Research Article

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Chemical composition and bioactive properties of *Cichorium spinosum* L. in relation to nitrate/ammonium nitrogen ratio

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Abstract

BACKGROUND: Nitrogenous fertilizers may affect the yield and quality of leafy vegetables via the application rate and nitrogen form. In the present study, the effect of the nitrate:ammonium nitrogen ratio in the nutrient solution on the chemical composition and bioactive properties of *Cichorium spinosum* leaves was evaluated. For this purpose, *C. spinosum* plants were fertigated with nutrient solution containing different ratios of nitrate: ammonium nitrogen: (i) 100:0 NO_3-N:NH_4-N; (ii) 75:25 NO_3-N:NH_4-N; (iii) 50:50 NO_3-N:NH_4-N; (iv) 25:75 NO_3-N:NH_4-N; and (v) 0:100 NO_3-N:NH_4-N of total nitrogen; as well as (vi) 100% ureic nitrogen.

RESULTS: The only detected tocopherol isoforms were α- and δ-tocopherol, which were positively affected by nitrate nitrogen (100:0 NO_3-N:NH_4-N). Similar results were observed for individual and total organic acids. The main detected sugars were fructose, glucose and sucrose, with a varied effect of nutrient solution composition on their content, whereas total sugar concentration was positively affected by a balanced or a slightly increased proportion of NH_4-N (50:50 and 25:75 NO_3-N:NH_4-N). The fatty acids profile was beneficially affected by the highest NH_4-N ratio (0:100 NO_3-N:NH_4-N), whereas higher amounts of NO_3^- than NH_4^+ nitrogen (75:25 NO_3-N:NH_4-N) resulted in a higher content of total phenolic compounds. Finally, no cytotoxic effects were observed against non-tumor (PLP2, HeLa) and tumor (HepG2, MCF-7, NCI-H460) cell lines for any of the studied nutrient solutions.

CONCLUSION: The modulation of NO_3-N:NH_4-N ratio in the nutrient solution supplied to *C. spinosum* may enhance the content of desirable health-promoting compounds and reduce the content of antinutrients, thus increasing the overall quality of the final product without compromising yield.

Keywords: bioactive properties; *Cichorium spinosum* L.; cytotoxicity; nitrogen form; nitrate:ammonium nitrogen; nutrient solution composition; omega-3 fatty acids; phenolic compounds

INTRODUCTION

Cichorium spinosum L. is a wild edible species that has received much recent attention as a potential alternative/complementary crop. Similar to other wild edible plants that are part of the traditional culinary heritage of the Mediterranean rural communities,[1–3] *C. spinosum* is an ingredient of the so-called 'Mediterranean diet', which is associated with several beneficial health effects.[4] There is a growing trend with respect to its commercial cultivation, which is usually based on cultivation practices applied on similar domesticated species (endives, chicory, lettuce) because of the scarce information available in the literature. Recently, our team has published information regarding the effect of salinity,[5] nutrient solution composition[6] and harvesting stage[7] on the nutritional value and chemical composition of the plant edible leaves, whereas a chemical characterization of various *C. spinosum* ecotypes has been also reported.[8]

Nitrogenous fertilizers are pivotal for the achievement of high yields in vegetable crop production, although their use has raised concerns with regard to leafy vegetable production because excessive amounts of nitrogen may have implications for human health.[9] Several studies have reported the importance of nitrogen form rather than the amount of total nitrogen, with significant effects not only on total yield, but also on marketable quality

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of the final vegetable products.[6,10–12] Therefore, modulation of nutrient solution composition can be a cost-effective means for the improvement of vegetable quality either by decreasing antinutritional factors[13] or by increasing beneficial compounds content.[14–16] Phenolic compounds and omega-3 fatty acids are of particular interest and an increase in their content in the final product is crucial for the achievement of high-added value products.[12,17] Especially for phenolic compounds, it has been well-established via *in vitro* and *in vivo* studies that they possess several functional properties including antitumoral acitivities by inhibition of tumorigenesis and chemoprevention.[18–20] Other important compounds with bioactive properties are tocopherols, which are present in plant tissues in various isoforms, and their content may be also regulated through the nutrient solution composition.[6,21] However, despite the beneficial effects of the various bioactive compounds detected in wild edible plants, several toxicity symptoms have been reported; therefore, cytotoxicity studies against normal and cancer cells have to be carried out prior to suggesting the consumption of such species.[22,23]

In a previous study[6], the effect of increasing ammonium nitrogen rates on plant growth and chemical composition of *C. spinosum* leaves was evaluated. In the present study, our aim was to further evaluate the effects of gradual nitrate:ammonium nitrogen ratios in nutrient solution on the chemical composition and bioactive properties of *C. spinosum* leaves. Accordingly, five fertilizer treatments with different ratios of nitrate: ammonium nitrogen were applied: (i) 100:0 NO_3-N:NH_4-N; (ii) 75:25 NO_3-N:NH_4-N; (ii) 50:50 NO_3-N:NH_4-N; (iv) 25:75 NO_3-N:NH_4-N; and (v) 0:100 NO_3-N:NH_4-N of total nitrogen; for comparison purposes, an extra treatment (vi) with 100% ureic nitrogen was also applied.

MATERIALS AND METHODS

Plant material and experimental procedures

The experiment was conducted at the experimental farm of the University of Thessaly, Greece. The plant material has been described previously.[6,24] Briefly, seeds of *C. spinosum* L. (Asteraceae) were sown on 17 September 2016 in seed trays containing peat. When plants reached the stage of three or four true leaves (14/02/2017), young seedlings were transplanted in 2-L pots containing peat (Klassman-Deilmann KTS2) (1.0 L) and perlite (1.0 L).[25] Fifteen pots, each containing one plant, were used for each fertilization treatment (90 pots in total). Fertilization of plants was carried out through the irrigation water with six fertilizer treatments (1–6) containing the same amount of nitrogen (200 mg L^{-1}). Fertilizer treatments differed in nitrogen source (ammonium, nitrate or ureic nitrogen) and their relative ratios: (i) 100:0 NO_3-N:NH_4-N; (ii) 75:25 NO_3-N:NH_4-N; (iii) 50:50 NO_3-N:NH_4-N; (iv) 25:75 NO_3-N:NH_4-N; and (v) 0:100 NO_3-N:NH_4-N of total nitrogen; as well as (vi) 100% ureic nitrogen. Nutrient solutions were prepared as described previously[6] and as reported in Table 1 using the following fertilizers: (i) ammonium nitrate (34.5-0-0; N-P_2O_5-K_2O); (ii) potassium nitrate (13.5-46.2-0; N-P_2O_5-K_2O); (iii) magnesium nitrate (11-0-0; N-P_2O_5-K_2O + 15 MgO); (iv) ammonium sulphate (21-0-0; N-P_2O_5-K_2O + 24 SO_3); (v) monopotassium phosphate (0-52-34; N-P_2O_5-K_2O); (vi) potassium sulphate (0-0-50; N-P_2O_5-K_2O); (vii) monoammonium phosphate (12-61-0; N-P_2O_5-K_2O); and (viii) 100% ureic nitrogen (46% nitrogen in urea form).

Harvesting of plants was carried out once during the growing period (on 26 April 2017) and when the rosettes of leaves had reached marketable size. After harvest, samples of fresh leaves were put in freezing conditions, then lyophilized, ground with a mortar and pestle, placed in air-sealed food bags, and stored at deep freezing conditions (−80 °C) until further analyses.

Chemical analyses

Tocopherols

As described previously by Barros *et al.*,[26] hexane solutions of butyl-hydroxy-toluene (10 mg mL^{-1}; 100 μL) and tocol (internal standard, 400 μL at 50 μg mL^{-1}), were added to 500 mg of the sample prior to extraction. The combination was then homogenized with 4 mL of methanol by vortex mixing (1 min), followed by 4 mL of hexane (vortex mixed for 1 min). After sample homogenization, saturated NaCl aqueous solution (2 mL) was added, the mixture was combined (vortex for 1 min), centrifuged (5 min at 4000 × *g*) and the clear upper layer carefully transferred to a vial. Sample extraction with hexane was performed an additional two times. The combined extracts (clear layer) were taken to dryness under a nitrogen stream, dissolved in 2 mL of *n*-hexane, dehydrated with anhydrous sodium sulphate, filtered through a 0.2-μm nylon filter (Whatman; GE Healthcare, Little Chalfont, UK), transferred into a dark injection vial and analyzed by high-performance liquid chromatography (HPLC) (Smartline system 1000; Knauer, Berlin, Germany) coupled to a fluorescence detector (FP-2020; Jasco, Easton, MD, USA).

Soluble sugars

For soluble sugars determination, a maceration with 1 g of dry sample spiked with melezitose (internal standard, 25 mg mL^{-1}) and 40 mL of aqueous ethanol (80%, v/v) at 80 °C was performed. Afterwards, the sample was filtered and the solvent was evaporated.[26] Identification and quantification of the individual sugars was performed via HPLC in conjunction with a refraction index detector and data were analyzed using Clarity, version 2.4 (DataApex, Prague, The Czech Republic). The results were expressed as g kg^{-1} dry weight (dw).

Organic acids

The samples were extracted by macerating 1 g of the sample and 25 mL of metaphosphoric acid (4.5%) during 20 min at room temperature. After sample filtration, the samples were analyzed by ultra-fast LC coupled to a photodiode array detector using 215 nm and 245 nm (for ascorbic acid) as preference wavelengths (UFLC-PDA; Shimadzu Coperation, Kyoto, Japan), as described previously by Barros *et al.*[26] The results were expressed as g kg^{-1} dw.

Fatty acids

As described previously by Barros *et al.*,[26] fatty acid content was investigated after trans-esterification of the lipid fraction obtained through Soxhlet extraction. The samples were analyzed by gas-LC with flame ionization detection/capillary column and the results were recorded and processed using CSW, version 1.7 (DataApex) and expressed as a relative percentage for each fatty acid.

Phenolic compounds characterization

The hydroethanolic extract was obtained by maceration, using aqueous ethanolic solution (80%, v/v; 30 g mL^{-1}) at 25 °C for 60 min. After filtration (Whatman no. 4 filter), the sample was re-extracted once more using the same conditions. Afterwards, the solvent was evaporated at 40 °C, under reduced pressure, in a rotary evaporator (model R-210; Büchi, Flawil,

Table 1. Macronutrient concentrations (mg L^{-1}) of the nutrient solution for each treatment

$NO3^-$:$NH4^+$	Total nitrogen	NO_3^-	NH_4^+	Urea	K	P	Ca	Mg	S
100:0	200	200	0	0	200	200	0	178.3	0
75:25	200	150	50	0	200	200	0	136.3	0
50:50	200	100	100	0	200	200	0	0	0
25:75	200	50	150	0	200	200	0	0	136.4
0:100	200	0	200	0	200	200	0	0	252.8
Urea	200	0	0	200	200	200	0	0	24

Switzerland) and the residual aqueous extract was freeze dried (FreeZone 4.5 model 7 750 031; Labconco, Kansas City, MO, USA).

The dry extracts were re-suspended at a concentration of 10 mg mL^{-1} using aqueous ethanol (50%, v/v) and filtered (0.2 µm disposable LC filter disk, 30 mm, nylon). Afterwards, the phenolic profile of *C. spinosum* leaves was determined by LC with a diode-array detector (wavelengths of 280, 330 and 370 nm) coupled with electrospray ionization mass spectrometry operating in negative mode (Dionex Ultimate 3000 UPLC and Linear Ion Trap LTQ XL; Thermo Scientific, San Jose, CA, USA), as described previously.[27] The phenolic compounds were identified according to their chromatographic characteristics by comparison with those obtained using standard compounds and also with the literature. Calibration curves of appropriate standards were obtained in the range 200–5 µg mL^{-1} for the quantitative analysis. The results were expressed as g kg^{-1} extract.

Cytotoxicity

The extracts descrbed above were re-dissolved in water at a concentration of 8 mg mL^{-1} and further diluted in the range from 400 to 6.25 µg mL^{-1}. The cytotoxic properties were evaluated using four human tumor cell lines: MCF-7 (breast adenocarcinoma); NCI-H460 (non-small cell lung cancer); HeLa (cervical carcinoma); and HepG2 (hepatocellular carcinoma). A non-tumor cell line (PLP2) was evaluated using a procedure described previously by Abreu *et al.*[28] A sulforhodamine B assay was carried out in accordance with the methods described by Barros *et al.*,[21] with ellipticine being used as a positive control. The results were expressed as GI_{50} values (i.e. the concentration that inhibited 50% of cell proliferation).

Statistical analysis

Plant growth measurements were recorded on 15 plants per fertilizer treatment ($n = 15$). For chemical analyses and for each fertilizer treatment, three samples of leaves ($n = 3$) were analyzed. All chemical composition assays were carried out in triplicate. Data were analyzed via one-way analysis of variance and, when significant differences were observed, a comparison of means was performed using Tukey's honestly significant difference test ($P = 0.05$). SPSS, version 21 (IBM Corp., Armonk, NY, USA) was used for the data analyses.

For a visual representation of the results, data on yield, dry matter (DM) content and all of the nutritional parameters examined were subject to principal component analysis (PCA) using PAST3.[29] Before carrying out the PCA, means were standardized [(x – mean)/SD). The data matrix subject to PCA was constituted by 18 samples (6 treatments × 3 replicates) for each of the 36 variables examined.

RESULTS AND DISCUSSION

Previous studies investigating the impact of nitrogen form on the yield and quality of *C. spinosum* revealed a relatively low response to NH_4-N in terms of fresh and dry biomass production.[6,30] Nevertheless, in both studies, the highest proportion of NH_4-N tested was approximately 50% of the total N and a different response was observed based on the crop growth stage, with higher tolerance to NH_4-N in the first *versus* the second growth period.[6] In the present study, the NO_3-N:NH_4-N ratio tested covered the full range of variation from 100% NO_3-N to 100% NH_4-N, including 75:25, 50:50 and 25:75 NO_3-N:NH_4-N ratios, as well as 100% ureic nitrogen as a control treatment. As previously reported by Kolovou *et al.*,[24] in the present study, the form of N applied had a significant impact on *C. spinosum* plant growth. Total number of leaves and total fresh weight were positively influenced by a higher proportion of NO_3-N, with the 100:0 and 75:25 NO_3-N:NH_4-N ratios providing the highest plant fresh weight (45.8 g) and number of leaves (43) per plant, respectively. However, as observed previously in a similar study by Petropoulos *et al.*,[6] also in this case, a positive response to a higher proportion of NH_4-N was observed at the first and second harvest, whereas a negative effect on plant growth was observed at the third harvest. The lowest plant fresh biomass and number of leaves at all stages were produced by plants nourished with urea, which was noxious for *C. spinosum* plants.[24] The NO_3-N:NH_4-N ratio of the nutrient solution influenced also the DM content of *C. spinosum* leaves, causing an increase in DM with an increasing proportion of NH_4-N over the total N up to the 25:75 NO_3-N:NH_4:N ratio.[24] These results suggest that the form of N may affect not only the dry biomass accumulation of *C. spinosum*, but also its water content and thus the fresh biomass. Despite producing the lowest dry biomass, having the highest water content, plants fertilized exclusively with NO_3-N achieved the highest fresh yield.[24] Similar to other leafy vegetables, the higher fresh yield of plants nourished with NO_3-N may be the result of a higher accumulation of NO_3^- in the leaf tissues. Because of its osmotic effect, the accumulation of NO_3^- in plant tissues enhances the plant water uptake; indeed, an inverse relationship has been observed between the accumulation of NO_3^- and the DM content of leafy vegetables.[31,32] Besides the effect on plant growth, the form of N supplied also had an impact on the nutritional profile of *C. spinosum* plants, influencing the content of all the metabolites analyzed in the present study.

Effect of the NO_3-N:NH_4-N ratio on sugars and organic acids

The *C. spinosum* leaf sugar concentration was positively influenced by a balanced or a slightly increased proportion of NH_4-N in the nutrient solution (Table 2). Compared with the 100% NO_3-N nutrient solution, increasing NH_4-N up to 50% and 75% of the total-N determined an increase of 25.8% and 28.8% of the total sugar content, respectively. Such results were consistent with the

Table 2. Composition in sugar (g kg^{-1} dw) of *Cichorium spinosum* leaves in relation to nutrient solution (NS) composition (mean ± SD)

NO_3^-:NH_4^+	Fructose	Glucose	Sucrose	Total sugars
100:0	15.1 ± 0.3 b	19.9 ± 0.4 d	24 ± 1 d	59 ± 1 c
75:25	16.4 ± 0.5 a	26 ± 1 b	26.9 ± 0.3 c	69 ± 1 b
50:50	14.4 ± 0.6 bc	24.9 ± 0.8 b	34.8 ± 0.1 a	74.2 ± 0.1 a
25:75	16.9 ± 0.7 a	27.9 ± 0.3 a	31.5 ± 0.9 b	76 ± 2 a
0:100	14.7 ± 0.5 bc	21 ± 1 c	31 ± 2 b	67 ± 4 b
Urea	14.1 ± 0.8 c	21 ± 1 c	34.6 ± 0.3 a	69 ± 2 b

In each column, different lowercase letters indicate significant differences between samples according to Tukey's test ($P = 0.05$).

observation of Flore *et al.*[33] and could be the result of a higher energy efficiency of plants nourished with moderately higher proportions of NH_4-N. Sucrose represented the prevalent sugar (on average 44%), followed by glucose (on average 34%) and fructose (on average 22%). The highest concentration of sucrose was observed in plants grown with a 50:50 NO_3-N:NH_4-N ratio or with urea, mostly at the expense of fructose, which showed the lowest concentration in the same plants. The highest and lowest concentrations of glucose were observed in plants grown with 75:25 and 100:0 NO_3-N:NH_4-N ratios, respectively. Plants grown with 75:25 and 25:75 NO_3-N:NH_4-N ratios had the highest concentration of fructose. Examining the sugar profile, no positive or negative relationships were observed between the concentration of the two monosaccharides and the disaccharide sucrose. Overall, total sugar concentrations were similar or within the lower range of values observed by Petropoulos *et al.*[8] and Petropoulos *et al.*[6] for *C. spinosum* under similar growing conditions.

On the other hand, the concentration of total organic acids was highest in plants of *C. spinosum* grown with the highest proportion of NO_3-N (Table 3). In accordance with the observation of Petropoulos *et al.*,[6] oxalic and quinic acid were the main organic acids detected, followed by malic and citric acid, along with traces of fumaric acid. The concentration of all the organic acids detected, including oxalic, quinic, malic and citric acid, was higher in the leaves of plants fertigated with 100% NO_3-N nutrient solution compared to all of the other plants fertilized with different NO_3-N to NH_4-N ratios, except for those grown with 25:75 NO_3-N:NH_4-N ratio, which showed an equivalent concentration of oxalic acid. The lowest value of total organic acids were observed in plants fertigated with a 25:75 NO_3-N:NH_4-N ratio, followed by those nourished with 100% ureic nitrogen, and the 50:50 NO_3-N:NH_4-N ratio. Such results are consistent with the findings of previous studies in which a reduction of organic acids was observed, especially of oxalic acid, with an increasing proportion of NH_4-N *versus* NO_3-N in the nutrient solution in the case of purslane,[14] spinach[34] and *C. spinosum*.[6] The higher accumulation of organic acids observed in the leaf tissues with an increasing proportion of NO_3-N may be explained by the need of the plant to counterbalance the increase in pH associated with the uptake of NO_3-N.[35] From a nutritional standpoint, a lower oxalic acid concentration is highly desirable in leafy vegetables with respect to avoiding the issues associated with the formation of oxalates. Oxalates, the salts formed between oxalic acid and Ca or other minerals, can limit the assimilation of essential elements (Fe, Ca, Mg) and cause the formation of insoluble crystals and subsequently stones in the kidneys or in the urinary tract.[36] From this perspective, as proposed for other vegetables, an increase in the proportion of NH_4-N in the nutrient solution may enhance the nutritional properties of *C. spinosum* by reducing the concentration of oxalic acid and limiting the potential formation of oxalates.

As for other food products, the profile of sugars and organic acids is a strong determinant of the sensory properties of leafy vegetables. The ratio of total sugar to total organic acids may be used as an indicator of the sensory properties of leafy greens. Examining the relationship between the NO_3-N:NH_4-N ratio of the nutrient solution and the ratio of total sugar to total organic acids, large variations were observed, with values ranging from 0.29 in plants fertigated exclusively with NO_3-N up to 0.56 in those fertigated with a 50:50 NO_3-N:NH_4-N ratio (Tables 2 and 3). In this perspective, future studies are warranted to investigate the potential of enhancing the flavor and sensory properties of leafy vegetables by modulating the NO_3-N:NH_4-N ratio of the fertigating solution.

Effect of the NO_3-N:NH_4-N on tocopherols

Cichorium spinosum plants fertigated with 100:0 NO_3-N:NH_4-N ratio had the highest content of both α- and δ-tocopherols, resulting in the highest content of total tocopherols for the same treatment (Table 4). Plants receiving nutrient solution with a NO_3-N:NH_4-N ratio of 75:25 and urea as the sole source of nitrogen had the lowest α- and δ-tocopherols, respectively, resulting in a 36.2% decrease on average of total tocopherols content compared to plants grown exclusively with NO_3-N. These results are partially in contrast with a previous study reorting an increase in α-tocopherols with a 75:25 NO_3-N:NH_4-N ratio *versus* 100% NO_3-N, in two out of three genotypes of *Portulaca oleracea*.[21] Intermediate total tocopherols concentrations were observed when 50%, 75% or 100% of total nitrogen was applied with the form of NO_3-N. Overall, when expressed on a fresh weight basis, the total tocopherol concentration values measured were similar to those reported by Petropoulos *et al.*[6] for first and second harvest of *C. spinosum* grown under similar conditions, as well as beiong similar to the values of total tocopherols reported for the wild ecotypes collected *in situ* and analyzed by Petropoulos *et al.*[8]

Effect of the NO_3-N:NH_4-N on the fatty acids profile

Twenty-four different fatty acids including saturated fatty acids (SFA) (12.9%), monounsaturated fatty acids (MUFA) (2.6%) and polyunsaturated fatty acids (PUFA) (84.5%) were detected by gas chromatography and a significant impact of the nutrient solution NO_3-N:NH_4-N ratio was observed on the fatty acids profile of *C. spinosum* leaves (Table 5). Palmitic acid (C16:0) and palmitoleic acid (C16:1) constituted, on average, 74.8% and 75% of the SFA and MUFA, respectively; oleic acid (C18:1*n*9c) was the second most abundant (22.7% on average) MUFA, whereas α-linolenic acid (C18:3*n*3) constituted the primary (80.8%) PUFA and fatty acid in general, followed by linoleic acid (C18:2*n*6c), which represented 18.8% of the PUFA. Overall, the observed fatty acid composition was similar to the fatty acid profile reported for different ecotypes of *C. spinosum*[8] and *C. spinosum* harvested at different times.[37] Although a lower omega-6/omega-3 fatty acids ratio was observed in the present study compared to that reported by Petropoulos *et al.*[8] mainly as a consequence of a lower content of linoleic acid (C18:2*n*6c), for all the tested treatments, the ratio values were lower than 4.0, which, according to Guil *et al.*,[38] indicates a high nutritional value. When examining the effect of the NO_3-N:NH_4-N ratio of the nutrient solution on the fatty acids composition, it was observed that an equal proportion of NO_3-N and

Table 3. Composition in organic acids (g kg^{-1} dw) of *Cichorium spinosum* leaves in relation to nutrient solution (NS) composition (mean ± SD)

NO_3^-:NH_4^+	Oxalic acid	Quinic acid	Malic acid	Citric acid	Fumaric acid	Total organic acids
100:0	56.1 ± 0.1 a	98.4 ± 0.7 a	31.8 ± 0.1 a	18.6 ± 0.1 a	tr	205.0 ± 0.8 a
75:25	53.1 ± 0.3 b	90.6 ± 0.2 b	28.5 ± 0.3 d	16.0 ± 0.2 b	tr	188.2 ± 0.1 b
50:50	52.5 ± 0.2 c	44.5 ± 0.4 d	30.0 ± 0.1 b	15.6 ± 0.1 c	tr	142.7 ± 0.6 d
25:75	56.3 ± 0.1 a	45 ± 1 d	19.4 ± 0.2 f	15.1 ± 0.1 d	tr	136 ± 1 f
0:100	48.0 ± 0.1 d	72 ± 1 c	20.5 ± 0.4 e	12.6 ± 0.1 f	tr	153 ± 1 c
Urea	52.3 ± 0.2 c	43 ± 1 e	29.5 ± 0.6 c	13.6 ± 0.1 e	tr	138.0 ± 0.7 e

In each column, different lowercase letters indicate significant differences between samples according to Tukey's test ($P = 0.05$).

Table 4. Composition in tocopherols (mg kg^{-1} dw) of *Cichorium spinosum* leaves in relation to the form of nitrogen in the nutrient solution (mean ± SD)

NO_3^-:NH_4^+	α-Tocopherol	δ-Tocopherol	Total tocopherols
100:0	20.9 ± 0.1 a	26.0 ± 0.2 a	46.9 ± 0.1 a
75:25	11.9 ± 0.1 f	17.8 ± 0.9 c	30 ± 1 d
50:50	20.5 ± 0.4 b	14.1 ± 0.1 d	35 ± 1 c
25:75	18.3 ± 0.1 c	18.5 ± 0.1 b	36.8 ± 0.1 b
0:100	17.4 ± 0.1 d	17.8 ± 0.5 c	35 ± 1 c
Urea	17.0 ± 0.2 e	12.8 ± 0.5 e	29.8 ± 0.6 d

In each column, different lowercase letters indicate significant differences between samples according to Tukey's test ($P = 0.05$).

NH_4-N (50:50 NO_3-N:NH_4-N) caused a significant increase in the SFA fraction. The percentage of MUFA remained stable in plants fertigated with nutrient solutions containing from 50% to 100% of NO_3-N and decreased when 100% ureic nitrogen or a higher proportion of NH_4-N was used. Instead, the exclusive use of NH_4-N caused an increase in eicosatrienoic acid (C20:3*n*3) and especially of α-linolenic acid (C18:3*n*3), thereby determining an increase in the percentage of PUFA and a higher PUFA/SFA ratio, suggesting that 100% NH_4-N fertigating solutions may be used to fortify the nutritional value of *C. spinosum* by increasing the concentration of omega-3 fatty acids and the PUFA/SFA ratio. The lowest percentage of α-linolenic acid (C18:3*n*3) and PUFA were observed in plants grown with a 50:50 NO_3-N:NH_4-N ratio, followed by those receiving 100% ureic nitrogen. The leaves of plants fertigated with a 25:75 NO_3-N:NH_4-N ratio were characterized by the highest omega-6/omega-3 fatty acids ratio as a result of a higher concentration of both linoleic (C18:2*n*6c) and γ-linolenic (C18:3*n*6) acid.

Effect of the NO_3-N:NH_4-N on phenolic compounds

The chromatographic characteristics of the tentatively identified compounds and the quantification results of *C. spinosum* leaves are presented in Table 6. Compounds were identified based on their retention time, UV-visible and mass spectra characteristics. Thirteen compounds were identified in total, including four phenolic acids (hydroxycinnamic acid derivatives) and nine flavonoid glycoside derivatives, of which eight were flavonols (mainly quercetin, kaempferol and isorhamnetin derivatives) and one was flavone (apigenin derivative). All the identified compounds have been previously identified in *C. spinosum*.[5,6,37,39]

The nutrient solution NO_3-N:NH_4-N ratio had a significant impact on the phenolic profile of *C. spinosum* leaves (Table 6). The main phenolic compounds detected were chicoric acid (on average, 53% *cis*- and 47% *trans*-chicoric acid) and 5-*O*-caffeoylquinic acid among the phenolic acids, and kaempferol-*O*-glucuronide, quercetin-3-*O*-glucuronide and kaempferol-3-*O*-glucuronide among the flavonoid compounds. These results are consistent with that reported in previous studies on *C. spinosum*, except for the lower proportion of total flavonoids (TF) compared to total phenolic acids (TPA).[6,39] TPA ranged between 31.9% and 46.9% of the TPC, and TF ranged between 53.1% and 68.1% of the total phenolic compounds (TPC), whereas a higher percentage of TPA was observed by Petropoulos *et al.* [6,37] The highest content of TPA, TF and TPC was observed in plants supplied with a 75:25 NO_3-N:NH_4-N ratio fertigating solution. Compared to plants fertigated with 100% ureic nitrogen, the high value of TPA observed with the 75:25 NO_3-N:NH_4-N ratio was primarily the result of an increase in *cis* chicoric acid and 5-*O*-caffeoylquinic acid, whereas the higher TF was mainly a consequence of the increase in quercetin-3-*O*-glucuronide and kaempferol-*O*-glucuronide. Plants fertigated with a 50:50 NO_3-N:NH_4-N ratio and with 100% NO_3-N had the second highest content of TPA and TF, respectively. The lowest TPA, TF and TPC values were observed in plants fertigated with a 25:75 NO_3-N:NH_4-N ratio solution, followed by plants fertilized with 100% urea.

Several studies have shown that the biosynthesis of phenolic compounds is highly influenced by N fertilization practices and, in general, a lower availability of N is associated with an increase in phenolic compounds.[40,41] Instead, there is limited and contrasting evidence available with respect to how the biosynthesis of phenolic compounds is affected by the form of N. In a study conducted on onion, Mollavali *et al.*[42] observed an increased concentration of quercetin-3,4′-di-*O*-β-D-glucoside and quercetin-4′-*O*-β-D-glucoside in plants grown with additional predominant NH_4-N. The increased flavonol concentration observed was also associated with a higher phenylalanine ammonia lyase (PAL) in plants grown predominantly with NH_4-N. Such results were consistent with the findings obtained in other studies demonstrating that the enzyme PAL along with the glutamine synthase plays a key role in the assimilation and recycling of NH_4^+, contributing to its detoxification in plant tissues.[43,44] The role that NH_4^+ may play in triggering a stress response stimulating the biosynthesis of phenolic compounds was also demonstrated by the higher expression of superoxide dismutase and soluble peroxidase enzymes activated in presence of oxidative stress.[45] The higher TPC observed in the present study with the supply of nutrient solutions with 75:25, 50:50 and 0:100 NO_3-N:NH_4-N ratios compared to the exclusive supply of NO_3-N is consistent with previous studies. An increase in TPC content with increasing the proportion of NH_4-N in nutrient solution up to 50% was observed also by Chatzigianni *et al.*[30] and Petropoulos *et al.*[6] However, the lower content of TPC observed using a 25:75 NO_3-N:NH_4-N ratio and 100% ureic nitrogen suggests

Table 5. . Fatty acids composition (%) of *Cichorium spinosum* leaves in relation to nutrient solution (NS) composition (mean ± SD)

	NO_3^-:NH_4^+					
	100:0	75:25	50:50	25:75	0:100	Urea
C6:0	0.058 ± 0.002 b	0.058 ± 0.002 b	0.065 ± 0.004 a	0.047 ± 0.001 c	0.035 ± 0.003 d	0.065 ± 0.005 a
C8:0	0.012 ± 0.001 c	0.021 ± 0.001 a	0.021 ± 0.001 a	0.012 ± 0.001 c	0.012 ± 0.001 c	0.019 ± 0.001 b
C10:0	0.021 ± 0.001 a	0.020 ± 0.001 a	0.021 ± 0.001 a	0.015 ± 0.001 b	0.012 ± 0.001 c	0.017 ± 0.001 b
C11:0	0.101 ± 0.001 c	0.126 ± 0.004 a	0.13 ± 0.01 a	0.098 ± 0.001 c	0.102 ± 0.002 c	0.118 ± 0.003 b
C12:0	0.031 ± 0.001 b	0.031 ± 0.001 b	0.033 ± 0.001 ab	0.023 ± 0.001 c	0.020 ± 0.001 c	0.035 ± 0.001 a
C14:0	0.329 ± 0.003 c	0.40 ± 0.02 a	0.433 ± 0.001 a	0.298 ± 0.001 d	0.30 ± 0.02 d	0.382 ± 0.008 b
C15:0	0.114 ± 0.001 a	0.109 ± 0.001 b	0.117 ± 0.001 a	0.105 ± 0.001 b	0.096 ± 0.003 c	0.114 ± 0.001 a
C16:0	9.42 ± 0.03 d	9.99 ± 0.03 c	10.60 ± 0.03 a	8.97 ± 0.03 f	9.08 ± 0.01 e	10.08 ± 0.08 b
C16:1	1.92 ± 0.01 b	1.86 ± 0.04 c	2.07 ± 0.01 a	1.87 ± 0.01 c	1.77 ± 0.01 d	2.07 ± 0.01 a
C17:0	0.130 ± 0.004 b	0.123 ± 0.004 c	0.176 ± 0.005 a	0.132 ± 0.006 b	0.114 ± 0.001 d	0.170 ± 0.002 a
C18:0	0.711 ± 0.001 c	0.89 ± 0.05 a	0.819 ± 0.003 b	0.617 ± 0.006 d	0.70 ± 0.04 c	0.83 ± 0.03 b
C18:1n9c	0.691 ± 0.009 b	0.80 ± 0.05 a	0.577 ± 0.004 c	0.459 ± 0.001 e	0.47 ± 0.04 e	0.50 ± 0.05 d
C18:2n6c	16.35 ± 0.01 b	16.89 ± 0.01 a	16.53 ± 0.01 c	15.08 ± 0.01 e	14.9 ± 0.2 f	15.6 ± 0.1 d
C18:3n6	0.060 ± 0.001 c	0.086 ± 0.001 a	0.077 ± 0.005 b	0.058 ± 0.001 c	0.054 ± 0.001 d	0.051 ± 0.004 d
C18:3n3	68.14 ± 0.02 c	66.59 ± 0.04 e	66.46 ± 0.04 e	70.10 ± 0.08 b	70.6 ± 0.3 a	67.6 ± 0.4 d
C20:0	0.160 ± 0.006 d	0.171 ± 0.001 c	0.20 ± 0.02 a	0.149 ± 0.005 e	0.145 ± 0.002 e	0.189 ± 0.004 b
C20:1	0.038 ± 0.002 a	0.038 ± 0.001 a	0.030 ± 0.001 b	0.032 ± 0.001 b	0.029 ± 0.001 b	0.031 ± 0.001 b
C20:2	0.087 ± 0.002 a	0.069 ± 0.001 bc	0.067 ± 0.006 c	0.072 ± 0.003 b	0.056 ± 0.002 d	0.067 ± 0.00 c
C21:0	0.041 ± 0.001 c	0.047 ± 0.002 b	0.048 ± 0.001 a	0.042 ± 0.003 c	0.028 ± 0.002 e	0.034 ± 0.003 d
C20:3n3	0.206 ± 0.006 a	0.188 ± 0.005 bc	0.178 ± 0.006 c	0.196 ± 0.009 b	0.21 ± 0.02 a	0.22 ± 0.01 a
C22:0	0.286 ± 0.008 d	0.30 ± 0.01d	0.34 ± 0.02 c	0.57 ± 0.05 a	0.29 ± 0.0 d	0.413 ± 0.002 b
C22:1	0.033 ± 0.001 b	0.033 ± 0.001 b	0.037 ± 0.003 a	0.024 ± 0.001 c	0.027 ± 0.001 c	0.030 ± 0.001 b
C23:0	0.297 ± 0.004 c	0.29 ± 0.02 c	0.32 ± 0.02 b	0.308 ± 0.009 bc	0.33 ± 0.01 b	0.63 ± 0.06 a
C24:0	0.78 ± 0.02 b	0.89 ± 0.04 a	0.66 ± 0.03 d	0.73 ± 0.02 c	0.65 ± 0.02 d	0.69 ± 0.06 d
SFA	12.49 ± 0.03 d	13.46 ± 0.03 c	13.97 ± 0.03 a	12.12 ± 0.08 e	11.91 ± 0.02 f	13.8 ± 0.2 b
MUFA	2.68 ± 0.01 a	2.72 ± 0.01 a	2.71 ± 0.01 a	2.38 ± 0.01 c	2.30 ± 0.04 d	2.63 ± 0.03 b
PUFA	84.83 ± 0.03 c	83.82 ± 0.04 d	83.31 ± 0.04 e	85.50 ± 0.09 b	85.79 ± 0.01 a	83.6 ± 0.3 d
PUFA/SFA	6.79 ± 0.03 c	6.23 ± 0.04 d	6.00 ± 0.03 e	7.05 ± 0.08 b	7.20 ± 0.03 a	6.06 ± 0.03 e
n6/n3	0.240 ± 0.002 c	0.254 ± 0.005 a	0.249 ± 0.003 b	0.215 ± 0.007 e	0.211 ± 0.003 e	0.231 ± 0.008 d

Caproic acid (C6:0); caprylic acid (C8:0); capric acid (C10:0); undecylic acid (C11:0); lauric acid (C12:0); myristic acid (C14:0); pentadecylic acid (C15:0); palmitic acid (C16:0); palmitoleic acid (C16:1); margaric acid (C17:0); stearic acid (C18:0); oleic acid (C18:1*n*9c); linoleic acid (C18:2*n*6c); γ-linolenic acid (C18:3n6); α-linolenic acid (C18:3*n*3); arachidic acid (C20:0); paulinic acid (C20:1); dihomo-linoleic acid (C20:2); heneicosylic acid (C21:0); eicosatrienoic acid (C20:3n3); behenic acid (C22:0); erucic acid (C22:1); tricosylic acid (C23:0); lignoceric acid (C24:0); SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids; *n*6/*n*3: omega-6/omega-3 fatty acids.
In each row, different lowercase letters indicate significant differences between samples according to Tukey's test ($P = 0.05$).

that other factors may be involved in modulating the biosynthesis of phenols and that a source of stress or a toxic effect such as that likely caused when using ureic nitrogen may not determine an increase in phenolic compounds.

Effect of the NO_3-N:NH_4-N on cytotoxic and antitumor activity

The N composition of the nutrient solution had no effect on the cytotoxicity of *C. spinosum* leaves (data not shown). Expressed as the sample concentration responsible for 50% inhibition of growth (GI_{50} μg mL^{-1}) in primary culture of selected non-tumor (PLP2, HeLa) and tumor (HepG2, MCF-7, NCI-H460) cell lines, the *C. spinosum* leaf cytotoxicity was more than 400 μg mL^{-1}, regardless of the composition of the nutrient solution.

PCA

The results of the PCA are presented in Fig. 1 and allow a clear distinction of the effects of nitrogen form on yield, DM and the nutritional profile of *C. spinosum* leaves. The first two principal components (PCs) accounted for 67.6% of the total variance, attributing 44.6% to PC1 and 23.1% to PC2. Most of the nutritional variables examined were positively correlated with PC1, and only a few parameters were negatively correlated with PC1. The variables with the highest positive correlation coefficient were phenolic compounds, including kaempferol-3-*O*-(6′-*O*-acetyl)-glucoside (0.97), quercetin-3-*O*-glucuronide (0.97), kaempferol-3-*O*-glucuronide (0.92), kaempferol-*O*-glucuronide (0.90), isorhamnetin-3-*O*-glucuronide (0.90), apigenin-7-*O*-glucuronide (0.90), quercetin-7-*O*-(6′-*O*-acetyl)-glucoside (0.84) and isorhamnetin-3-*O*-(6″-*O*-acetyl)-glucoside (0.75) among the flavonoids, as well as *cis* chicoric acid (0.95) and caftaric acid (0.87) among the phenolic acids. A high positive correlation with PC1 was observed also for linoleic acid (C18:2*n*6c, 0.83) and for the total organic acids (0.69). The PC1 was negatively correlated with *C. spinosum* leaf DM (−0.74) and linolenic acid (C18:3*n*3) (−0.69) content. The PC1 allowed a clear separation of plants grown with a higher proportion of NO_3-N (100:0; 75:25) or with a 50:50 NO_3-N:NH_4-N ratio from those grown with prevalent NH_4-N (0:100; 25:75) or with 100% ureic nitrogen placed, respectively, on the right and

Table 6. Retention time (Rt), wavelengths of maximum absorption in the visible region (λ_{max}), mass spectral data, and tentative identification and quantification of phenolic compounds (g kg^{-1}) of *Cichorium spinosum* leaves in relation to nutrient solution (NS) composition

Compounds	Rt (min)	λ_{max} (nm)	Molecular ion $[M\text{-}H]^-$ (*m/z*)	MS^2 (*m/z*)	Tentative identification	NO_3^-:NH_4^+ 100:0	75:25	50:50	25:75	0:100	Urea
1	4.23	328	311	179 (85), 149 (54), 135 (100)	Caftaric acid[1]	0.70 ± 0.01 d	1.198 ± 0.004 a	1.02 ± 0.01 b	0.211 ± 0.001 e	0.856 ± 0.005 c	0.87 ± 0.04 c
2	6.37	328	353	191 (100), 179 (71), 135 (43)	5-*O*-Caffeoylquinic acid[2]	0.72 ± 0.01 e	2.10 ± 0.04 b	2.19 ± 0.06 a	1.74 ± 0.02 c	1.71 ± 0.04 c	0.83 ± 0.03 d
3	11.78	330	473	313 (68), 293 (83), 219 (13), 179 (93), 149 (100), 135 (42)	*cis* Chicoric acid[1]	1.09 ± 0.03 c	1.99 ± 0.02 a	1.22 ± 0.03 b	0.30 ± 0.01 f	0.798 ± 0.008 d	0.58 ± 0.02 e
4	12.45	330	473	313 (68), 293 (83), 219 (13), 179 (93), 149 (100), 135 (42)	*trans* Chicoric acid[1]	0.81 ± 0.02 cd	0.91 ± 0.01 c	1.50 ± 0.09 a	0.334 ± 0.001 e	1.15 ± 0.04 b	0.73 ± 0.02 d
5	18.19	358	477	301 (100)	Quercetin-3-*O*-glucuronide[3]	1.095 ± 0.003 b	1.35 ± 0.01a	1.033 ± 0.007 c	0.726 ± 0.007 e	1.00 ± 0.05c	0.90 ± 0.01 d
6	18.67	350	461	285 (100)	Kaempferol-*O*-glucuronide[3]	1.16 ± 0.01 b	1.38 ± 0.01 a	1.062 ± 0.007 c	0.854 ± 0.005 e	1.18 ± 0.02 b	0.982 ± 0.003 d
7	20.27	356	505	463 (10), 301 (100)	Quercetin-7-*O*-(6″-*O*-acetyl)-glucoside[3]	0.543 ± 0.001 d	0.632 ± 0.004 a	0.569 ± 0.003 c	0.508 ± 0.001 e	0.584 ± 0.001 b	0.542 ± 0.002 d
8	21.14	348	593	285 (100	Kaempferol-3-*O*-rutinoside[3]	0.506 ± 0.001 e	0.518 ± 0.001 c	0.546 ± 0.001 a	0.514 ± 0.001 d	0.521 ± 0.001 b	0.501 ± 0.001 f
9	22.03	348	461	285 (100	Kaempferol-3-*O*-glucuronide[3]	1.059 ± 0.003 b	1.079 ± 0.004 a	0.935 ± 0.005 d	0.714 ± 0.001 f	0.977 ± 0.001 c	0.812 ± 0.005 e
10	23.1	336	445	269 (100)	Apigenin-7-*O*-glucuronide[4]	0.755 ± 0.007 c	0.96 ± 0.02 a	0.82 ± 0.02 b	0.658 ± 0.007 d	0.80 ± 0.02 b	0.71 ± 0.02 c
11	23.51	358	491	315 (100)	Isorhamnetin-3-*O*-glucuronide[3]	0.785 ± 0.006 b	0.797 ± 0.001 a	0.686 ± 0.002 d	0.573 ± 0.002 f	0.734 ± 0.001 c	0.646 ± 0.006 e
12	24.71	338	489	285 (100)	Kaempferol-3-*O*-(6″-*O*-acetyl)-glucoside[3]	0.552 ± 0.006 bc	0.569 ± 0.002 a	0.555 ± 0.001 b	0.492 ± 0.002 e	0.546 ± 0.004 c	0.526 ± 0.001 d
13	25.95	358	519	315 (100)	Isorhamnetin-3-*O*-(6″-*O*-acetyl)-glucoside[3]	0.501 ± 0.001 b	0.500 ± 0.001 b	0.496 ± 0.001 c	0.466 ± 0.001 e	0.510 ± 0.002 a	0.479 ± 0.001 d
					Total phenolic acids	**3.324 ± 0.005 d**	**6.20 ± 0.05 a**	**5.94 ± 0.08 b**	**2.58 ± 0.03 f**	**4.51 ± 0.09 c**	**3.01 ± 0.03 e**
					Total flavonoids	**6.958 ± 0.002 b**	**7.78 ± 0.03 a**	**6.70 ± 0.03 d**	**5.503 ± 0.0 02 f**	**6.857 ± 0.00 1 c**	**6.10 ± 0.02 e**
					Total phenolic compounds	**10.282 ± 0.006 d**	**13.98 ± 0.02 a**	**12.6 ± 0.1 b**	**8.08 ± 0.03 f**	**11.37 ± 0.09 c**	**9.11 ± 0.02 e**

Calibration curves used: 1-caffeic acid ($y = 388\,345x + 406\,369$; $r^2 = 0.999$); 2-chlorogenic acid ($y = 168\,823x - 161\,172$; $r^2 = 0.999$); 3-quercetin-3-*O*-glucoside ($y = 34\,843x - 160\,173$; $r^2 = 0.999$); 4-apigenine-7-*O*-glucoside ($y = 10\,683x - 45\,794$; $r^2 = 0.997$).
In each row, different lowercase letters indicate significant differences between samples according to Tukey's test ($P = 0.05$).

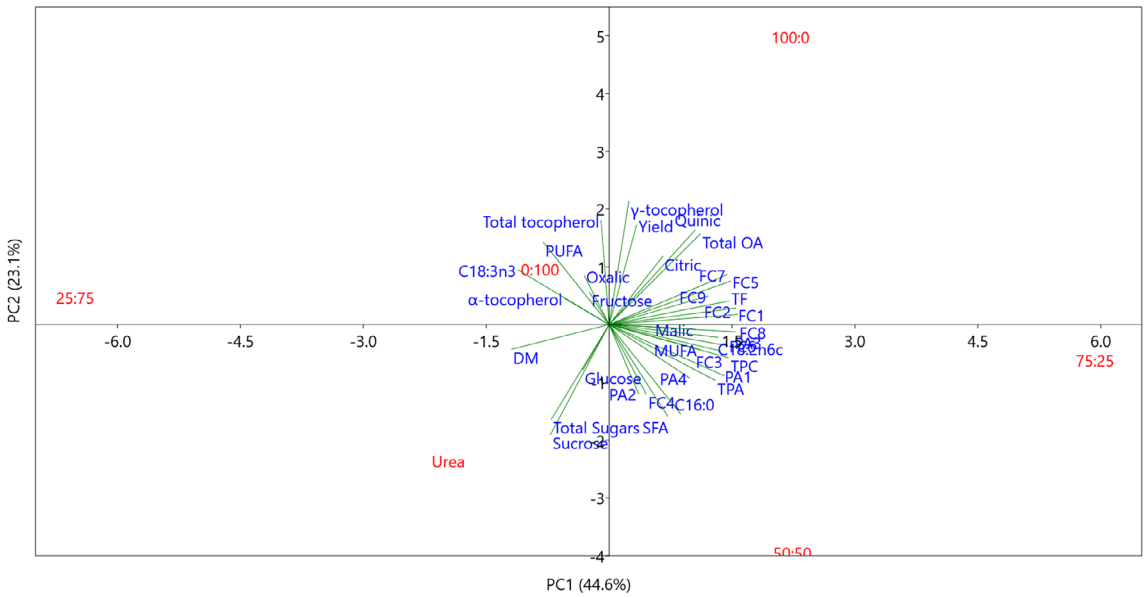

Figure 1. Principal component analysis biplot (PC1 *versus* PC2) showing the spatial distribution of the nutritional parameters, yield and dry matter content (DM) of leaves of *C. spinosum* fertigated with nutrient solutions characterized by different NO_3-N:NH_4-N ratios (100:0, 75:25, 50:50, 25:75, 0:100) or by urea as the sole source on nitrogen. Parameters considered include: yield and DM previously reported by Kolovou *et al.*[24]; oxalic, quinic, malic, citric acid and total organic acids (Total OA) fructose, glucose, sucrose and total sugars, α-tocopherol, γ-tocopherol, total tocopherol caftaric acid (PA1), 5-*O*-caffeoylquinic acid (PA2), *cis* chicoric acid (PA3), *trans* chicoric acid (PA4), quercetin-3-*O*-glucuronide (FC1), kaempferol-*O*-glucuronide (FC2), quercetin-7-*O*-(6′-*O*-acetyl)-glucoside (FC3), kaempferol-3-*O*-rutinoside (FC4), kaempferol-3-*O*-glucuronide (FC5), apigenin-7-*O*-glucuronide (FC6), isorhamnetin-3-*O*-glucuronide (FC7), kaempferol-3-*O*-(6′-*O*-acetyl)-glucoside (FC8), isorhamnetin-3-*O*-(6″-*O*-acetyl)-glucoside (FC9), total phenolic acids (TPA), total flavonoids (TF) and total phenolic compounds (TPC) palmitic acid (C16:0), linoleic acid (C18:2*n*6c) linolenic acid (C18:3*n*), saturated fatty acids (SFA), monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA).

left side of PC1, suggesting that the former, and especially those grown with a 75:25 NO_3-N:NH_4-N ratio, were characterized by a higher TPC and linoleic acid (C18:2*n*6c) (Tables 5 and 6). The latter were characterized by a relatively lower TPC (Table 6), lower oxalic acid (Table 3) and higher linolenic acid (C18:3n3) content, especially in the case of plants grown with a 0:100 NO_3-N:NH_4-N ratio (Table 5), and a higher DM content, especially in the case of plants grown with a 25:75 NO_3-N:NH_4-N ratio. The PC2 was positively correlated with γ-tocopherol (0.95), total tocopherols (0.81), yield (0.77), quinic acid (0.73) and total organic acids (0.70), as well as negatively correlated with the content of total sugars (−0.73) and especially sucrose (−0.85), and also with SFA (−0.71). The PC2 clearly separated plants grown exclusively with NO_3-N characterized by a higher yield, total organic acids (Table 3) and total tocopherols content (Table 4) from those grown with a 50:50 NO_3-N:NH_4-N ratio and with urea characterized by a higher sucrose (Table 2) and SFA content (Table 5).

CONCLUSIONS

Analyzing the variation of the nutritional profile of *C. spinosum* in response to fertigation testing the full range of NO_3-N:NH_4-N (100:0, 75:25, 50:50, 25:75, 0:100) and 100% ureic-N, the present study revealed that the form of N provided via fertigation has a considerable impact on the content of both nutritional and anti-nutritional compounds. Tocopherols (α- and δ-tocopherol) and organic acids (quinic, malic, citric and oxalic acid) content were maximized in plants grown exclusively with NO_3-N (100:0). Fertigation with a 75:25 NO_3-N:NH_4-N ratio allowed maximization of the content of phenolic compounds, including both flavonoids and phenolic acids, as well as the concentration of linoleic acid (C18:2n6c), although the main phenolic compound content (5-*O*-caffeoylquinic and chicoric acid) was the highest for the solution with a balanced ratio of NO_3^- and NH_4^+ nitrogen (50:50 NO_3^-:NH_4-N). On the other hand, the exclusive use of NH_4-N (0:100) ensured the highest content of linolenic acid (C18:3*n*3) and polyunsaturated fatty acids and, at the same time, the lowest content of oxalic acid, which is considered to be an antinutrient. The exclusive use of ureic-N was toxic for plants of *C. spinosum* and did not enhance its nutritional profile. When examining the impact of the form of N on the cytotoxicity effect of *C. spinosum* leaf extracts against non-tumor (PLP2, HeLa) and tumor (HepG2, MCF-7, NCI-H460) cell lines, no significant effects were observed for all of the N treatments tested. It is concluded that, by modulating the NO_3-N:NH_4-N ratio of the nutrient solution supplied to *C. spinosum*, it is possible to enhance the content of desirable health-promoting compounds and reduce the content of antinutrients, thus increasing the overall quality of the final product.

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CONFLICT OF INTERESTS

The authors declare that they have no conflicts of interest.

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