Industrial Crops and Products 83 (2016) 359–363

Contents lists available at ScienceDirect

Industrial Crops and Products

journal homepage: www.elsevier.com/locate/indcrop

Optimization of ultrasound-assisted extraction and LC-ESI–MS/MS analysis of phenolic acids from *Brassica oleracea* L. var. *sabellica*

Anna Oniszczuk[a,*], Marta Olech[b]

[a] Department of Inorganic Chemistry, Medical University of Lublin, Chodźki 4a, 20-093 Lublin, Poland
[b] Department of Pharmaceutical Botany, Medical University of Lublin, Chodzki 1, 20-093 Lublin, Poland

ARTICLE INFO

Article history:
Received 9 July 2015
Received in revised form 4 January 2016
Accepted 9 January 2016
Available online 25 January 2016

Keywords:
Ultrasound-assisted extraction
Phenolic acids
Brassica oleracea L. var. *sabellica*
High performance liquid chromatography–mass spectrometry

ABSTRACT

Vegetables belonging to the *Brassica oleracea* group are an important source of bioactive metabolites, especially phenolic acids and flavonoids. A literature search did not yield any reference about earlier reports on the ultrasound-assisted extraction (USAE) of phenolic compounds from *B. oleracea* L. var. *sabellica* (kale). Therefore, the aim of this review is to optimize USAE for LC-ESI–MS/MS analysis of these compounds from kale.

In the experiment four solvents, (ethanol and ethanol, 80% aqueous ethanol, methanol and 80% aqueous methanol) and two extraction times, (40 and 60 min) with ultrasound frequency 20 kHz and power 100 W, were examined. The most effective conditions for the isolation of phenolic acids are 80% aqueous ethanol and extraction time 60 min. USAE can offer high yield of analyzed compounds in short time, simple manipulation and reduced volume of solvent. Techniques used in experiment proved to be repeatable methods, as evidenced by the RSD% values.

1. Introduction

Brassica genus (family *Brassicaceae*) belongs to 37 species and originates from the regions between the Mediterranean and Saharan (Gómez-Campo, 1980). Annuals and biennials, belong to the genus, ranging from weedy, wild plants to domestic crops (Card et al., 2015; Rakow, 2004). Some species also grow as invasive weeds in the Americas (North and South) and Australasia. Many crops from *Brassica* are vegetables cultivated throughout the world, for example: *Brassica carinata* (cabbage), *Brassica napus* (rape), *Brassica narinosa* (spinach mustard), *Brassica oleracea* (cauliflower, broccoli, brussels sprout, kale) (Stuart et al., 2015).

Vegetables belonging to the *B. oleracea* group have attracted much attention because of their potential application on physiological functioning (Hafidh et al., 2013). They are known for their abundant supply of health-promoting substances that reduce the risk of diseases (Park et al., 2014). These vegetables are an important source of bioactive metabolites, especially phenolic acids and flavonoids (Fiol et al., 2012; Vale et al., 2015) as well as vitamin C, E, glucosinolates, anthocyanidins, carotenoids and amino acids (Mattila and Hellstrom, 2007; Park et al., 2014).

B. oleracea L. var. *sabellica* (kale) is a leafy crop grown mainly in Central and Northern Europe and North America (Neugart et al., 2013). This plant is rich in hydroxycinnamic acid and hydroxybenzoic acid derivatives as well as flavonol glycosides (Cartea et al., 2011; Olsen et al., 2009; Schmidt et al., 2010). The main glycosides are quercetin and kaempferol derivatives (Schmidt et al., 2010). They could be acylated with *p*-coumaric acid, caffeic acid, ferulic acid, hydroxyferulic acid and sinapic acid.

Phenolic acids are an aromatic secondary plant metabolites. These are very important compounds due to their antibacterial, antiviral, anti-inflammatory and anti-vasodilatory properties (Duthie et al., 2000). A large number of studies revealed that the hydroxycinnamic acid derivatives were considered to can be strong antioxidant agents (Mattila and Hellstrom, 2007). Furthermore they are applied in the prevention of cancer and cardiovascular diseases (Luthria and Mukhopadhyay, 2006; Ness and Powles, 1997). In general, the aims of extraction of plant metabolites are isolation and identification of the natural compounds that positively correlate to a human's health (Fiol et al., 2012).

Different extraction methods have been used for extracting phenolic acids from herbal medicine, including: maceration (Diouf et al., 2009), Soxhlet extraction and heat reflux extraction (Jun et al.,

* Corresponding author. Fax: +48 81535 7378.
E-mail address: anoniszczuk@o2.pl (A. Oniszczuk).

http://dx.doi.org/10.1016/j.indcrop.2016.01.015
0926-6690/

2011; Waksmundzka-Hajnos et al., 2007), microwave-assisted extraction (Dahmoune et al., 2014; Dahmoune et al., 2015) and accelerated solvent extraction (Luthria, 2008; Oniszczuk et al., 2014; Oniszczuk and Podgórski, 2015). The extraction of phenolic acids from several *Brassicaceae*, including Brussels sprouts, cauliflower broccoli (Podsedek, 2007), cabbage (Kaulmann et al., 2014; Park et al., 2014), and broccoli (Kaulmann et al., 2014; Podsedek, 2007) were examined in a number of recent studies. A few of these also include kale (Fiol et al., 2012). Available literature did not yield any references about earlier reports on the ultrasound-assisted extraction (USAE) of phenolic compounds from *B. oleracea* L. var. *sabellica*. Therefore the aim of this review is to optimize USAE for LC-ESI–MS/MS analysis of these compounds from kale.

2. Materials and methods

2.1. Chemicals and instruments

Standards of phenolic acids were purchased from Sigma–Aldrich Fine Chemicals (St. Louis, MO, USA). All the chemicals were of analytical grade. LC grade methanol (MeOH) was purchased from J.T. Baker (Phillipsburg, USA). LC grade water was prepared using a Millipore Direct-Q3 purification system (Bedford, MA, USA).

2.2. Plant materials

B. oleracea L. var. *sabellica* was purchased from "*Klasa*" industrial (Kurów, Poland). Plant was dried in the convection dryer at average temperature 39.0 °C Before the extraction the dry plant material was milled and sieved.

2.3. Extraction procedures

Ultrasound assisted extraction was carried out in an ultrasonic bath (J.P. Selecta, Barcelona, Spain; frequency 20 kHz, power 100 W) with a thermostat. The process was performed with 2 g of ground sample at constant temperature 60°C with 40 mL of solvent in each cycle. Four different solvent systems (ethanol, methanol, 80% aqueous ethanol and 80% aqueous methanol) and two extraction times (40 min – two cycles for 20 min and 60 min – two cycles for 30 min) were evaluated for the extraction of phenolic acids from kale. Extracts were filtered, combined and evaporated to dryness. The residues were dissolved in 10 mL of methanol. The whole procedure was repeated three times for each sample. The extracts were then filtered through a 0.45 μm nylon syringe filter (Millex-HN, Ireland) before chromatographic analysis.

2.4. HPLC-ESI–MS/MS analysis of phenolic acids

The samples were analyzed according to the method previously described by Nowacka et al. (2014) with some modifications. For chromatographic separation, an Agilent 1200 Series HPLC system (Agilent Technologies, USA) equipped with a binary gradient solvent pump, a degasser, an auto-sampler and column oven was used. Samples were separated on a Zorbax SB-C18 column (2.1 × 50 mm, 1.8-μm particle size; Agilent Technologies, USA) maintained at 25 °C, using 3 μl injections. The solvents used were: water containing 0.1% HCOOH (solvent A) and methanol containing 0.1% HCOOH (solvent B). The following gradient elution program at a flow rate of 400 μL min^{-1} was applied: 0–1 min—5% B; 2–4 min—20% B; 8–9.5 min—70% B; 11.5–15 min—5% B. MS detection was performed in a 3200 QTRAP Mass spectrometer (AB Sciex, USA) (Applied Biosystems, Darmstadt, Germany) equipped with an electrospray ionisation source (ESI) and a triple, quadrupole-ion trap mass analyzer that was controlled by the Analyst 1.5 software. ESI worked in the negative-ion mode and the optimum values of the source parameters were: capillary temperature 400 °C, curtain gas 30 psi, nebulizer gas 60 psi, source voltage −4500 V. Nitrogen was used as curtain and collision gas. For each compound, the optimum conditions of Multiple Reaction Mode (MRM) were determined in the infusion mode. Triplicate injections were made for each standard solution and sample. The analytes were identified by comparing retention time and m/z values obtained by MS and MS^2 with the mass spectra from corresponding standards, tested under the same conditions. The calibration curves obtained in MRM mode were used for quantification of all analytes. The identified phenolic acids were quantified on the basis of their peak areas and comparison with a calibration curve obtained with the corresponding standards. Linearity ranges for calibration curves were specified.

The limits of detection (LOD) and quantification (LOQ) for phenolic compounds were determined at a signal-to-noise ratio of 3:1 and 10:1, respectively, by injecting a series of dilute solutions with known concentrations.

3. Results and discussion

The goal of the presented study was to determine the effects of different factors of ultrasound treatment on the extraction yield of phenolic acids from kale. This method can offer high yield of analyzed compounds in short times, simple manipulation, reduced volume of solvent, lower energy input, high reproducibility and meets the requirements of "Green Chemistry (Chemat et al., 2008). The enhancement of extraction efficiency of organic compounds by ultrasound is attributed to the cavitation. Cavitation can cause locally high temperatures and pressures which may accelerate isolation of extracted compounds (Khan et al., 2010; Rostagno et al., 2003; Sun et al., 2011). Moreover, ultrasound can penetrate the matrix material, rupturing the cell walls (Wang and Weller, 2006) resulting in extracted compounds being more easily released from the matrix into the extraction medium (Sun et al., 2011).

Several parameters that could potentially affect the extraction efficiency were evaluated and optimized. In the experiment, ethanol (EtOH), 80% aqueous ethanol (80% EtOH), methanol (MeOH) and 80% aqueous methanol (80% MeOH) were used as extractants. As a results of LC-ESI–MS/MS analysis protocatechuic, 4-OH-benzoic, vanilic, *trans*-caffeic, *cis*-caffeic, *trans*-*p*-coumaric, *cis*-*p*-coumaric, 3-OH-cinnamic, *trans*-ferulic, *cis*-ferulic, salicylic, *trans*-sinapic and *cis*-sinapic acids were identified in extracts.

80% aqueous ethanol appears to be the superior solvent for isolation of 4-OH-benzoic, *trans*-*p*-coumaric, *cis*-*p*-coumaric, 3-OH-cinnamic, *trans*-ferulic, *cis*-ferulic, salicylic, *trans*-sinapic and *cis*-sinapic acids, while 80% aqueous methanol was the best extractant for protocatechuic, vanilic, *trans*-caffeic and *cis*-caffeic acids from *B. oleracea* L. var. *sabellica* (Table 1). Although the differences in individual phenolic acids yield are not significant, compared to 80% aqueous methanol, 80% EtOH offers several advantages, especially insignificant toxicity and environmental compatibility. Ethanol and its dilutions are recommended by the US Food and Drug Administration for extraction purposes (Khan et al., 2010). The high extraction yield of analyzed compounds by 80% aqueous ethanol using USAE may be due to its physical properties of this solvent—surface tension, viscosity, and relatively high vapor pressure (the most important factor during ultrasound extraction) (Hemwimol et al., 2006). These properties, combined with its molecular affinity with phenolic acids, make 80% aqueous ethanol the best extractant for the phenolic acids from kale. Due