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

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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

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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,