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Characterization and Differentiation of Wild and Cultivated Berries Based on Isotopic and Elemental Profiles

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Abstract: The isotopic content ($\delta^{13}C$, $\delta^{2}H$, $\delta^{18}O$) and concentrations of 30 elements (Li, Na, Mg, P, Ca, Ti, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, Ga, As, Rb, Sr, Ag, Cd, Ba, Pb, La, Ce, Pr, Nd, Sm, Eu, Gd, and Tb) were determined in different wild and cultivated berries (raspberry, seaberry, blackberry, cranberry, and blueberry). Partial least squares discriminant analysis (PLS-DA) was applied in order to develop models for differentiating berries according to their botanical origin and growing system. $\delta^{13}C$, $\delta^{2}H$, $\delta^{18}O$, Li, Na, Mg, P, Ca, V, Mn, Co, Ni, Zn, As, Rb, Sr, Ba, and Eu were identified as significant elements for the differentiation of berry species, based on which an 85% PLS-DA model accuracy was obtained. Similarly, the PLS-DA model developed for the growing system differentiation correctly classified 94.4% of the cultivated berries and 77.2% of the wild ones, based on the main predictors: $\delta^{13}C$, $\delta^{18}O$, Li, Na, Ca, Cr, Mn, Ni, Rb, and Ba. The developed PLS-DA model for the discrimination of wild blueberries from cultivated ones showed excellent levels of sensitivity (100%), specificity (100%), and accuracy (100%).

Keywords: wild berries; cultivated berries; isotopic content; elemental profile; PLS-DA

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1. Introduction

Due to consumer awareness regarding the consumption of health-beneficial foods with a positive effect on health, numerous plant-based foods are a part of the human diet. In this context, different species of berries have been recognized as being useful for human nutrition and health for thousands of years. They are some of the most essential and high-valued plant-based foods, representing one of the most important modern-day "superfoods" [1]. These forest fruits are versatile, being consumed fresh in juices and beverages, frozen, dried, or as extracts. Berry fruits are good sources of phenolic compounds (phenolic acid, flavonoids anthocyanins, and tannins), vitamins (ascorbic acid), and minerals [2], their consumption leading to a positive impact on human health. Fruits and herbal supplements containing berries have antimicrobial, anti-inflammatory, and antioxidant properties [3], and are used for the alleviation of indications related to the gastrointestinal tract and diabetes. Due to these properties, they are highly appreciated and consumed worldwide, their consumption having a trend of stable increase.

Thus, in recent years, berry trade and production are expanding globally, being in a period of continuous and rapid growth [4]. For example, between 2010 and 2019, blueberry production almost doubled, growing from 439,000 tons to around 1 million [5]. In addition, from 2010 to 2019, the world raspberry production has grown by 80%, from 373,000 tons to 684,000 tons [6]. In Romania, on the sunny slopes of the Carpathians, significant quantities of berries grow. In addition, more and more berry farms are appearing; Romania currently has over 350 berry growers but it also has an annual production that allows for export, especially since in recent years there has been a very high demand for these products. According to the Payments and Intervention Agency for Agriculture, the area cultivated

with forest fruits such as raspberries, blueberries, and currants has increased seven times since 2010 and reached 5000 hectares. Consumers prefer wild berries versus cultivates ones for many reasons: wild berries have a more intense flavor and less sugar compared with their cultivated counterpart; wild berries maintain their shape, color, and texture when used in baking or undergoing manufacturing processes. They freeze very well and maintain their quality for over 2 years when frozen [7].

Given the importance of the consumption of berry fruits, there is a need for studies regarding the quality and authenticity of different species of berries. This issue is important both for consumers and producers. Over the last few decades, different analytical methods, such as isotope ratio mass spectrometry (IRMS), inductively coupled plasma mass spectrometry (ICP-MS) or inductively coupled plasma optical emission spectrometry (ICP-OES), gas chromatography mass spectrometry (GS-MS), and high-performance liquid chromatography (HPLC) have been employed for the investigation of berry fruits based on isotopic fingerprint [3,8,9], elemental profile [3,10,11], volatile compounds [12–14], and phenolic compounds content [15–17]. In addition, the analytical results corroborated with chemometric methods (e.g., PCA, LDA, PLS-DA, etc.) represent important tools for the authenticity and quality control of berries, and also for tracing similarities or differences between different fruit species [1,8–11,18–20]. The aim of this preliminary study was to characterize the isotopic and elemental composition ($\delta^{13}C$, $\delta^{2}H$, $\delta^{18}O$, Li, Na, Mg, P, Ca, Ti, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, Ga, As, Rb, Sr, Ag, Cd, Ba, Pb, La, Ce, Pr, Nd, Sm, Eu, Gd, and Tb), as well as to develop models for differentiating berry samples coming from the Romanian market according to the botanical origin (raspberry, seaberry, blackberry, cranberry, and blueberry) and growing system (wild and cultivated) using partial least squares discriminant analysis (PLS-DA). Additionally, the significant variables—those having the most differentiation potential—were identified.

2. Materials and Methods

2.1. Sample Description

A total of 40 berry samples were analyzed by isotope ratio mass spectrometry (IRMS) and inductively coupled plasma mass spectrometry (ICP-MS) techniques. The sample set consisted of five different types of berries: raspberries (*Rubus idaeus*, n = 9), seaberries (*Hippophae goniocarpa*, n = 6), blackberries (*Rubus fruticosus L.*, n = 6), cranberries (*Vaccinum vitis-idaea*, n = 9), and blueberries (*Vaccinum caesariense*, n = 10). The samples were collected from the Romanian market, 18 of them coming from commercial production (cultivated berries), and 22 being wild berries (Figure 1). After purchase, the berry samples were transported to the laboratory, and were prepared within a maximum of 24 h for further investigations.

2.2. Sample Preparation and Analysis

An amount of 0.5 kg of fresh fruit was purchased for each berry variety. From the entire homogenized quantity of each sample, a specimen of 5 g was taken for the preparation process. The water was extracted from each berry sample using a protocol based on cryogenic distillation under vacuum [21]. The isotopic fingerprints of ^{2}H and ^{18}O were determined from the obtained water. The dry fraction resulting from the cryogenic distillation procedure was divided into two parts: one was prepared in order to obtain the content of ^{13}C by isotopic determination; the other was prepared by a specific protocol for elemental analysis.

2.2.1. Sample Preparation and Stable Isotope Analysis

Using dry combustion (550 °C) in excess oxygen for 3 h, 5 mg of each berry sample was converted to CO_2. The resulting CO_2 was isolated from the other combustion gases by a cryogenic separation and then measured by isotope ratio mass spectrometry (IRMS).

Figure 1. The map of berry samples provenance.

The isotopic results are reported in "so-called" δ notation (isotopic composition or isotopic fingerprint/signature) relative to international standards, in compliance with Equation (1) [22]:

$$\delta^i X = \frac{R_{sample}}{R_{standard}} - 1, \quad (1)$$

where i is the mass number of the heavier isotope of the element X (e.g., ^{13}C, ^{2}H, ^{18}O), R_{sample} is the isotope number ratio of a sample ($^{13}C/^{12}C$, $^{2}H/^{1}H$, $^{18}O/^{16}O$), and $R_{standard}$ is that of the international standard (V_{PDB}—Vienna Pee Dee Belemnite for $\delta^{13}C$, and V_{SMOW}—Vienna Standard Mean Ocean Water for $\delta^{2}H$ and $\delta^{18}O$). The delta values are multiplied by 1000 and are expressed in units "per mil" (‰).

To obtain the ^{13}C isotopic fingerprint of CO_2 resulting from berries, an isotope ratio mass spectrometer (Delta V Advantage, Thermo Scientific, Waltham, MA, USA) in line with a dual inlet system was used. Every day, before the samples' analysis, one working standard was measured. Its calibration was performed versus NBS-22 oil (IAEA—International Atomic Energy Agency)-certified reference material ($\delta^{13}C_{VPDB} = -30.03$‰). All berry samples were measured in duplicate. The uncertainty was ±0.3‰.

A liquid-water isotope analyzer (DLT–100, Los Gatos Research, San Jose, CA, USA) was used to record the isotopic fingerprint of $\delta^{2}H$ and $\delta^{18}O$ from the water previously extracted from berries. A set of five working standards was available (working standard 1, $\delta^{18}O = -19.57 \pm 0.1$‰ and $\delta^{2}H = -154.1 \pm 1$‰; working standard 2, $\delta^{18}O = -15.55 \pm 0.1$‰ and $\delta^{2}H = -117.0 \pm 1$‰; working standard 3, $\delta^{18}O = -11.54 \pm 0.1$‰ and $\delta^{2}H = -79.0 \pm 1$‰; working standard 4, $\delta^{18}O = -7.14 \pm 0.1$‰ and $\delta^{2}H = -43.6 \pm 1$‰; working standard 5, $\delta^{18}O = -2.96 \pm 0.1$‰ and $\delta^{2}H = -9.8 \pm 1$‰). The uncertainty was ±0.2‰ for $\delta^{18}O$ and ±1‰ for $\delta^{2}H$.

2.2.2. Sample Digestion Procedure and Multi-Element Analysis

The dried berries were ground using an agate mortar and pestle. Approximately 0.2 g of berry powder was digested using 7 mL of 65% HNO_3 and 1 mL of 30% H_2O_2. Digestions were performed in closed PTFE vessels at 200 °C for 30 min using a high-

pressure microwave (Speed ENTRY by Berghof®). The digested solutions were left to cool at room temperature, then diluted with ultrapure water (resistivity 18 MΩ cm^{-1}, Millipore, Bedford, MA, USA water purification system) to a final volume of 50 mL. Elemental concentrations (Li, Na, Mg, P, Ca, Ti, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, Ga, As, Rb, Sr, Ag, Cd, Ba, Pb, La, Ce, Pr, Nd, Sm, Eu, Gd, and Tb) were determined by ICP–MS, using an ELAN RDC (e) mass spectrometer (PerkinElmer SCIEX®, USA) equipped with a Meinhart nebulizer. Multi-element calibration standard 3 (PerkinElmer Pure Plus) and multi-element calibration standard 2 (PerkinElmer Pure Plus) were used to prepare intermediate multi-element standard solutions for ICP-MS measurements.

2.3. Chemometric Analysis

Analysis of variance (ANOVA) was used in this study to assess whether there were statistically meaningful variations in the elemental concentrations (Li, Na, Mg, P, Ca, Ti, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, Ga, As, Rb, Sr, Ag, Cd, Ba, Pb, La, Ce, Pr, Nd, Sm, Eu, Gd, and Tb) from different species of berries, such as raspberry, seaberry, blackberry, cranberry, and blueberry. With the aim of developing differentiation models for classifying berry samples according to the botanical origin (raspberry, seaberry, blackberry, cranberry, and blueberry) and growing system (wild and cultivated), partial least squares discriminant analysis (PLS-DA) was applied on isotopic and elemental data using the software SOLO 8.9.1, 2021 (Eigenvector Research Incorporated, 2022 Manson, WA, USA). PLS-DA is based on the PLS regression method, which searches for latent variables (LVs) with maximum covariance with the y-variables. The number of latent variables chosen for the PLS-DA models followed the criterion of lowest classification error average in cross-validation [23]. The performance of the differentiation models was evaluated by means of 10-fold cross-validation and expressed as accuracy scores (i.e., the number of correctly classified samples divided by the total number of samples) [24].

3. Results and Discussion

IRMS and ICP-MS analyses of different types of berries were performed: firstly, to determine their isotopic and elemental profile, and, secondly, to develop differentiation models with respect to the botanical origin (berry species) and growing system (wild and cultivated) based on the isotopic signature and elemental content.

3.1. Isotopic Results

The isotopic composition of ^{13}C in terrestrial plants ranges as a function of the photosynthetic pathway. There are two main groups of photosynthesis: C3 (Calvin cycle) and C4 (Hatch–Slack cycle) [25], depending on the different enzyme involved in the carboxylation process, atmospheric CO_2 first being incorporated into one three-carbon or four-carbon compound. Most of the plants on Earth follow a C3 cycle (fruits, vegetables, the majority of cereals), having $\delta^{13}C$ values between −30 and −23‰ [26]. C4 plants (maize, sugarcane, millet, sorghum) present a higher isotopic signature of ^{13}C, ranging from −14 to −12‰.

The isotopic fingerprint of ^{13}C for the investigated berries was compared with those recorded by other authors for C3 plants [8,27]. The $\delta^{13}C$ values of studied samples varied between −32 and −23.2‰, in the range of those reported by Klavins et al. (2021) [3] for blueberries and bilberries (from −28.6 to −23.9‰). Raspberry samples presented the highest $\delta^{13}C$ values (mean of −25.7‰), and blackberries had a similar ^{13}C isotopic signature (mean of −26.1‰) (Figure 2).

Blueberries showed the lowest values (mean of −28.2‰). The recorded values for blueberry samples are supported by the results of Perini et al. (2018) [8], who obtained lower values (mean of −26.2‰) for blueberries coming from Eastern Europe (Romania and Poland) versus Italian blueberries ($\delta^{13}C$ = −24.2‰). These differences could be explained by plant physiology processes and the agricultural practices used in growing regimes (open field, greenhouse) [28,29], taking into account that the ^{13}C isotopic signature of C3 terrestrial plants preserves valuable data related to: (i) internal physiologic characteristics,

and (ii) changes in external environment that could influence carbon fixation during the photosynthetic process [30,31].

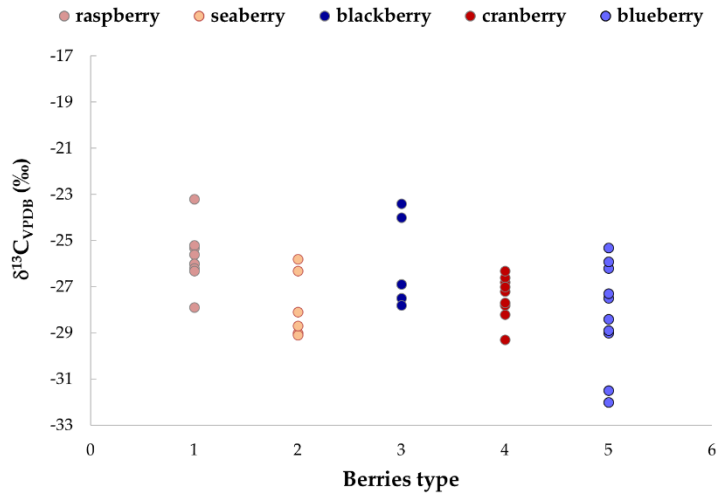


Figure 2. The ^{13}C signature of the investigated berries.

Plants take up the water from the soil during the growing process and incorporate it into their tissues. Therefore, the ^{2}H and ^{18}O isotopic signatures of extracted water from plants (fruits, vegetable, cereals, etc.) will reflect the isotopic fingerprint of water from the soil. This water is practically the ground-water, which is well correlated with meteoric water (precipitation) [32]. The isotope composition of water varies greatly by temperature, latitude, altitude, and precipitation amount [33]. By analyzing the extracted water from berries, data linked to the water source and sample origin could be obtained. In this regard, for all of the investigated berry samples, the $\delta^{2}H$ values ranged between −49.0 and −17.0‰, and for $\delta^{18}O$ between −4.8 and 4.9‰ (Table 1).

Table 1. Isotopic signature of ^{2}H and ^{18}O for studied samples.

Berry Type	$\delta^{2}H_{VSMOW}$ (‰)			$\delta^{18}O_{VSMOW}$ (‰)		
	Min	Max	Mean	Min	Max	Mean
Raspberry	−49.0	−31.6	−38.9	−4.8	0.4	−1.8
Seaberry	−38.2	−24.1	−31.7	−3.5	4.3	0.1
Blackberry	−36.2	−18.8	−29.4	−2.3	3.5	0.7
Cranberry	−37.2	−19.5	−28.7	−3.2	0.8	1.0
Blueberry	−34.3	−17.0	−25.3	−1.2	4.9	2.0

The sample with the highest content of hydrogen and oxygen is a cultivated blueberry, from a farm located at 891 m altitude (46°44′42″ N 23°06′19″ E, Rasca village, Cluj County). These enriched values could be explained by the progressive process of evapo-transpiration, depending on temperature and humidity: as the blueberry sample is ripe, mature, approaching harvest, or in the process of harvest, the lighter isotopes in the berries' water will evaporate more quickly than heavier isotopes [8,34].

The lowest isotopic signatures of ^{2}H and ^{18}O were recorded for two raspberry samples coming from conventional production, suggesting a colder region for the water source [35] used to irrigate these cultivars. This explanation is consistent with the mention on the label: the production site of these two samples is a farm located in the mountain area, at a higher altitude, 1400 m a.s.l. (46°34′32″ N 23°28′03″ E, Băişoara village, Cluj, Romania).

To emphasize the isotopic difference between wild and cultivated berries, Figure 3 shows the groups of blueberries that grew in a natural environment versus those coming from conventional production. In Romania, in the Transylvanian region, where the investigated samples were collected, wild blueberries grow naturally in spontaneous flora,

in forested areas, and exposed rock outcroppings found on hills or mountains, without irrigation, cultivation, or use of additional fertilizers. For these reasons, wild blueberries have a more intense flavor and are much smaller in size than cultivated ones [7]. In contrast, cultivated blueberries contain much more water (to keep roots moist throughout the growing season) than do the wild berries, preferring acidic soil and full sun [36].

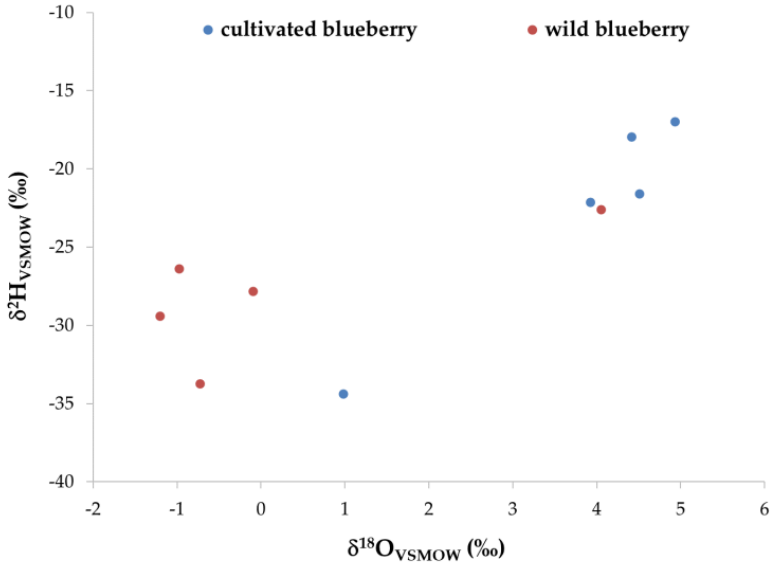


Figure 3. δ^2H versus $\delta^{18}O$ for the studied berries.

It can be observed that one blueberry sample from the cultivated group fits the isotopic signature of the wild blueberries group. This sample, as indicated on the label, comes from a farm located at the foothills of the Fagaras Mountains, Breaza village (45°42′20″ N 24°53′00″ E), Brasov County, at 610 m altitude (Figure 3).

In this region, the vegetation is characterized by an altitudinal layering, well expressed, but with obvious local contrasts, determined by the lithological, edaphic, and topo climatic particularities [37]. For this cultivated sample, the natural environmental conditions overlapped with those specific to a farmed blueberry (cool, humid climate and can be cultivated up to altitudes of 600–800 m; supports low temperatures down to −25 °C in winter, preferring well-drained soils, without excess moisture) [38].

Additionally, in Figure 3, it can be observed that another blueberry sample, sold as "wild", overlaps with the cultivated blueberry group. According to the data on the label, this sample was collected on the same day and location as its "neighbor" sample on the graph, having similar isotopic values. Thus, it can be considered that for this sample there is a false declaration of the growing regime.

3.2. Multi-Element Analysis of Investigated Berries Samples

The results concerning the elemental analysis (Li, Na, Mg, P, Ca, Ti, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, Ga, As, Rb, Sr, Ag, Cd, Ba, Pb, La, Ce, Pr, Nd, Sm, Eu, Gd, and Tb) in different species of berries are summarized in Table S1, presented in the supplementary files.

The multi-element analysis of the investigated samples was used to compare firstly, the composition of the berries according to their botanical origin, and secondly, the elemental content of the berries according to their growing conditions (cultivated vs. wild).

The concentrations of macro-, micro-, and trace elements in five species of berries (raspberries, seaberries, blackberries, cranberries, and blueberries) were compared. Our results showed that the investigated berries are a rich source of macro-elements, such as Na, Ca, P, and Mg. The highest levels were found for Mg and P in raspberries (with mean values of 601.57 mg/kg, d.w. and 1531.38 mg/kg, d.w., respectively), then in the investigated blackberry samples (with mean values of 593.43 mg/kg, d.w. and 1473.18 mg/kg, d.w.,

respectively), and the lowest content in blueberries samples (332.35 mg/kg, d.w. for Mg and 730.34 mg/kg, d.w. for P, respectively). The results of Na analysis, expressed as the mean value, showed that the highest mean concentration of this element was in seaberries samples (187.77 mg/kg, d.w.), followed by blueberries (39.55 mg/kg, d.w.), blackberries (28.22 mg/kg, d.w.), cranberries (27.50 mg/kg, d.w.), and raspberries (26.11 mg/kg, d.w.). In the present study, the Ca concentration in blackberries was higher than the other investigated berries. The general pattern of accumulation of this macro-mineral in the berries studies was in the order of blackberries (866.07 mg/kg, d.w.) > cranberries (623.01 mg/kg, d.w.) > raspberries (606.47 mg/kg, d.w.) > blueberries (559.58 mg/kg, d.w.) > seaberries (245.15 mg/kg, d.w.).

From the micro- and trace elements profile analysis, the mean concentration decreased in the following order: Mn, Fe, Cu, Zn, Ba, Ti, Cr, Ni, Pb, As, Li, Co, V, and Cd (for cranberries); Mn, Fe, Cu, Zn, Ba, Cr, Ti, Ni, Pb, Li, Co, As, V, and Cd (for blueberries); Fe, M, Zn, Cu, Cr, Ti, Ni, Ba, As, Co, Cd, Pb, Li, and V (for raspberries); Fe, Cu, Zn, Mn, Ti, Ni, Cr, Ba, Li, As, Pb, V, Co, and Cd (for seaberries); and Fe, Mn, Cu, Zn, Cr, Ti, Ba, Ni, As, Co, Li, Pb, V, and Cd (for blackberries).

The study of Klavins et al. (2021) [3] reported the elemental concentrations (mg/kg d.w.) in different varieties of blueberries, as variation ranges: Ca (239–432), Mg (38–361), Na (57–208), P (658–865), Ba (0.64–1.46), Cu (1.65–4.28), Fe (16.1–21.7), Mn (10.4–19.1), Sr (0.49–1.37), Zn (3.7–4.8), As (0.12–0.31), Cd (<LOD-0.059), Co (0.02–0.07), Cr (<LOD-0.08), Ni (0.03–0.18), Pb (0.03–0.15), and V (<LOD-0.067). The same authors indicated the concentrations (mg/kg) of elements in blueberries from different countries, as follows: Spain (477 for Ca, 388 for Mg, 25 for Na, 907 for P, 0.54 for Ba, 1.54 for Cu, 14.9 for Fe, 15.6 for Mn, 0.36 for Sr, 5.1 for Zn, 0.01 for As, 0.005 for Cd, 0.02 for Co, 0.01 for Cr, 0.04 for Ni, 0.04 for Pb, and 0.04 for V); Germany (550 for ca, 342 for Mg, 22 for Na, 533 for P, 2.96 for Ba, 2.03 for Cu, 14.4 for Fe, 7.2 for Mn, 0.8 for Sr, 3.7 for Zn, 0.30 for As, 0.03 for Cd, 0.01 for Co, <LOD for Cr, 0.12 for Ni, 0.13 for Pb, and 0.028 for V); Poland (360 for Ca, 275 for Mg, 45 for Na, 875 for P, 0.68 for Ba, 1.58 for Cu, 16.0 for Fe, 7.2 for Mn, 0.80 for Sr, 2.8 for Zn, 0.04 for As, 0.022 for Cd, 0.02 for Co, 0.04 for Cr, 0.22 for Ni, 0.06 for Pb, and 0.03 for V); Peru (1293 for Ca, 459 for Mg, 90 for Na, 586 for P, 2.74 for Ba, 1.90 for Cu, 31.7 for Fe, 27.5 for Mn, 3.77 for Sr, 3.5 for Zn, 0.15 for As, 0.03 for Cd, 0.03 for Co, 0.10 for Cr, 0.42 for Ni, 0.12 for Pb, and 0.13 for V); Chile (520 for Ca, 423 for Mg, 38 for Na, 804 for P, 2.82 for Ba, 7.91 for Cu, 13.3 for Fe, 21.5 for Mn, 1.76 for Sr, 6.5 for Zn, 0.03 for As, <LOD for Cd, 0.02 for Co, 0.29 for Cr, 0.60 for Ni, <LOD for Pb, and <LOD for V); and Argentina (1028 for Ca, 438 for Mg, 41 for Na, 882 for P, 8.54 for Ba, 3.90 for Cu, 26.0 for Fe, 117.2 for Mn, 3.70 for Sr, 5.2 for Zn, 0.14 for As, 0.073 for Cd, 0.02 for Co, 0.06 for Cr, 0.26 for Ni, 0.16 for Pb, and 0.025 for V).

The concentration of heavy metals (mg/kg d.w.) in raspberry samples coming from conventional and wild crops was reported by Kotula et al. (2022) [39]. The concentration values recorded for raspberry fruits in conventional crops were: 0.4 for As, 0.04 for Cd, 0.04 for Pb, 3.76 for Cr, 27.59 for Zn, 2.08 for Co, 4.78 for Cu, 29.00 for Mn, 0.02 for V, 4.10 for Sr, and 0.88 for Ti. For wild-growing raspberry fruits, the metals concentration of samples was: 0.4 for As, 1.00 for Cd, 0.07 for Pb, 3.60 for Cr, 35.76 for Zn, 1.95 for Co, 5.96 for Cu, 340.70 for Mn, 0.03 for V, 3.03 for Sr, and 1.3 for Ti. According to Akimov et al. (2021) [40], the mean concentration in mg/kg of macro- and microelements investigated in raspberry fruits was: 23.7 for Na, 385 for Ca, 255 for Mg, 6.0 for Fe, 0.44 for Cu, and 3.5 for Zn.

Figure 4 presents the box plots of the statistically significant elemental compositions of berries, as obtained by applying the ANOVA test, with respect to the botanical origin.

The mean contents of macro-, micro-, and trace elements in wild and cultivated berries (raspberry, seaberry, blackberry, cranberry, and blueberry) are presented in Figure 5.

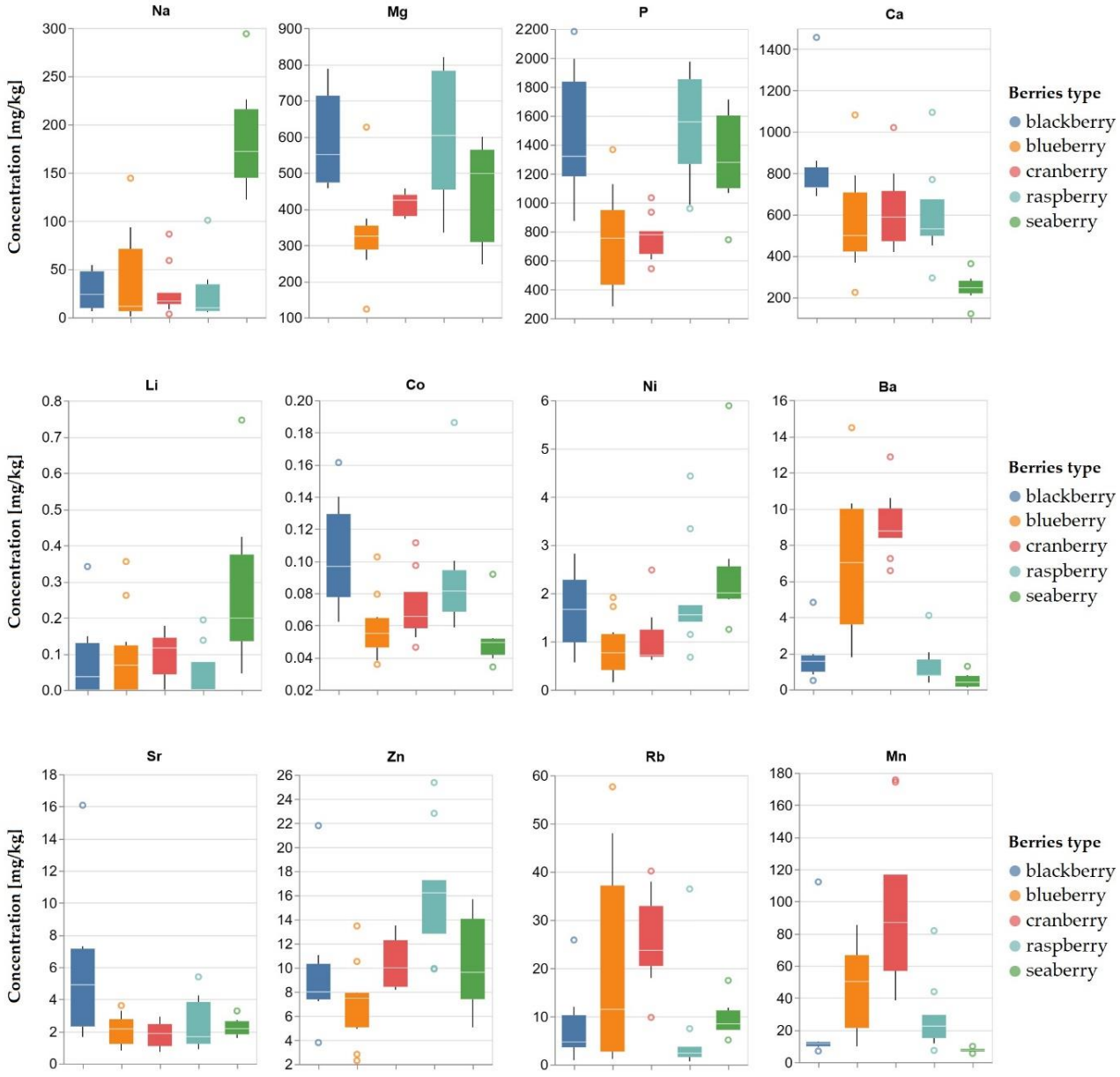


Figure 4. Box plots of statistically significant elements concentrations in investigated berries according to botanical origin.

In the investigated wild berries, the average reported concentrations reflected the following trend: for Na, seaberries (200.87 mg/kg, d.w.) > blackberries (30.40 mg/kg, d.w.)~cranberries (29.09 mg/kg, d.w.) > blueberries (22.99 mg/kg, d.w.) > raspberries (8.63 mg/kg, d.w.); for Mg, (blackberries (622.54 mg/kg, d.w.)~raspberries (617.80 mg/kg, d.w.) > seaberries (414.77 mg/kg, d.w.) > cranberries (409.08 mg/kg, d.w.) > blueberries (394.27 mg/kg, d.w.); for P, blackberries (2087.81 mg/kg, d.w.) > raspberries (1893.15 mg/kg, d.w.) > seaberries (1210.14 mg/kg, d.w.) > blueberries (999.32 mg/kg, d.w.) > cranberries (749.16 mg/kg, d.w.); and for Ca, blackberries (1092.44 mg/kg, d.w.) > raspberries (812.53 mg/kg, d.w.) > blueberries (718.28 mg/kg, d.w.) > cranberries (642.03 mg/kg, d.w.) > seaberries (245.50 mg/kg, d.w.).

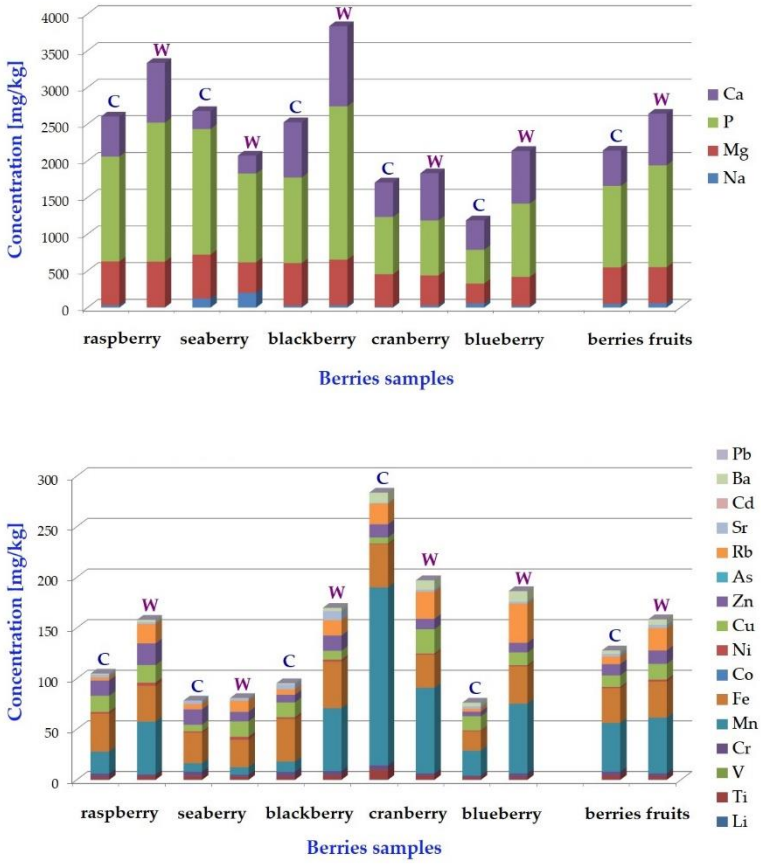


Figure 5. The mean concentration of macro-, micro-, and trace elements in mg/kg dry weight (d.w) in cultivated (C) and wild berries (W) (raspberry, seaberry, blackberry, cranberry, and blueberry).

Among the investigated cultivated berries, seaberries samples contained the highest content of Na (122.21 mg/kg, d.w.), followed by blueberries (56.09 mg/kg, d.w.), raspberries (31.10 mg/kg, d.w.), blackberries (27.12 mg/kg, d.w.), and cranberries (14.67 mg/kg, d.w.). Seaberries, raspberries, and blackberries contain similar values in terms of the average concentration of Mg (599.09 mg/kg, d.w., 596.93 mg/kg, d.w., and 578.89 mg/kg, d.w., respectively), while blueberries contain the lowest concentration of Mg (270.41 mg/kg, d.w., mean value). Seaberry samples contain the highest concentration of P, followed by raspberries, blackberries, cranberries, and blueberries. The Ca content of the investigated berries was the highest in the blackberry samples (752.88 mg/kg, d.w.), while the seaberries contain the lowest concentration of Ca (243.37 mg/kg, d.w., as mean value). Raspberries contain an average value of 547.59 mg/kg, d.w., cranberries of 470.82 mg/kg, d.w., and blueberries of 400.87 mg/kg, d.w.

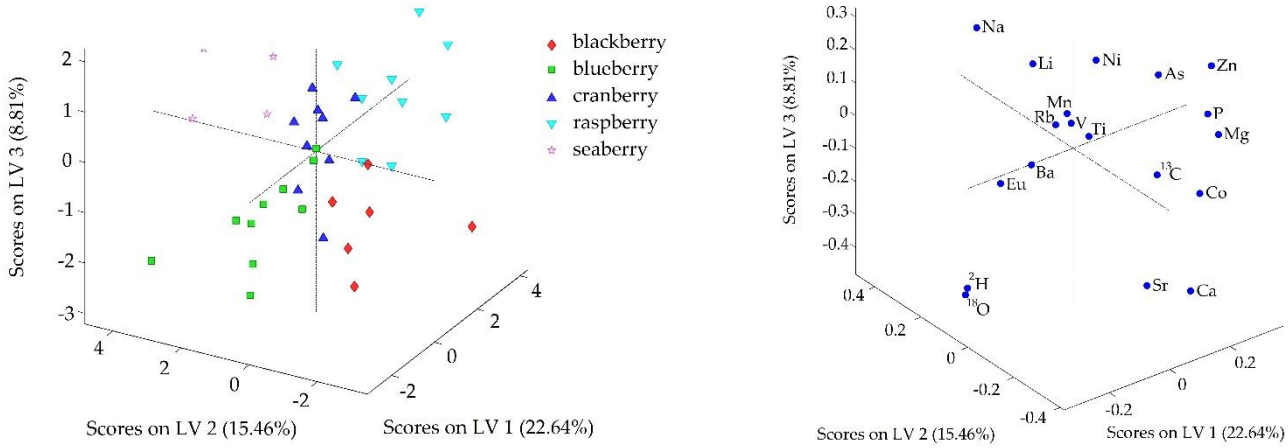
Unlike cultivated fruits, wild varieties of berries survive and grow in natural conditions, without direct human influence by the application of organic or synthetic fertilizers. Furthermore, wild berries are flavorful, delicious, and yield fruits with high nutritive value and excellent quality.

3.3. Differentiation Models of Berries According to Botanical Origin and Growing System

In order to differentiate the berry samples according to the botanical origin (raspberry, seaberry, blackberry, cranberry, and blueberry) and growing system (wild and cultivated), the PLS-DA supervised method was performed on the data associated with the isotopic content ($\delta^{13}C$, $\delta^{2}H$, $\delta^{18}O$) and multi-element concentration (Li, Na, Mg, P, Ca, Ti, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, Ga, As, Rb, Sr, Ag, Cd, Ba, Pb, La, Ce, Pr, Nd, Sm, Eu, Gd, and Tb). First, the measured isotope and elemental concentrations were preprocessed through the auto scale method. After that, to identify the variables with the highest influence on

the botanical/growing system differentiation of berries, a model-based feature selection method built on PLS was applied.

The determined parameters which potentially have the highest influence on berries' differentiation according to botanical origin were: $\delta^{13}C$, $\delta^{2}H$, $\delta^{18}O$, Li, Na, Mg, P, Ca, V, Mn, Co, Ni, Zn, As, Rb, Sr, Ba, and Eu. The PLS-DA differentiation model constructed based on these features was characterized by four LVs, as it illustrated the lowest cross-validation classification error average. The performance of the model corresponded to an 85% accuracy in the cross-validation procedure, which was obtained after correctly predicting 66%, 70%, 88%, 100%, and 100% of the blackberry, blueberry, cranberry, raspberry, and seaberry samples, respectively. The score and loading plots (LV1 vs. LV2 vs. LV3) associated with the PLS-DA model are displayed in Figure 6a,b, respectively, illustrating and supporting the influence of the identified parameters for differentiating berries according to botanical origin.



(**a**) (**b**)

Figure 6. PLS-DA score (**a**) and loading plot (**b**) associated with the differentiation of berries according to botanical origin.

The PLS-based feature selection method indicated that the variables that are the most significant for classifying berry samples according to the growing system are: $\delta^{13}C$, $\delta^{18}O$, Li, Na, Ca, Cr, Mn, Ni, Rb, and Ba. The PLS-DA model constructed based on these 10 variables was characterized by a single LV and conducted the correct assessment (in the cross-validation procedure) of 94.4% and 77.2% of the cultivated and wild berries, respectively. Therefore, a model accuracy of 85% was obtained. One cultivated berry sample was assigned as a wild sample, while five samples from the wild class were predicted as cultivated berries. The score plot of the PLS-DA model is presented in Figure S1, in the Supplementary Materials.

Nonetheless, PLS-DA was applied for a particular case, namely for the differentiation of wild blueberries from cultivated ones. Firstly, the PLS-DA model was developed, based on the entire set of data (33 variables, corresponding to all of the isotopic and elemental contents measured). In this case, an accuracy of 80% was obtained in the cross-validation procedure. One blueberry sample from the wild group was assigned to the cultivated group and one cultivated blueberry sample was predicted as a sample from the wild group. When the model-based feature selection method was applied, the variables that significantly contributed to the discrimination model were: $\delta^{13}C$, $\delta^{18}O$, Mg, P, Ca, Mn, Zn, Ga, Rb, and Ba. To refine the classification model of blueberry samples according to the growing system, the most significant parameters identified by the feature selection method were used to build a final PLS-DA model. The developed PLS-DA model illustrated the use of 5 LVs and showed excellent levels of sensitivity (100%) and specificity (100%), both in the calibration

and cross-validation methods. Therefore, an accuracy of 100% for the differentiation of wild blueberries from cultivated ones was achieved.

4. Conclusions

In this work, the isotopic and elemental profiles of 40 berry samples—from different botanical origins (raspberry, seaberry, blackberry, cranberry, and blueberry) and from two growing regimes (wild and cultivated)—were assessed. The composition of berries depends on their species, growing conditions (cultivated or wild), and type of soil, with observable variability in both the stable isotopic ratios and elemental content. In order to discriminate berries according to the botanical origin and growing system, PLS–DA models were built. Moreover, a variable selection approach was applied to determine the variables that have the highest discrimination power with respect to each classification criteria. Thus, $\delta^{13}C$, $\delta^{2}H$, $\delta^{18}O$, Li, Na, Mg, P, Ca, V, Mn, Co, Ni, Zn, As, Rb, Sr, Ba, and Eu were identified as the most relevant attributes for the berry species, while $\delta^{13}C$, $\delta^{18}O$, Li, Na, Ca, Cr, Mn, Ni, Rb, and Ba corresponded to the growing regime markers. By applying PLS-DA on the most significant features identified, correct classification rates of 94.4% (for cultivated berries) and 77.2% (for wild berries) were obtained in the cross-validation procedure, leading to a model accuracy of 85%. Similarly, an 85% accuracy was achieved through the PLS-DA model built for the varietal discrimination of the samples. Additionally, the developed PLS-DA model for the discrimination of wild blueberries from cultivated ones showed excellent levels of sensitivity (100%), specificity (100%), and accuracy (100%).

This preliminary study indicated the fact that the corroboration of analytical methods (IRMS and ICP-MS), followed by chemometric treatment using PLS-DA, as a statistical supervised method, could be utilized as an authenticity testing tool for berries according to growing system. For future studies, the sample's data set will be enlarged to provide more significant results. In addition, different statistical models will be developed to authenticate the geographical origin of different types of berries based on isotopic and elemental fingerprints.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/app13052980/s1.

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