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Supercritical carbon dioxide extraction of antioxidant fractions from selected Lamiaceae herbs and their antioxidant capacity

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ABSTRACT

Antioxidant fractions from four herb spices belonging to the Lamiaceae family: rosemary (*Rosmarinus officinalis*), sage (*Salvia officinalis*), thyme (*Thymus vulgaris*) and hyssop (*Hyssop officinalis*) were isolated using supercritical CO_2 at 35 MPa and 100 °C. The antioxidant fractions were characterized chemically by HPLC-DAD/ESI-ToF-MS. Antioxidant activity of obtained extracts was determined by measuring their ability to scavenge stable 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical and reactive hydroxyl radical during the Fenton reaction trapped by 5,5-dimethyl-1-pyroline-N-oxide (DMPO), using electron spin resonance (ESR) spectroscopy. The antioxidant activity of the extracts was compared to the activity of butylated hydroxyanisole (BHA) and Flavor' Plus™ water-soluble rosemary extract. In DPPH radical assay the order from the strongest to the weakest antioxidant activity was: BHA, thyme extract, Flavor' Plus™, rosemary and sage extracts, and hyssop extract, while in hydroxyl radical assay order was: Flavor' Plus™, sage extract, rosemary extract, hyssop extract, BHA and thyme extract.

Industrial relevance: Spices and herbs have been used not only for flavoring food but also for improving the overall quality of the product and to extend the shelf-life of foods. The present investigation relates to the field of food additives, and particularly to an antioxidant fractions from four herb spices which belong to the Lamiaceae family: rosemary (*Rosmarinus officinalis*), sage (*Salvia officinalis*), thyme (*Thymus vulgaris*) and hyssop (*Hyssop officinalis*). Butylated hydroxyanisole (BHA) and Flavor' Plus™ are used in food industry as antioxidants due to their ability to prolong the shelf-life of foodstuffs by protecting them against deterioration caused by oxidation, such as fat rancidity, colour changes, degradation of the flavor and loss of nutrient value. Synthetic antioxidants such as BHA now being replaced by natural antioxidants because of their possible toxicity and due to a suspected action as promoters of carcinogens. The present study confirms that investigated herb spices belonging to the Lamiaceae family present important sources for the production of food additives.

1. Introduction

Herbs of the Lamiaceae family (previously known as Labiatae), like rosemary, sage, oregano and thyme are well-known for their antioxidant activity. Antioxidants are important in the food industry not only because of their usefulness as a preservation method but also because of their beneficial effects on human health (Madhavi, Despande, & Salunkhe, 1996). The utilisation of synthetic antioxidants is limited because consumers are increasingly demanding additive-free or natural products (Ahn, Grün, & Fernando, 2002). Therefore, the application of spices and herbs as sources of many effective antioxidants is a promising alternative to the use of synthetic antioxidants. The majority of natural antioxidants are phenolic compounds or polyphenols and the antioxidant activity of many natural extracts is due to such phenolic compounds.

The leaves of the plant *Rosmarinus officinalis* L. are commonly used as a spice, flavoring agent, and naturally occurring antioxidant. Phenolic compounds present in rosemary extracts can be classified into three groups: phenolic diterpenes, flavonoids and phenolic acids (Cuvelier, Richard, & Berset, 1996). Among the herbs of the Lamiaceae family, rosemary has been more extensively studied as a natural antioxidant source (Yanishlieva, Marinova, & Pokorný, 2006).

Sage, *Salvia officinalis* L., has been most commonly known not only as a culinary herb for flavoring and seasoning, but has been also of

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great medicinal importance such as anti-lactation, anti-inflammation, anti-sore throat, and anti-dyspepsia (Ninomiya et al., 2004). Together with *R. officinalis* L., *S. officinalis* L. has been shown to have the strongest antioxidant activities among herbs. It was previously reported that the antioxidant activity of extract obtained by supercritical carbon dioxide extraction from rosemary and sage leaves was comparable with the activity of synthetic antioxidants, such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT), without the cytotoxic and carcinogenic risk of synthetic antioxidants (Huang et al., 1994; Ito, Fukushima, Hagiwara, Shibata, & Ogiso, 1983; Djarmati, Jankov, Schwirtlich, Djulinac, & Djordjevic, 1991). Djarmati et al. (1991) reextracted ethanol extract of sage with supercritical carbon dioxide and isolated antioxidant compound (rosmanol-9-ethyl ether) with activity much greater than BHT. Highly active compounds in rosemary and sage extracts were found to be phenolic diterpenes such as carnosol and carnosic acid (Schwarz & Waldemar, 1992), royleanonic acid and 7-methoxyrosmanol (Ninomiya et al., 2004), rosmadial (Nakatani & Inatani, 1983), rosmanol (Inatani, Nakatani, Fuwa, & Seto, 1982; Nakatani & Inatani, 1983), epirosmanol and isorosmanol (Nakatani & Inatani, 1984), methyl carnosate (Cuvelier, Berset, & Richard, 1994), rosmanol-9-ethyl ether (Djarmati et al., 1991) flavonoids such as genkwanin, cirstimaritin and scutellarein and phenolic acids such as rosmarinic acid (Cuvelier et al., 1996; Senorans, Ibanez, Cavero, Tabera, & Reglero, 2000; Cavero et al., 2005).

Thyme (*Thymus vulgaris* L.) has been used commonly as a culinary herb for adding flavor and as cough medicine, to treat dyspepsia and other gastrointestinal disturbances. In particular, thyme is valued for its antiseptic and antioxidant properties (Miura & Nakatani, 1989; Schwarz, Ernst, & Ternes, 1996; Economou, Oreopoulo, & Thomopoulos, 1991), deodorizing (Nakatani, Miura, & Inagaki, 1989) and anti-platelet activity (Okazaki, Kawazoe, & Takaishi, 2002). A recent investigation (Simandi et al., 2001) has demonstrated antioxidant activity of thyme extract obtained by supercritical fluid extraction.

Hyssop (*Hyssopus officinalis* L.) as a food ingredient has its own importance in the flavor industry and also in sauce formulations (Kazazi, Rezaei, Ghotb-Sharif, Emam-Djomeh, & Yamini, 2007). As a medicinal plant, hyssop has also been used as a carminative, emmenagogue, stimulant, stomachic, and tonic. Among the medicinal and aromatic plants, hyssop is a plant that has not been studied very much. Dapkevicius, Venskutonis, van Beek, and Linssen (1998) reported that antioxidant activity of deodorized hyssop extracts was very low in comparison with rosemary, thyme, marjoram and sage. Djarmati et al.(1991) isolated potent antioxidant rosmanol-9-ethyl ether from the alcoholic extract of the hyssop.

Supercritical fluid extraction (SFE) with carbon dioxide is considered to be the most suitable method for producing natural antioxidants to be used in the food industry. Carbon dioxide is safe (GRAS), non-toxic, non-carcinogenic, non-flammable, has modest critical conditions (31.1 °C, 7.38 MPa) and inexpensive. This emerging clean technology provides solvent free extracts and the selectivity of the supercritical CO_2 can be adjusted by varying temperature and pressure to obtain the fractions consisting of desirable compounds. Antioxidants from rosemary have been extracted and de-aromatized by supercritical CO_2 for use in natural supplements and several reports have been published. For instance, Lopez-Sebastian et al. (1998) utilized supercritical CO_2 for the deodorization of rosemary extracts obtained by solvent extraction, while Ibanez et al. (1999) fractionated rosemary essential oil and antioxidant fraction.

The method describing isolation of antioxidants by SFE at high pressures was patented in USA in 1991 (Nguyen, Frankman, & Evans, 1991). Nguyen et al. (1991) reported extraction of antioxidants by supercritical CO_2 from rosemary, sage, oregano and thyme at pressures above 35 MPa and temperatures in range 90–110 °C. At extraction temperature above 110 °C heat damage of the extracted components could occur. Further, the authors reported that it was preferred that the supercritical fluid used in process was pure CO_2 without the addition of cosolvents such as ethanol or propane. Cosolvents increased the yield by coextraction of other compounds (without antioxidant properties) resulting in reduced antioxidant activity of the obtained extract when compared with the activity of the extract obtained with pure CO_2.

The aim of the present study was to investigate isolation of antioxidant fractions from rosemary, sage, thyme and hyssop by fractional supercritical carbon dioxide extraction, as well as to determine the antioxidant activity of the obtained extracts by measuring their ability to scavenge DPPH free radical and hydroxyl radical using electron spin resonance (ESR) spectroscopy. Chemical analysis of obtained antioxidant extracts was performed using LC-MS. Carnosol and carnosic acid, among the main compounds present in examined extracts, were quantified with regard to pure standard. Identification of the other compounds was tentative. To the best of our knowledge, there is no data available in the open literature on chemical composition of SFE extracts of investigated plants obtained at 35 MPa and 100 °C. Also there is no data at all on the chemical composition of thyme and hyssop extracts obtained by SFE.

2. Materials and methods

2.1. Samples and chemicals

Dried leaves of selected herbs belonging to the Lamiaceae family: rosemary (*R. officinalis*), sage (*S. officinalis*), thyme (*T. vulgaris*) and hyssop (*Hyssop officinalis*) originated from the southern Balkan region were used for the study.

Commercial carbon dioxide (99% purity, Tehno-gas, Novi Sad, Serbia) was used for the SFE. DPPH, DMPO and BHA were from Sigma Chemical Co., St. Louis, USA, and Flavor' Plus™ was from Naturex, France. Methanol for HPLC was from Burdick & Jackson, Mashegon, MI, USA, acetonitrile was from Merck KgaA, Darmstadt, Germany, formic acid was from Lach-Ner, s.r.o., Neratovice, Czech Republic and Milli Q water 18.2 MΩ cm was obtained from purification system. Millipore Simplicity 185 were used for the study. The standard compounds used for the chemical analyses were carnosic acid and carnosol (Sigma Chemical Co. (St. Louis, USA)). These chemicals were of analytical reagent grade. Other used chemicals and solvents were of the highest analytical grade and obtained from "Zorka" Šabac (Serbia).

2.2. Supercritical CO_2 extraction

In order to isolate antioxidant fractions, method of fractional supercritical extraction with carbon dioxide was applied. Essential oil fraction was extracted first at pressure of 11.5 MPa and temperature of 40 °C. Extraction of antioxidant fraction followed at pressure of 35 MPa and temperature of 100 °C. Extraction conditions (100 °C and 35 MPa) were chosen on the basis of previous investigations. Nguyen et al. (1991) showed that the temperature range 90–110 °C was the most preferable for the isolation of antioxidant fraction from Lamiaceae family species. Further Rižnar, Čelan, Škerget, and Knez (2008) reported that carnosol (from rosemary extract) solubility had the highest value at the pressure of 35 MPa and increased with the temperature increasing from 40 °C to 80 °C.

Plant material was ground and sieved. The fraction of the average particle diameter of 0.400 mm was used for further study. The flow rate of SC-CO_2 was 0.3 kg/h in all experiments. The initial used mass of the plant samples was 56 g for rosemary, 56.5 g for sage, 54 g for thyme and 55 g for hyssop. Extractions with supercritical carbon dioxide (SC CO_2) were performed in the Autoclave Engineers Screening System previously described (Zizovic, Stamenic, Orlovic, & Skala, 2007). The simplified scheme of the laboratory plant is presented in Fig. 1. Liquid CO_2 is supplied from the CO_2 cylinder by a siphon tube and cooled in a cryostat between the cylinder outlet and