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Chemical composition and nutritive potential of *Cichorium intybus* L. leaves from Montenegro

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Abstract: The leaves of chicory (*Cichorium intybus* L.) plant have been used for centuries in Montenegro and other Mediterranean countries as a vegetable in salads, sauces and other types of appetizers and meals. The wild and cultivated chicory leaves from different locations in Montenegro were analysed regarding several nutrients, major and trace element as well as vitamin composition, using standard methods of analysis. The results of the study indicated that chicory leaves were rich in total dietary fiber and mineral content and had low energy value. Also, they were potential sources of useful nutrients such as potassium, calcium, manganese, iron, and vitamin A, with the average content of 391.9, 164.7, 0.55, 2.33 and 0.47 mg/100 g in fresh leaves, respectively. Wild plants were superior to the cultivated ones regarding carbohydrate, calcium and manganese content. Origin of the chicory leaves significantly influenced most of the analyzed parameters.

Keywords: chicory; nutrients; minerals; vitamins.

INTRODUCTION

Indigenous plants play important role in human diet because they contain significant amounts of nutrients and trace elements. Despite the wide-spread consumption of cultivated vegetables, some indigenous plants have been reported as more nutritious and less expensive. In Montenegro many indigenous plants are traditionally collected from the nature, but precise and valid data for their nutritional value is not available.

Wild chicory (*Cichorium intybus* L.) is an erect perennial herb, 80–90 cm in height usually with bright blue flowers, rarely white or pink. It has a fleshy taproot up to 75 cm in length. Chicory is a member of *Asteraceae* family. The genus *Cichorium* consists of six species mainly distributed in the areas of Europe

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and Asia.[1] Several *Cichorium* species have been used for centuries as part of the traditional diet in Mediterranean countries.[2] Mostly used plant parts are leaves (as salads, cooked vegetable and meat dishes), chicons (blanched buds), or roots (var. *sativum*, which are baked, ground, and used as a coffee substitute and inulin source). Most of the information on plant chemical composition refers to the roots and seeds, but leaves have been rarely investigated.[2-4]

Chicory leaves are usually bitter. Their bitterness is highly appreciated in certain cuisines, such as in Italy and in Spain, Greece and Turkey. The use of this herb is also common in the diet of people living in the Montenegrin part of the Adriatic coast, especially in the Boka Bay region. Recently, due to the growing demand, this vegetable crop has been cultivated in certain Montenegro areas. The goal of this study was to estimate nutritive potential of chicory leaves growing in Montenegro and to compare selected nutritional parameters of wild and cultivated plants. In Montenegro, this is the first study of chicory leaves composition.

EXPERIMENTAL

Details about sample collection and preparation are given in Supplementary material to this paper.

Nutritional analyses

The recommended methods by the Association of Official Analytical Chemists were used for the determination of moisture, ash, lipids, dietary fiber and nitrogen content.[5-9] At least three replicate determinations were made for each sample.

Proteins were estimated by multiplying the sample percentage nitrogen content by factor 6.25. Available carbohydrate was calculated by subtracting total sum of proteins, lipids, dietary fiber and ash from 100 % sample.

Total energy values were calculated in kcal* multiplying the amounts of protein and carbohydrate by the factor 4 and fat by the factor 9.[10]

Determination of mineral content

Each fresh plant material (500 mg) was accurately weighted (±0.1 mg) and digested using closed microwave assisted digestion (Berghof MWS-4, Germany) with 5 cm^3 of nitric acid (≥69%, puriss. *p.a.*, Sigma Aldrich) and 2 cm^3 of hydrogen peroxide (≥30%, for trace analyses, Sigma Aldrich). Digested samples were filtered and transferred into volumetric flasks by adding deionised water.[11,12] All analyses were performed in triplicates.

Working standards for measurements of major and trace elements were prepared from Sigma Aldrich solutions of 1000 mg dm^{-3} each. Atomic absorption measurements were carried out on Shimadzu AA-6800 (Japan). For analyses of iron, manganese, zinc and copper flame AAS was used and for analyses of calcium, magnesium, sodium, potassium and phosphorus ICP-OES Thermo iCAP 6300 (UK) was used. The reliability of the analytical method was evaluated by using Certified Standard Reference Material NCS DC73348 (Bush Branches and Leaves) from the China National Analysis Center for Iron and Steel, Beijing.

* 1 kcal = 4184 J

Vitamin analyses

The vitamin content (B_1, B_2 and B_6) was determined after acid hydrolysis of the samples in autoclave. After adjusting pH to 4.5 with CH_3COONa solution, the samples were filtered through membrane filter and were ready for the determination of vitamin B_2 and B_6. The determination of vitamin B_1 was carried out with the pre-column oxidation of thiamine to tiochrom with oxidizing reagent (alkaline solution of potassium ferricyanide) and filtration through membrane filter. Sample analysis was performed using HPLC technique under the following conditions: column – LiChrospher 100 RP-18 (5 μm) LiChroCART 250-4; detector-RF-535 Shimadzu Fluorescence HPLC monitor; eluent for vitamin B_1 and B_2: 450 ml CH_3OH+620 ml 5 mM CH_3COONH_4; eluent for vitamin B_6: 250 ml CH_3OH+770 ml 5 mM hexansulfonic acid; flow rate 0.8 ml min^{-1}; injected volume 20 μl; column temperature 20° C; wavelengths - B_1: λ_{ex} = 370 nm and λ_{em} = 430 nm; B_2: λ_{ex} = 450 nm and λ_{em} = 530 nm; B_6: λ_{ex} = = 286 nm and λ_{em} = 392 nm. Standard solutions of vitamins B_1, B_2 and B_6 were purchased from Sigma. Methanol HPLC grade, ammonium-acetate and hexansulphonic acid were all purchased from Merck.

β-Carotene was determined using extraction with acetone and hexane in volume ratio of 70:30, followed by the separation of the other colouring matter on a chromatographic column. The absorbance of samples was measured at 430 nm.[13]

Vitamin C was analyzed by volumetric method with 2,6-dichloro-phenol-indophenol.[14] In this procedure, sample was first extracted with distilled water.

For vitamins, at least three replicates for each sample were made.

Statistics

Statistical difference between averages of the two groups (wild and cultivated plants) was determined using Student's *t*-test – two sample assuming unequal variances, and *p* value <0.05 was considered statistically significant.

RESULTS AND DISCUSSION

Nutrient composition

The results of the nutrients' content and estimated energy values obtained for the nine samples of *Cichorium intybus* L. leaves are presented in Table I.

Determined average values for dry matter were 12.42 % for the leaves of wild plants and 8.85% for the cultivated plant leaves. The total mineral content expressed as ash content was 1.36 % for the wild plants and 1.16 % for the cultivated plants. The lipid contents varied between 0.22 and 0.49 % for the wild plant leaves, while the average content in cultivated plants was 0.44 %. The lipid content results indicated that the chicory leaves are poor sources of plant lipids, which is in agreement with general observation that leafy vegetables are low fat containing food.[15]

The dietary fiber content in the samples varied considerably between 2.90 and 6.16 % and represented about 35 % of dry matter in average. Our results showed that plants grown on different locations had different carbohydrate content, but also different protein content. The protein content was the highest in those from Pricelje (2.78 %) and the lowest in those from Podgor (1.63 %).

TABLE I. Nutritive composition and energy value of *Cichorium intybus* L. leaves; data are expressed per 100 g fresh weight and presented as mean±*SD* of three independent determinations; a: significant difference was stated at $p < 0.05$

Location	Content, %						Energy value kcal/100 g
	Dry matter	Ash	Lipids	Dietary fiber	Proteins	Available carbohydrate	
Wild plants							
Zoganje	11.94±0.40	1.54±0.03	0.45±0.09	4.99±0.14	2.19±0.10	2.77±0.25	24
Risan	14.31±0.28	1.58±0.04	0.49±0.10	4.35±0.20	1.90±0.12	5.99±0.17	36
Podgor	11.83±0.73	1.39±0.02	0.35±0.05	4.11±0.15	1.63±0.08	4.35±0.22	27
Tivat	11.00±0.34	1.37±0.04	0.41±0.11	3.01±0.16	1.89±0.14	4.32±0.26	29
Pricelje	14.47±0.19	1.42±0.04	0.45±0.10	3.39±0.14	2.78±0.06	6.43±0.38	41
Plavnica	11.46±0.72	1.20±0.05	0.33±0.14	3.27±0.14	2.75±0.06	3.90±0.14	30
Pljevlja	11.93±0.34	1.05±0.05	0.22±0.07	6.16±0.19	2.19±0.08	2.31±0.25	20
Average	12.42±1.39	1.36±0.19	0.39±0.09	4.18±1.11	2.19±0.44	4.30±1.52	30±7
Cultivated plants							
Komani	9.48±0.18	1.23±0.02	0.48±0.06	4.29±0.15	2.16±0.11	1.32±0.19	18
Susanj	8.21±0.09	1.08±0.04	0.39±0.10	2.90±0.11	2.28±0.11	1.56±0.16	19
Average	8.85±0.90[a]	1.16±0.11	0.44±0.06	3.60±0.98	2.22±0.08	1.44±0.14[a]	19±1[a]

Compared to the literature data on composition of the most-commonly consumed leafy vegetable species in Montenegro e.g. spinach (*Spinacia oleracea)* and lettuce (*Lactuca sativa*), an average value of dry matter in analysed chicory leaves was 1.4 and 2.5 times higher, respectively.[16,17] The reason is the evident difference in dietary fiber and carbohydrate content between the analysed samples and the other leafy vegetables. Also, the average protein content in chicory was about 25 % higher than in lettuce.[18]

On the basis of the estimated nutrient composition, energy value of one portion (100 g) of fresh chicory leaves was calculated. The average value was 27 kcal/100 g, which was 30% higher than in lettuce.[18] The highest and lowest energy values were 41 kcal/100 g (Pricelje) and 18 kcal/100 g (Komani).

The difference between dry matter and carbohydrate content in the leaves of wild and cultivated plants was significant ($p < 0.05$). Cultivated plants had 30 % lower dry matter content than wild types. Carbohydrate content in the wild plants was three times higher compared to the cultivated plants.

Our results for basic nutritive composition of fresh chicory leaves from Montenegro are in good correlation with the data obtained for cultivated chicory leaves in Holland.[19]

Minerals

The results of the major mineral content obtained for nine samples of chicory leaves are presented in Table II.

TABLE II. Major mineral composition of *Cichorium intybus* L. leaves; data are calculated based on the original weight and presented as mean±*SD* of three independent measurements; a: significant difference was stated at $p < 0.05$

Location	Content of minerals, mg/100 g				
	K	Na	Ca	Mg	P
Wild plants					
Zoganje	445.5±5.1	49.3±2.1	223.4±3.1	32.1±1.4	48.8±6.1
Risan	439.1±4.2	52.1±2.0	275.4±2.4	41.0±2.0	28.8±7.0
Podgor	511.2±4.0	15.3±2.2	146.0±2.1	25.8±1.9	35.1±7.4
Tivat	383.4±4.1	60.4±3.4	158.8±3.9	28.1±1.9	38.4±6.0
Pricelje	391.1±3.1	31.0±3.1	281.0±1.2	41.4±4.1	31.7±5.1
Plavnica	335.9±3.2	45.0±2.4	149.2±1.4	24.8±2.2	23.2±4.1
Pljevlja	311.4±4.2	10.8±1.3	88.8±0.9	20.1±1.9	32.8±5.1
Average	402.5±68.6	37.7±19.1	188.9±72.5	30.5±8.2	34.1±8.1
Cultivated plants					
Komani	383.1±5.2	29.1±2.2	87.8±1.8	24.2±1.8	22.8±4.1
Susanj	326.5±3.0	9.3±1.9	72.0±2.1	17.3±2.1	20.4±3.2
Average	354.8±40.0	19.2±14.0	79.9±11.2[a]	20.8±4.9	21.6±1.7

All samples had low amount of Na (average 33.6 mg/100 g), but relatively high concentration of K (average 391.9 mg/100 g). Variations in Ca content were in the range 72.0–281.0 mg/100 g. Mg content in fresh *Cychorium* leaves was low and in the range 17.3–41.4 mg/100 g, while P content was from 20.4 mg/100 g in the samples from Susanj to 48.8 mg/100 g in the samples from Zoganje. K content was about 40% higher than in lettuce, while the average value of Ca (164.7 mg/100 g) was similar as in spinach, but five to ten times higher than in lettuce.[18–20]

These results proved that fresh *Cychorium* leaves are poor P and Mg dietary sources, while Ca and K are present in higher amounts, which is in good correlation with the findings for organically grown forage chicory leaves from New Zealand.[22]

The results of trace mineral content in fresh chicory leaves are presented in Table III.

The values for Cu content in fresh chicory leaves were low and in the range 0.02–0.12 mg/100 g. Zn average content in chicory leaves was 0.68 mg/100 g. The lowest (0.26 mg/100 g) and the highest (1.07 mg/100 g) Mn contents were recorded in the leaves from Komani and Pricelje, respectively. The average value for Fe content was 2.33 mg/100 g. The obtained value for average Zn content was about seven times higher than in lettuce. Also, chicory leaves had 30 % more Fe, but 50 % less Cu than spinach.[20,21]

K, Mg, P, Cu and Fe content did not show significant differences between wild and cultivated plants. Difference in Ca, Zn and Mn content in the leaves of wild and cultivated plants was significant ($p < 0.05$). The wild plants were about

2.5 times richer in Ca, 4 times richer in Zn and 2 times richer in Mn than the cultivated ones. Although it is known that plant mineral content varies with the environmental and maturity conditions, these results indicate that wild growing plants can have more beneficial mineral profile than cultivated ones and these findings should be further investigated.

TABLE III. Content of Cu, Zn, Mn and Fe in *Cichorium intybus* L. leaves; data are calculated based on the original weight and presented as mean±*SD* of three independent measurements; a: significant difference was stated at $p < 0.05$

Location	Content of minerals, mg/100 g			
	Cu	Zn	Mn	Fe
		Wild plants		
Zoganje	0.12±0.02	1.14±0.02	0.56±0.01	1.64±0.07
Risan	0.02±0.02	0.97±0.01	0.69±0.02	2.94±0.08
Podgor	0.08±0.02	0.82±0.02	0.53±0.01	1.71±0.06
Tivat	0.09±0.02	0.77±0.01	0.70±0.02	2.21±0.07
Pricelje	0.08±0.01	0.96±0.02	1.07±0.03	4.51±0.09
Plavnica	0.10±0.01	0.63±0.02	0.41±0.01	1.99±0.05
Pljevlja	0.04±0.02	0.38±0.01	0.33±0.01	2.60±0.07
Average	0.08±0.03	0.81±0.25	0.61±0.24	2.51±1.00
		Cultivated plants		
Komani	0.05±0.02	0.22±0.01	0.26±0.01	1.60±0.06
Susanj	0.03±0.02	0.18±0.01	0.36±0.01	1.80±0.07
Average	0.04±0.01	0.20±0.03[a]	0.31±0.07[a]	1.70±0.14

Vitamins

The results for the vitamins B_1, B_2, B_6, β-carotene and vitamin C content in fresh chicory leaves are given in Table IV.

The mean value of vitamin B_1 content in fresh chicory leaves was 138.6 μg/100 g, while values for vitamin B_2 content were in the range 85.1–181.6 μg/100 g, and for vitamin B_6 137.0–252.6 μg/100 g. The values of β-carotene content in chicory leaves were 1.9-3.8 mg 100 g^{-1} and for vitamin C varied from 3.2 to 6.0 mg/100 g.

Our results for vitamin B_6 content were about twenty times higher than results published for spinach and even 100 times higher than for lettuce.[23]

Content of vitamin C in chicory leaves was about forty times higher than in lettuce, but compared to spinach four times lower.[23]

Interestingly, the content of analyzed vitamins was not different between wild and greenhouse produced plants, while the total carbohydrate and mineral content (calcium, zinc and manganese) were significantly influenced by the production method.

Some health workers and consumers prefer wild plants than cultivated ones when given a choice, due to the notion that intensive plant production could

result in lower content of certain valuable components. Several investigations confirmed this hypothesis when polyphenols and essential oils were investigated.[24,25] Our results proved the hypothesis that wild chicory leaves have better nutritional profile of selected nutrients than cultivated ones.

TABLE IV. Content of the vitamins B_1, B_2, B_6 and β-carotene and vitamin C in chicory leaves; data are expressed based on the original weight and presented as mean±*SD* of three independent measurements

Location	Content of vitamins, µg / 100 g				
	B_1	B_2	B_6	β-carotene	C
Wild plants					
Zoganje	82.2±0.3	85.1±1.5	140.8±2.4	2.7±0.1	6.0±0.3
Risan	137.8±1.2	118.5±0.7	161.6±1.4	3.6±0.2	4.6±0.4
Podgor	126.7±1.6	99.4±2.2	161.6±1.3	2.7±0.1	3.5±0.3
Tivat	215.8±1.7	115.7±0.9	148.9±1.3	3.8±0.1	3.5±0.3
Pricelje	81.5±2.3	83.6±1.2	137.0±1.4	3.5±0.1	3.9±0.4
Plavnica	160.3±2.9	181.6±1.1	252.6±1.1	1.9±0.1	3.2±0.1
Pljevlja	129.4±1.4	129.1±1.5	178.7±2.5	2.6±0.2	3.2±0.2
Average	133.4±46.4	116.1±33.6	168.7±39.6	3.0±0.7	4.0±1.0
Cultivated plants					
Komani	106.4±3.4	120.4±0.9	160.3±2.4	2.2±0.1	3.6±0.1
Susanj	206.9±8.1	104.4±1.1	165.3±1.0	2.5±0.1	3.3±0.1
Average	156.7±71.1	112.4±11.3	162.8±3.5	2.4±0.2	3.5±0.2

Table V shows the results obtained for the content of major and trace minerals, as well as vitamins in one portion (100 g of fresh chicory leaves) compared to the reference daily intake values (*RDI*).

TABLE V. Content of major and trace minerals, and vitamins in 100 g fresh leaves compared to reference daily intake (*RDI*[10])

Major element	*RDI* / %	Trace element	*RDI* / %	Vitamin	*RDI* / %
K	19.2	Zn	6.8	B1	12.5
Ca	20.6	Cu	8.0	B2	8.2
Mg	7.5	Mn	27.5	B6	11.9
P	4.4	Fe	16.6	A[a]	58.8
				C	4.8

[a]Calculated from beta-carotene (1 µg of vitamin A = 6 µg of beta-carotene)

Considering the amount of analysed vitamins and minerals in one edible portion of fresh chicory leaves (100 g) and expressed as percentage of *RDI* values, it could be concluded that K, Ca, Mn, Fe, and vitamin A (calculated from β-carotene) contents were >15% of *RDI*, which is the requirement for foods to carry a nutritional statement "source of".[26]

CONCLUSION

Based on the results of our analyses it can be concluded that chicory leaves, which have been used as food in Montenegro for centuries, are valuable sources of several nutrients, minerals and vitamins and therefore could be regarded as healthy foods in well-balanced diets. Chicory leaves are especially rich in carbohydrates, Ca, Mn, Fe and vitamins B_1, B_2 and B_6. They could also be used in low caloric diets because of their low energy value. Better knowledge of traditional foods' nutritive potential can serve as a basis for their more intensive inclusion in modern dietary patterns. The case of chicory plant in Montenegro also proves that revitalization of selected wild plants, either by harvesting them from nature or by cultivating process, is the best way for preserving traditional foods in certain geographical regions.

SUPPLEMENTARY MATERIAL

Details about sample collection and preparation are available electronically at the pages of journal website: http://www.shd.org.rs/JSCS/, or from the corresponding author on request.

ИЗВОД

ХЕМИЈСКИ САСТАВ И НУТРИТИВНИ ПОТЕНЦИЈАЛ ЛИШЋА *Cichorium Intybus* L. ИЗ ЦРНЕ ГОРЕ

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Лишће биљке цикорије (*Cichorium intybus* L.) вековима се користило као поврће у салатама, сосевима и другим врстама предјела и јела у Црној Гори и другим медитеранским земљама. Лишће самоникле и гајене цикорије са различитих локација у Црној Гори анализирано је у погледу састава нутријената, макро- и микроелемената и витамина коришћењем стандардних метода. Резултати испитивања су показали да је лишће цикорије богато дијеталним влакнима и минералима и да има ниску енергетску вредност. Такође, оно је значајан извор корисних нутријента као што су калијум, калцијум, манган, гвожђе и витамин А, са средњом вредности садржаја од 391,9, 164,7, 0,55, 2,33 и 0,47 mg/100 g свежег лишћа, редом. Самоникле биљке су биле богатије од гајених у погледу садржаја угљених хидрата, калцијума и магнезијума. Порекло лишћа цикорије имало је значајан утицај на већину анализираних параметара.

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