Separation and Purification Technology 93 (2012) 42–47

Contents lists available at SciVerse ScienceDirect

Separation and Purification Technology

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

Ultrasound assisted extraction of polyphenols from black chokeberry

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ARTICLE INFO

Article history:
Received 4 January 2012
Received in revised form 16 March 2012
Accepted 21 March 2012
Available online 1 April 2012

Keywords:
Black chokeberry
Aronia melanocarpa
Ultrasound assisted extraction
Polyphenols
Antioxidants
Extraction kinetics

ABSTRACT

Ultrasound assisted extraction (UAE) of antioxidant polyphenols from *Aronia melanocarpa* berries was studied. The influence of various parameters (time and temperature of extraction, solvent composition, solid–solvent ratio, particle size, and ultrasonic irradiations) on the extraction kinetics and yields was evaluated. Very clear effect of ultrasound was observed (up to 85% increase of the yield of extracted polyphenols). High temperature and the presence of ethanol in the solvent improved also greatly the extraction process. The high antioxidant activity of the extracts determined by DPPH tests confirmed suitability of UAE for the preparation of antioxidant-rich plant extracts. A very good correlation between the concentration of polyphenols in the extracts and the corresponding antioxidant activity was observed.

1. Introduction

Phenolic compounds are commonly found in both edible and nonedible plants. Crude extracts of fruits, herbs, vegetables, cereals, and other plant materials rich in phenolics are increasingly of interest in the food industry because of their multiple biological effects, including antioxidant activity [1]. Polyphenols protect against the oxidation of high-density lipids and therefore improve the quality and nutritional value of food [2]. Among all common fruits and vegetables in the diet, berries, especially those with dark blue or red colors, have the highest antioxidant capacities [3]. Many of these fruits, including chokeberries, have a long tradition in European and North American folk medicine [4,5]. *Aronia melanocarpa* (black chokeberry) fruits are ones of the richest plant sources of phenolic substances, mainly anthocyanins [3–5]. The high content of phenolics seems to correlate with the antioxidant activity reported for these berries [1,6]. *Aronia* juice exhibits the highest antioxidant capacity among the polyphenol-rich beverages [4]. Chokeberries and their extracts are useful for the prevention and treatment of cardiovascular disease [7] and colon cancer [8]. Antidiabetes and antimutagenic effects of phenolics form black chokeberry were also reported [6]. Black chokeberries have been also highlighted as a suitable and rather cheap source of a food colorant [9,10]. At present, there are no data about any unwanted and toxic effects of *A. melanocarpa* fruits, juice and extracts [5].

Till now mainly the composition and the antioxidant properties of the extracts of black chokeberry [1,3,4,11,12], as well as purification of the extracts [10,13,14] have been studied. Despite of the interesting properties of the black chokeberry and its extracts the optimal conditions for the extraction of polyphenols are not yet well investigated. One of the objectives of the present study was to elucidate the influence of the main extraction parameters (time and temperature of the extraction, solid–solvent ratio, type of solvent, particle size) on the yields of extracted polyphenols. The other objective of this work was to evaluate the effect of ultrasound on the extraction kinetics and yields and to outline the potential of ultrasound assisted extraction (UAE) in the preparation of aronia extracts rich in antioxidant polyphenols. The use of ultrasonic means for extraction purposes in raw materials is considered as an economical alternative to traditional extraction processes, this being a demand by industry for a sustainable development [15]. Ultrasound assistance has already demonstrated an important effect on the extraction of phenolics from other vegetal sources [16–19].

2. Materials and methods

2.1. Sample preparation

Dried berries of *A. melanocarpa* grown in the region of Elena (Bulgaria) were used. The berries were stored at room temperature. The size of the berries was 6.0 ± 0.5 mm and their moisture content was 9.0 ± 1.5%. To enhance the extraction of polyphenols,

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http://dx.doi.org/10.1016/j.seppur.2012.03.024

the berries were pretreated in two different ways. In the first case, the berries were firstly additionally dried for 4 h at 95 °C and then ground in portions of about 3 g for 15 s in a laboratory grinder (Yellow line, A10, IKA-Werke, Staufen, Germany). The obtained particles were separated by sieving into five particle classes: (d < 0.5 mm), (d = 0.5–1.0 mm), (d = 1.0–1.4 mm), (d = 1.4–2.0 mm) and (d > 2.0 mm). Vibratory Sieve Shaker AS 200 Basic (Haan, Germany) was used for this purpose. The samples were packed and stored at room temperature (T = 20 ± 2 °C) before extraction. The second type of pretreatment consisted in cutting of the berries in half just before the extraction process. This type of pretreatment is interesting from technological point of view because it do not need previous drying of the source and the separation of the obtained native extract form the exhausted vegetal source after extraction process is easier than in the case of extraction from fine ground particles.

2.2. Reagents and standards

As extraction solvents ultra pure water (prepared using a Milli-Q system), ethanol (95%) or ethanol–water mixtures were used. Gallic acid (purity: >98%), Folin–Ciocalteu phenol reagent (2N), 2,2-diphenyl-1-picrylhydrazyl (DPPH) and sodium carbonate (purity: >99%) were supplied by Sigma–Aldrich (France); methanol (purity: >99%) and ethanol (purity: 95%) were provided by Flandre Chimie (France).

2.3. Experimental equipment and procedures

The extraction of polyphenols was studied in an agitated glass contactor of 1 L, equipped of a generator of ultrasounds (SinapTec, France). The experimental setup used is depicted schematically in Fig. 1. The extraction temperature was maintained constant by a circulation of water in an external jacket connected to a thermostat. In all experiments, the agitation speed was fixed to 120 min^{-1}. In such a way the solid particles were suspended in the solvent media during the experimental runs. The solvent volume was 400 mL in the studies with ground berries and 600 mL in the studies with berries cut in half, respectively. The experiments were carried out at four temperature levels (20, 40, 60, 80 °C) and three levels of the initial solid–solvent ratio (1:10, 1:20, 1:40). The extraction from aronia berries was studied without or with ultrasound assistance. In the case of ultrasound assistance, the sonication was applied in continuous mode at frequency of 30.8 kHz and power of 100 W.

Extraction kinetics was studied for a period of 4 h. Samples were taken at regular intervals. The extracts were centrifuged for 10 min at 10,000g (Eppendorf Centrifuge 5804 R, Hamburg, Germany) and the supernatants were carefully removed for further analysis.

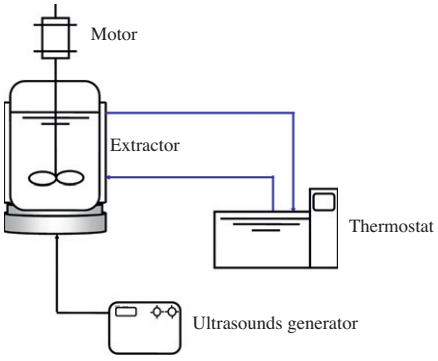

Fig. 1. Schematic representation of experimental setup for ultrasound assisted extraction.

As two lots of black chokeberry with different content of polyphenols were used, the main part of results was expressed as yield of extracted polyphenols (in%) on the basis of the total amount of polyphenols in the source. The content of polyphenols in the aronia berries (4.2% in the lot used in studies with berries cut in half and 2.8% in the lot used in studies with ground berries, respectively) was obtained after 4 h extraction of 5.0 g ground berries in 100 mL solvent (mixture of 50 mL water and 50 mL ethanol) at ebullition under reflux.

2.4. Total polyphenols

The concentration of total phenolic compounds in the extracts was determined using a spectrophotometer UVmini 1240 (Shimadzu France) following the method proposed by Singleton et al. [20]. The liquid extracts were diluted and mixed with Folin–Ciocalteu reagent (2N) and 200 g L^{-1} sodium carbonate solution and the absorbance of the obtained mixture was measured after 2 h at 765 nm. Since gallic acid was used as standard, the content of polyphenols was expressed as GAE (gallic acid equivalent). The following regression model expresses the relation between the concentrations of standard gallic acid solutions and the corresponding absorbances observed: $C_{GA} = (A_{765} + 0.0049)/0.0011$, where A_{765} is the absorbance at 765 nm and C_{GA} is the concentration of gallic acid (mg L^{-1}).

2.5. Antioxidant activity

The method of DPPH scavenging activity described by Brand-Williams et al. [21] was employed. Aliquots (50 μL) of extracts were added to 1950 μL of a methanolic solution (100 μM) of DPPH radical. After agitation, the reaction mixture was incubated in the dark at room temperature for 30 min and the absorbance was measured at 517 nm by spectrophotometer UVmini 1240 (Shimadzu France). The extracts reducing activities were estimated from the decrease in absorbance and the results were expressed in a percentage. The efficient concentration value (EC_{50}) was defined as the amount of extract that decreased to 50% the initial absorbance of the DPPH radical solution [22].

3. Results and discussion

Firstly, the influence of the main parameters of extraction was studied without ultrasound application and then the effect of ultrasound irradiation on the extraction of polyphenols was also evaluated at different operating conditions.

3.1. Effects of time and temperature of extraction

Time and temperature of extraction are important parameters to be optimised in order to minimise energy cost of the process. Kinetics of water extraction of polyphenols from black chokeberry cut in half is presented in Fig. 2. At every studied condition, the amount of extracted polyphenols increased continuously with the time. The rate of extraction decreased progressively but the extraction was not completed at the end of the experimental runs (after 4 h extraction).

Generally, the temperature has a positive effect on the extraction of phenolic compounds from vegetal sources [23–25]. The obtained results show also a very clear influence of the medium temperature on the extraction of polyphenols from aronia berries (Fig. 2). At 60 °C the yields of extracted polyphenols were tripled comparing to the yields obtained at 20 °C. The observed positive