CORE
Metadata, citation and similar papers at core.ac.uk
Provided by Digital.CSIC

1
2
3 # Kinetic study of the supercritical CO_2 extraction of
4 # different plants from *Lamiaceae* family
5
6
7 **Tiziana Fornari*, Alejandro Ruiz-Rodriguez, Gonzalo Vicente, Erika**
8 **Vázquez, Mónica R. García-Risco, Guillermo Reglero**
9
10
11 Instituto de Investigación en Ciencias de la Alimentación CIAL (CSIC-
12 UAM). C/Nicolás Cabrera 9, Universidad Autónoma de Madrid, 28049
13 Madrid, España.
14
15
16 **Running title:** CO_2 extraction of *Lamiaceae* plants.
17
18
19 *** Corresponding author:** Instituto de Investigación en Ciencias de la Alimentación
20 23 CIAL (CSIC-UAM). C/ Nicolás Cabrera 9. Universidad Autónoma de Madrid.
21 28049, Madrid, Spain Tel: +34661514186. E-mail address: tiziana.fornari@uam.es
22

23 **Abstract**

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

35

36

37

38

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

40

41

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

67 regions, used as a medicinal plant with healthy properties like its powerful anti-
68 bacterial and anti-fungical properties [6, 7]. The responsible of these activities in
69 oregano is the volatile oil, which contains thymol and carvacrol as the primary
70 components [8]. In these compounds, Puertas-Mejia et al. [9] also found some
71 antioxidant activity.
72 The supercritical extraction and fractionation of oregano has been studied and
73 reported in the literature [10 - 12]. Moderate conditions (solvent densities between
74 300 and 500 kg/m^3) were found to be sufficient for an efficient extraction of volatile
75 oil compounds. Although higher pressures increase the rate of extraction and yield of
76 the essential oil fraction, also significant amounts of waxes were co-extracted and,
77 consequently, the essential oil content in the extract decreased [12].
78 Thymol and carvacrol were also found in the essential oil of another *Lamiaceae* plant,
79 namely *Thymus*. The variety most studied is, indeed, *Thymus vulgaris* [13-14]. Yet,
80 particularly attention is focused on *Thymus zygis*, a thyme variety widespread over
81 Portugal and Spain, which extract has proved to be useful for food flavoring [15] and
82 in the pharmaceutical [16-17] and cosmetic industries [18]. Moldao-Martins et al. [19]
83 studied the supercritical extraction of *Thymus zygis* at different temperatures (300-323
84 K) and pressures (8-20 MPa) and reported a comprehensive comparison of the
85 extracts produced with those obtained from steam distillation.
86 Other *Lamiaceae* plants being intensively studied are the *"Officinalis"* ones (from
87 Latin meaning medicinal). Sage (*Salvia officinalis L.*) is a popular kitchen herb and
88 has been used in a variety of food preparations since ancient times, and has a
89 historical reputation for promotion of health and treatment of diseases [20]. Modern
90 day research has shown that sage essential oil can improve the memory and has
91 shown promise in the treatment of Alzheimer's disease [21]. In the past few decades

92 however, sage has been the subject of an intensive study for its phenolic antioxidant
93 components [22-24]. Supercritical extraction of sage demonstrated that when sage
94 leaves are ground in fine particles, the essential oil is easily accessible to the SC-CO_2
95 solvent (9-13 MPa and 298-323 K) and the extraction is controlled by phase
96 equilibrium [25]. That is, large part of the total essential oil contained in the plant
97 matrix is dissolved almost immediately in SC-CO_2. To extract high molecular and
98 polar compounds from sage, CO_2 with an ethanol-water mixture as co-solvent was
99 employed; antioxidant substances such as rosmarinic acid and carnosic compounds
100 were extracted, achieving a recovery of 55 % and 75 % respectively [26].
101 The supercritical extraction of rosemary (*Rosmarinus officinalis L.*), which has been
102 recognized as one of the plants with large antioxidant activity, also produced extracts
103 with large concentrations of phenolic antioxidants. Main substances associated with
104 the antioxidant activity of rosemary extract are the phenolic diterpenes such as
105 carnosol, rosmanol, carnosic acid, methyl carnosate, and phenolic acids such as the
106 rosmarinic and caffeic acids [27-31]. Among the large number of papers related with
107 the supercritical extraction and fractionation of rosemary and its effect on the
108 antioxidant activity of the extracts, the authors have recently presented two new
109 contributions [32, 33]. In the first work [32], the scaling of supercritical rosemary
110 extraction in terms of extraction kinetic and mass transfer coefficients was studied. In
111 the second contribution [33], on-line fractionation was considered with the target of
112 attaining a product with high yield and antioxidant activity.
113 Indeed, numerous variables have singular effect on the supercritical extraction yield
114 and on the composition and quality of extracts. Process conditions, such as extraction
115 pressure and temperature, type and amount of cosolvent, extraction time,
116 fractionation, raw material pre-treatment, plant location and harvesting time, greatly

117 affect not only yield but also composition of the extracted material. The different
118 process conditions applied, together with the variety of equipment and process scale
119 employed, complicate the comparison of the competence of supercritical CO_2
120 technology in the extraction of bioactive compounds from plant material.
121 Comparison of supercritical CO_2 extraction of different plant matrix maintaining
122 identical conditions is of relevance in order to study the extraction of mixed plants.
123 Furthermore, extraction of mixed herbs is of high processing interest from a cost-
124 effective point of view: many bioactive phytochemicals may act synergistically and
125 thus, may have much more effective response. In this case, the kinetic behavior of
126 each plant at a given extraction condition should be considered and compared in order
127 to attain a bioactive target in the extract.
128 In this paper we carried out the extraction of four *Lamiaceae* plant varieties, namely
129 oregano, thyme, sage and rosemary, using the same procedure for the preparation of
130 the raw materials (plant leaves), employing the same experimental pilot-plant device
131 and the same extraction conditions and procedure. Then, the kinetic behavior of the
132 extractions, considering both yield and composition of the fractions obtained, was
133 evaluated and compared.
134

135 **2. Materials and methods**

136 **2.1 Chemicals**

137 Carnosic acid (≥96%) were purchased from Alexis Biochemical (Madrid, Spain).
138 Thymol (99.5%), Camphor (>97%) and Linalool (>97%) were purchased from
139 SIGMA-ALDRICH (Madrid, Spain), whereas 1,8 cineole (98%) and Borneol (>99%)
140 were purchased from FLUKA (Madrid, Spain). Ethanol, acetonitrile and phosphoric
141 acid were all HPLC grade from Lab Scan (Dublin, Ireland).

142 **2.2 Preparation of plant leaves**

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

150 **2.3 Supercritical extraction method**

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

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

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

177 **2.4 HPLC analysis**

178 In order to quantify the carnosic acid content in the rosemary extracts, samples were
179 analyzed employing a HPLC (Varian Pro-star) equipped with a Nova Pack C18
180 column (Waters) of 15 mm × 4.6 mm and 3.5 μm particle size. The mobile phase
181 consisted of acetonitrile (solvent A) and 0.1% of phosphoric acid in water (solvent B)
182 applying the following gradient: 0–8 min, 23% A and 8-20 min, 75% A. This last
183 composition was kept until the end of the chromatogram and initial conditions were
184 gained in 5 min. Total time analysis was 40 minutes. The flow rate was constant at 0.7
185 mL/min. Injection volume was 20 μL and the detection was accomplished by using a
186 diode array detection system Varian storing the signal at a wavelength of 230, 280
187 and 350 nm.

188 **2.5 GC-MS analysis**

189 Oregano, sage and thyme extracts were analyzed by GC-MS in order to determine the
190 essential oil composition of the different fractions collected. In the case of oregano
191 and sage, a GC-2010 (Shimadzu, Japan) was employed, comprising a split/splitless

192 injector, electronic pressure control, AOC-20i auto injector, GCMS-QP2010 Plus
193 mass spectrometer detector, and GC-MS Solution software. The column used was a
194 ZB-5 (Zebron) capillary column, 30 m x 0.25 mm I.D. and 0.25 µm phase thickness.
195 For thyme extracts, a 7890A System (Agilent Technologies, U.S.A.) was employed,
196 comprising a split/splitless injector, electronic pressure control, G4513A auto injector,
197 a 5975C triple-Axis mass spectrometer detector, and GC-MS Solution software. The
198 column used was an Agilent 19091S-433 capillary column, 30 m x 0.25 mm I.D. and
199 0.25 µm phase thickness. For all the analysis, the chromatographic method was as
200 follows: oven temperature programming was 60 ºC isothermal for 4 min then
201 increased to 106 ºC at 2.5 ºC/min and from 106ºC to 130ºC at 1ºC/min and finally
202 from 130ºC to 250 ºC at 20 ºC/min, this temperature was kept constant for 10.2 min.
203 Sample injections (1 µL) were performed in split mode (1:20). Helium, 99.996% was
204 used as a carrier gas at a flow of 1 mL/min with an inlet pressure of 57.5 KPa. Injector
205 temperature was of 250ºC and MS ion source and interface temperatures were 230ºC
206 and 280ºC, respectively. The mass spectrometer was used in TIC mode, and samples
207 were scanned from 40 to 500 amu. Thymol, borneol, camphor, 1,8 cineole and
208 linalool were identified by comparison with standard mass spectra, obtained in the
209 same conditions and compared with the mass spectra from library Wiley 229. Rests of
210 the compounds were identified by comparison with the mass spectra from Wiley 229
211 library. A calibration curve was employed to quantify thymol, camphor and carnosic
212 acid content.
213
214 **3. Results and discussion**
215 Table 1 show the amounts of material recovered in each separator (S1 and S2) during
216 each interval of time (first interval: 0-1.5 h; second interval: 1.5- 3 h; and third

217 interval: 3-4.5 h) for the four plants extracted. Figure 1 show a comparison between
218 the global yields (S1 + S2) obtained for the different raw materials as a function of
219 extraction time. As can be deduced from the figure, sage and oregano were
220 completely extracted, with an estimated optimal extraction time of 1.76 h (see Figure
221 1). But in the case of rosemary and thyme, none of these plant materials were
222 completely exhausted during the 4.5 h of extraction. Moreover, very similar kinetic
223 behavior resulted for sage and oregano, so as for thyme and rosemary. Considering
224 the first period of time (t1: 0 - 1.5 h) it was estimated a removal velocity of around
225 0.004 g extract / g CO_2 in the case of sage and oregano, and almost half of this value
226 in the case of rosemary and thyme.

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

232 Despite the distinct fractionation behavior observed that definitely should be
233 attributed to the different substances that compose the extracts (extraction and
234 fractionation conditions were kept exactly the same), it is expected that the essential
235 oil compounds were selectively recovered in S2 separator for the four plant materials
236 studied.

237 The extraction yields and fractionation behavior observed in this work compare well
238 with data available in the literature. For example, Cavero et al. [11] reported a global
239 yield of 4.89 % for the extraction of oregano leaves at 35 MPa and 40°C. This value is
240 very similar to the global yield obtained in our work (4.77 %). Simandi et al. [10]
241 reported analogous results for the global yield and further, similarly to our results, on-

242 line fractionation resulted in a pasty extract in the first separator (8 MPa, 311 K) and
243 an oily fraction in the second separator (2 MPa, 298 K) with higher yield in S1 (ca.
244 3%) than in S2 (ca. 2%).
245 Additionally, previous work reported by the authors [32] demonstrated that global
246 yield achieved in the supercritical CO_2 extraction of rosemary accomplished in this
247 work was similar to the values obtained in analytical or low-scale equipment [4].
248 The extraction of sage leaves using CO_2 without cosolvents was reported by
249 Aleksovski and Sovová [25]. Extraction yields ranged between 2.7 and 4.8 % in
250 dependence on extraction conditions (pressure, 9-12.8 MPa; temperature, 298-323 K).
251 The global yield obtained in this work (4.62 %) corresponds with the higher yields
252 previously reported [25] and support the exhaustion of sage leaves observed in the
253 kinetic extraction curve (Figure 1).
254 Finally, extraction of *Thymus zygis* variety was previously studied by Moldao-Martins
255 et al. [19] reporting a yield around 8 % at 24 MPa, 313 K and CO_2 / plant load ratio of
256 120 g/g. This value is rather higher than the yield obtained in our work (2.61 %).
257 Indeed, this discrepancy should be attributed to the considerably lower CO_2 / plant
258 load ratio employed in our work (20 g/g). Furthermore, the kinetic curves depicted by
259 Moldao-Martins et al. [19] and Oszagyan et al. [38] indicate a yield of around 2-3.5 %
260 for a CO_2 / plant load ratio of 20 g/g and similar extraction temperature and pressure
261 employed in our work. These values are in accordance with the 2.61 % yield attained
262 in our work for thyme extraction.
263 As mentioned before, main bioactive substances in thyme and oregano leaves are
264 thymol and carvacrol, which have powerful anti-bacterial properties. These
265 substances are contained in the essential oil fraction of the plant and thus, the volatile
266 oil composition was investigated in the case of thyme and oregano extracts.

Also in the case of sage samples, the chemical analysis was focused on the volatile oil composition, taking into account the content of camphor, a substance with recognized strong anti-fungical properties. Phenolic compounds were also identified in sage extracts, but very low amounts were determined in the samples produced in this work. Previous works [26, 35] demonstrated that phenolic compounds were significantly extracted from sage only when a polar cosolvent was employed.

Tables 2, 3 and 4 present the essential oil compounds identified, respectively, in oregano, sage and thyme extracts, according to the GC-MS analysis. The tables provide the essential oil composition in terms of the percentage of peak area of each identified substance. As can be deduced from the tables, the main (more abundant) compounds identified in oregano were thymol, sabinene hydrate and carvacrol, in accordance with the literature [10]. In the case of sage extracts, the main substances detected were camphor and 1,8 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. Thymol and carvacrol are the main bioactive compounds identified in the volatile oil fraction of different varieties of thyme [5, 13, 19 and 38]. Further, considering the high pressure applied in the extraction, the N-II compound could be related with a high molecular weight paraffin-type compound (waxes) [13].

As expected, a concentration of the volatile oil compounds is selectively produced in S2 for oregano, sage and thyme. 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

292 compounds come to an agreement with the higher extractions yields obtained in this
293 separator in the case of sage and thyme. But it is clear that in oregano extraction, high
294 amounts of substances different from the volatile oil compounds are extracted and
295 precipitated in S1 separator. These substances could be related with waxy products
296 since, as reported by Simandi et al. [10], high extraction pressures significant increase
297 the amounts of co-extracted waxes. At pressures similar to the one accomplished in
298 our work, Simandi et al. [10] reported S1 and S2 yields of the same order that the
299 ones obtained in our work. Further, similarly to our work, a dark-yellow odorless
300 mass (waxes and resins) was collected in the first separator, and a brownish-yellow
301 liquid with a strong oregano odor (the essential oil) was recovered from the second
302 separator [10].

303 Figures 2 and 3 show the variation with time of the quantified areas obtained for the
304 main compounds identified in the S1 samples (Figure 2) and in the S2 samples
305 (Figure 3) of oregano, sage and thyme. In general, as expected, the concentration of
306 these compounds decrease with time both in S1 and S2 samples. Further, a noticeable
307 reduction in the extraction of these compounds is observed in the case of oregano and
308 sage, what agree with the fact that oregano and sage leaves are almost exhausted
309 during the first interval of extraction (0 - 1.5 h). But in the case of thyme extracts, the
310 decrease in the essential oil compounds extraction is much less pronounced, what
311 approves the delayed kinetic behavior observed in thyme leaves.

312 The concentrations (% weight) of some key components with recognized biological
313 activity were also determined and are given in Table 5: thymol in oregano and thyme
314 extracts, camphor in sage, and carnosic acid in rosemary.

315 Despite the fact that rosemary oleoresin contains bioactive substances (e.g. eucalyptol
316 and camphor) the volatile oil composition of rosemary samples was not investigated.

317 Phenolic compounds are the main bioactive (antioxidant) substances present in
318 rosemary and thus, carnosic acid was selected as key substance for chemical analysis
319 of rosemary fractions.
320 As expected, the % weight of the monoterpene compounds (thymol and camphor),
321 which are main constituents of the volatile oil fractions, decrease with extraction time.
322 But the concentration of carnosic acid in the rosemary fractions recovered, increase
323 with extraction time. Further, 72.4 % of the total antioxidant carnosic acid extracted
324 from rosemary was selectively recovered in the first separator.
325 Decreasing percentages of lighter compounds (terpenes and oxygenated terpenes)
326 were found as extraction time increase, while higher-molecular-weight compounds,
327 such as a phenolic diterpene, showed a continuous percentage increase at increasing
328 extraction times, as observed by Reverchon et al. [35]. As sake of comparison, it was
329 calculated that 97.6 % of the mass of camphor extracted from sage was precipitated in
330 S1 and S2 separators during the first interval of time (t1). Also high recoveries and
331 very similar values were obtained for the recovery of thymol during t1: 82.6 and 80.4
332 %, respectively, in the oregano and thyme extraction. All these values are
333 significantly higher than the recovery obtained for the carnosic acid extracted from
334 rosemary during t1 (41.4 %). Furthermore, these values agree with the order reported
335 in the literature [36, 37] for the solubility of these substances in supercritical CO_2
336 (camphor > thymol >> carnosic acid).
337 The concentration (% wt) reported in Table 5 for the different bioactive substances in
338 the different plant extracts compares reasonably well with data reported in the
339 literature. For example, Molda-Martins et al. [19] reported ca. 12 % wt of thymol in
340 global thyme (*Thymus zygis*) supercritical extracts, and a thymol content of 22.1 % wt
341 in thyme essential oil. The overall thymol composition obtained in our work (24.6

342 %wt) reveals that thyme extraction was not complete and thus, mainly the essential oil
343 of the plant was recovered. With respect to the content of carnosic acid in
344 supercritical rosemary extract, the data reported in the literature [4, 39, 40] ranged
345 from 0.5 to 20 % wt in dependence on extraction conditions. The fractions collected
346 in our work present concentrations from 1.8 to 19 % wt, with an overall content (S1 +
347 S2) of 9.9 % wt.
348 A comparison of the content of some volatile oil compounds identified in oregano,
349 sage and thyme is presented in Table 6. Total areas determined by GC-MS analysis
350 for these key compounds allowed calculating their relative amount in the different
351 plant extracts. The oregano/thyme and sage/thyme ratios given in Table 6 indicate that
352 the content of 1,8 cineole and camphor in sage was at least 8 times higher than in
353 thyme. Further, oregano and thyme contain similar amounts of linalool, with content
354 around 15 times higher than sage. Sabinene hydrates, α-terpineol, thymol, carvacrol
355 and caryophyllene were significantly more abundant in oregano than in thyme or sage
356 extracts (see oregano/thyme ratios in Table 6).
357
358 **Conclusion**
359 The supercritical extraction of *Lamiaceae* plants, namely oregano, sage, thyme and
360 rosemary, was carried out under identical conditions of raw material pre-treatment,
361 apparent density in the extraction cell, extraction pressure and temperature and
362 fractionation procedure. In this way, a thoughtful comparison of the extraction
363 kinetics could be observed with the target of ascertain adequate conditions for the
364 extraction of the mixed herbs.
365 Oregano and sage were much more rapid exhausted than thyme and rosemary,
366 presenting very similar kinetic behavior in terms of extraction yield. The fractionation

367 of the extract indicated that sage and thyme contains larger amounts of high volatile
368 or high CO_2 soluble substances than oregano or rosemary, since for sage and thyme
369 the yield obtained in S2 was almost double the yield obtained in S1. Thymol, a
370 monoterpene phenol which is one of the main components of oregano and thyme
371 plants, was highly extracted despite the plant variety: 82.6 and 80.4 % of the total
372 amounts of thymol present in, respectively, oregano and thyme extracts were
373 recovered during the first interval of extraction. On the other side, carnosic acid was
374 only 41.4 % recovered from rosemary in this extraction period. Thus, the weight
375 content of lighter compounds (thymol and camphor) were found to decrease with
376 extraction time, while the weight content of higher molecular weight and less soluble
377 substance (carnosic acid) showed a continuous increase at increasing extraction times.
378

379 **Acknowledges**

380 The authors gratefully acknowledge the financial support from the Comunidad
381 Autónoma de Madrid (ALIBIRD, project number S-2009/AGR-1469) and the
382 Ministerio de Educación y Ciencia (CONSOLIDER-INGENIO, CSD 2007-00063).

383 **References**

384

385 [1] E. Reverchon, I. De Marco, Supercritical fluid extraction and fractionation of
386 natural matter, J. of Supercritical Fluids 38 (2006) 146-166.
387 [2] S.M. Pourmortazavi, S.S. Hajimirsadeghi, Supercritical fluid extraction in plant
388 essential and volatile oil analysis, J. of Chromatography A 1163 (2007) 2-24.
389 [3] M. Herrero, A. Cifuentes, E. Ibañez, Sub- and supercritical fluid extraction of
390 functional ingredients from different natural sources: Plants, food-by-products, algae
391 and microalgae: A review, Food Chemistry 98 (2006) 136-148.
392 [4] R.N.Jr. Carvalho, L.S. Moura, P.T.V. Rosa, M.A.A. Meireles, Supercritical
393 fluid extraction from rosemary (Rosmarinus officinalis): Kinetic data, extract's global
394 yield, composition, and antioxidant activity. J. Supercrital. Fluids 35 (2005) 197-204.
395 [5] M.C. Díaz-Maroto, I.J. Díaz-Maroto Hidalgo, E. Sánchez-Palomo, M.S.
396 Pérez-Coello, Volatile Components and Key Odorants of Fennel (*Foeniculum*
397 *vulgare* Mill.) and Thyme (*Thymus vulgaris* L.) Oil Extracts Obtained by
398 Simultaneous Distillation-Extraction and Supercritical Fluid Extraction, J of
399 Agricultural and Food Chemistry 53 (2005) 5385-5389.
400 [6] M. Elgayyar, F.A. Draughon, D.A. Golden, J.R. Mount, Antimicrobial activity of
401 essentials oils from plants against selected pathogenic and saprophytic
402 microorganisms, J. of Food Protection. 64 (2001) 1019-1024.
403 [7] M. Sokovic, O. Tzakou, D. Pitarokili, M. Couladis, Antifungal activities of
404 selected aromatic plants growing wild in Greece, Molecular Nutrition Food Research,
405 Nahrung/Food 46 (2002) 317-320.
406 [8] S. Kokkini, R. Karousou, A. Dardioti, N. Krigas, T. Lanaras, Autumn essential
407 oils of Greek oregano, Phytochemistry 44 (1997) 883.

408 [9] M. Puertas-Mejia, S. Hillebrand, E. Stashenko, P. Winterhalter, In vitro radical
409 scavenging activity of essential oils from Columbian plants and fractions from
410 oregano (Origanum vulgare L.) essential oil, Flavour and Fragrance J. 17 (2002) 380-
411 384.
412 [10] B. Simandi, M. Oszagyan, E. Lemberkovics, A. Kery, J. Kaszacs, F. Thyrion,
413 T. Matyas, Supercritical carbon dioxide extraction and fractionation of oregano
414 oleoresin. Food Research International 31 (1998) 723-728.
415 [11] S. Cavero, M.R. García-Risco, F.R. Marín, L. Jaime, S. Santoyo, F.J.
416 Señoráns, G. Reglero, E. Ibañez, Supercritical fluid extraction of antioxidant
417 compounds from oregano Chemical and functional characterization via LC–MS and
418 in vitro assays. J. of Supercritical Fluids 38 (2006) 62–69
419 [12] F. Gaspar, J of Agricultural and Food Chemistry 51 (2003) 6604.
420 [13] Z. P. Zeković, Ž. D. Lepojević, S. G. Milošević. A. Š. Tolić, Modeling of the
421 thyme-liquid carbon dioxide extraction, Acta Periodica Technologica APTEFF 34
422 (2003) 1–148.
423 [14] B. Simandi, V. Hajdu, K. Peredi, B. Czukor, A. Nobik-Kovacs, A. Kery.
424 Antioxidant activity of pilot-plant alcoholic and supercritical carbon dioxide extracts
425 of thyme. European. J. of Lipid Science and Technology. 103 (2001) 355–358.
426 [15] Prakash V., Leafy Spices, CRC Press, Boca Raton, Florida, 1990, p. 99.
427 [16] L. Bravo, J. Cabo, A. Revert, A. Villar, Accion sobre duodeno de rata del
428 aceite esencial de diversas especies del genero Thymus, ARS Pharmacology. XVI 3
429 (1975) 345.
430 [17] C.M. Priestley, E.M. Williamson, K.A. Wafford, D.B. Sattelle, Thymol, a
431 constituent of thyme essential oil, is a positive allosteric modulator of human

432 GABAA receptors and a homo-oligomeric GABA receptor from Drosophila
433 melanogaster, British Journal of Pharmacology 140 (2003) 1363-1372.
434 [18] B.D. Mookherjee, R.A. Wilson, R.W. Trenkle, M.J. Zampino, K.P. Sands, R.
435 Teranishi, R.G. Buttery, F. Shahidi, Flavor Chemistry: Trends and Developments,
436 ACS Symposium Series, Washington (1989) p. 176.
437 [19] M. Moldao-Martins, A. Palavra, M. L. Beirao da Costa, M. G. Bernardo-Gil,
438 Supercritical CO2 extraction of *Thymus zygis L.* subsp. sylvestris aroma, Journal of
439 Supercritical Fluids 18 (2000) 25–34.
440 [20] S.E. Kintzios, Sage – the genus salvia. Amsterdam: Harwood Academic
441 (2000).
442 [21] E.K. Perry, A.T. Pickering, W.W. Wang, P.J. Houghton, N.S L. Perry,
443 Medicinal plants and Alzheimer's disease: from ethnobotany to phytotherapy, Journal
444 of Pharmacy and Pharmacology, 51 (2005) 527–534.
445 [22] A. Bisio, G. Romussi, G. Ciarallo, N. de Tommasi, Flavonoids and
446 triterpenoids from Salvia blepharophylla Brandegee ex Epling, Pharmazie 52 (1997)
447 330–331.
448 [23] J.R. Chipault, J.M. Hawkins, W.O. Lundberg, The antioxidant properties of
449 natural spices. Food Research 17 (1952) 46–54.
450 [24] M. Wang, J. Li, M. Rangarajan, Y. Shao, E.J. LaVoie, T.C. Huang,
451 Antioxidative phenolic compounds from sage (Salvia officinalis), Journal of
452 Agricultural and Food Chemistry 46 (1998) 4869–4873.
453 [25] S.A. Aleksovsk, H. Sovová, Supercritical CO2 extraction of Salvia officinalis
454 L. J. of Supercritical Fluids 40 (2007) 239-245.

455 [26] N.E. Durling, O.J. Catchpole, J.B. Grey, R.F. Webby, K.A. Mitchell, L.Y.
456 Foo, N.B. Perry, Extraction of phenolics and essential oil from dried sage (Salvia
457 officinalis) using ethanol–water mixtures. Food Chemistry 101 (2007) 1417–1424.
458 [27] E. Ibáñez, A. Oca, G. de Murga, S. López-Sebastian, J. Tabera, G. Reglero,
459 Supercritical Fluid Extraction and Fractionation of Different Preprocessed Rosemary
460 Plants. J of Agricultural and Food Chemistry. 47 (1999) 1400-1404.
461 [28] S. Cavero, L. Jaime, P. J. Martín-Alvarez, F.J. Señoráns, G. Reglero, E.
462 Ibáñez, In vitro antioxidant analysis of supercritical fluid extracts from rosemary
463 (Rosmarinus officinalis L.), European Food Research and Technology 221 (2005)
464 478-486.
465 [29] A. Szumny, A. Figiel, A. Gutierrez-Ortiz, A.A. Carbonell-Barrachina,
466 Composition of rosemary essential oil (Rosmarinus officinalis) as affected by drying
467 method, J. Food Engineering 97 (2010) 253-260.
468 [30] M.E. Napoli, G. Curcuruto, G. Ruberto, Srrening of the essential oil
469 composition of wild Sicilian rosemary. Biochemical Systematics and Ecology 38
470 (2010) 659–670.
471 [31] Y. Zaouali, T. Bouzaine, M. Boussaid, Essential oils composition in two
472 Rosmarinus officinalis L. varieties and incidence for antimicrobial and antioxidant
473 activities. Food and Chemical Toxicology 48 (2010) 3144–3152.
474 [32] M. R. García-Risco, E. J. Hernández, G. Vicente, T. Fornari, F. J. Señorans,
475 G. Reglero, Kinetic study of pilot-scale supercritical CO2 extraction of rosemary
476 (Rosmarinus officinalis) leaves, J. of Supercritical Fluids 55 (2011) 971–976
477 [33] G. Vicente, M.R. García-Risco, T. Fornari, G. Reglero. Supercritical
478 fractionation of rosemary extracts to improve antioxidant activity. Chemical
479 Engineering & Technology. Accepted November 2011

480 [34] M.R. García-Risco, G. Vicente, G. Reglero, T. Fornari. Fractionation of thyme
481 (*Thymus vulgaris L.*) by supercritical fluid extraction and chromatography, J. of
482 Supercritical Fluids 55 (2011) 949–954.
483 [35] E. Reverchon, R. Taddeo, G. Della Porta, Extraction of Sage Oil by
484 Supercritical CO2: Influence of Some Process Parameters. J. of Supercritical Fluids 8
485 (1995) 302-309.
486 [36] R. B. Gupta, J. J. Shim, Solubility in supercritical carbon dioxide. CRC Press,
487 Taylor and Francis Group. 2007.
488 [37] A. Chafer, T. Fornari, A. Berna, E. Ibañez, G. Reglero, J. Supercritical Fluids,
489 34 (2005) 323-329.
490 [38] M. Oszagyan, B. Simhdi, J. Sawinsky, A. Kery, E. Lemberkovics, J. Fekete.
491 Supercritical Fluid Extraction of Volatile Compounds from Lavandin and Thyme.
492 Flavour and Fragance J., 11(1996) 157-165.
493 [39] O. Y. Celiktas, E. Bedir, F. Vardar Sukan. In vitro antioxidant activities of
494 Rosmarinus officinalis extracts treated with supercritical carbon dioxide. Food
495 Chemistry, 101 (2007) 1457-1464.
496 [40] Chi-Huang Chang, Charng-Cherng Chyau, Chiu-Lan Hsieh, Yen-Ying Wu,
497 Yaw-Bee Ker, Hau-Yang Tsen, Robert Y. Peng. Relevance of phenolic diterpene
498 constituents to antioxidant activity of supercritical CO2 extract from the leaves of
499 rosemary. Natural Products Research, 22 (2008) 76-90.
500
501

502 **Table 1.** Mass (g) of material recovered and yield ((mass extracted / plant load)
503 obtained in each separator cell (S1 and S2) as a function of time in the extraction of
504 oregano, sage, thyme and rosemary at 30 MPa and 313 K.

505

		Mass recovered (g)		Extraction yield (%)	
	time (h)	S1	S2	S1	S2
oregano	1.5	15.51	7.21	2.59	1.20
	3.0	3.35	2.01	0.56	0.33
	4.5	0.20	0.33	0.03	0.05
	global yield	19.06 ± 2.41	9.55 ± 0.54	3.18 ± 0.40	1.59 ± 0.09
sage	1.5	6.79	16.36	1.13	2.73
	3.0	1.26	2.71	0.21	0.45
	4.5	0.27	0.31	0.04	0.05
	global yield	8.32 ± 1.08	19.38 ± 1.02	1.39 ± 0.18	3.23 ± 0.17
thyme	1.5	3.72	6.80	0.62	1.13
	3.0	1.22	1.93	0.20	0.32
	4.5	0.51	1.49	0.09	0.25
	global yield	5.45 ± 0.68	10.22 ± 0.44	0.91 ± 0.11	1.70 ± 0.07
rosemary	1.5	6.29	5.60	1.05	0.93
	3.0	2.08	2.75	0.35	0.46
	4.5	2.22	2.14	0.37	0.36
	global yield	10.59 ± 1.40	10.49 ± 0.57	1.77 ± 0.23	1.75 ± 0.10

506

507

508

509 **Table 2.** Essential oil compounds identified in the oregano extracts as a function of
510 time. t1, t2 and t3 correspond to the three intervals of time studied.

511

Retention time	Compound	Percentage area					
		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
	Total area (t1+t2+t3)			21693877			209741538

512

513

514 **Table 3.** Essential oil compounds identified in the sage extracts as a function of time.
515 t1, t2 and t3 correspond to the three intervals of time studied.
516

Retention time	Compound	Percentage area					
		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
	Total area (t1+t2+t3)			4556509			15793068

517
518
519

520 **Table 4.** Essential oil compounds identified in the thyme extracts as a function of
521 time. t1, t2 and t3 correspond to the three intervals of time studied.
522

523

Retention time	Compound	Percentage area					
		S1			S2		
		t1	t2	t3	t1	t2	t3
10.53	P-Cymene	1.69	-	-	5.31	0.51	0.29
10.89	1,8 cineole	-	-	-	0.77	0.29	0.44
13.30	Sabinene	-	-	-	0.60	0.38	0.46
14.89	Linalool	2.95	-	-	6.46	4.18	3.66
15.30	Trans-Sabinene Hidrate	-	-	-	0.53	0.60	1.50
17.28	Camphor	-	-	-	1.63	1.23	2.00
18.55	Borneol	3.73	3.54	3.52	5.67	5.54	5.26
18.70	α-Terpineol	-	-	-	0.63	0.49	0.46
24.30	Camphene	-	-	-	0.72	0.95	1.17
26.00	N-I	-	-	-	0.93	1.12	
27.00	Thymol	73.07	70.65	71.24	63.11	65.88	66.59
27.50	Carvacrol	4.87	4.82	4.28	5.31	5.05	4.95
35.20	E-Caryophyllene	-	-	-	1.38	0.85	0.84
37.36	N-II	13.68	20.99	20.96	6.94	12.93	12.39
	Total area (t1+t2+t3)			4627696			65510747

524
525
526

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

530

		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

531
532

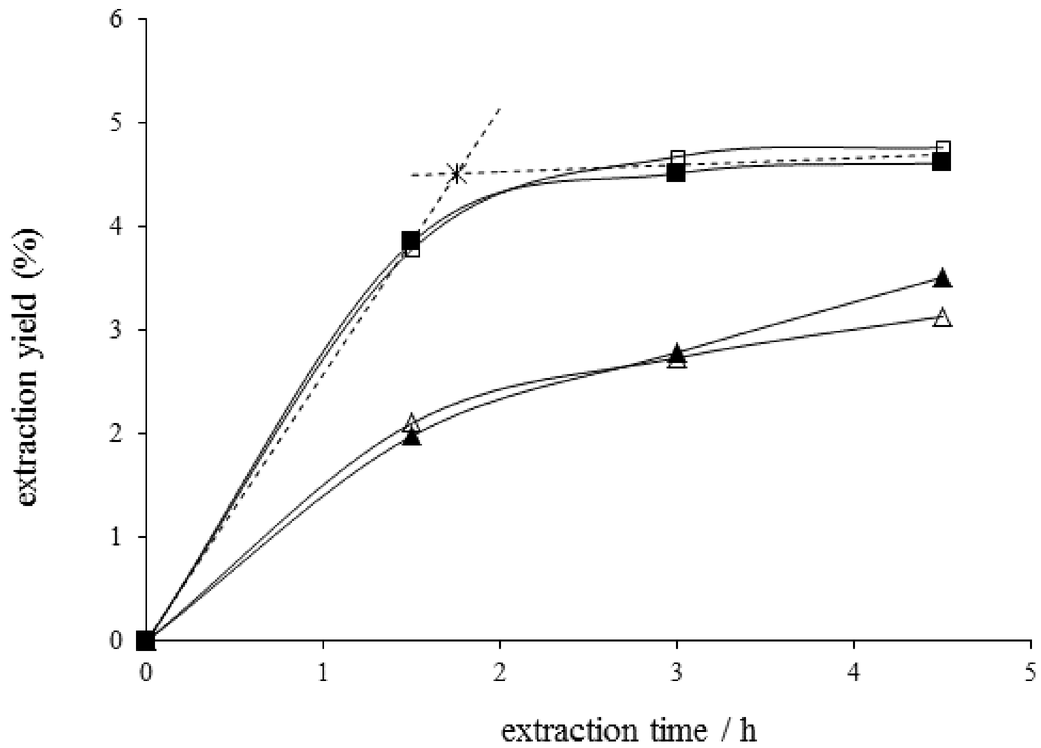
533 **Table 6.** Comparison of the total content (S1 + S2) of some volatile oil compounds
534 identified in oregano, sage and thyme extracts.

535

compound	total area determined in the samples [a]			ratio between total areas	
	oregano	sage	thyme	oregano/thyme	sage/thyme
1,8 cineole	n.i.	3092961	367182	-	8.42
Sabinene hydrate	98738980	382742	485797	203.25	0.79
Linalool	3161980	237435	3476055	0.91	0.07
Camphor	n.i.	8778453	1036757	-	8.47
Borneol	n.i.	1635085	3803945	-	0.43
α-terpineol	7390649	306102	363945	20.31	0.84
Linalyl acetate	3964877	957492	n.i.	-	-
Thymol	74510644	n.i.	45660581	1.63	-
Carvacrol	27282568	n.i.	3598557	7.58	-
E-caryophyllene	5108137	384253	731776	6.98	0.53

536 [a] n.i.: non identified
537
538
539
540

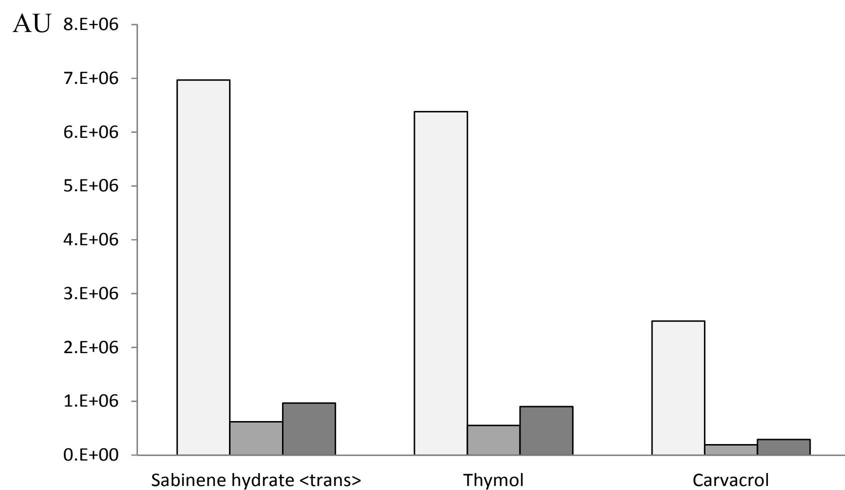
541
542
543
544
545



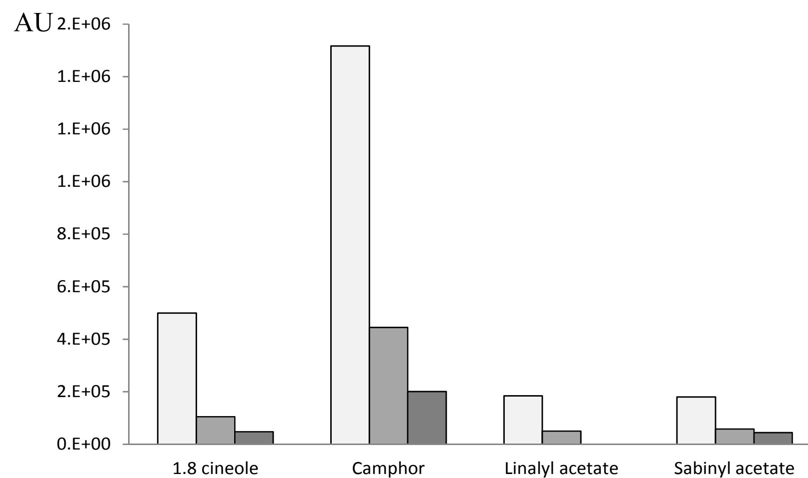
546
547
548

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

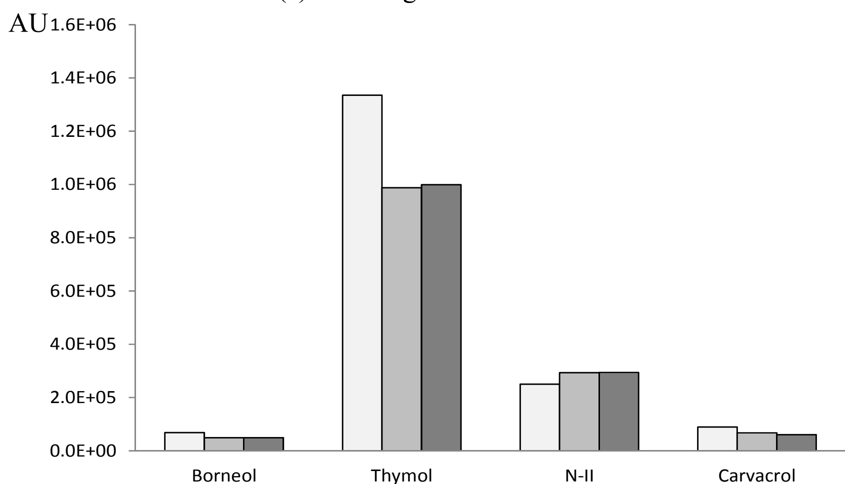
552
553
554



(a) S1 - oregano extraction

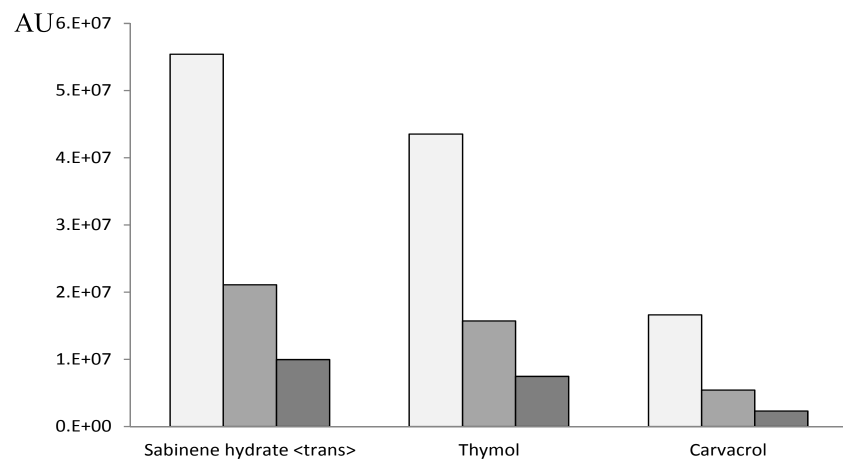


(b) S1 - sage extraction

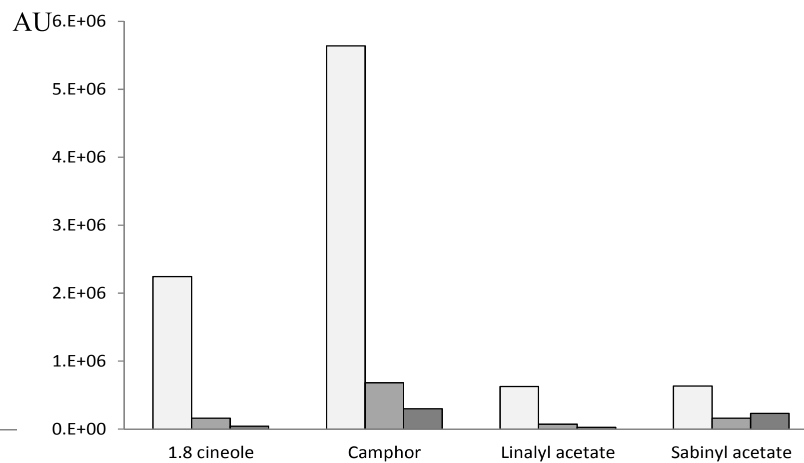


(c) S1 - thyme extraction

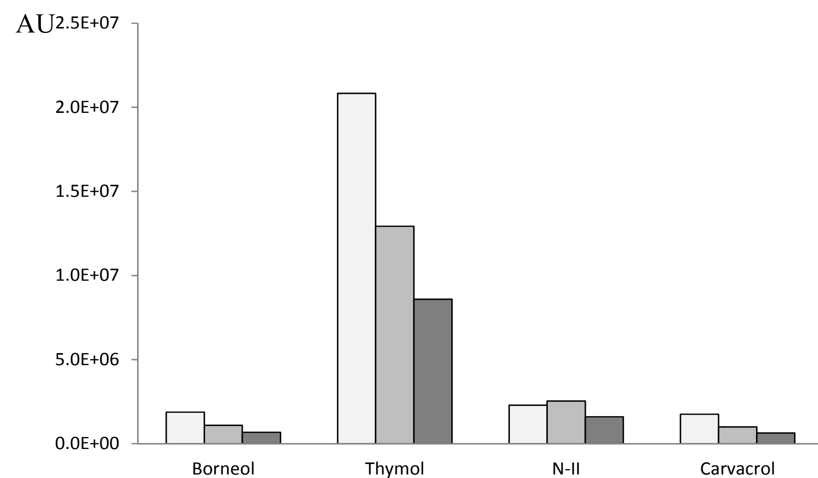
Figure 2. Kinetic behavior in the recovery of the main essential oil compounds identified in (a) oregano, (b) sage and (c) thyme S1 extracts. AU: area units.



(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. AU: area units.