Biotechnology

Content of Mineral Elements in Wild-Growing Blueberry Leaf Teas

Sophio Papunidze^{*}, Nino Seidishvili^{*}, Zurab Mikeladze^{*}, Iamze Chkhartishvili^{*}, Guram Papunidze^{*}, Nunu Kutaladze^{*}

* Agrarian and Membrane Technologies Institute, Batumi Shota Rustaveli State University, Batumi, Georgia

(Presented by Academy Member Vano Papunidze)

The concentrations of thirteen mineral elements (Ca, Cu, Fe, K, Mg, Mn, Na, Zn, Ni, Pb, Cd, Co and Cr) were determined in four different types of wild-growing blueberry leaf tea such as black tea, red tea, green tea and yellow tea. Plasmaatomic emission spectrometer ICPE-9820 was used for qualitative and quantitative determination of the elements in the required concentration range, because of high sensitivity, wide dynamic range and high sample throughput of this spectrometer. The amount of essential elements slightly varied from one tea to the other, showing the unique mineral profile for each tea. In the blueberry leaf teas high content of Ca (13100 to 18800 mg/kg), K (7600 to 15300 mg/kg), Mg (4340 to 5900 mg/kg), Mn (2025 to 3350 mg/kg), Fe (1185 to 2335 mg/kg) and Na (1725 to 2285 mg/kg) was observed. Low concentrations were noted for Cu and Zn (34.0-42.0 mg/kg, 26.8-59.5 mg/kg, respectively). Such elements as Cr and Ni were found under the limit of quantitation, whereas Pb, Cd and Co were under the limit of detection in all tea samples. © 2023 Bull. Georg. Natl. Acad. Sci.

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The wild-growing blueberry (*Vaccinium myrtillus* L) is one of the most popular wild-harvested plant in Georgia, its berries and leaves are traditionally used as healthy food product and also in folk medicine. Blueberries production has increased in the last few years boosted by World Health Organization (WHO) approvals for their contribution to a healthy diet and the evidence that they act against different diseases.

Compared with carefully studied fruits [1-3], blueberry leaves are generally ignored and

discarded as by-products of blueberry product. Blueberry leaves contain higher levels of nutrients and phytochemicals than the fruits, which can serve as an effective antioxidant resource or functional food ingredient, which indicates their ability to scavenge free radicals is higher in comparison to the fruits, also confirmed by previous findings [4-6]. While the content of bioactive compounds is quite often determined and compared, the concentrations of essential mineral elements, which are also the important components of the leaves, are rarely reported. In humans mineral elements accomplish decisive functions to maintain human health. They play an important role in the activation of enzymatic systems or have involvement in the metabolism of biomolecules. Basically, they act as cofactors for various enzymatic systems or possess regulatory activity. Deficiency in essential mineral elements lead to undesirable pathological conditions that can be prevented or reversed by adequate supplementation [7,8].

Tea, produced from the leaves of the plant Camellia sinensis is the most widely consumed beverage in the world. Tea leaves as a source of polyphenols, catechins, a crucial group for their antioxidative activity, mineral elements have been found to be responsible for numerous health benefits of teas [9]. With similar chemical components and bioactivity to C. sinensis, blueberry leaves have great potential to be further explored as new kind of tea [10,11]. Lacking the relevant study, the research of different types of blueberry leaves tea is promising, and changes in chemical compositions during the process of making tea are worth careful studying. This research will contribute to the development of modern knowledge on the comprehensive use of blueberry leaf waste as a potentially valuable resource, as well as the systematic development of new varieties of tea.

The objective of the present study was to investigate and compare the mineral composition of four different types of blueberry leaf teas (black tea, red tea, green tea and yellow tea) using a Shimadzu ICPE-9820 simultaneous ICP atomic emission spectrometer.

Materials and Methods

Fresh leaves of wild-growing blueberry (*Vaccinium myrtillus* L.) were collected from Adjara region mountains of Western Georgia (at 1700-2200 meters above sea level) growing on publicly accessible lands from late April to late October. The shoots with one bud and few leaves were selected

from the top of the newly grown branches by hand and immediately delivered to the laboratory for the analyses and tea preparation. Four different kinds of blueberry leaf tea samples: black tea, red tea, green tea and yellow tea were made. The process of making the tea samples was performed on the basis of corresponding traditional tea processing method of C. sinensis [12-14].

The ICPE-9820 spectrometer (Shimadzu, Japan) was used for the analysis of thirteen elements (Ca, Cu, Fe, K, Mg, Mn, Na, Zn, Ni, Pb, Cd, Co and Cr) in blueberry leaf tea samples. The operating parameters of the spectrometer were set in accordance with the manufacturer's recommendations, i.e., the radio frequency power - 1.20 kW; the argon auxiliary gas flow rate of 0.60 L/min, the carrier gas - 0.70 L/min; the plasma gas - 7.00 L/min; the gas purity - 99.95%, detector - CCD (charge coupled device); view direction - Radial (RD)/Axial (AX). The intensity measurements were repeated three times with an integration time of 1 s. The most prominent analytical lines for determination of each element were selected, i.e., Ca - 315.887 nm, Cu - 327.396 nm, Fe - 259.940 nm, K - 797.395 nm, Mg - 285.213 nm, Mn -344.297 nm, Na - 589.592 nm, Zn -213.856 nm, Ni - 231.604 nm, Pb - 220.353 nm, Cd - 226.502 nm, Co - 237.862 nm, Cr - 206.149 nm. For spectrometry measurements, series of calibration solutions with proper concentrations were made. For calibration solution preparations following standards (Sigma-Aldrich, Switzerland) were used: multielement Standard Solution 6 for ICP, 100mg/L each element in 5% HNO3; Internal Yttrium Standard (Y) for ICP, 1001mg/L±4 mg/L in 2% HNO₃. 1% HNO₃ was used to prepare calibration standards immediately before usage. Concentrations of calibrations were from 5µg/L to 5mg/L for every element and 0.1mg/L for an internal yttrium standard. Deionized water with the maximum resistivity of 18.2 M Ω /cm obtained from the Purity Labwater system D340 (Oxfordshire, United Kingdom) were used for sample pretreatment and Sophio Papunidze, Nino Seidishvili, Zurab Mikeladze...

dilution. All the solutions were prepared in highdensity polyethylene containers and were of analytical reagent grade [15].

For the determination of elemental composition of teas, it is required to properly prepare the samples [16]. 5g of dried, ground tea sample was weighed to an accuracy of 0.1mg into a porcelain crucible. The crucible was placed on a hot plate at 100°C. As soon as fumes cease to evolve, the sample was placed in a muffle furnace at 450 -500°C for three hours until all evidence of carbon was gone. The fully ashed sample gives a light grey ash. Removed the crucible from the muffle furnace. When cool, the ash was dissolved by adding 2mL of 4% (v/v) nitric acids. Gently warmed to speed up the dissolution of the ash. The dissolved ash solution was then brought to 25mL of nitric acid. The containers used for storage or treatment of the samples were cleaned to avoid contamination with any metals. The containers were treated with nitric acid and washed with deionized water. Mineral element concentrations in tea samples were expressed as mg/kg of dry weight [17].

Results and Discussion

Thirteen mineral elements were identified in blueberry leaf tea samples by plasma-atomic emission spectrometer (ICPE-9820). The analytical results of elements of black, red, green and yellow tea samples are presented in Table.

The results indicated that the contents of essential elements like calcium, potassium, magnesium, manganese, sodium and iron are abundant in all tea samples with Ca, K, Mg being present in the highest concentration: Ca - 13100 to 18800 mg/kg (Table), K - 7600 to 15300 mg/kg (Table), Mg - 4340 to 5900 mg/kg (Table). The content of Mn, Na and Fe measured in tea samples is 2025 to 3350 mg/kg (Table), 1725 to 2285 mg/kg (Table) and 1185 to 2335 mg/kg (Table), respectively. Other essential elements such as Cu and Zn present at low concentrations: 34.0-42.0 mg/kg (Table) and 26.8-59.5 mg/kg (Table),

respectively. The concentration levels of these elements in this study are close to the values found in teas from Camellia sinensis plant by other researchers [18, 19]. According to The Commission of the European Communities [20], non-essential (toxic) elements such as chromium and nickel have extremely low concentrations, under the limit of quantitation (ULOQ) whereas plumbum, cadmium and cobalt are under the limit of detection (ULOD) in all tea samples (Table). This can be explained by the limited industrialization in the area where blueberry was cultivated. Hence, none of teas from blueberry leaf would represent a risk to human health from this point of view.

Ca, K, Mg content is the highest in all tea samples. Mn and Na content of black, red and green tea is higher than those of yellow tea, whereas Fe and Zn content of black, green and yellow tea is higher than those of red tea. Cu content of red, green and yellow tea is higher than that of black tea.

The red tea showed the highest level of Ca (18800 mg/kg), Cu (42.0 mg/kg), Mg (5900 mg/kg) and Mn (3350 mg/kg) and the lowest level of Fe (1185 mg/kg) and Zn (26.8 mg/kg), whereas, in the yellow tea the lowest Ca, Cu, Mg and Mn concentrations (13100 mg/kg, 37.1 mg/kg, 4340 mg/kg, 2025 mg/kg, respectively) and the highest Fe and Zn concentrations (2335 mg/kg and 59.5 mg/kg, respectively) were found. For the green tea the highest K (15300 mg/kg) and Na (2285 mg/kg) content was observed. On the other hand, the lowest K (7600 mg/kg) and Na (17245 mg/kg) content was detected in the black and yellow tea, respectively.

Generally, the total average mineral content in black, red and green teas is in the order Ca>K>Mg>Mn>Na>Fe>Cu>Zn, while in yellow tea - Ca>K>Mg>Fe>Mn>Na>Zn>Cu. Based on the amount of concentrations in tea samples, the elements are classified into four categories: Ca, K, Mg, Mn, Na and Fe – elements with high concentrations; Cu and Zn – elements with low concentrations; Cr and Ni – under the limit of quantitation; Pb, Cd and Co – under the limit of detection.

The present paper adds significant knowledge to the field of blueberry leaf teas via mineral element profiles. All the studied tea could be qualified as a valuable source of essential mineral elements in human nutrition with health-promoting and disease-preventing properties. Differences in mineral profiles between the tea samples can be due to the different technologies with these samples were produced.

 Table. Analytical results of mineral elements in tea

 samples (unit: mg/kg)

Element	Black	Red	Green	Yellow
	tea	tea	tea	tea
Ca	15450	18800	17350	13100
Cu	34.0	42.0	38.4	37.1
Fe	1400	1185	1525	2335
K	7600	12200	15300	8850
Mg	4745	5900	5650	4340
Mn	2145	3350	3195	2025
Na	1960	1975	2285	1725
Zn	32.7	26.8	29.6	59.5
Cr	ULOQ	ULOQ	ULOQ	ULOQ
Ni	ULOQ	ULOQ	ULOQ	ULOQ
Pb	ULOD	ULOD	ULOD	ULOD
Cd	ULOD	ULOD	ULOD	ULOD
Со	ULOD	ULOD	ULOD	ULOD

ULOQ – under the limit of quantitation ULOD – under the limit of detection

Conclusions

Plasma-atomic emission spectrometer (ICPE-9820) has been used for qualitative and quantitative deter-

mination of thirteen mineral elements (Ca, Cu, Fe, K, Mg, Mn, Na, Zn, Ni, Pb, Cd, Co and Cr) in four tea samples (black tea, red tea, green tea and yellow tea) produced from wild-growing blueberry leaves collected from Adjara region mountains of Western Georgia.

All examined blueberry leaf tea samples showed considerable levels of essential mineral elements with Ca (13100 to 18800mg/kg), K (7600 to 15300 mg/kg) and Mg (4340 to 5900 mg/kg) being abundant in all tea samples. The level of Mn and Na content of black, red and green teas was higher than that of yellow tea, whereas Fe and Zn content of black, green and yellow teas was higher than that of red tea. Cu content of red, green and yellow teas was higher than that of black tea. Nonessential (toxic) elements such as Cr and Ni were under the limit of quantitation. Pb, Cd and Co were under the limit of detection in all tea samples.

The results of the conducted studies indicate that the blueberry leaf teas in terms of the content of essential mineral elements are promising sources for the creation of new tea varieties as functional food sources.

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ბიოტექნოლოგია

ველურად მზარდი მოცვის ფოთლის ჩაის მინერალური ელემენტების შემცველობა

ს. პაპუნიძე*, ნ. სეიდიშვილი*, ზ. მიქელაძე*, ი. ჩხარტიშვილი*, გ. პაპუნიძე*, ნ. კუტალაძე*

* ბათუმის შოთა რუსთაველის სახელმწიფო უნივერსიტეტი, აგრარული და მემბრანული ტექნოლოგიების ინსტიტუტი, ბათუმი, საქართველო

(წარმოდგენილია აკადემიის წევრის ვ. პაპუნიძის მიერ)

აჭარის მთიან რეგიონში გავრცელებული ველურად მზარდი მოცვის ფოთლის ჩაის ოთხ სხვადასხვა სახეობაში (შავი, წითელი, მწვანე და ყვითელი ჩაი) განისაზღვრა 13 ბიოლოგიურად აუცილებელი მინერალური ელემენტი (Ca, Cu, Fe, K, Mg, Mn, Na, Zn, Ni, Pb, Cd, Co და Cr). მინერალური ელემენტების ხარისხობრივი და რაოდენობრივი განსაზღვრისათვის გამოყენებულ იქნა პლაზმური ატომურ-ემისიური სპექტრომეტრი (ICPE-9820), რომელიც გამოირჩევა მაღალი მგრმნობელობით, ფართო დინამიკური დიაპაზონითა და ნიმუშების მაღალი გამტარუნარიანობით. ჩაის სხვადასხვა სახეობაში ელემენტების რაოდენობა განსხვავებულია, რაც აჩვენებს თითოეული ჩაის უნიკალურ მინერალურ შემადგენლობას. მოცვის ფოთლის ჩაიში დაფიქსირდა მირითადი ელემენტების მაღალი შემცველობა Ca (13100 - 18800 მგ/კგ), K (7600 -15300 მგ/კგ), Mg (4340 - 5900 მგ/კგ), Mn (2025 - 3350 მგ/კგ), Fe (1185 - 2335 მგ/კგ) და Na (1725 -2285 მგ/კგ), Cu და Zn გამოვლინდა დაბალ კონცენტრაციებში (34,0-42,0 მგ/კგ, 26,8-59,5 მგ/კგ). Cr და Ni აღმოჩნდა რაოდენობრივი განსაზღვრის ზღვარის ქვემოთ, Pb, Cd და Co კი - ჩაის ყველა ნიმუშში გამოვლენილი ზღვრის ქვემოთ.

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