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Profiling chicory sesquiterpene lactones by high resolution mass spectrometry

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ABSTRACT

Sesquiterpene lactones (SLs) are the main determinants of radicchio bitterness and the control of their concentrations is a key point for the market value of this product. An innovative analytical approach based on two complementary mass spectrometers, Orbitrap-HRMS and MS/MS, was used for quantitative analysis of SLs in aerial part of four different varieties of chicory. Data highlighted the presence of eight SLs: 11β,13-dihydrolactucin, lactucin, 8-deoxy-lactucin, dihydro-8-deoxylactucin, dihydrolactucopicrin, lactucopicrin, lactuside C (jaquinellin glucoside) and dihydro-lactucopicrin oxalate. Significant varietal differences were found. The highest amount of SLs was found in the radicchio "Treviso Precoce" variety (189.71 μg/g), the lowest amount in "Treviso Tardivo" variety (45.78 μg/g). Lactucopicrin was the most abundant compound with concentration ranged between 99.36 in "Treviso Precoce" and 13.50 μg/g in "Treviso Tardivo" while dihydro-lactucopicrin oxalate was the less abundant in all analyzed varieties with an average concentration of about 1% on the total amount of SLs.

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1. Introduction

Diet rich in vegetables and fruit has been linked with lower rates of cancer and coronary disease (Drewnowski & Gomez-Carneros, 2000; Ou, Huang, Hampsch-Woodill, Flanagan, & Deemer, 2002; Vinson, Hao, Su, & Zubik, 1998; Yan-Hwa, Chang, & Hsu, 2000). Plant-based phenols, flavonoids, isoflavones, glucosinolates, terpenes and other compounds that are present in the everyday diet have antioxidant and anticarcinogenic properties and a wide spectrum of tumor-blocking activities (Drewnowski & Gomez-Carneros, 2000; Higdon, Delage, Williams, & Dashwood, 2007). These compounds are known as phytochemicals or phytonutrients and were often bitter, acrid or astringent (Craig, 1997; Drewnowski & Gomez-Carneros, 2000; Lesschaeve & Noble, 2005).

Unfortunately, bitter taste lowered consumer acceptability despite the fact that bitter compounds were often potentially helpful to human health (Ames, Profet, & Gold, 1990). Bitter taste also posed considerable problems at industrial level because de-bittering processes are complex and can reduce the overall quality, especially for vegetable food (Rousseff, 1990; Roy, 1990). Therefore, the control and modulation of the bitter taste in food played a pivotal role in order to match health quality and marketing needs. Genetic improvement techniques or innovative farming practices can be used to handle the bitter taste (Martínez-Ballesta et al., 2008). However, rapid and precise analytical methods to identify and quantify the chemical components responsible for the bitter taste are needed. In this framework, chicory species and in particular radicchio (*Cichorium intybus* L., group *rubifolium*) represented a peculiar case: in fact, they are well-known for their bitter taste which is differently desired for consumers. Radicchio is one of the most popular and expensive type of chicory also due to the smart red colored leaves, which makes it very eye-catching among leafy vegetables. The bitter taste of chicory originates from sesquiterpene lactones (SLs) mainly present in the latex (Poli et al., 2002).

SLs are C-15 terpenoids which occur as hydrocarbons or in oxygenated forms such as alcohols, ketones, aldehydes, acids or lactones in nature. The principal SLs found in species of *Lactuca* and chicory were lactucin, lactucopicrin, 8-deoxylactucin and derivatives such as 11,13 dihydro-analogs. The SLs lactucin, 8-deoxylactucin, and lactucopicrin were also intensely bitter, so their presence within salad lettuce and chicory should be carefully controlled to avoid product depreciation (Poli et al., 2002; Price, Dupont, Shepherd, Chan, & Fenwick, 1990). Previous studies also reported the presence of SL glycosides such as 15-glycosyl conjugate of 11,13-dihydrolactucopicrin that has been identified in roots of *Lactuca tatarica*, *Lactuca aculeata* and *C. intybus* (Beharav et al., 2010; Kisiel & Zielińska, 2001; Kisiel & Barszcz, 1997). This glycoside and others such as picriside A (lactucin 15-glycoside) and crepidiaside A (8-deoxylactucin-15-glycoside), have also been identified in other members of the Lactuceae species (Adegawa et al., 1985; Nishimura et al., 1986). The presence of 15-oxalates and 8-sulfates as additional major conjugates of lactucin, 8-deoxylactucin, and lactucopicrin in lettuce and chicory has also been reported by Sessa, Bennett, Lewisi, Mansfield, and Bealei (2000). The most frequently used

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analytical techniques for the separation and analysis of SLs are reversed-phase HPLC (RP-HPLC) with UV detection, supercritical fluid chromatography, micellar electrokinetic chromatography (MEKC), gas chromatography and thin-layer chromatography (Merfort, 2002). Among these, HPLC-UV, mainly on reversed phase HPLC was the most applied analytical method using mostly acetonitrile–water or methanol–water gradients, less numerous were data reporting SL analysis performed by mass spectrometry. Sessa et al. (2000) studied chicory SL profile by HPLC-UV and electrospray mass spectral data for each HPLC peak were obtained from analysis of components of individual peaks collected from preparative HPLC. Ferioli and D'Antuono (2012), reported the identification of chicory SLs by HPLC-mass spectrometry using a single quadrupole mass spectrometer and Selected Ion Monitoring (SIM) acquisition. The use of two complementary mass spectrometry techniques such as high resolution mass spectrometry (HRMS) and ion spray triple quadrupole could be very useful for qualitative analysis of target compounds and can be adopted for the identification and quantification of the maximum number of SLs. LC/MS/MS in MRM (Multiple Reaction Monitoring) acquisition allows one to obtain high specificity for each selected analyte, even in complex matrices. The measured compounds are limited to those targeted by MRM events programmed in the method. In addition, quantitative performance decreases with an increasing number of MRM scan events (Nagy, Redeuil, Bertholet, Steiling, & Kussmann, 2009; Sanchez-Rabaneda et al., 2003; Xie, Zhang, Kong, & Rexit, 2011).

Moreover, with Orbitrap a generic full scan method can be used, looking for every compound in the selected scan range. Ion-specific chromatograms are generated for all observed ion signals, using a 5 ppm *m/z* window. The number of compounds that can be detected is virtually unlimited (Scigelova & Makarov, 2006).

In this work, we adopted an innovative analytical technique based on combination of mass spectrometry (MS) techniques, liquid chromatography/electrospray ionization HRMS (LC/ESI-HRMS) and liquid chromatography/electrospray ionization tandem mass spectrometry (LC/ESI-MS/MS) on a triple quadrupole, for the identification and quantification of SLs in four different chicory varieties.

2. Materials and methods

2.1. Plant material and chemicals

The marketable aerial part of four different varieties of chicory "Cicoria Catalogna" (*C. intybus* L. var *dentatum*), radicchio "Rosso di Chioggia", radicchio "Rosso di Treviso Precoce" and radicchio "Rosso di Treviso Tardivo" (*C. intybus* L. *Rubifolium* Group) was considered. Plants were cultivated in Northern-East of Italy (Veneto Region), transplanted on loamy soil in the second half of July following normal agronomical practices. For each chicory type three plots 25 m^2 (5 × 5 m) were singled out and 35 representative plants were uprooted from each of these at harvest time. The harvest was carried out at marketable maturity in November for "Cicoria Catalogna" and in December for radicchio cultivars. After harvest radicchio "Rosso di Treviso Tardivo" was subjected to the usual forcing process before commercialization as reported by Nicoletto and Pimpini (2009, 2010). For all varieties, the marketable material of each plot was cut in pieces, immediately frozen at −80 °C overnight, freeze-dried and ground before extraction. All reagents and solvents of HPLC grade were purchased from Merck (Darmstadt, Germany). Santonin used to quantify SLs was obtained from Sigma (Milano, Italy).

2.2. Extraction of sesquiterpene lactones (SLs)

0.5 g of freeze-dried sample was exactly weighed in a screw-cap plastic centrifuge tube to which was added 100 μg of santonin (internal standard, methanolic solution) and 15 mL of 2% (*v/v*) formic acid in methanol/water 4/1 (*v/v*). The sample was then shaken on a vortex stirrer for 1 min, sonicated at room temperature for 10 min and then centrifuged at 4000 *g* for 10 min. After collection of the supernatant fraction, the extraction procedure was repeated. The extracts were pooled, dried under reduced pressure at 35 °C, subjected to nitrogen flux for 5 min, and recovered with 2 mL of methanol. Before SPE, the SL fraction was centrifuged at 4000 g for 10 min to remove solid particles.

SLs were purified from phenols and other interfering compounds by SPE, employing silica cartridges (3 mL reservoir, 500 mg sorbent mass). The cartridges were conditioned with 6 mL of dichloromethane/isopropanol 1/1 (v/v), equilibrated with 6 mL of dichloromethane and, after sample loading, eluted with 6 mL of dichloromethane/ethyl acetate 3/2 (v/v). Both the loading and elution fractions were collected, dried under reduced pressure at 35 °C, recovered with 1 mL of methanol/water 1/1 (*v/v*), filtered in a HPLC glass vial through a nylon syringe filter (diameter: 13 mm; pore dimension: 0.45 μm) and stored at −18 °C until mass spectrometry analyses.

2.3. LC/MS/MS analysis

Chromatographic separation was performed using an HPLC apparatus equipped with two micropumps Series 200 (Perkin Elmer, CanadaShelton, USA) and Luna C18, 250 × 4.6 mm, 5 μm (Phenomenex, Torrance, CA, USA) (Phenomenex, CA, USA). The solvent system consisted of (A) water and (B) acetonitrile. The gradient program was as follows: 10–42% B (30 min), 10% B (5 min), constant to B (5 min), at a constant flow of 1 mL/min. Injection volume was 20 μL. These conditions were also used for preparative HPLC. MS/MS analyses of SLs were performed on an API 3000 triple quadrupole mass spectrometer (Applied Biosystems, Canada) equipped with a Turbo Ion Spray source. The LC flow was split and 0.2 mL/min was sent to the mass spectrometer. The declustering potential (DP) and the collision energy (CE) were optimized for each compound infusing directly into the mass spectrometer standard solutions (10 μg/mL) at a constant flow rate of 10 μL/min using a model 11 syringe pump (Harvard Apparatus, Holliston, MA, USA).

Analysis was performed in the positive and negative ion mode in MRM (Multiple Reaction Monitoring). Drying gas (air) was heated to 350 °C and the capillary voltage (IS) was set to +5000 V in positive ion mode and −4000 V in negative ion mode. Data acquisition and processing were performed using Analyst software version 1.4.2.

2.4. High resolution mass spectrometry (HRMS) analysis

LC–MS data were acquired on an Accela U-HPLC system coupled to an Exactive mass spectrometer (Thermo Fisher Scientific, San Jose, CA). The Accela system consisted of a quaternary pump, an autosampler and a column oven. The analysis was performed using the same column and chromatographic condition reported in LC/MS/MS section. The injection volume was 10 μL and the partial loop was used as injection technique. The Exactive Orbitrap MS equipped with a heated electrospray interface (HESI) was operated in the full spectral acquisition mode, in the positive and negative ionization mode in the mass range of *m/z* 150–900. The resolving power was set to 25,000 full width at half-maximum resulting in a scan time of 0.5 s. An automatic gain control target was set into high dynamic range, and the maximum injection time was 250 ms.

The interface parameters were as follows: the spray voltage was +3500 V and −3000 V, respectively in positive and negative ion mode, the tube lens was at 20 V, the capillary voltage was 30 V, the capillary temperature was 275 °C, and a sheath and auxiliary gas flow of 45 and 15 arbitrary units were used. Chromatographic data acquisition, peak integration, and quantification were performed using Xcalibur software (Thermo Fisher Scientific, San Jose, USA). Xcalibur software (Thermo Fisher Scientific, San Jose, USA), with a specifically modified Excel macro was used. Quantitative results were obtained using santonin as internal standard.