






Пищевая ценность микрозелени и зрелого салата (*Lactuca sativa*), выращенных в условиях фитотрона городского типа (ISR 0.2)

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Аннотация. Салат листовой (*Lactuca sativa*) является основным листовым овощем и обычно используется в свежем виде в салатных смесях и бутербродах. Следовательно, данная культура может внести существенный вклад в повышение питательной ценности потребляемой овощной продукции и формирование здоровых диет. Одной из существенных проблем питания, требующей особого внимания, является минеральное (в частности, Fe, Zn) недоедание, от которого страдают более двух третей населения мира, проживающих в странах с разным экономическим статусом. Кроме того, потребление салатов из микрозелени и проростков многих овощных культур приобретает все большую популярность как кулинарная тенденция благодаря особому вкусу продукции, высокому содержанию минеральных элементов и возможности производства практически в любом регионе. В настоящей работе проведено сравнительное изучение накопления фитонутриентов (минеральных элементов, аскорбиновой кислоты, полифенолов) и суммарной антиоксидантной активности салата листового в двух периодах онтогенеза – в фазу технической спелости и микрозелени, выращенных в условиях гидропоники на субстрате из минеральной ваты. Из 10 исследованных минеральных веществ по большинству из них (P, K, S, Ca, Mg, Mn, Cu, Zn, Fe, N) в микрозелени содержалось значительно большее количество всех питательных веществ, чем в зрелом салате (кроме Ca и K). Микрозелень легко выращивается не только в производственных, но и домашних условиях, что дает возможность потреблять непосредственно после уборки свежую продукцию, содержащую большее количество полезных фитонутриентов, чем взрослые растения в фазе технической спелости.

Ключевые слова: фитохимические вещества, минеральное содержание, минеральная недостаточность, микрозелень, гидропоника, фитотрон

The nutritional profile of microgreen and mature lettuce (*Lactuca sativa*) grown under urban-type phytotron (ISR 0.2) conditions

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Abstract. Lettuce (*Lactuca sativa*) is a major leafy vegetable and is commonly used in salad mixes and sandwiches. Therefore, lettuce can contribute significantly to the nutritional content of healthy diets. One specific nutritional problem that needs attention is mineral (e.g., Fe, Zn) malnutrition, which impacts over two-thirds of the World's people living in countries of every economic status. Also, consumption of salads Microgreens, the edible cotyledons of many vegetables has been gaining popularity as a culinary trend due to its flavor and density of minerals that can be sustainably produced in almost any locale. In this study, the nutrient contents of both mature and microgreen oakleaf lettuce grown on rockwool mat were assessed and compared to each other together with the phytonutrient contents like ascorbic acid, total antioxidant capacity and total phenolic content. Of the 10 nutrients examined (P, K, S, Ca, Mg, Mn, Cu, Zn, Fe, N), lettuce microgreens had significantly larger quantities of all nutrients than mature lettuce except for the Ca and K. As microgreens can be grown easily in one's home using the methods used in this study, they may provide a means for consumer access to larger quantities of nutrients per gram plant biomass relative to store-bought mature lettuce, which had relatively lower nutrient contents than microgreens with respect to most nutrients examined.

Keywords: phytochemicals, mineral content, mineral malnutrition, microgreens, hydroponics, phytotron

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Introduction

One-third of the World's people, living in countries of every economic status, is overweight and/or undernourished [1, 2]. This dichotomous problem of nutritional excess and insufficiency is the product of processes associated with food production, distribution, and consumption [1]. The reliance of urban populations on long food chains that begin in distant rural areas limits accessibility to produce that has short shelf-lives and, therefore, poor transportability. As a result, many urban populations reside in areas classified as "food deserts", where people do not have ready access to a complete complement of required nutrients and depend primarily on heavily processed and packaged foods [3]. Therefore, in addition to creating problems of nutritional excess and insufficiency, current food systems are detrimental to the very environment on which the production of nutritious food depends [1].

Microgreens can be grown at a small scale, by individuals for home use, or at a large scale, in industrial production systems, for commercial marketing. Growing, harvesting and postharvest handling may have a considerable effect on the accumulation and degradation of phytonutrients in microgreens. Regarding the cultivating conditions, microgreens are a versatile product. They may be grown in greenhouse or indoor, with natural or artificial light sources, in soil or in soilless systems.

However, there are some disadvantages associated with microgreens production. One major limitation to the growth of the microgreen industry is rapid quality deterioration postharvest and the risk of contamination with pathogens and public health [4]. This could be resulted with a high prices and might restricts commerce to local sales.

One specific nutritional problem that is common in both developed and developing countries is mineral malnutrition with over 60% and 30% of the World's seven billion people, being Fe and Zn deficient respectively [3]. Rates of mineral malnutrition are especially high in Asia and Africa [5], where soil degradation is especially severe and has significantly decreased the nutritional value of crops [6]. However, nutritionists consider mineral malnutrition as the most important global challenges to humankind that can be solved and prevented [7]. Current efforts to mitigate mineral malnourishment are focused on developing biofortification methods and genetically engineering crops for maximal nutrient uptake from soils [8].

However, a newly emerging crop that may be a dense source of nutrition in the absence of biofortification and genetic engineering and has the potential to be produced in just about any locale

is microgreens. Microgreens are edible seedlings of vegetables, herbs and some flowers that are usually harvested 7–14 days after germination, when they have two fully developed cotyledon leaves [9]. They are used to add texture and flavor to various dishes and they are earning a reputation as dense sources of nutrition even though only a few studies have examined their vitamin, nutrient and carotenoid contents [10, 11]. Due to their favourable contents in micronutrients and bioactive compounds, microgreens have been proposed as "super foods".

The potential nutritional benefits of microgreens combined with their ease of cultivation in one's home has piqued consumer interest in cultivating microgreens, especially given that they are not widely available for retail sale. The impact of commonly recommended cultivation methods on the nutritional value of microgreens remains to be assessed, but could assist consumers in making educated decisions about how to grow microgreens in their own homes. Vitamins and their precursors are another nutrient class lending nutritional value to microgreens. Relevant amounts of α -tocopherol (vitamin E), β -carotene (pro-vitamin A), ascorbic acid (vitamin C) and phyloquinone (vitamin K1) were reported in recent investigations, though high variability was observed when different species and cultivars were compared [9, 12, 13, 14]. Other phytochemicals reported in microgreens are phenolic antioxidants, anthocyanins, glucosinolates and carotenoids [14].

This study compares the nutrient content including minerals and phytochemicals of lettuce microgreens grown on rockwool growing mats (mineral wool pads) and mature lettuce of the same variety grown hydroponically on pots of the same substrate.

Materials and methods

Oakleaf lettuce (*Lactuca sativa* var. Dubachek MC) was cultivated in phytotron ISR-0.2 conditions designed by the (Institute for Development Strategies, Moscow, Russian Federation). The experiment was carried out in G.V. Plekhanova. Moscow in 12th February 2020. Both microgreen lettuce and mature lettuce were cultivated in media substrate based on mineral wool, for mature lettuce, three seeds were sown in each growing pot and cultivated for 34 days. For the production of microgreen lettuce, exactly weighted 4 grams of lettuce seeds were sown into 5 trays containing mineral wool with dimensions of (17.5*40*6 cm) and harvested 11 days after germination using ethanol cleaned scissors by cutting the cotyledon stems as close to the growth substrate as possible. The following amounts of nutrients were available for watering in mg. L⁻¹: № 145, P2O5 41, K2O – 275, Ca 100, Mg 24, S 30, Fe 0.94, Mn 0.14, B 0.16, Cu 0.03, Zn 0.13, Mo 0.03.

The pH of the nutrient solution ranged between 5.5–5.8, and the electrical conductivity was maintained between 1.8–2.0 mS.cm⁻¹. To reduce the salt content from the tap water, we used a combination of distilled and tap water in a proportion of 70:30 respectively. Photoperiod of 16 h and 22/15 °C (day/night) temperature. The rockwool mats utilized are compostable and may be especially convenient for consumers who wish to grow microgreens in relatively small urban dwellings and avoid purchasing or working with a soil matrix.

Total antioxidant capacity Leaf samples for this assay were collected at the same time as for the determination of total phenolic concentration. The total amount of antioxidants in lettuce samples was determined using a coulometric analyzer MVI-01–44538054–07 "EXPERT-006". Bromine was generated at a constant current of 50 mA from a 0.2 M aqueous solution of KBr in a 0.1 M solution of H₂SO₄ with the determination of the end of titration by a voltmetric indication with two polarized electrodes made of an inert metal. Then 40 cm³ of the buffer solution was poured into a Becher, the electrodes were lowered, and the generator circuit was switched on. Then an aliquot of the test sample was added to the cell (1 g of macerated lettuce). The end point of titration was fixed when the initial value of the indicator potential was reached. During the reaction time, all substances with antioxidant properties reacted with an excess of bromine. After the mixing time was completed, the device automatically filtered the bromine outflow, which was numerically equal to the number of antioxidant substances introduced in the aliquot. At the same time, the device showed the total content of antioxidants in milligrams in aliquot. Results are expressed in mg/g of fresh lettuce [15].

Total phenols content Total phenolic content was determined using the Folin-Ciocalteu method. A fresh sample with weight of 0.05g was grounded with 1.5 cm³ 96% ethanol, extraction of phenol compounds was carried out for 45 minutes at 45 °C with periodic stirring (every 15 minutes) and subsequent centrifugation for 2 minutes at rotation speed of 16,000 rpm, from obtained extract samples are taken, with volume of 0.075 cm³, adding to them at 0.075 cm³ Folin-Ciocalteu reagent diluted 5-fold is mixed, after 3 min 0.15 cm³ 20% solution of sodium carbonate and 1.2 cm³ distilled water, closed with a cover, stirred and left at room temperature, and after 1 hour, the optical density of the formed tungsten blue is measured at wavelength 725 nm, the length of the optical path is 1 cm. Total content of PC is expressed in mg-equivalent gallic acid per g fresh raw material weight [16, 17].

Ascorbic acid determination The content of the free form of vitamin C (Ascorbic acid) by capillary electrophoresis system (Капель "capel" 105M – Russian Federation) under positive high voltage polarity (internal diameter of the capillary 50/60 µm, total length 75 cm) was used Buffer: 10 mM sodium tetraborate, 40 mM, (pH 9.2), Sample injection 450 mbar.s⁻¹, Voltage: +20 kV, Detection 254 nm or 200 nm, at 23 °C.

Analysis were done up to the method suggested by with some modification. A 5g of fresh sample was diluted to 100 cm³ and well shaken for 10 minutes in the dark then it was filtered and placed in Eppendorf

tube and centrifuged under 15000 rpm twice to avoid any impurities. The supernatant was replaced into the device for analysis.

Mineral content Mineral content of microgreens and mature lettuce was determined following a slightly modified wet digestion method as suggested by Havlin and Soltanpour (1980) [18]. In short, following the method described by Lee et al. (2015) [19], exactly 0.1 g of dried shoot was analyzed with an ICP-OES spectrophotometer (PlasmaQuant 9100 ICP-OES Analytik jena. Jena, Germany) for P, K, Ca, Mg, S, Fe, Mn, Zn, and Cu. The macro and micro minerals contents were represented as milligrams (mg) and micrograms (µg) per g of dry weight, respectively.

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Results and discussion

Dry matter content No significant differences were observed in the content of dry matter for both lettuce microgreens and mature lettuce (6.1 ± 0.3% and 6.8 ± 0.34%, respectively). The average weight of microgreens and mature lettuces reached 2.08 ± 0.22 g for microgreens while for mature lettuce 42 ± 5.9 g was recorded for fresh weight per plant.

Mineral content The results of this experiment carried on oakleaf lettuce (*Lactuca sativa* var Dubachek MC) indicate that Based on nutrient mass per gram dry plant material grown on rock wool substrate are significantly more nutrient-rich than hydroponically-grown oakleaf lettuce of the same variety. The mean mineral content of both microgreens and mature lettuce are expressed on a dry weight basis (Table 1). The most abundant macro elements in microgreen samples were, in the order, K and P, followed by Mg. However, the proportions of macro elements in mature lettuce differs. Results shows that Mature lettuce contain significantly higher N, Ca and K content than microgreen lettuce. Based on microgram per gram dry plant material we determined the following micro elements (Zn, Fe, Cu and Mn). the results shows that they are presented at significantly higher levels in microgreen lettuce than mature lettuce of the same sort.

Table 1.
Average mineral content (n=5, (standard deviation)), for hydroponically grown mature and microgreen oakleaf lettuce vegetable, Number of repetitions (n=5).

Element	Mature lettuce	Microgreen lettuce	Ratio microgreen/ mature lettuces
	mg.g-1 (DW)		
Ca	9.48 ± 2.22	1.89 ± 0.08	0.2
P	6.58 ± 2.32	12.27 ± 1.88	1.8
K	65.76 ± 4.7	12.82 ± 2.74	0.2
Mg	3.69 ± 0.1	5.59 ± 1.75	1.5
S	2.72 ± 0.8	3.42 ± 0.05	1.25
µg.g-1 (DW)			
Zn	35.8 ± 9.89	167.39 ± 17	4.6
Fe	87.7 ± 19.3	290.2 ± 15.5	3.3
Cu	10.79 ± 2.7	27.5 ± 2.1	2.5
Mn	31.1 ± 2.81	58.85 ± 2.2	1.9
mg.kg-1 (FW)			
N	527.25 ± 21.25	109.32 ± 10.2	0.36
DW (dry weight), FW (Fresh weight).			

Nitrate content: As presented in (Table 1) higher nitrates contents were observed in mature lettuces. Nitrates can accumulate in lettuce leaves, leading to the toxic effects for the human being. The European Union establishes the maximum permissible levels from 3,500 to 4,500 mg of residual nitrates per kg fresh weight for the winter season and 2,500 mg of residual nitrates per kg for the summer season. Nitrates content of both microgreens and mature lettuce was below this limit; however, in our experiment the results revealed that mature oakleaf lettuces provides five-fold as much nitrates – than microgreens. The accumulation of nitrates in leafy vegetables is reported in previous researches [20, 21, 22]. Some researches attributed the development of methaemoglobinaemia [23, 24] in both infants and young children due to the consumption of vegetables high with nitrates [25]. Microgreens could be considered as a good alternative to reduce the intake of nitrates.

Phenolic and antioxidant content: Lettuce contains various health-promoting phytochemicals, including vitamins and phenolic compounds with antioxidant properties. In recent decades, phenolic-rich natural diets with antioxidant activity have fostered interest in nutrition and food science [26, 27]. Phenolic compounds are good electron donors because their hydroxyl groups can directly contribute to antioxidant action [28, 29]. Among the major phenolic compounds in lettuce known to have health-promoting qualities are chlorogenic acid, caffeic acid, and chicoric acid [30, 31]. Furthermore, some of them stimulate the synthesis of endogenous antioxidant molecules in the cell [32]. According to multiple reports in the literature,

phenolic compounds exhibit free radical inhibition, peroxide decomposition, metal inactivation or oxygen scavenging in biological systems and prevent oxidative disease burden [33]. Total phenolic content of the oakleaf lettuce plants were measured using the Folin-Ciocalteu method, and the results are shown in (Table 2). The total phenolic content of microgreen plants ranged around 53.54 ± 7.3 mg GAE/g, which is up to 1.95 folds than its content in mature lettuce plants. Taking into account the important role of antioxidants for the plant itself and for providing healthy nutrition for the human being, we determined the total content of antioxidants in both microgreen and mature lettuce (Table 2). The maximum value of total antioxidant capacity (TAC) reached 22.37 ± 3.32 mg/g in microgreen lettuce, which is 2.4 times higher than their level in the mature lettuce – 9.151 ± 2.45 mg/g.

Table 2.
Average dry weight in (%), ascorbic acid in mg/100g fresh weight, Total phenolic content in (mg GAE)/g dry weight and total antioxidant capacity in mg/g fresh weight for hydroponically grown mature and microgreen oakleaf lettuce vegetable, Number of repetitions (n=5)

Phytonutrient	Mature lettuce	Microgreen lettuce	Ratio microgreen/ mature lettuces
Dry weight, (%)	6.3 ± 0.2	6.6 ± 0.24	1.1
Ascorbic acid, (mg/100g)	3.88 ± 0.4	20.74 ± 4.78	5.3
TPC, (mg GAE)/g DW	27.36 ± 0.25	53.54 ± 7.3	1.95
TAC, (mg/g)	9.151 ± 2.45	22.37 ± 3.32	2.4
DW (dry weight), FW (Fresh weight), TPC (Total phenolic content), TAC (Total antioxidant capacity), GAE (Gallic acid equivalent).			

Conclusion

Some consider that there are not enough scientific researches to prove the higher level of nutrients in microgreens than in mature plants. But as revealed from this study, we assume that the production of microgreens is a great addition in horticulture. The results presented in this experiment indicate that microgreens could provide a means for consumer-access to larger quantities of nutrients per gram plant biomass including macro and micro minerals like P, Mg, Fe, Mn, Zn, S and Cu relative to store-bought mature vegetables. However, mature lettuce of the same variety possesses higher amount of Ca, K and nitrates. Moreover, the nitrates content of microgreens was incredibly lower than mature lettuces. Which make microgreens to be safely consumed by human being, in particular for young children, to fulfil their daily mineral requirements.

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