Food and Bioproducts Processing 108 (2018) 69–80

Contents lists available at ScienceDirect

Food and Bioproducts Processing

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

Ultrasound-negative pressure cavitation extraction of phenolic compounds from blueberry leaves and evaluation of its DPPH radical scavenging activity

Tong Wang[a,b], Na Guo[a,b], Sui-Xin Wang[a,b], Ping Kou[a,b], Chun-Jian Zhao[a,b], Yu-Jie Fu[a,b,*]

[a] Key Laboratory of Forest Plant Ecology, Ministry of Education, Northeast Forestry University, Harbin 150040, PR China

[b] Engineering Research Center of Forest Bio-preparation, Ministry of Education, Northeast Forestry University, 150040 Harbin, PR China

ARTICLE INFO

Article history:
Received 14 September 2017
Received in revised form 24 December 2017
Accepted 15 January 2018

Keywords:
Blueberry leaves
Ultrasound-negative pressure cavitation extraction
Phenolics
Flavonoids
Proanthocyanidin
Mass transfer kinetics
DPPH radical scavenging activity

ABSTRACT

Traditional extraction methods for natural products from biomass have many drawbacks such as low extraction yields, time-consuming, plenty of organic solvent consumption and being harmful to environment and human beings. In the present study, we developed an efficient extraction method called ultrasound-negative pressure cavitation extraction (U-NPCE) for extraction of bioactive compounds from blueberry leaves. The optimization of the extraction process was based on single factor experiments and Box–Behnken design (BBD). The optimal extraction conditions were ethanol concentration (v/v) 68.61%, ultrasonic power 0.36 W/cm^2, negative pressure −0.07 Pa, extraction time 15 min, solid/liquid ratio 1:30 g/mL and extraction temperature 50 °C. Under the optimal extraction condition, the mass transfer kinetics model indicated that U-NPCE has higher extraction yields for total phenols (TP), total flavonoids (TF) and total procyanidins (TPA) and the time consumption were 3.33 and 3.67 times shorter than that of ultrasound-assisted extraction (UAE) and negative pressure cavitation extraction (NPCE) respectively. Moreover, extracts of U-NPCE (22.5 μg/mL) have stronger DPPH radical scavenging activity than those of UAE (30 μg/mL) and NPCE (32.5 μg/mL). The results indicated that U-NPCE was more efficient than UAE and NPCE for extraction of polyphenols from blueberry leaves and extracts has much higher TP, TF and TPA contents and DPPH radical scavenging activity.

1. Introduction

Blueberry (genus *Vaccinium*) fruit is popular around the world. In the past years, the blueberry cultivation area has been increased worldwide. As a main by-product, plenty of blueberry leaves has been discarded. Traditionally, blueberry leaves was used to treat many diseases such as cataract, diabetes, premature aging and anemia and had obviously curative effect (Allen, 1927; Watson, 1928). Recent studies suggested that blueberry leaf extracts show excellent biological activities, including antioxidant activity (And and Prior, 2001), antileukemic activity (Skupień et al., 2006), activity of suppressing hepatitis C virus (Nakama et al., 2011; Takeshita et al., 2009), hypolipidemic activity (Li et al., 2011), antimicrobial activity (Gurjar et al., 2012), hypotensive activity (Sakaida et al., 2014), and activity of anticancer (Agarwal et al., 2000; Singh et al., 2011). These activities are attributed to the types and quantities of phytochemicals in the leaves. Studies reported that blueberry leaves contain abundant polyphenols and procyanidins (And and Prior, 2001; Cyboran et al., 2013; Naczk et al., 2006; Vyas et al., 2013), such as chlorogenic acid, quercetin glycosides (Harris et al., 2007) and oligomeric proanthocyanidins (Wang et al., 2015; Yosuke et al., 2010). So the blueberry leaves could be a kind of cheap raw

* *Corresponding author at*: Key Laboratory of Forest Plant Ecology, Ministry of Education, Northeast Forestry University, Harbin 150040, PR China.
E-mail address: yujie_fu@163.com (Y.-J. Fu).
https://doi.org/10.1016/j.fbp.2018.01.003

materials with favourable biological activities and abundant phenolic compounds used in pharmaceuticals, cosmetics, food additives and functional food. However, there were less any studies about the extraction of polyphenols from blueberry leaves.

The extraction method can significantly influence the types and quantities of compounds in extracts (Farhat et al., 2017). Thus, extracts by using different extraction methods are diverse in bioactive intensity (Zheng et al., 2014). So an efficient extraction method can improve the quality of the extracts, at the same time, decrease organic solvent and energy consumption. To overcome the drawbacks such as laboriousness, time and hazardous organic solvents consumption, thermo-sensitive degradation and low extraction yields when using conventional extraction methods. (Roohinejad et al., 2014), several more efficient extraction methods have been developed (Espinosa-Pardo et al., 2016; Lohani et al., 2016; Viganó et al., 2016; Vorobiev and Lebovka, 2013). As an efficient and green extraction methods, ultrasound-assisted extraction (UAE) has been widely used for the extraction of natural products from biomass (Upadhyay et al., 2015; Upadhyay et al., 2012; Wang et al., 2017a; Wang et al., 2017b; Wang et al., 2017c). The mechanism of UAE is shown in Fig. 1A. When the ultrasound wave passes through an elastic medium, the solvent molecules move in longitudinal, resulting in a succession of compression and rarefaction phases. During the compression phases, the solvent molecules will collide with the surrounding molecules. During the rarefaction phase, exerted negative pressure pulls the molecules apart and generates cavitation bubbles in the liquid. The cavitation bubbles will grow while the dissolved gas enters the bubble. When the bubbles collapse, hotspots would be created, the temperature and the pressure could be estimated to be up to 5000 K and 5000 atm in an ultrasonic bath at room temperature. The hotspots would destroy the cell walls of the plant matrix and the compounds would release to the solvent (Iii et al., 1999; Mcclements, 1995). However, in the process of UAE, the plant materials were deposited on the bottom of the container. So we hypothesized that if the plant materials were evenly distributed in the solvent, then the contact area of plant materials with hotspots would increase. Thus the cavitation effect, thermal effect and mechanical effect of ultrasound could be fully applied to the plant materials, causing severe disruption of the cell wall and the intensification of mass transfer. The extraction efficiency would be improved greatly.

Negative pressure cavitation extraction (NPCE) was designed in our laboratory and used for extraction of natural products from plants (Liu et al., 2009; Roohinejad et al., 2016). As shown in the Fig. 1B, in the extraction tank, negative pressure was created by a vacuum pump. The nitrogen pressure was greater than that of the solvent in the extraction tank. So nitrogen entered into the solvent and nitrogen bubbles formed due to the low solubility in the solvent. Under negative pressure conditions, the component was easier to diffuse into the solvent, and the extraction efficiency was enhanced. Moreover, turbulence, collision generated when the bubbles moved from the bottom to the top acutely, caused the plant materials evenly distribute in the solvent and the contact area between solvent and plant materials were increased. All the mentioned factors could improve extraction efficiency in the process of NPCE. However, the temperature and the pressure released by nitrogen bubbles collapse were very weak compared with the UAE which could not destroy plant materials strongly. As shown in Fig. 1C, in present study a novel extraction method called U-NPCE was designed by combination of UAE and NPCE, not only could overcome the problems of small contact area between materials and hotspots in UAE process but also could overcome the problem of weak energy release which could not destroy the materials strongly in NPCE process. Therefore, the extraction efficiency would be enhanced significantly.

Based on mentions above, in the present study, a new extraction method called ultrasound-negative pressure cavitation extraction (U-NPCE) was developed and optimized for the extraction of total phenols (TP), total flavonoids (TF) and total procyanidins (TPA) from blueberry leaves. The mass transfer kinetics model of U-NPCE, UAE and NPCE was established. The comparisons of U-NPCE, UAE and NPCE were based on the extraction yields of TP, TF and TPA and DPPH radical scavenging activity of the extracts.

2. Materials and methods

2.1. Materials and chemicals

Blueberry leaves were collected from Grand Khingan, HeiLongJiang province in November. The samples were identified by Professor Zi-Jun Mao from the Key Laboratory of Forest Plant Ecology, Ministry of Education, Northeast Forestry University, PR China. The fresh leaves were cleaned and dried to constant weight, and then pulverized by a disintegrator. The pulverized materials were sieved (40 mesh), and then stored in darkness at room temperature prior to use.

The standards of gallic acid, rutin, catechin, were purchased from Yuanye Chemical Reagent Co. (Shanghai, China). Methanol was purchased from J & K Chemical Ltd. (China). Ethanol of analytical grade for extraction was bought from Tianjin Kemel Reagents Co. (Tianjin, China). Deionized water was purified by a Milli-Q Water. Purification system was purchased from Millipore (MA, USA). $Al(NO_3)_3$, $NaNO_2$, and NaOH were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China) which were of analytical grade. 2,2-Diphenyl-1-picrylhydrazyl (DPPH), Folin–Ciocalteu and vanillic acid were purchased from Sigma–Aldrich (Steinheim, Germany).

2.2. Ultrasound-negative pressure cavitation extraction (U-NPCE)

As shown in the schematic diagram Fig. 1C, the extraction process was briefly as follows: firstly, the extraction solvent was poured into the extraction tank from the sample inlet, and the solvent was heated to the setting temperature. Then, 1 g plant materials were added into the tank from the sample inlet, and the vacuum pump and the ultrasonic transmitter adjusted to required power. Turn on the air inlet valve adjusted the negative pressure to the setting value. Start the time until the scheduled time was achieved. After extraction, cut off the air supply and turn off the power supply. Then, the filtered extraction solution was collected.

2.2.1. Optimization of extraction parameters by single factor experiment

To investigate the effect of factors on the extraction yields of TP, TF and TPA, the extraction process was done as showed in Section 2.2. The detail conditions for single factor experiments were listed in the Table 1.

2.2.2. Optimization of U-NPCE by response surface

Based on single factor experiments, ethanol concentration, negative pressure and ultrasonic power were the most significant factors which influenced the extraction efficiency of TP, TF and TPA. Box–Behnken design (BBD) was used to optimize three independent variables: ethanol concentration (v/v) (60–80%), ultrasonic power (0.3–0.4 W/cm^2), and negative pressure (−0.06 to −0.08 Pa) at three levels (−1, 0, +1). The arrangement and results of experiment were shown in Table 2. All experiments were performed in triplicates. Design-Expert Ver. 8.01 (Stat-Ease Inc., Minneapolis, MN, USA) was used for analysis of the BBD experiment results.

2.3. Determination of the phenolics content

The total phenolics content of leaf extracts was assayed using Folin–Ciocalteu method with minor modification (Singleton