Comparison of the Phenolics Profiles of Forced and Unforced Chicory (*Cichorium intybus* L.) Cultivars

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Summary

Modern and sustainable production of safe and healthy high quality vegetables for human consumption is nowadays the goal of many producers. Chicory (*Cichorium intybus* L.) has become an important crop used as food in many countries over the past decade. In this work field and glasshouse trials were conducted to compare phenolic profiles of hydroponically forced leaves and leaves produced as common agronomic practice of five chicory cultivars. Total phenolic content of hydroponically forced samples ranged from 60 to 140 mg/100 g fresh weight, and those of unforced grown leaves varied between 117 and 386 mg/100 g fresh weight. Red cultivar 'Treviso' shows the highest phenolic content for both, unforced and forced leaves extracts. In the unforced leaves a total of 33 and in those of forced a total of 44 compounds (peaks) were used for the discrimination study.

Key words

chicon, forcing, HPLC, hydroxycinnamic acids, phenolic profile

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Introduction

Cultivated chicory (*Cichorium intybus*) types belong to var. *foliosum* includes all cultivar groups whose commercial products are the leaves. Main cultivation groups for fresh or cooked vegetables can be divided to leaf chicory and forced chicons (Street et al., 2013). The productive cycle of forced chicory may be divided in two distinct phases. The first has aim to obtain well developed roots which are in the second phase hydroponically forced in the dark, ending up with the production of the vegetative apical buds known as chicons (Lucchin et al., 2008).

Polyphenols are secondary metabolites of plants and large group of natural effective antioxidants with the potential health benefits for humans. They are well known due to their protection against several diseases, including cancer and cardiovascular diseases (Pandey and Rizvi, 2009; Ullah and Khan, 2008). Phenolic components of chicory proved to be potential antioxidants (Farrukh et al., 2006; Liu et al., 2013; Llorach et al., 2004). Red coloured, red-spotted and fully green chicory varieties are characterized by the presence of large amount of hydroxybenzoic and hydroxycinnamic acids, whereas the red colour is due to cyanidin glycosides (Rossetto et al., 2005). Cichoric acid has been identified as the major compound in methanol extracts of chicory (Carazzone et al., 2013). The aim of this preliminary work was to gain knowledge about the variability of phenolic profiles of the leaves within the species *Cichorium intybus*. For this purpose five chicory cultivars were produced according to two agronomic practices, namely as common leaf chicory and hydroponically forced chicons. Phenolic profile was analysed by means of high-performance liquid chromatography (HPLC).

Materials and methods

Plants of following *Cichorium intybus* L. var. *foliosum* cultivars have been studied: three red coloured ('Treviso', 'Verona' and 'Anivip'), a red-spotted ('Castelfranco'), and a green ('Monivip'). Seeds were purchased from a commercial seed companies ('Treviso', 'Verona' and 'Castelfranco' from Semenarna Ljubljana, Slovenia; 'Anivip' and 'Monivip' from L'ortolano, Cesena, Italy). The chicory roots for forcing process were produced on a moderate soil in the Posavje region (Slovenia) using basic fertilization (500 kg ha^{-1} Multicomb 13-11-20 + micro-elements). The soil properties before ploughing were analysed (6.3, pH; 27.1 mg/100 g, P_2O_3; 8.6 mg/100 g, K_2O; 5.8%, organic matter; 3.4%, carbon). The field management practices in 2011 was performed as follows: sowing on 29th of June; thinning and hoeing on 3rd of August and harvesting of developed roots on 19th of November. First fertilization was carried out on 5th of August (300 kg ha^{-1} N-P-K 12-0-42) and second on 20th of August (200 kg ha^{-1} calcium nitrate). After harvest, the undercut roots were left to wilt for 10 days. Before being placed for forcing, the leaves were cut about 2 cm above the root crown and trimmed to the length of 15 cm. The hydroponically forcing process was conducted for 25 days from December 2011 to January 2012 in the glasshouse experimental station (46° 04′ N, 14° 31′ W; 320 m above sea level). The twelve roots of each cultivar were placed in forcing box filled with distilled water. Air temperature was maintained at 10°C during the first forcing period (15 days) and was gradually increased to 15°C during the second period (10 days). On the 16th of January, 2012 the new leaves named chicons (forced) were harvested.

The common cultivation method was set up to produce the leaf chicory (unforced) in first half of 2012 in the glasshouse experimental station. Five plastic planting pots were filled with seven litters of virgin soil that has not been cultivated before. Sowing of chicories took place on 30th of January, 2012 and the pots were placed on the rolling benches in heated conditions (13°C to 17°C). Until germination, all pots were covered with black foil and watered when needed. After four weeks the thinning of seedlings was carried out in order to have six plants per pot. The harvesting of leaves was performed on the 10th of June, 2012.

The ten randomly chosen leaves of each cultivar from both cultivation methods were lyophilised and ground to a fine powder using a ball mill. The leaves from all of the plants were dried in a laboratory oven at 80°C for 28 h to determine the dry matter (%DM) content. For the extraction of phenolic compounds from unforced leaves a sample powder (100 mg) was mixed with 500 µl of HCO_2H (5% in MeOH) containing 0.05 mg/ml flavone as internal standard. The extraction was performed in an ultrasonic bath at 4°C for 30 min, and after centrifugation (1120*g*, 10 min, 4°C) the supernatant was used for HPLC analysis. The extraction of phenolic compounds from forced leaves was carried out from dry powder (150 mg) with the addition of 600 µl of HCO_2H (5% in MeOH) and 150 µl of HCO_2H (5% in MeOH) containing 0.1 mg/ml eriodyctiol as internal standard. The extraction was performed for 30 min in a cooled ultrasonic bath at 4°C. After centrifugation (1120*g*, 10 min, 4°C), the supernatant was evaporated, the residue was re-dissolved in 100 µl HCO_2H (5% in MeOH) and 10 µl were injected on to HPLC column. Separation of compounds was performed according to method described previously by Treutter et al. (1994). The reverse phase HPLC system consisted of two pumps (model 422, Kontron Instruments, Germany), an automatic sample injector (Modell 231, Gilson Abimed Systems, Germany), and a diode-array detector – DAD (Kontron 540+, BioTek Kontron Instruments). Phenolic compounds were separated using an analytical column Nucleosil ®120-3 RP-C18 (250 × 4 mm, 120A, particle size 3 µm, Macherey-Nagel) following a stepwise gradient mixtures of HCO_2H (5% in H_2O) and MeOH from 95:5 (v/v) to 10:90 (v/v) with a flow rate of 0.5 ml/min. The injection volume for all samples was 10 µl. The diode-array detector running time was 195 min, followed by 40 min equilibration. The DAD scanned from 250 to 600 nm with four discrete channels at 280, 320, 350 and 540 nm. The software Geminyx-III was applied for the integration and quantification of phenolic compounds. Phenolic compounds were grouped into five categories and monitored at related wavelengths: 280 nm (unknown phenolic compounds, UPCs), 320 nm (hydroxycinnamic acids, HCAs; and flavones), 350 nm (flavonols) and 540 nm (anthocyanins). Quantification was performed using the internal standard method after having calculated response factors for the authentic standards available at each concentration point on the calibration curve within the linear range. As the value of the response factors may change for different concentrations, the average values were used for quantification: chlorogenic acid (320 nm) 1.03×10^{-5}; apigenin (320 nm) 7.8×10^{-6}; rutin (350 nm) 2.23×10^{-5}, and cyanidin-3-glucoside (540 nm) 2.4×10^{-5}. The calculation was done on the basis of specific response factors for reference compounds. UPCs and HCAs were calculated as chlorogenic acid, flavones as apigenin, flavonols as rutin and anthocyanins as cyanidin 3-glucoside.

Anthocyanins were detected and quantified only in unforced red cultivar 'Verona' in small amount (0.18 mg/g dry weight); therefore, thise was taken into account in the calculations of the total phenolic concentration (TPC), but is is not discussed as an individual phenolic class in the present study. The HCAs were grouped according to their HPLC retention times (R_t) as monomeric (R_t <100 min) and/or oligomeric (R_t >100 min) HCAs.

Results and discussion

Hydroponic forcing treatment process of developed chicory roots led to the formation of novel compounds in the leaf tissue. In general, the chromatograms of tested leaf chicory cultivars were similar between themselves and those of chicons as well. Figure 1 shows the chromatograms recorded at 280 nm of five chicory cultivars produced by two agronomic methods as common cultivation method (unforced) and hydroponic forcing of developed roots to obtain chicons (forced). There is clearly seen that forcing process has impact on different phenolic profile compared to unforced leaves within the same cultivar. The peaks in chromatograms belong to main phenolic classes of HCAs (monomeric or oligomeric), flavones, flavonols and UPC according to their UV-absorbance and chromatographic behaviour. The concentrations of those main classes and total phenolic concentrations (mg/g DW) of five chicory cultivars are reported in Figure 2, specifically for unforced and forced leaf extracts. The total phenolic concentrations (TPC) ranged from 10 mg/g DW to 33 mg/g DW. The highest TPC was found for the unforced red cv. 'Treviso' (33.15 mg/g DW), followed by the unforced red 'Verona' (22.63 mg/g DW) and forced 'Treviso' (21.76 mg/g DW). This results are in agreement with the published results of green 'Catalogna' and red 'Palla Rossa di Chioggia' chicory cultivars (Ferioli and D'Antuono, 2012). The TPCs of unforced chicory cultivars amounts to as follows: 'Treviso' > 'Verona' > 'Monivip' > 'Castelfranco' > 'Anivip'; and those of forced: 'Treviso' > 'Verona'

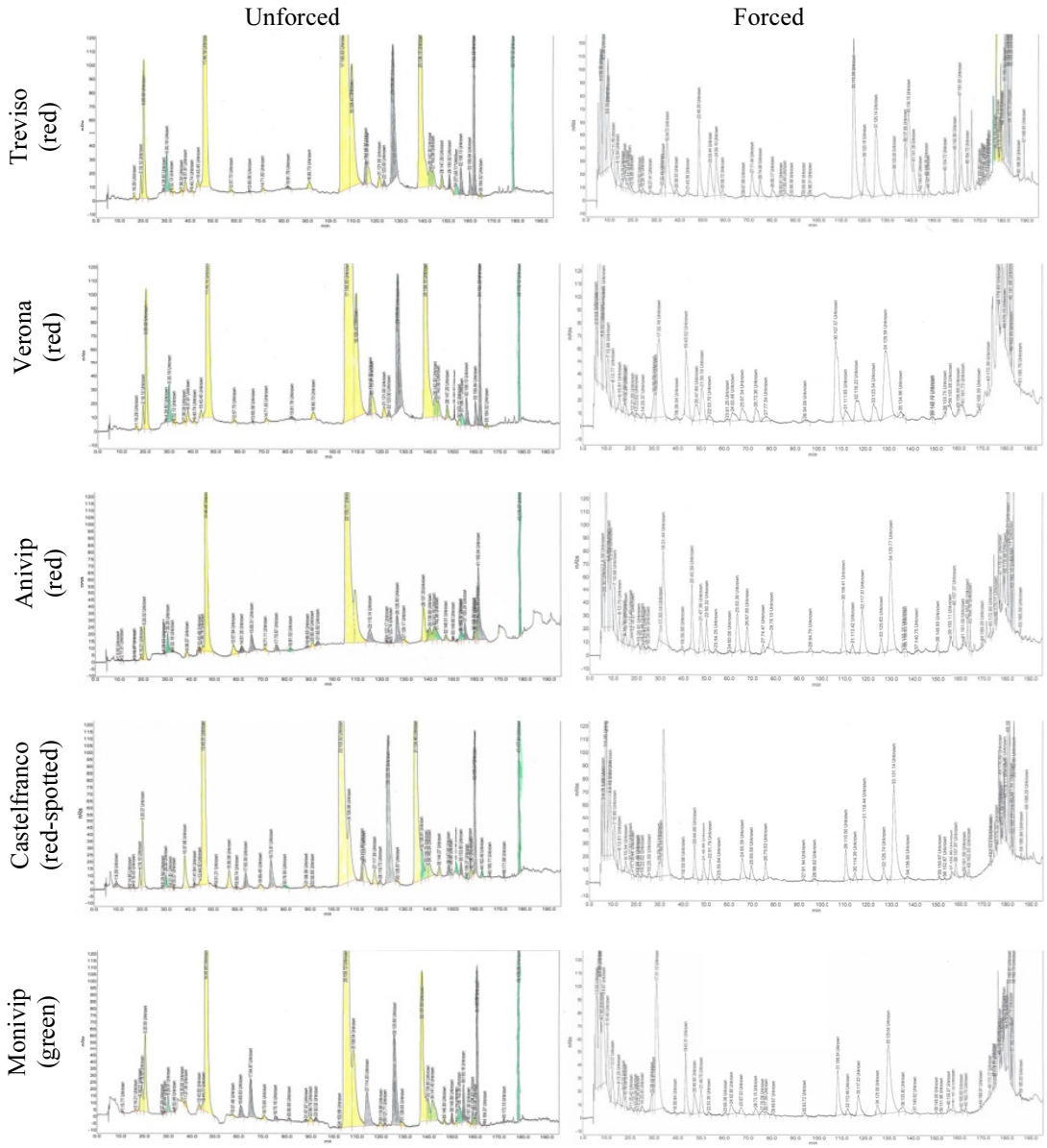

Figure 1. Chromatograms acquired by HPLC-DAD (280 nm) of five chicory cultivars from common cultivation method (unforced) and hydroponic forcing of developed roots (forced).

> 'Monivip' > 'Anivip' > 'Castelfranco'. The lowest TPC was found for unforced red 'Anivip' (10.45 mg/g DW) and forced red-spotted 'Castelfranco' (11.61 mg/g DW). Unforced 'Treviso' thus has twice the TPC as compared to the unforced 'Castelfranco' and three times the unforced 'Anivip'. For the forced leaves the differences are smaller between cultivars; 'Treviso' has about twice the TPC as compared to the 'Castelfranco'. D'evoli et al. (2013) determined a high TPC in 'Treviso' cultivar as well, but TPC of forced chicons have not been reported in previous studies yet. Due to the growth from reserve substances of roots the effect of hydroponic forcing process resulted in the reduction of TPC for 12% in cv. 'Monivip', 20% in 'Verona', 34% in 'Treviso' and 38% in 'Castelfranco'; the exception was cv. 'Anivip' where TPC thus increased for 31%.

The HCAs (collectively as monomeric and oligomeric HCAs) are the most represented main phenolic classes (81% to 90%) in unforced chicory leaves (Figure 2), followed by the UPCs, flavonols and flavones as minor classes. The further distribution analysis shows that there are less monomeric HCAs than oligomeric HCAs in unforced leaves (Figure 2). These data show that

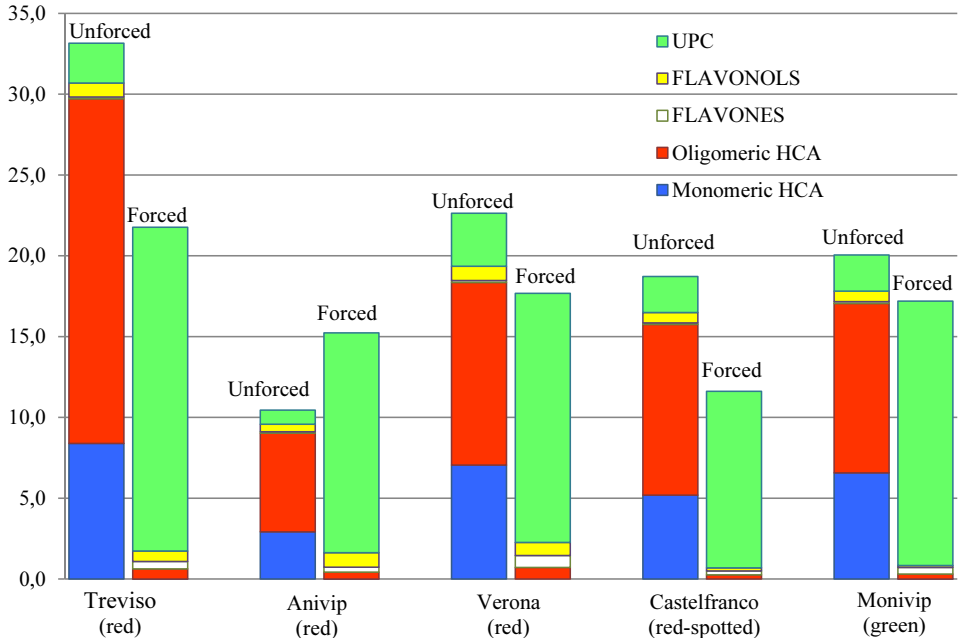

Figure 2.
Main phenolic classes comparison of five chicory cultivars (mg/g dry weight) from two different agronomic cultivation methods.
Legend: UPC, unknown phenolic compounds; HCA, hydroxycinnamic acids.

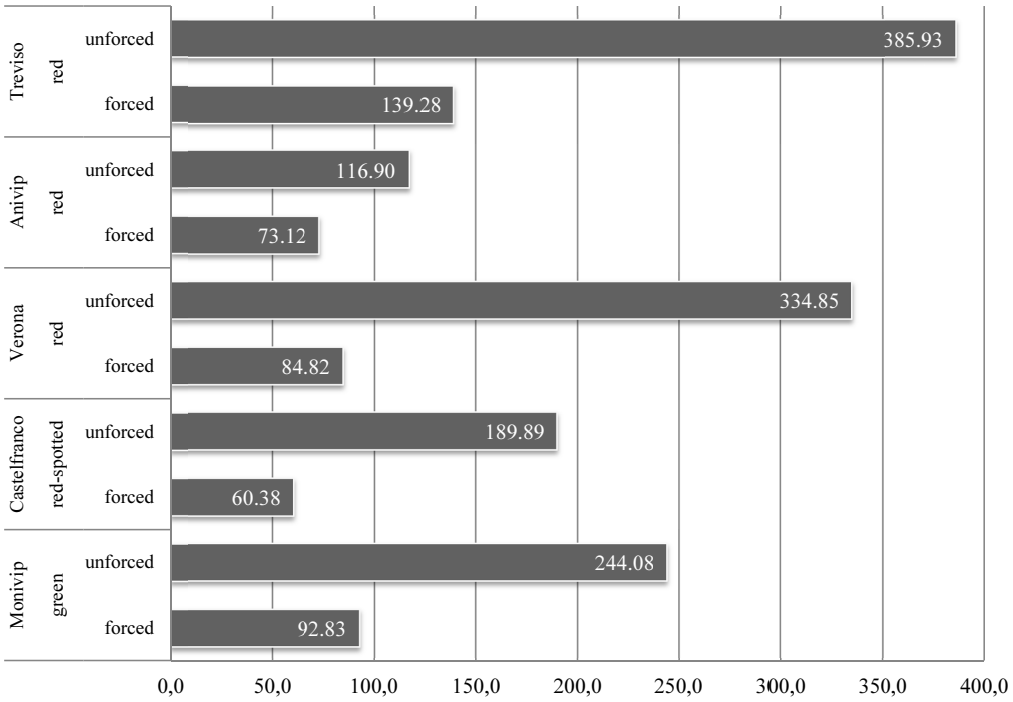

Figure 3.
Total phenolic content expressed as mg/100 g fresh weight of five chicory cultivars produced from two different cultivation methods.

'Treviso' had higher total HCA content, as well as monomeric and oligomeric, compared to the other cultivars studied. In the forced chicon leaves the UPCs represent the highest phenolic class (87% to 95%), followed by other minor classes (flavonols, oligomeric HCAs, flavones) which concentrations ranked between 0.12 mg/g DW to 0.90 mg/g DW (Figure 2). The results demonstrate that the forcing process influences the TPC, especially significantly modulate the HCAs to novel unknown phenolic compounds of five studied chicory cultivars.

The dry matter content (%DM) of leaves of unforced cultivars was twice as high as those obtained by forcing process; for the forced chicon leaves ranked from 4.8% to 6.4% and for unforced chicory leaves between 10.2% and 14.8%. Total phenolic concentrations expressed as mg/100 g fresh weight (FW) for the analysed chicory cultivars are presented in Figure 3. TPCs of unforced leaves ranked from 116.90 mg/100 g FW ('Anivip') to 385.93 mg/100 g FW ('Treviso'); and those of forced from 60.38 mg/100 g FW ('Castelfranco') to 139.28 mg/100 g FW ('Treviso'). As expected, the red cultivars (except for 'Anivip') had significantly higher TPCs as compared to the red-spotted and green cultivar, which is in agreement with previous studies carried out on other cultivars (Innocenti et al., 2005; Nicoletto and Pimpini, 2010). All forced chicon leaves contained from 25% to 62% TPCs as compared to leaves from common cultivation.

Conclusions

Combination of different agricultural practices to cultivate the chicory used as fresh food could allow consumer's year-round supply with this vegetable. The hydroponic process to force developed roots is especially interesting for the winter months when the availability of fresh leafy vegetables is limited. Results of this preliminary study show chicory as well as its forced chicons as the promising future crop with concentration of polyphenols comparable to those in blackberry, strawberry, raspberry or red wine. However, the forcing process results in reduce content of total phenolic compounds as compared to common cultivation.

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