Supercritical CO_2 extraction of oregano and clove buds mixture-synergistic effect and chemical composition of extract

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Abstract

The goal of present study was to investigate kinetics of supercritical carbon dioxide (SC CO_2) extraction of essential oils from dried clove buds and oregano leaves. Total extract yields after exhaustion of plant material at 10 MPa and 40°C (after consumption of 94.3 $kgCO_2/kg_{\text{ plant material}}$) were 18.2% and 1.0 %, respectively. Synergistic effect in the extraction process was also investigated using mixture of clove buds and oregano leaves with initial mass ratio of clove buds to oregano leaves of 1:18.2. This mass ratio was applied assuming that it will give the extract with equivalent masses of clove and oregano extract (50:50) and that expected total yield will be 1.89 %. However, the experimentally determined total yields of extract in three repeated experiments were in the range of 2.10-2.66 % and it was higher than expected.

The experimental data of extract yield *versus* CO_2 consumption were used to obtain the parameters of Sovová model and to propose logical interpretation of SFE extraction from oregano leaf and clove bud mixture.

Chemical analyses of extracts were accomplished by GC/FID and GC/MS analytical methods. Extract obtained by SFE from mixture of oregano leaf and clove bud contains a lower amount of less and higher amount of heavier soluble monoterpene and sesquiterpene type compounds (eugenol, eugenol acetate and *trans*-caryophyllene) than expected on the basis of separate SFE from oregano and clove buds. Such results indicate that some synergistic effect in the SFE process really exists.

***Key words*:** Supercritical extraction; Oregano; Clove buds; Synergistic effect; Modelling.

INTRODUCTION

The clove (*Syzygium aromaticum*, syn. *Eugenia aromaticum* or *Eugenia caryophyllata*), is a tree of the family Myrtaceae, is indigenous to Indonesia, Madagascar, Sri Lanka and the south of China grown primarily for the unopened flower buds which are dried to produce the commercial product. Clove buds contain 15 to 21% by weight of volatile oil rich in eugenol (up to 95%), eugenol acetate and *trans*-caryophyllene. Clove oil and eugenol are well-known for antibacterial, antifungal, insecticidal and antioxidant properties. Eugenol is used as anti-microbial agent against oral bacteria in preparations for dental care and periodontal disease, while sesquiterpenes found in clove have been investigated as potential anticarcinogenic agents [1-3]. The essential oils from clove buds are traditionally obtained by steamdistillation and hydrodistillation [3, 4]. Clove extracts can be isolated by conventional extraction with organic solvents [3, 4], by sub-critical water extraction [4] and supercritical fluid extraction (SFE) [3-9].

Oregano (*Origanum vulgare* L; belongs to family Lamiaceae) originates from the Mediterranean region. The dried leaves, the tincture and the essential oils are used in the flavor industry in various liqueur formulations, in baked goods, tomato sauces, condiments and salad dressings. Extract or essential oil from oregano contains carvacrol and thymol as the major components and they have been studied for its powerful antibacterial, antifungal and antioxidant properties [10-13]. The essential oils from oregano leaves are isolated by steam-distillation and hydro-distillation [12,13]. Active principles from oregano leaves can be also isolated by solvent extraction [13] and extraction with supercritical carbon dioxide (SC CO_2) [10, 14-16]. Recently, some new techniques such as solvent-free microwave extraction and sub-critical water extraction for isolation of bioactive compounds from oregano have been proposed as well [17, 18].

The SFE has been established as an environmentally benign technique for isolation of bioactive compounds from the herbs and spices. Unlike steam-distillation and hydro-distillation the extraction with SC CO_2 provides isolation of essential oil rich extracts from plant material under mild temperature conditions (40°C-60°C), thus avoiding thermal degradation of active compounds as well as hydrolysis and water solubilization of some fragrance constituents. Using a high pressure and mild temperature enables adjusting of dissolving power of supercritical fluid as well as its simple, fast and complete separation from the solute unlike conventional solvent extraction processes. Therefore, SFE could be used for isolation of high quality solvent-free extracts with organoleptic characteristics of the starting vegetable material particularly favorable for application in food industry as well as for preparation of pharmaceutical and cosmetic products.

Since simulation of the SFE processes is of great importance for design purposes, various models have been developed within the past two decades. Three different approaches have been proposed for the mathematical modelling of SFE: empirical models, models based on heat and mass transfer analogy, and models based on differential mass balances integration. The most used and the most proper analysis is obtained from the integration of the differential mass balances, whereby time dependent concentration profiles are obtained for fluid and solid phase [19]. Several models based on differential mass balances integration have been proposed for description of SFE of clove buds [5,9, 20-23] and oregano leaves [24, 25].

The goal of present work was to investigate kinetics of SFE from dried oregano leaves and clove buds as well as kinetics of simultaneous SFE from mixture of clove and oregano. To the best of our knowledge, simultaneous supercritical fluid extraction (SFE) from clove buds and oregano leaves was investigated for the first time in order to observe and estimate potential synergistic effect in the SFE process with respect to quantity and quality of the given supercritical extract. The mixture of 1:18.2 initial mass ratio of clove buds and oregano leaves was used assuming that it will give the extract with equivalent quantities of clove and oregano isolates obtained in the case of separate extraction. Identification and quantification of bioactive compounds in supercritical extracts were accomplished by GC/FID and GC/MS analysis.

The study was intended to explore and compare the capacity and efficiency of carbon dioxide in the extraction of valuable compounds from oregano leaves and clove buds and to determine the parameters of semi-empirical mathematical model of Sovová [22, 23] that has been used in the correlation of experimental data.

MATERIALS AND METHODS

Raw material

Dried clove buds (*Syzygium aromaticum*, syn. *Eugenia aromaticum* or *Eugenia caryophyllata*) imported from Canary Islands (Spain) and dried leaves of oregano (*Origanum vulgare* L.) grown in Zrenjanin-Čenta (Northern Serbia) were supplied from local markets in Belgrade (Serbia). Plant material was ground and sieved, whereby particles with mean diameter of 0.400 mm were selected for the SFE experiments. The moisture content of the air-dried plant material determined by Karl Fischer volumetric titration was 8.78 mass % and 9.70 mass % for clove buds and oregano leaves, respectively.

The supercritical fluid extraction (SFE)

Extractions with SC CO_2 were performed in Autoclave Engineers SCE Screening System with a 150 cm^3 extraction cell previously described elsewhere [26]. The extractions were performed at mild conditions (10 MPa; 313.15 K; 630 kg/m^3) in order to isolate essential oil rich extracts [3, 15]. The initial mass of the plant material was 31.5 g for separate extractions from oregano leaves and clove buds and 35.0 g for simultaneous extractions from the mixture of clove buds and oregano leaves. Plant material was milled and sieved and the fraction of the average particle diameter of 0.400 mm was used for the further study. After milling, plant material (oregano leaves) was exposed to SC CO_2 at extraction conditions for an hour, as a pre-treatment, prior to continuous flow extraction. Chosen pretreatment of herbaceous matrix enhanced the rate of the SFE due to the secretory structure (glandular trichomes) cracking during the exposure to supercritical fluid. Unlike oregano oil, clove bud oil is located in secretory cavities and cells and the extraction process from this type of secretory structures depends dominantly on the grinding efficiency [21, 24].

In order to investigate synergistic effect in the extraction process, mixture of clove buds and oregano leaves with initial mass ratio of clove buds and oregano of 1:18.2 (1.82 g of clove buds and 33.18 g of oregano leaves) was used. This mass ratio was applied assuming that it will give the extract with equivalent quantities of clove and oregano isolates. Since starting mixture of plant materials used for the simultaneous SFE contained 94.8 mass % of oregano leaves, above explained pre-treatment procedure of plant material was applied in order to provide the same condition for extraction of oregano oil. During the simultaneous SFE a differential quantity of extract was collected in order to monitor the changes of the chemical composition with respect to CO_2 consumption. Several fractions were collected after consumption of 20, 50 and 94.3 $kgCO_2/kg_{\text{plant material}}$ in order to determine their composition profile during the period of fast extraction, the transitional period and the period of slow extraction.

The mass flow rate of the CO_2 was 0.56-0.64 kg/h. All extractions were carried until exhaustion of the plant material and the total extraction yields were estimated after consumption of 94.3 $kgCO_2/kg_{\text{plant material}}$ (after 5.5 h of extraction). The extracts obtained during the SFE process were weighed in an analytical balance and the extraction yield (mass %) was calculated from the mass of plant material charged in the extractor and plotted against SC CO_2 consumption ($kgCO_2/kg_{\text{plant material}}$). All experiments were carried out in duplicate (separate SFEs) or triplicate (simultaneous SFEs from mixture; three experiments were noted as Experiment I, Experiment II and Experiment III).

Mathematical modeling

The experimental data of extract yield *versus* CO_2 consumption were used to obtain the parameters of an extended Lack's plug flow model developed by Sovová [23] which was applied for description of experimental extraction curves. The model of Sovová is based on following assumptions: (1) extract is a single compound; (2); axial dispersion in extractor is negligible; (3) temperature, pressure, solvent density and flow rate are constant along the layer of plant material (bed); (4) solvent is solute-free at the entrance to the extractor; and (5) solid bed is homogenous. Basic assumption of the model is that a part of the cells (hypothetical oil-containing units) was opened by milling. The easily accessible solute from the cells opened by milling is extracted first, and the slower extraction of the solute protected by the cell walls follows. Eqs. (1) and (2) describe the mass balances in the fluid and solid phases, respectively:

$$-\rho_s(1-\varepsilon)\frac{\partial x}{\partial t} = J(x,y) \tag{1}$$

$$\rho U\frac{\partial y}{\partial h} = J(x,y) \tag{2}$$

The boundary conditions are:

$$x\,(h, t{=}0){=}x_0 \tag{3}$$

$$y\,(h{=}0, t){=}0 \tag{4}$$

where: h, bed height (m); t, extraction time (s); x, oil concentration in solid phase (kg/m^3); x_0, initial oil concentration in solid phase (kg/m^3); y, oil concentration in fluid phase (kg/m^3); ρ_s, solid specific mass (kg/m^3); ρ, fluid specific mass (kg/m^3); ε, bed porosity; U, interstitial velocity (m/s).

The accessible solute, whose transfer depends only on the diffusion resistance in the solvent, is extracted first. When the oil concentration in solid phase decreases to x_k, the concentration of oil (extract) in fluid phase depends on the rate of its diffusion in the solid phase. The expressions used for mass transfer rates are:

$$J(x > x_k, y) = k_f a_0 \rho(y_r - y) \text{ at } x{>}x_k \tag{5}$$

$$J(x \le x_k, y) = k_s a_0 \rho_s x \text{ at } x{<}x_k \tag{6}$$

where: a_0, solid matrix specific surface area (m^2/m^3); k_f, mass transfer coefficient in fluid phase (m/s); k_s, mass transfer coefficient in solid phase (m/s); y, oil concentration in fluid phase (kg/m^3); y_r, oil solubility in fluid phase (kg/m^3).

The volumetric mass transfer coefficient in the CO_2 phase is:

$$k_f a_0 = 2.7\cdot(U/\varepsilon)^{0.54} \tag{7}$$

The concentration profiles in the solid and solvent phases are calculated from Eqs. (1) and (2) by integration after substituting for the rate of mass transfer $J(x,y)$. Extraction curve is determined as:

$$e(t) = e(q = \dot{q}\,t) = x_0 - \int_0^H x(h,t)dh \tag{8}$$

Introducing dimensionless variables:

$$r = \frac{x}{x_k}, Y = 1-\frac{y}{y_r}, Z = \frac{k_f a_0}{U}\cdot h, \tau = \frac{k_f a_0 \rho y_r}{(1-\varepsilon)\rho_s x_k}\cdot t \tag{9}$$

into Eqs. (1) and (2) with boundary conditions (3) and (4) yields a set of equations:

$$\frac{\partial r}{\partial \tau} = \frac{\partial Y}{\partial z} = -J^*(r,Y) \tag{10}$$

$$r(z,\tau=0)=r_0 \tag{11}$$

$$Y(z=0,\tau)=1 \tag{12}$$

where,

$$J^*(r,Y)=J(x,y)/(k_f a_0 \rho y_r) \tag{13}$$

The extraction curve is given by the relation that includes expressions for three extraction periods: the first period is extraction of easily accessible solute released by grinding, the second one is a transitional period when the easily accessible solute is still extracted in one section of the fixed bed, while the extraction from inside of particles takes place in the other section and, third extraction period is characterized by extraction of inaccessible solute inside the solid particles. Analytical expressions for these periods are:

$$e=\begin{cases}(x_{\mathrm{k}}\dfrac{\tau}{Z})[1-\exp(-Z)] & \text{for } \tau<\tau_m \\ (\dfrac{x_{\mathrm{k}}}{Z})\left[\tau-\tau_{\mathrm{m}}\exp(z_w-Z)\right] & \text{for } \tau_m\le\tau<\tau_n \\ x_0-(\dfrac{x_k}{kZ})\ln\left\{1+[\exp(r_0kZ)-1]\exp[k(\tau_m-\tau)]/r_0\right\} & \text{for } \tau\ge\tau_{\mathrm{n}}\end{cases} \tag{14}$$

where,

$$Z=\frac{k_{\mathrm{f}}a_0H}{U}\ ;\ k=\frac{k_{\mathrm{s}}\rho_s x_{\mathrm{k}}}{\mathrm{k_f}\rho y_{\mathrm{r}}}\ ;\ \tau_m=r_0-1;\ \tau_n=\tau_m+\frac{1}{k}\ln\frac{1+\tau_m\exp(r_0kZ)}{1+\tau_m} \tag{15}$$

The easily accessible solute becomes exhausted at the solvent entrance at the time τ_m, when a transition period between the fast and the slow extraction begins. In this period, the easily accessible solute is still extracted in one section of the fixed bed, while the extraction inside of particles takes place in the other section. The coordinate of the boundary between both sections is:

$$z_w=\frac{1}{kr_0}\ln\frac{r_0\exp[k(\tau-\tau_m)]-1}{r_0-1}\qquad \text{for } \tau_m\le\tau\le\tau_n \tag{16}$$

The overall mass transfer coefficients in the solid phase (k_s), inaccessible oil content per oil-free solid bases (x_k) and solubility of oil in the CO_2 (y_r), were used as adjustable parameters.

Analytical procedures

Gas chromatography analysis of the extracts was carried out on a HP-5890 Series II GC apparatus [Hewlett-Packard, Waldbronn (Germany)], equipped with split-splitless injector and automatic liquid sampler, attached to HP-5 column (25 m x 0.32 mm, 0.52 μm film thickness) and fitted to flame ionization detector (FID). Carrier gas flow rate (H_2) was 1 ml/min, split ratio 1:30, injector temperature was 250 °C, detector temperature 300 °C, while column temperature was linearly programmed from 40-260 °C (at rate of 4 °C/min), and then kept isothermally at 260 °C for further 10 minutes. Solutions of samples in ethanol (or mixture of ethanol and chloroform, 50:50) (~1 mass%) were consecutively injected in amount of 1 μl. Area percent reports, obtained as result of standard processing of chromatograms, were used as base for the quantification analysis.

The same analytical conditions as those mentioned for GC/FID were employed for GC/MS analysis, along with column HP-5MS (30 m x 0.25 mm, 0.25 μm film thickness),

using HP G 1800C Series II GCD system [Hewlett-Packard, Palo Alto, CA (USA)]. Helium was used as carrier gas. Transfer line was heated at 260 °C. Mass spectra were acquired in EI mode (70 eV); in m/z range 40-450. The amount of 0.2 µl of sample solution in ethanol (or mixture of ethanol and chloroform, 50:50) (~1 mass %) was injected.

The components of the oil were identified by comparison of their mass spectra to those from Wiley 275 and NIST/NBS libraries, using different search engines. The experimental values for retention indices were determined by the use of calibrated Automated Mass Spectral Deconvolution and Identification System software (AMDIS ver.2.1.) [27], compared to those from available literature (Adams, 2007) [28] and used as additional tool to approve MS findings.

RESULTS AND DISCUSSION

The kinetic of SFE and the process modelling

The extraction conditions of single-stage SFE of clove buds and oregano leaves (10 MPa, 313.15 K, CO_2 density of 630 kg/m^3) has been chosen in order to obtain supercritical extracts with a high content of volatile and aromatic compounds, primary phenolic monoterpene alcohols (eugenol and carvacrol) and their derivatives which are considered to be the most responsible for antibacterial activity of clove and oregano. The SFE at pressures higher than 10 MPa can result in higher yield of clove extract as result of extraction of other high-molecular weight compounds present in clove buds (e.g., fatty acids, fatty acids methyl esters, sterols, etc.) but the eugenol content of the clove oil does not change obviously [3]. On the other hand, the increase of temperature from 40°C to 50°C (at 10 MPa) doesn't influence the extraction yield but higher temperature increases the content of antibacterial compounds in the clove extract (eugenol). From the same reasons, similar conditions (8-12 MPa and 40 °C) were suggested for isolation of supercritical extract from oregano leaves rich in volatiles (carvacrol) [15, 16].

In this study, separate SFE from clove and oregano as well as simultaneous SFE from the mixture of clove and oregano experiments were realized and extracts yield *versus* specific amount of SC CO_2 consumed in the process of SFE ($kgCO_2/kg_{plant\ material}$) is shown in **Fig. 1**. The experiments were two or three times repeated (in the case of simultaneous SFE from mixture of oregano and clove) and the average experimental yield are within the experimental error of about 2-5 % (experimental error out of this range was observed only for experiment I) (**Fig. 1**).

The supercritical extract isolated from the clove buds was liquid and light yellow colour. The yield of essential oil rich extract from clove buds measured at the end of an exhaustive extraction was 18.2 mass % by weight of the material charged in the extractor. This was in accordance with previously reported yields of clove supercritical extracts ranged from 13-20.8 mass % [3, 5-8, 22]. Unlike, the yield of oregano supercritical extract (1.0 mass%) reported in this study was somewhat lower than previously reported in literature [14, 15]. This can be partially explained either by different extraction conditions or origin of the plant material used in the previous studies [14-16]. As can been seen in the **Fig. 1**, about 90 % of oregano extract can be isolated after one hour of extraction at given conditions with relatively low consumption of SC CO_2 (20 $kgCO_2/kg_{\ plant\ material}$). Unlike, within the same working condition and same amount of SC CO_2 consumed (20 $kgCO_2/kg_{\ plant\ material}$), only 35 % of the clove oil was extracted.

Therewith, an interesting effect has been observed during the process of simultaneous SFE from the mixture of oregano leaves and clove buds. Starting plant material with 94.8 mass % of oregano leaves (oregano leaves to clove buds mass ratio of 18.2:1) has been chosen to give extract with equivalent quantity of oregano and clove isolates in the final extract. This assumption was made on the basis of the total yields of oregano and clove obtained by separate supercritical extractions at given extraction conditions. All three experiments have shown significant increase of extraction yield as well as higher extraction rate in the first extraction period. Determined extraction yields were 2.66 mass%, 2.10 mass % and 2.16 mass % for three repeated experiments (I, II and III, respectively) which are for 40.7%, 11.1% and 14.3 % higher then expected on the basis of results of separate SFE from oregano and clove buds (expected 1.89 mass % after 5.5 h of extraction and supercritical fluid consumption of 94.3 kg CO_2/kg $_{\text{plant material}}$). Theoretically expected yield has been achieved after shorter extraction time (3.5 h) and lower consumption of SC CO_2 (60 kg CO_2/kg $_{\text{plant material}}$). This observation indicated that some kind of synergistic effect occurs during the process of simultaneous SFE from oregano and clove mixture

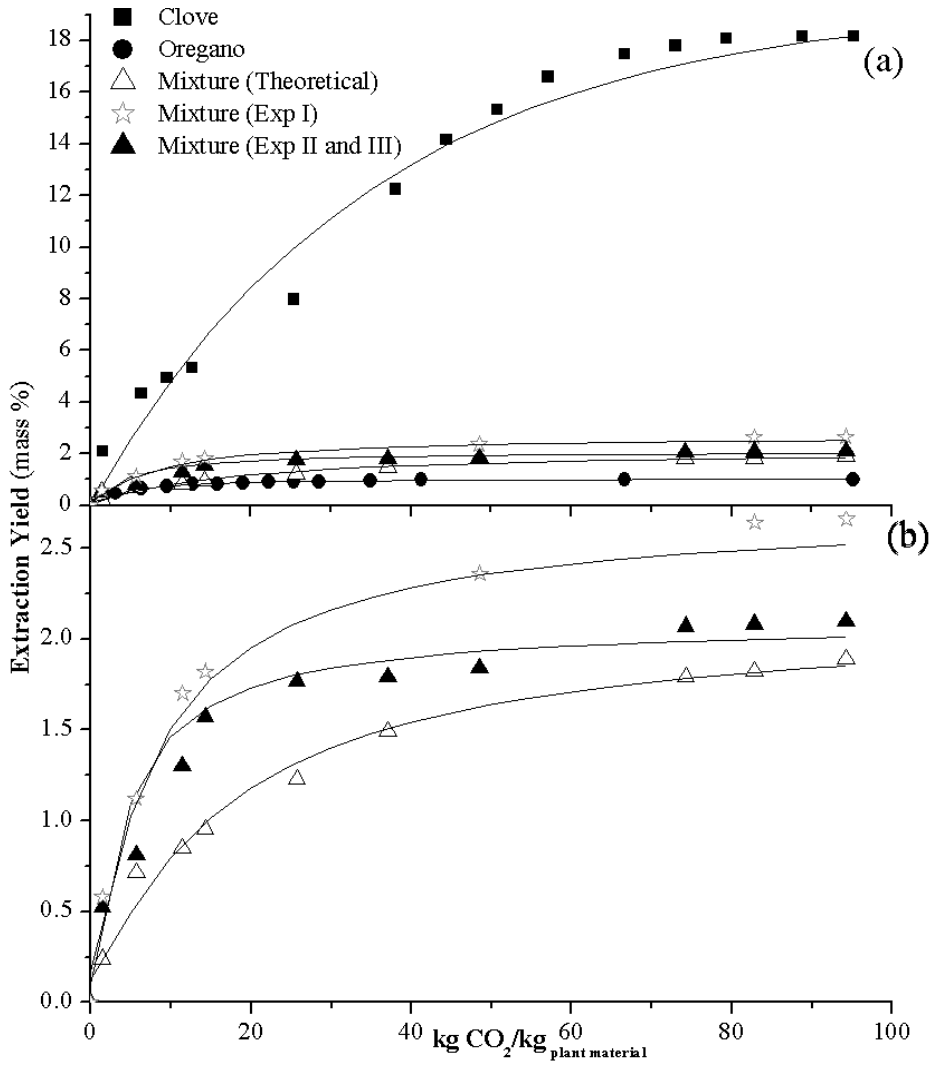

Fig. 1. The extraction yield of supercritical extracts (—: Fitting curves)

With a goal to explain observed effect, the results of following analysis were used: a) mathematical simulation of extraction process using model proposed by Sovová [22, 23] for

interpretation differences between the mass transfer rates in the given extraction processes and, b) GC and GC/MS and chemical composition of the extracts obtained from clove buds and oregano leaves as well as from their mixture to find the influence of specific compounds on overall extraction rate.

The standard deviation between experimental and calculated data ($J = (1/m)\sum_{1}^{m}(Y_j - Y_{j,\exp})^2$) as well as optimized values of adjustable parameters for the model of Sovová are given in the **Table 1**, while experimentally determined and calculated extraction curves are shown in **Fig. 2**.

Table 1. Parameters for model of Sovová

Plant material	Parameter	Units	Clove	Mixture (Exp I)	Mixture (Exp II /Exp III)	Oregano
Experimental and calculated* parameters	Porosity	ε	0.45	0.45	0.45	0.45
	Particle diameter	d_p (mm)	0.4	0.4	0.4	0.4
	Flow rate of CO_2	$q_{CO2} \cdot 10^4$ (kg/s)	1.56	1.67	1.70	1.78
	Density of CO_2	ρ_{CO2} (kg/m)	630	630	630	630
	Superficial velocity	$U \cdot 10^4$ (m/s)	3.46	3.70	3.77	3.95
	Binary diffusion coefficient*	$D_{12} \cdot 10^8$ (m^2/s)	1.46	1.46	1.46	1.50
	Solvent-phase mass transfer coefficient	$k_f \cdot \cdot 10^5$ (m/s)	5.15	5.32	5.39	5.27
	volumetric mass transfer coefficient in fluid phase	$k_f \cdot \cdot a_0\ 10^2$ (1/s)	5.62	5.80	5.90	6.04
	Specific interfacial area*	$a_0 \cdot 10^{-3}$ (1/m)	1.09	1.10	1.10	1.14
Fitting parameters	Inaccessible oil content per oil-free solid bases	$x_k \cdot 10^2$	15.0	0.82	0.65	0.38
	Solid-phase mass transfer coefficient	$k_s \cdot 10^7$(m/s)	1.0	0.52	0.55	2.0
	Overall mass transfer coefficient in solid phase	$k_s \cdot a_0$(1/s) $\cdot 10^4$	1.09	0.57	0.60	2.28
	Solubility	$y_r \cdot 10^3$ ($kg_{extract}$/kg_{CO2})	4.55	1.5	2.20	3.80
Standard deviation	to experimental data	J	0.55	$3.1 \cdot 10^{-3}$	$5.54 \cdot 10^{-3}$	$2.09 \cdot 10^{-3}$

The carvacrol and eugenol were used as pseudo components for calculation the binary diffusion coefficient and modelling the SFE from oregano leaves and clove buds, respectively. Eugenol was also used as pseudo component for calculation the binary diffusion coefficient for simultaneous SFE from oregano and clove mixture.

In the case of SFE from oregano, one reason for a higher value of external mass transfer is the rate of diffusion of carvacrol (expressed by corresponding binary diffusion coefficient) compared to SFE from clove buds where eugenol was used as pseudo component representing extract. Other is of course the origin of plant matrix and its structure. Thus, the resistance to mass transfer in the fluid phase for the simultaneous SFE from oregano and clove mixture was analogically higher than for the pure oregano SFE due to presence of eugenol and carvacrol at the same time.

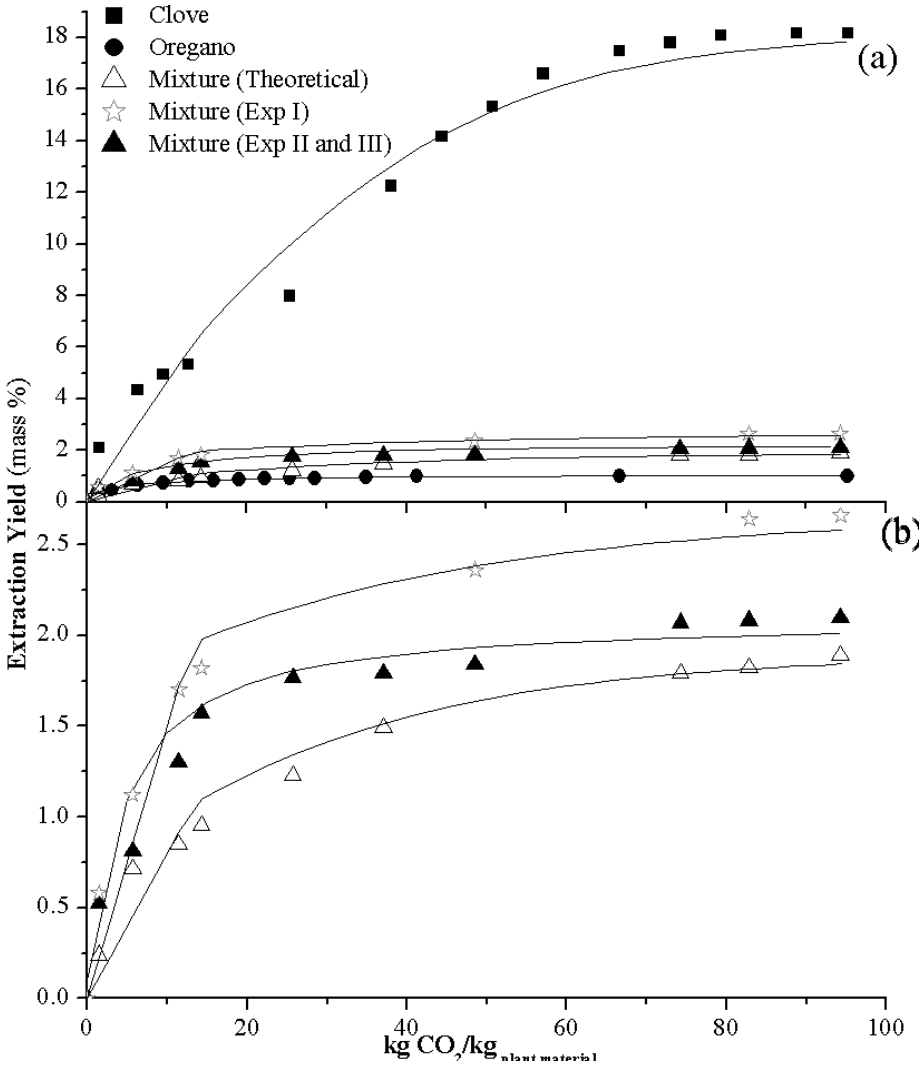

Fig. 2. Yield of extract as a function of the specific amount of SC CO_2 for SFE at 10 MPa and 313.15 K (—: results of mathematical modelling)

Carvacrol as main component of oregano is more soluble in SC CO_2 compared to solubility of eugenol in SC CO_2 which is used as main constituent of clove extract [29]. However, one of adjusted parameters (y_r; kg $_{extract}$/kg $_{CO2}$; **Table 1**) used for determination the extraction curve by Sovová model in this study, indicated a higher solubility of clove extract and similar values for the oregano extract. One can expect that the value of this parameter for

the mixture of clove and oregano will be of the same order but it was app. two times less determined as adjusted and optimal value (experiments I-III, **Table 1**). At this moment there is not possible to give a reasonable explanation for such observed effect and only some evidence is useful being pointed out. Namely, the applied pre-treatment of plant material (exposure of the ground plant material to SC CO_2) leads to faster exhaustion of oil from oregano; the fraction of inaccessible solute in intact glands was much lower than in case of SFE from clove (**Table 1**). Lower inaccessible oil content per oil-free solid bases indicates that the most of the oregano oil is released from glandular trichomes (on the surface of leaves) by grinding and due to cracking during the exposure of the plant material to SC CO_2. It was previously suggested that trichomes disruption during the exposure to supercritical fluid prior to continuous SFE for an hour can result in significant minimization of the internal mass transfer resistance [21]. In accordance to these remarks, further extraction of "inaccessible" oil from oregano requires a higher overall mass transfer coefficient in the solid phase (k_s adjusted parameter **Table 1**). Thus, it seems that product of k_s and x_k which can be defined as an "effective" overall mass transfer coefficient could be the best parameter used for explanation the rate of third or slow period of extraction. This product, $k_s^{eff} = k_s . x_k$, for pure clove is of order 10^{-8} (m/s) but for mixture of clove and oregano as well as for pure oregano if for at least one order less and about 10^{-9}-10^{-10} (m/s). Further study with different initial ratio of clove bud and oregano will be tested to prove such an assumption and calculate the value of "effective" overall mass transfer coefficient.

Chemical analysis

GC/FID and GC/MS analytical methods were used for identification and quantification of the major compounds in obtained supercritical extracts. The clove extract comprised extremely high content of oxygenated monoterpenes, primarily eugenol (63.43%), eugenyl acetate (19.74%) and *trans*-caryophyllene (12.84%) (**Table 2**).

Table 2. The major compounds identified in the supercritical extracts (mass %)

Components	KI	RT/MS	Clove	Oregano	Exp I	Exp II	Adjusted	Theoretical
Thymoquinone (TQ)	1248	18.15		13.13	8.38	1.39	6.03	6.56
Thymol (T)	1289	19.67		5.92	3.13	0.51	2.66	2.96
Carvacrol (C)	1298	20.04		56.51	29.3	17.46	26.14	28.07
Eugenol (E)	1356	21.93	63.43		30.7	44.15	35.28	31.72
trans-Caryophyllene (TC)	1417	23.65	12.84	0.99	5.37	8.2	7.41	6.92
Eugenyl acetate (EA)	1521	27.08	19.74	0.1	8.2	13.58	9.73	9.92
ID%			96.01	76.65	85.08	85.29	87.25	86.15

KI, Kovats index; RT/MS, retention time of corresponding constituent obtained by GC/MS; ID%, percent of given identified compounds

Similar composition profiles of the supercritical extracts from clove buds obtained within the pressure range of 10-30 MPa and at temperatures of 30-55°C have been previously reported in the literature (**Table 3**). The major compounds in the supercritical extracts of oregano were monoterpene alcohols and their derivatives, carvacrol (56.51%), thymol (5.92%) and thymoquinone (13.13%) (**Table 2**). In the previously published data on chemical composition of supercritical extraction of oregano, the main carvacrol and thymol are the most abundant components but their content oscillate due to different extraction conditions [14-16] and origin of row material [15].

Table 3. The composition profiles of supercritical extracts from clove buds and oregano leaves

Origin of plant material	p (MPa);t(°C);time (h); $kg_{CO2}/kg_{plant\ material}$	Major compounds (mass %)	References
Clove buds			
Canary Islands	10 MPa; 40°C; 5.35 h; 94.3	Eugenol (65.43) Eugenyl acetate (19.74) β-Caryophyllene (12.84)	in this study
China	10-30 MPa; 30-50°C; 2 h Optimal: 10 MPa; 50°C; 6160	Eugenol (58.77); Eugenyl acetate (19.6)	[3]
Madagascar	20 MPa; 55°C; 5.5 h; 55	Eugenol (16.42) Eugenyl acetate (3.39); β-Caryophyllene (1.73)	[4]
Italy	9 MPa; 50°C (1^{st} : 9 MPa; -10°C 2^{nd}: 1.5 MPa; 10°C); 10.5 h; 54	Eugenol (65.87); Eugenyl acetate (19.0); β-Caryophyllene (11.1)	[5, 6]
Italy	15 MPa; 25°C; 1.5 h; 51	Eugenol (79.4); β-Caryophyllene (10.2); α-Humulene (12.0); Eugenyl acetate (9.2)	[7]
Brazil	10 MPa; 25°C; 2 h; 5.75	Eugenol (72.03); Eugenyl acetate (15.61) β-Caryophyllene (10.92); α-Humulene (1.44)	[8]
Brazil	6.447-6.97 MPa; 10-12°C;-	Eugenol (48.41-58.62); Eugenyl acetate (19.50-28.19) β-Caryophyllene (19.77-19.91); α-Humulene (1.60-2.49)	[9]
Oregano leaves			
Serbia	10 MPa; 40°C; 4.5 h; 94.3	Carvacrol (56.51); Thymoquinone (13.13); Thymol (5.92)	in this study
Estonia	17.2-25.5 MPa; 45°C; 2.5-7% ethanol; 1 h; -	Thymol (7.2-11.2); Carvacrol (8.0-10.9); Thymol acetate (0.1-12.4)	[14]
Turkey and Hungary	Fractional extraction: 8-30 MPa; 40°C (1^{st}: 8 MPa; 33-37°C 2^{nd}: 2 MPa; 20-25°C); 6-7 h; 90-250	Turkish oregano: Carvacrol (86.1-90.5); p-Cymene (1.50-5.90); Linalool (1.30-2.60) Hungarian oregano: Carvacrol (77.85); Thymol (9.15)	[15]
Spain	12 MPa; 40-60°C; 0.42 h; -	Linalool (43.6); Thymol (21.7); Carvacrol (6.4)	[16]

As expected, the composition mixture of oregano and clove extracts obtained by separate SFE (1:1) was similar to theoretically expected on the basis of the separate extractions from oregano and clove. The extract obtained by simultaneous SFE from oregano and clove mixture obtained in the first experiment comprised almost equally quantities of carvacrol and eugenol unlike the extract obtained in the second experiment which comprised almost three times higher content of eugenol. Since the experiment I had shown significant disagreement with the Experiment II and Experiment III with respect to kinetic data and chemical

composition analyses, it can not be regarded as representative. The increase of total extract's yield of 12.7 % is calculated as mean value from Experiment II and III and used for further analyses.

The quantities of the main components in the representative extract obtained by simultaneous SFE were compared to expected quantities of particular components in accordance to chemical analysis of clove and oregano extracts obtained by separate SFEs. Therewith, the higher amount of the main components of clove (eugenol, *trans*-caryophyllene and eugenol acetate) and lower amount of the main components of oregano extract than expected on the basis of the separate SFEs were observed (**Table 4**). An increase of eugenol, *trans*-caryophyllene and eugenol acetate prevails over decrease of carvacrol, thymol and thymoquinone and result in the increase of the total extract obtained after exhaustive simultaneous SFE at given conditions.

Table 4. Quantitative analysis of the extracts

Components	**Clove** (mass%)	**Oregano** (mass%)	**Mixture** (mass%)	**Mass** (mg/100g plant material)	**Mixture (theoretical)** (mass%)	**Mass** (mg/100g plant material)	**Increase** (%)
Yield (g/100g of plant material)	**18.2**	**1.0**	**2.13**	**2.13**	**1.89**	**1.89**	**12.7**
TQ	-	13.13	1.39	29.61	6.56	124.00	-76
T	-	5.92	0.51	10.86	2.96	55.90	-81
C	-	56.51	17.46	371.90	28.26	534.11	-30
E	63.43	-	44.15	940.40	31.72	599.51	**57**
TC	12.84	0.99	8.20	174.66	6.92	130.79	**33**
EA	19.74	0.1	13.58	289.17	9.92	187.49	**55**

The evaluation of differential and cumulative yield (calculated with respect to mass of the extract) of major compounds during simultaneous SFE from oregano and clove mixture at 10 MPa and 40°C with respect to SC CO_2 consumption ($kgCO_2/kg_{\text{plant material}}$) (**Fig. 3**.) has been made in order to study the dynamics of extraction of the major compounds present in the extract. Differential yield of the carvacrol, thymol and thymoquinone (major components of the oregano) gradually increases in the first and transitional extraction period of SFE (up to consumption of 50 $kgCO_2/kg_{\text{plant material}}$ or 3h). After 3 hours of extraction only negligible quantity of these compounds was still extracted but not in expected quantities which might be observed after their complete exhaustion. Unlike, the constant differential yield decrease was observed for the extraction of eugenol, *trans*-caryophyllene and eugenyl acetate during the extraction (**Fig. 3a**). Slower extraction of these components occurs since the most of the clove oil remains in the intact secretory cells and cavities.

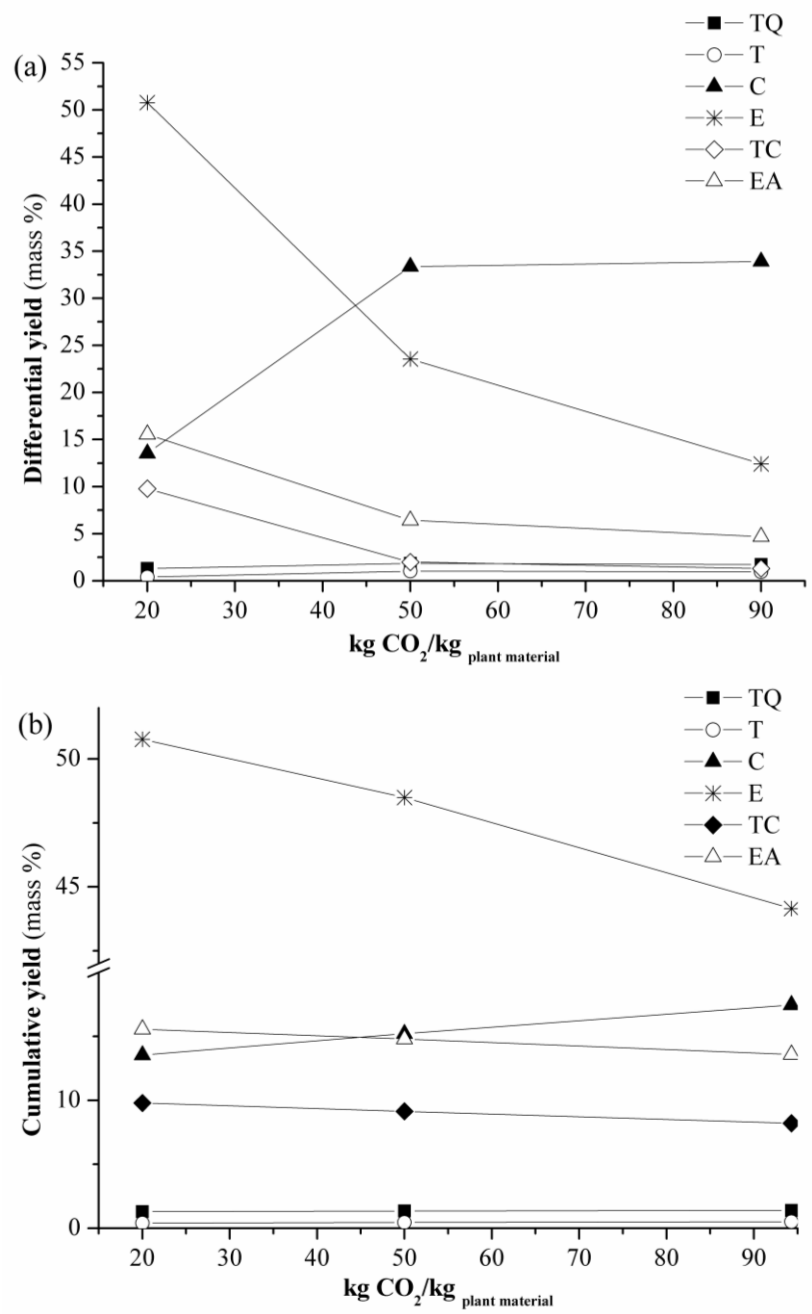

Fig. 3. Differential and cumulative yield of major compounds during simultaneous SFE from mixture of clove buds and oregano leaves (mass %)

Cumulative yields of some compounds shown in **Fig. 3b** remain almost constant during extraction. It was not expected that slight increase of cumulative yields for oregano main component (carvacrol) and decrease for clove main component (eugenol) could be result of simultaneous extraction of clove and oregano. Namely, the fast extraction of compounds which belong to oregano from the easily accessible fraction (carvacrol, thymol and thymoquinone) is expected at the beginning of extraction, while an increase of cumulative yield of other compounds from clove must prevail in the later stage of extraction. In the previous studies, yield of eugenol during the clove buds SFE at similar extraction conditions constantly increases with extraction time and starts to decrease after long time of extractions (after 6.5 h) [4,6]. In this study, decrease of cumulative yield eugenol in the later extraction

period during simultaneous SFE from the oregano and clove mixture can be result of increased rate of extraction of eugenol from herbaceous matrix of clove in the presence of carvacrol.

The following explanation for observed effect could be given. Due to applied pre-treatment of the plant material, the most of the oregano extractible substances (carvacrol) are easily accessible to SC CO_2 and readily dissolved in it. During the exposure of the plant material to SC CO_2, supercritical fluid with already dissolved oregano components diffuse into secretory cavities and cells of oregano. The readily dissolved carvacrol influences solubility power of SC CO_2 acting like co-solvent and promoting extraction of the heavier soluble compounds such eugenol, eugenyl acetate and *trans*-caryophyllene. One part of the oregano extract (carvacrol) probably stays in the finest secretory cavities while in the same time increased quantity of eugenol, eugenol, eugenyl acetate and *trans*-caryophyllene is dissolved in SC CO_2 inside the matrix. The increased quantity of eugenol, eugenol, eugenyl acetate and *trans*-caryophyllene now needs less time to diffuse from solid herbaceous matrix into CO_2 phase. This could explain by lower values of resistance to internal mass transfer during simultaneous SFE of clove and oregano mixture with respect to SFE from clove buds alone.

These assumptions still need to be confirmed by further investigations on simultaneous SFE of the same system (oregano and clove) with various initial composition or to use some other and similar mixture of plant material.

CONCLUSION

The exhaustive SFE extractions from oregano leaves and clove buds as well as simultaneous extraction of mixture of this plant materials (10 MPa; 40 ^{o}C SC CO_2) were analyzed in this study. Extraction curves for pure plants or their mixture were analyzed using model proposed by Sovová. Calculated mass transport phenomena and determined parameters of model were used for description of the experimentally obtained curve for simultaneous SFE from mixture. The results of chemical composition and eugenol and carvacrol solubility data in the SC CO_2 have also used for describing experimental data.

Presented results indicated that increase of the eugenol, *trans*-caryophyllene and eugenyl acetate content in extract and decrease of the carvacrol, thymol and thymoquinone content in the extract comparing to the expected and theoretical contents after exhaustion of the mixture of clove bud and oregano could be result of decreased internal mass transfer resistance caused by different rate of diffusion of eugenol in the SC CO_2 in the presence of carvacrol. Moreover, it is suggested that main components of oregano (primarily carvacrol and thymol) change the solubility power of the SC CO_2 in the first period of extraction (acting like modifier of SC CO_2) and thus increase the solubility of heavier and less soluble compounds in the mixture (eugenol).

The present study reveals possibility of optimization of SFE process with respect to extraction rate, as well as quantity and quality of extracts by co-extraction of plant materials with different structure and chemical compositions.

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