J. of Supercritical Fluids 64 (2012) 1–8

Contents lists available at SciVerse ScienceDirect

The Journal of Supercritical Fluids

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

Kinetic study of the supercritical CO_2 extraction of different plants from *Lamiaceae* family

Tiziana Fornari*, Alejandro Ruiz-Rodriguez, Gonzalo Vicente, Erika Vázquez, Mónica R. García-Risco, Guillermo Reglero

Instituto de Investigación en Ciencias de la Alimentación CIAL (CSIC-UAM), C/Nicolás Cabrera 9, Universidad Autónoma de Madrid, 28049 Madrid, Spain

ARTICLE INFO

Article history:
Received 20 November 2011
Received in revised form 12 January 2012
Accepted 12 January 2012

Keywords:
Supercritical extraction
Carbon dioxide
Oregano
Sage
Rosemary
Thyme

ABSTRACT

The supercritical CO_2 extraction of four different plants from *Lamiaceae* family, namely oregano (*Origanum vulgare*), thyme (*Thymus zygis*), sage (*Salvia officinalis*) and rosemary (*Rosmarinus officinalis*) was carried out in an experimental pilot-plant comprising an extraction cell of 2 L capacity. 600 g of leaves of each plant material, with the same pre-treatment, were extracted at the same pressure and temperature (30 MPa and 313 K) and using 2.4 kg/h of CO_2. Further, the same fractionation procedure in a two on-line decompressing separators at, respectively, 10 MPa and 0.1 MPa was employed. In this way, a thoughtful comparison of the extraction kinetic was established and discussed, in terms of the extraction yields attained in the separators, the variation of the essential oil composition with time and the content of key bioactive substances identified in the different fractions.

1. Introduction

In the European market there are a lot of products derived from natural plants, commonly recognized with biological properties, such as antioxidant, antiseptic, diuretic, stimulating the central nervous system, sedative, expectorant, digestive, etc. Some of these plants have been used in traditional medicine since ancient times and are available on market as infusions, tablets and/or extracts.

Natural sources of bioactive substances, as well as new industrial approaches to extract and isolate these substances from raw materials, are gaining much attention in the food and pharmaceutical research field. Indeed, among innovative process technologies, supercritical CO_2 (SC-CO_2) extraction and fractionation is the most widely studied application. The production of supercritical plant extracts has received increasing interest in recent decades [1–3] and has brought a wide variety of products that are being intensively investigated due to their favorable effects on diversity human diseases. Different authors compared supercritical extracts with those obtained using liquid solvents (ethanol and hexane) or hydrodistillation, and described superior quality (better functional activity) of the supercritical extracts [4,5].

Among the different vegetable raw materials considered, several plants from the *Lamiaceae* family were subject of intensive study. In general, the essential oils of these plants are recognized to contain the substances for which the plant is used in the pharmaceutical, food or fragrance industries. Essential oils represent a small fraction of the plant composition; the main compounds are terpenes and sesquiterpenes, and several oxygenated derivative compounds (alcohols, aldehydes, ketones, acids, phenols, ethers, esters, etc.) all of them responsible for the characteristic plant odor and flavor [2].

Particularly, *Origanum vulgare L.* is an herbaceous plant native of the Mediterranean regions, used as a medicinal plant with healthy properties like its powerful anti-bacterial and anti-fungical properties [6,7]. The responsible of these activities in oregano is the volatile oil, which contains thymol and carvacrol as the primary components [8]. In these compounds, Puertas-Mejia et al. [9] also found some antioxidant activity.

The supercritical extraction and fractionation of oregano has been studied and reported in the literature [10–12]. Moderate conditions (solvent densities between 300 and 500 kg/m^3) were found to be sufficient for an efficient extraction of volatile oil compounds. Although higher pressures increase the rate of extraction and yield of the essential oil fraction, also significant amounts of waxes were co-extracted and, consequently, the essential oil content in the extract decreased [12].

Thymol and carvacrol were also found in the essential oil of another *Lamiaceae* plant, namely *Thymus*. The variety most studied is, indeed, *Thymus vulgaris* [13,14]. Yet, particularly attention is focused on *Thymus zygis*, a thyme variety widespread over Portugal

* Corresponding author at: Instituto de Investigación en Ciencias de la Alimentación, 23 CIAL (CSIC-UAM), C/Nicolás Cabrera 9, Universidad Autónoma de Madrid, 28049 Madrid, Spain. Tel.: +34 661514186; fax: +34 914978255.
E-mail address: tiziana.fornari@uam.es (T. Fornari).

0896-8446/$ – see front matter © 2012 Elsevier B.V. All rights reserved.
doi:10.1016/j.supflu.2012.01.006

and Spain, which extract has proved to be useful for food flavoring [15] and in the pharmaceutical [16,17] and cosmetic industries [18]. Moldao-Martins et al. [19] studied the supercritical extraction of *Thymus zygis* at different temperatures (300–323 K) and pressures (8–20 MPa) and reported a comprehensive comparison of the extracts produced with those obtained from steam distillation.

Other *Lamiaceae* plants being intensively studied are the "*Officinalis*" ones (from Latin meaning medicinal). Sage (*Salvia officinalis L.*) is a popular kitchen herb and has been used in a variety of food preparations since ancient times, and has a historical reputation for promotion of health and treatment of diseases [20]. Modern day research has shown that sage essential oil can improve the memory and has shown promise in the treatment of Alzheimer's disease [21]. In the past few decades however, sage has been the subject of an intensive study for its phenolic antioxidant components [22–24]. Supercritical extraction of sage demonstrated that when sage leaves are ground in fine particles, the essential oil is easily accessible to the SC-CO_2 solvent (9–13 MPa and 298–323 K) and the extraction is controlled by phase equilibrium [25]. That is, large part of the total essential oil contained in the plant matrix is dissolved almost immediately in SC-CO_2. To extract high molecular and polar compounds from sage, CO_2 with an ethanol–water mixture as co-solvent was employed; antioxidant substances such as rosmarinic acid and carnosic compounds were extracted, achieving a recovery of 55% and 75%, respectively [26].

The supercritical extraction of rosemary (*Rosmarinus officinalis L.*), which has been recognized as one of the plants with large antioxidant activity, also produced extracts with large concentrations of phenolic antioxidants. Main substances associated with the antioxidant activity of rosemary extract are the phenolic diterpenes such as carnosol, rosmanol, carnosic acid, methyl carnosate, and phenolic acids such as the rosmarinic and caffeic acids [27–31]. Among the large number of papers related with the supercritical extraction and fractionation of rosemary and its effect on the antioxidant activity of the extracts, the authors have recently presented two new contributions [32,33]. In the first work [32], the scaling of supercritical rosemary extraction in terms of extraction kinetic and mass transfer coefficients was studied. In the second contribution [33], on-line fractionation was considered with the target of attaining a product with high yield and antioxidant activity.

Indeed, numerous variables have singular effect on the supercritical extraction yield and on the composition and quality of extracts. Process conditions, such as extraction pressure and temperature, type and amount of cosolvent, extraction time, fractionation, raw material pre-treatment, plant location and harvesting time, greatly affect not only yield but also composition of the extracted material. The different process conditions applied, together with the variety of equipment and process scale employed, complicate the comparison of the competence of supercritical CO_2 technology in the extraction of bioactive compounds from plant material.

Comparison of supercritical CO_2 extraction of different plant matrix maintaining identical conditions is of relevance in order to study the extraction of mixed plants. Furthermore, extraction of mixed herbs is of high processing interest from a cost-effective point of view: many bioactive phytochemicals may act synergistically and thus, may have much more effective response. In this case, the kinetic behavior of each plant at a given extraction condition should be considered and compared in order to attain a bioactive target in the extract.

In this paper we carried out the extraction of four *Lamiaceae* plant varieties, namely oregano, thyme, sage and rosemary, using the same procedure for the preparation of the raw materials (plant leaves), employing the same experimental pilot-plant device and the same extraction conditions and procedure. Then, the kinetic behavior of the extractions, considering both yield and composition of the fractions obtained, was evaluated and compared.

2. Materials and methods

2.1. Chemicals

Carnosic acid (≥96%) was purchased from Alexis Biochemical (Madrid, Spain). thymol (99.5%), camphor (>97%) and linalool (>97%) were purchased from Sigma–Aldrich (Madrid, Spain), whereas 1,8 cineole (98%) and borneol (>99%) were purchased from FLUKA (Madrid, Spain). Ethanol, acetonitrile and phosphoric acid were all HPLC grade from Lab Scan (Dublin, Ireland).

2.2. Preparation of plant leaves

Plant material consisted of dried leaves obtained from an herbalist's producer (Murcia, Spain). A kitchen-type knife mill was employed to carry out grinding of the leaves. The mill was adapted so as to break up the row material under cryogenic conditions (using carbon dioxide). The particle size distribution was determined with a vibratory sieve shaker. Sieves were selected in order to have high yield in the grinding process (>85%). Particle size obtained was in the range of 500–1000 μm. The samples were stored at −20 °C until use.

2.3. Supercritical extraction method

Extractions were carried out in a pilot-plant scale supercritical fluid extractor (Thar Technology, Pittsburgh, PA, USA, model SF2000) comprising a 2 L cylinder extraction cell and two different separators (S1 and S2), each of 0.5 L capacity, with independent control of temperature and pressure. The extraction vessel has a height/diameter ratio of 5.5 (0.42 m height, 0.076 m internal diameter). A detail explanation of the experimental device can be found elsewhere [34].

For each experiment, the cell was filled with 0.6 kg of plant raw material. The extractions were performed at a pressure constant of 30 MPa. Fractionation of the extract was accomplished maintaining S1 at 10 MPa and S2 at ambient pressure (0.1 MPa). Extraction and fractionation temperature was set to be 313 K in all experimental assays. Further, CO_2 flow rate was set to 2.4 kg/h in all experiments (CO_2/plant = 20 kg/kg). For each plant variety extractions were carried out by duplicate, but only in the first assay samples were collected from both separators at intervals of 1.5 h during 4.5 h. The second assay was employed to estimate the uncertainties in the global extraction yields, which were lower than 13.2% of the mass collected in S1 and 5.6% of the mass collected in S2.

The samples recovered in S1 were solid and pasty. Fractions collected in S2 were also solid, but oily appearance. In this separator, after the first interval of time (1.5 h of extraction) a small amount of an aqueous fraction was also observed. This fraction was separated from the solid material and was not considered in the analysis. The solid fractions obtained in S1 and S2 were recuperated and placed in vials. In order to ensure an accurate determination of extraction yield with time, separators were washed with ethanol and the residual material recovered in each case was mixed with the corresponding solid fraction. Ethanol was eliminated by evaporation (35 °C) and then, homogeneous solid samples were obtained and kept under N_2 at −20 °C in the dark until analysis.

2.4. HPLC analysis

In order to quantify the carnosic acid content in the rosemary extracts, samples were analyzed employing a HPLC (Varian