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Simultaneous HPLC-ELSD determination of sugars and cyclitols in different parts of *Phacelia tanacetifolia* Benth.

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ABSTRACT

Three cyclitols (*allo*-inositol, *scyllo*-inositol and myo-inositol) and seven sugars (xylose, D-mannose, D-glucose, sucrose, D-(+)-turanose, D-(+)-melezitose monohydrate and D-(+)-raffinose pentahydrate) were determined in different morphological parts of lacy phacelia by high performance liquid chromatography-evaporative light scattering detector (HPLC-ELSD) method in 30 min. The applied analytical method is rapid, simple, sensitive, low cost, reliable and suitable for profiling of major sugars and cyclitols in *Phacelia tanacetifolia* Benth. (lacy phacelia) parts. Pressurized liquid extraction and solid phase extraction were used as extraction and purification methods. The obtained results showed significant differences in the quantities and distribution of cyclitols and sugars found in the investigated morphological parts. The quantities of the identified cyclitols in lacy phacelia ranged between 1.07 and 4.68 mg/g of dried plant, while for sugars in the range of 1.07–25.3 mg/g. However, flowers were richer in cyclitols and sugars compared to the seeds.

1. Introduction

Plants are an important source of many natural bioactive compounds which have many proved health-promoting activities like antioxidant, antibacterial, antihypertensive, anti-inflammatory ones etc. Cyclitols are one of the most widely occurring groups of phytochemicals, which are secondary-metabolites in plants and play an important role in cell functioning and self-defense against unfavorable environmental conditions such as salt or water stress (Das-Chatterjee et al., 2006). Additionally, several researchers reported that this group of phytochemicals possesses a variety of biological activities such as antioxidant (Sivakumar and Subramanian, 2009), anti-inflammatory (Wu et al., 2015), anti-diabetic (Hernández-Mijares et al., 2013) and anti-cancer (Shamsuddin et al., 1996). Among other important groups of phytochemicals found in plants are sugars. Sugars are an essential component of plant nutrition, play an important role in plant growth and development, and play a key role in the cell-wall structure. In addition, they play a significant role in protecting or stimulating the immune system against various disorders and act as osmoregulators in maintaining physiological functions of the plant under different abiotic stresses such as water deficiency, saline water, or soils with high salt concentrations (Gómez-González et al., 2010). In food science, although the main reason for the use of sugar is its sweet taste, sugars have many other functions. Sugar added to food acts as a sweetener, preservative, texture modifier, fermentation substrate, flavoring and coloring agent, or bulking agent (Koivistoinen and Hyvönen, 1985).

Phacelia tanacetifolia Benth. commonly known as lacy phacelia or blue tansy, belongs to the Boraginaceae family. It is a flowering annual herb which grows to a maximum height of nearly 100 cm and has high carbohydrate and protein content (Ates et al., 2014; Daniel and Zobelt, 1986). It is native to the southwest region of the United States and Mexico, but it is now used extensively in Europe in agriculture as a cover crop (catch crop), green manure for protecting against soil erosion, for intercropping for its bio-control benefits as well as bee forage as honey bee plant (Howes, 1945; Stevenson, 1991; Yakimovich et al., 2017). *Phacelia* is listed among the top 20 pollen plants that produce flowers for honeybees, and is very attractive to pollinator insects, including bees and hoverflies (Petanidou, 2003). The honey of phacelia has been demonstrated to have healing properties such as antibacterial, anti-Staphylococcal, antifungal and anti-inflammatory, and therefore, it is used for treating inflammation of the mouth and throat (Grecka et al., 2018; Popović et al., 2017).

The traditional extraction methods such as maceration and Soxhlet extraction were the first methods used for extraction of soluble sugars

Abbreviations: ASE, Accelerated solvent extraction; ELSD, evaporative light scattering detector; PLE, pressurized liquid extraction; MAE, microwave-assisted extraction; SFE, supercritical fluid extraction; SPE, Solid phase extraction; UAE, ultrasound assisted extraction

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and cyclitols from the plant material using proper solvent like water, methanol and/or ethanol. Nowadays, different modern extraction procedures (green techniques) such as pressurized liquid extraction (PLE) or accelerated solvent extraction (ASE), supercritical fluid extraction (SFE), ultrasound assisted extraction (UAE) and microwave assisted extraction (MAE), have been developed for isolation of various bioactive compounds including cyclitols and sugars (Al-Suod et al., 2017; Ratiu et al., 2018). The new techniques have gained an increasing interest over the last decades and have almost completely replaced traditional procedures. These techniques offer reduction of extraction times, lower solvent consumption, lower energy consumption and higher extract quality and quantity (Al-Suod et al., 2017).

Pressurized liquid extraction (PLE) is one of the most commonly used green techniques for cyclitols extraction (Raks et al., 2018; Ratiu et al., 2018; Ruiz-Aceituno et al., 2014), because of its high efficiency, inexpensive equipment, simple operation and high selectivity when applied in proper conditions. The principle of PLE is simple and based on using solvents under high temperature and pressure without reaching the critical point. Different research has demonstrated the potential of PLE in extraction of cyclitols from different plant materials such as pine nuts, oak wood and mulberry leaves (Alañón et al., 2009; Rodrìguez-Sánchez et al., 2013; Ruiz-Aceituno et al., 2014).

Different analytical techniques have been applied in analysis of cyclitols and sugars. Among of them, gas chromatography (GC) and liquid chromatography (LC) in combinations with different detectors are the two methods most commonly used for identifying and quantifying these compounds (Ligor et al., 2018). Matrix-assisted laser desorption ionisation technique with time-of-flight and mass spectrometry (MALDI-TOF-MS) has been applied for the first time for identification of three cyclitols in different parts of alfalfa (Al-Suod et al., 2018a,b). Other methods including nuclear magnetic resonance (NMR) and infrared (IR) have also been used (Jawla et al., 2013).

To the best of our knowledge, there is no report on determination of sugars and cyclitols in lacy phacelia. The aim of the present work was the using of HPLC-ELSD for determination the profiles of sugars and cyclitols isolated for the first time by pressurized liquid extraction (PLE) from different morphological parts of lacy phacelia.

2. Material and methods

2.1. Chemicals and equipment

Standards (*allo*-inositol, *scyllo*-inositol, D-pinitol, D-*chiro*-inositol, *myo*-inositol, xylose, D-fructose, D-mannose, D-glucose, D-(+)-turanose, and D-(+)-melezitose monohydrate, D-(+)-raffinose pentahydrate) with purity ≥95% were all purchased from Sigma–Aldrich (St. Louis, MO, USA). Sucrose (purity ≥98%) was purchased from Avantor (Gliwice, Poland). Methanol (HPLC grade, ≥99.9) was obtained from Sigm–Aldrich (Steinheim, Germany). Acetonitrile (HPLC grade, ≥99.9) was purchased from J.T. Baker (The Netherlands). HPLC-grade ammonium formate was purchased from Tedia (USA). Ultra-pure water was obtained from a Milli-Q water system (Millipore, Bedford, MS, USA). CHROMABOND® C18 ec cartridges (Macherey-Nagel, Düren, Germany) and OASIS®HLB cartridges (Waters, Milford, Massachusetts USA) were used for solid phase extraction (SPE).

2.2. Plant samples collection

Leaves, stems, flowers, seeds and roots of lacy phacelia were collected from an agricultural field, without the use of any cultivation treatment in Bobrowniki, Kuyavian-Pomeranian Voivodeship, Poland. Plants were harvested from agricultural meadows in August 2016. The collected plant samples were dried in shade at room temperature for two weeks, grounded with a laboratory mill to fine powder, and stored in glass vials, in darkness.

2.3. Extraction of soluble sugars and cyclitols

All the assays were carried out using Dionex ASE 350 system (Thermo Scientific, Waltham, MA, USA) equipped with an auto-sampler carousel and a collection tray that allowed for sequential extraction of 24 samples. Portions of 500 mg of lacy phacelia powdered samples (leaves, stems, flowers, seeds and roots) were weighed successively, placed in a 10 mL stainless steel extraction cell and mixed with sea sand in a 1:2 ratio to avoid sample compaction. The extraction was performed at 50 °C, 10 MPa, in three cycles (18 min each one), according to the Ruiz-Aceituno et al., 2014 procedure, which found these parameters to be the most suitable for inositols extraction. Water was used as a solvent in this experiment due the results of our previous work (Ratiu et al., 2018), where water was demonstrated to be the most efficient solvent for extraction of sugars and cyclitols.

2.4. Sample preparation

A sequential solid-phase extraction method was applied in order to achieve a group separation (fractionation) of the complex mixture of compounds. For this purpose, CHROMABOND® C18ec and OASIS®HLB SPE cartridges were used to eliminate non-polar compounds, phenolic compounds and other possible interferents of phacelia extracts to clean-up sugars and cyclitols (Al-Suod et al., 2018a,b; Başkan et al., 2016; Loos et al., 2003; Pérez-Magariño et al., 2008). This process was carried out in two stages. In the first step for eliminating chlorophyll and some phenolic compounds, the water extract was passed through a C18 SPE cartridge. Phenolic compounds (especially flavonoids) in the sample were retained on the column while sugars and cyclitols passed. The cartridge was then washed with 3 mL of water. Followed by a combination of the column outflow (A) and wash water (B). This solution contained sugars, cyclitols and other unretained compounds. In the second step, the combined solution was passed through OASIS®HLB SPE cartridge (Fraction C) in order to retain other phenolic compounds and phenolic acids not retained by the C18 column. The cartridge was then washed with 3 mL of water (Fraction D) to make sure that all sugars and cyclitols were eluted from the column. Finally, fraction C and D were combined in a vial. For HPLC analysis, 100 μL of faction (C + D) was diluted with methanol in (1:1 v/v) ratio and 10 μL was injected into the HPLC-ELSD system in duplicate. Both the C18 and HLB sorbents were activated and conditioned first with 5 mL methanol and next with 5 mL water. All these procedures are summarized in Fig. 1.

2.5. HPLC instrumentation and chromatographic conditions

All analytical measurements were performed using an Agilent 1260 Infinity Series LC system (Agilent Technologies, Germany) consisting of a gradient pump, a degassing system, a autosampler, a thermostated column and coupled with an Agilent evaporative light scattering detector (ELSD-380) (Agilent Technologies, UK). The data were acquired and quantified with the use of OpenLab software. The stationary phase used for the separation was a Silec Obelisc N (150 × 4.6 mm, 5 μm; 100 Å) column from (Bujno Chemicals, Warsaw, Poland) kept at temperature of 35 °C. Chromatographic separation was performed by a gradient elution with (A) 10 mM Ammonium formate (pH = 3.0) and (B) acetonitrile (ACN) and the gradient program was as follows: 15% A, held for 10 min, and then a linear gradient increasing to 25% A for 10–15 min and isocratic at 25% A for 15–20 min. At 21 min the gradient was programmed to initial conditions (15%A) to re-equilibrate the column for 9 min, giving a total cycle time of 30 min. Obelisc N has anions close to the surface separated from cationic groups by a hydrophilic chain. This column can work in three different systems: the reverse phase (RP), the normal phase and HILIC. Mobile phase composition changes the conformation of the long hydrophilic chain. In our research, we used the column Obelisc N and aqueous ammonium formate for the separation of sugars and cyclitols. PH, low buffer