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Iron content in leafy plants cultivated in Bosnia and Herzegovina

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RESEARCH ARTICLE

Abstract

Iron deficiency anemia is one of the main health issues that has an impact on cognitive function, physical ability, immune function, and reproductive performance. Therefore, this study focused on the determination of iron content in leafy plants cultivated in Bosnia and Herzegovina, and the average daily intake of iron *via* consumption of these plants. The plant samples were prepared by wet digestion with nitric acid (HNO_3), and iron content was determined using flame atomic absorption spectrometry (FAAS). The mean concentration of Fe ranged from 41.97 mg/kg for *Brassica oleracea var. acephala* to 338.73 mg/kg for *Spinacia oleracea*. Daily intakes for different leafy plants were also calculated. The leafy plants were arranged by daily iron intake in the following decreasing order *Spinacia oleracea* > *Beta vulgaris* > *Atriplex hortensis* > *Urtica dioica* > *Brassica oleracea var. acephala*. Results from this study indicate that leafy plants can be a significant source of iron. The findings conclusively suggest that our local leafy plants are good source of iron through diet.

Keywords: leafy plants, FAAS, daily iron intake.

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INTRODUCTION

Iron is an essential microelement for living organisms due to its role in numerous metabolic processes. It is required for various highly complex processes, including the transport of oxygen from the lungs to tissues *via* red blood cell hemoglobin, DNA synthesis, electron transfers, and as a significant component of enzyme systems in various tissues (Pandrangi et al., 2022). Iron is also involved in many crucial biochemical processes, such as the synthesis of amino acids, lipids, deoxyribonucleotides, and sterols (Sun et al., 2022). Many diseases are related to abnormal iron metabolism, which results in iron overload or deficiency. Iron deficiency usually results in anemia, which is defined as lower blood hemoglobin levels than normal (Roemhild et al., 2021). Many studies have found a connection between iron deficiency anemia and poor motor and mental performance in children, as well as poor pregnancy outcomes and low work productivity in adults (Horton and Ross, 2003). Intake of more heme iron from food of animal origin can significantly reduce the risk of anemia. The main natural sources of iron in food are red meat (liver and beef), fish, and eggs. However, at the same time, consuming additional food of animal origin can cause hypertension, gastrointestinal cancer, diabetes, and chronic diseases (Kruger and Zhou, 2018; de La Pomélie et al., 2019). Leafy plants are also a rich natural source of iron Leafy plants such as garden orache, spinach, swiss chard, nettle, and collard green have occupied a unique place in the Bosnian-Herzegovinian diet because of their flavor, color, and health benefits. In addition to iron, these plants are rich in a wide range of nutrients, including zinc, magnesium, calcium, and others, on top of being an affordable source of antioxidants, vitamins, and dietary fibers (Caunii et al., 2010; Gavrilaș et al., 2018). Most leafy plants are short duration crops that accumulate a large amount of biomass in short periods of time.

The garden orache (*Atriplex hortensis*) is grown as a cultivated plant from this genus. It is also called mountain spinach, French spinach, or arrach and is considered one of the oldest cultivated plants, valued mainly for its leaves. The young stems and leaves are used as food. Orache is rich in vitamins and biologically active compounds. It also has dietary and medicinal properties. When used in food, it promotes the absorption of nutrients from food and stimulates digestion (Zeipiņa et al., 2015). Nettle (*Urtica dioica*) is an annual wild plant in the botanical family Urticaceae. It is well known that this plant has exceptional biological activity and has positive effects on human health. Fresh nettles are used on arthritic or paralyzed limbs to stimulate blood circulation and warm the joints and extremities. Various studies have demonstrated antioxidant, antimicrobial, anti-inflammatory, anti-ulcer and analgesic properties of nettle extracts as well as many other biological activities. In addition to its use in medicine, this herb is also used in human nutrition as food or tea and is also commercially harvested due to its high content of chlorophyll, which is used as a green colorant in food and medicines (Upton, 2013). Spinach (*Spinacia oleracea*) is an edible flowering leafy plant consumed in the human diet for its high nutritional value and growing in huge amounts. Nowadays it is an important horticultural crop as its leaves are used in human nutrition worldwide. It is rich in antioxidants, vitamins (A, C, E, K, B2, and B6), and minerals (magnesium, manganese, calcium, phosphorus, iron, zinc, and copper). A versatile plant, spinach is eaten both raw (in salads and smoothies) and cooked (e.g., steamed, casseroles, soups) (Roberts and Moreau, 2016). The green biennial leafy plant known as Swiss chard or chard (*Beta vulgaris*) is available all year long. It belongs to the Amaranthaceae family and can be harvested over a long period of time as it is planted in the spring and summer of each year. In addition to phenolic acids like p-coumaric acid, caffeic acid, and syringic acid, and minerals like iron, calcium, potassium, magnesium, manganese, and phosphorus, swiss chard stems and leaves also contain significant amounts of vitamins A, B, and C, and flavonoids like the glycosides apigenin, quercetin, and kaempferol. (Ali et al., 2021). Collard green (*Brassica oleracea var. acephala*) occupies an important place in the cuisine and diet of Europe, Asia, and American populations. It is traditionally grown by farmers on small acreages, primarily for family consumption, either as food or animal feed. The recent trend and promotion of collard green as a 'superfood' has brought this plant onto the menus of many restaurants in the USA, especially those specializing in healthy food. Because it is tolerant to low temperatures, the plants can survive the winter and serve as fresh vegetables from late autumn to early spring of the next season. The younger and more tender leaves of collard green are used for human consumption, while the older leaves are more suitable as animal feed. Leaves are usually eaten fresh in salads and as collard green juice and prepared in various soup dishes, omelets, and stir-fries. Collard green has calcium, folate, riboflavin, vitamin K content, and vitamin C amount much higher than in other vegetables (Šamec et al., 2019).

A number of studies focusing on the determination of iron content in leafy plants have established that iron occurs in a wide range of concentrations from several tens to several hundred/thousands of mg/kg of dry plant weight (Caunii et al., 2010; Chatterjee et al., 2016). In many cases, this level is determined by genetic factors, morphological characteristics of a plant, geographical region, geochemical soil characteristics, and the ability of plants to selectively accumulate iron (Alloway, 2013; Briat and Lobréaux, 1997).

Due to lack of data, this study aimed to assess the content of iron in five commonly consumed green leafy plants in Bosnia and Herzegovina (B&H), namely garden orache (*Atriplex hortensis*), spinach (*Spinacia oleracea*), swiss chard (*Beta vulgaris*), nettle (*Urtica dioica*) and collard green (*Brassica oleracea var. acephala*). Furthermore, the obtained data was used for the calculation of average daily iron intake.

MATERIALS AND METHODS

Sampling

Organically grown leafy plants - garden orache, spinach, swiss chard, nettle, and collard green, were collected in Tuzla, B&H (during the year 2020). The sampling site is significantly far from the urban part, thus reducing the influence of plant pollution on the results of total iron contents. The local and botanical names of samples are given in Table 1. Parts of the sample that had been visibly contaminated from soil, insects, or mechanically damaged were removed. the selected samples were then washed in distilled water. Only the leaves of the samples were dried in a dark and airy place. The leaf is most often used in diet, so it was used for analysis. The University of Sarajevo's Faculty of Science's Laboratory for Plant Systematics used keys and iconographies to verify the authenticity of plant samples (Domac, 1994; Hayek, 1927; Jávorka and Csapody, 1991).

Sample preparation

Samples were prepared using a modified wet digestion method (Pazalja et al., 2021). In a Teflon beaker, 1 g (with an accuracy of ± 0.1 mg) of dried and ground samples was weighed in triplicates. Following that, 25 mL of analytical-grade 65% HNO_3 was added. The beakers were then covered with watch glasses and left overnight. The samples were digested for 12 hours at 70-80 ^oC. After digestion, the residue was filtered through a white band filter paper

(Whatman No. 42). The samples were then diluted to 50 mL with Milli-Q water. A Blank digest was carried out in the same way.

Table 1. Leafy plant samples with their local and botanical names

English name	Local name (B&H)	Botanical name	Used part
Garden orache	Loboda	*Atriplex hortensis*	Leaf
Spinach	Špinat	*Spinacia oleracea*	Leaf
Nettle	Kopriva	*Urtica dioica*	Leaf
Swiss chard	Blitva	*Beta vulgaris*	Leaf
Collard greens	Raštika	*Brassica oleracea* var. *acephala*	Leaf

Reagent and standard solutions

The reagents used in the experiments were analytical grade, while distilled and deionized water (Milli-Q, Millipore) was used for sample dilution and labware washing. A standard solution of iron (1000 mg/L, Certipur, Merck, Darmstadt) was used to create the calibration diagram. The calibration solutions were prepared over a concentration range of 0 – 20 mg/L Fe. The absorbance was measured for the calibration solution and analyzed solutions (the calibration curves demonstrated adequate linearity, $R^2 = 0.99$). The calibration curve is presented in Figure 1.

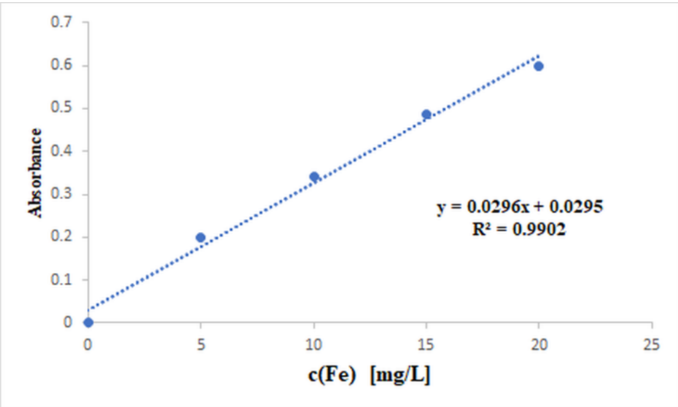

Figure 1. The calibration curve of iron.

FAAS analysis of iron

Iron content of commonly consumed leafy plants in B&H was determined by FAAS (Varian AA240FS). The standard deviation was calculated after the sample and blank analyses were completed in triplicate. The value of the detection limit (LOD) and quantification limit (LOQ) for Fe were 83.85 µg/L and 111.2 µg/L, respectively. Spiked samples were used to test the methods at different analyte concentrations, and the percent recovery was calculated. The recovery of known additions was acceptable (85-104%). Iron concentration is expressed as the mean value (mg/kg) ± standard deviation.

Daily intake for leafy plants

Daily intake for iron *via* leafy plants was calculated based on the assumption that the body mass is 60 kg, and the ingestion rate of leafy plants is 200 g. The daily intake was calculated based on equation (1) (Sharma et al., 2009; Singh et al., 2010):

$$\text{Daily intake Fe (mg kg}^{-1}\text{ day}^{-1}) = \frac{c_{Fe} \cdot I_R}{1000 \cdot BW}$$

Where, c_{Fe} – concentration of iron in analyzed leafy plants, I_R – ingestion rate of leafy plants and BW – body weight.

RESULTS AND DISCUSSIONS

A number of edible leafy plants species, including garden orache, spinach, swiss chard, nettle, and collard greens, can be found in Bosnia and Herzegovina due to the country's diverse natural environments, changing climatic conditions, and seasonal variations. These leafy plants are inexpensive, easy to prepare, and full of nutrients that are vital for human health, including copper, manganese, zinc and iron. Therefore, in this study, the total iron content was determined in organically cultivated leafy plants from Tuzla (B&H). Certain leafy plant species are a good source of iron, but they can also contain specified inhibitors and promoters of iron absorption. Depending on whether plant contains promoters or inhibitors, the bioavailability of iron differs. Additionally leafy plants are an excellent source of some minerals, essential oils, glycosides, pigments etc. which stimulate appetite (Caunii et al, 2010).

The iron contents in analyzed leafy plants, which are commonly used in the diet in B&H, are presented in Table 2 as mean values of three replicate determinations.

Table 2. Iron content (mg/kg ± SD, dry weight) in leafy plants cultivated in B&H

Plant species	English name plant	Iron content (mg/kg ± SD)	Iron content, literature values (mg/kg)	References
Atriplex hortensis	Garden orache	85 ± 0.21	100-215	(Carlsson and Hallqvist, 1981; Rinchen and Singh, 2015)Hallqvist, 1981; Rinchen and Singh, 2015)
Spinacia oleracea	Spinach	338.73 ± 0.32	200-930	(Carlsson and Hallqvist, 1981)
Beta vulgaris	Swiss chard	217.18 ± 0.11	461-527	(Itanna, 2002)
Urtica dioica	Nettle	51.30 ± 0.18	34-303	(Said et al., 2015)
Brassica oleracea var. *acephala*	Collard Green	41.97 ± 0.08	15.10-25.5	(Estevão and Poltronieri, 2012)

The obtained results showed that iron content in the analyzed samples ranged from 41.97 to 338.78 mg/kg. The lowest iron content was obtained for *Brassica oleracea var. acephala* and the highest for *Spinacia oleracea*. For comparison, the literature values of iron content in leafy plants by different authors are presented in Table 2. Rinchen and Singh (2015) reported that the iron content of *Artiplex hortensis* grown in Ladakh (Region of India) was 100 mg/kg, which is similar to our results. The iron content for *Spinacia oleracea* and *Beta vulgaris* according to literature is in the range of 200-930 mg/kg, 461-527 mg/kg, respectively (Carlsson and Hallqvist, 1981; Itanna, 2002). Iron content for *Spinacia oleracea* was satisfactory, and for *Beta vulgaris* lower than the literature data. The comparison of our results for *Urtica dioica* and *Brassica oleracea var. acephala* with those of other authors showed that the iron content was similar or higher (Said et al., 2015; Estevão and Poltronieri, 2012). Deviations from the literature values can be due to various factors such as the type of leafy plant, different analysis method and preparation of the samples.

It can be concluded from the results, that the highest content of iron can be found in leafy plants of the Amaranthaceae family (*Spinacia oleracea*, *Beta vulgaris*, and *Atriplex hortensis*). Different studies have shown that amaranth plants are a rich source of iron (Yamada et al., 2023; Carlsson, 1997). Therefore, these leafy plants may be suitable for people with significantly increased iron needs, including infants and women during pregnancy.

The humans need a certain daily intake of iron. Recommended daily intake of iron has different values for different countries, and according to the Institute of Medicine (U.S.) Panel on Micronutrients guidelines, it varies from 0.27-11 mg for infants, 10 mg for children 4-8 years, 11 mg for adolescents up to 8 mg for men and 18 mg for women (Institute of Medicine (US) Panel on Micronutrients). Dietary iron bioavailability is low in populations consuming monotonous plant-based diets. In Bosnia and Herzegovina, several studies have shown that anemia is present in different age groups. Alibabić et al. (2016) reported that most Bosnian women have a significant prevalence of low iron intake. The average daily dietary iron intakes were 7.62 mg/day. Also, Bajraktarević reported that anemia due to iron deficiency is very common in pregnant and lactating women in BiH and it represents a very serious health problem. Also, a high frequency of anemia was recorded in the population of people aged 65 and over, and the causes were the presence of various diseases and low intake of iron-rich foods (Lazarević and Prodan, 2014). The obtained results indicate a significant deficiency in iron content in the collective diet of the population in B&H and also suggest a need to create food composition tables for food being consumed in our region.

Figure 2 shows the calculated average daily iron intake *via* the consumption of leafy plants cultivated in B&H.

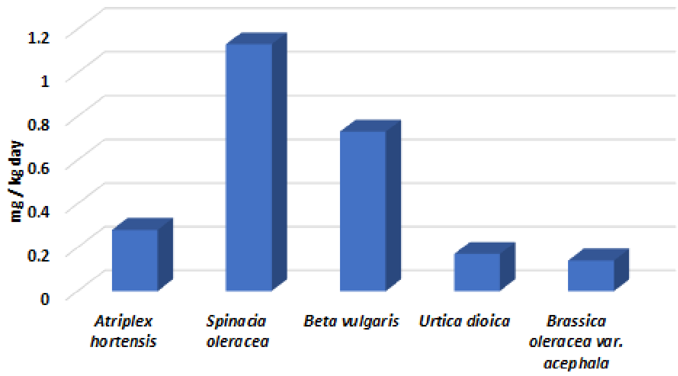

Figure 2. Daily intake of iron via leafy plants.

According to our results of iron content in the analyzed leafy plants, and comparing these results with the recommended daily intake of iron, the consumption of about 200 g of *Atriplex hortensis*, 66 g *Spinacia oleracea*, 100 g *Beta vulgaris*, 350 g *Urtica dioica* and 500 g *Brassica oleracea var. acephala* should provide the total daily iron requirement for non-pregnant and non-lactating women between the ages of 19 and 50 (Institute of Medicine (US) Panel on Micronutrients).

CONCLUSIONS

Although iron is abundant in the biosphere, organisms can be exposed to iron deficiency, leading to fatal consequences. Certain leafy plants are a good source of iron. The bioavailability of iron depends on the presence of iron absorption promoters or inhibitors in food. The iron content in leafy plants cultivated and commonly consumed in B&H was determined by FAAS spectrometry. In general, the acquired results showed that analyzed leafy plants were a good source of iron, especially plants from the family Amaranthaceae (*Spinacia oleracea*, *Beta vulgaris*, and *Atriplex hortensis*). To overcome various nutritional issues like deficiency in iron and other minerals, these green leafy plants should be a frequent part of the diet.

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Conflicts of Interest

The authors declare that they do not have any conflict of interest.

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