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Optimization of polyphenols extraction from dried chokeberry using maceration as traditional technique

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ABSTRACT

Traditional maceration method was used for the extraction of polyphenols from chokeberry (*Aronia melanocarpa*) dried fruit, and the effects of several extraction parameters on the total phenolics and anthocyanins contents were studied. Various solvents, particle size, solid–solvent ratio and extraction time have been investigated as independent variables in two level factorial design. Among examined variables, time was not statistically important factor for the extraction of polyphenols. The optimal extraction conditions were maceration of 0.75 mm size berries by 50% ethanol, with solid–solvent ratio of 1:20, and predicted values were 27.7 mg GAE/g for total phenolics and 0.27% for total anthocyanins. Under selected conditions, the experimental total phenolics were 27.8 mg GAE/g, and total anthocyanins were 0.27%, which is in agreement with the predicted values. In addition, a complementary quantitative analysis of individual phenolic compounds was performed using HPLC method. The study indicated that maceration was effective and simple technique for the extraction of bioactive compounds from chokeberry fruit.

1. Introduction

Chokeberry (*Aronia melanocarpa* [Michx] Elliot, Rosaceae) is a perennial shrub native to North America, and it was introduced to Eastern Europe, Scandinavia, and Russia in early 20th century (Kokotkiewicz, Jaremicz, & Luczkiewicz, 2010; Kulling & Rawel, 2008). Fully ripened chokeberry fruits contain different phenolic compounds such as proanthocyanidins, flavan-3-ol and flavonol glycosides, and phenolic acids (Rugina et al., 2012; Suiero et al., 2006). Moreover, chokeberry represents one of the richest plant sources of anthocyanins exhibiting strong antioxidant activity (Braunlich et al., 2013). Anthocyanins and proanthocyanidins play important role in human nutrition and the growing interest for their utilization is mainly due to their antioxidant potential and the association between their consumption and the prevention of cancer, coronary heart disease, diabetes and other degenerative disorders (Kokotkiewicz et al., 2010; Ovando, Hernandez, Hernandez, Rodriguez, & Galan-Vidal, 2009; Sainova et al., 2012).

The main source of chokeberry fruit on the market originate from plantations, and beside fresh fruits various processed products like juices, jams, jellies as well as extracts and dietary supplements are available (Gonzales-Molina, Moreno, & Garcia-Viguera, 2008; Kokotkiewicz et al., 2010; Kulling & Rawel, 2008). For the products of pharmaceutical, cosmetic and food industry, quality of the extracts as component of the products is critical and increment of the amount of biologically active phenolics in extracts is a challenging task. Extraction is the first and important step in isolation and purification of bioactive components from plant material. Various extraction techniques can be applied for polyphenol recovery from plants, and generally these techniques can be divided into traditional and modern ones. The traditional extraction methods include maceration, maceration assisted with stirring, and Soxhlet extraction. In recent years a new techniques have been used for the extraction of bioactive compounds including ultrasound-assisted extraction, microwave-assisted extraction, sub- and supercritical fluid extraction and accelerated solvent extraction (Khoddami, Wilkes, & Roberts, 2013). Each technique has its own advantages and disadvantages, but the main goal of the chosen method is the achievement of complete extraction of the compounds of interest and avoidance of their chemical modification. Extraction efficiency is influenced by several factors such as type and concentration of solvent, solid–solvent ratio, time, temperature, pH, etc. However, in the most studies the influence of a single factor has been explained while the interactions between the factors have not been examined thoroughly. Therefore, in order

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to optimize the extraction conditions it would be useful to investigate the influence of different factors on extraction of chokeberry polyphenols using experimental design strategy. Factorial design is a good and simple statistical tool that allows the simultaneous study of the effects that several factors may have on an optimization of a particular process. It also allows measuring the interaction between each factor in order to achieve the best overall optimization of the process.

Many studies have reported extraction of polyphenols from different plant material, but literature data concerning optimization of polyphenol extraction from chokeberry is very scarce. Only optimization of ultrasound-assisted extraction of polyphenols from chokeberry and chokeberry by-products has been studied previously (Galvan D'Alessandro, Dimitrov, Vauchel, & Nikov, 2014; Galvan D'Alessandro, Kriaa, Nikov, & Dimitrov, 2012; Ramić et al., 2015). Considering that phenolic molecules possesses beneficial effects on human health, and the industry set up demands for reduction of production costs, it is worthwhile to investigate the optimal conditions for the efficient extraction of chokeberry polyphenols.

In the present study, the effects of four factors (time, solid–solvent ratio, solvent type and particle size) on the extraction efficiency of polyphenols from chokeberry dried fruit were analyzed using factorial design in order to optimize experimental procedure using maceration as traditional technique for the extraction. Moreover, quantitative analysis of individual phenolic compounds, i.e. flavonoids and anthocyanins by HPLC was also performed.

2. Materials and methods

2.1. Plant material

Berries were collected in August 2013, at fully ripened stage from plantation located at mountain Suvobor, Serbia (44°08′16.04″N, 20°10′56.28″E, 779 m a.s.l.). Soil type was Calcocambisol, weakly skeleted with rock (<25%), well drained, with slightly acidic reaction (pH = 6.2). Climate was continental and characterized with average rainfall precipitation of 50 mm and average minimal–maximal temperature range of 3.3–27.3 °C during vegetation period (March–September). Beside pruning and regular weeding no other agro-technical measurements were applied. Collected berries were dried in a tunnel dryer at 40 °C and moisture content was 10.65 ± 1.39%.

Dried berries were grounded by industry mill and obtained particles were separated using sieves into 5 particle sizes according to Yugoslav Pharmacopoeia (Ph Yug V, 2000). The samples were stored at room temperature before the extraction.

2.2. Reagents and standards

Folin–Ciocalteu phenol reagent (Sigma–Aldrich, Steinheim, Germany), sodium carbonate, methanol, formic acid, and orthophosphoric acid were purchased from (Sigma–Aldrich Chemie GmbH, Munich, Germany). Ethanol was of analytical grade, acetonitrile (Merck, Germany) was of HPLC grade, and ultra pure water was prepared using a Milli-Q purification system (Millipore, France). The anthocyanin standards cyanidin-3-*O*-galactoside, cyanidin-3-*O*-glucoside, cyanidin-3-*O*-arabinoside, and flavonoids quercetin-3-*O*-rutinoside (rutin), quercetin-3-*O*-galactoside (hyperoside) and quercetin-3-*O*-glucoside (isoquercetin) were purchased from Extrasynthese (Cedex, France). Gallic acid was obtained from Sigma–Aldrich (Steinheim, Germany).

2.3. Extraction procedures

Maceration in an Erlenmeyer flask (100 mL) was performed on a shaker (Unimax 1010, Heidolph, Germany) with agitation fixed on 170 rpm, at ambient temperature. An initial step was carried out in order to determine the optimum and rational duration of extraction, because maceration usually requires performance over a longer period of time. The experiments were performed under selected conditions for polyphenols extraction based on the literature data. Duration of extraction was studied with 50% ethanol (Galvan D'Alessandro et al., 2012; Ramić et al., 2015), 1:20 solid–solvent ratio (Cacace & Mazza, 2003; Galvan D'Alessandro et al., 2012), and 2 mm particle size (as a medium sized particle). The samples were collected at 15 min, 30 min, 60 min, 90 min, 2 h, 2.5 h, 5 h, 10 h and 18 h. As the extraction time of more then 90 min had no significant influence on the amount of phenolics (data not shown), the optimization of the extraction was further monitored at 15, 30, 60 and 90 min.

In the next set of experiments, the influence of each factor on the extraction process has been studied and the results are shown in the preliminary screening. Each factor in all levels was separately tested in combinations with other investigated factors. To find out the suitability of the solvent for extraction of polyphenols, four different solvents levels, 25 mL of distilled water, 50%, 70% and 96% mixture ethanol–water were used. Three levels of the solid–solvent ratio (1:10, 1:20 and 1:30) were studied in order to select out suitable ratio for extraction, and five levels of particle classes (6, 3, 2, 1, and 0.75 mm) were extracted to find out the optimal particle size.

Ultrasound-assisted extraction was performed in an ultrasonic bath (bath power 35 W, continuous mode at frequency of 40 kHz, Maget, Bela Palanka, Serbia) for 30 and 60 min. The generator of ultrasounds was placed on the lateral sides of the bath, and an Erlenmeyer flask (100 mL) was positioned in the same distance from three sides. The berry extract in the Erlenmeyer was 5 cm below the surface of water in the bath and sonicated without agitation. The solvent volume (50% ethanol) was 25 mL, solid–solvent ratio was 1:20, and 0.75 mm sieve was used.

2.4. Design of experiments

2.4.1. Preliminary screening of process levels

The selection of the process levels of each factor that have significant influence on the extraction of total phenolics (TP) and total anthocyanins (TA) has been performed. Statistical significance among factor levels has been estimated on triplicate samples through one-way ANOVA followed by Duncan's multiple range test at $p < 0.05$ level. Data in charts were presented as means coupled with vertical bars which denote 0.95 confidence intervals of triplicate measurements. Means followed by different letters in charts and tables differ significantly, based on Duncan's test at $p < 0.05$ level. Selected two levels with the highest yields of both observed quality parameters (TP and TA) were subjected to further factorial designs.

2.4.2. Factorial design

Two experimental design methods were used for the screening and optimization of process factors. In the first factorial design (Plackett–Burman design), four independent variables time, solid–solvent ratio, solvent type and particle size, each at two levels, were screened forming the 2^4 full factorial design (Table 1). The purpose of this step is to identify which variable have significant effect on the total phenolics and total anthocyanins contents.

Based on the results obtained in the first factorial design, a new 2^3 full factorial design was employed to investigate the effect and