""><td>0.07±0.02</td><td>0.20±0.02</td><td>0.07±0.02</td><td>0.03±0.02</td><td>0.06±0.02</td><td>0.04±0.02</td></lod<></td></lod<>	<lod< td=""><td>0.07±0.02</td><td>0.20±0.02</td><td>0.07±0.02</td><td>0.03±0.02</td><td>0.06±0.02</td><td>0.04±0.02</td></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	$0.60 \pm 0.144$	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< td=""><td>0.11±0.01</td><td>0.16±0.031</td><td>0.13±0.02</td><td><lod< td=""><td>0.13±0.044</td><td>0.06±0.01</td><td>0.05±0.01</td></lod<></td></lod<>	0.11±0.01	0.16±0.031	0.13±0.02	<lod< td=""><td>0.13±0.044</td><td>0.06±0.01</td><td>0.05±0.01</td></lod<>	0.13±0.044	0.06±0.01	0.05±0.01
Se***	0.24±0.20	<lod< td=""><td>0.37±0.02</td><td>0.08±0.01</td><td>0.30±0.01</td><td><lod< td=""><td>0.08±0.01</td><td>0.21±0.01</td><td>0.13±0.01</td></lod<></td></lod<>	0.37±0.02	0.08±0.01	0.30±0.01	<lod< td=""><td>0.08±0.01</td><td>0.21±0.01</td><td>0.13±0.01</td></lod<>	0.08±0.01	0.21±0.01	0.13±0.01
V***	0.130±0.02	<lod< td=""><td>0.111±0.01</td><td>0.025±0.01</td><td>0.105±0.01</td><td>0.04±0.01</td><td>0.028±0.01</td><td>0.03±0.01</td><td>0.02±0.01</td></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 197 natural forest stands in the Baltic Sea region countries (Latvia, Lithuania, Finland) and 198 Norway demonstrates more variability than nationwide sampling in Latvia (Table 3). 199 However, elemental composition of bilberries from Northern Europe shows differences 200 in element concentrations in berries sampled in Latvia, Lithuania and Finland on one 201 hand and in Norway on the other hand (Table 3). In bilberries from Norway, higher con-202 centrations of major elements (K, Na, Ca, Mg, Mn, Fe, P) was found compared to berries 203 sampled in the studied Baltic Sea region countries 204





**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 208 significant; \*0.05; \*\*0.01; \*\*\*0.001. Values are expressed in mg/kg. 209

Considering the significant effect of origin on the composition of blueberries and bil-211 berries, development of origin authentication methods would be of importance to exclude 212 the forgery. As a prospective approach can be suggested the use of elemental composition 213 analyses as well as light stable element isotope ( $\delta^{13}$ C,  $\delta^{15}$ N,  $\delta^{18}$ O) ratio analyses, used for 214 authentication of wine and table grape origin [27], origin of goji berries [28] and other 215 berries [29]. Figure 2 shows light stable element isotope ( $\delta^{13}$ C,  $\delta^{15}$ N,  $\delta^{18}$ O) ratio values in 216 commercial blueberries samples from different countries. The most significant changes 217 appear in  $\delta^{15}$ N values (-12,9 – 3,7 ‰). Less but still significant changes are seen in  $\delta^{18}$ O 218 values (25,7 – 38,3 ‰) and the lowest changes are in  $\delta^{13}$ C values (-23,9 – -28,6 ‰).  $\delta^{15}$ N 219 most commonly is used as nitrogen cycle indicator in different plants [30].  $\delta^{15}N$  value is 220 dependent on many factors, for example on the availability of nitrogen in soil or nitrogen 221 source in plants, atmosphere, soil, fertilizer as well as nitrogen uptake processes in plant 222 itself [31]. However, for plants it indicates fertilization practices.  $\delta^{15}$ N values are not char-223 acteristic for large geographical areas but is more specific to each location (farm). The 224 comparison of  $\delta^{15}N$  values in commercially available blueberries on our study (Figure 2) 225 demonstrates differing patterns of blueberry fertilization. In some samples,  $\delta^{15}N$  values 226 varies from -6 to – 12 ‰, while in cases of organic farming with minimal fertilizer appli-227 cation,  $\delta^{15}$ N values are in range from -2 to 5 ‰ as it was found in case of different blue-228 berry cultivars from one farm in Latvia (Figure 3). Also, in wild bilberries from different 229 places in Latvia (sampled in forests),  $\delta^{15}$ N values are in range from -2 to 5 ‰. Thus, this 230 interval of  $\delta^{15}N$  values can be considered as indicative for natural berry cultivation 231 (growth) conditions. 232



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

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δ<sup>18</sup>O values are more characteristic to the geographical location and usually are asso-236 ciated with the climatic conditions, such as temperature [32]. From  $\delta^{18}$ O values in blueber-237 ries from different countries, somewhat similar pattern as in the case of  $\delta^{18}$ O in water can 238 be seen. Because of natural water cycle and precipitations heavy oxygen isotope (18O) 239 tends to concentrate more in oceans [33], the lower  $\delta^{18}$ O values indicate distance from the 240 ocean. Similar pattern can be seen in Figure 4; countries with distance from ocean (Latvia, 241 Poland, continental China) have lower d<sup>18</sup>O values (25 – 29 ‰) while countries closer to 242 oceans and warmer climate, the d<sup>18</sup>O value is larger than 30‰. 243

 $\delta^{13}$ 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. 250



**Figure 3.** Light stable element isotope ( $\delta^{13}$ C,  $\delta^{15}$ N,  $\delta^{18}$ O) ratio values in different varieties of blueberry from one location in Latvia. 254

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**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 265 from Finland, Latvia, Lithuania, and Norway, was analysed using principal components 266 analysis to visualize the possible differences among the regions of berry harvest. Samples 267 gathered in their respective countries clustered together within the matrix, and the ana-268 lysed data explained 61.1% of the data variability (Figure 5). The loadings plot indicates 269 presence of certain metals in higher or lower concentration in the respective countries, 270 which could be explained by the differences in the geochemical composition. Clusters of 271 Lithuania and Latvia overlap (Figure 5) and these neighbouring countries (Baltic States) 272 are known to share closely related types of soils [26]. The use of multivariate methods for 273 distinguishing among groups of parameters and their variables could be successfully used 274 to identify specific regions of berry product origin, however, to identify specific countries, 275 even more detailed analysis would be necessary. IRMS data has been previously success-276 fully used to identify the origin of meat [34], extra virgin olive oil [35], fruits and vegeta-277 bles [36, 37]. However, an approach where in addition to stable isotope ratios, also metal 278 analysis are used, could provide more specific method to determine the place of origin in 279 greater detail. Additionally, similarly to previous studies where IRMS has been used to 280 determine adulteration in fruit juices [38], essential oils [39], instant coffee [40], such ap-281 proach could be used for bilberry products, which are often diluted with much cheaper, 282 cultivated blueberries, or even other fruits. 283

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