Industrial Crops and Products 44 (2013) 558–565

Contents lists available at SciVerse ScienceDirect

Industrial Crops and Products

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

Optimization of ultrasonic-assisted extraction of antioxidant compounds from blackberry leaves using response surface methodology

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ARTICLE INFO

Article history:
Received 29 June 2012
Received in revised form
26 September 2012
Accepted 26 September 2012

Keywords:
Ultrasonic-assisted extraction
Blackberry leaves
Response surface methodology
Antioxidant capacity
Phenolic compounds

ABSTRACT

Response surface methodology was used to optimize experimental conditions for ultrasonic-assisted extraction of phenolic compounds from blackberry leaves. The Box-Behnken design (BBD) was employed for the optimization of extraction parameters in terms of total phenolics and antioxidant capacity. The optimal conditions for results of ABTS and CUPRAC were HCl concentration 0.41 and 0.45 M, methanol concentration 61 and 64% (v/v), extraction temperature 66 and 68 °C and extraction time 105 and 117 min, respectively. The experimental values agreed with those predicted values within a 95% confidence level, thus indicating the suitability of response surface methodology in optimizing the ultrasound-assisted extraction of phenolic compounds from blackberry leaves. The results showed that phenolic compounds present in blackberry leaves exhibited significant antioxidant properties. Seven phenolic compounds such as ellagic acid, caffeic acid, chlorogenic acid, quercetin, myricetin, kaempferol and kaempferol 3-β-D-glucopyranoside were determined in blackberry leaves by HPLC-DAD after extraction at optimum conditions.

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1. Introduction

Blackberry leaves are widely used as a tea substitute. The advantages of blackberry raw material are primarily determined by its phenolic composition. It is known that phenolic compounds are the major bioactive compounds of tea (Melkadze et al., 2008). However, data on the chemical composition of this plant are scanty. It has been reported that the blackberry leaves contain high level of chlorogenic acid, caffeic acid, coumaric acid, ferulic acid, gallic acid, rutin (Eruçar, 2006), ellagic acid, quercetin-3-D-glucoside, (+)-catechin, (−)-epicatechin, epicatechingallate and procyanidin B1 (Buřičová et al., 2011). Many research studies have demonstrated that blackberry leaves contain various components with antioxidant activity, which are responsible for their beneficial health effects. (Martini et al., 2009; Tavares et al., 2010). Blackberry leaves present in herbal mixtures have been used for curing nervous disorders, atherosclerosis, hypertension, and radiation diseases (Wang and Lin, 2000; Melkadze et al., 2008).

The selection of different extraction methods would mainly depend on the advantages and disadvantages of the processes, such as the extraction yield, complexity, cost of the process, environmental effects, and safety (Zhang et al., 2011). Many factors contribute to the efficiency of solvent extraction, such as the type of solvent, the concentration of solvent, the pH, the extraction temperature/time, the pressure and the particle size of plant (Juntachote et al., 2006). Therefore, it is appropriate to choose the optimal pretreatment method according to the chemical structures and properties of the analyzed compounds. The method used is dependent upon the particular class of phenolic compound that are soluble in the solvent system used on the nature of the matrix (Ryan et al., 2001; Türkben et al., 2010). Conventional techniques such as organic extraction and acid extraction have been used to isolate phenolic compounds (Zhang et al., 2011). The main disadvantages are the loss of phenolic compounds due to oxidation, ionization and hydrolysis during extraction as well as the long extraction time (Li et al., 2005). Other techniques, which include ultrasonic assisted extraction, microwave assisted extraction, enzymatic extraction and supercritical carbon dioxide extraction have also become of interest as alternatives to these conventional methods. Ultrasonic extraction has proven to be equally or more efficient than other extraction methods. The major advantages of this method are the reproducibility of the technique, the applicability of the method to a range of sample sizes, the dramatic reduction in time needed to perform highly efficient extractions, and efficient extraction of polar organic compounds (Zhang et al., 2011).

In recent years, chemometric tools have been frequently applied to the optimization of analytical methods, considering their advantages such as a reduction in the number of experiments that need be executed resulting in lower reagent consumption and considerably less laboratory work (Ferreira et al., 2007). Response surface methodology (RSM) enables the evaluation of the effects

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0926-6690/$ – see front matter © 2012 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.indcrop.2012.09.022

of many factors and their interactions on response variables. The main advantage of RSM is the reduced number of experimental trials needed to evaluate multiple parameters and their interactions; therefore, it is less laborious and time-consuming than other approaches required to optimize a process (Lee et al., 2000; Bezerra et al., 2008; Aybastıer and Demir, 2010). The most common designs, i.e. central composite design (CCD) and Box-Behnken design (BBD), of the principal response surface methodology have been widely used in various experiments. Box-Behnken, a spherical and revolving design, has been applied in optimization of chemical and physical processes because of its reasoning design and excellent outcomes (Donga et al., 2009).

In the present study, optimization of temperature, time, solvent and acid concentration for extraction of antioxidant compounds from blackberry leaves was carried out using ultrasound-assisted extraction and response surface methodology. A three level, four-variable BBD was employed in order to maximize simultaneously the total phenolic content and antioxidant capacity.

2. Material and methods

2.1. Plant material

Dried blackberry leaves were obtained from local market in Bursa–Turkey. The purchased samples were milled into uniform dry powder by rondo and stored at 4 °C.

2.2. Chemicals and reagents

Folin–Ciocalteu (F–C) phenol reagent, neocuproine (2,9-dimethyl-1,10-phenanthroline), gallic acid, kaempferol 3-β-D-glucopyranoside, (+)-catechin hydrate, (−)-epicatechin hydrate, kaempferol, quercetin hydrate, rosmarinic acid, rutin hydrate, luteolin, vanillic acid, chlorogenic acid were purchased from Sigma–Aldrich (St. Louis, USA); Trolox [(±)-6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid] was purchased from Aldrich (Aldrich Chemicals Company, Steinheim, Germany); ellagic acid, myricetin, trans-cinnamic acid and ABTS [2,2′-azinobis(3-ethylbenzothiazoline-6-sulphonic acid) diammonium salt] were purchased from Fluka (Buchs, Switzerland). Methanol, caffeic acid, ferulic acid, *p*-coumaric acid, *p*-hydroxybenzoic acid, protocatechuic acid was purchased from Merck (Darmstadt, Germany). All standard solutions were prepared in methanol (Merck, Darmstadt, Germany). HPLC grade formic acid and acetonitrile were purchased from Merck (Darmstadt, Germany).

2.3. Ultrasound-assisted extraction

Ultrasound-assisted extraction was performed in a temperature controlled ultrasonic cleaner. Furthermore, temperature was also controlled with thermometer. The blackberry leaves powder (0.5 g) was placed into a glass vial (45 mL), added acidic methanol solvent of 30 mL and then placed ultrasonic cleaning bath (United) at 40 kHz. The extract was filtered and filtrate was analyzed.

2.4. Experimental design

A three level, four-variable BBD was employed in this study, requiring 30 experiments for the optimization of extraction parameters. The parameters and their levels are: HCl concentration (0.4–1.6 M), methanol concentration (20–80%, v/v), extraction temperature (30–70 °C) and extraction time (20–120 min). Total phenolic content (mg gallic acid equivalent/g dried plant) and antioxidant capacity (mg trolox equivalent/g dried plant) were taken as the response of the design experiments (y). Total phenolic content was determined by Folin–Ciocalteu method and antioxidant capacity was determined by ABTS and CUPRAC methods. The actual and coded levels of the independent variables are given in Table 1. Thirty experiments were augmented with six times and carried out at the center points to evaluate the pure error.

Table 1
Range of coded and actual values for Box-Behnken design.

Factor	Level		
	−1	0	1
HCl concentration (M)	0.4	1.0	1.6
Methanol concentration (%, v/v)	20	50	80
Extraction temperature (°C)	30	50	70
Extraction time (min)	20	70	120

Second-order polynomial Eq. (1) which includes all interaction terms was used to calculate the predicted response:

$$y = b_0 + \sum_{i=1}^{4} b_i x_i + \sum_{i=1}^{4} b_{ii} x_i^2 + \sum_{i=1}^{3} \sum_{j=i+1}^{4} b_{ij} x_i x_j \quad (1)$$

where y is response, b_0 is the offset term, b_i is the linear effect, b_{ii} is the squared effect, b_{ij} is the interaction effect and x_i and x_j are independent variables.

The data were analyzed using Design Expert program (7.0.0 version) and the coefficients were interpreted using *F* test. Three main analytical steps: analysis of variance (ANOVA), regression analysis and plotting of response surface plots were performed to establish optimum conditions for total phenolic content and antioxidant capacities.

2.5. HPLC-DAD analysis

An Agilent Technologies 1200 HPLC system (Waldbronn, Germany), consisting of a vacuum degasser, binary pump, autosampler and a diod-array detector, was used for determination of phenolic compounds in extract. Chromatographic separations were carried out using an XBridge C18 (4.6 × 250 mm, 3.5 μm) column from Waters. Mobile phase consists of 1% formic acid in water (solvent A) and acetonitrile (solvent B). Gradient conditions are as follows; 0–10 min 13% B, 10–20 min 41.5% B, 20–25 min 70% B, 25–35 min 10% B, total run time is 35 min. Flow rate was 0.5 mL/min and injection volume was 10 μL. Data acquisition and preprocessing was done with Chemstation for LC (Agilent Technologies). The monitoring wavelengths were 280 nm for ellagic acid, 320 nm for caffeic acid, chlorogenic acid and 360 nm for quercetin, myricetin, kaempferol and kaempferol 3-β-D-glucopyranoside. Peaks were identified on the basis of comparison of retention times and UV spectra with standards of caffeic acid, chlorogenic acid, ellagic acid, quercetin, myricetin, kaempferol and kaempferol 3-β-D-glucopyranoside.

2.6. Folin–Ciocalteu method

The total phenolic content by Folin–Ciocalteu reagent was carried out according to the procedure reported in our previous work (Şahin et al., 2011). The absorbance was measured by spectrophotometer (Varian Cary 50, Australia) at 750 nm. Total phenols were expressed as mg of gallic acid equivalent (GAE) per g of dried weight.

2.7. ABTS method

The antioxidant capacity of extract was determined with ABTS method as described in our previous work (Sarıburun et al., 2010). The absorbance was recorded at 734 nm against blank after 6 min.

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