https://doi.org/10.17113/ftb.61.04.23.8119 original scientific paper

How to Increase the Nutritional Quality of Stinging Nettle Through Controlled Plant Nutrition[§]

Running head: Increasing Nutritional Quality of Stinging Nettle

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Received: 11 February 2023
Accepted: 28 July 2023

SUMMARY

Research background. As food production faces major challenges, modern agricultural practices are increasingly focused on conserving resources, reducing negative environmental impacts, and sustainably producing foods high in health-promoting phytochemicals. During the production process, many factors can influence the quality and chemical composition of a final food product. Proper selection of cultivating conditions, especially a balanced nutrition, can significantly increase nutritional value resulting in foods with strong biological and functional properties. Stinging nettle is a rich source of minerals, vitamins, pigments, phenols and other bioactive compounds (BC) and can be consumed as a green leafy vegetable with beneficial effects on human health. Therefore,

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[§]This paper was presented at the 10th International Congress of Food Technologists, Biotechnologists and Nutritionists, 30 November-2 December 2022, Zagreb, Croatia

the aim of this study was to determine the nutritional quality and antioxidant capacity of stinging nettle leaves under the influence of different nutrient solution (NS) treatments during three mowings.

Experimental approach. The experiment was performed in a floating hydroponic system, with different treatments of NS application during three mowings. After each mowing, the following treatments were differed: treatment 1 – depletion with water, treatment 2 - supplementation with standard NS, and treatment 3 - correction with nutrients. Of the BC minerals, ascorbic acid, phenols, and photosynthetic pigments content, as well as antioxidant capacity were analyzed spectrophotometrically, while individual phenols were determined by liquid chromatography.

Results and conclusions. Different nutrition solution treatments and the number of mowings had a significant influence on the content of the analyzed BC. The highest contents of total phenols (377.04 mg GAE/100 g fm), total flavonoids (279.54 mg CTH/100 g fm), ascorbic acid (112.37 mg/100 g fm), and pigments (total chlorophylls 1.84, and total carotenoids 0.36 mg/g), as well as the highest antioxidant capacity (35.47 µmol TE/g) were recorded in the third mowing, with nutrient solution supplementation.

Novelty and scientific contribution. This is the first time that stinging nettle leaves are produced in a floating hydroponic system by controlled plant nutrition. We establish this type of nutrition manipulation during multiple mowings as an innovative technique for the production of novel food with high and improved nutritional value suitable for consumption as green leafy vegetables.

Keywords: sustainable food production; food quality; plant nutrition; bioactive compounds; antioxidant capacity

INTRODUCTION

Food production faces numerous problems and global challenges due to the pronounced consequences of climate change which has led to more frequent droughts, floods, natural disasters, soil degradation and arable land losses (*1*). Today, food production is one of the main contributors to global warming through greenhouse gasses emission, and soil and water pollution through the misuse of chemical fertilizers and pesticides (*2,3*). Thus, there is increasing urgency for a stronger focus on adapting the food production chain to sustainable solutions. One of the main objectives of the strategy of European Union and Circular Economy Action Plan (CEAP) (*4*) is to reduce pressure on natural resources and ensure sustainable production. Another challenge for food production is to provide a growing population with an adequate amount of nutrient-rich food.

The nutritional, functional, and biological properties of foods of plant origin primarily depend on the content and composition of phytochemicals that possess significant antioxidant activity.

Stinging nettle (*Urtica dioica* L.) is a plant species known for its nutritional and medicinal properties since ancient times (*5*). Nettle has great potential in food processing for the production of food supplements, tinctures and capsules (*6*), and as a source of green pigments for food coloring (*7*). It is also used in the pharmaceutical, cosmetic, and textile industries. All parts of the plant contain large amounts of different bioactive compounds (BC), but leaves are the richest source which is why they are intended for consumption as a green leafy vegetable (*6*). Nettle leaves contain: carbohydrates, dietary fiber, protein, fat, fatty acids (mostly palmitic and linoleic acids), vitamins (A, B group vitamins, C, D, E, and K), minerals (especially P, Ca, Mg, and Fe), photosynthetic pigments (chlorophyll a and b, carotenoids), amino acids, organic acids, tannins, terpenoids, and phenols (*8,9*). Determined individual phenolic compounds are: caffeic, coumaric, chlorogenic, hydroxybenzoic, vanillic, and quinic acid, as well as caffeic and quinic acid derivatives and rutin, quercetin, and kaempferol (*8-10*). The complex chemical composition and antioxidant capacity are responsible for the proven specific biological effects of stinging nettle. Pharmacological studies showed antioxidant, antimicrobial, anti-inflammatory, antidiabetic, hepatoprotective, antitumor (*5,8,9,11*) and other properties of stinging nettle. However, the chemical composition of nettle leaves can vary significantly depending on the origin of the plant material. Nowadays, nettle is mostly collected traditionally from the wild, but such plant material is of questionable quality and has uneven chemical composition. In addition, nettle has the ability to absorb increased amounts of heavy metals and nitrates from the soil, which makes it unfavorable for consumption (*9,12*). Control of abiotic and biotic factors as well as management of production conditions can significantly improve nettle quality and safety.

The floating hydroponics is a production technique that offers solutions to overcome the above-mentioned problems related to food production. It allows less use of fertilizers and pesticides which can greatly affect on food safety and prevent food contamination. Continuous control of abiotic factors and more efficient use of water and nutrients can result in higher nutritional quality of raw material by ensuring favorable chemical composition (*9,12*). Floating hydroponics is a soilless technique of growing plants using a nutrient solution, that provides precise and balanced plant nutrition according to the specific needs of the plant species. Controlled nutrition can affect the amount of nitrogen (N), which is important because excessive amounts of N can represent the environmental problem, but also negatively affect food nutritional quality by reducing the accumulation of phenolic compounds, ascorbic acid and other BC (*13-15*). A very important advantage of floating hydroponics is also the possibility of multiple mowings, which ultimately leads to greater food production while preserving the nutritional value of the raw material.

Given the aforementioned, the aim of this study was to determine the BC content and antioxidant capacity under the influence of different NS management during three mowings.

MATERIALS AND METHODS

Plant material

Fresh leaves of stinging nettle were collected from cultivated nettle grown at the Department of Vegetable Crops, University of Zagreb Faculty of Agriculture in Croatia. The experiment was conducted in a floating hydroponic system during the fall-winter growing season 2021/2022, in a heated greenhouse. The amount of 50 nettle seeds (B&T World Seeds, France) was sown per plate opening in polystyrene boards filled with inert perlite (Europerl d.o.o., Samobor, Croatia), on August 26, 2021. After 6 days of germination under optimal conditions (temperature: 20–25 °C; relative humidity: 60– 70 %), the boards were relocated into 3 basins filled with nutrient solution prepared according to Johnson's recipe (salts: KNO_3 – 250.99, KH_2PO_4 – 142.7, K_2SO_4 – 0, $Ca(NO_3)_2$ x $4H_2O$ – 501.5, $MgSO_4$ x $7H_2O$ – 256.25, FeEDTA 13 % –12.8, H_3BO_3 – 1.32, $CuSO_4$ x $5H_2O$ – 0.026, $MnSO_4$ x $4H_2O$ – 0.79, $ZnSO_4$ x $7H_2O$ – 0.11, and Na_2MoO_4 x $2H_2O$ – 0.018 mg /L; EC 1.5 mS/cm, pH 5.8–6.2). Johnson nutrient solution (JNS) was used to prevent excessive accumulation of nitrates because it has a lower nitrate content than other commercial nutrient solutions. Originally, basin 1 and basin 2 were filled with JNS prepared independently of the water chemical composition, and basin 3 was filled with JNS adjusted according to the water chemical analysis. Since stinging nettle has the ability to regrow after mowing, three mowings were conducted: on November 3, 2021; January 11, 2022, and March 10, 2022. Mowing of the stinging nettle plants was performed manually, before flowering. After each mowing, different NS treatments were applied by refilling basins to replace the drop level of JNS. In the first nutrient solution treatment (NS1) water was added to basin one (depletion), in the second nutrient solution treatment (NS2) the initial JNS was added to basin two (supplementation), and in the third nutrient solution treatment (NS3), a chemical analysis was performed, based on which the amount of nutrients was added to basin three to match the composition of the initial solution (correction). Due to depletion with water, NS1 contained less minerals, especially nitrogen (N), while the supplementation and correction treatments contained higher concentrations of N and other minerals. Also, greenhouse abiotic factors (air temperature and relative humidity) were monitored regularly throughout the experiment, so plants were grown under controlled conditions. After each mowing plant raw material was cleaned from mechanical impurities, the leaves were separated from the stems and the fresh leaves were immediately analyzed.

Determination of physico-chemical properties

According to the CIELAB method, the chromaticity parameters of the fresh nettle leaves (*L**, *a**, *b**, *C*, *h*◦) were measured using a colorimeter (ColorTec PCM+, PCE Instruments, Southampton, UK). 15 leaves were measured, with 5 leaves representing one replicate, for a total of three replicates per treatment. The *L** value represents the lightness of the sample from black to white on a scale from 0 to 100 where 0–50 indicates dark and 51–100 indicates light. The *a** and *b** values represent chromaticity, *i.e.*, the presence of red or green and blue or yellow, respectively. A negative a* value corresponds with green, and positive a* with red, a negative b* corresponds with blue and positive b* with yellow color (*16*).

Using a standard laboratory procedure according to the Association of Officiating Analytical Chemists (AOAC) (*17*), by drying in an oven at 105 °C to constant mass, total dry matter content (DM, %) was determined. Potentiometric titration with sodium hydroxide solution (*c*=0.1 mol/L) was used to determine total acid content (TA, %) according to AOAC (*17)*. To determine total dry matter and acid content, six measurements were made with two measurements representing a replicate, for a total of three replicates per treatment.

Determination of mineral composition

The dry matter of plant leaves was determined gravimetrically in a drying oven (INKO, ST-360T, Croatia) to constant mass at 105 °C (*18*). The dry samples were digested in a microwave oven (ETHOS ONE, Milestone, Italy) with concentrated HNO_3 and $HClO_4$ acids. In digested samples calcium, magnesium, iron, zinc, manganese, copper and molybdenum, were determined by atomic absorption spectrometer (Thermo Scientific, Solar, England), while boron was determined spectrophotometrically using azomethine-H method. (*19*). All analyses were performed in triplicates.

Determination of ascorbic acid content

Titration with 2,6-dichloroindophenol (DCPIP) was used to determine the ascorbic acid (AsA) content according to the standard AOAC method (*19*). The plant material (approximately 3.5 g of the samples) was mixed and homogenized with 100 mL of 2 % (*V/V*) oxalic acid and filtered through Whatman filter paper. A volume of 10 mL of the filtrate was titrated with freshly prepared DCPIP until a specific pink coloration appeared, which must be stable for several minutes. Analyses for all treatments were performed in three replicates. The final AsA content of tested samples was calculated according to Equation /1/:

$$\text{AsA (mg/100 g fm)} = (V \times F / D) \times 100 \qquad /1/$$

where V is the volume of used DCPIP (mL), F is the factor of the DCPIP and D is the sample mass in the filtrate used for titration (g).

Determination of phenolic compounds

For the purpose of determining the total phenolic compounds, the plant material was first extracted with 80 % ethanol. The extraction process was performed according to the following procedure: samples (10±0.01) g were homogenized with 40 mL of 80 % EtOH (*V/V*), heated to boiling point, and refluxed for 10 min. After filtration, another 50 mL of 80 % EtOH (*V/V*) was added and the process was repeated as described in Dujmović *et al.* (20) The extracts were used for the determination of total phenolics (TPC), total flavonoids (TFC), total non-flavonoids (TNFC) content, and antioxidant capacity.

According to a method described by Ough and Amerine (*21*) polyphenolic compounds were determined based on the reaction with Folin–Ciocalteu (FC) reagent and performed in triplicate. For TPC determination, the reaction was prepared in a 50 mL volumetric flask in the following order: 0.5 mL of the extracts, 30 mL of distilled water (dH_2O), 2.5 mL of the freshly prepared FC reagent diluted with dH_2O (1:2, *V/V*) and 7.5 mL of saturated sodium carbonate solution (Na_2CO_3) and made up to the mark with dH_2O. The prepared reaction stood for 2 h at ambient temperature, with occasional shaking. Final TP contents and absorbances of the extracts were determined spectrophotometrically (Shimadzu, 1900i, Kyoto, Japan) at a wavelength of 750 nm using distilled water as a blank. Gallic acid was used as an external standard, and TPC was expressed as milligrams of gallic acid equivalents per 100 g fresh mass (mg GAE/100 g fm).

For the determination of TNFC, chemical reactions were performed as follows: into a 25 mL volumetric flask, 10 mL of the ethanolic extract, 5 mL of HCl in EtOH (1:4, *V/V*) and 5 mL of formaldehyde (p.a.) were added and obtained solutions were blown with nitrogen (N_2). The further procedure took place according to the protocol as described in the study by Dujmović *et al.* (20). TFC was calculated as the difference between the amount of TPC and TNFC and expressed as milligrams of catechol equivalents per 100 g fresh mass (mg CTH/100 g fm).

Identification and quantification of individual phenols by high-performance liquid chromatography (HPLC).

Following the modified method described by Otles and Yalcin (*22*) high-performance liquid chromatography (HPLC) was used for the separation, identification and quantification of the individual phenolic compounds from fresh stinging nettle leaves. Of the individual phenolic compounds caffeic acid, coumaric acid, ellagic acid, ferulic acid, and naringin were analyzed. Phenols were extracted by

homogenizing (1±0.01) g of fresh leaves with 10 mL of 80 % MeOH (*V/V*) using a laboratory homogenizer (IKA, UltraTurax T-18, Staufencity, Germany) and further homogenized in closed vessels in an ultrasonic bath (Bandelin RK 103H, Berlin, Germany) for 30 min at 50 °C. After the first filtration of the extracts through Whatman filter paper, they were filtered again through Chromafil PA filters. The HPLC analyzes of phenolic compounds were performed using LC Nexera (Shimadzu, Kyoto, Japan) equipped with a photodiode array and fluorescent detector (PDA-RF), an automatic injector, and LabSolution software. Separation of selected individual phenolic compounds and their standards was performed on a NUCLEOSIL 100-5 C18 (5 µm, 250×4.6 mm i.d.) column (Macherey-Nagel, GmbH, Düren, Germany). Analytical conditions, with minor modifications, were set up according to Repajić *et al.* (*23*) parameters using the same mobile phases, flow rate, and applied sample volume The analyses were performed at a temperature of 23 °C and the duration of the single run was 45 min. The gradient profile (A % / B %) was as follows: at 0 min 90/10, at 25 min 60/40, at 30 min 30/70 and then after 35 min to 45 min 90/10. All determinations were performed in duplicate. Phenols were detected at wavelengths ranging from 220 to 360 nm and identified based on their retention times compared to commercial standards (Sigma Aldrich, Steinheim, Germany). Quantification of individual phenolic compounds was carried out by calculating the external standard using the equation based on the calibration curves (Table 1) and were expressed as mg/100 g. The calibration curves were generated using different concentrations (2, 10, 40, and 100 µg/mL) of the standard solution mixture, also injected in duplicate.

Table 1

Determination of the photosynthetic pigment content

For the determination of photosynthetic pigments, approximately 0.2 g of fresh leaves were weighed and mixed with a total volume of 15 mL of acetone (p.a.) in three steps using a laboratory homogenizer (IKA, UltraTurax T-18, Staufencity, Germany) as previously described in the article by Dujmović *et al.* (20). Pigment compounds were determined according to the method described by Holm (*24*) and Wettstein (*24*). . Total chlorophylls (TCh), chlorophyll a (Chl_a), chlorophyll b (Chl_b) and total carotenoids (TCa) were determined in three replicates and absorbance values were measured spectrophotometrically (Shimadzu, 1900i, Kyoto, Japan) at wavelengths of 662, 644 and 440 nm using acetone (p.a.) as a blank. The pigment content was calculated by including measured absorbance values in the Holm–Wettstein Equations /2/-/5/ and the results were expressed in mg/g.

$$\text{Chl\_a} = 9.784 \times A_{662} - 0.990 \times A_{644} \text{ (mg/L)} \quad /2/$$

$$\text{Chl\_b} = 21.426 \times A_{644} - 4.65 \times A_{662} \text{ (mg/L)} \quad /3/$$

$$\text{TCh} = 5.134 \times A_{662} + 20.436 \times A_{644} \text{ (mg/L)} \quad /4/$$

$$TCa = 4.695 \times A_{440} - 0.268 \times TCh \text{ (mg/L)} \quad /5/$$

Determination of antioxidant capacity

Antioxidant capacity was determined by two different methods: the ABTS assay previously described by Miller *et al.* (*26*) and the FRAP assay according to Benzie and Strain (*27*). The same ethanolic extracts prepared for the detection of phenols were also used for antioxidant capacity determination. Measurements for both methods were performed in three repetitions. All chemical materials: ABTS (2,2'-azinobis (3-ethylbenzothiazoline-6-sulfonic acid)), TPTZ (2,4,6-tripyridyl-S-triazine), and Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) were purchased from Sigma-Aldrich (St. Louis, MO, USA).

The ABTS assay is a colorimetric method that measures the ability of antioxidants to scavenge the generated ABTS•+ radical cation compared with a Trolox (water-soluble vitamin E analogue) as an equivalent antioxidant standard. Trolox stock standard (2.5 mM) was prepared in 80 % ethanol (*V/V*). The ABTS•+ radical cation was pre-generated a day before analyses, by mixing 88 µL of a 140 mM $K_2S_2O_8$ and 5 mL of a 7 mM ABTS solution and allowed to stand at room temperature in the dark overnight for 16 h. On the day of analyzes, a ABTS solution was prepared as described in Dujmović *et al.* (*20*). The addition of the antioxidants (sample extracts), reduced the ABTS•+ radical cation to ABTS and the reaction was manifested by discoloration of the blue–green solution. After 5 min of incubation at ambient temperature, the absorbances were measured spectrophotometrically (Shimadzu, 1900i, Kyoto, Japan) at 734 nm. As a blank, 96 % ethanol was used and final results were calculated based on a calibration curve and expressed as µmol TE/g^1 (micromole Trolox equivalent per gram).

The ferric reducing antioxidant power (FRAP) assay measures antioxidant potential in samples through the reduction of ferric iron (Fe^{3+}) to ferrous iron (Fe^{2+}) at low pH (3.6), reducing colourless ferric-tripyridyltriazine (Fe^{3+}-TPTZ) to an intensely blue coloured ferrous-tripyridyltriazine complex (Fe^{2+}-TPTZ). Fresh working FRAP reagent was prepared in a ratio of 10:1:1 by mixing 0.3 M acetate buffer, a solution of 10 mM TPTZ reagent in 40 mM HCl, and 20 mM ferric chloride ($FeCl_3$ x $6H_2O$). For reaction, distilled water (960 µL), sample extracts (320 µL) and FRAP reagent (8320 µL) were well mixed. The same reactions were prepared for the blank, except that 80 % EtOH (*V/V*) was used instead of the sample extract. The prepared mixtures were incubated for 5 min at 37 °C in a water bath, while absorbance was measured spectrophotometrically (Shimadzu, 1900i, Kyoto, Japan) at 593 nm. As the antioxidant standard Trolox was used and stock standard (2 mM) was prepared in 80 % ethanol (*V/V*). Results were expressed as µmol TE/g.

Statistical analyses

The cultivating experiment was set according to the randomized complete block design with 3 replicates and all laboratory analyses were also performed in triplicate. The obtained results were statistically analysed using PROC GLM in SAS software system, version 9.3. (*28*), subjected to two-way analysis of variance (ANOVA), and expressed as mean values. Means were compared by t-test (LSD) and considered significantly different at $p \leq 0.05$. Different letters indicate significant statistical differences between samples.

RESULTS AND DISCUSSION

Physico-chemical properties of fresh stinging nettle leaves

Analysed chromaticity parameters showed the highest *L** values measured for nettle leaves during depletion with water (NS1) at the second and third mowing with an average value of 45.85 (Table 2). These leaves were significantly lighter than all other samples. Negative *a** values represent green, with the greenest leaves recorded for all three treatments at the first mowing and in the depletion treatment (NS1) at the third mowing with an average value of 15.06. Positive *b** values were recorded in all treatments, indicating the presence of yellow colour. The results of this study showed that among the tested factors, only NS treatment had a significant influence on *L** values, while both individual factors, NS treatment and mowing number (NS and M) had a significant influence on *a** and *b** values. The determination of chromaticity parameters is important because colour is one of the first properties of food products consumers notice to determine sensory characteristics, flavour, ripeness, and freshness (*29*), as well as being an initial indicator of photosynthetic pigment accumulation.

Table 2

Dry matter (DM) is accumulated through photosynthesis by which plants produce sugars, that are then converted to a diverse organic compound that constitutes about 95 % of plant dry mass (*30*). In this study, the highest DM content was observed in the second mowing in NS2 (supplementation, 23.41 %) and NS3 (correction, 23.96 %) treatment (Table 3). The lowest values were noted at the first mowing during all NS treatments suggesting DM content can be increased by applying multiple mowings which is confirmed by Westwood and Mulcock (*31*). Analysis of the significance of the factors showed that both individual factors, nutrient solution (NS) and mowing number (M) and their interaction (NS x M) had a significant effect on DM content. The DM content of plant tissues can be influenced by several other factors, such as genetic characteristics, ecological factors (temperature and humidity), precipitation, pollution (*32*), and mostly growing conditions, especially biogenic mineral nutrition. Paulauskienė *et al.* (*33*) established that leaves of wild collected stinging nettle contained 20.48–24.41 % DM depending on the harvest time, while Radman *et al.* (*34*) reported DM amount of

20.11 % DM for cultivated nettle leaves. Both mentioned DM values are similar to those obtained in this research for the second and third mowing, while in first mowing DM content was lower regardless of the NS content. This trend might be consequence of lower accumulation of nutrients such as N and P which is in accordance with the study of Akter *et al.* (*35*). In terms of green leafy vegetables stinging nettle can be considered to have high DM content in regards to other species such as spinach (6.3–11.2 %) (*36*), leaf turnip (13.4–14.1 %) and kale (14.7–20.6 %) (*31*). Since DM content is an indicator of nutritional potential and food quality, the high DM value obtained in our research shows high nutrient content of nettle leaves. It is worth mentioning that plant materials with a higher DM, *i.e.*, lower water content, have a longer shelf life, better storability and sensory characteristics.

Organic acids from plants are a class of nutrients with acidic properties that have been attributed with numerous beneficial effects on human health, such as anti-inflammatory, immunoprotective, calcium absorption stimulation and prevention of neurodegenerative diseases Shi *et al.* (*37*). The results of this study showed that regardless of the NS treatment, the highest total acid content (TA) was observed in the second, and the lowest in the first mowing (Table 3). The NS treatment had a significant influence on TA content with the highest values recorded in NS3 (correction) after the second mowing (0.11 % fm). Diverse results can be explained by complex and different nutrient solution mineral's affinity to the acidic environment whereas organic acids can make P and Fe complexes more soluble and available to plants, but also reduce nitrate uptake (*38*). The composition and level of organic acids varies in different food and plant tissues depending on the species, cultivar, age of the plant, tissue type, environment pH and stress conditions (*37*). This study showed that organic acid content can also be influenced by different NS treatments and number of mowings. The analysis of the significance of different factors and their interactions showed that both individual factors (NS and M) and the combination of these two factors (NS x M) had a significant effect on the TA values.

Table 3

Mineral composition of fresh stinging nettle leaves

Minerals are important components of the diet responsible for maintaining health, normal growth and development (*39*), and their specific amounts are required for the proper functioning of body systems. Since they must be consumed through the diet, it is necessary to pay attention to the mineral-rich sources, as their deficiency can have noticeable adverse effects on human health (*40*). Leafy vegetables are generally considered a rich source of minerals (*39*), but their amount and bioavailability depend strongly on the vegetable species. Table 4 shows the results of the content of selected biogenic elements (minerals) in fresh nettle leaves expressed in fresh mass. Ca and Mg are

important minerals involved in muscles contraction, bones and teeth formation, lowering blood pressure and LDL cholesterol, preventing osteoporosis, are cofactors in many enzymatic reactions necessary for protein synthesis, nerve function, and many others (*41*). The highest Ca requirement is during the growth phase, *e.g.*, in childhood and during lactation. Regardless of the NS treatment, the highest levels of Ca (2106.35 mg/100 g fm) and Mg (333.34 mg/100 g fm) in nettle leaves were found at the second, while the lowest levels were found at the first mowing. NS treatment had a significant effect on Ca and Mg content in fresh stinging nettle leaves, with the highest values measured during NS1 (depletion). Ca and Mg tend to accumulate in more mature than in young plants (*42*), while studies also show that Ca and Mg content in plant tissues is strongly dependent on water availability and salinity. Under conditions of lower water availability and higher salinity, Ca uptake is lower due to reduced absorption and transpiration (*43*). It is worth noting that according to FAO and WHO (*44*), the recommended Ca intake for adult men and women is 1000 mg/day, which can be provided by consuming 47.5–130 g of the nettle leaves obtained in this study. The recommended daily Mg intake is 190–220 for adult women, and 224–260 mg/day for adult men (*44*) which can be fulfilled by consuming 100 g of nettle leaves obtained from all treatments of second and third mowings. Some rich Ca sources from leafy vegetables are kale and watercress with average Ca values of 100 and 150 mg/100 g, respectively (*41*), and good Mg sources are spinach and kale with average values of 53 and 30 mg/100 g, respectively (*45*) which are significantly lower compared to the results for Ca and Mg in fresh stinging nettle leaves obtained in this research. Thus, nettle leaves can also be highlighted as a rich source of Ca and Mg.

The iron (Fe) content in fresh nettle leaves was significantly affected by the number of mowing and the NS treatments (Table 4), with the highest Fe content, without significant difference, found in the second and third mowing in NS2 (supplementation) and NS3 (correction) with an average value of 2.21 mg/100 g fm. Fresh spinach and kale, as sources known for high Fe content, contain on average 2.8 and 1.7 mg/100 g Fe, respectively (*45*), which are similar values compared to the nettle leaves from this research. However, it should be noted that Fe from vegetables has low bioavailability. But although some dietary factors, such as ascorbic acid and proteins, can enhance Fe bioavailability, others, such as Ca, phytates and polyphenols inhibit Fe absorption (*46*). Regardless of its bioavailability, Fe is an essential element for almost all living organisms as it participates in a variety of metabolic processes, including oxygen transport, DNA synthesis, and electron transport (*40*). Another mineral essential for human metabolism is zinc (Zn), whose content in nettle leaves also significantly varied given the number of mowings and NS treatments. Zn content in nettle leaves was significantly higher in the second and third mowings than in the first mowing regardless of NS

treatment. Given the NS treatment, Zn content was the highest during all mowings in NS1 (0.41, 0.60 and 0.91 mg/100 g fm), in which the lack of water was only supplemented. As other research states, Fe and Zn content in plants strongly depends on several factors, such as water availability, pH, salinity, temperature, maturity stage, *etc.* (*42*), while the content of both mainly increases with higher water availability and lower salinity. For comparison, the nettle leaves produced in this study are a much better source of Zn than some other leafy vegetables such as kale (0.44 mg/100 g) and spinach (0.7 mg/100 g) (*45*). In addition to Fe and Zn, there are other trace elements that the human organism needs in smaller quantities, but which play an important role: manganese (Mn), copper (Cu), boron (B) and molybdenum (Mo). According to the results, Mn (1.27 mg/100 g fm) and Cu (0.23 mg/100 g fm) had the highest values in the second mowing during NS3 (correction). The highest levels of B and Mo were also found at the second mowing. The highest values for B was 1.15 mg/100 g fm in NS2 (supplementation), and for Mo 0.05 mg/100 g fm in NS3 (correction). Some other researchers studied the mineral content of stinging nettle and also pointed out the high value of nettle leaves as a rich source of minerals (*9,34*). This study showed that the tested factors (NS and M) and their combination (NS x M) significantly affected the content of each mineral analyzed, except for Mo where NS had no influence on Mo content. The uptake of mineral nutrients by plants can also be influenced by the type of cultivation, temperature, light, oxygen concentration, nutrient concentration, plant development, and others. It is important to emphasize that, in addition to these factors, antagonisms between individual macro- and micronutrients and genetic characteristics also play an important role (*39,42,43*).

Table 4

Ascorbic acid content of fresh stinging nettle leaves

Ascorbic acid (AsA) is an important free radical scavenger that can prevent their harmful effects on organisms and is a cofactor for numerous enzymes in plant and human metabolism. The strong antioxidant activity of AsA is the most known property, as it is often used to prevent different diseases and as an additive in food storage (*47*). The accumulation of AsA in plants can be influenced by various factors such as climatic conditions (especially light intensity and temperature), environmental stress, genotypic differences, cultivation practices, maturity, harvesting methods (*47*) and plant nutrition.

The results of this study showed that regardless of NS treatment, the highest AsA content was observed in the third mowing, while the lowest in the second (Table 5). Considering NS treatments, the highest AsA content was detected after the third mowing in NS2 (supplementation) in the amount of 112.37 mg/100 g fm. This value was about 139 % higher than the lowest observed values at the

second mowing in NS2 (47.00 mg/100 g fm) and NS3 (48.12 mg/100 g fm). These results can be explained by the effect of N on AsA content. As several studies suggest (*13,15*), a higher amount of N results in the decrease of AsA. The highest content of AsA at NS2 (supplementation) at the third mowing may be due to other minerals present in nutrient solution, since AsA is positively related to the concentration of Mg and P (*48*). Also, Radman *et al.* (*15*) obtained multiple harvests of field cultivated nettle, and the results confirmed that an increased number of harvests led to an increase of AsA content, just as in this present study. Their results showed AsA values ranging from 16.00 to 112.80 mg/100 g fm, with the highest AsA content matching the highest value obtained in our study. The analysis of the significance and interaction of the tested factors showed that the NS treatment had no significant effect on the content of AsA, but the number of mowings (M) and the combination of both factors (NS x M) showed a very high statistical significance. Based on the results of this study it is evident that stress caused by multiple mowings had a greater impact than plant nutrition. Levels of AsA also can vary a lot depending on origin of the raw material. In wild harvested nettles AsA content varied from 0.58–25.66 mg/100 g (*33*), while Shonte *et al.* (*49*) reported values of 94.7 mg/100 g of AsA for field cultivated nettle which are significantly lower values than in our treatments. According to the Dietary Reference Intakes (*50*) developed by the Food and Nutrition Board (Institute of Medicine) the recommended daily intake (RDI) of vitamin C is 75–90 mg/day for adult women and men which can be fulfilled by consuming 67–80 g of nettle leaves collected after the third mowing supplemented with initial NS as established in this study.

Phenolic compounds of fresh stinging nettle leaves

Phenolic compounds play an important protective and reproductive role in plants, but when ingested through the food, they also exhibit a series of properties beneficial to human health. Their effects are manifested in antioxidant, anti-inflammatory, antimicrobial, anticancer, cardioprotective, and antithrombotic properties, and as protection against chronic and neurodegenerative diseases (*51*). Therefore, it is essential to determine the content and find measures to help increase the phenolic quantity of plant-based foods. The phenolic composition of plants is affected by many factors, such as climate, soil, variety, phenophase, harvest time, cultivation treatment, and processes applied (*52*).

According to obtained results, both tested factors (NS and M), as well as their interaction (NS x M), had a significant impact on total phenolic (TPC), flavonoid (TFC) and non-flavonoid (TNFC) content (Table 5). Regardless of the NS treatments, the highest TPC and TFC content was detected at the third mowing with average values of 280.25 for TPC and 181.26 mg GAE/100 g for TFC. Considering that bioactive substances are increasingly synthesized and accumulated in stressful

conditions as a defense mechanism of the plant, it is likely that the amount of TPC and TFC was induced by stress due to multiple mowings. Values for TPC ranged from 163.63–377.04 mg GAE/100 g, with the highest TPC (377.04 mg GAE/100 g) and TFC (279.54 mg GAE/100 g) recorded in NS2 (supplementation) after the third mowing. For TPC, this was a 130 % higher value than in the lowest observed treatments: first mowing NS1 and third mowing NS3. Due to the addition of initial NS and the specific amount of nutrients, suplementation (NS2) and correction (NS3) treatments contained higher amounts of N and other minerals. Higher N levels can lead to reduction in phenolic content (*14*) which was the case for TPC, TNFC and TFC in the first two mowings. Radman *et al.* (*15*) investigated the phenolics content of conventionally open-field cultivated nettle which ranged from 348.68–962.70 mg GAE/100 g fm for TPC which is in agreement with our results. In different studies, TPC of wild collected nettle leaves varied a lot (*8,11,33*) confirming that environmental factors and stress conditions strongly influence phenolic composition.

Table 5

Besides the total phenolic compounds, some of the selected individual phenols were identified and quantified: caffeic, coumaric, ferulic acids (group of hydroxycinnamic acid) ellagic acid (group of ellagitannins) and naringin (group of flavanone-7-O-glycoside) (Table 6). According to the results, caffeic acid was not determined in stinging nettle leaves in this research. Low values of coumaric acid were detected and content was in the range of 0.001–0.71 mg/100 g with the highest amount recorded in the first mowing at NS1. In samples from the second mowing coumaric acid was not determined. For ellagic acid the highest content (2.24 mg/100 g) was found in the first mowing during NS3 (correction) which was about 8-fold higher than NS1 and NS3 in the second mowing. Ferulic acid content was not significantly different at first mowing in NS1 and NS2 and at third mowing in NS2, with an average concentration of 5.5 mg/100 g. The highest naringin content was observed after the second mowing at NS1 (8.37 mg/100 g). Of the individual phenolic compounds determined naringin was the dominant compound, probably because it plays an important role in leaves development (*53*). Statistical significance of the tested factors showed that different NS manipulations had a significant influence on ellagic and ferulic acid and naringin content. The number of mowings had a great impact on coumaric, ellagic and ferulic acid, and the combination of factors (NS x M) significantly affected ellagic, ferulic acid and naringin.

Some authors identified a wide spectrum of individual phenols in nettle with high amounts of chlorogenic, 2-O-caffeoylmalic, protocatechuic and α-resorcylic acid, as well as kaempferol and rutin (*8,9,11,23*). Repajić *et al.* (*8*) identified a total of 41 compounds with cinnamic acids as the most abundant polyphenols group in wild nettle leaves showing that nettle is a very rich source of specific individual phenolic compounds. It is important to point out that plant origin and environmental factors

can strongly influence the composition and quantity of individual phenolic compounds in nettle leaves (*8,23*).

Hydroxycinnamic acids (such as ferulic, caffeic, sinapic, and *p*-coumaric acids), ellagic acid and naringin are a group of compounds known to possess potent antioxidant, anti-inflammatory, antimutagenic, anticarcinogenic, antimicrobial, and cardioprotective activities (*53,54*). Thus, the enhancement of food with phenolic compounds, which are one of the most abundant antioxidants present in the human diet, is desirable due to their beneficial medicinal properties towards human health. Further studies concerning the additional improvement of nettle phenolic content can be recommended.

Table 6

Photosynthetic pigments content of fresh stinging nettle leaves

In this research, from pigments chlorophyll a (Chl_a), chlorophyll b (Chl_b), total chlorophylls (TCh) and total carotenoids (TCa) were quantified in the stinging nettle leaves (Table 7). Apart from their use as food and pharmaceutical colorants (E140) (*7*), studies have shown that consumption of chlorophylls and their derivatives may have health-promoting activities, and provide protection against a variety of diseases. Carotenoids also show antioxidant activity and are major precursors of vitamin A in humans (*55*). Green leaves are the main plant tissue that is the best source of chlorophyll. Based on obtained results it can be pointed that, regardless of the NS treatment, the highest TCh and TCa levels were recorded at third mowing (Table 7), which can be explained by the plant's struggle by accumulating antioxidants to overcome the stress of multiple mowings. The experimental data also showed that the content of all photosynthetic pigments, regarding NS treatment, demonstrated the highest values after the third mowing in NS2 (supplementation). The highest content of Chl_a (1.21 mg/g) was around 98 % higher than the value observed in NS1 (depletion) at the second (0.62 mg/g) and third mowings (0.61 mg/g). The quantity of TCh was twofold higher in treatment NS2 than NS1 during the third mowing. After each mowing, the plant had to regrow back completely, and the increased accumulation of pigments most likely enabled easier photosynthesis and faster growth. The addition of water in NS1 reduced the amount of N, and N deficiency disturbed the nutrient balance and homeostasis of the plants, thus decreasing photosynthetic pigment levels (*56*). So, due to the increased amount of N in NS2 and NS3, which is necessary for photosynthesis and synthesis of pigments, increased accumulation of TCh and TCa probably occurs. The studies by Paulauskienė *et al.* (*33*) and Huang *et al.* (56) confirm that pigment compound content can strongly depend on abiotic factors such as harvest and nutrient solution concentration, which is in accordance with the present results.

Table 7

Antioxidant capacity of fresh stinging nettle leaves

Dietary antioxidants prevent oxidative damage caused by increased accumulation of reactive oxygen species (ROS) and nitrogen species (RNS) thus helping in disease prevention and contributing to many other beneficial effects on human health (*57*). Bioactive compounds present in leafy vegetables and other foods are responsible for their antioxidant properties. All bioactive substances analysed in this research are well-recognized as significant antioxidants. Antioxidant compounds found in plants and foods are hydrophilic to lipophilic, i.e., polar and nonpolar substances, therefore, two different methods were used in this study to determine antioxidant capacity. The ABTS tests can easily detect both hydrophilic and lipophilic antioxidants while the FRAP assay is more suitable for hydrophilic antioxidants.

The highest value for antioxidant capacity measured during the third mowing and NS2 (supplementation) was 35.47 μmol TE/g according to FRAP method (Fig. 1). This value was 183 % higher than the lowest recorded (first mowing NS1). The highest value obtained while using FRAP method was determined during the same mowing and treatment as the highest values of AsA, total phenolics and photosynthetic pigments confirming them as very powerful antioxidants. Quite different results were observed when using the ABTS assay where the highest antioxidant capacity was determined during NS3 (correction), after the second mowing with a value of 25.09 μmol/TE g. Different treatments with NS, number of mowings and their combination had a very significant influence on the antioxidant capacity of nettle leaves according to FRAP method. The ABTS test was influenced by the individual factors, but not by their interaction.

Both FRAP and ABTS values were much higher compared with the research of Radman *et al.* (15). Stinging nettle also exhibited the highest antioxidant activity in ABTS and FRAP assays among the other *Urtica* species (*Urtica urens*, and *Urtica membranacea*) in the study by Carvalho *et al.* (*58*). Considering the high antioxidant capacity, which can be further improved by nutrition manipulation with multiple mowings, as presented in our research, stinging nettle can be considered as a rich source of bioactive compounds with high antioxidant potential.

CONCLUSIONS

Considering the challenges and harmful effects that food production leaves on the planet, it is necessary to use sustainable and controlled production techniques. There are many factors throughout the production process that can affect the nutritional composition and quality of food. Proper plant nutrition may be one of the most important first steps in manipulating the nutritional value

of the final product resulting in foods with significant biological and pharmacological properties. This study showed that proper nutrition management can improve the nutritional quality of stinging nettle leaves. To achieve the highest levels of AsA, TPC, TFC, photosynthetic pigments and antioxidant capacity, it can be recommended to obtain three mowings and supplementation with NS. Based on the results, it is not necessary to perform a chemical analysis of NS after each mowing, yet it is enough to add the same initial solution to ensure the highest BC content. However, to ensure the highest content of essential macronutrients important for proper body function (Ca and Mg) water depletion during second mowing proved to be the best treatment. Also, for the consumption of nettle as a green leafy vegetable it is recommended to cultivate it under controlled conditions to ensure the safety and quality of the plant material available throughout the whole year.

FUNDING

This research was funded by Croatian Science Foundation (Hrvatska zaklada za znanost—www.hrzz.hr) under the project IP-2019-04-3325 URTICA-BioFuture—Nutritional and functional value of nettle (*Urtica dioica* L.) by application of modern hydroponic cultivation techniques.

CONFLICT OF INTEREST

Authors have no conflict of interest to declare relevant to the content of this study.

AUTHORS' CONTRIBUTION

M. Dujmović participated in conceptualization of the work, performing experiment, data collection, data analysis and interpretation, and drafting the article. N. Opačić, S. Radman and S. Fabek Uher provided plant material, helped with conception and design of the work, participated in data collection. L. Čoga and M. Petek participated in performing the analysis and data collection. S. Voća supervised the work. J. Šic Žlabur conceptualized and supervised the work, helped with the data interpretation, provided critical revision of the article along with its editing and final approval of the version to be published.

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Table 1. Equations of the calibration curves for the mixture of individual phenolic standards

Standard	Calibration Curve Equation	R^2 Value
Caffeic acid	y = 13159.9x + 12112.7	0.9998
Coumaric acid	y = 2551.11x + 2349.01	0.9999
Ellagic acid	y = 33829.1x – 6862.97	1.0000
Ferulic acid	y = 27461.5x – 90059.3	0.9870
Naringin	y = 3941.28x – 30329.7	0.9914

Table 2. Chromaticity parameters of fresh stinging nettle leaves cultivated under the different nutrient solution treatments during three mowings

Treatment	*L**	*a**	*b**	*C*	*h*°
First mowing					
NS1	(44.12±2.26)^{ab}	(-15.33±0.73)^{c}	(25.27±1.89)^{a}	(29.58±1.96)^{a}	(121.33±1.13)^{cd}
NS2	(43.18±2.37)^{abc}	(-14.69±0.58)^{c}	(22.64±1.85)^{a}	(27.00±1.84)^{a}	(123.03±1.34)^{bc}
NS3	(43.34±1.46)^{abc}	(-15.54±0.90)^{c}	(23.62±2.00)^{a}	(28.27±2.13)^{a}	(122.72±1.65)^{bc}
Second mowing					
NS1	(45.91±4.56)^{a}	(-13.49±1.46)^{bc}	(25.31±4.40)^{a}	(28.72±4.18)^{a}	(118.36±4.16)^{e}
NS2	(40.30±1.98)^{bc}	(-11.55±2.57)^{ab}	(16.08±3.13)^{b}	(19.80±4.04)^{b}	(125.56±0.88)^{a}
NS3	(40.86±2.00)^{bc}	(-11.88±2.01)^{ab}	(17.56±3.90)^{b}	(21.20±4.35)^{b}	(124.06±1.33)^{ab}
Third mowing					
NS1	(45.78±1.85)^{a}	(-14.66±0.04)^{c}	(25.23±0.96)^{a}	(29.18±0.82)^{a}	(120.18±0.99)^{de}
NS2	(39.90±1.63)^{c}	(-11.67±1.26)^{ab}	(17.51±2.08)^{b}	(21.04±2.43)^{b}	(123.72±0.31)^{abc}
NS3	(40.48±1.04)^{bc}	(-11.07±1.08)^{a}	(15.72±1.79)^{b}	(19.22±2.08)^{b}	(125.19±0.52)^{ab}
ANOVA	0.0447	0.0059	0.0007	0.0011	0.0003
LSD	4.0681	2.4125	4.6124	5.0578	2.499
NS	0.0031	0.0242	≤0.0001	0.0002	≤0.0001
M	NS	0.0006	0.0043	0.0023	NS
NS x M	NS	NS	NS	NS	0.0206

Results are expressed as mean±standard deviation. Different letters indicate significant differences between mean values. *L**=lightness, *a**=green–red components, *b**=blue–yellow components, *C**=chroma, *h*◦=hue, NS1=first nutrient solution treatment (depletion), NS2=second nutrient solution treatment (supplementation), NS3=third nutrient solution treatment (correction); significance of factors and their interactions: NS=nutrient solution, M=number of mowings, NS x M=nutrient solution and number of mowings; NS=non-significant

Table 3. Total dry matter and total acid content of stinging nettle cultivated under the different nutrient solution treatments during three mowings

Treatment	DM/%	TA/%
First mowing		
NS1	$(13.49\pm0.27)^{f}$	$(0.05\pm0.01)^{ef}$
NS2	$(15.50\pm0.31)^{e}$	$(0.06\pm0.01)^{de}$
NS3	$(15.05\pm0.26)^{e}$	$(0.08\pm0.01)^{bc}$
Second mowing		
NS1	$(21.73\pm0.24)^{c}$	$(0.09\pm0.01)^{bc}$
NS2	$(23.41\pm0.65)^{a}$	$(0.07\pm0.01)^{cd}$
NS3	$(23.96\pm0.27)^{a}$	$(0.11\pm0.01)^{a}$
Third mowing		
NS1	$(21.58\pm0.17)^{c}$	$(0.09\pm0.01)^{ab}$
NS2	$(22.45\pm0.44)^{b}$	$(0.04\pm0.01)^{f}$
NS3	$(17.84\pm0.15)^{d}$	$(0.07\pm0.02)^{cd}$
ANOVA	≤0.0001	≤0.0001
LSD	0.5782	0.0173
NS	≤0.0001	≤0.0001
M	≤0.0001	≤0.0001
NS x M	0.0003	0.0003

Results are expressed as mean ± standard deviation. Different letters indicate significant differences between mean values. DM=total dry matter content, TA=total acid content, NS1=first nutrient solution treatment (depletion), NS2=second nutrient solution treatment (supplementation), NS3=third nutrient solution treatment (correction); significance of factors and their interactions: NS=nutrient solution, M=number of mowings, NS x M=nutrient solution and number of mowings

Table 4. Mineral content of fresh stinging nettle leaves cultivated under the different nutrient solution treatments during three mowings

Treatment	*w*(mg/100 g)							
	Ca	Mg	Fe	Zn	Mn	Cu	B	Mo
First mowing								
NS1	(767.00±16.02)^{d}	(114.71±5.46)^{f}	(1.33±0.01)^{e}	(0.41±0.01)^{d}	(0.13±0.01)^{h}	(0.19±0.01)^{c}	(0.66±0.05)^{d}	(0.025±0.01)^{e}
NS2	(863.21±62.70)^{d}	(129.92±0.21)^{ef}	(1.65±0.08)^{d}	(0.29±0.01)^{f}	(0.50±0.02)^{f}	(0.17±0.01)^{c}	(0.71±0.02)^{d}	(0.026±0.01)^{e}
NS3	(812.72±8.73)^{d}	(136.80±0.17)^{e}	(1.97±0.03)^{b}	(0.37±0.01)^{e}	(1.08±0.02)^{b}	(0.21±0.02)^{b}	(0.85±0.01)^{c}	(0.031±0.01)^{de}
Second mowing								
NS1	(2106.35±146.74)^{a}	(333.34±6.74) ^{a}	(1.79±0.02)^{c}	(0.60±0.01)^{b}	(0.16±0.01)^{g}	(0.21±0.01)^{b}	(0.79±0.06)^{c}	(0.047±0.01)^{ab}
NS2	(1725.36±174.06)^{b}	(278.79±12.72)^{b}	(2.19±0.01)^{a}	(0.45±0.01)^{c}	(0.85±0.01)^{c}	(0.21±0.01)^{b}	(1.15±0.03)^{a}	(0.044±0.01)^{bc}
NS3	(1670.80±49.42)^{b}	(290.99±2.99)^{b}	(2.20±0.07)^{a}	(0.45±0.01)^{c}	(1.27±0.01)^{a}	(0.23±0.01)^{a}	(1.09± 0.03)^{ab}	(0.052±0.01)^{a}
Third mowing								
NS1	(1328.15±25.28)^{c}	(247.16±8.62)^{c}	(1.00±0.03)^{f}	(0.91±0.03)^{a}	(0.80±0.01)^{d}	(0.13±0.01)^{d}	(0.70±0.01)^{d}	(0.036±0.01)^{d}
NS2	(1236.56±11.28)^{c}	(219.60±6.40)^{d}	(2.05±0.03)^{b}	(0.39±0.02)^{de}	(0.60±0.02)^{e}	(0.07±0.01)^{f}	(1.03±0.01)^{b}	(0.038±0.01)^{cd}
NS3	(813.36±8.56)^{d}	(229.21±7.71)^{d}	(2.24±0.02)^{a}	(0.37±0.01)^{e}	(0.80±0.02)^{d}	(0.10±0.01)^{e}	(0.84±0.01)^{c}	(0.032±0.01)^{de}
ANOVA	≤0.0001	≤0.0001	≤0.0001	≤0.0001	≤0.0001	≤0.0001	≤0.0001	≤0.0001
LSD	194.4	15.369	0.1017	0.0323	0.0297	0.0148	0.0748	0.0077
NS	≤0.0001	≤0.0001	≤0.0001	≤0.0001	≤0.0001	≤0.0001	≤0.0001	NS
M	≤0.0001	≤0.0001	≤0.0001	≤0.0001	≤0.0001	≤0.0001	≤0.0001	≤0.0001
NS x M	≤0.0001	≤0.0001	≤0.0001	≤0.0001	≤0.0001	≤0.0001	≤0.0001	0.0145

Results are expressed as mean ± standard deviation. Different letters indicate significant differences between mean values. NS1=first nutrient solution treatment (depletion), NS2=second nutrient solution treatment (supplementation), NS3=third nutrient solution treatment (correction); significance of factors and their interactions: NS=nutrient solution, M=number of mowings, NS x M=nutrient solution and number of mowings; NS=non-significant

Table 5. Ascorbic acid and phenolic compounds content of fresh stinging nettle leaves cultivated under the different nutrient solution treatments during three mowings

Treatment	AsA/ (mg/100 g)	TPC/ (mg GAE/100 g)	TNFC/ (mg GAE/100 g)	TFC/ (mg CTH/100 g)
		First mowing		
NS1	(72.93±1.99)^{d}	(163.94±0.75)^{f}	(88.05±1.93)^{g}	(75.88±2.27)^{f}
NS2	(67.09±0.95)^{d}	(194.79±2.84)^{e}	(99.06±0.72)^{ef}	(95.73±2.13)^{de}
NS3	(84.12±2.49)^{c}	(202.00±0.87)^{d}	(104.58±0.84)^{d}	(97.42±0.55)^{d}
		Second mowing		
NS1	(56.11±4.92)^{e}	(204.50±1.49)^{d}	(115.81±0.24)^{c}	(88.68±1.50)^{e}
NS2	(47.00±7.75)^{f}	(262.14±1.22)^{c}	(128.12±0.82)^{b}	(134.02±2.03)^{c}
NS3	(48.12±1.20)^{f}	(267.00±1.99)^{c}	(139.36±0.71)^{a}	(127.64±2.51)^{c}
		Third mowing		
NS1	(95.42±3.35)^{b}	(300.09±1.79)^{b}	(100.63±1.76)^{e}	(199.45±3.55)^{b}
NS2	(112.37±4.75)^{a}	(377.04±11.26)^{a}	(97.50±0.67)^{f}	(279.54±11.35)^{a}
NS3	(94.64±8.05)^{b}	(163.63±0.80)^{f}	(98.97±1.21)^{ef}	(64.66±1.79)^{g}
ANOVA	≤0.0001	≤0.0001	≤0.0001	≤0.0001
LSD	7.5147	6.9634	1.759	7.6369
NS	NS	≤0.0001	≤0.0001	≤0.0001
M	≤0.0001	≤0.0001	≤0.0001	≤0.0001
NS x M	≤0.0001	≤0.0001	≤0.0001	≤0.0001

Results are expressed as mean±standard deviation. Different letters indicate significant differences between mean values. AsA=ascorbic acid, TPC=total phenolic content, TNFC=total non-flavonoid content, TFC=total flavonoid content, NS1=first nutrient solution treatment (depletion), NS2=second nutrient solution treatment (supplementation), NS3=third nutrient solution treatment (correction); significance of factors and their interactions: NS=nutrient solution, M=number of mowings, NS x M=nutrient solution and number of mowings; NS=non-significant

Table 6. Individual phenolic compounds of fresh stinging nettle leaves cultivated under the different nutrient solution treatments during three mowings

Treatment	*w*/(mg/100 g)				
	Caffeic acid	Coumaric acid	Ellagic acid	Ferulic acid	Naringin
First mowing					
NS1	nd	(0.71±0.13)^{a}	(1.05±0.05)^{b}	(5.62±0.26)^{a}	(7.96±0.05)^{bc}
NS2	nd	(0.24±0.24)^{abc}	(0.80±0.11)^{bcd}	(5.39±0.06)^{a}	(7.87±0.04)^{c}
NS3	nd	(0.61±0.61)^{ab}	(2.24±0.18)^{a}	(5.11±0.21)^{ab}	(8.25±0.05)^{ab}
Second mowing					
NS1	nd	nd	(0.26±0.01)^{d}	(3.50±0.13)^{c}	(8.37±0.03)^{a}
NS2	nd	nd	(0.29±0.01)^{cd}	(3.46±0.02)^{c}	(7.96±0.02)^{bc}
NS3	nd	nd	(0.28±0.01)^{d}	(3.43±0.05)^{c}	(7.98±0.03)^{bc}
Third mowing					
NS1	nd	(0.14±0.14)^{bc}	(0.85±0.37)^{bc}	(3.85±0.50)^{c}	(8.27±0.09)^{ab}
NS2	nd	(0.23±0.23)^{abc}	(0.39±0.17)^{cd}	(5.49±0.35)^{a}	(8.15±0.29)^{abc}
NS3	nd	(0.001±0.01)^{c}	(0.69±0.48)^{bcd}	(4.62±0.37)^{b}	(8.13±0.33)^{abc}
ANOVA	-	0.0014	≤0.0001	≤0.0001	0.0223
LSD	-	0.4738	0.5562	0.673	0.3722
NS	-	NS	0.0002	0.0053	0.0352
M	-	0.0006	≤0.0001	≤0.0001	NS
NS x M	-	NS	0.0001	0.0001	0.0155

Results are expressed as mean ± standard deviation. Different letters indicate significant differences between mean values. NS1=first nutrient solution treatment (depletion), NS2=second nutrient solution treatment (supplementation), NS3=third nutrient solution treatment (correction); significance of factors and their interactions: NS=nutrient solution, M=number of mowings, NS x M=nutrient solution and number of mowings; nd=not determined; NS=non-significant

Table 7. Photosynthetic pigments content of fresh stinging nettle leaves cultivated under the different nutrient solution treatments during three mowings

Treatment	w/(mg/g)			
	Chl_a	Chl_b	TCh	TCa
First mowing				
NS1	(0.80±0.01)^{e}	(0.40±0.01)^{c}	(1.20±0.02)^{e}	(0.24±0.01)^{d}
NS2	(0.87±0.02)^{c}	(0.36±0.01)^{e}	(1.22±0.01)^{de}	(0.27±0.01)^{c}
NS3	(0.88±0.01)^{c}	(0.36±0.01)^{e}	(1.23±0.01)^{cd}	(0.30±0.01)^{b}
Second mowing				
NS1	(0.62±0.01)^{g}	(0.38±0.01)^{d}	(1.00±0.01)^{g}	(0.21±0.01)^{f}
NS2	(0.95±0.01)^{b}	(0.48±0.01)^{b}	(1.43±0.01)^{b}	(0.27±0.01)^{c}
NS3	(0.84±0.01)^{d}	(0.40±0.01)^{c}	(1.24±0.01)^{c}	(0.27±0.01)^{c}
Third mowing				
NS1	(0.61±0.01)^{g}	(0.26±0.01)^{g}	(0.88±0.01)^{h}	(0.23±0.01)^{e}
NS2	(1.21±0.01)^{a}	(0.62±0.01)^{a}	(1.84±0.02)^{a}	(0.36±0.01)^{a}
NS3	(0.72±0.01)^{f}	(0.33±0.01)^{f}	(1.05±0.01)^{f}	(0.24±0.01)^{d}
ANOVA	≤0.0001	≤0.0001	≤0.0001	≤0.0001
LSD	0.0141	0.0112	0.0192	0.0033
NS	≤0.0001	≤0.0001	≤0.0001	≤0.0001
M	≤0.0001	≤0.0001	≤0.0001	≤0.0001
NS x M	≤0.0001	≤0.0001	≤0.0001	≤0.0001

Results are expressed as mean±standard deviation. Different letters indicate significant differences between mean values. Chl_a=chlorophyll a, Chl_b=chlorophyll b, TCh=total chlorophylls, TCa=total carotenoids, NS1=first nutrient solution treatment (depletion), NS2=second nutrient solution treatment (supplementation), NS3=third nutrient solution treatment (correction); significance of factors and their interactions: NS=nutrient solution, M=number of mowings, NS x M=nutrient solution and number of mowings

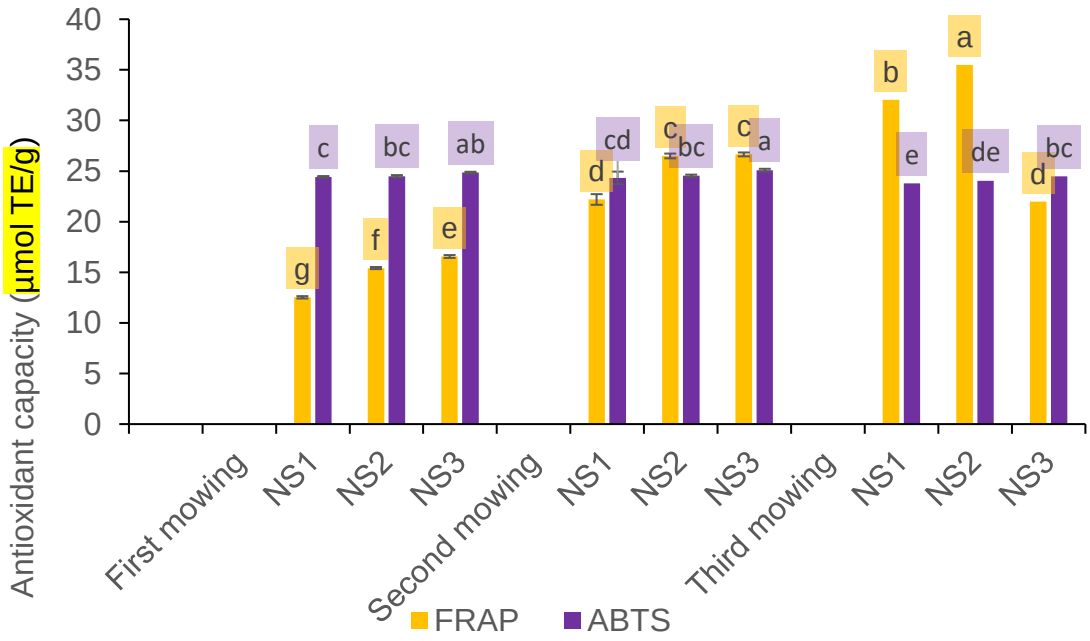

Fig. 1. Antioxidant capacity (µmol TE/g) of fresh stinging nettle leaves cultivated under the different nutrient solution treatments during three mowings. Results are expressed as mean±standard deviation. Different letters indicate significant differences between mean values. NS1=first nutrient solution treatment (depletion), NS2=second nutrient solution treatment (supplementation), NS3=third nutgent solution treatment (correction)