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Subcritical water extraction as a cutting edge technology for the extraction of bioactive compounds from chamomile: Influence of pressure on chemical composition and bioactivity of extracts

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ABSTRACT

The study was designed to determine the relationship between chemical structure, bioactivity and pressure during the subcritical water extraction (SCW) of chamomile. Extraction was carried out at isothermal conditions (100 °C) at five different pressures (10, 30, 45, 60 and 90 bar). Twenty three polyphenolic compounds were identified in the extracts, whereby apigenin was found to be the dominant compound (61.53–1344.99 mg/kg). Results suggest that the lowest applied pressure has negligible effect on phenolic recovery from chamomile, but also the use of pressures above than 45 bar was proven as needlessly. By using *in vitro* assays, influence of pressure on antioxidant, cytotoxic and enzyme-inhibitory activities of the extracts was evaluated. Extracts obtained at 30, 45 and 60 bar exhibited stronger bioactivities than at 10 and 90 bar. It was concluded that pressure exert a significant influence on chemical composition of extracts, and thus on biological activity of chamomile extracts.

1. Introduction

German chamomile is one of the most popular and well documented plants in the world. Its flower-heads are usually used in the form of tea, tisane and herb beer (McKay & Blumberg, 2006). Additionally, fresh or dried chamomile flowers are used in soups and salads to improve their flavour and enhance nutritional value (Das, 2014). Due to its aromatic and pleasant taste, the plant is used in different foodstuffs, confectionery, alcoholic/nonalcoholic beverages, candies, jellies and tobacco (Furia & Bellanca, 1975; Gašić, Lukić, Adamović, & Đurković, 1989). Essential oil from chamomile can be used as food colorant as well (Emongor, Chweya, Keya, & Munavi, 1990; Mann & Staba, 1986). Proven antibacterial, antiviral, antifungal and antioxidant activities (Cvetanović, Švarc-Gajić, Zeković, et al., 2015; Cvetanović, Švarc-Gajić, Mašković, Savić, & Nikolić, 2015; Franke & Schilcher, 2005; Lis-Balchin, Deans, & Eaglesham, 1998; McKay & Blumberg, 2006), make chamomile extracts an interesting ingredient for functionalizing foods. Caleja et al. (2015) showed that the incorporation of phenolic rich chamomile extracts into cheese can increase nutritional value of such product. Moreover, chamomile extracts could be used as food preservatives, since their polyphenols inhibit strong antioxidant activity.

Phenolics isolated from chamomile have been investigated extensively within the last 10 years (Cvetanović, 2016) and different extraction methods have been used for their isolation. The most commonly used techniques required ethanol, methanol, chloroform or other organic solvents (Haghi, Hatami, Safaei, & Mehran, 2014; Srivastava, Pandey, & Gupta, 2009; Srivastava, Shankar, & Gupta, 2010). However, in order to ensure safe and healthy product it is necessary to avoid usage of such solvents. From this perspectives, utilization of green and safe extraction techniques is of utmost importance. In light of the above challenges, subcritical water extraction (SCW) offers numerous advantages being a completely green and economically viable technique whereby its selectivity can be drastically varied and optimised.

Basically, SCW is a technique based on the extraction with hot water at temperatures over its boiling point and below its critical point while maintaining high pressures in order to keep the water in a liquid state

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during the whole extraction process (Adachi, 2009). These conditions alter physicochemical properties of water, influencing its solvating properties. The selectivity of subcritical water can be tuned owing to changes in the polarity of pressurized water with the extraction conditions. Temperature affects greatly water polarity, but also viscosity, surface tension and density. The influence of pressure in SCW is generally considered to be of minor influence (Cvetanović et al., 2017). Beside its major role, to keep water in its liquid form, elevated pressure allows better filing of the matrix pores with the solvent. Application of high pressures also causes disruption of matrix cells resulting in enhanced mass transfer processes. In addition, elevated pressures help to control the problems related to the existence of air bubbles within the matrix. Presence of air bubbles has a negative influence on mass transfer hindering the solvent to reach desired compounds (Mustafa & Turner, 2011). Such conditions often boost solubility of the compounds and desorption kinetics as well. On the other hand, studies on evaluation of the chemical composition of SCW extracts as a function of pressure have not been reported. Unlike temperature, which influence on extraction efficiency in SCW has been well investigated, the detailed influence of pressure is still unknown.

SCW of phenolic compounds from chamomile has been reported in the literature where advantages of using this novel extraction approaches have been demonstrated. Furthermore, it has been reported that extraction of chamomile under mild subcritical conditions could be excellent tool for phenolic isolation, even better than some other extraction techniques (ultrasound, microwave and Soxhlet). Additionally, SCW extracts showed improved antimicrobial, anticancer and antioxidant activities (Cvetanović, Švarc-Gajić, Mašković, et al., 2015). This could be of particular importance for the design of new functional foods in which chamomile could be used as additive or preservative. In order to use the full potential of the process, understanding the relationship between extraction parameters and chemical composition of extracts are necessary. Available literature offer data only in terms of total phenols and total flavonoids (measured spectrophotometrically). Cvetanović et al. (2017) studied the influence of pressure on the yield of apigenin. The authors reported a 90% increase in apigenin yield and more than 100% in total phenolics by changing the pressure from 10 to 45 bar. However, except for the apigenin, pressure influence on individual phenolic compounds from chamomile has not been reported. Moreover, there is dearth of data about its influence on the biological activity of plant extracts.

The main objective of this work was to explore the influence of the pressure on the chemical composition of chamomile extracts in SCW. Likewise, we endeavoured to define the relationship between this parameter and bioactivity of the extracts. The obtained data could be used to fully characterize the mechanisms involved in the SCW of polyphenols and further to produce chamomile extracts with improved bioactivity. Such extracts could be further used not only in a plethora of food products but also in pharmaceutical or cosmetical products.

2. Material and methods

2.1. Chemicals and reagents

Kojic acid, 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid), ascorbic acid, trichloroacetic acid, iron(II) sulfate hydrate, butylated hydroxytoluene, ferrozine, sodium dodecyl sulfates (SDS), essential medium (MEM) eagle, 2-deoxy-D-ribose, α-amylase solution (ex-porcine pancreas, EC 3.2.1.1), α-glucosidase solution (from Saccharomyces cerevisiae, EC 3.2.1.20), L-glutathione, tyrosinase, 3,4-Dihydroxy-L-phenylalanine (L-DOPA), acarbose and polyphenolic standards (analytical grade; purity ≥99%) were purchased from Sigma-Aldrich (St. Louis, MO, Missouri, USA). PNPG (4-*N*-trophenyl-α-D-glucopyranoside), methanol (HPLC grade) acetonitrile and acetic acid (both of MS grade) were purchased from Merck Co (Darmstadt, Germany). Potassium ferricyanide and ferric chloride were obtained from Zorka (Šabac, Serbia). Etylenediaminetetraacetic acid (EDTA) was purchased from Centrohem (Stara Pazova, Serbia). *Cis*-diamminedichloroplatinum (*cis*-DDP) was purchased from Tedia Company (USA). MTT reagent (3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyltetrazolium bromide) was obtained from Fluka (Buchs, Switzerland). Dulbecco's modification of Eagle's medium was obtained from Alfa Aesar (Karlsruhe, Germany). Ultrapure water (Thermofisher Scientific, Bremen, Germany) was used to prepare standard solutions and blanks. Syringe filters (13 mm, PTFE membrane 0.45 μm) were purchased from Supelco (Bellefonte, PA, USA). All other chemicals and reagents were analytical reagent grade.

2.2. Plant material

Plant material was produced by the Institute of Field and Vegetable Crops, Bački Petrovac, Serbia. Taking into account that phenolic compounds are mainly contained in chamomile ligulate flowers (CLF), the extractions were performed with these parts of chamomile flos. Chamomile flos was collected in the end of April and were dried at the temperature of 40 °C. The process of drying was performed until the moisture content of 12%. Thereafter, CLF were separated from the tubular parts by sieving, packed in paper bags and stored in the dark until further use.

2.3. Subcritical water extraction

Subcritical water extraction of CLF was performed using homemade subcritical water extractor/reactor previously described (Cvetanović et al., 2017). A sample (10.0 g) was placed in reaction vessel and 300 mL of double distilled water was added. Extraction was performed within 30 min after the temperature was reached. Temperature (100 °C) was kept constant during the all extraction runs, while five different pressures were used (10, 30, 45, 60 and 90 bar). The mass transfer process was increased by stirring process which also prevented local overheat on the inner walls of extractor. After filtration, obtained liquid extracts were evaporated by vacuum evaporator (Devarot, Slovenia) and dried at 40 °C. Dry extracts, thus obtained, were stored in the dark place at 4 °C until analysis.

2.4. UHPLC–DAD MS/MS analysis of polyphenolic compounds

The separation, determination and quantification of the polyphenolic compounds in SCW extract were performed using a Dionex Ultimate 3000 UHPLC system equipped with a diode array detector (DAD) that was connected to TSQ Quantum Access Max triple-quadrupole mass spectrometer (ThermoFisher Scientific, Basel, Switzerland). The elution was performed at 40 °C on a Syncronis C18 column (100 × 2.1 mm, 1.7 μm particle size) from ThermoFisher Scientific. The mobile phase consisted of water + 0.01% acetic acid (A) and acetonitrile (B), which were applied in the following gradient elution: 5% B in the first 2.0 min, 2.0–12.0 min 5–95% B, 12.0–13.0 min from 95% to 5% B, and 5% B until the 20th min. The flow rate was set to 0.3 mL/min and the detection wavelengths to 254 and 280 nm. The injection volume was 5 μL.

Stock methanolic solutions of polyphenolics in the concentration of 1000 mg/L were prepared. The stock solutions were mixed and diluted with water in order to obtain working solutions (concentrations of 0.01, 0.05, 0.10, 0.25, 0.50, 0.75, and 1.00 mg/L).

A TSQ Quantum Access Max triple-quadrupole mass spectrometer equipped with an heated electrospray ionization (HESI) source was used with the vaporizer temperature kept at 250 °C, and the ion source settings as follows: spray voltage 4500 V, sheet gas (N_2) pressure 27 AU, ion sweep gas pressure 0 AU and auxiliary gas (N_2) pressure 7 AU, capillary temperature 275 °C, skimmer offset 0 V, and capillary offset −35 V. The mass spectrometry data were acquired in the negative ionization mode, in the *m/z* range from 100 to 1000. Multiple mass