Industrial Crops & Products 120 (2018) 305–312

Contents lists available at ScienceDirect

Industrial Crops & Products

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

Chemical profile and antioxidant activity of sage herbal dust extracts obtained by supercritical fluid extraction

Branimir Pavlić[a], Oskar Bera[a], Nemanja Teslić[b], Senka Vidović[a], Giuseppina Parpinello[b], Zoran Zeković[a,*]

[a] University of Novi Sad, Faculty of Technology, Bulevar Cara Lazara 1, 21000 Novi Sad, Serbia
[b] University of Bologna, Department of Agricultural and Food Sciences, Piazza Goidanich 60, 47521 Cesena (FC), Italy

ARTICLE INFO

Keywords:
Sage herbal dust
Supercritical fluid extraction
Monoterpenes
Antioxidant activity
Artificial neural networks

ABSTRACT

The main aims of this research were chemical profiling of essential oil and lipid extracts of sage herbal dust obtained by traditional (hydrodistillation and Soxhlet extraction) and modern extraction techniques (supercritical fluid extraction – SFE), as well as optimization of the SFE process using Artificial Neural Networks (ANNs). Chemical profile of lipid extracts obtained at different set of SFE pressure (100–300 bar), temperature (40–60 °C) and CO_2 flow rate (0.2–0.4 kg/h) were compared with essential oil and lipid extracts obtained by conventional techniques. The most abundant compounds in all samples were oxygenated monoterpenes (camphor, α-thujone and eucalyptol), sesquiterpenes (viridiflorol) and diterpene polyphenols (epirosmanol). SFE provided significant advantages in terms of monoterpene yield and selectivity comparing to traditional techniques. Antioxidant activity of lipid extracts obtained by SFE was determined by DPPH and FRAP assays. Furthermore, multi-response optimization of SFE process was performed by ANNs, and optimized conditions for maximized total extraction yield and antioxidant activity were pressure of 297.52 bar, temperature of 44.39 °C and CO_2 flow rate of 0.40 kg/h.

1. Introduction

Plants from *Lamiaceae* family, such as rosemary, thyme, sage, oregano and peppermint, have been recognized for their potent antioxidant activity (Babovic et al., 2010). Therefore, they have been utilized for production essential oils and extracts which could be used in either food additives or pharmaceutical formulations. Sage (*Salvia officinalis* L.) has particularly rich history of usage as medicinal and aromatic plant, due to its interesting chemical profile. The most abundant compounds in sage essential oil are monoterpene ketones, such as α-thujone, β-thujone and camphor (Aleksovski and Sovová, 2007), however, its high antioxidant potential has been mainly attributed to presence of diterpene polyphenols (epirosmanol, carnosol and carnosic acid) (Babovic et al., 2010). It should be also pointed out that sage contains high content of bioactive polyphenols (Lu and Yeap Foo, 2002), which could possess different polarity comparing to essential oil compounds. Therefore, it is essential to apply appropriate extraction procedure with adequate set of process parameters in order to recover target compounds in obtained extracts.

Various extraction techniques have been applied for extraction of sage bioactives. Hydrodistillation and supercritical fluid extraction (SFE) have been commonly used for essential oil (EO) recovery (Aleksovski and Sovová, 2007; Occhipinti et al., 2014; Pavlić et al., 2015), while polyphenolic fraction has been extracted by different emerging techniques such as ultrasound-assisted (Valachovic et al., 2001; Zeković et al., 2017a), microwave-assisted (Putnik et al., 2016; Zeković et al., 2017a) and subcritical water extraction (Pavlić et al., 2016). Conventionally used techniques for essential oil recovery such as hydrodistillation and extraction with organic solvents are followed with certain disadvantages. Heat exposure in hydrodistillation can lead to degradation of thermo-labile compounds (Aleksovski and Sovová, 2007), which results in alteration of chemical profile in obtained essential oil. Furthermore, hydrodistillation procedure demands huge energy consumption (Pourmortazavi and Hajimirsadeghi, 2007). In case of essential oil extraction with organic solvents, toxicity of solvent residue and its poor selectivity represent the main drawbacks. Therefore, recent trends of "green" chemistry demand application of "green" extraction techniques (Chemat et al., 2012). Furthermore, it has been reported that green extraction techniques provided certain advantages in terms of chemical profile and selectivity comparing to conventional in recovery of coriander essential oil (Pavlić et al., 2015). Accordingly, the initial hypothesis of this work was that sage extracts obtained by

* Corresponding author.
E-mail address: zzekovic@tf.uns.ac.rs (Z. Zeković).

https://doi.org/10.1016/j.indcrop.2018.04.044
Received 20 November 2017; Received in revised form 13 March 2018; Accepted 15 April 2018

SFE would higher monoterpene yield comparing to hydrodistillation and Soxhlet extraction.

Another aspect which should be addressed was recent utilization of food industry by-products and agricultural waste as raw material for recovery of bioactive compounds. Therefore, biorefinery has recently emerged as promising approach which could lead towards sustainable concept (Silalertruksa et al., 2017). Due to ever-increasing market of functional foods the search for new natural bioactive components is a hot topic on which a lot of research effort is being focused currently (Herrero et al., 2015). Food industry by-products and waste streams have been characterized as valuable source of bioactive compounds including polyphenols, proteins, alkaloids, sugars and lipids (de los Ángeles Fernández et al., 2018) and appropriate concepts for bioactives recovery should be established. Recently, few laboratory concepts for recovery polyphenols (Pavlić et al., 2017a) and lipids (Pavlić et al., 2017b) from sage herbal dust obtained as by-product from filter tea factory have been developed.

The aim of this work was to study quality and quantity of EO and lipid extracts of sage herbal dust obtained by traditional (hydrodistillation and Soxhlet extraction) and modern extraction techniques (SFE). Obtained results are compared in order to estimate whether novel techniques could provide a satisfactory yields in terms of total extracts and dominant compounds. Furthermore, another goal was to determine influence of SFE parameters on monoterpene yield and antioxidant activity and to perform Artificial Neural Networks (ANN) optimization which should provide extract with the best properties.

2. Materials and methods

2.1. Plant material

Sage (*Salvia officinalis* L.) originated from Montenegro was kindly donated by local filter tea factory, Fructus DOO (Bačka Palanka, Serbia). Herbal dust fraction with particle size < 0.315 mm was generated as by-product in filter tea processing. Discarded plant material was collected and used as raw material in this research.

2.2. Chemicals

Commercial carbon dioxide (Messer, Novi Sad, Serbia) with > 99.98% (w/w) purity was used for laboratory scale SFE. 1,1-Diphenyl-2-picrylhydrazyl (DPPH), α-thujone, TPZT (2,4,6-tris(2-pyridil)-s-triazine), iron(III) chloride and Iron(II) sulfate heptahydrate and potassium persulfate were purchased from Sigma (Sigma-Aldrich GmbH, Steinheim, Germany). Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) was purchased from Sigma–Aldrich (Milano, Italy). Sodium acetate and hydrochloric acid were obtained from Merck (Darmstadt, Germany). Formic acid was obtained from VWR International (Milan, Italy). The standard compounds for GC analysis (GC purity) were purchased from Ehrenstorfen, Germany and Carl Roth, Germany. Acetonitrile (HPLC-grade) was purchased from J. T. Baker (Deventer, The Netherlands), and HPLC standards were supplied from Sigma (Sigma-Aldrich GmbH, Steinheim, Germany). All other chemicals used were of analytical reagent grade.

2.3. Supercritical fluid extraction

The supercritical fluid extraction (SFE) experiments were performed on laboratory scale high pressure extraction plant (HPEP, NOVA, Swiss, Effretikon, Switzerland) described in detail by Pekić et al. (1995). The main plant parts and properties, by manufacturer specifications were: gas cylinder with CO_2, the diaphragm type compressor with pressure range up to 1000 bar, extractor with heating jacket for heating medium with internal volume 200 mL, maximum operating pressure of 700 bar, separator with heating jacket for heating medium (with internal volume 200 mL, maximum operating pressure of 250 bar), pressure control valve, temperature regulation system and regulation valves. The separator conditions were 15 bar and 25 °C, while extraction conditions varied for each experimental run.

Table 1
Experimental design for applied SFE, hydrodistillation and Soxhlet extraction.

Sample	Pressure [bar]	Temperature [°C]	CO_2 flow rate [kg/h]	Density [g/cm^3]
Box-Behnken experimental design				
SFE-1	100	60	0.3	0.290
SFE-2	300	50	0.2	0.871
SFE-3	200	40	0.4	0.840
SFE-4	200	60	0.2	0.724
SFE-5	200	40	0.2	0.840
SFE-6	300	60	0.3	0.830
SFE-7	100	50	0.4	0.384
SFE-8	100	50	0.2	0.384
SFE-9	300	40	0.3	0.910
SFE-10	200	60	0.4	0.724
SFE-11	200	50	0.3	0.784
SFE-12	100	40	0.3	0.629
SFE-13	200	50	0.3	0.784
SFE-14	200	50	0.3	0.784
SFE-15	300	50	0.4	0.871
Additional experiments				
SFE-16	300	50	0.3	0.871
SFE-17	100	50	0.3	0.384
Conventional techniques				
EO	Hydrodistillation		< 100 °C, 2 h	
SOX-MeCl	Soxhlet extraction		Boiling point approx. 40 °C	Methylene chloride
SOX-Hex			Boiling point approx. 69 °C	Hexane

Experimental design used for this research was previously described by Pavlić et al. (2017b). Briefly, design consisted of 15 runs of Box-Behnken experimental design with pressure (100, 200 and 300 bar), temperature (40, 50 and 60 °C) and CO_2 flow rate (0.2, 0.3 and 0.4 kg/h) as independent variables. Two more experiments were added in order to provide more detailed information about influence of SFE parameters on total extraction yield, chemical profile and antioxidant activity (Table 1). Total extraction yield (Y) was expressed as grams of total extractable compounds per 100 g of plant material (g/100 g), i.e. percentage (%).

2.4. Hydrodistillation

The essential oil (EO) of sage herbal dust was obtained using the official hydrodistillation procedure (*Ph. Jug.* IV).

2.5. Soxhlet extraction

Sage herbal dust (10.0 g) was separately extracted by methylene chloride and hexane (120 mL each) using Soxhlet apparatus. After 15 exchanges of the extract (approximately 6 h), which was enough for discoloration, solvent was evaporated under vacuum and obtained extract was further dried at 40 °C for 24 h in laboratory dryer (Sutjeska, Serbia). Obtained dry extracts (SOX-MeCl and SOX-Hex) were used in further analysis.

2.6. GC–MS and GC-FID analysis

GC–MS analysis was used for identification of volatile compounds in lipid extracts/essential oil obtained from sage herbal dust, and it was performed on Agilent GC890N system coupled to mass spectrometer model Agilent MS 5759, equipped with HP-5MS column (30 m length, 0.25 mm inner diameter and 0.25 μm film thickness). Helium flow rate was 2 mL/min. Obtained extracts were dissolved in methylene chloride (about 1 mg/mL) and injected volume of solution was 5 μL with split