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Nutrient solution composition and growing season affect yield and chemical composition of *Cichorium spinosum* plants

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ABSTRACT

Nitrogen fertilizer form may affect quality and yield of leafy vegetables. In the present study, the effect of ammonium nitrogen rates on yield and chemical composition of *Cichorium spinosum* L. was examined. Five fertilizer treatments with different amounts of ammonium nitrogen (F1: 14%, F2: 24%, F3: 34%, F4: 43% and F5: 53% NH_4-0N of total N) were applied. Fertilizer treatments had a significant effect on both plant fresh weight and chemical composition, depending on growing period and harvest stage. For both harvests of the 1st growing period, yield was higher in treatments F4 (43% NH_4-N) and F5 (53% NH_4-N), whereas in the 2nd growing period yield was higher for treatments F1, F2 and F3. Moreover, the highest content of total phenolics were recorded in the 2nd growing period. Antioxidant properties were also affected by fertilization treatments and growing periods, with antioxidant potency being higher in the 2nd growing period and for treatments F1 and F2. According to the results of the present study, nitrogen fertilizer form should be considered together with growing period and harvest stage as a useful means towards increasing the quality of the final product without compromising total yield.

1. Introduction

Cichorium spinosum L. is a perennial species that forms a spiny shrub, more or less erect, depending on the ecotype. It is a basic ingredient of Mediterranean diet with people from rural communities usually hand picking the rosettes and use them in many traditional dishes (Melliou et al., 2003; Petropoulos et al., 2016). During the first year of growth, only one rosette of leaves is formed on each plant, while at the end of the first growth cycle a spiny flowering stem appears. The following years, more auxiliary buds are developed and many rosettes of leaves are formed.

The use of fertilizers has rapidly increased yield and farmers' income during the last decades; however the irrational use of nitrogen fertilizers has many negative implications for the environment and consumers' health, since excessive rates of nitrogen fertilizers can increase the risk of gastrointestinal cancer and methemoglobinemia (Hord et al., 2009). Moreover, nitrogen form may also affect the quality of the final product, since it is involved in the biosynthesis of various phytonutrients such as organic and fatty acids (Fontana et al., 2006; Szalai et al., 2010). Conesa et al. (2009) have also reported that nitrate/ammonium ratio in nutrient solution is essential for the yield and quality of baby leaf spinach and bladder campion plants, especially regarding oxalate content, which are considered anti-nutritional factors due to their association with kidney stone formation and deficiencies in calcium, copper, iron and magnesium (Zhang et al., 2005). According to Liu et al. (2015), the increase of ammonium nitrogen in nutrient solution did not increase oxalate content in spinach leaves, whereas oxalate accumulation is highly associated with nitrate nitrogen uptake. Furthermore, Zhang et al. (2005) suggested that a nitrate: ammonium nitrogen ratio of 0.5 can result in the lowest total oxalate in spinach leaves without compromising yield. Palaniswamy et al. (2004) have also reported that nitrate: ammonium nitrogen ratios may also affect the omega-3 and total fatty acids content in purslane leaves, especially α-linolenic acid which was higher at ratios of 0.5:0.5 of nitrate: ammonium nitrogen.

Apart from nutrient solution, growing period may also affect chemical composition and quality of leafy vegetables. According to Fallovo et al. (2009), the increase in nutrient solution concentration resulted in

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an increase of biomass production and nitrate and chlorophyll content in lettuce plants, while growing plants during spring decreased yield and growth but increased quality in terms of carbohydrates and nitrates content, comparing to the summer growing period. Moreover, Bonasia et al. (2017) have reported seasonal differences in visual quality and nutritional profile of wild rocket, with colder periods (autumn-winter) resulting in better visual quality, whereas warmer periods (winter-spring) were beneficial to nutritional profile in terms of nitrates, total phenols, ascorbic acid and glucosinolates content. According to Becker et al. (2014), light intensity and growth stage may affect phenolic compounds composition, such as cyanidin and quercetin glucosides in lettuce plants grown under shading nets, while Becker et al. (2013) reported that the reduction of flavonoid glycosides content under low light intensities is fully compensated when plants are subsequently subjected to high photosynthetic photon flux density. Moreover, other phenolic compounds such as caffeic acid derivatives were not affected by shading conditions (Becker et al., 2014), which indicates that apart from environmental factors, ontogeny is also important for chemical composition of leafy vegetables. Fu et al. (2017) and Stagnari et al. (2015) have also demonstrated the significance of light intensity on quality of leafy vegetables, especially regarding nitrates and vitamin C content which are crucial quality features for lettuce, while Petropoulos et al. (2017a) have highlighted the effect of growing period on *C. spinosum* nutritional value and chemical composition.

Although chemical composition and nutritional profile of *C. spinosum* has been already described (Petropoulos et al., 2016; Zeghichi et al., 2003), scarce literature regarding the effect of cultivation practices on these parameters is available so far. Therefore, the aim of the present study was to evaluate the effect of ammonium nitrogen rates on plant yield, and chemical composition and nutritional profile of *C. spinosum* leaves, especially on quality features such as phenolic compounds content and fatty acids composition.

2. Materials and methods

2.1. Experimental design, plant material and growing conditions

Plants were grown in an unheated plastic greenhouse at the experimental farm of the University of Thessaly in Velestino, Greece (Latitude: 39° 38′ 86″ N; Longitude: 22° 94′ 14″ E). More specifically, seeds collected from Crete island (Greece) were sown in seed trays on September 2nd 2015 and December 15th 2015 (growing period 1 and 2, respectively) containing peat by Vianame S.A. (Timpaki, Greece) (Anesti et al., 2016). Seedlings of *Cichorium spinosum* L. (Vianame S.A.; Timpaki, Greece) were transplanted at the stage of 3 true leaves in 2 L pots containing peat (Klassman-Deilmann KTS2, 1.0 L) and perlite (1.0 L).

The experiment was set up as a factorial with three factors [1st Factor: growing period with two levels (G1: transplanting on December 5th, 2015, G2: transplanting February 15th, 2016); 2nd factor: number of harvests with three levels (H1: 1st harvest, H2: 2nd harvest, H3: one harvest at the same day of 2nd harvest with no previous cutting); 3rd Factor: fertilization treatments (F1: 14% NH_4-N, F2: 24% NH_4-N, F3: 34% NH_4-N, F4: 43% NH_4-N, F5: 53% NH_4-N of total nitrogen)], and laid out in a Completely Randomized design with 10 pots per treatment (n = 10).

Regarding the fertilization treatments, plants were fertilized with the same amount of nitrogen, phosphorus and potassium through the nutrient solution, twice a week at the start of the experiment (50 mL per pot) and after transplantation of seedlings and thrice a week at later growth stages and when temperatures increased (300 mL per pot). Nutrient solution for each fertilization treatment (Table 1) was composed to simulate commercial growing conditions in terms of total nitrogen amount, while pH was adjusted at 6.5 for all treatments. Therefore only commercial fertilizers were used as previously described by Anesti et al. (2016), namely: a) 20-20-20 (N-P-K) with nitrogen consisting of urea (10%), NO_3-N (5.6%) and NH_4-N (4.4%), b) ammonium nitrate (34.5% total nitrogen, with a ratio of 1:1 for NO_3-N: NH_4-N), c) calcium nitrate (15.5% nitrogen, [NO_3-N (14.4%) and NH_4-N (1.1%)], and 26.5% CaO), d) urea (46% nitrogen in urea form), e) ammonium sulphate (21% of nitrogen in NH_4-N form, and 24% sulfur. Concerning the harvest treatments, for the 1st growing period and H1 and H2 treatments, the harvest was carried out at 82 (25/02/2016) and 118 days (01/04/2016) after transplantation (DAT), respectively, while for H3 treatment, harvest took place at the same day as the second harvest (118 DAT; 01/04/2016). For growing period 2, only one harvest (H1 treatment) took place at 78 DAT (03/05/2016), since climate conditions induced early flowering. After harvest fresh and dry weight of leaves was recorded, while samples of raw leaves were stored at −80 °C and lyophilized for further analyses. Climate conditions during the experimental period are presented in Fig. 1. Data for temperatures inside the greenhouse were obtained from Onset HOBO RH/Temp data logger (Onset Computer Corporation, MA, USA), while solar radiation data were obtained from the meteorological station of the University of Thessaly, located near the experimental site.

Table 1
Nutrient solution composition expressed in % of nitrogen.

Elements	Treatments				
	1	2	3	4	5
Total N (ppm)	299.95	300.13	300.40	300.01	299.97
NO3-N	36.3	46.3	49.5	43.7	37.0
NH4-N	13.7	23.7	33.7	43.0	53.0
Urea	50.0	30.0	16.8	13.3	10.0

2.2. Standards and reagents

Acetonitrile (99.9%) was of HPLC grade (Fisher Scientific, Portugal). Formic acid was purchased from Panreac Química S.L.U. (Barcelona, Spain). L-ascorbic acid, fatty acid methyl esters reference standard (standard 47885-U), fatty acids isomers, organic acids, sugars and tocopherol standards were purchased by Sigma-Aldrich Quimica S.L. (Madrid, Spain). Phenolic standards were from Extrasynthèse (Genay, France). Water was treated in a Milli-Q water purification system (TGI Pure Water Systems, USA).

2.3. Chemical composition analyses

Free sugars and tocopherols were determined following procedures previously described by Guimarães et al. (2013). Free sugars analysis was performed by high performance liquid chromatography with a refraction index detector (HPLC-RI; Knauer, Smartline system 1000, Germany). Chromatographic separation was achieved using a Eurospher 100-5 NH2 column (4.6 × 250 mm, 5 mm, Knauer), operating at 35 °C (7971 R Grace oven). Elution was performed with acetonitrile/water, 70:30 (v/v) at a flow rate of 1 mL/min and controlled by Clarity 2.4 Software (DataApex, Czech Republic). Sugars were identified by comparing their retention times with standard compounds and quantification was conducted by comparison with dose–response curves constructed from authentic standards, using the internal standard (IS, melezitose) method.

Chromatographic separation of tocopherols was achieved using an HPLC equipment, with a fluorescence detector (FP-2020; Jasco, USA), programmed for excitation at 290 nm and emission at 330 nm. The compounds were identified by chromatographic comparisons with authentic standards. Tocopherols were identified by comparing their retention times with standard compounds and quantification was conducted by comparison with dose–response curves constructed from authentic standards, using the IS (tocol) method.

Organic acids were determined following a procedure previously