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Optimization of subcritical water extraction of antioxidants from *Coriandrum sativum* seeds by response surface methodology

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ABSTRACT

Subcritical water extraction (SWE) of antioxidants from *Coriandrum sativum* seeds (CSS) was optimized by simultaneous maximization of the total phenolics (TP) and total flavonoids (TF) yield and antioxidant activity, using IC_{50} value. Box–Behnken experimental design (BBD) on three levels and three variables was used for optimization together with response surface methodology (RSM). Influence of temperature (100–200 °C), pressure (30–90 bar) and extraction time (10–30 min) on each response was investigated. Experimentally obtained values were fitted to a second-order polynomial model and multiple regression. Analysis of variance (ANOVA) was used to evaluate model fitness and determine optimal conditions. Moreover, three-dimensional surface plots were generated from employed mathematical model. The optimal SWE conditions obtained in simultaneous optimization were temperature of 200 °C, pressure of 30 bar and extraction time of 28.3 min, while obtained values of TP and TF yields and IC_{50} value at this experimental point would be 2.5452 g GAE/100 g CSS, 0.6311 g CE/100 g CSS and 0.01372 mg/ml, respectively. Moreover, good and moderate linear correlation was observed between antioxidant activity (IC_{50} value) and total phenolics content (R^2 = 0.965), and total flavonoids content (R^2 = 0.709) which indicated that these groups of compounds are responsible for antioxidant activity of *C. sativum* extracts.

1. Introduction

Coriander (*Coriandrum sativum* L.) is aromatic plant which is widely distributed and cultivated in Mediterranean countries. The coriander seeds contain an essential oil (up to 1%) [1], where monoterpenoid linalool is the main compound (>50%), and limonene, camphor and geraniol are present in significant quantity [2,3]. It has the advantage of being more stable and of retaining its agreeable odor longer than any other oil of its class [4]. Leaves and seeds are employed as condiment in food industry, being used to flavor various commercial foods such as liqueurs, teas, meat products and pickles [5]. Besides aromatic, coriander seeds are recognized for their medicinal properties. The seeds and aerial parts of the plant were extensively used in traditional medicine for various ailments such as spasm, neuralgia gastric complaints, dysentery, dyspepsia and giddiness [6]. Studies have demonstrated hypolipidemic action and effects of carbohydrate metabolism of *C. sativum* seeds [7]. Seeds have been also recognized due to their antimicrobial potential against different pathogen bacteria and yeasts [8,9]. Both hydrophilic and lipophilic extracts of coriander have demonstrated significant antioxidant activities in in vitro and in vivo studies [10,11].

Conventional solvent extractions with organic solvents and hydrodistillation have been widely used for the isolation of volatile and nonpolar compounds from plant material. In order to overcome certain disadvantages of conventional techniques such as extraction time, use of organic solvent and thermal degradation, modern extraction techniques have been developed. Supercritical fluid extraction (SFE) with non-toxic carbon dioxide (CO_2) is becoming more common for the extraction of flavours and fragrances, and can often yield more rapid extractions than hydrodistillation, as well as recovering some species that are not recovered by hydrodistillation [12]. More recently, subcritical or superheated water extraction (SWE) has been developed as a new technique based on the use of water, at temperatures between 100 and 374 °C and pressure high enough to maintain the liquid state [13]. Dielectric constant of water which controls solubility of the solute in water is directly connected with temperature which allows modification of water selectivity with change of temperature. The dielectric constant of water is 78.4 at room temperature, however at 200 °C it

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is 35.6, which is enough for quantitative extraction of less polar compounds [14]. SWE demonstrated ability to selectively extract different classes of compounds, with the more polar organics being extracted at lower temperatures and the less polar organics being extracted at higher temperatures [15]. The most important advantages of SWE over conventional extraction techniques are shorter extraction time, higher quality of the extract, lower costs of the extracting agent, and the environmental compatibility [16].

The most common and often used approach on the process optimization uses one-factor-at-a-time, where influence of independent variables on responses are investigated one by one, while all other factors are kept under constant values. This approach could be time-consuming and expensive for certain experiments. Moreover, possible interaction effects between variables may not be evaluated. In order to overcome these disadvantages, response surface methodology (RSM) could be applied. RSM is a collection of statistical and mathematical techniques useful for developing, improving and optimizing processes in which a response of interest is influenced by several variables, and the objective is to optimize this response [17]. Analyzing the effects of the independent variables, this experimental methodology generates a mathematical model which describes the chemical processes within the experimental range [18].

The main objectives of present work were to investigate effects of SWE conditions (temperature, extraction time and pressure), and to apply RSM approach in order to optimize these conditions to obtain the highest polyphenolics content and highest antioxidant activity of obtained liquid extracts of dried *C. sativum* seeds.

2. Materials and methods

2.1. Chemicals

1,1-Diphenyl-2-picryl-hydrazyl-hydrate (DPPH), Folin–Ciocalteu reagent and (±)-catechin were purchased from Sigma (Sigma-Aldrich GmbH, Sternheim, Germany). Gallic acid was purchased from Sigma (St. Luis, MO, USA). All other chemicals and reagents were of analytical reagent grade.

2.2. Plant material

Coriander (*C. sativum*), i.e. coriander seeds, were produced by the Institute of Field and Vegetable Crops, Novi Sad, Serbia (year 2012). Seeds were air-dried, milled and mean particle size (0.466 mm) was determined by sieve set (CISA Cedaceria Industrial, Spain).

2.3. SWE procedure

Subcritical water extraction (SWE) was performed in batch-type high-pressure extractor (Parr Instrument Company, USA) with internal volume 450 ml and maximum operating pressure of 200 bar and temperature 350 °C, connected with temperature controller (4838, Parr Instrument Company, USA). Extraction procedure was carried out by the scheme from Fig. 1. In all experimental runs, 10.0 g of coriander seeds sample were mixed with 100 ml of water in extractor (1). The operating pressure was reached with the injection of nitrogen in extractor from gas cylinder (2) through valve (3), and measured with pressure indicator (4). Nitrogen was used in order to prevent possible oxidation on high temperatures in the presence of oxygen from air. Extractor vessel was heated with electric heating jacket (5) and temperature was measured and controlled on controller (6), connected with temperature indicator (7). Magnetic stirrer (8) (750 rpm) was used for the stirring in order to increase mass and heat transfer and prevent local overheat on the inner walls of extractor. After the extraction, extractor was immediately cooled in ice-bath at 30 °C, and nitrogen was discharged from extractor through valve (9).

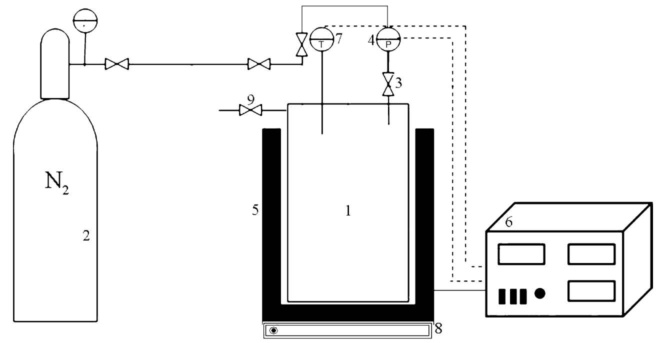

Fig. 1. Schematic diagram of subcritical water extraction system: (1) extractor; (2) nitrogen cylinder; (3) input gas valve; (4) pressure indicator; (5) electric heating jacket; (6) digital controller; (7) temperature indicator; (8) magnetic stirrer; (9) output gas valve.

Temperature (100–200 °C), pressure (30–90 bar) and extraction time (10–30 min) were independent variables. Temperature profiles of the extraction on different process temperature are presented on Fig. 2. The first part of all three curves describes approximately linear heating of extractor which lasted for 11, 16 and 21 min for extraction at 100, 150 and 200 °C, respectively. During extraction period, temperature was held constant (stationary phase) for different extraction time depending on experimental run. After the extraction, extractor was cooled in ice-bath during approximately 5 min to reach room temperature. After extraction, extracts were immediately filtered through filter paper under vacuum. Extracts were collected into glass flasks and stored at 4 °C until the analysis.

2.4. Determination of total phenols content

The total phenolics content (TP) in obtained *C. sativum* extracts was determined by Folin–Ciocalteu procedure [19,20] using gallic acid as a standard. Absorbance was measured at 750 nm. Content of phenolic compounds was expressed as grams of gallic acid equivalent (GAE) per 100 g of *C. sativum* seeds (g GAE/100 g CSS). All experiments were performed in three replicates.

2.5. Determination of total flavonoids content

The total flavonoids content (TF) was determined using aluminum chloride colorimetric assay [21]. Results were expressed as grams of catechin equivalents (CE) for 100 g of dry *C. sativum*

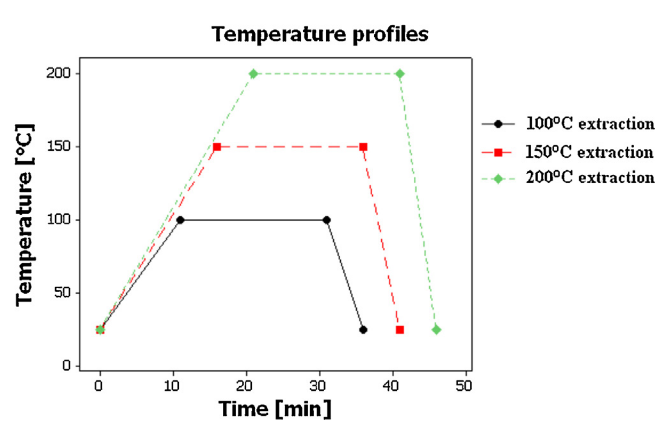

Fig. 2. Temperature profiles of the extraction at different process temperature.