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Comprehensive two-dimensional gas chromatography with mass spectrometry applied to the analysis of volatiles in artichoke (*Cynara scolymus* L.) leaves

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ABSTRACT

Artichoke (*Cynara scolymus* L.) is well known due to its medicinal properties and, as a result, a large number of studies have been conducted to determine the chemical constituents produced by the plant. However, investigations were mainly focused on the non-volatile compounds, while the volatile constituents remained largely neglected. This study was aimed at obtaining a deeper understanding of the volatile composition of artichoke. For this propose, comprehensive two-dimensional gas chromatography coupled to a rapid scanning quadrupole mass spectrometer (GC × GC/qMS) and retention indices were used to improve the chemical characterization of volatiles from leaves. A total of 130 compounds were found, 109 of which are reported for the first time in *C. scolymus* L., including oxygenated monoterpenes, sesquiterpenes, oxygenated sesquiterpenes, norisoprenoids, lactones, alcohols, ketones and aldehydes. The major compounds were 1-octen-3-one (3.85%), (*E*)-2-hexenal (3.75%), benzene acetaldehyde (2.90%), 2,2-dimethyl-4-pentenal (2.81%), β-ionone (1.94%), furfural (1.65%), (*E*)-β-damescenone (1.59%), α-methyl-γ-butirolactone (1.53%), benzaldehyde (1.47%) and dihydroactinidiolide (1.44%). The comprehensive GC × GC/qMS approach enabled a greater number of analytes to be identified, approximately four times higher than that obtained for GC/qMS. Additionally, the results imply that artichoke leaves are a potential source of volatile bioactive compounds.

1. Introduction

Artichoke (*Cynara scolymus* L.) is a herbaceous perennial crop that originates from the Mediterranean area and is widely cultivated around the world. Since ancient times, artichoke extracts have been used in herbal medicine because of their recognized therapeutic effects. Several studies have demonstrated hepatoprotective, anticarcinogenic, antioxidative, antibacterial, antifungal, anti-HIV, urinative, anticholesterol and glycaemia reduction pharmacological activities (Adzet et al., 1987; Brown and Rice-Evans, 1998; Cairella and Vecchi, 1969; Clifford, 2000; Clifford and Walker, 1987; Gebhardt, 1997; Preziosi, 1969; Rondanelli et al., 2011; Rodriguez et al., 2002; Zhu et al., 2005). Water and polar organic solvents were typically used in these studies. Such a broad range of therapeutic activities was ascribed to several active compounds providing synergistic pharmacological effects (Romano et al., 2005; Zhu et al., 2004).

Because of these diverse beneficial properties, the literature contains numerous studies on non-volatile artichoke constituents, particularly the phenolic compounds (as reviewed by Lattanzio et al., 2009). However, there are few investigations on their volatile constituents. Some researchers have reported that monoterpenes, sesquiterpenes, alcohols, aldehydes and ketones are the main volatile constituents of the leaves and heads of *C. scolymus* L. (Buttery et al., 1978; Ghanem et al., 2009; Guillén-Ríos et al., 2006; Hădărugă et al., 2009; MacLeod et al., 1982; Nassar et al., 2013).

Plant volatiles are usually complex samples that contain hundreds of compounds, including alcohols, aldehydes, esters, ketones, phenols, lactones, phenylpropanoids and terpenoids (Brielmann et al., 2006; Rowshan et al., 2013). One-dimensional gas chromatography (1D-GC) has been used for many years as the standard tool for separating volatiles from plants. However, obtaining the best component separation via 1D-GC is difficult due

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to the insufficient resolution power of a single column (Purcaro et al., 2009). This problem persists despite the continuous development of chromatographs, techniques and analytical methodologies (Mateus et al., 2010). Overlapping peaks usually significantly complicate compound identification and accurate quantification (Zhu et al., 2007). In addition, plant volatiles are present in a wide range of concentrations, and the trace analytes that are occasionally the biologically active matrix components may not be detected, particularly if they are co-eluted with high concentration compounds (Mateus et al., 2010).

Comprehensive two-dimensional gas chromatography (GC × GC) has emerged as a powerful separation technique for overcoming these limitations and is widely used to characterize complex samples. GC × GC uses two orthogonal mechanisms to separate the sample constituents through two columns connected in series with different stationary phases and a transfer device, defined as the modulator. The modulator continuously isolates, reconcentrates, and introduces small portions of the primary column effluent to a secondary column. The time required to complete this process is defined as the modulation period (Marriott et al., 2012; Mondello et al., 2008). The chromatographic resolution is greatly enhanced by this technology (Marriott et al., 2004).

The detectors used in GC × GC represent an additional challenge because high spectral acquisition rates are required for correct peak assignment and quantification (Adahchour et al., 2005). The time-of-flight mass spectrometer (TOFMS) can be used to obtain such data; however, its high cost limits its laboratory utilization (Mondello et al., 2005). In contrast, a quadrupole mass spectrometer (qMS) is much less expensive and more user-friendly, and several authors have reported the use of qMS hyphenated to GC × GC (Adahchour et al., 2008; Mondello et al., 2008). Recent studies have shown the effectiveness of GC × GC in combination with qMS operating in the rapid scanning mode to achieve satisfactory data acquisition rates (Cordero et al., 2007; Purcaro et al., 2010; Tranchida et al., 2013).

The aim of this study was to investigate the volatile constituents of artichoke leaves via comprehensive two-dimensional gas chromatography coupled to a rapid scanning quadrupole mass spectrometer. The linear temperature programmed retention indices (LTPRI) were used to confirm the peak assignments. To the best of the authors' knowledge, this is the first report detailing volatile compounds in artichoke leaves using GC × GC.

2. Materials and methods

2.1. Plant material

Artichoke (*C. scolymus* L.) was collected in the municipal district of Riozinho (29°38′27″ S and 50°27′10″ W), Rio Grande do Sul State, Brazil, in February 2011 and identified by the specialist botanist Dr. Eduardo Pasini (Bio-sciences Institute, Department of Botany, UFRGS, Rio Grande do Sul, Brazil). A voucher specimen (ICN: 166985) was deposited in the Herbarium of UFRGS, Rio Grande do Sul, Brazil. The leaves were dried at 35 °C until their weight was constant, and they were then stored in dark bags to protect them from humidity and light.

2.2. Chemicals and solvents

All of the solvents and standards (linear alkanes) used were purchased from Sigma–Aldrich (St. Louis, MO, USA). All water was purified using a Milli-Q system (Millipore, Bedford, MA, USA). Other unmarked reagents were of analytical grade.

2.3. Volatile compounds extraction

Dried artichoke leaves (100 g) were hydrodistilled for 4 h using a Clevenger-type apparatus following the method recommended by the Brazilian Official Pharmacopoeia V (2010). The obtained distillate was extracted with dichloromethane and dried over anhydrous sodium sulfate. The organic layer was transferred into dark vials and stored at 4 °C until its analysis.

2.4. GC/qMS analysis

The GC/qMS measurements were performed on a Shimadzu GC/qMS system, consisting of a GC2010 Gas Chromatograph and a QP2010 Plus Mass Spectrometer (qMS) (Kyoto, Japan). The GC was equipped with an AOC-20i auto-injector (split/splitless). The separation was performed on a ZB-5MS (5% phenyl, 95% dimethylpolysiloxane) column with 60 m length × 0.25 mm I.D. × 0.25 μm film thickness (Phenomenex, Torrance, CA, USA). The GC oven started at 40 °C, was heated at 2 °C/min to 300 °C, and then maintained for 20 min. The injector temperature was 300 °C, and the injection was performed in the splitless mode using 0.5 μL. High purity helium (99.99%, Linde Gases, Canoas, Porto Alegre, RS, Brazil) at a flow rate of 0.91 mL/min was used as the carrier gas. The interface and ion source temperature was 300 °C. The mass spectrometer operated in the electron impact mode (EI) at 70 eV and scanned from 40 to 500 *m/z* in a full scan acquisition mode. The data were acquired using GCMS-solution software version 2.6 (Shimadzu, Kyoto, Japan). The mass spectrum of each detected compound was compared with those in the NIST-05 mass spectral library, using similarity matches of at least 80% for identification. This identification was supported by the experimentally determined linear temperature programmed retention index (LTPRI) values and compared with the values reported in the bibliography when available (Adams, 2007; NIST 11, 2013). The LTPRI values were determined using a C_6–C_{30} *n*-alkane series and calculated from the van den Dool and Kratz equation (van den Dool and Kratz, 1963). The relative amounts (%) for each individual component in the sample were expressed as percent peak areas relative to the total peak area.

2.5. GC × GC/qMS analysis

The GC × GC/qMS was performed using a Shimadzu GC × GC system consisting of a GC2010 gas chromatograph and a QP2010 Ultra mass spectrometer (qMS) (Kyoto, Japan). The GC was equipped with an AOC-20i auto-injector (split/splitless) and dual-stage loop-type modulator ZX1-GC × GC (Zoex Corporation, Houston, TX, USA). Cryogenic modulation occurred every 5 s, with a hot jet duration of 0.5 s. The first-dimension chromatographic separation was performed on a OV-5 (5% phenyl, 95% dimethylpolysiloxane) column with 60 m length × 0.25 mm I.D. × 0.10 μm film thickness (Ohio Valley Specialty Company, Marietta, OH, USA). A DB-17 (50% phenyl and 50% dimethylpolysiloxane) column with 2.15 m × 0.18 mm × 0.18 μm (J&W Scientific, Agilent Technologies, Palo Alto, CA, USA) was used as the second dimension. The GC conditions are the same as for GC/qMS. The data were acquired using the GC Image software version 2.2 (ZOEX Corporation, Houston, TX, USA). The identification methods were the same as those used previously. The relative amounts (%) for each individual component in sample were expressed as the percent of the peak volume relative to the total peak volume. The sum of the retention time from the first and second dimensions were used to identify each peak. This procedure was validated by von Mühlen et al. (2008).