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The Study of the Elemental and Amino Acid Composition of *Cetraria Islandica* (L.) Ach. Thalli Batches Harvested in Ukraine

Abstract

Aim. To study the elemental and amino acid composition of *Cetraria islandica* (L.) Ach. thalli batches harvested in Ukraine.

Materials and methods. Seven batches of *C. islandica* thalli harvested in different regions of Ukraine in late summer/early fall 2019 were used for the study. The elemental composition of the raw material was studied by atomic absorption spectrometry with photographic registration. The component composition of free and bound amino acids in the raw material was determined by the HPLC method.

Results and discussion. The presence of at least 19 macro-, trace and ultra-trace elements was determined in 7 batches of the raw material. The predominance of the following elements was found: potassium 190 mg/100 g (batch 6) – 325 mg/100 g (batch 7); silicon 30 mg/100 g (batch 1) – 115 mg/100 g (batch 4); calcium 37 mg/100 g (batch 6) – 86 mg/100 g (batch 4). It was determined that the quantitative content of molybdenum and cobalt did not exceed 0.03 mg/100 g, the content of cadmium, astatine and mercury did not exceed 0.01 mg/100 g. The quantitative content of lead met the requirements of the monograph of the State Pharmacopoeia of Ukraine (SPhU) 2.0. The total ash content in the batches of the raw material ranged from 0.61 ± 0.02 % to 1.43 ± 0.05 %, meeting the requirements of the monograph of the SPhU 2.0. The presence of 7 amino acids was determined; 5 of them were nonessential (aspartic and glutamic acids, serine, arginine, and alanine) and 2 were essential (threonine and valine). The quantitative content of the total amount of free amino acids was 0.794 μg mg^{-1}, the total amount of bound ones was 3.276 μg mg^{-1}.

Conclusions. For the first time, the elemental composition of 7 batches of *C. islandica* thalli harvested in Ukraine was determined. In each batch, potassium was the predominant element (from 190 mg/100 g to 325 mg/100 g depending on the batch). The component composition of free and bound nonessential and essential amino acids was determined (the quantitative content of the total amount of free amino acids was 0.794 μg mg^{-1}, the total amount of bound ones was 3.276 μg mg^{-1}); alanine (0.289 μg mg^{-1}) was the dominant component among free amino acids, while arginine (0.993 μg mg^{-1}) prevailed among bound ones. The results obtained will be used for further studies of the raw material of *C. islandica* harvested in Ukraine.

Keywords: *Cetraria islandica*; thalli; mineral composition; free and bound amino acids

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Вивчення елементного та амінокислотного складу серій слані *Cetraria islandica* (L.) Ach., заготовлених в Україні

Анотація

Мета. Дослідити елементний та амінокислотний склад серій слані *Cetraria islandica* (L.) Ach., заготовлених в Україні.

Матеріали та методи. Для дослідження було використано 7 серій слані *C. islandica*, заготовлених у різних регіонах України наприкінці літа – восени 2019 року. Елементний склад сировини досліджували методом атомно-абсорбційної спектрометрії з фотографічною реєстрацією. Компонентний склад вільних і зв'язаних амінокислот у сировині визначали методом ВЕРХ.

Результати та їх обговорення. Визначено наявність у 7 серіях сировини щонайменше 19 макро-, мікро- та ультрамікроелементів. Серед елементів виявлено переважання калію: 190 мг/100 г (серія 6) – 325 мг/100 г (серія 7); силіцію: 30 мг/100 г (серія 1) – 115 мг/100 г (серія 4); кальцію: 37 мг/100 г (серія 6) – 86 мг/100 г (серія 4). Визначено, що кількісний вміст молібдену та кобальту не перевищував 0,03 мг/100 г, вміст кадмію, астату та ртуті не перевищував

0,01 мг/100 г. Кількісний вміст плюмбуму відповідав вимогам монографії Державної фармакопеї України (ДФУ) 2.0. Вміст загальної золи в серіях сировини становив від 0,61 ± 0,02 % до 1,43 ± 0,05 %, що відповідає вимогам монографії ДФУ 2.0. Визначено наявність 7 амінокислот, з яких 5 замінні (аспарагінова і глутамінова кислоти, серин, аргінін та аланін) та 2 незамінні (треонін і валін). Кількісний вміст суми вільних амінокислот становив 0,794 мкг мг$^{-1}$, суми зв'язаних – 3,276 мкг мг$^{-1}$.

Висновки. Уперше визначено елементний склад 7 серій слані *C. islandica*, заготовлених в Україні. У кожній серії домінантним елементом був калій (від 190 мг/100 г до 325 мг/100 г залежно від серії). Визначено компонентний склад вільних і зв'язаних замінних і незамінних амінокислот (кількісний вміст суми вільних амінокислот – 0,794 мкг мг$^{-1}$, суми зв'язаних – 3,276 мкг мг$^{-1}$), домінантним компонентом серед вільних амінокислот був аланін (0,289 мкг мг$^{-1}$), серед зв'язаних – аргінін (0,993 мкг мг$^{-1}$). Отримані результати будуть використані для подальших досліджень сировини *C. islandica,* заготовленої в Україні.

Ключові слова: *Cetraria islandica*; слань; мінеральний склад; вільні та зв'язані амінокислоти

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Introduction

Cetraria islandica (L.) Ach. is a foliose lichen of the *Parmeliaceae* family, which has been used in the European and Asian folk medicine for centuries, in particular for the respiratory and digestive diseases [1]. Due to the content of many groups of biologically active substances (BAS) in the raw material and a wide spectrum of their pharmacological activity, including antibacterial, expectorant, antioxidant and anti-inflammatory, the study of the raw material of *C. islandica* does not lose its relevance nowadays [2, 3].

As of May 2022, the raw material of *C. islandica* is a component of 5 medicinal products presented at the pharmaceutical market of Ukraine, such as Pectolvan phyto Iceland moss (JSC Farmak, Ukraine); pastilles Isla-Moos and Isla-Mint (Engelhard Arzneimittel GmbH& Co. KG, Germany), Herbion Iceland moss syrup (KRKA, Slovenia) and Complex expectorant extract (Phytopharm Klenka SA, Poland) [4]. It is also contained in dietary supplements in the form of tablets, pastilles, syrups and herbal teas, which are used for the inflammatory processes of the respiratory system treatment and help to regulate the respiratory function [5].

The pharmacological action of lichens is due to the ability of myco- and photobiont to form primary and secondary metabolites belonging to different groups of BAS [6]. A comprehensive study of their component composition is important for the systematic study of the raw material and for the search for optimal ways of their complex processing as well [3]. Lichens have a high ability to absorb mineral substances from the lichen substrate, air, and precipitation [7]. Mineral substances accumulated by the mycobiont can form complex compounds with such primary lichen metabolites as amino acids, proteins, polysaccharides, vitamins [8].

The study of the elemental composition of the raw material not only allows to prevent the use of the raw material which can be exposed to environmental pollution, but it is also important for determining the influence of elements on the pharmacological effect of the drugs based on this raw material [8, 9].

The monographs on *C. islandica* are included in the pharmacopoeias of many countries, including the State Pharmacopoeia of Ukraine (SPhU) 2.0 [10]. The monograph "Cetraria islandica" of the SPhU 2.0 does not contain a national part and does not regulate the quantitative content of BAS in the raw material. In the "Tests" section of the monograph of the SPhU 2.0, it is specified that the content of lead in the raw material of *C. islandica* must not exceed 0.0010% (10.0 ppm) [10].

In one of the first studies of the elemental composition of *C. islandica* thalli, Finnish scientists (*Airaksinen* et al., 1986) studied the toxicity of the raw material of lichens *C. islandica* and *Cladonia rangiferina.* It has been traditionally used in this region in the food industry, as emergency food and as livestock fodder. In the study, a high content of heavy metals, in particular lead, was determined, and the necessity of additional processing of the raw material for further safe application was shown [11].

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The results of the elemental analysis of the raw material of *C. islandica* of Italian origin (*Meli* et al., 2017) demonstrate a high content of calcium, silicon, and potassium in the raw material [12]. Similar data on the elemental composition of *C. islandica* thalli were obtained for the raw material harvested in the South-Eastern Siberia, the Pre-Urals, Karelia and other regions of the boreal forest and tundra (*Vershinina* et al., 2010), as well as on the Taymyr Peninsula (*Kaiser* et al., 2020) where the dominance of potassium and calcium was noted [13–14]. Ukrainian scientists (*Vladymyrova* et al., 2019) also found the predominance of potassium, silicon, and calcium in the raw material of *C. islandica* when studying the elemental composition of the raw material of various medicinal plants in thyroid gland diseases [15]. The analysis of the raw material of *C. islandica* and medicines and food supplements on its basis represented at the European market showed the absence of a strong correlations between the origin of the raw material and its elemental composition (*Giordani* et al., 2017) [16].

Recent data on the study of the amino acid composition of *C. islandica* thalli are mainly devoted to the research of the raw material harvested in different regions of the boreal forest and tundra. Their results demonstrate the presence of 14 to 16 amino acids in the raw material, including a high content of glutamine, phenylalanine, and valine (*Kaiser* et al., 2020); alanine, ornithine (*Tabalenkova* et al., 2017); aspartic and glutamic acids, alanine, lysine, and leucine (*Vershinina* et al., 2010) [13, 14, 17].

An earlier study conducted by Serbian scientists (*Grujic-Injac*, 1976) demonstrated the presence of 17 amino acids in the raw material of *C. islandica*, among which aspartic and glutamic acids, alanine and leucine predominated [18].

Therefore, in the literature sources available to us no data on the elemental and amino acid composition of *C. islandica* thalli harvested in Ukraine were found. For this reason, the aim of the current work was to determine the elemental and amino acid composition of *C. islandica* (L.) Ach thalli batches harvested in Ukraine.

■ Materials and methods

For the study, 7 batches of the raw material of *C. islandica* harvested in late summer/early fall 2019 were used (batch 1 – Volyn region; batch 2 – Zakarpattia region; batch 3 – Zakarpattia region; batch 4 – Zakarpattia region; batch 5 – Ivano-Frankivsk region; batch 6 – Chernivtsi region; batch 7 – Rivne region).

The total ash content in the batches of the raw material was determined according to the requirements of the monograph 2.4.16 “Total ash” of the SPhU 2.0 [19].

The elemental composition of the raw material was studied by atomic absorption spectrometry with photographic registration according to the generally accepted method [20]. The research was conducted in the SSI “Institute of Single Crystals” of the National Academy of Sciences of Ukraine (Kharkiv).

The component composition of free and bound amino acids in the raw material was determined by the HPLC method in batch 2 of the raw material. The chromatographic separation was performed on an Agilent 1200 liquid chromatograph (Agilent technologies, USA). A Zorbax AAA column, 150 mm long, with a 4.6 mm internal diameter and 3 µm sorbent grain diameter was used. The mobile phase A was 40 mM Na_2HPO_4 with pH 7.8; B – MeCN/MeOH/H_2O (45:45:10, *v*/*v*/*v*). The separation mode was gradient with a constant flow rate of 1.5 mL min^{-1}:

Chromatography time, min	Mobile phase A	Mobile phase B
0:00	100	0
2:00	100	0
18:00	43	57
19:00	0	100
23:00	0	100
26:00	100	0

The temperature of the thermostat column was 40 °C. The pre-column derivatization was performed in the automatic programmed mode with the FMOC reagent (Agilent 5061-3337) and the OPA reagent (Agilent 5061-3335). The derivatized amino acids was detected with a fluorescent detector [21].

Sample preparation

For the extraction of free amino acids, a portion of the sample of the raw material ground to a powdery state was placed in a vial, 2 mL of 1N hydrochloric acid was added, and the vial was kept in an ultrasonic bath at 50°C for 3 hours. For the extraction of general amino acids, a portion of the sample of the raw material ground to a powdery state was placed in a vial, 2 mL of 6N hydrochloric acid was added, and the vial was placed in a thermostat at 110 °C. Hydrolysis was carried out for 24 hours.

Then 0.5 mL of the centrifuged extract was evaporated on a rotary evaporator, and the residue was washed with three portions of distilled water to eliminate hydrochloric acid. The extract was re-suspended in 0.5 mL of distilled water and filtered through regenerated cellulose membrane filters with 0.2 µm pores. The fluorescent derivatives were obtained in an automatic programmed mode before the sample injection into the chromatographic column.

The identification of amino acids was performed by comparing retention times with a mixture of amino acid standards (Agilent 5061-3334). The quantitative content of amino acids was calculated from the value of the peak area of the amino acids comparing to that one of the amino acid standards. The quantitative content of bound amino acids was determined by subtracting the value of the quantitative content of free amino acids from the value of their total quantitative content [22].

Results and discussion

The results of the determination of the total ash content (%) in the batches of the raw material are shown in Figure 1. The total ash content was ranged from 0.61 ± 0.02% (batch 5) to 1.43 ± 0.05% (batch 4) and did not exceed 3% specified in the monograph of the SPhU 2.0 [10].

The presence of at least 19 macro-, trace, and ultra-trace elements was determined in the batches of the raw material. The results of the determination of the macroelemental composition of the batches of the raw material of *C. islandica* harvested in Ukraine are shown in Figure 2. The quantitative content of macroelements, such as potassium, calcium, silicon, magnesium, sodium, and phosphorus was determined. The potassium content was the highest among the macronutrients in all batches and ranged from 190 mg/100 g (batch 6) to 325 mg/100 g (batch 7). There was a sufficiently high content of silicon – from 30 mg/100 g (batch 1) to 115 mg/100 g (batch 4) and calcium – from 37 mg/100 g (batch 6) to 86 mg/100 g (batch 4) (Figure 2).

The results of determining the trace elemental composition of the batches of the raw material are shown in Figure 3. Among the trace elements the quantitative content of zinc, aluminum, manganese, iron, strontium, copper, and molybdenum was determined.

Compared to other trace elements, a significant part was occupied by zinc – from 5.4 mg/100 g (batch 7) to 28.6 mg/100 g (batch 4) and aluminum – from 4.6 mg/100 g (batch 6) to 12.2 mg/100 g (batch 5).

The pattern of accumulation of the elements for the each batch of the raw material studied was as follows:

- batch 1: K > Ca > Si > Mg > Na > P > Zn > Al > Mn > Fe > Sr > Cu > Mo;
- batch 2: K > Ca > Si > Mg > P > Na > Zn > Al > Fe > Mn > Sr > Cu > Mo;
- batch 3: K > Si > Ca > Mg > Na > P > Zn > Fe > Al > Mn > Sr > Cu > Mo;
- batch 4: K > Si > Ca > Mg > Na > P > Zn > Al > Mn > Fe > Sr > Cu > Mo;
- batch 5: K > Si > Ca > Na > Mg > P > Al > Zn > Fe > Mn > Cu > Sr > Mo;
- batch 6: K > Si > Ca > Mg > Na > P > Zn > Al > Fe > Mn > Sr > Cu > Mo;
- batch 7: K > Ca > Si > Mg > P > Na > Al > Zn > Fe > Mn > Sr > Cu > Mo.

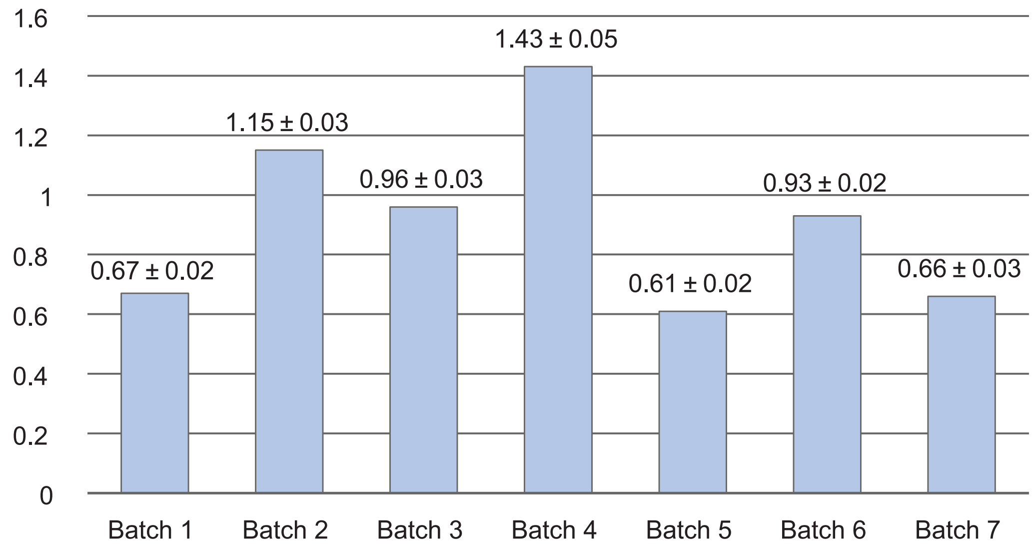

Figure 1. The total ash content (%) in the batches of the raw material of *C. islandica* harvested in Ukraine

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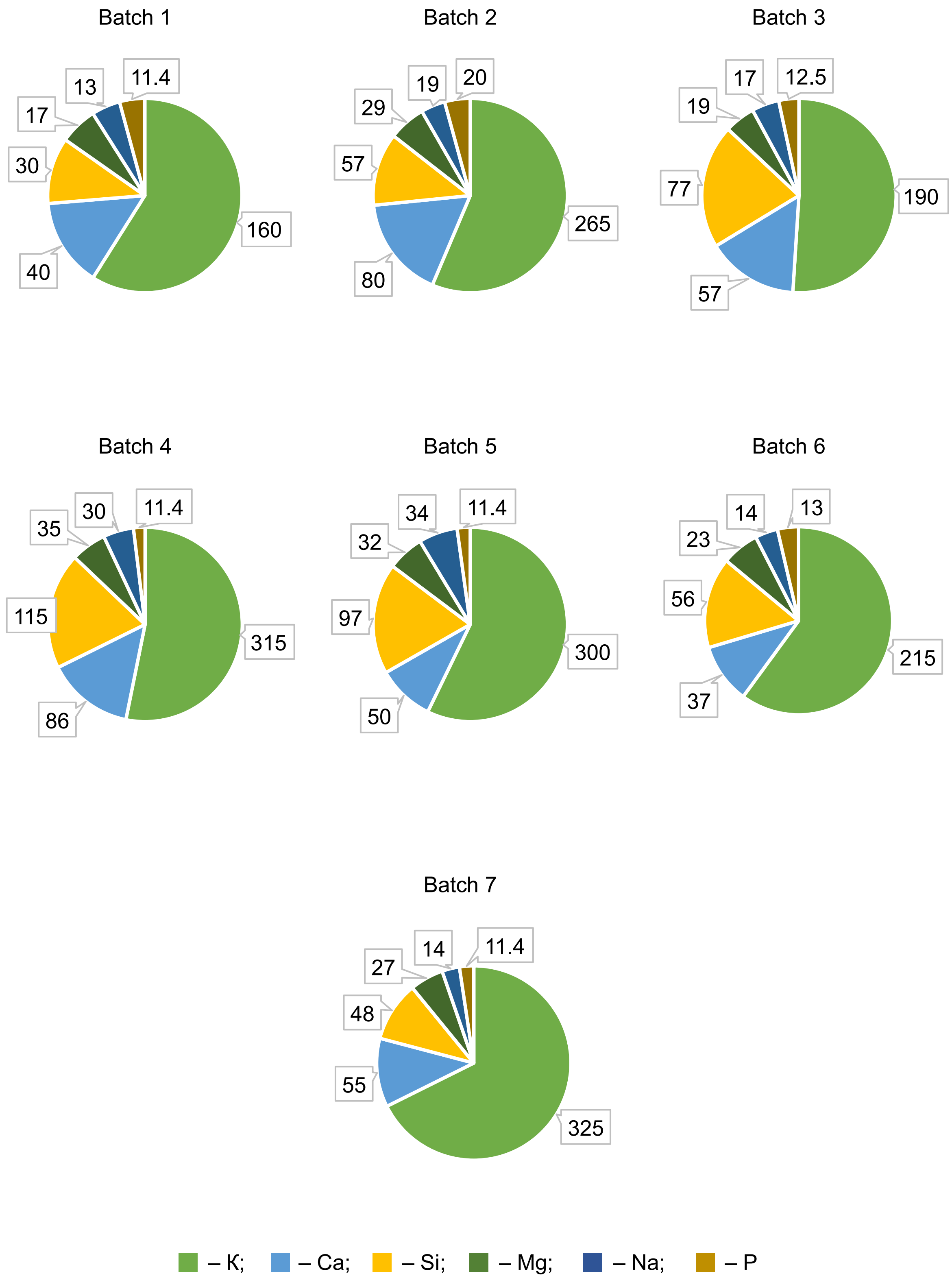

Figure 2. The macroelemental composition of the batches of the raw material of *C. islandica* (mg/100 g)

In all batches of the raw material the quantitative content of molybdenum and cobalt was less than 0.03 mg/100 g, the content of cadmium, astatine and mercury was less than 0.01 mg/100 g. The quantitative content of nickel did not exceed 0.128 mg/100 g.

It is important to note that the quantitative content of lead in the raw material ranged from 0.067 mg/100 g or 0.67 ppm (batch 1) to 0.18 mg/100 g or 1.8 ppm (batch 4), which did not exceed 10.0 ppm specified in the monograph of the SPhU.2.0 [10].

The results of determination of the component composition of free and bound nonessential and essential amino acids by the HPLC method are shown in Table 1 and Figure 4.

Figure 3. The trace element composition of the batches of the raw material of *C. islandica* (mg/100 g)

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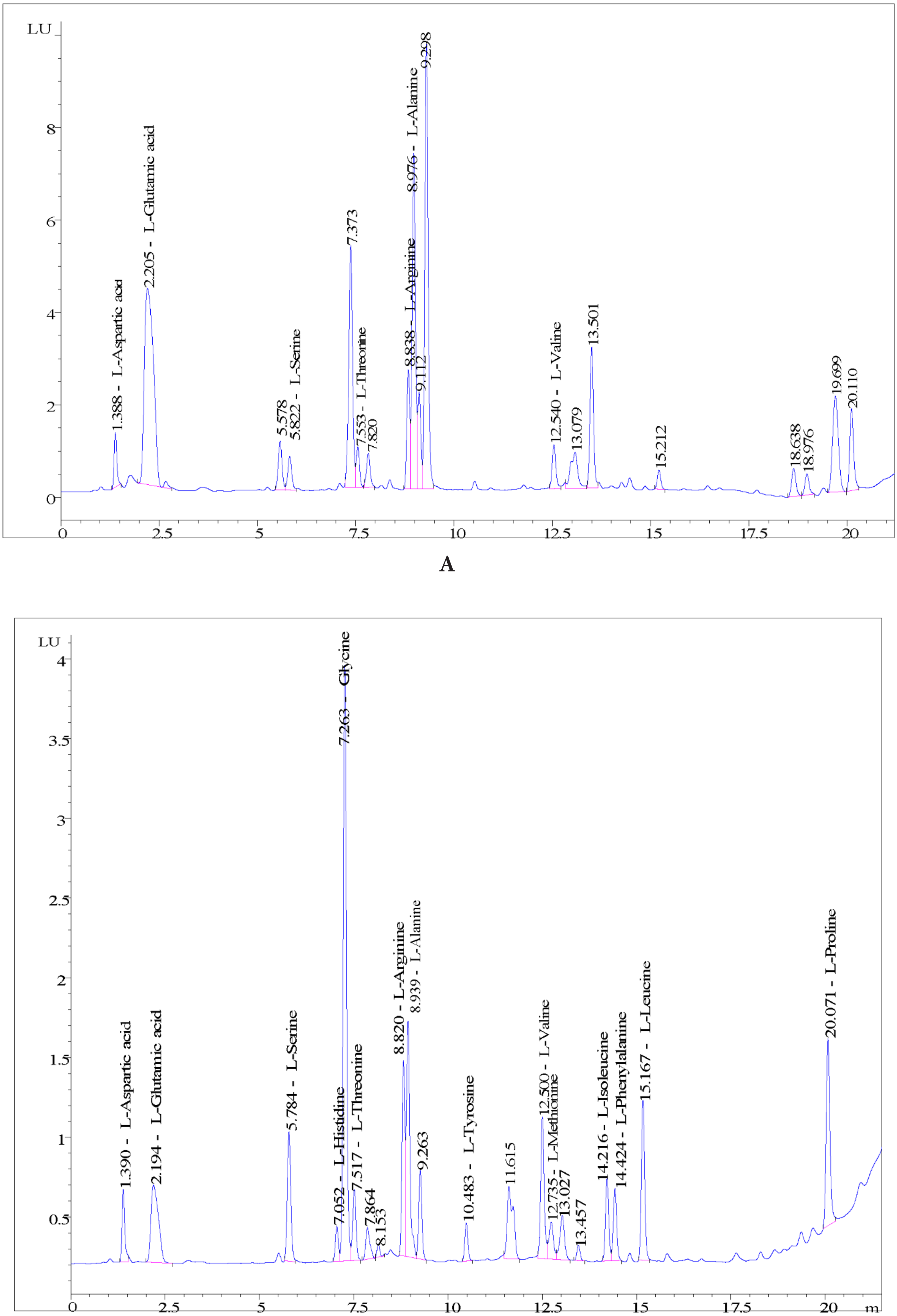

Figure 4. The HPLC chromatogram of free (A) and bound (B) amino acids in the raw material of *C. islandica*

Table 1. The quantitative content of amino acids in the raw material of *C. islandica*

	Free, μg mg^{-1}	Bound, μg mg^{-1}
Nonessential amino acids		
Aspartic acid	0.075	0.326
Glutamic acid	0.115	0.029
Serine	0.043	0.550
Glycine	–[a]	–
Arginine	0.175	0.993
Alanine	0.289	0.556
Tyrosine	–	–
Proline	–	–
Essential amino acids		
Threonine	0.050	0.304
Valine	0.047	0.518
Methionine	–	–
Histidine	–	–
Phenylalanine	–	–
Isoleucine	–	–
Leucine	–	–
Lysine	–	–
In total	0.794	3.276

Note: [a] "–" means that a component was not found

The presence of at least 7 amino acids was determined in the raw material studied, 5 of them were nonessential (aspartic acid, glutamic acid, serine, arginine, and alanine) and 2 acids were essential (threonine and valine). The quantitative content of the total amount of free nonessential amino acids in the raw material was 0.697 μg mg^{-1}, the bound ones – 2.454 μg mg^{-1}. The quantitative content of the total amount of free essential amino acids was 0.097 μg mg^{-1}, the bound ones – 0.822 μg mg^{-1}. The total quantitative content of the total amount of free amino acids in the raw material of *C. islandica* was 0.794 μg mg^{-1}, the total amount of bound amino acids was 3.276 μg mg^{-1}.

Conclusions

For the first time, the elemental composition of 7 batches of *C. islandica* thalli harvested in Ukraine was determined. In each batch, potassium was the predominant element (from 190 mg/100 g to 325 mg/100 g depending on the batch). The component composition of free and bound nonessential and essential amino acids was determined (the quantitative content of the total amount of free amino acids was 0.794 μg mg^{-1}, the total amount of bound ones was 3.276 μg mg^{-1}). Alanine (0.289 μg mg^{-1}) was the dominant component among free amino acids, while arginine (0.993 μg mg^{-1}) prevailed among bound ones. The results obtained will be used for further studies of the raw material of *C. islandica* harvested in Ukraine.

References

1. Crawford, S. D. Lichens Used in Traditional Medicine. In *Lichen Secondary Metabolites: Bioactive Properties and Pharmaceutical Potential*; Ranković, B., Ed.; Springer International Publishing: Cham, 2015; pp 27–80. https://doi.org/10.1007/978-3-319-13374-4_2.
2. Goga, M.; Elečko, J.; Marcinčinová, M.; Ručová, D.; Bačkorová, M.; Bačkor, M. Lichen Metabolites: An Overview of Some Secondary Metabolites and Their Biological Potential. In *Co-Evolution of Secondary Metabolites*; Mérillon, J.-M.; Ramawat, K. G., Eds.; Springer International Publishing: Cham, 2020; pp 175–209. https://doi.org/10.1007/978-3-319-96397-6_57.
3. Zhao, Y.; Wang, M.; Xu, B. A comprehensive review on secondary metabolites and health-promoting effects of edible lichen. *Journal of Functional Foods* **2021,** *80*, 104283. https://doi.org/10.1016/j.jff.2020.104283.
4. Ministry of Health of Ukraine. Derzhavnyi reiestr likarskykh zasobiv Ukrainy [State Register of Medicines of Ukraine, *in Ukrainian*]. http://www.drlz.com.ua/ibp/ddsite.nsf/all/shlist?opendocument (accessed May 2, 2022).
5. Kompendium – likarski preparaty [Compendium – medicines, *in Ukrainian*]. https://compendium.com.ua/ (accessed May 7, 2022).
6. Ranković, B.; Kosanić, M. Lichens as a Potential Source of Bioactive Secondary Metabolites. In *Lichen Secondary Metabolites: Bioactive Properties and Pharmaceutical Potential*; Ranković, B., Ed.; Springer International Publishing: Cham, 2019; pp 1–29. https://doi.org/10.1007/978-3-030-16814-8_1.
7. McDonough, A. M.; Bird, A. W.; Luciani, M. A.; Todd, A. K. Establishing trace element concentrations for lichens and bryophytes in the ring of fire region of the Hudson Bay Lowlands, Ontario, Canada. *Environ. Monit. Assess.* **2022,** *194* (3), 226. https://doi.org/10.1007/s10661-022-09890-0.
8. Calcott, M. J.; Ackerley, D. F.; Knight, A.; Keyzers, R. A.; Owen, J. G. Secondary metabolism in the lichen symbiosis. *Chem. Soc. Rev.* **2018,** *47* (5), 1730–1760. https://doi.org/10.1039/C7CS00431A.
9. Skrebtsova, K. S.; Fedchenkova, Yu. A.; Khvorost, O. P. The elemental composition of leaves of promising species of decorative plants [*in Ukrainian*]. *Current issues in pharmacy and medicine: science and practice* **2019**, *12* (1), 21–24. https://doi.org/10.14739/2409-2932.2019.1.158955.
10. *Derzhavna farmakopeia Ukrainy*: v 3 tomakh, 2 vydannia [The State Pharmacopoeia of Ukraine: in 3 volumes, 2nd ed., *in Ukrainian*]; State Enterprise "Ukrainian Scientific Pharmacopoeial Center for Quality of Medicines": Kharkiv, 2014; Vol. 3.
11. Airaksinen, M. M.; Peura, P.; Antere, S. Toxicity of Iceland Lichen and Reindeer Lichen. *Toxic Interfaces of Neurones, Smoke and Genes*, Proceedings of the European Society of Toxicology Meeting, Kuopio, June 16–19, 1985; Springer, Berlin, Heidelberg: Berlin, Heidelberg, 1986; pp 406-409.
12. Meli, M. A.; Desideri, D.; Cantaluppi, C.; Ceccotto, F.; Feduzi, L.; Roselli, C. Elemental and radiological characterization of commercial Cetraria islandica (L.) Acharius pharmaceutical and food supplementation products. *Sci. Total Environ.* **2018,** *613-614*, 1566–1572. https://doi.org/10.1016/j.scitotenv.2017.08.320.

ISSN 2308-8303 (Print) / 2518-1548 (Online)

13. Vershinina, S. E.; Vershinin, K. E.; Kravchenko, O. Yu. Compositional analysis of plant material Cetraria laevigata Rassad. 1945, C. islandica (L.) Ach. 1803 (Parmeliaceae, Lichens) [*in Russian*]. *Vestnik Irkutskoj gosudarstvennoj sel'skohozyajstvennoj akademii* **2010**, 41, 13 – 21.
14. Kayzer, A. A.; Kayzer, G. A.; Korniyenko, I. P.; Yevdokimova, M. O. Biochemical Composition and Food and Drug-Value of lichen (Cetraria islandica) Growing of Taymyr [*in Russian*]. *Agrarnye problemy Gornogo Altaya i sopredel'nyh regionov*, Materialy Vserossijskoj nauchno-prakticheskoj konferencii, posvyashchennoj 90-letiyu Gorno-Altajskogo NII sel'skogo hozyajstva i 100-letiyu Ministerstva sel'skogo hozyajstva Respubliki Altaj, Barnaul, 2020; 53 – 63.
15. Vladymyrova, I.; Georgiyants, V.; Savelieva, E. Pharmacotherapeutic action analysis of mineral substances of medicinal plants, which are used in thyroid gland diseases. Bulletin of National Academy of Sciences of the Republic of Kazakhstan **2019**, 1, 6 – 13. https://doi.org/10.32014/2019.2518-1467.1.
16. Giordani, P.; Minganti, V.; Brignole, D.; Malaspina, P.; Cornara, L.; Drava, G. Is there a risk of trace element contamination in herbal preparations? A test study on the lichen Cetraria islandica. *Chemosphere* **2017,** *181*, 778 – 785. https://doi.org/10.1016/j.chemosphere.2017.04.140.
17. Tabalenkova, G. N.; Dalke, I. V.; Zakhozhiy, I. G. Amino acids composition of some lichen species in taiga zone of European north-east of Russia [*in Russian*]. *Izvestiya Samarskogo nauchnogo tsentra Rossiyskoy akademii nauk* **2017**, *19* (2(3)), 556 – 560.
18. Grujic-Injac, B.; Kolarski, D.; Stefanovic, D. K. The chemical investigation of the proteins from oak (*Everni prunastri*) and the islandic (*Cetraria islandica*) lichens. *Bull. Acad. Serbe Sci. Arts* **1976**, *54* (14), 9 – 12.
19. *Derzhavna farmakopeia Ukrainy*: v 3 tomakh, 2 vydannia [The State Pharmacopoeia of Ukraine: in 3 volumes, 2nd ed., *in Ukrainian*]; State Enterprise "Ukrainian Scientific Pharmacopoeial Center for Quality of Medicines": Kharkiv, **2015**; Vol. 1.
20. Imbrea, I. M.; Radulov, I.; Nicolin, A. L.; Imbrea, F. Analysis of Macroelements Content of some Medicinal and Aromatic Plants using Flame Atomic Absorption Spectrometry (FAAS). *Romanian Biotechnological Letters* **2016**, *21* (4).
21. Jámbor, A.; Molnár-Perl, I. Quantitation of amino acids in plasma by high performance liquid chromatography: Simultaneous deproteinization and derivatization with 9-fluorenylmethyloxycarbonyl chloride. *J. Chromatogr. A* **2009,** *1216* (34), 6218 – 6223. https://doi.org/10.1016/j.chroma.2009.06.083.
22. Jámbor, A.; Molnár-Perl, I. Amino acid analysis by high-performance liquid chromatography after derivatization with 9-fluorenylmethyloxycarbonyl chloride: Literature overview and further study. *J. Chromatogr. A* **2009,** *1216* (15), 3064 – 3077. https://doi.org/10.1016/j.chroma.2009.01.068.

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