Food Chemistry 237 (2017) 83–90

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Successive harvesting affects yield, chemical composition and antioxidant activity of *Cichorium spinosum* L.

Spyridon Petropoulos [a,*], Ângela Fernandes [b], Anestis Karkanis [c], Georgia Ntatsi [d], Lillian Barros [b], Isabel C.F.R. Ferreira [b,*]

[a] Laboratory of Vegetable Production, University of Thessaly, Fytokou Street, 38446 N. Ionia, Magnissia, Greece
[b] Centrode Investigação de Montanha (CIMO), ESA, Instituto Politécnico de Bragança, Campus de Santa Apolónia, 5300-253 Bragança, Portugal
[c] Laboratory of Weed Science, University of Thessaly, Fytokou Street, 38446 N. Ionia, Magnissia, Greece
[d] Laboratory of Vegetable Production, Agricultural University of Athens, Iera Odos 75, 11855 Athens, Greece

ARTICLE INFO

Article history:
Received 29 March 2017
Received in revised form 16 May 2017
Accepted 17 May 2017
Available online 18 May 2017

Keywords:
Cichorium spinosum L.
Harvesting time
Nitrates
Organic acids
Phenolic compounds
Successive harvests
Sugars
Tocopherols

ABSTRACT

In the present study, the effect of successive harvesting on yield, chemical composition and antioxidant activity of *Cichorium spinosum* plants was examined. *C. spinosum* plants were grown from seeds sown in seed trays containing peat and young seedlings were transplanted in 2L pots containing peat and perlite (1:1 v/v). Plants were harvested two or three times during two consecutive growing periods. Total fresh weight and number of leaves were higher for successive harvests in both growing periods comparing to a single harvest. The application of more than two harvests resulted in quality loss during the 1st growing period, while in the 2nd growing period the overall chemical composition, antioxidant properties and phenolic compounds content was higher than the 1st period. In conclusion, cultivation practices such as sowing date and successive harvesting may be useful tools towards the production of high quality end-product with increased bioactive properties without compromising total yield.

1. Introduction

Cichorium spinosum L. is a perennial plant, native in the Mediterranean basin, which is usually consumed for its edible leaves, either as a cooked vegetable or pickled for the periods when no fresh leaves are available in the market. In recent years, the ever-growing market needs and the high product prices have create a prosperous niche and many farmers have started up commercial farms for raw and/or processed products. In natural conditions, harvest of leaves is made by hand-picking and is limited to one or two harvests from each plant within the growing period of the species (winter to early spring). However, when plants are cultivated under commercial growing systems multiple harvests are usually applied (9–10 harvests after the first year of establishment or for older plants), thus allowing for higher total yields comparing to wild plants.

According to Csizinszky (1999) and Kmiecik and Lisiewska (1999) multiple harvests in leafy vegetables may result in significantly higher total yield comparing to a single harvest regime, as soon as the apical meristem remains intact after each harvest. However, growing conditions are crucial for the application of multiple harvests in leafy vegetables, since depending on the species and cultivar, improper day length (short day or long day plants) and/or temperatures (high or low) may induce transition from vegetative to flowering stage and therefore quality reduction of the edible parts is critical (Ventura et al., 2011).

Apart from growing conditions and genotype, secondary metabolites content in plants is depended on growth stage, which in the case of leafy vegetables could affect quality of the marketable product. According to Omezzine, Bouaziz, Simmonds, and Haouala (2014), total phenolics, flavonoids, flavones and flavonols content in aerial parts of fenugreek (*Trigonella foenum-graecum* L.) was higher at vegetative stage comparing to flowering and fruiting stage. Moreover, Pokkaew et al. (2013) have reported significant variation during the vegetative stage of *Arachis hypogaea* L. plants, with total phenolics, epicatechin and caffeic acids content of leaves being higher at the second harvest of plants grown in soil and soilless cultivation systems and harvested every 10 days. In a previous

* Corresponding authors at: University of Thessaly, School of Agricultural Sciences, Fytokou Street, 38446 N. Ionia, Magnissia, Greece (S. Petropoulos). Instituto Politécnico de Bragança, Campus de Santa Apolónia, 1172, 5300-253 Bragança, Portugal (I.C.F.R. Ferreira).

E-mail addresses: fangio57gr@gmail.com (S. Petropoulos), iferreira@ipb.pt (I.C.F.R. Ferreira).

http://dx.doi.org/10.1016/j.foodchem.2017.05.092
0308-8146/

study, Zeghichi, Kallithraka, and Simopoulos (2003) evaluated chemical composition and antioxidant activity of cultivated *C. spinosum* leaves at four different growth stages and concluded that harvest of leaves 40–50 days after planting is the optimal stage for higher content in minerals, antioxidants and alpha-linolenic acid.

Harvest stage may also affect sensory quality of vegetables and could be essential for the marketability and consumer acceptance of the final product and its retail price. There are many studies regarding the effect of maturity stage on vegetable fruit sensorial properties and chemical composition (Gajewski & Arasimowicz, 2004; Pinto, Almeida, Aguiar, & Ferreira, 2014), as well as the effect of harvest stage and growing season (Moura, Carlos, De Oliveira, Martins, & Da Silva, 2016), and growing systems on leafy vegetables quality (Petropoulos, Chatzieustratiou, Constantopoulou, & Kapotis, 2016). Another important issue regarding the quality of leafy vegetables is nitrate content of edible parts, since species such as spinach and lettuce are considered nitrate accumulators and may contain significant amounts of nitrates under specific conditions (high nitrogen application rates, growing during winter, harvest time during the day and growth stage) (Petropoulos, Constantopoulou, Karapanos, Akoumianakis, & Passam, 2011; Petropoulos, Olympios, & Passam, 2008; Petropoulos et al., 2016).

Chemical composition and nutritional value of the *C. spinosum* is already described (Petropoulos et al., 2017; Zeghichi et al., 2003) and a recent study by the authors (Petropoulos, Ntatsi, Levizou, Barros, & Ferreira, 2016) showed significant differences between various ecotypes and growing systems, regarding the chemical composition and nutrition value of the edible parts of the plant. To the best of our knowledge, so far there is no available literature regarding the chemical composition of leaves when multiple harvests are applied, which is the common practice for commercial cultivation. Therefore, the aim of the present study was the evaluation of chemical composition and bioactive compounds content of aerial parts of *C. spinosum* when successive harvests during the growing period are applied, in comparison with plants where no previous harvest have taken place. The results from this study could be important for the identification of the optimum harvest stage and number of harvests in order to obtain the highest quality without compromising total yield.

2. Materials and methods

2.1. Plant material and growing conditions

Seedlings of *Cichorium spinosum* L. (Asteraceae) were obtained from Vianame S.A. (Timpaki, Greece). Plants were grown from seeds as previously described by Anesti et al. (2016). More specifically, seeds were sown in seed trays on September 2nd 2015 and December 15th 2015 (growing period 1 and 2, respectively) containing peat. Young seedlings were transplanted when they reached the stage of 3–4 true leaves on December 1st, 2015 and March 3rd, 2016 [90 and 81 days after sowing (DAS) for growing period 1 and 2, respectively] in 2 L pots containing peat (Klassman-Deilmann KTS2, 1.0 L) and perlite (1.0 L) (Anesti et al., 2016). Plants were fertilized throughout the experiment with nutrient solution containing the same amount of nitrogen (300 mg L^{-1}) with amounts of 50 mL per pot and up to 300 mL per pot at the end of the growth cycle.

Harvest was carried out three and two times during growing period 1 and 2 (133, 175 and 195, and 124 and 147 DAS, for growing period 1 and 2 respectively). Each time, harvest took place when plants reached the marketable size in order to examine the effect of successive harvesting on total yield and chemical composition of the aerial parts. Especially for growing period 2, the last harvest took place at flower initiation and when flowering stem elongation took place. Therefore, only two harvests were carried out since due to climate conditions (high temperatures and large day-length) the transition from vegetative growth to flowering was very rapid and did not allow for more harvests. On each day of harvest, fresh and dry weight of leaves was measured. Harvest took place between 10:30 and 12:30 on each harvest day, in order to avoid fluctuations in nitrate content in leaves due to diurnal variation (Petropoulos et al., 2011).

For dry weight evaluation, samples of fresh leaves were oven dried at 72 °C to a constant weight (approximately for 48 h) (Anesti et al., 2016).

2.2. Chemical composition analyses

For chemical composition, raw samples of leaves were stored at deep freezing conditions (−80 °C) and freeze-dried prior to analysis. Free sugars analysis was performed by high performance liquid chromatography with a refraction index detector (HPLC-RI; Knauer, Smartline system 1000, Berlin, Germany), using a Eurospher 100-5 NH2 column (4.6 × 250 mm, 5 μm, Knauer), operating at 35 °C (7971 R Grace oven) for chromatographic separation (Barros et al., 2013). The isocratic elution was performed with acetonitrile/water (70:30, *v/v*), at a flow rate of 1 mL/min and controlled by a Clarity 2.4 Software (DataApex, Podohradska, Czech Republic). Identification and quantification of the sugars were performed respectively, by comparing their retention times with standard compounds and by comparison with dose–response curves constructed from authentic standards, using the internal standard (IS, melezitose) method.

Organic acids analysis was performed using a Shimadzu 20A series UFLC (Shimadzu Coperation, Kyoto, Japan), using a Sphere-Clone reverse phase C_{18} column (4.6 × 250 mm, 5 μm, Phenomenex, Torrance, CA, USA), operating at 35 °C (Pereira, Barros, Carvalho, & Ferreira, 2013) for chromatographic separation and controlled by LabSolutions multi LC-DAD software (Shimadzu Coperation, Kyoto, Japan). The isocratic elution was performed with sulphuric acid 3.6 mM at a flow rate of 0.8 mL/min. Organic acids identification was performed using standard compounds, when available, by comparison with their retention times and UV–vis spectra. For quantitative analysis, a calibration curve was constructed for each available organic acid standard (Sigma-Aldrich, St. Louis, MO, USA), based on the UV signals.

Fatty acids were analyzed with a DANI 1000 gas chromatographer coupled to a flame ionization detector (GC-FID, Dani Instruments, Milan, Italy), after a transesterification procedure described by (Barros et al., 2013). Results were recorded and processed using Clarity 4.0.1.7 Software (DataApex, Podohradska, Czech Republic) and the fatty acids methyl esters (FAMEs) were identified by comparing their retention time with authentic standards.

Tocopherols analysis was achieved using the HPLC equipment described above for free sugars, with a fluorescence detector (FP-2020; Jasco, Easton, MD, USA), programmed for excitation at 290 nm and emission at 330 nm (Barros et al., 2013). The compounds were identified by chromatographic comparisons with authentic standards and quantified by comparison with dose–response curves using authentic standards, performed with an IS (tocol) methodology.

For mineral composition, samples of leaves tissues were dried in a forced-air oven at 72 °C to constant weight. Dried leaves were ground to powder and after dry ashing at 550 °C they extracted with 1 N HCl for mineral content determination (Anesti et al., 2016). Ca, Mg, Fe, Mn, Zn, and Cu content were determined by atomic absorption spectrophotometry (Perkin Elmer 1100B,

Waltham, MA, USA) and Na and K content by flame photometry (Sherwood Model 410, Cambridge, UK).

Nitrate content was assessed colorimetrically by the nitration of salicylic acid, following the procedure previously described by Cataldo, Maroon, Schrader, and Youngs (1975), using a Perkin Elmer Model Lambda 1A spectrophotometer (Perkin Elmer, Waltham, Mass.).

2.3. Antioxidant activity assays

The extract was obtained using a methanolic/water (80:20, *v/v*) mixture with one gram of lyophilized material. The extractions was performed twice during 1 h and by using a magnetic stirrer plate (25 °C at 150 rpm), with 30 mL of methanol/water (80:20, *v/v*), filtered through a Whatman No. 4 paper and vacuum-dried (rotary evaporator Büchi R-210, Flawil, Switzerland) at 40 °C to remove the methanol. The extracts were further frozen, lyophilized and then re-dissolved in methanol/water (80:20, *v/v*) mixture to obtain a stock solution of 20 mg/mL, which were further diluted to obtain a range of concentrations.

The antioxidant activity was evaluated using four different assays: DPPH radical-scavenging, reducing power, inhibition of beta-carotene bleaching and TBARS inhibition assays, as previously described (Petropoulos, Fernandes, Barros, Ferreira, & Ntatsi, 2015). The results were expressed in EC_{50} values (sample concentration providing 50% of antioxidant activity or 0.5 of absorbance in the reducing power assay) and trolox (6-hydroxy-2,5,7,8-tetramethyl chroman-2-carboxylic acid, Sigma-Aldrich, St. Louis, MO, USA) was used as a positive control.

2.4. Phenolic compounds analysis

The above-mentioned extracts were re-dissolved in methanol/water (80:20, *v/v*) at a final concentration of 30 mg/mL, filtered through a Whatman syringe filter (0.45 μm) and transferred to amber color HPLC vial for phenolic compound analysis. The phenolic profile was performed by LC-DAD-ESI/MSn (Dionex Ultimate 3000 UPLC, Thermo Scientific, San Jose, CA, USA), using a double online detection with a DAD (280, 330 and 370 nm as preferred wavelengths) and a mass spectrometer (Linear Ion Trap LTQ XL mass spectrometer, ThermoFinnigan, San Jose, CA, USA) equipped with an ESI source, as previously described by Bessada, Barreira, Barros, Ferreira, and Oliveira (2016). Phenolic compounds were identified by using standard compounds (comparison with their retention times, UV–vis and mass spectra), when available, and by comparing the obtained information with available data reported in the literature. A calibration curve for each available phenolic standard (Extrasynthèse, Genay, France) was constructed based on the UV signal and for compounds with no available commercial standards, the quantification was performed using calibration curves of the most similar available compound.

2.5. Statistical analyses

The experiment was laid out in a Completely Randomized Blocks design with 10 pots per treatment and n = 3. Statistical analysis was carried out with Statgraphics 5.1.plus (Statistical Graphics Corporation). Data were evaluated by analysis of variance for the main effects (H: number of harvests; G: growing period), whereas the means of values were compared by Tukey's HSD Test at $p < 0.05$. Chemical composition analyses were performed in three samples for treatment, while all analyses were carried out in triplicate. The results are presented as mean values and standard deviations (SD), while statistical analysis was performed with SPSS v. 22.0 program (IBM Corp., Armonk, NY, USA) by using one-way analysis of variance (ANOVA), followed by Tukey's HSD Test at $p < 0.05$.

3. Results and discussion

The effect of successive harvests and growing period on fresh and dry weight and number of leaves is presented in Table 1. Fresh weight of the aerial parts and the number of leaves of *C. spinosum* plants was higher when multiple harvests were applied, comparing to only a single harvest practice at early stages of growth cycle, while total yield was higher in the 1st growing period due to higher total number of harvested leaves (Table 1). The higher fresh weight is due to the higher number of leaves that plants form when multiple harvests take place during the cultivation period. The same results have been observed for other leafy vegetables that form rosettes and have the ability to regrow after harvest, allowing for higher total yields especially for vegetables (i.e. parsley) that are marketed as leaf bunches (Csizinszky, 1999; Kmiecik & Lisiewska, 1999). The only limitation for this harvesting practice is the meristem to remain intact by cutting leaves a few cm above ground. However, sowing date and consequently growing period should also be carefully selected according to climate conditions of growing regions, since according to the results of the present study high temperatures and/or long days could induce bolting. Moreover, earlier sowing could allow for larger growth cycle and consequently for more harvests that could further increase total leaf yield per plant.

Successive harvests and growing periods, both significantly affected the sugar composition of leaves (Table 2). The main detected sugars were glucose and sucrose, followed by trehalose and fructose, whereas total sugars content was higher in the 2nd than the 1st growing period (Table 2). Considering the bitter taste of *C. spinosum* leaves, high total sugars content may increase marketability of the final product, since sugar content is highly correlated with better taste (less bitter). In the present study, successive harvests resulted in a decrease of total and individual sugars content during the 1st growing period, where it seems that the application of more than two harvests has a detrimental effect on sugars content and consequently on leaves taste and consumer's acceptability (Table 2). However, the same trend was not observed during the 2nd growing period, where glucose, trehalose and total sugars content increased at the second harvest. The higher sugars content in the 2nd growing period of the present study could be attributed to higher light intensity and consequently to higher photosynthetic and biosynthetic rates comparing to the 1st one (Wojciechowska,

Table 1
Fresh and dry weight (expressed in g/plant and % of dry matter, respectively) and number of leaves of *Cichorium spinosum* plants (mean ± SD) for each harvest and growing period.

Harvest	Fresh weight (g plant^{-1})	Dry weight (%)	Number of leaves
1st growing period			
1st	19.2 ± 3.7b	8.7 ± 0.7a	21.8 ± 3.2b
2nd	29.7 ± 6.5a	9.0 ± 0.2a	38.8 ± 7.1a
3rd	11.4 ± 0.6c	8.4 ± 0.3a	18.9 ± 3.3b
Total	60.3	n/a	79.5
Harvest	Fresh weight (g plant^{-1})	Dry weight (%)	Number of leaves
2nd growing period			
1st	19.2 ± 4.2a	12.0 ± 0.8a	21.9 ± 3.6a
2nd	17.3 ± 3.2b	11.8 ± 0.6a	19.7 ± 2.5a
Total	36.5	n/a	41.6

n/a: not available.
Means of the same column and growing period are separated by Tukey HSD test at $P < 0.05$. Latin letters indicate significant differences between means of the same growing period.

Table 2
Composition in sugars and organic acids (g/kg fw) of *Cichorium spinosum* leaves (mean ± SD) in relation to harvest stage and growing period.

H*	Fructose	Glucose	Sucrose	Trehalose	Total Sugars	Oxalic acid	Quinic acid	Malic acid	Ascorbic acid	Citric acid	Total organic acids
1st growing period											
1st	1.48 ± 0.01a	2.32 ± 0.25a	2.20 ± 0.12a	0.49 ± 0.01b	6.5 ± 0.2a	4.22 ± 0.02c	2.64 ± 0.02c	1.58 ± 0.02b	tr	0.46 ± 0.01c	8.9 ± 0.1c
2nd	0.374 ± 0.002b	0.79 ± 0.01c	1.25 ± 0.01b	1.358 ± 0.004a	3.780 ± 0.001b	7.2 ± 0.1a	5.8 ± 0.2a	1.770 ± 0.004a	tr	1.19 ± 0.03a	16.0 ± 0.3a
3rd	0.30 ± 0.01c	1.388 ± 0.004b	1.05 ± 0.01c	0.363 ± 0.002c	3.10 ± 0.01c	4.7 ± 0.03b	5.01 ± 0.1b	0.73 ± 0.02c	0.0100 ± 0.0002	0.99 ± 0.02b	11.5 ± 0.02b
2nd growing period											
1st	0.61 ± 0.06a	1.61 ± 0.05b	5.09 ± 0.07a	0.87 ± 0.03b	8.2 ± 0.1b	6.5 ± 0.1b	4.8 ± 0.1b	2.92 ± 0.01a	0.0100 ± 0.0001a	0.78 ± 0.01a	15.0 ± 0.2b
2nd	0.32 ± 0.04b	3.37 ± 0.10a	4.97 ± 0.03b	1.102 ± 0.007a	9.8 ± 0.1a	9.8 ± 0.1a	7.08 ± 0.04a	2.680 ± 0.001b	0.010 ± 0.001b	0.76 ± 0.01a	20.4 ± 0.2a

Means of the same column and growing period are separated by Tukey HSD test at $P < 0.05$. Latin letters indicate significant differences between means of the same growing period.
* H: Harvest.

Dugosz-Grochowska, Koton, & Zupnik, 2015), since leaves were harvested according to rosette's size and not according to growth stage. Moreover, Poli et al. (2002) have reported a significant variation in total sugar content during cultivation and between inner and outer leaves of *C. intybus*.

Organic acids content may have a significant effect on quality and taste of *C. spinosum* leaves, while high oxalic acid content may have adverse health effects. Despite the fact that oxalic acid was the main detected organic acid for all the harvests and growing periods, its content was not as high as other leafy vegetables such as spinach, while only in the 3rd harvest of the 1st growing season oxalic acid: calcium ratio was higher than 2.5 which is consider the upper threshold for a food source to be considered as good source of calcium (Table 2; Guil, Torija, Giménez, Rodrí guez-García, & Giménez, 1996). Moreover, the highest content of individual and total organic acids was observed in the second harvest of the 1st growing period, except for the case of ascorbic acid, while in the 2nd growing period only oxalic and malic acid increased with successive harvesting. It should be also mentioned that ascorbic acid content is negatively correlated with glucose content, since the specific sugar is one of the sugars used in ascorbic acid biosynthetic pathways (Loweus, 1999). Therefore, its fluctuation and decrease during the 1st and 2nd growing periods, respectively, could be explained by the opposite trends in glucose content that was observed in the present study, and consequently on growth conditions and high light intensity (Poli et al., 2002; Wojciechowska et al., 2015).

Wild edible greens are considered a valuable source of polyunsaturated fatty acids, especially ω-3 and ω-6 fatty acids, which is one of the main reasons they are considered an important ingredient of the Mediterranean diet. The main detected fatty acids were alpha-linolenic (C18:3n3; ranging between 57.2 and 74.6%), linoleic (C18:2n6c; ranging between 14.2 and 17.8%) and palmitic acid (C16:0; ranging between 7.3 and 16.1%) (Table 3). Polyunsaturated fatty acids (PUFA) and a-linolenic acid in particular, comprised the highest amount of total fatty acids (Table 3). Total saturated fatty acids content (SFA) was higher in the 2nd growing period, especially at the 2nd harvest where the highest amount of palmitic acid was observed. The same trend was also observed for monounsuturated fatty acids content (MUFA); however, their overall content was very low comparing to the other fatty acid types. In both growing periods and for all the harvests, PUFA/SFA and n-6/n-3 ratios were higher than 0.45 and lower than 4.0, respectively; however, leaves of the 1st growing period and especially those of the second harvest showed the best values of these ratios (9.01 and 0.19, respectively) indicating a higher nutritional value of leaves comparing to the other harvests. Zeghichi et al. (2003) have observed similar trends regarding the main fatty acids of *C. spinosum* leaves (linoleic and alpha-linolenic acids), although they reported significantly lower amounts of palmitic acid. The gradual decrease of the main fatty acids, and especially the lower content of alpha-linolenic acid and PUFAs in general that was observed during the 2nd growing period (Table 3) could be associated with the decrease of antioxidants compounds such as tocopherols which have been suggested to protect lipids from oxidation and modulate PUFAs metabolism (Cao et al., 2015; Maeda, Sage, Isaac, Welti, & Dellapenna, 2008).

Both harvests and growing periods also significantly affected the tocopherol content. In particular, in the 1st growing period successive harvests resulted in a significant decrease of all individual tocopherols and consequently of total tocopherols content, while the lowest content was observed in the last harvest (3rd harvest). Moreover, gamma- and alpha-tocopherols were the main detected tocopherols and had the highest contribution to total tocopherol content, regardless of the number of harvests. The same trend was also observed in the 2nd growing period, with only exception beta-tocopherols content which increased with successive harvesting, while the 2nd growing period was more favorable to tocopherol biosynthesis than the 1st growing period (Table 3). According to Márkus, Daood, Kapitány, & Biacs (1999), earlier harvesting of red pepper (unripe fruit) resulted in higher tocopherols content comparing to overripe fruit where biosynthesis of tocopherols is retarded, whereas Dhillon et al. (2016) observed a higher tocopherols content in bitter gourd at harvesting 14–20 days after fruit set (DAFS), comparing to 12 DAFS. Zeghichi et al. (2003), have also reported that alpha-tocopherol content was higher at the earliest stages of growth of *C. spinosum* plants (40 days after planting comparing to 43 days after planting in our study); however no further details about growing period and sowing date are available for this study in order to make safe comparisons.

Mineral composition of leaves is presented in Table 4. The first two harvests in the 1st growing period showed the highest content in macro and micro minerals, whereas in the 2nd growing period where only two harvests were applied, micro minerals were detected in higher amount in the 1st harvest. Zeghichi et al. (2003) have observed a similar trend for macro (except for Ca) and micro minerals (except for Mn), with higher contents being detected at 40 days after planting and a gradual decrease after that stage.

The lowest nitrate contents in leaf tissues were observed only in the first harvest of each growing condition, suggesting that the early harvested products are more safe, considering health concerns associated with high nitrates daily consumption. However, in any case of the present study nitrates content did not exceed the limits set by EU regulations, especially when considering that the final product is an ingredient in gourmet dishes and is usually consumed in small amounts on a daily or less regular basis. The effect of growing period on nitrate content of leafy vegetables has been also reported by Petropoulos et al. (2008, 2016); however, although in these studies late harvesting and cultivation during

Table 3
Fatty acids (relative percentage) and tocopherol (mg/kg fw) composition of *Cichorium spinosum* leaves for each harvest and growing period (mean ± SD).

	Harvest				
	1st growing period			2nd growing period	
	1st	2nd	3rd	1st	2nd
C6:0	0.031 ± 0.001	0.057 ± 0.002	0.117 ± 0.001	0.010 ± 0.001	0.004 ± 0.001
C8:0	0.019 ± 0.001	0.043 ± 0.002	0.046 ± 0.004	0.026 ± 0.001	0.030 ± 0.001
C10:0	0.026 ± 0.001	0.043 ± 0.001	0.060 ± 0.003	0.071 ± 0.005	0.035 ± 0.001
C12:0	0.032 ± 0.001	0.045 ± 0.001	0.039 ± 0.001	0.072 ± 0.004	0.059 ± 0.002
C14:0	0.186 ± 0.007	0.195 ± 0.007	0.237 ± 0.003	0.41 ± 0.02	0.915 ± 0.008
C15:0	0.34 ± 0.01	0.289 ± 0.009	0.271 ± 0.005	0.356 ± 0.010	0.769 ± 0.009
C16:0	7.86 ± 0.10	7.33 ± 0.06	7.31 ± 0.01	9.08 ± 0.09	16.10 ± 0.06
C16:1	0.345 ± 0.001	0.305 ± 0.001	0.275 ± 0.002	0.406 ± 0.006	0.62 ± 0.01
C17:0	0.103 ± 0.006	0.090 ± 0.002	0.087 ± 0.004	0.110 ± 0.002	0.384 ± 0.004
C18:0	0.79 ± 0.01	0.648 ± 0.004	0.769 ± 0.002	0.985 ± 0.004	1.75 ± 0.01
C18:1n9c	0.837 ± 0.001	0.60 ± 0.01	0.975 ± 0.008	1.19 ± 0.01	1.49 ± 0.01
C18:2n6c	15.07 ± 0.01	14.21 ± 0.01	14.32 ± 0.01	16.39 ± 0.01	17.76 ± 0.04
C18:3n3	72.71 ± 0.12	74.64 ± 0.08	73.64 ± 0.06	69.18 ± 0.10	57.16 ± 0.02
C20:0	0.207 ± 0.006	0.175 ± 0.002	0.17 ± 0.01	0.23 ± 0.01	0.77 ± 0.01
C20:1	0.027 ± 0.001	0.029 ± 0.001	0.032 ± 0.001	0.024 ± 0.001	0.034 ± 0.001
C20:2	0.120 ± 0.001	0.106 ± 0.004	0.106 ± 0.008	0.118 ± 0.004	0.064 ± 0.001
C20:3n3	0.127 ± 0.007	0.150 ± 0.004	0.137 ± 0.004	0.124 ± 0.010	0.122 ± 0.006
C21:0	0.021 ± 0.001	0.017 ± 0.001	0.022 ± 0.001	0.024 ± 0.001	0.13 ± 0.01
C20:5n3	0.069 ± 0.003	0.063 ± 0.001	0.094 ± 0.008	0.101 ± 0.001	0.16 ± 0.01
C22:0	0.294 ± 0.008	0.259 ± 0.006	0.403 ± 0.009	0.348 ± 0.012	0.622 ± 0.009
C23:0	0.096 ± 0.006	0.089 ± 0.007	0.077 ± 0.001	0.101 ± 0.001	0.22 ± 0.01
C24:0	0.700 ± 0.008	0.628 ± 0.001	0.83 ± 0.03	0.63 ± 0.01	0.82 ± 0.01
Total SFA (% of total FA)	10.70 ± 0.13a	9.90 ± 0.07c	10.43 ± 0.05b	12.46 ± 0.12b	22.59 ± 0.05a
Total MUFA (% of total FA)	1.21 ± 0.01b	0.93 ± 0.01c	1.28 ± 0.01a	1.62 ± 0.01b	2.15 ± 0.01a
Total PUFA (% of total FA)	88.10 ± 0.13c	89.16 ± 0.08a	88.29 ± 0.05b	85.92 ± 0.11a	75.26 ± 0.06b
alpha-Tocopherol	3.49 ± 0.05a	1.440 ± 0.00b	0.343 ± 0.005c	8.68 ± 0.01a	8.55 ± 0.04b
beta-Tocopherol	0.082 ± 0.002a	0.086 ± 0.005a	0.008 ± 0.001c	0.136 ± 0.001b	0.254 ± 0.005a
gamma-Tocopherol	6.04 ± 0.01a	4.08 ± 0.02b	0.99 ± 0.01c	3.50 ± 0.01a	1.81 ± 0.01b
delta-Tocopherol	0.022 ± 0.001a	0.023 ± 0.002a	0.019 ± 0.001b	0.019 ± 0.001a	0.007 ± 0.001b
Total Tocopherols	9.64 ± 0.06a	5.63 ± 0.01b	1.356 ± 0.005c	12.34 ± 0.01a	10.63 ± 0.03b

Means of the same raw and growing period are separated by Tukey HSD test at $P < 0.05$. Latin letters indicate significant differences between means of the same growing period.

Table 4
Mineral composition and nitrates content (mg/kg fw) of *Cichorium spinosum* leaves (mean ± SD) for each harvest and growing period.

H*	K	Na	Ca	Mg	Zn	Mn	Fe	NO_3^-
1st growing season								
1st	4132 ± 202a	770 ± 179a	2550 ± 607b	380 ± 52a	4.1 ± 0.1a	6.2 ± 0.7b	6.8 ± 0.4a	532 ± 196b
2nd	4140 ± 360a	726 ± 73b	2924 ± 471a	358 ± 35b	2.4 ± 0.1b	7.2 ± 0.8a	5.6 ± 0.6b	722 ± 158a
3rd	3588 ± 257b	566 ± 184c	1516 ± 187c	272 ± 20c	1.6 ± 0.1c	5.8 ± 0.5c	5.2 ± 0.2b	711 ± 208a
2nd growing season								
1st	3984 ± 526b	667 ± 106a	3263 ± 431b	450 ± 54b	2.3 ± 0.4a	8.6 ± 0.8a	8.5 ± 0.3a	1392 ± 549b
2nd	4461 ± 115a	446 ± 118b	5626 ± 595a	715 ± 73a	1.6 ± 0.2b	7.7 ± 0.1b	8.2 ± 1.6b	1906 ± 270a

Means of the same column and growing period are separated by Tukey HSD test at $P < 0.05$. Latin letters without parenthesis indicate significant differences between means of the same growing period, while letters in parenthesis differences between means of different growing periods.
* H: Harvest.

spring resulted in lower nitrates content, this was not the case in the present study where late harvests were associated with high nitrate content. Considering that *C. spinosum* is a halophyte, Na^+ usually has the main osmoregulatory role, especially under saline conditions where Na tends to accumulate in leaf tissues (Petropoulos et al., 2017). The fact that in the present study Na content in leaf tissues was significantly lower than previously reported for the species by Petropoulos et al. (2017) could suggest that plants tends to accumulate nitrates to serve as osmoregulatory factors under spring conditions where high temperatures increase water requirements and water uptake. Moreover, according to Konstantopoulou et al. (2012), the application of 260 mg L^{-1} of N, resulted in a slight increase of nitrates content in lettuce leaves during spring comparing to winter growing period, while leaf parts and position may also affect nitrate content (Konstantopoulou et al., 2010). In addition, Sareer, Bernstein, Ahmad, and Umar (2016) have reported that nitrate content in *Andrographis paniculata* increased significantly with plant development, while Masclaux-Daubresse et al. (2010) suggested that increased nitrate contents in leaves could be attributed to nitrogen remobilization to expanding leaves. Considering that successive harvesting induces formation of new leaves, could be the reason for the high nitrates content in leaves at late harvest stages in the present study, despite the favorable conditions for nitrates reduction.

Antioxidant properties of wild edible greens and *C. spinosum* in particular are highly appreciated in ethnobotanical studies (Tardío, Pardo-de-santayana, & Morales, 2006). In the present study, the 2nd growing period was more favorable in terms of EC_{50} values, with 1st harvest having the lowest EC_{50} values for all the tested

Table 5
Antioxidant properties of *Cichorium spinosum* leaves (mean ± SD) for each harvest and growing period.

H*	Reducing power	Radical scavenging activity		Lipid peroxidation inhibition
	Ferricyanide/Prussian blue (EC_{50}; mg/mL)	DPPH scavenging activity (EC_{50}; mg/mL)	Beta-carotene/linoleate (EC_{50}; mg/mL)	TBARS (EC_{50}; mg/mL)
1st growing period				
1st	0.709 ± 0.004b	2.30 ± 0.10c	0.71 ± 0.03b	0.43 ± 0.01b
2nd	1.24 ± 0.01a	3.24 ± 0.01a	0.96 ± 0.03a	0.39 ± 0.02c
3rd	1.24 ± 0.02a	3.11 ± 0.03b	0.73 ± 0.01b	0.454 ± 0.007a
2nd growing period				
1st	0.337 ± 0.001b	0.388 ± 0.021b	0.349 ± 0.009b	0.163 ± 0.009b
2nd	0.491 ± 0.002a	0.793 ± 0.012a	0.68 ± 0.03a	0.362 ± 0.003a

The antioxidant activity was expressed as EC_{50} values, what means that higher values correspond to lower reducing power or antioxidant potential. EC_{50}: Extract concentration corresponding to 50% of antioxidant activity or 0.5 of absorbance in reducing power assay. Trolox EC_{50} values: 41 μg/mL (reducing power), 42 μg/mL (DPPH scavenging activity), 18 μg/mL (β-carotene bleaching inhibition) and 23 μg/mL (TBARS inhibition).
Means of the same column and growing period are separated by Tukey HSD test at $P < 0.05$. Latin letters indicate significant differences between means of the same growing period.
* H: Harvest.

Table 6
Retention time (Rt), wavelengths of maximum absorption in the visible region (λ_{max}), mass spectral data, tentative identification and quantification of phenolic compounds in *Cichorium spinosum* leaves (mg/g extract, mean ± SD) in relation to harvest stage and growing period.

Peak	Rt (min)	λ_{max} (nm)	$[M-H]^-$ (*m*/*z*)	MS^2 (*m*/*z*)	Tentative identification	Harvests				
						1st growing period			2nd growing period	
						1st	2nd	3rd	1st	2nd
1	7.1	325	353	191(100),179(5),173 (3),161(3),135(3)	5-*O*-Caffeoylquinic acid[1]	2.37 ± 0.02	2.31 ± 0.02	2.65 ± 0.04	8.17 ± 0.04	4.64 ± 0.02
2	12.0	313	337	191(100),173(3),163 (10),145(3),119(3)	*p*-Coumaroylquinic acid[2]	0.150 ± 0.003	0.315 ± 0.003	0.341 ± 0.006	0.478 ± 0.006	0.233 ± 0.001
3	12.4	328	473	311(100),293 (94),219(3),179 (7),149(3),135(3)	Chicoric acid[3]	5.83 ± 0.03	2.98 ± 0.02	3.973 ± 0.009	19.90 ± 0.02	11.7 ± 0.2
4	13.6	327	367	193(10),191 (100),173(5),143 (3),134(3)	5-*O*-Feruolyquinic acid[4]	nd*	0.090 ± 0.002	nd	nd	nd
5	16.5	328	193	178(20),134 (100),117(8)	Ferulic acid[4]	0.117 ± 0.004	nd	0.063 ± 0.001	nd	nd
6	18.2	342	477	301(100)	Quercetin-3-*O*-glucuronide[5]	0.73 ± 0.01	0.316 ± 0.001	0.414 ± 0.003	2.591 ± 0.011	2.39 ± 0.03
7	18.7	348	461	285(100)	Kaempferol-*O*-glucuronide[6]	0.854 ± 0.002	0.796 ± 0.001	0.85 ± 0.03	3.39 ± 0.02	2.82 ± 0.05
8	20.5	360	505	463(26),301(100)	Quercetin-7-*O*-(6″-*O*-acetyl)-glucoside[5]	0.219 ± 0.001	0.183 ± 0.001	0.201 ± 0.001	0.412 ± 0.002	0.740 ± 0.005
9	20.9	329	515	353(100),191 (97),179(48),173 (5),161(3),135(7)	3,5-*O*-Dicaffeoylquinic acid[1]	0.187 ± 0.002	nd	nd	0.451 ± 0.039	nd
10	22.2	345	461	285(100)	Kaempferol-3-*O*-glucuronide[6]	0.902 ± 0.001	0.423 ± 0.006	0.532 ± 0.007	2.28 ± 0.02	2.23 ± 0.03
11	23.4	337	447	269(100)	Apigenin-*O*-glucuronide[7]	0.207 ± 0.001	0.220 ± 0.001	0.225 ± 0.001	0.267 ± 0.001	0.362 ± 0.003
12	23.7	350	491	315(100)	Isorhamnetin-3-*O*-glucuronide[5]	0.248 ± 0.001	0.214 ± 0.001	0.242 ± 0.001	0.643 ± 0.003	0.65 ± 0.01
13	25.0	345	489	285(100)	Kaempferol-3-*O*-(6″-*O*-acetyl)-glucoside[6]	0.066 ± 0.001	0.0056 ± 0.0001	0.049 ± 0.001	0.305 ± 0.004	0.69 ± 0.02
14	26.3	334	519	477(5),315(100)	Isorhamnetin-3-*O*-(6″-O-acetyl)-glucoside[5]	0.167 ± 0.001	nd	nd	0.209 ± 0.008	nd
					Total phenolic acids	**8.66 ± 0.05a**	**5.69 ± 0.05c**	**7.03 ± 0.03b**	**29.00 ± 0.05a**	**16.5 ± 0.2b**
					Total flavonoids	**3.399 ± 0.004a**	**2.16 ± 0.01c**	**2.52 ± 0.01b**	**10.095 ± 0.001a**	**9.9 ± 0.2a**
					Total phenolic compounds	**12.05 ± 0.04a**	**7.85 ± 0.05c**	**9.55 ± 0.04b**	**39.09 ± 0.05a**	**26.4 ± 0.4b**

* nd-not detected. Calibration curves used: A – chlorogenic acid ($y = 168823x - 161172$; $R^2 = 0.999$); B – *p*-coumaric acid ($y = 301950x + 6966.7$; $R^2 = 0.999$); C – caffeic acid ($y = 388345x + 406369$; $R^2 = 0.994$); D – ferulic acid ($y = 633126x - 185462$; $R^2 = 0.999$); E – quercetin-3-*O*-glucoside ($y = 34843x - 160173$; $R = 0.999$); F – kaempferol-3-*O*-rutinoside ($y = 11117x + 30861$; $R^2 = 0.999$); G – apigenin-7-*O*-glucoside ($y = 10683x - 45794$; $R^2 = 0.996$). Means of the same raw and growing period are separated by Tukey HSD test at $P < 0.05$. Latin letters indicate significant differences between means of the same growing period.

assays (Table 5). Similar trends were observed in the 1st growing period, except for the lipid peroxidation inhibition assay where the 2nd harvest showed the best results, indicating that the first two harvests in a successive harvesting regime are the most appropriate for high quality end-product. Zeghichi et al. (2003) have reported similar results for DPPH assay in *C. spinosum* leaf extracts

with 2nd harvest (comparable to 1st harvest of our study) having the lowest EC_{50} values. The decrease in antioxidant activity during the growing period could be associated with the lower content in tocopherols and other bioactive compounds, such as phenolic acids and flavonoids, which was also observed in the present study.

Compound characteristics (retention time, wavelength of maximum absorption in the visible region and mass spectral data), tentative identities and quantitative results of leaf tissues of *C. spinosum* are presented in Table 6. Compounds were identified based on their chromatographic, UV and mass spectra characteristics. Fourteen compounds were identified from which six were phenolic acids and eight flavonoid glycoside derivatives. The detected phenolic acids corresponded to hydroxycinnamic acid derivatives, namely chlorogenic acids, a family of esters formed between certain cinnamic acids, most commonly caffeic, *p*-coumaric and ferulic acids and quinic acid (IUPAC, 1976), while the flavonoids corresponded to quercetin (λ_{max} around 350 nm and an MS^2 fragment at *m*/*z* 301), kaempferol (λ_{max} around 348 nm, MS^2 fragment at *m*/*z* 285) and isorhamnetin (λ_{max} around 350 nm, MS^2 fragment at *m*/*z* 315) glycoside derivatives.

With the exception of compounds 2, 4 and 5, the remaining peaks have been previously identified in *C. spinosum* by the authors, in plants grown under different saline conditions (Petropoulos et al., 2017) and in different ecotypes of *C. spinosum* (Petropoulos et al., 2016), as well as in leaves of *C. intybus* (Carazzone, Mascherpa, Gazzani, & Papetti, 2013; Dalar & Konczak, 2014; Heimler, Isolani, Vignolini, & Romani, 2009; Sinkovič et al., 2015). Peaks 2 ($[M-H]^-$ at *m*/*z* 337) and 4 ($[M-H]^-$ at *m*/*z* 367), as well as the other previously detected hydroxycinnamoylquinic acid isomers were named using the recommended IUPAC numbering system (IUPAC, 1976) and the hierarchical keys previously developed by Clifford, Johnston, Knight, and Kuhnert (2003) and Clifford, Knight, and Kuhnert (2005). Both peaks were identified as 5-*p*-coumaroylquinic acid (peak 2) and 5-*O*-feruolyquinic acid (peak 4), while compound 5 (ferulic acid) was positively identified according to its retention, mass and UV–vis characteristics by comparison with a commercial standard.

The most abundant compounds were 5-*O*-caffeoylquinic and chicoric acids, followed by the two kaempferol glucuronides (peaks 7 and 11), which were detected in lower amounts (Table 6). In general, the 1st growing period revealed lower phenolic contents when compared to the 2nd growing period. To the best of our knowledge, this is the first study that reveals the effect of successive harvests and growing periods on phenolic composition of *C. spinosum* plants.

4. Conclusions

Therefore, although successive harvests may increase significantly total yield and consequently farmers' income, the loss of quality should also be taken into consideration, especially when more than two harvests are applied where nutritional quality loss is significant regarding antioxidant activity, and sugars and bioactive compounds content (tocopherols, phenolic compounds). Moreover, although the 2nd growing period did not allow for more harvests than the 1st one, the overall chemical composition, antioxidant properties and phenolic compounds content of leaves indicates a higher quality of the marketable product compensating for lower total yield. The great differences in chemical composition and antioxidant properties of *C. spinosum* leaves between harvests and growing periods detected in the present study indicate that cultivation practices such as sowing date and successive harvests may be useful tools for farmers and the food industry towards the production of high quality end-product with increased bioactive properties.

Acknowledgments

The authors would like to thank Miss Constantina Fassoli and Stavroula Anesti for their technical assistance throughout the experiment. The authors are also grateful to the Foundation for Science and Technology (FCT, Portugal) and FEDER under Programme PT2020 for financial support to CIMO (UID/AGR/00690/2013) and L. Barros (SFRH/BPD/107855/2015) grant.

References

Anesti, S., Fasoli, K., Petropoulos, S., Ntatsi, G., Antoniadis, V., & Ferreira, I. C. F. R. (2016). Effect of ammonium fertilizer on growth and quality of *Cichorium spinosum* plants. In VII international scientific agriculture symposium "Agrosym 2016" (pp. 827–833).

Barros, L., Pereira, E., Calhelha, R. C., Dueñas, M., Carvalho, A. M., Santos-Buelga, C., & Ferreira, I. C. F. R. (2013). Bioactivity and chemical characterization in hydrophilic and lipophilic compounds of *Chenopodium ambrosioides* L.. *Journal of Functional Foods, 5*(4), 1732–1740. http://dx.doi.org/10.1016/j.jff.2013.07.019.

Bessada, S. M. F., Barreira, J. C. M., Barros, L., Ferreira, I. C. F. R., & Oliveira, M. B. P. P. (2016). Phenolic profile and antioxidant activity of *Coleostephus myconis* (L.) Rchb.f.: An underexploited and highly disseminated species. *Industrial Crops and Products, 89*, 45–51. http://dx.doi.org/10.1016/j.indcrop.2016.04.065.

Cao, J., Li, H., Xia, X., Zou, X.-G., Li, J., Zhu, X.-M., & Deng, Z.-Y. (2015). Effect of fatty acid and tocopherol on oxidative stability of vegetable oils with limited air. *International Journal of Food Properties, 18*(4). http://dx.doi.org/10.1080/10942912.2013.864674.

Carazzone, C., Mascherpa, D., Gazzani, G., & Papetti, A. (2013). Identification of phenolic constituents in red chicory salads (*Cichorium intybus*) by high-performance liquid chromatography with diode array detection and electrospray ionisation tandem mass spectrometry. *Food Chemistry, 138*(2–3), 1062–1071. http://dx.doi.org/10.1016/j.foodchem.2012.11.060.

Cataldo, D. A., Maroon, M., Schrader, L. E., & Youngs, V. L. (1975). Rapid colorimetric determination of nitrate in plant tissue by nitration of salicylic acid 1. *Communications in Soil Science & Plant Analysis, 6*(1), 71–80. http://dx.doi.org/10.1080/00103627509366547.

Clifford, M. N., Johnston, K. L., Knight, S., & Kuhnert, N. (2003). Hierarchical scheme for LC-MSn identification of chlorogenic acids. *Journal of Agricultural and Food Chemistry, 51*(10), 2900–2911. http://dx.doi.org/10.1021/jf026187q.

Clifford, M. N., Knight, S., & Kuhnert, N. (2005). Discriminating between the six isomers of dicaffeoylquinic acid by LC-MSn. *Journal of Agricultural and Food Chemistry, 53*(10), 3821–3832. http://dx.doi.org/10.1021/jf050046h.

Csizinszky, A. A. (1999). Yield response of herbs to nitrogen and potassium in sand in multiple harvests. *Journal of Herbs, Spices & Medicinal Plants, 6*(4), 11–22. http://dx.doi.org/10.1300/J044v06n04.

Dalar, A., & Konczak, I. (2014). Cichorium intybus from Eastern Anatolia: Phenolic composition, antioxidant and enzyme inhibitory activities. *Industrial Crops and Products, 60*, 79–85. http://dx.doi.org/10.1016/j.indcrop.2014.05.043.

Dhillon, N., Lin, C., Sun, Z., Hanson, P., Ledesma, D., Habicht, S., & Yang, R. (2016). Varietal and harvesting stage variation in the content of carotenoids, ascorbic acid and tocopherols in the fruit of bitter gourd (Momordica charantia L.). *Plant Genetic Resources*. http://dx.doi.org/10.1017/S147926211500057X.

Gajewski, M., & Arasimowicz, D. (2004). Sensory quality of eggplant fruits (*Solanum melongena* L.) as affected by cultivar and maturity stage. *Polish Journal of Food and Nutrition Sciences, 1354*(3), 249–254.

Guil, J. L., Torija, M. E., Giménez, J. J., Rodríguez-García, I., & Giménez, A. (1996). Oxalic acid and calcium determination in wild edible plants. *Journal of Agricultural and Food Chemistry, 44*(7), 1821–1823. http://dx.doi.org/10.1021/jf950472a.

Heimler, D., Isolani, L., Vignolini, P., & Romani, A. (2009). Polyphenol content and antiradical activity of *Cichorium intybus* L. from biodynamic and conventional farming. *Food Chemistry, 114*(3), 765–770. http://dx.doi.org/10.1016/j.foodchem.2008.10.010.

IUPAC (1976). Nomenclature of cyclitols. *Biochemical Journal, 153*, 23–31.

Kmiecik, W., & Lisiewska, Z. (1999). Comparison of leaf yields and chemical composition of Hamburg and leafy types of parsley. *Folia Horticulturae, 11*(1), 53–63.

Konstantopoulou, E., Kapotis, G., Salachas, G., Petropoulos, S. A., Chatzieustratiou, E., Ioannis, C., & Passam, H. C. (2012). Effect of nitrogen application on growth parameters, yield and leaf nitrate content of greenhouse lettuce cultivated during three seasons. *Journal of Plant Nutrition, 35*, 1246–1254.

Konstantopoulou, E., Kapotis, G., Salachas, G., Petropoulos, S. A., Karapanos, I. C., & Passam, H. C. (2010). Nutritional quality of greenhouse lettuce at harvest and after storage in relation to N application and cultivation season. *Scientia Horticulturae, 125*(2), 93–94. http://dx.doi.org/10.1016/j.scienta.2010.03.003.

Loweus, F. A. (1999). Biosynthesis and metabolism of ascorbic acid in plants and of analogs of ascorbic acid in fungi. *Phytochemistry, 52*, 193–210. http://dx.doi.org/10.1111/j.1749-6632.1975.tb29265.x.

Maeda, H., Sage, T. L., Isaac, G., Welti, R., & Dellapenna, D. (2008). Tocopherols modulate extraplastidic polyunsaturated fatty acid metabolism in Arabidopsis at low temperature. *The Plant Cell, 20*(2), 452–470. http://dx.doi.org/10.1105/tpc.107.054718.

Márkus, F., Daood, H. G., Kapitány, J., & Biacs, P. A. (1999). Change in the carotenoid and antioxidant content of spice red pepper (Paprika) as a function of ripening and some technological factors. *Journal of Agricultural and Food Chemistry, 47*(1), 100–107.

Masclaux-Daubresse, C., Daniel-Vedele, F., Dechorgnat, J., Chardon, F., Gaufichon, L., & Suzuki, A. (2010). Nitrogen uptake, assimilation and remobilization in plants: Challenges for sustainable and productive agriculture. *Annals of Botany, 105*(7), 1141–1157. http://dx.doi.org/10.1093/aob/mcq028.

Moura, L. D. O., Carlos, L. D. A., De Oliveira, K. G., Martins, L. M., & Da Silva, E. C. (2016). Physicochemical characteristics of purple lettuce harvested at different ages. *Revista Caatinga, 29*(2), 489–495.

Omezzine, F., Bouaziz, M., Simmonds, M. S. J., & Haouala, R. (2014). Variation in chemical composition and allelopathic potential of mixoploid *Trigonella foenum-graecum* L. with developmental stages. *Food Chemistry, 148*, 188–195. http://dx.doi.org/10.1016/j.foodchem.2013.10.040.

Pereira, C., Barros, L., Carvalho, A. M., & Ferreira, I. C. F. R. (2013). Use of UFLC-PDA for the analysis of organic acids in thirty-five species of food and medicinal plants. *Food Analytical Methods, 6*(5), 1337–1344. http://dx.doi.org/10.1007/s12161-012-9548-6.

Petropoulos, S. A., Chatzieustratiou, E., Constantopoulou, E., & Kapotis, G. (2016). Yield and quality of lettuce and rocket grown in floating culture system. *Notulae Botanicae Horti Agrobotanici Cluj-Napoca, 44*(2), 603–612. http://dx.doi.org/10.15835/nbha44210611.

Petropoulos, S. A., Constantopoulou, E., Karapanos, I., Akoumianakis, C. A., & Passam, H. C. (2011). Diurnal variation in the nitrate content of parsley foliage. *International Journal of Plant Production, 5*(4), 431–438.

Petropoulos, S. A., Fernandes, Â., Barros, L., Ferreira, I. C. F. R., & Ntatsi, G. (2015). Morphological, nutritional and chemical description of "Vatikiotiko", an onion local landrace from Greece. *Food Chemistry, 182*, 156–163. http://dx.doi.org/10.1016/j.foodchem.2015.03.002.

Petropoulos, S. A., Levizou, E., Ntatsi, G., Fernandes, Â., Petrotos, K., Akoumianakis, K., & Ellipsis Ferreira, I. C. F. R. (2017). Salinity effect on nutritional value, chemical composition and bioactive compounds content of *Cichorium spinosum* L.. *Food Chemistry, 214*, 129–136. http://dx.doi.org/10.1016/j.foodchem.2016.07.080.

Petropoulos, S. A., Ntatsi, G., Levizou, E., Barros, L., & Ferreira, I. C. F. R. (2016). Nutritional profile and chemical composition of *Cichorium spinosum* ecotypes. *LWT Food Science and Technology, 73*, 95–101. http://dx.doi.org/10.1016/j.lwt.2016.05.046.

Petropoulos, S. A., Olympios, C. M., & Passam, H. C. (2008). The effect of nitrogen fertilization on plant growth and the nitrate content of leaves and roots of parsley in the Mediterranean region. *Scientia Horticulturae, 118*(3), 255–259. http://dx.doi.org/10.1016/j.scienta.2008.05.038.

Pinto, E., Almeida, A. A., Aguiar, A. A. R. M., & Ferreira, I. M. P. L. V. O. (2014). Changes in macrominerals, trace elements and pigments content during lettuce (*Lactuca sativa* L.) growth: Influence of soil composition. *Food Chemistry, 152*, 603–611. http://dx.doi.org/10.1016/j.foodchem.2013.12.023.

Pokkaew, R., Wang, S. H., Liu, C. D., Huang, F. L., Chang, J. C., Lo, C. Y., & Chiou, R. Y. Y. (2013). Properties and characterization of antioxidant and antiglycative activities for the multiple harvests of aquatic- and field-cultivated peanut leaves and stems. *Journal of Functional Foods, 5*(1), 327–336. http://dx.doi.org/10.1016/j.jff.2012.11.003.

Poli, F., Sacchetti, G., Tosi, B., Fogagnolo, M., Chillemi, G., Lazzarin, R., & Bruni, A. (2002). Variation in the content of the main guaianolides and sugars in *Cichorium intybus* var. "Rosso di Chioggia" selections during cultivation. *Food Chemistry, 76*(2), 139–147. http://dx.doi.org/10.1016/S0308-8146(01)00254-0.

Sareer, O., Bernstein, N., Ahmad, S., & Umar, S. (2016). Genetic, developmental and temporal variability in nitrate accumulation and nitrate reductase activity in medicinal herb *Andrographis paniculata*. *Pedosphere, 26*(6), 839–847. http://dx.doi.org/10.1016/S1002-0160(15)60089-3.

Sinkovič, L., Demšar, L., Žnidarčič, D., Vidrih, R., Hribar, J., & Treutter, D. (2015). Phenolic profiles in leaves of chicory cultivars (*Cichorium intybus* L.) as influenced by organic and mineral fertilizers. *Food Chemistry, 166*, 507–513. http://dx.doi.org/10.1016/j.foodchem.2014.06.024.

Tardío, J., Pardo-de-santayana, M., & Morales, R. (2006). Ethnobotanical review of wild edible plants in Spain. *Botanical Journal of the Linnean Society, 152*, 27–71.

Ventura, Y., Wuddineh, W. A., Shpigel, M., Samocha, T. M., Klim, B. C., Cohen, S., & Ellipsis Sagi, M. (2011). Effects of day length on flowering and yield production of *Salicornia* and *Sarcocornia* species. *Scientia Horticulturae, 130*(3), 510–516. http://dx.doi.org/10.1016/j.scienta.2011.08.008.

Wojciechowska, R., Dugosz-Grochowska, O., Koton, A., & Zupnik, M. (2015). Effects of LED supplemental lighting on yield and some quality parameters of lamb's lettuce grown in two winter cycles. *Scientia Horticulturae, 187*, 80–86. http://dx.doi.org/10.1016/j.scienta.2015.03.006.

Zeghichi, S., Kallithraka, S., & Simopoulos, A. P. (2003). Nutritional composition of molokhia (*Corchorus olitorius*) and stamnagathi (*Cichorium spinosum*). *World Review in Nutrition and Dietetics, 91*.