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

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Running title: CO_2 extraction of *Lamiaceae* plants.

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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 two liters 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.

Keywords: supercritical extraction; carbon dioxide; oregano; sage; rosemary; thyme.

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 derivatives 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 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 25-50 °C) 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].

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.

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%) were 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 to 1000 μm. The samples were stored at -20ºC 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 pressure 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). Samples were collected from both separators at intervals of 1.5 h during 4.5 h. 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 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 Pro-star) equipped with a Nova Pack C18 column (Waters) of 15 mm × 4.6 mm and 3.5 μm particle size. The mobile phase consisted of acetonitrile (solvent A) and 0.1% of phosphoric acid in water (solvent B) applying the following gradient: 0–8 min, 23% A and 8-20 min, 75% A. This last composition was kept until the end of the chromatogram and initial conditions were gained in 5 min. Total time analysis was 40 minutes. The flow rate was constant at 0.7

mL/min. Injection volume was 20 μL and the detection was accomplished by using a diode array detection system Varian storing the signal at a wavelength of 230, 280 and 350 nm.

2.5 GC-MS analysis

Oregano, sage and thyme extracts were analyzed by GC-MS in order to determine the essential oil composition of the different fractions collected. In the case of oregano and sage, a GC-2010 (Shimadzu, Japan) was employed, comprising a split/splitless injector, electronic pressure control, AOC-20i auto injector, GCMS-QP2010 Plus mass spectrometer detector, and GC-MS Solution software. The column used was a ZB-5 (Zebron) capillary column, 30 m x 0.25 mm I.D. and 0.25 μm phase thickness. For thyme extracts, a 7890A System (Agilent Technologies, U.S.A.) was employed, comprising a split/splitless injector, electronic pressure control, G4513A auto injector, a 5975C triple-Axis mass spectrometer detector, and GC-MS Solution software. The column used was an Agilent 19091S-433 capillary column, 30 m x 0.25 mm I.D. and 0.25 μm phase thickness. For all the analysis, the chromatographic method was as follows: oven temperature programming was 60 ºC isothermal for 4 min then increased to 106 ºC at 2.5 ºC/min and from 106ºC to 130ºC at 1ºC/min and finally from 130ºC to 250 ºC at 20 ºC/min, this temperature was kept constant for 10.2 min. Sample injections (1 μL) were performed in split mode (1:20). Helium, 99.996% was used as a carrier gas at a flow of 1 mL/min with an inlet pressure of 57.5 KPa. Injector temperature was of 250ºC and MS ion source and interface temperatures were 230ºC and 280ºC, respectively. The mass spectrometer was used in TIC mode, and samples were scanned from 40 to 500 amu. Thymol, borneol, camphor, 1,8 cineole and linalool were identified by comparison with standard mass spectra, obtained in the same conditions and compared with the mass spectra from library Wiley 229. Rests of

the compounds were identified by comparison with the mass spectra from Wiley 229 library. A calibration curve was employed to quantify thymol, camphor and carnosic acid content.

3. Results and discussion

Table 1 show the amounts of material recovered in each separator (S1 and S2) during each interval of time (first interval: 0-1.5 h; second interval: 1.5- 3 h; and third interval: 3-4.5 h) for the four plants extracted. Figure 1 show a comparison between the global yields (S1 + S2) obtained for the different raw materials as a function of extraction time. As can be deduced from the figure, salvia and oregano were completely extracted, with an estimated optimal extraction time of 1.76 h (see Figure 1). But in the case of rosemary and thyme, none of these plant materials were completely exhausted during the 4.5 h of extraction. Moreover, very similar kinetic behavior resulted for salvia and oregano, so as for thyme and rosemary. Considering the first period of time (t1: 0 - 1.5 h) it was estimated a removal velocity of around 0.004 g extract / g CO_2 in the case of salvia and oregano, and almost half of this value in the case of rosemary and thyme.

With respect to the fractionation of the extracted material, the performance is quite different considering the diverse plants studied (see Table 1). In the case of oregano, the amount of material recovered in S2 is almost half the amount recovered in S1. Just the opposite behavior is observed for sage and thyme, while in the case of rosemary extraction similar amounts of extract were recovered in both S1 and S2.

Despite the distinct fractionation behavior observed that definitely should be attributed to the different substances that compose the extracts (extraction and fractionation conditions were kept exactly the same), the essential oil compounds

were selectively recovered in S2 separator for the four plant materials studied.

Tables 2, 3 and 4 present the essential oil compounds identified, respectively, in oregano, sage and thyme extracts, according to the GC-MS analysis. These tables provide the total area determined for each compound (Tables 2a, 3a and 4a) and the essential oil composition in terms of the percentage of area of each identified substance (Tables 2b, 3b and 4b). As can be deduced from the tables, the main compounds identified in oregano were thymol, sabinene hydrate and carvacrol. In the case of sage extractions, the main substances detected were camphor and cineole, following by borneol and sabinyl and linalyl acetates. Finally, for thyme extracts the main compounds identified were thymol and N-II (a non-identified compound with a retention time of 49.09 min) following by carvacrol and borneol.

As mentioned before, a concentration of the volatile oil compounds is selectively produced in S2 for all plants studied. The ratio between the total area quantified in S2 and the total area quantified in S1 (S2/S1) is, respectively, 9.7, 3.4 and 14.2 for oregano, sage and thyme (see Tables 2 to 4). This means that 90.6, 77.6 and 93.4 % of the volatile oil compounds identified, respectively, in oregano, sage and thyme were recovered in S2 separator. This selectively recovery in S2 of the essential oil compounds come to an agreement with the higher extractions yields obtained in this separator in the case of sage and thyme. But it is clear that in oregano extraction, high amounts of substances different from the volatile oil compounds are extracted and precipitated in S1 separator (i.e. the high yield obtained in S1 oregano extraction is not due to the volatile oil removal).

Figures 2 and 3 show the variation with time of the quantified areas obtained for the main compounds identified in the S1 samples (Figure 2) and in the S2 samples (Figure 3) of oregano, sage and thyme. In general, as expected, the concentration of

these compounds decrease with time both in S1 and S2 samples. Further, a noticeable reduction in the extraction of these compounds is observed in the case of oregano and sage, what agree with the fact that oregano and sage leaves are almost exhausted during the first interval of extraction (0 - 1.5 h). But in the case of thyme extracts, the decrease in the essential oil compounds extraction is much less pronounced, what approves the delayed kinetic behavior observed in thyme leaves.

The concentrations (% weight) of some key components with recognized biological activity were also determined and are given in Table 5: thymol in oregano and thyme extracts, camphor in sage, and carnosic acid in rosemary. As expected, the % weight of the monoterpene compounds (thymol and camphor), which are main constituents of the volatile oil fractions, decrease with extraction time. But the concentration of carnosic acid in the rosemary fractions recovered, increase with extraction time. Further, 72.4 % of the total antioxidant carnosic acid extracted from rosemary was selectively recovered in the first separator.

Decreasing percentages of lighter compounds (terpenes and oxygenated terpenes) were found as extraction time increase, while higher-molecular-weight compounds, such as a phenolic diterpene, showed a continuous percentage increase at increasing extraction times, as observed by Reverchon et al. [35]. As sake of comparison, it was calculated that 97.6 % of the mass of camphor extracted from sage was precipitated in S1 and S2 separators during the first interval of time (t1). Also high recoveries and very similar values were obtained for the recovery of thymol during t1: 82.6 and 80.4 %, respectively, in the oregano and thyme extraction. All these values are significantly higher than the recovery obtained for the carnosic acid extracted from rosemary during t1 (41.4 %). Furthermore, these values agree with the order reported in the literature [36, 37] for the solubility of these substances in supercritical CO_2

(camphor > thymol >> carnosic acid).

Conclusion

The supercritical extraction of *Lamiaceae* plants, namely oregano, sage, thyme and rosemary, was carried out under identical conditions of raw material pre-treatment, apparent density in the extraction cell, extraction pressure and temperature and fractionation procedure. Oregano and sage were much more rapid exhausted than thyme and rosemary, presenting very similar kinetic behavior in terms of extraction yield. The fractionation of the extract indicated that sage and thyme contains larger amounts of high volatile or high CO_2 soluble substances than oregano or rosemary, since for sage and thyme the yield obtained in S2 was almost double the yield obtained in S1. Thymol, a monoterpene phenol which is one of the main components of oregano and thyme plants, was highly extracted despite the plant variety: 82.6 and 80.4 % of the total amounts of thymol present in, respectively, oregano and thyme extracts were recovered during the first interval of extraction. On the other side, carnosic acid was only 41.4 % recovered from rosemary in this extraction period. Thus, the weight content of lighter compounds (thymol and camphor) were found to decrease with extraction time, while the weight content of higher molecular weight and less soluble substance (carnosic acid) showed a continuous increase at increasing extraction times.

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Table 1. Mass (g) of material recovered in each separator cell (S1 and S2) as a function of time in the extraction of oregano, sage, thyme and rosemary at 30 MPa and 313 K.

	time (h)	S1 (g)	S2 (g)
oregano	1.5	15.513	7.211
	3.0	3.346	2.006
	4.5	0.203	0.325
	global yield	19.062	9.542
sage	1.5	6.794	16.359
	3.0	1.261	2.708
	4.5	0.269	0.311
	global yield	8.324	19.378
thyme	1.5	3.720	6.800
	3.0	1.220	1.930
	4.5	0.510	1.490
	global yield	5.45	10.22
rosemary	1.5	6.287	5.599
	3.0	2.083	2.750
	4.5	2.220	2.135
	global yield	10.59	10.484

Table 2. Chromatographic GC-MS areas of the essential oil compounds identified in the oregano extracts as a function of time. t1, t2 and t3 correspond to the three intervals of time studied.

(a) *Absolute areas*

Retention time	Compound	S1			S2		
		t1	t2	t3	t1	t2	t3
13.35	Limonene	-	-	-	145257	123798	107069
14.94	γ-Terpinene	59200	-	-	45281	103523	-
15.38	cis-Sabinene hydrate	307051	-	-	2080399	920461	433226
17.18	trans-Sabinene hydrate	6968774	617088	965065	55412749	21087412	9946755
17.36	Linalool	184257	13823	39405	1699025	800394	425076
21.75	Terpineol	439285	34796	46977	3975567	1355249	633420
22.54	α-terpineol	505669	43278	60984	4644332	1453346	683040
25.68	Thymyl methyl ether	104106	-	-	1180444	463215	207421
26.20	Sabinene hydrate acetate	119882	-	-	1870980	452084	186150
26.43	Linalyl acetate	165154	-	-	2677209	779175	343339
28.70	Thymol	6378950	546737	898247	43532669	15690062	7463979
29.28	Carvacrol	2487757	189595	286686	16611808	5399309	2307413
37.85	E-caryophyllene	231111	-	-	3481108	938718	457200
	Total area (t1+t2+t3)			21693877			209741538

(b) *Percentage area*

Retention time	Compound	S1			S2		
		t1	t2	t3	t1	t2	t3
13.35	Limonene	-	-	-	0.11	0.25	0.46
14.94	γ-Terpinene	0.33	-	-	0.03	0.21	-
15.38	cis-Sabinene hydrate	1.71	-	-	1.51	1.86	1.87
17.18	trans-Sabinene hydrate	38.82	42.70	42.01	40.34	42.54	42.88
17.36	Linalool	1.03	0.96	1.72	1.24	1.61	1.83
21.75	Terpineol	2.45	2.41	2.04	2.89	2.73	2.73
22.54	α-terpineol	2.82	2.99	2.65	3.38	2.93	2.94
25.68	Thymyl methyl ether	0.58	-	-	0.86	0.93	0.89
26.20	Sabinene hydrate acetate	0.67	-	-	1.36	0.91	0.80
26.43	Linalyl acetate	0.92	-	-	1.95	1.57	1.48
28.70	Thymol	35.53	37.83	39.10	31.69	31.65	32.18
29.28	Carvacrol	13.86	13.12	12.48	12.09	10.89	9.95
37.85	E-caryophyllene	1.29	-	-	2.53	1.89	1.97

Table 3. Chromatographic GC-MS areas of the essential oil compounds identified in the sage extracts as a function of time. t1, t2 and t3 correspond to the three intervals of time studied.

(a) *Absolute areas*

Retention time	Compound	S1			S2		
		t1	t2	t3	t1	t2	t3
13.30	1.8 cineole	499567	104213	47435	2241357	159084	41305
15.38	Cis sabinene hydrate	40740	-	-	118065	-	-
17.18	Trans Sabinene hydrate	14122	-	-	65411	38836	105568
17.36	Linalool	62288	-	-	175147	-	-
19.60	Cis sabinol	78268	-	-	310751	54752	29098
19.75	Camphor	1516240	444963	200370	5639369	679951	297560
21.05	Borneol	241000	78901	47798	954417	192116	120853
21.75	Terpineol		-	-	83930	-	-
22.54	α-terpineol	48370	-	-	183878	43913	29941
26.32	Geraniol	51713	-	-	151456	52063	17125
26.43	Linalyl acetate	184062	49984	-	626072	71727	25647
28.17	Endobornyl acetate	117396	36275	-	350615	55674	15919
28.68	Sabinyl acetate	179528	57611	43841	633299	156566	231019
32.58	α-terpinenyl	117363	47660		428938	59980	-
37.85	E-caryophyllene	80540	-	-	259271	44442	-
40.62	α-humulene	54515	-	-	186375	-	-
43.03	Geranyl propionate	66497	-	-	169758	-	-
51.18	Spathulenol	57021	-	-	147270	42472	-
51.47	Caryophillene oxide	-	-	-	107806	-	-
52.05	Viridiflorol	79729	-	-	259112	82749	52411
	Total area (t1+t2+t3)			4556509			15793068

(b) *Percentage areas*

Retention time	Compound	S1			S2		
		t1	t2	t3	t1	t2	t3
13.30	1.8 cineole	14.32	13.50	13.97	17.12	9.17	4.27
15.38	Cis sabinene hydrate	1.17	-	-	0.90	-	-
17.18	Trans Sabinene hydrate	0.40	-	-	0.50	2.24	10.92
17.36	Linalool	1.79	-	-	1.34	-	-
19.60	Cis sabinol	2.24	-	-	2.37	3.16	3.01
19.75	Camphor	43.46	57.64	59.03	43.07	39.21	30.79
21.05	Borneol	6.91	10.22	14.08	7.29	11.08	12.50
21.75	Terpineol	-	-	-	0.64	-	-
22.54	α-terpineol	1.39	-	-	1.40	2.53	3.10
26.32	Geraniol	1.48	-	-	1.16	3.00	1.77
26.43	Linalyl acetate	5.28	6.48	-	4.78	4.14	2.65
28.17	Endobornyl acetate	3.36	4.70	-	2.68	3.21	1.65
28.68	Sabinyl acetate	5.15	7.46	12.92	4.84	9.03	23.90
32.58	α-terpinenyl	3.36	-	0.00	3.28	3.46	-
37.85	E-caryophyllene	2.31	-	-	1.98	2.56	-
40.62	α-humulene	1.56	-	-	1.42	-	-
43.03	Geranyl propionate	1.91	-	-	1.30	-	-
51.18	Spathulenol	1.63	-	-	1.12	2.45	-
51.47	Caryophillene oxide	-	-	-	0.82	-	-
52.05	Viridiflorol	2.29	-	-	1.98	4.77	5.42

Table 4. Chromatographic GC-MS areas of the essential oil compounds identified in the thyme extracts as a function of time. t1, t2 and t3 correspond to the three intervals of time studied.

(a) *Absolute areas*

Retention time	Compound	S1			S2		
		t1	t2	t3	t1	t2	t3
16.45	P-Cymene	30852	-	-	1751398	99903	37246
16.90	1,8 cineole	-	-	-	254488	56062	56632
19.47	Sabinene	-	-	-	197383	74813	58804
21.17	Linalool	53929	-	-	2129639	820577	471910
21.50	Trans-Sabinene Hidrate	-	-	-	174697	117889	193211
24.97	Camphor	-	-	-	537949	240541	258267
26.20	Borneol	68169	49514	49361	1871812	1087149	677940
26.52	α-Terpineol	-	-	-	209063	96019	58863
32.36	Camphene	-	-	-	237107	187021	150831
34.00	N-I	-	-	-	307110	218910	
35.00	Thymol	1335059	987930	999037	20822212	12928508	8587835
35.61	Carvacrol	89066	67342	60093	1753178	990554	638324
45.04	E-Caryophyllene	-	-	-	456217	166961	108598
49.09	N-II	249940	293465	293939	2289516	2538140	1597470
	Total area (t1+t2+t3)			4627696			65510747

(b) *Percentage areas*

Retention time	Compound	S1			S2		
		t1	t2	t3	t1	t2	t3
16.45	P-Cymene	1.69	-	-	5.31	0.51	0.29
16.90	1,8 cineole	-	-	-	0.77	0.29	0.44
19.47	Sabinene	-	-	-	0.60	0.38	0.46
21.17	Linalool	2.95	-	-	6.46	4.18	3.66
21.50	Trans-Sabinene Hidrate	-	-	-	0.53	0.60	1.50
24.97	Camphor	-	-	-	1.63	1.23	2.00
26.20	Borneol	3.73	3.54	3.52	5.67	5.54	5.26
26.52	α-Terpineol	-	-	-	0.63	0.49	0.46
32.36	Camphene	-	-	-	0.72	0.95	1.17
34.00	N-I	-	-	-	0.93	1.12	
35.00	Thymol	73.07	70.65	71.24	63.11	65.88	66.59
35.61	Carvacrol	4.87	4.82	4.28	5.31	5.05	4.95
45.04	E-Caryophyllene	-	-	-	1.38	0.85	0.84
49.09	N-II	13.68	20.99	20.96	6.94	12.93	12.39

Table 5. Concentration (% weight) of bioactive compounds identified in oregano, sage, thyme and rosemary extracts. t1, t2 and t3 correspond to the three intervals of time studied.

		t1	t2	t3
% weight thymol in oregano extracts				
	S1	0.55	0.28	-
	S2	10.36	7.97	1.92
% weight camphor in sage extracts				
	S1	4.65	1.36	0.61
	S2	17.28	2.08	0.91
% weight thymol in thyme extracts				
	S1	3.19	2.41	5.58
	S2	43.9	24.13	15.82
% weight carnosic acid in rosemary extracts				
	S1	12.03	15.54	19.05
	S2	1.82	7.55	12.30

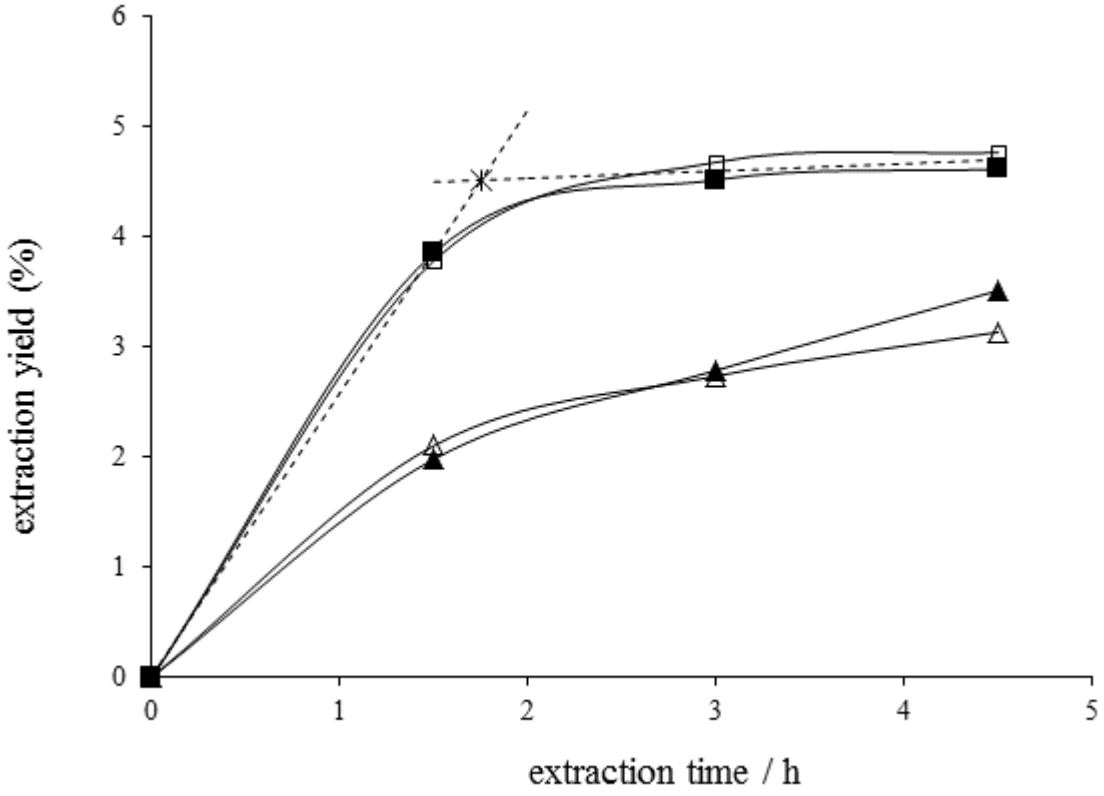
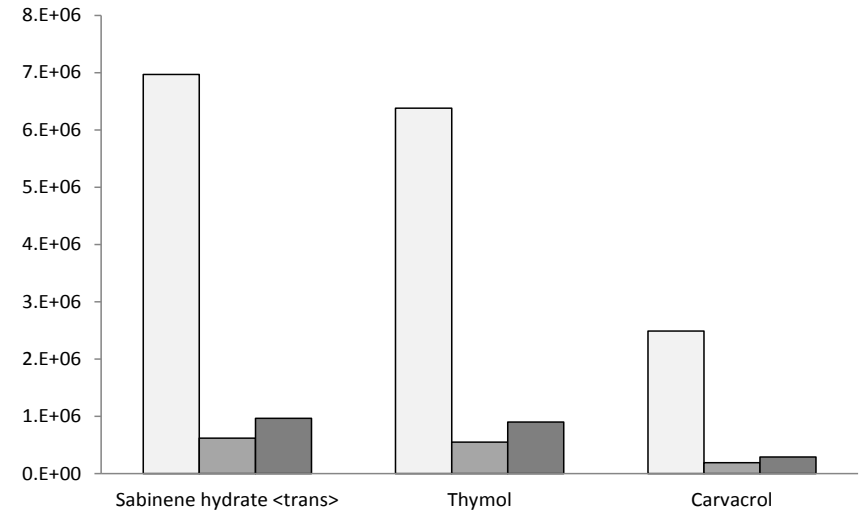
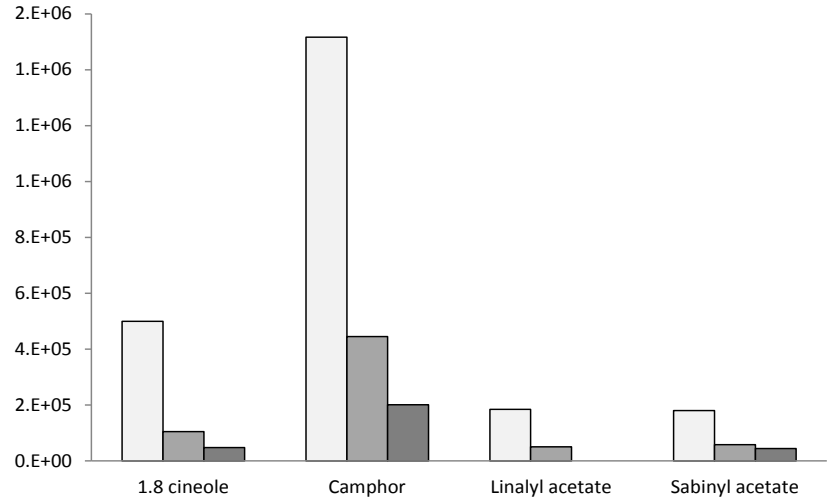


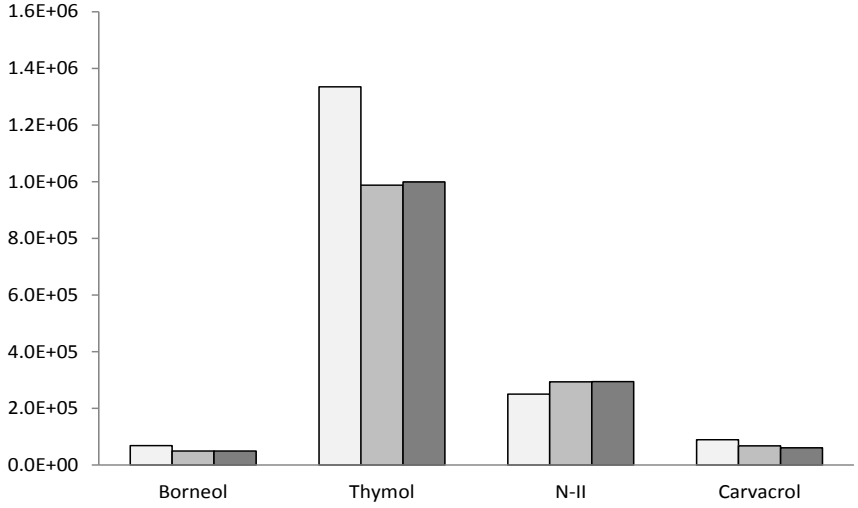
Figure 1. CO_2-SFE at constant pressure (30 MPa) of oregano (□), sage (■), thyme (△) and rosemary (▲). (∗) Estimated optimal extraction time in the case of sage and oregano extraction.



(a) S1 - oregano extraction

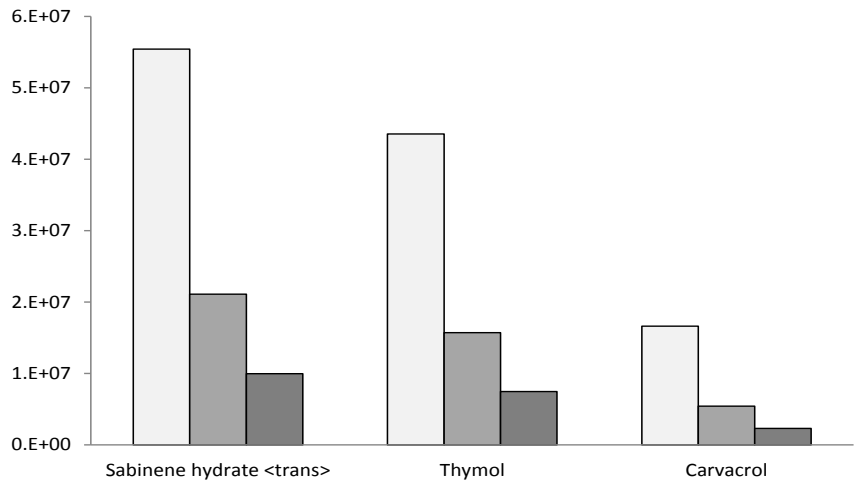


(b) S1 - sage extraction

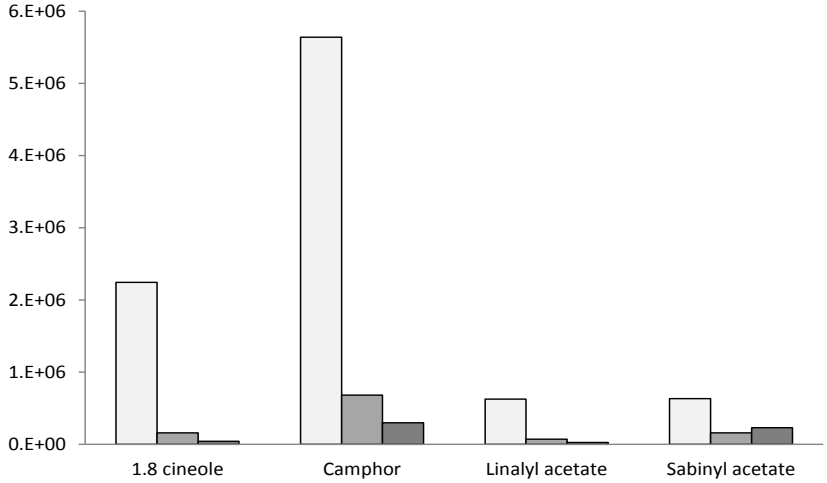


(c) S1 - thyme extraction

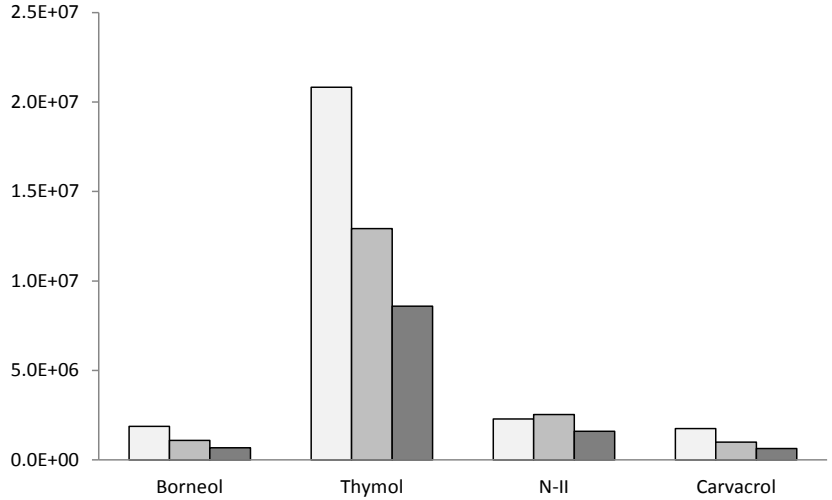
Figure 2. Kinetic behavior in the recovery of the main essential oil compounds identified in (a) oregano, (b) sage and (c) thyme S1 extracts.



(a) S2 – oregano extraction



(b) S2 – sage extraction



(c) S2– thyme extraction

Figure 3. Kinetic behavior in the recovery of the main essential oil compounds identified in (a) oregano, (b) sage and (c) thyme S2 extracts.

Graphical Abstract

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

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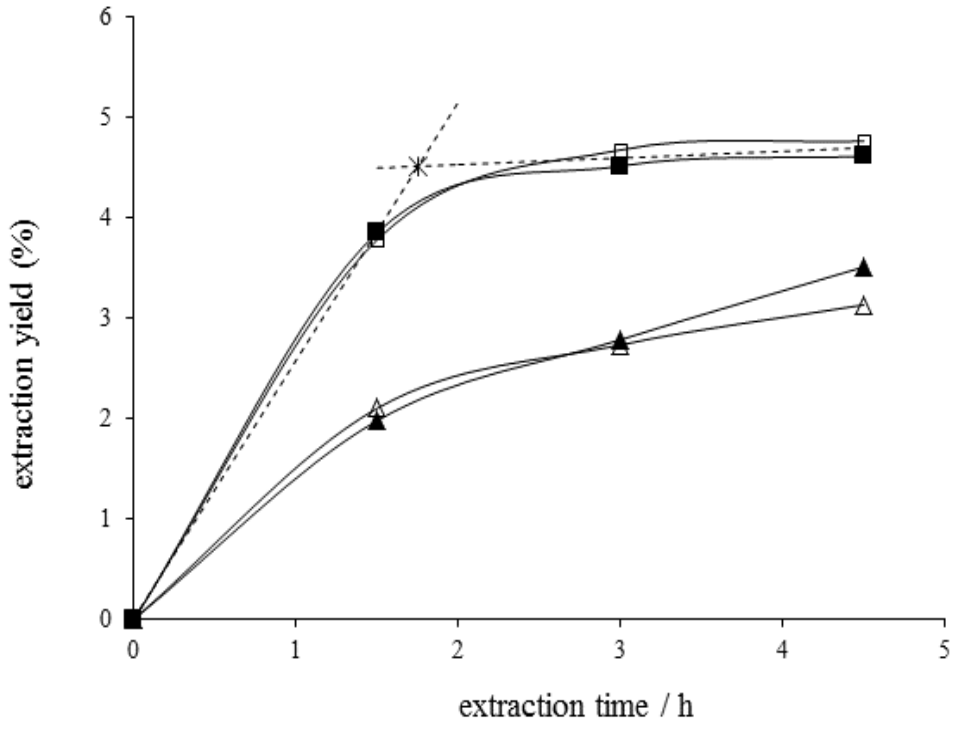
Supercritical CO_2 extraction of four different plants from *Lamiaceae* family, namely oregano (*Origanum vulgare*), thyme (*Thymus zygis*), sage (*Salvia officinalis*) and rosemary (*Rosmarinus officinalis*) was carried out in an experimental pilot-plant at 30 MPa and 313 K. Comparison of the kinetic performance reveals very similar behavior of oregano (□) and sage (■) extraction, so as for thyme (△) and rosemary (▲) extraction. A comparison between the extraction of the different plants was discussed, in terms of the extraction yields, the variation of the essential oil composition with time and the content of key bioactive substances identified in the different fractions.



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

Highlights

- Supercritical CO_2 extraction of four different plants from *Lamiaceae* family was accomplished employing the same raw material pre-treatment, extraction and fractionation (two on-line decompressing separators) conditions.
- Comparison of the kinetic behavior reveals a removal velocity for thyme and rosemary almost half of the value corresponding to salvia and oregano.
- Oregano extract in mainly recovered in the first separator while the opposite behavior is observed for sage and thyme. In the case of rosemary extraction similar amounts of extract were recovered in both separators.
- Oregano, sage and thyme volatile oil compounds were selectively recovered (90.6, 77.6 and 93.4 %, respectively) in the second separator, while 72.4 % of the total antioxidant carnosic acid extracted from rosemary was selectively recovered in the first separator.
- The % weight of the monoterpene compounds (thymol and camphor) decrease with extraction time, while higher-molecular-weight compounds, such as phenolic diterpenes, increase with extraction time.