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Isolation of essential oil from different plants and herbs by supercritical fluid extraction

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24 **Abstract**

25 Supercritical fluid extraction (SFE) is an innovative, clean and environmental friendly
26 technology with particular interest for the extraction of essential oil from plants and herbs.
27 Supercritical CO₂ is selective, there is no associated waste treatment of a toxic solvent, and
28 extraction times are moderate. Further supercritical extracts were often recognized of superior
29 quality when compared with those produced by hydro-distillation or liquid-solid extraction.

30 This review provides a comprehensive and updated discussion of the developments and
31 applications of SFE in the isolation of essential oils from plant matrices. SFE is normally
32 performed with pure CO₂ or using a cosolvent; fractionation of the extract is commonly
33 accomplished in order to isolate the volatile oil compounds from other co-extracted
34 substances. In this review the effect of pressure, temperature and cosolvent on the extraction
35 and fractionation procedure is discussed. Additionally, a comparison of the extraction yield
36 and composition of the essential oil of several plants and herbs from *Lamiaceae* family,
37 namely oregano, sage, thyme, rosemary, basil, marjoram and marigold, which were produced
38 in our supercritical pilot-plant device, is presented and discussed.

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46 **Keywords:** supercritical extraction; carbon dioxide; essential oil; *Lamiaceae* plants;
47 bioactive ingredients.

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60 **1. Introduction**

61 Essential oils extracted from a wide variety of plants and herbs have been traditionally
62 employed in the manufacture of foodstuffs, cosmetics, cleaning products, fragrances,
63 herbicides and insecticides. Further, several of these plants have been used in traditional
64 medicine since ancient times as digestives, diuretics, expectorants, sedatives, etc., and are
65 actually available in the market as infusions, tablets and/or extracts.

66 Essential oils are also popular nowadays due to aromatherapy, a branch of alternative
67 medicine that claims that essential oils and other aromatic compounds have curative effects.
68 Moreover, in the last decades, scientific studies have related many biological properties
69 (antioxidant, anti-inflammatory, antiviral, antibacterial, stimulators of central nervous system,
70 etc.) of several plants and herbs, to some of the compounds present in the essential oil of the
71 vegetal cells [1-5]. For example, valerenic acid, a sesquiterpenoid compound, and its
72 derivatives (acetoxvalerenic acid, hydroxyvalerenic acid, valeranone, valeranal) of valerian
73 extract are recognized as relaxant and sedative; lavender extract is used as antiseptic and anti-
74 inflammatory for skin care; menthol is derived from mint and is used in inhalers, pills or
75 ointments to treat nasal congestion; thymol, the major component of thyme essential oil is
76 known for its antimicrobial activity; limonene and eucalyptol appear to be specifically
77 involved in protecting the lung tissue. Therefore, essential oils have become a target for the
78 recovery of natural bioactive substances. For example, nearly 4000 articles in which
79 “essential oil” or “volatile oil” appears as keyword were published in the literature since year
80 2000 up today (<http://www.scirus.com/>); around 3000 also include the word “bioactive” or
81 “bioactivity” in the article text.

82 Essential oils are composed by lipophilic substances, containing the volatile aroma
83 components of the vegetal matter, which are also involved in the defense mechanisms of the
84 plants. The essential oil represent a small fraction of plant composition, and is comprised
85 mainly by monoterpenes and sesquiterpenes, and their oxygenated derivatives such as
86 alcohols, aldehydes, ketones, acids, phenols, ethers, esters, etc. The amount of a particular
87 substance in the essential oil composition varies from really high proportions (e.g. around 80-
88 90 %w/w of δ -limonene is present in orange essential oil) to traces. Nevertheless,
89 components present in traces are also important, since all of them are responsible for the
90 characteristic natural odor and flavor. Thus, it is important that the extraction procedure
91 applied to recover essential oils from plant matrix can maintain the natural proportion of its
92 original components [6].

93 New effective technological approaches to extract and isolate these substances from raw
94 materials are gaining much attention in the research and development field. Traditional
95 approaches to recover essential oil from plant matrix include steam- and hydro-distillation,
96 and liquid-solvent extraction. One of the disadvantages of steam-distillation and hydro-
97 distillation methods is related with the thermolability of the essential oil constituents, which
98 undergo chemical alteration due to the effect of the high temperatures applied (around the
99 normal boiling temperature of water). Therefore, the quality of the essential oil extracted is
100 extremely damaged [6].

101 On the other side, the lipophilic character of essential oils requires solvents such as paraffinic
102 fractions (pentane and hexane) to attain an adequate selectivity of the extraction. Further,
103 liquid solvents should have low boiling points, in order to be easily separated from the extract
104 and re-utilized. In this sense, the main drawback is the occurrence of organic toxic residues in
105 the extracted product.

106 Among innovative process technologies, supercritical fluid extraction (SFE) is indeed the
107 most widely studied application. In practice, SFE is performed generally using carbon
108 dioxide (CO₂) for several practical reasons: CO₂ has moderately low critical pressure (74 bar)
109 and temperature (32°C), is non-toxic, non-flammable, available in high purity at relatively
110 low cost, and is easily removed from the extract. Supercritical CO₂ has a polarity similar to
111 liquid pentane and thus, is suitable for extraction of lipophilic compounds. Thus, taking into
112 account the lipophilic characteristic of plant essential oils, it is obvious that SFE using CO₂
113 emerged as a suitable environmentally benign alternative to the manufacture of essential oil
114 products.

115 The commercial production of supercritical plant extracts has received increasing interest in
116 recent decades and has brought a wide variety of products that are actually in the market. As
117 mentioned before, supercritical plant extracts are being intensively investigated as potential
118 sources of natural functional ingredients due to their favorable effects on diverse human
119 diseases, with the consequent application in the production of novel functional foods,
120 nutraceuticals and pharmacy products. The reader is referred to several recent works [7-10] in
121 which is reviewed the supercritical extraction and fractionation of different type of natural
122 matter to produce bioactive substances. The general agreement is that supercritical extracts
123 proved to be of superior quality, i.e. better functional activity, in comparison with extracts
124 produced by hydro-distillation or using liquid solvents [11-14]. For example, Vági et al. [11]
125 compared the extracts produced from the extraction of marjoram (*Origanum maorana L.*)

126 using supercritical CO₂ (50°C and 45 MPa) and ethanol Soxhlet extraction. Extraction yields
127 were, respectively, 3.8 and 9.1%. Nevertheless, the supercritical extract comprised 21% of
128 essential oil, while the alcoholic extract contained only 9% of the volatile oil substances.
129 Furthermore, studies related with the antibacterial and antifungal properties of the extract
130 revealed better activity for the supercritical product. Another example of improved biological
131 activity exhibit by supercritical extracts was reported by Glisic et al. [14], demonstrating that
132 supercritical carrot essential oil was much more effective against *Bacillus cereus* than that
133 obtained by hydro-distillation.

134 Indeed, numerous variables have singular effect on the supercritical extraction and
135 fractionation process. Extraction conditions, such as pressure and temperature, type and
136 amount of cosolvent, extraction time, plant location and harvesting time, part of the plant
137 employed, pre-treatment, greatly affect not only yield but also the composition of the
138 extracted material.

139 Knowledge of the solubility of essential oil compounds in supercritical CO₂ is of course
140 necessary, in order to establish favorable extraction conditions. In this respect, several studies
141 have been reported [15-18]. Nevertheless, when the initial solute concentration in the plant is
142 low, as is the case of essential oils, mass transfer resistance can avoid that equilibrium
143 conditions are attained. Therefore, pretreatment of the plant become crucial to break cells,
144 enhancing solvent contact, and facilitating the extraction. In fact, moderate pressures (9-12
145 MPa) and temperatures (35-50°C) are sufficient to solubilize the essential oil compounds [15-
146 18]. Yet, in some cases, higher pressures are applied to contribute to the rupture of the
147 vegetal cells and the liberation of the essential oil. However, other substances such as
148 cuticular waxes are co-extracted and thus, on-line fractionation can be applied to attain the
149 separation of the essential oil from waxes and also other co-extracted substances.

150 In this review, on the basis of data reported in the literature and own experience, a detailed
151 and thorough analysis of the supercritical extraction and fractionation of plants and herbs to
152 produce essential oils is presented. Furthermore, the supercritical CO₂ extraction of several
153 plants (oregano, sage, thyme, rosemary, basil, marjoram and marigold) from *Lamiaceae*
154 family was accomplished in our supercritical pilot-plant at 30 MPa and 40°C. High CO₂
155 density was applied in order to ensure a complete extraction of the essential oil compounds.
156 Then, on-line fractionation in a cascade decompression system comprising two separators
157 was employed to isolate de essential oil fraction. Yield and essential oil composition was
158 determined and compared.

160 2. The essential oil of plants and herbs

161 Essential oils could be obtained from roots and rhizomes (such as ginger), leaves (mint,
162 oregano and eucalyptus), bark and branches (cinnamon, camphor), flowers (jasmine, rose,
163 violet and lavender) and fruits and seeds (orange, lemon, pepper, nutmeg). In general,
164 essential oil represents less than 5% of the vegetal dry matter. Although all parts of the plant
165 may contain essential oils; their composition may vary with the part of the plant employed as
166 raw material. Other factors such as cultivation, soil and climatic conditions, harvesting time,
167 etc. can also determine the composition and quality of the essential oil [19, 20]. For example,
168 Celiktas et al. [21] studied different sources of variability in the supercritical extraction of
169 rosemary leaves, including location (different cities of Turkey) and harvesting time
170 (December, March, June and September). They demonstrated that even applying the same
171 raw material pre-treatment and the same process conditions, extracts obtained from leaves
172 collected in different locations and harvesting times have rather different composition. For
173 example, the concentration of carnosic acid, one of the most abundant antioxidant substances
174 present in rosemary, varied from 0.5 to 11.6 % w/w in the extracts obtained from the different
175 samples of plant matrix. Furthermore, they observed that the plants harvested in September
176 had antioxidant capacities superior to those collected at other harvesting times. Of course,
177 geographical coordinates and local climate should be evaluated to consider this conclusion;
178 for example, high temperatures occur in September (average values around 25-29°C) in the
179 Turkish locations. Accordingly, Hidalgo et al. [22] reported that for rosemary plants
180 harvested from Cordoba (Spain), the carnosic acid content increased gradually during the
181 spring and peaked in the summer months.

182 The main compounds of plant essential oils are terpenes, which are also called isoprenes
183 since derived from isoprene (2-methyl-1,3-butadiene, chemical formula C_5H_8) (see Figure 1).
184 Main hydrocarbon terpenes present in plant essential oil are monoterpenes (C_{10}), which may
185 constitute more than 80% of the essential oil, and sesquiterpenes (C_{15}). They can present
186 acyclic structures, so as mono-, bi- or tricyclic structures (see Figure 2). Terpenoids are
187 derived from these hydrocarbons, for example by oxidation or just reorganization of the
188 hydrocarbon skeleton. Terpenoids present in essential oils comprise a wide variety of
189 chemical organic functions, such as alcohols, aldehydes, ketones, acids, phenols, ethers,
190 esters, etc.

191 The chemical structure of some popular essential oil compounds are depicted in Figure 2:
192 limonene, a cyclic hydrocarbon, and citral, an acyclic aldehyde, are main terpenes present in
193 citrus peel; menthol is a cyclic alcohol and the characteristic aroma compound of mint
194 (*Mentha* varieties); linalool is a acyclic alcohol that naturally occur in many flowers and spice
195 plants and has many commercial applications due to its pleasant fragrance; thymol and
196 carvacrol (positional isomers) are phenolic alcohols with strong antiseptic properties; α -
197 pinene, a bicyclic hydrocarbon, is found in the oils of many species of coniferous trees,
198 particularly the pine; sabinene, also a bicyclic hydrocarbon, is one of the chemical
199 compounds that contributes to the spiciness of black pepper and is a major constituent of
200 carrot seed oil; camphor is a bicyclic ketone present in abundance in camphor tree and in the
201 essential oil of several *Lamiaceae* plants, such as sage and rosemary; and valerenic acid is a
202 sesquiterpenoid constituent of the essential oil of the valerian (*Valeriana officinalis*) and is
203 thought to be at least partly responsible for the sedative effects of the plant.

204 In general, terpenes and terpenoids are chemically instable (due to the C=C bonds) and thus
205 molecules present different chemical reorganizations (isomerization). Further, substances
206 comprising essential oils have similar boiling points and are difficult to isolate. The normal
207 boiling point of terpenes varies from 150°C to 185°C; while the normal boiling point of
208 oxygenated derivatives is in the range 200-230°C. Extraction and fractionation of these
209 substances should be carried out at moderate temperatures, in order to prevent thermal
210 decomposition. In fact, this is the main drawback of steam- and hydro-distillation. Besides
211 the breakdown of thermally labile components, Chyau et al. [23] observed incomplete
212 extraction of the essential oil compounds of *G. tenuifolia* and promotion of hydration
213 reactions when steam-distillation is employed. Furthermore, the removal of water from the
214 product is usually necessary after steam- or hydro-distillation.

215 In general, terpenes contribute less than terpenoids to the flavor and aroma of the oil.
216 Additional, they are easily decomposed by light and heat, quickly oxidize and are insoluble in
217 water. Thus, the removal of terpenes from essential oil leads to a final product more stable
218 and soluble. In this respect, supercritical fluid fractionation in countercurrent packed columns
219 was employed to accomplish the deterpenation of essential oils [24-26].

220 For example, Benvenuti et al. [25] studied the extraction of terpenes from lemon essential oil
221 (terpenoids/terpene ratio = 0.08) using a semi-continuous single-stage device at 43°C and
222 8.0-8.5 MPa and developed a model (based in Peng-Robinson equation of state) to simulate
223 the process. Then, the model was applied to study the steady state multistage countercurrent

224 process and a terpenoids/terpene ratio around 0.33 (4-fold increase) was obtained in the
225 raffinate. A similar result (5-fold increase of terpenoids in raffinate) was obtained by
226 Espinosa et al. [26] in the simulation and optimization of orange peel oil deterpenation. The
227 low terpenoids/terpene ratio of the original essential oil requires high solvent flow and high
228 recycle flow rate in order to achieve moderate terpenoids concentration in the raffinates.

229 With respect to the solubility of essential oil compounds in supercritical CO₂, it could be
230 stated in general that the solubility of hydrocarbon monoterpenes is higher than the solubility
231 of monoterpenoids. For example, the reported solubility of limonene at 9.6 MPa and 50°C is
232 2.9 % w/w; at the same pressure and temperature conditions the solubility of thymol and
233 camphor are, respectively, 0.9 and 1.6 % w/w [18]. Moreover, these values are considerably
234 higher than the solubility of other extractable compounds present in plants and herbs, such as
235 phenolic compounds, waxes, carotenoids and chlorophylls. As it is well-known phenolic
236 compounds present in plants constitute a special class of bioactive substances due to their
237 recognized antioxidant activity [27]. For example, Murga et al. [28, 29] reported that the
238 solubility of protocatechuic acid, methyl gallate and protocatechualdehyde (phenolic
239 compounds present in grapes) in pure supercritical CO₂ measured at different temperatures
240 (40-60°C) and pressures up to 50 MPa were lower than 0.02 % w/w. Furthermore, also low
241 solubilities were reported for carotenoids [30].

242 On the other side, the solubility of *n*-alkanes C₂₄-C₂₉ in supercritical CO₂ is in the range of
243 0.1-1 %w/w at rather low pressures (8-25 MPa) [31]. These values are quite close to the
244 solubility values referred above for several monoterpene compounds and thus, waxes are in
245 general the main substances co-extracted with essential oils. Thus, fractionation schemes are
246 target towards an efficient separation of essential oil constituents from high molecular weight
247 hydrocarbons and waxy esters.

248 Figure 3 compares the solubility in supercritical CO₂ of several substances, representing
249 different family of compounds present in vegetal natural matter. Solubilities are represented
250 as a function of pressure, for temperatures in the range 35-50°C. Particularly, the figure
251 shows the solubility of main monoterpenes of grape essential oil, namely α -pinene, limonene
252 and linalool; the solubility reported for some low molecular weight phenolic compounds
253 (protocatechuic acid, methyl gallate and *p*-cumaric acid) also present in grapes; and the
254 solubility of β -carotene and *n*-C₂₈, as representatives, respectively, of pigments and waxy
255 compounds. As can be observed in Figure 3, the solubility of main constituents of essential
256 oil (monoterpenes) of grapes is considerably higher than the solubility of the phenolic

257 compounds present in grapes. That is, low extraction pressures would extract grape essential
258 oil but would not promote the extraction of its phenolic compounds. Further, pigments and
259 chlorophylls also require high solvent pressures to be readily extracted. But waxes solubilities
260 are quite close to monoterpene solubilities and thus, this type of compounds are readily co-
261 extracted when extraction pressure is somewhat increased.

262 Table 1 presents a list of several plants which have been subject of SFE to produce essential
263 oils. Also given in the table are the main compounds identified in the references cited in the
264 table. As can be observed, several plants from *Lamiaceae* family, namely oregano, thyme,
265 sage, rosemary, mint, basil, marjoram, etc. were focus of intensive study.

266 Among *Origanum* genus, oregano (*Origanum vulgare*) is an herbaceous plant native of the
267 Mediterranean regions, used as a medicinal plant with healthy properties like its powerful
268 antibacterial and antifungal properties [32, 33]. It has been recognized that the responsible of
269 these activities in oregano is the essential oil, which contains thymol and carvacrol as the
270 primary components [34]. In these compounds, Puertas-Mejia et al. [35] also found some
271 antioxidant activity. Also marjoram (*Origanum maorana*) essential oil, which represent
272 around 0.7-3.0% of plant matrix, was recognized to have antibacterial and antifungal
273 properties [36, 37]. Popularly, the plant was used as carminative, digestive, expectorant and
274 nasal decongestant. Main compounds identified in marjoram essential oil are cis-sabinene, 4-
275 terpineol, α -terpineol and γ -terpinene [11, 38-40].

276 Thymol and carvacrol isomers were also found in the essential oil of another *Lamiaceae*
277 plant, namely Thymus. The variety most studied is, indeed, *Thymus vulgaris* [41, 42]. Yet,
278 particularly attention is focused on *Thymus zygis*, a thyme variety widespread over Portugal
279 and Spain, which extract has proved to be useful for food flavoring [43] and in the
280 pharmaceutical [44, 45] and cosmetic industries [46].

281 Other *Lamiaceae* plants being intensively studied are the “*Officinalis*” ones (from Latin
282 meaning medicinal). Sage (*Salvia officinalis*) is a popular kitchen herb (preserves a variety of
283 foods such as meats and cheeses) and has been used in a variety of food preparations since
284 ancient times. Further, sage has a historical reputation for promotion of health and treatment
285 of diseases [47]. Modern day research has shown that sage essential oil can improve the
286 memory and has shown promise in the treatment of Alzheimer’s disease [48]. Main
287 constituents of sage essential oil are camphor and eucalyptol (1,8 cineole). Depending on
288 harvesting, sage oil may contain high amounts of toxic substances, such us α - and β -thujone
289 [49, 50], which content is regulated in food and drink products. In the past few decades

290 however, sage has been the subject of an intensive study due to its phenolic antioxidant
291 components [51-53]. Although main studies related with rosemary (*Rosmarinus officinalis*)
292 extracts are related with its high content of antioxidant substances (mainly carnosic acid,
293 carnosol, and rosmarinic acid) [54-56], the essential oil of this plant contains high amounts of
294 eucalyptol and camphor, and is also recognized as an effective anti-bactericide [56-58].

295 Basil (*Ocimum basilicum L.*) is an aromatic plant also belonging to the group of *Lamiaceae*
296 family. It has been used in traditional medicine as digestive, diuretic, against gastrointestinal
297 problems, intestinal parasites, headaches, and even as a mild sedative due to its activity as
298 depressant of the central nervous system. Basil essential oil has been recognized to have
299 antiseptic and analgesic activity and thus, it has been used to treat eczema, warts and
300 inflammation [59]. Main monoterpenes present in basil essential oil are linalool, 1,8-cineole
301 and α -terpineol, and also sesquiterpenes such as α -bergamotene, epi- α -cadinol y α -cadinene
302 [60-65].

303 In the case of marigold (*Calendula officinalis L.*) the essential oil is mainly comprised in the
304 flower petals (0.1-0.4%). Traditionally it has been used externally to treat wounds or sores.
305 The essential oil contains monoterpenes, such as eugenol and γ -terpineno, and sesquiterpenes,
306 such as γ - and δ -cadinene. Furthermore, marigold is highly regarded for the important content
307 of lutein [59].

308

309 **3. Supercritical fluid extraction (SFE) of essential oils**

310 A basic extraction scheme for SFE of solid materials is shown in Figure 4. The equipment
311 design implies a semi-continuous procedure. A continuous feeding and discharging of the
312 solid to obtain the continuous process was studied and developed [66] but design and
313 operation of this alternative is neither cheap nor simple and thus, in practice is not commonly
314 employed.

315 The central piece in the SFE device of Figure 4 is the extraction vessel (EV) charged with the
316 raw matter to be extracted. The raw matter (dried and grinded) is generally loaded in a basket,
317 located inside the extractor, and allows a fast charge and discharge of the extraction vessel.
318 The extraction vessel is commonly cylindrical; as a general rule the ratio between length and
319 diameter is recommended to be 5-7.

320 From the bottom of the extraction vessel the supercritical solvent is continuously loaded; at
321 the exit of the extractor the supercritical solvent with the solutes extracted flows through a

322 depressurization valve (V) to a separator (S1) in which, due to the lower pressure, the extracts
323 are separated from the gaseous solvent and collected. Some SFE devices contain two or more
324 separators, as is the case of the scheme shown in Figure 4. In this case, it is possible to
325 fractionate the extract in two or more fractions (on-line fractionation) by setting suitable
326 temperatures and pressures in the separators.

327 In the last separator of the cascade decompression system the solvent reaches the pressure of
328 the recirculation system (generally around 4-6 MPa). Then, after passing through a filter (F),
329 the gaseous solvent is liquefied (HE1) and stored in a supplier tank (ST). When the solvent is
330 withdrawn from this tank is pumped (P1) and then heated (HE2) up to the desired extraction
331 pressure and temperature. Before pumping, precooling of the solvent is generally required
332 (HE3) in order to avoid pump cavitation. If a cosolvent is employed an additional pump is
333 necessary (P2). Usually, the cosolvent is mixed with the solvent previously to introduction to
334 HE2 as is depicted in Figure 4.

335 **3.1 Effect of matrix pretreatment and packing**

336 The particular characteristics of the plant species is, indeed, a decisive factor in the
337 supercritical extraction kinetics. Recently, Fornari et al. [67] presented a comparison of the
338 kinetics of the supercritical CO₂ extraction of essential oil from leaves of different plant
339 matrix from *Lamiaceae* family. In their work, identical conditions of raw material
340 pretreatment, particle size, packing and extraction conditions (30 MPa, 40°C and no co-
341 solvent) were maintained. Figure 5 show a comparison between the global yields obtained for
342 the different raw materials as a function of extraction time. As can be deduced from the
343 figure, sage (*Salvia officinalis*) and oregano (*Origanum vulgare*) were completely extracted
344 in less than 2 h, while rosemary (*Rosmarinus officinalis*) and thyme (*Thymus zygis*) were not
345 completely exhausted after 4.5 h of extraction. Moreover, very similar kinetic behavior
346 resulted for sage and oregano, so as for thyme and rosemary. Considering the first period of
347 extraction (1.5 h) it was estimated a removal velocity of around 0.004 g extract / g CO₂ in the
348 case of sage and oregano, and almost half of this value in the case of rosemary and thyme.

349 With respect to the fractionation of the extracted material, a depressurization cascade system
350 comprised of two separators (similar to that depicted in Figure 4) was employed, and it was
351 observed that the performance is quite different considering the diverse plants studied. In the
352 case of oregano, the amount of material recovered in the second separator (S2) is almost half
353 the amount recovered in the first one (S1). Just the opposite behavior is detected for sage and

354 thyme, while in the case of rosemary extraction similar amounts of extract were recovered in
355 both S1 and S2. This distinct fractionation behavior observed should be attributed to the
356 different substances co-extracted with the essential oil compounds (extraction and
357 fractionation conditions were kept exactly the same), since the isoprenoid type compounds
358 were selectively recovered in S2 separator for the four plant materials studied [67]. GC-MS
359 analysis of the essential oil compounds present in S1 and S2 samples resulted that ca. 91, 78,
360 93 and 86% of the volatile oil compounds identified, respectively, in oregano, sage, thyme
361 and rosemary were recovered in S2 separator. A comparison of the content of some common
362 volatile oil compounds identified in oregano, sage and thyme was also given by Fornari et al.
363 [67] and is resumed in Table 2. The oregano/thyme and sage/thyme ratios given in Table 2
364 indicate that the content of 1,8 cineole and camphor in sage was at least 8 times higher than
365 in thyme. Further, oregano and thyme contain similar amounts of linalool, and around 15
366 times higher than sage. Sabinene, α -terpineol, carvacrol and caryophyllene were significantly
367 more abundant in oregano than in thyme or sage extracts [67].

368 Also the part of the plant employed as raw material is an important factor to be considered,
369 since may greatly affect the composition of the extracted essential oil. For example, Bakó et
370 al. [68] investigate the carotenoid composition of the stems, leaves, petals and pollens of
371 *Calendula officinalis* L. and concluded that in the petals and pollens, the main carotenoids
372 were flavoxanthin and auroxanthin while the stem and leaves mostly contained lutein and β -
373 carotene. Moreover, with respect to essential oil composition, minor qualitative and major
374 quantitative variations were determined with respect to the substances present in the different
375 parts of the plant. For example, Chalchat et al. [69] examined the chemical composition of
376 the essential oil produced by hydro-distillation of flowers, leaves and stems from basil
377 (*Ocimum basilicum* L.). They conclude that the essential oil obtained from flowers and leaves
378 contained more than 50-60% of estragole and around 15-20% of limonene, while only 16%
379 of estragole and 2.4% of limonene were present in the essential oil extracted from stems.
380 Furthermore, dillapiole was the main substance identified in stems (\approx 50%) and very low
381 amounts of this compound were found in flowers and leaves.

382 Despite the lipophilic character of essential oil compounds, the water present in the vegetable
383 matrix may interfere in the solute-CO₂ interaction (particularly in the case of terpenoids
384 which are most polar than terpenoids) and produce a decrease of extraction yield. For this
385 reason, drying of the raw material is recommended.

386 Generally, the vegetable matrix should not have water content higher than 12%; the presence
387 of water can cause other undesirable effects such as formation of ice in pipelines due to the
388 rapid depressurization provoked to precipitate the solutes, hydrolysis of compounds, etc. In
389 turn, it is obvious that drying may influence the content of volatile oil compounds. Oca et al.
390 [70] studied the influence of different drying processes on the essential oil composition of
391 rosemary supercritical extracts. Three different methods of drying were investigated: freeze-
392 drying, oven-drying and vacuum rotary evaporation. They conclude that the highest quantity
393 of rosemary essential oil was achieved when freeze-drying was utilized, due to the low
394 temperatures applied and thus, less aroma compounds were lost. Although rotary evaporation
395 was carried out at lower temperature (35°C) than oven-drying (45°C), the absence of light in
396 the second method produced less damage in the composition of rosemary essential oil.

397 Beyond the specific characteristics of the plant variety and the part of the plant employed for
398 extraction, cell disruption is a crucial factor in solvent extraction processes and thus, in SFE.
399 Essential oil compounds are found in intracellular spaces, more than on the surface of the
400 vegetal cell. Thus, in order to attain an adequate contact with the solvent, a pretreatment to
401 produce cell disruption (comminuting, grinding) is critical. Then, the efficiency of the
402 extraction process is improved by a decreasing of mass transfer resistance. Indeed, particle
403 size greatly affects process duration and both variables are interconnected with CO₂ flow rate.
404 The selection of these parameters has the target of producing the exhaustion of the desired
405 compounds in the shorter time.

406 Particle size plays an important role in SFE processes; if internal mass transfer resistances
407 could be reduced, the extraction is controlled by equilibrium conditions and thus, short
408 extraction times are required. For example, Aleksovsk and Sovová [49] proved that in the
409 SFE of sage leaves ground in small particles, the essential oil was easily accessible to the
410 supercritical CO₂ solvent at moderate conditions (9-13 MPa and 25-50°C) and the extraction
411 was controlled by phase equilibrium. The same readily SFE of sage was observed by Fornari
412 et al. [67] while a delayed kinetic (controlled by mass diffusion) was deduced for thyme and
413 rosemary supercritical extraction [67, 71] although the same grinding method, particle size
414 and packing procedure was applied for the three plants.

415 Decreasing particle size improves SFE rate and yield. For example, Damjanovic et al. [72]
416 reported that a decrease of fennel particles from 0.93 to 1.48 mm produced a significant
417 increase in the essential oil yield (from 2.15% to 4.2%). Moreover, very small particles could
418 result in low bed porosity (tight packing) and problems of channeling can arise inside the

419 extraction bed. Also, during grinding, the loss of volatile compounds could be produced. In
420 this respect, several authors have studied the effect of cooling during grinding [73, 74].

421 Almost 99% of input energy in grinding is dissipated as heat, rising the temperature of the
422 ground product. In spice grinding temperature rises to the extent of 42 - 93°C [75] and this
423 causes the loss of volatile oil and flavor constituents. The temperature rise of the vegetal
424 matter can be minimized to some extent by circulating cold air or water around the grinder.
425 But this technique is generally not enough to significantly reduce the temperature rise of the
426 solid matrix. The loss of volatiles can be significant reduced by the cryogenic grinding
427 technique, using liquid nitrogen or liquid carbon dioxide that provides the refrigeration (by
428 absorbing heat generation during grinding) needed to pre-cool the spices and maintain the
429 desired low temperature. Meghwal and Goswami [73] present a comprehensive study of
430 black pepper grinding. They compare the grinding using a rotor mill at room temperature
431 without any refrigeration and cryogenic grinding using liquid nitrogen. They proved that the
432 volatile oil content in powder obtained after the cryogenic grinding was higher (ca. 1.98 to
433 2.15 ml / 100 g of powder) than that obtained from ambient grinding (0.87 to 0.96 ml / 100 g
434 of powder). Further, the authors also demonstrated cryogenic grinding improved the
435 whiteness and yellowness indices of the product obtained, whereas ambient grinding
436 produces ash colored powder with high whiteness and low yellowness indices.

437 **3.2 Effect of extraction conditions**

438 The most relevant process parameter in SFE from plant matrix is the extraction pressure,
439 which can be used to tune the selectivity of the supercritical solvent. With respect to
440 extraction temperature, in the case of thermolabile compounds such as those comprising
441 essential oils, values should be set in the range 35-50°C; e.g., in the vicinity of the critical
442 point and as low as possible to avoid degradation.

443 Essential oils can be readily extracted using supercritical CO₂ at moderate pressures and
444 temperatures. That is, from an equilibrium point of view rather low pressures are required to
445 extract essential oils from plant matrix (9-12 MPa) (see Figure 3). Yet, higher pressures are
446 also applied in order to take advance of the compression effect on the vegetal cell, what
447 enhances mass transfer and liberation of the oil from the cell. High pressures produce the co-
448 extraction of substances other than essential oil. The general rule is: the higher is the
449 pressure, the larger is the solvent power and the smaller is the extraction selectivity. Thus,
450 when high pressures are applied, on-line fractionation scheme with at least two separators is

451 required to isolate the essential oil from the other co-extracted substances. For example,
452 moderate conditions (solvent densities between 300 and 500 kg/m³) were found to be
453 sufficient for an efficient extraction of essential oil from oregano leaves [76]. Although
454 higher pressures increase the rate of extraction and yield, also significant amounts of waxes
455 were co-extracted and, consequently, the essential oil content in the extract decreased [67]. In
456 the case of marigold extraction, when high pressures are applied (50 MPa and 50°C) main
457 compounds extracted are triterpenoid esters [77], while lower pressures (20 MPa and 40°C)
458 produce extracts rich in aliphatic hydrocarbons, acetyl eugenol and guaïol [78].

459 Supercritical CO₂ is a good solvent for lipophilic (non-polar) compounds, whereas, it has a
460 low affinity with polar compounds. Thus, a cosolvent can be added to CO₂ to increase its
461 solvent power towards polar molecules. Since essential oils are comprised by lipophilic
462 compounds, the addition of a cosolvent to attain a suitable recovery of essential oils is not
463 necessary. This is an important advantage of SFE essential oil production, since subsequent
464 processing for solvent elimination (and recuperation for recycling) is not required. Moreover,
465 several studies are reported in which ethanol and other low molecular weight alcohols are
466 employed in the SFE of plants and herbs. But in these cases, antioxidant compounds were
467 generally the target. For instance, Leal et al. [79] studied the SFE of basil using water at
468 different concentrations (1, 10 and 20 %) as cosolvent of CO₂. They conclude that the
469 extraction yield increases as the percentage of cosolvent increases, but also a reduction of the
470 content of terpene compounds while an increase of phenolic acids content is observed in the
471 extracted product. Menaker et al. [63] and Hamburger et al. [80] also observed an increase in
472 the extraction yield when ethanol is employed as co-solvent in the SFE of basil, but a
473 substantial decrease of the essential oil components when the amount of co-solvent and CO₂
474 density increases, while the extract is enriched in flavonoid-type compounds.

475 Table 3 show the effect of ethanol as cosolvent in the supercritical extraction of rosemary
476 leaves. Although different extraction pressures were employed (data obtained in our SFE
477 pilot-plant) is evident that the amount of essential oil extracted, which is represented in the
478 table by the main constituents of rosemary essential oil, is not significantly increased when
479 ethanol is employed as cosolvent, while ca. 4 and 6 fold increase in the extraction of,
480 respectively, carnosic acid and carnosol is observed. That is, the major effect of employing
481 ethanol as cosolvent in the CO₂ SFE of rosemary is observed on the recovery of its phenolic
482 antioxidant compounds but not in the extraction of essential oil substances.

483

484 **3.3 Fractionation alternatives**

485 Another technological alternative that can be very useful to improve the selectivity of SFE to
486 produce essential oils is fractionation of the extract, what means the separation of the solutes
487 extracted from the plant matrix in two or more fractions. This strategy can be used when it is
488 produced the extraction of several compound families from the same matrix, and they show
489 different solubilities in supercritical CO₂ (see Figure 3). Fractionation techniques take
490 advantage of the fact that the supercritical solvent power can be sensitively varied with
491 pressure and temperature.

492 Two different fractionation techniques are possible: an extraction accomplished by successive
493 steps (multi-step fractionation) and fractionation of the extract in a cascade decompression
494 system (on-line fractionation).

495 In the case of multi-step fractionation, the conditions applied in the extraction vessel are
496 varied step by step, increasing CO₂ density in order to obtain the fractional extraction of the
497 soluble compounds contained in the organic matrix. Thus, the most soluble solutes are
498 recovered in the first fraction, while substances with decreasing solubility in the supercritical
499 solvent are extracted in the successive steps. Essential oils generally constitute the first
500 fraction of a multi-step fractionation scheme due to their good solubility in supercritical CO₂.

501 For example, multi-step fractionation arrangement may consist in performing a first
502 extraction step at low CO₂ density ($\approx 300 \text{ kg/m}^3$) followed by a second extraction step at high
503 CO₂ density ($\approx 900 \text{ kg/m}^3$). Then, the most soluble compounds are extracted during the first
504 step (for example, essential oils) and the less soluble in the second one (e.g. antioxidants).
505 Fractionation of rosemary extract was first reported by Oca et al. [70]: two successive
506 extraction steps resulted in a low-antioxidant but essential oil rich fraction in the first step (10
507 MPa and 40°C, CO₂ density = 630 kg/m^3) and a high-antioxidant fraction in the second step
508 (40 MPa and 60°C, CO₂ density = 891 kg/m^3).

509 Multi-step fractionation was also employed by the authors (data non published) to produce
510 the complete exhaustion of rosemary essential oil using pure CO₂ in a first step, and a
511 fraction with high antioxidants content using CO₂ and ethanol as co-solvent in the second
512 step. But in this case, high CO₂ density was applied first (30 MPa and 40°C, CO₂ density =
513 911 kg/m^3) in order to produce the complete deodorization of plant matrix. Despite the fact
514 that some antioxidants were also co-extracted in this step, the high pressures applied ensured
515 the complete exhaustion of essential oil substances from plant matrix. Then, a step using

516 ethanol cosolvent was applied at lower CO₂ densities (15 MPa and 40°C, CO₂ density = 781
517 kg/m³). This second step produced an extract (5% yield) containing 33 %w/w of antioxidants
518 (carnosic acid plus carnosol) and less than 2.5 %w/w of volatile oil compounds.

519 On-line fractionation is another fractionation alternative which allows operation of the
520 extraction vessel at the same conditions during the whole extraction time, while several
521 separators in series (normally, no more than two or three separators) are set at different
522 temperatures and decreasing pressures. The cascade depressurization is achieved by means of
523 back pressure regulators valves (see the scheme depicted in Figure 4). The scope of this
524 operation is to induce the selective precipitation of different compound families as a function
525 of their different saturation conditions in the supercritical solvent. This procedure has been
526 applied with success in the SFE of essential oils as it was well established by Reverchon and
527 coworkers in the 1990s [50, 81-83].

528 A different on-line fractionation alternative to improve the isolation of antioxidant
529 compounds from rosemary has been recently presented by the authors [55]. The experimental
530 device employed in the study is similar to the one schematized in Figure 4, comprising two
531 separators (S1 and S2) in a cascade decompression system. The SFE temperature and
532 pressure were kept constant (30 MPa and 40°C) but the depressurization procedure adopted
533 to fractionate the material extracted was varied with respect to time. At the beginning (first
534 period) on-line fractionation of the extract was accomplished; due to the lower solubility of
535 the antioxidant compounds in comparison to the essential oil substances it is apparent that the
536 antioxidants would precipitate in S1, while the essential oil would mainly be recovered in S2.
537 Nevertheless, when the amount of volatile oil remained in the plant matrix is significantly
538 reduced, no further fractionation is necessary. Then, during the rest of the extraction (second
539 period) S1 pressure is lowered down to CO₂ recirculation pressure and all the substances
540 extracted were precipitated in S1, and mixed with the material that had been recovered in this
541 separator during the first period of extraction. The authors varied the extend of the first
542 extraction period and determine the optimum in order to maximize antioxidant content and
543 yield in the product collected in S1. In this way, a fraction was produced with a 2-fold
544 increase of antioxidants in comparison with a scheme with no fractionation, and with a yield
545 almost five times higher than that obtained when on-line fractionation is accomplished during
546 the whole extraction time. With respect to rosemary volatile oil a 2.5-4.5 fold increase was
547 observed for several substances (1,8 cineol, camphor, borneol, linalool, terpineol, verbenone

548 and β -caryophyllene) in the sample collected in S2 with respect to the antioxidant fraction
549 collected in S1 [55].

550

551 **3.4 Ultrasound assisted SFE**

552 Since high pressures are used in SFE, mechanical stirring is difficult to be accomplished.
553 Thus, application of ultrasound assisting the extraction may produce important benefits to
554 improve mass transfer processes.

555 The use of ultrasound to enhance extraction yield has started in the 1950s with laboratory
556 scale equipment. Traditional solvent extraction assisted by ultrasound has been widely used
557 for the extraction of food ingredients such as lipids, proteins, essential oils, flavonoids,
558 carotenoids and polysaccharides. Compared with traditional solvent extraction methods,
559 ultrasound can improve extraction rate and yield and allow reduction of extraction
560 temperature [84].

561 The enhancement produced by the application of ultrasonic energy in the extraction of plants
562 and herbs was recognized in several works [85, 86]. Ultrasound causes several physical
563 effects such as turbulence, particle agglomeration and cell disruption. These effects arise
564 principally from the phenomenon known as cavitation, i.e. the formation, growth and violent
565 collapse of microbubbles due to pressure fluctuations. Cavitation in conventional solvent
566 extraction is well established. However, in the case of pressurized solvents, the intensity
567 required producing cavitation increases and thus it is expected that the effect of ultrasound
568 application to high pressure processes is much limited [87].

569 Riera et al. [88] study the effect of ultrasound assisting the supercritical extraction of almond
570 oil. Trials were carried out at various pressures, temperatures, times and CO₂ flow rates. At
571 pressures around 20 MPa the improvement in the yield was low (\approx 15%) probably because
572 the solubility of almond oil in supercritical CO₂ is rather low. However, at higher extraction
573 pressures larger improvements between extraction curves with and without ultrasounds were
574 achieved (around 40-90%).

575 Balachandran et al. [89] studied the influence of ultrasound on the extraction of soluble
576 essences from a typical herb (ginger) using supercritical CO₂. A power ultrasonic transducer
577 with an operating frequency of 20 kHz was connected to an extraction vessel and the
578 extraction of gingerols (the pungent compounds of ginger) from freeze-dried ginger particles

579 was monitored. In the presence of ultrasound, both extraction rate and yield increased. The
580 recovery of gingerols was significantly increased up to 30%, in comparison with the
581 extraction without sonication. This higher extraction rate observed was attributed to
582 disruption of the cell structures and an increase in the accessibility of the solvent to the
583 internal particle structure, which enhances the intra-particle diffusivity. While cavitation
584 would readily account for such enhancement in ambient processes, the absence of phase
585 boundaries should exclude such phenomena at supercritical conditions.

586

587 **4. Supercritical chromatography fractionation of essential oils**

588 Supercritical fluid chromatography (SFC) is also a novel procedure employed in the food and
589 nutraceutical field to separate bioactive substances. SFC embraces many of the features of
590 liquid and gas chromatography, and occupies an intermediate position between the two
591 techniques. Because solubility and diffusion can be optimized by controlling both pressure
592 and temperature, chromatography using a supercritical fluid as the mobile phase can achieve
593 better and more rapid separations than liquid chromatography.

594 Natural products have also been subjected to application of SFC. First studies in this field
595 were the separation of tocopherols from wheat germ [90] and the isolation of caffeine from
596 coffee and tea [91]. More recent works are related with the fractionation of lipid-type
597 substances and carotenoids. As examples, the reader is referred to the work of Sugihara et al.
598 [92], in which SFE and SFC are combined for the fractionation of squalene and phytosterols
599 contained in the rice bran oil deodorization distillates, and the work of Bamba et al. [93] in
600 which an efficient separation of structural isomers of carotenoids was attained.

601 With respect to essential oils, Yamauchi et al. [94] reported the SFC fractionation of lemon
602 peel oil in different compounds such as hydrocarbons, alcohols, aldehydes or esters.
603 Desmortreux et al. [95] studied the isolation of coumarins from lemon peel oil and Ramirez et
604 al. [96, 97] reported the isolation of carnosic acid from rosemary extract both in analytical
605 and semi-preparative scale.

606 Recently, the authors [98] studied the fractionation of thyme (*Thymus vulgaris L.*) essential
607 oil using semi-preparative SFC. The essential oil was produced by supercritical extraction at
608 15 MPa and 40°C (no co-solvent). In the SFC system a silica- packed column (5 µm particle
609 diameter) placed in an oven was employed, and was coupled to a UV/Vis detector. The SFC
610 system comprises six collector vessels in which the sample can be fractionated, with a

611 controlled flow of solvent (also ethanol) to ensure completely recovery of injected material.
612 Figure 6 shows a scheme of the supercritical SFC device employed. Different conditions
613 were explored, including the use of ethanol as cosolvent, to produce a fraction enriched in
614 thymol, the most abundant antimicrobial substance present in thyme essential oil.

615 Figure 7 shows the SFC chromatogram obtained at 50°C, 15 MPa and using 3 % ethanol
616 cosolvent. Chromatogram A on Figure 7 corresponds to the injection of 5 mg/ml concentrate
617 of supercritical thyme extract and chromatogram B corresponds to injections carried out at 20
618 mg/ml. In both cases, a distinct peak at similar elution time of thymol (2.8 min) can be
619 observed in the figure. Figure 7 also shows the intervals of time selected to fractionate the
620 thyme extract sample; three different fractions (F1, F2 and F3) were collected. As a result,
621 around a 2 fold increase of thymol was obtained in F2 fraction (from 29 % to 52 % w/w) with
622 a thymol recovery higher than 97%.

623

624 **5. Comparison of the SFE extraction of essential oil from different plant matrix**

625 Supercritical CO₂ extraction of several plants from *Lamiaceae* family were extracted and
626 fractionated in a supercritical pilot-plant comprising an extraction cell of 2 l of capacity. The
627 SFE system (Thar Technology, Pittsburgh, PA, USA, model SF2000) is similar to that
628 schematized in Figure 4. Plant matrix consisted in dried leaves of oregano (*Origanum*
629 *vulgare*), thyme (*Thymus vulgaris*), sage (*Salvia officinalis*), rosemary (*Rosmarinus*
630 *officinalis*), basil (*Ocimum basilicum*) and marjoram (*Origanum majorana*), while dried
631 petals were employed in the case of marigold (*Calendula officinalis*) extraction. All plant
632 matrixes were ground in a cooled mill and were sieving to 200-600 µm of particle size.

633 The extraction cell was loaded with 0.50-0.55 kg of vegetal matter. The extractor pressure
634 was 30 MPa and temperature of the extraction cell and separators was maintained at 40°C.
635 CO₂ flow rate was 60 g/min and extraction was carried out for 5 h. Fractionation of the
636 extracted material was accomplished by setting the pressure of the first separator (S1) to 10
637 MPa, while the second separator (S2) was maintained at the recirculation system pressure (5
638 MPa). The same extraction conditions were applied for all plant varieties. A comparison of
639 the extraction yield, fractionation behavior and essential oil composition was established.

640 The essential oil compounds of samples were determined by GC-MS-FID using 7890A
641 System (Agilent Technologies, U.S.A.), as described previously [67]. The essential oil
642 substances were identified by comparison with mass spectra from library Wiley 229.

643 Table 4 shows the extraction yield (mass extracted / mass loaded in the extraction cell x 100)
644 obtained in the separators S1 and S2 for all plant matrix processed. The lower overall
645 extraction yields were achieved for basil, thyme and marjoram ($\approx 2\%$) while higher yields
646 were obtained for the rest of plants. Oregano is the only raw material for which extraction
647 yield was significantly higher in S1 than in S2. As mentioned before, this behavior in oregano
648 supercritical extraction was previously explained by the high amounts of waxes co-extracted
649 when high extraction pressures were employed [76]. For the rest of plant matrix, similar
650 extraction yields were achieved both in S1 and S2 (rosemary and marigold) or S2 yields were
651 higher than S1 yields (sage, thyme, basil and marjoram).

652 Table 5 present the essential oil composition of the different fractions collected (S1 and S2
653 samples) in terms of the percentage of total area identified in the GC-MS analysis. Figures 8
654 and 9 show, respectively, the chromatogram obtained for basil and marigold extracts.

655 Total chromatographic area quantified in the GC analysis allowed an estimation of the
656 percentage of essential oil compounds recovered in S2 fractions, with respect to the total
657 essential oil recovered in S1 and S2 fractions. As can be observed in Table 4, almost all
658 essential oil substances were recovered in S2 fraction ($> 70\%$) for all plant matrixes studied.
659 That is, on-line fractionation was a suitable technique to achieve the isolation of the plant
660 essential oil in the second separator.

661 Furthermore, it can be stated in general that although the amounts of essential oil compounds
662 recovered in S1 were rather lower than those recovered in S2, the essential oil compositions
663 (% area of identified compounds) of both fractions were quite similar (see Table 5). That is,
664 differences between both fractions were more quantitative than qualitative. Some exceptions
665 were the larger % area of linalool observed in basil S2 fraction with respect to basil S1
666 sample, the high % area of a non-identified compound (NI in Table 5) present in thyme S1
667 extract, and the larger concentrations of 1,8 cineole observed in sage and rosemary S1
668 samples in comparison with the corresponding S2 samples.

669 According to the results given in Table 5, some common substances such as linalool,
670 sabinene, terpineol and caryophyllene were found in all samples in different concentrations.
671 High concentrations of sabinene were found only in oregano and marjoram, linalool in
672 marigold and basil, and caryophyllene in rosemary. Hydrocarbon monoterpenes (pinene,
673 camphene, cymene, and limonene) were found in low % area in oregano, thyme, sage and
674 rosemary. Further, in the case of marigold, marjoram and basil these substances were not
675 detected. As expected, thyme and oregano extracts were the ones with the larger

676 concentrations of thymol and carvacrol. Also, high amounts of 1,8 cineole, borneol and
677 camphor were found in rosemary and sage. The content of borneol and camphor were,
678 respectively, 3 and 5 times higher in rosemary, while the content of 1,8 cineole was around
679 2.5 times higher in sage.

680

681 **Conclusion**

682 Essential oils of plants and herbs are important natural sources of bioactive substances and
683 SFE is an innovative, clean and efficient technology to produce them. The lipophilic
684 character of the substances comprising essential oils guarantees high solubility in CO₂ at
685 moderate temperatures and pressures. Further, the use of polar cosolvents is not necessary
686 and the subsequent processing for solvent elimination is not required. The low processing
687 temperatures result in non-damaged products, with superior quality and better biological
688 functionality. Higher extraction pressures produce the co-extraction of substances with lower
689 solubilities and fractionation alternatives allow the recovery of different products with
690 different composition and biological properties. More recent studies revealed the ultrasound
691 assisted supercritical extraction may increase both extraction rate and yield.

692 These favorable features in the production of supercritical essential oils from plants gained
693 commercial application in the recent decades and a wide variety of products are available in
694 the market at present. Moreover, the increasing scientific evidence which links essential oil
695 components with favorable effects on human diseases, permit to predict an increase of the
696 application of supercritical fluid technology to extract and isolate these substances from plant
697 matrix, with the consequent application in the production of functional foods, nutraceuticals
698 and pharmacy products.

699

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704 1469).

705 **References**

- 706 [1] L. K. Chao, K. F. Hua, H. Y. Hsu, S. S. Cheng, J. Y. Liu, S. T. Chang, *J. Agr. Food*
707 *Chem.* 53 (2005) 7274.
- 708 [2] T. Gornemann, R. Nayal, H. H. Pertz, M. F. Melzig, *J. Ethnopharmacol.* 117 (2008)
709 166.
- 710 [3] L. Jirovetz, G. Buchbauer, I. Stoilova, A. Stoyanova, A. Krastanov, E. Schmidt, *J.*
711 *Agr. Food Chem.* 54 (2006) 6303.
- 712 [4] N. Mimica-Dukic, B. Bozin, M. Sokovic, N. Simin, *J. Agr. Food Chem.* 52 (2004)
713 2485.
- 714 [5] C. I. G. Tuberoso, A. Kowalczyk, V. Coroneo, M. T. Russo, S. Dessì, P. Cabras, *J.*
715 *Agr. Food Chem.* 53 (2005) 10148.
- 716 [6] C. Anitescu, V. Doneanu, Radulescu, *Flavour Fragr. J.* 12 (1997) 173.
- 717 [7] E. Reverchon, I. De Marco. *J. Supercritic. Fluid.* 38 (2006) 146.
- 718 [8] S.M. Pourmortazavi, S.S. Hajimirsadeghi. *J. of Chromatography A* 1163 (2007) 2.
- 719 [9] M. Herrero, A. Cifuentes, E. Ibañez. *Food Chem.* 98 (2006) 136.
- 720 [10] C. G. Pereira, M. A. A. Meireles, *Food Bioprocess. Technol.* 3 (2010) 340.
- 721 [11] E. Vági, B. Simándi, Á. Suhajda, É. Héthelyi. *Food Res. Int.* 38 (2005) 51.
- 722 [12] R. N. Jr. Carvalho, L. S. Moura, P. T. V. Rosa, M. A. A. Meireles. *J. Supercrit. Fluid.*
723 35 (2005) 197.
- 724 [13] M. C. Díaz-Maroto, I. J. Díaz-Maroto Hidalgo, E. Sánchez-Palomo, M. S. Pérez-
725 Coello. *J. Agr. Food Chem.* 53 (2005) 5385.
- 726 [14] S. B. Glisic, D. R. Misic, M. D. Stamenic, I. T. Zizovic, R. M. Asanin, D. U. Skala,
727 *Food Chem.* 105 (2007) 346.
- 728 [15] C. Raeissi, C. J. Peters, *J. Supercrit. Fluid.* 33 (2005) 115.
- 729 [16] C. Raeissi, C. J. Peters, *J. Supercrit. Fluid.* 35 (2005) 10.
- 730 [17] H. Sovová, R. P. Stateva, A. A. Galushko, *J. Supercrit. Fluid.* 20 (2001) 113.
- 731 [18] R. B. Gupta, J. J. Shim, *Solubility in supercritical carbon dioxide.* CRC Press, Taylor
732 and Francis Group New York, USA. 1st Edition. 2007.
- 733 [19] C. G. Pereira, I. P. Gualtieri, N. B. Maia, M. A. A. Meireles, *J. Agr. Sci. Technol.* 35
734 (2008) 44.
- 735 [20] M.E. Napoli, G. Curcuruto, G. Ruberto, *J. Agr. Sci. Technol.*, 35 (2010) 44.
- 736 [21] O. Y. Celiktas, E. Bedir, F. Vardar Sukan, *Food Chem.* 101 (2007) 1457.
- 737 [22] P. J. Hidalgo, J. L. Ubera, M. T. Tena, M. Valcarcel, *J. Agr. Food Chem.* 46 (1998)
738 2624.

- 739 [23] C. Chyau, S. Tsai, J. Yang, C. Weng, C. Han, C. Shih, J. Mau, *Food. Chem.* 100
740 (2007) 808.
- 741 [24] F. Gironi, M. Maschietti, *Chem. Eng. Sci.* 63 (2008) 651.
- 742 [25] F. Benvenuti, F. Gironi, L. Lamberti, *J. Supercrit. Fluid.* 20 (2001) 29.
- 743 [26] S. Espinosa, S. Diaz, E. A. Brignole, *Lat. Am. Appl. Res.* 35 (2005) 321.
- 744 [27] M. Suhaj, *J. Food Compos Anal.* 19 (2006) 531.
- 745 [28] R. Murga, M. T. Sanz, S. Beltran, J. L. Cabezas, *J. Supercrit. Fluid.* 23 (2002) 113.
- 746 [29] R. Murga, M. T. Sanz, S. Beltran, J. L. Cabezas, *J. Supercrit. Fluid.* 27 (2003) 239.
- 747 [30] J. Shia, G. Mittal, E. Kimb, S. J. Xue, *Food Rev. Int.* 23 (2007) 341.
- 748 [31] A. S. Teja, V. S. Smith, T. S. Sun, J. Mendez-Santiago, *Solids Deposition in Natural*
749 *Gas Systems; Research Report, GPA (GAs processor association) Project 171 (2000)*
750 *905.*
- 751 [32] M. Elgayyar, F. A. Draughon, D. A. Golden, J. R. Mount, *J. of Food Protection.* 64
752 (2001) 1019.
- 753 [33] M. Sokovic, O. Tzakou, D. Pitarokili, M. Couladis, *Mol. Nutr. Food Res.* 46 (2002)
754 317.
- 755 [34] S. Kokkini, R. Karousou, A. Dardioti, N. Krigas, T. Lanaras, *Phytochem.* 44 (1997)
756 883.
- 757 [35] M. Puertas-Mejia, S. Hillebrand, E. Stashenko, P. Winterhalter, *Flavour Fragr. J.* 17
758 (2002) 380-384.
- 759 [36] M. T. Baratta, H. G. D. Dorman, S. G. Deans, A. C. Figueiredo, J. G. Barroso, G.
760 Ruberto, *Flavour Fragr. J.* 13 (1998) 235.
- 761 [37] M. Charai, M. Mosaddak, M. Faid, *J. Essent. Oil Res.* 8 (1996) 657.
- 762 [38] M. R. A. Rodrigues, E. B. Caramão, J. G. dos Santos, C. Dariva, J. V. Oliveira, J.
763 *Agric. Food Chem.* 51 (2003) 453.
- 764 [39] M. E. Komaitis, *Food Chem.* 45 (1992)117.
- 765 [40] M. B. Hossain, C. Barry-Ryan, A. B. Martin-Diana, N. P. Bruton, *Food Chem.* 126
766 (2011) 339.
- 767 [41] Z. P. Zeković, Ž. D. Lepojević, S. G. Milošević. A. Š. Tolić, *Acta Periodica*
768 *Technologica APTEFF* 34 (2003) 1.
- 769 [42] B. Simandi, V. Hajdu, K. Peredi, B. Czukor, A. Nobik-Kovacs, A. Kery, *Eur. J. Lipid*
770 *Sci. Tech.* 103 (2001) 355.
- 771 [43] V. Prakash, *Leafy Spices*, CRC Press, Boca Raton, Florida, 1990.
- 772 [44] L. Bravo, J. Cabo, A. Revert, A. Villar. *ARS Pharmacology.* 3 (1975) 345.

- 773 [45] C. M. Priestley, E. M. Williamson, K. A. Wafford, D. B. Sattelle, *Brit. J. Pharmacol.*
774 140 (2003) 1363.
- 775 [46] B. D. Mookherjee, R. A. Wilson, R. W. Trenkle, M. J. Zampino, K. P. Sands, R.
776 Teranishi, R.G. Buttery, F. Shahidi, *Flavor Chemistry: Trends and Developments*,
777 ACS Symposium Series, Washington (1989) 176.
- 778 [47] S. E. Kintzios, *Sage – the genus salvia*. Amsterdam: Harwood Academic, 2000.
- 779 [48] E. K. Perry, A. T. Pickering, W. W. Wang, P. J. Houghton, N. S L. Perry, *J. Pharm.*
780 *Pharmacol.* 51 (2005) 527.
- 781 [49] S. A. Aleksovsk, H. Sovová, *J. Supercrit. Fluid.* 40 (2007) 239.
- 782 [50] E. Reverchon, R. Taddeo, G. Della Porta, *J. Supercrit. Fluid.* 8 (1995) 302.
- 783 [51] A. Bisio, G. Romussi, G. Ciarallo, N. de Tommasi, *Pharmazie* 52 (1997) 330.
- 784 [52] J. R. Chipault, J. M. Hawkins, W. O. Lundberg, *Food Res.* 17 (1952) 46.
- 785 [53] M. Wang, J. Li, M. Rangarajan, Y. Shao, E. J. LaVoie, T. C. Huang, *J. Agr. Food*
786 *Chem.* 46 (1998) 4869.
- 787 [54] S. Cavero, L. Jaime, P. J. Martín-Alvarez, F. J. Señoráns, G. Reglero, E. Ibáñez, *Eur.*
788 *Food Res. Technol.* 221 (2005) 478.
- 789 [55] G. Vicente, M.R. García-Risco, T. Fornari, G. Reglero, *Chem. Eng. Technol.* 35
790 (2012) 176.
- 791 [56] Y. Zaouali, T. Bouzaine, M. Boussaid, *Food Chem. Toxicol.* 48 (2010) 3144.
- 792 [57] A. Szumny, A. Figiel, A. Gutierrez-Ortiz, A. A. Carbonell-Barrachina, *J. Food Eng.*
793 97 (2010) 253.
- 794 [58] M. E. Napoli, G. Curcuruto, G. Ruberto, *Biochem. Syst. and Ecol.* 38 (2010) 659.
- 795 [59] B. Vanaclocha, S. Cañigüeral. *Fitoterapia. Vademécum de prescripción*. Editorial
796 Elsevier, Barcelo, España, 4th ed. 2003.
- 797 [60] O. Politeo, M. Jukic, M. Milos, *Food Chem.* 100 (2007) 374.
- 798 [61] A. I. Hussain, F. Anwar, S. Tufail, H. Sherazi, R. Przybylski, *Food Chem.* 108 (2008)
799 986.
- 800 [62] M. C. Díaz-Maroto, M. S. Pérez-Coello, M. D. Cabezudo, *J. Chromatogr. A.* 947
801 (2002) 23.
- 802 [63] A. Menaker, M. Kravets, M. Koel, A. Orav, *C. R. Chimie.* 7 (2004) 629.
- 803 [64] S. J. Lee, K. Umamo, T. Shibamoto, K. G. Lee, *Food Chem.* 91 (2005) 131.
- 804 [65] Y. Yang, B. Kayan, N. Bozer, B. Pate, C. Baker, A. M. Gizir, *J. Chromatogr. A.* 1152
805 (2007) 262.

- 806 [66] R. Eggers, S.K. Voges, Ph.T. Jaeger, Solid bed properties in supercritical processing,
807 in: I. Kikic, M. Perrut (Eds.), Proceedings of the 9th Meeting on Supercritical Fluids,
808 Trieste, Italy (2004) E11.
- 809 [67] T. Fornari, A. Ruiz-Rodriguez, G. Vicente, E. Vázquez, M. R. García-Risco, G.
810 Reglero, J. Supercritic. Fluid. 64 (2012) 1.
- 811 [68] E. Bako, J. Deli, G. Toth, J. Biochem. Biophys. Methods 53 (2002) 241.
- 812 [69] J-C. Chalchat, M. M. Ozcan. Food Chemistry 110 (2008) 501-503.
- 813 [70] E. Oca, A. Ibanez, G. Murga, S.L.d. Sebastian, J. Tabera, G. Reglero, J. Agric. Food
814 Chem. 47 (1999) 1400.
- 815 [71] M. R. García-Risco, E. J. Hernández, G. Vicente, T. Fornari, F. J. Señorans, G.
816 Reglero. J. Supercrit. Fluid. 55 (2011) 971.
- 817 [72] B. Damjanovic, A. Tolic, Z. Lepojevic, Proceedings of the 8th Conference on
818 Supercritical Fluids and Their Applications, ISASF, Nancy, France (2006) 125.
- 819 [73] M. Meghwal, T. K. Goswami, Continental J. Food Science and Technology 4 (2010)
820 24.
- 821 [74] P. Masango, J. Clean. Prod. 13 (2005) 833.
- 822 [75] K.K. Singh, T.K. Goswami, Studies on cryogenic grinding of spices. IIT Kharagpur,
823 India (1997).
- 824 [76] B. Simandi, M. Oszagyan, E. Lemberkovics, A. Kery, J. Kaszacs, F. Thyron, T.
825 Matyas, Food Res. Inter. 31 (1998) 723.
- 826 [77] M. Hamburger, S. Adler, D. Baumann, A. Förg, B. Weinreich, Fitoterapia. 14 (2003)
827 328.
- 828 [78] L. Danielski. L. M. A. S. Campos, L. F. V. Bresciani, H. Hense, R. A. Yunes, S. R. S.
829 Ferreira, Chem. Eng. Process. 46 (2007) 99.
- 830 [79] P. F. Leal, N. B. Maia, Q. A. C. Carmello, R. R. Catharino, M. N. Eberlin, M. A. A.
831 Meireles, Food Bioprocess. Technol. 1 (2008) 326.
- 832 [80] M. Hamburger, D. Baumann, S. Adler, Phytochem. Anal. 15 (2004) 46.
- 833 [81] E. Reverchon, J. Supercrit. Fluid. 5 (1992) 256.
- 834 [82] E. Reverchon, G. Della Porta, J. Supercrit. Fluid. 9 (1996) 199.
- 835 [83] G. Della Porta, S. Porcedda, B. Marongiu, E. Reverchon, Flavour Fragr. J. 14 (1999)
836 214.
- 837 [84] K. Vilku, R. Mawson, L. Simons, D. Bates, Innov. Food Sci. Emerg., 9 (2008) 161.
- 838 [85] S. Albu, E. Joyce, L. Paniwnyk, J.P. Lorimer, T.J. Mason. Ultrasonics Sonochemistry
839 11 (2004) 261.

- 840 [86] F. Chemat, Zill-e-Huma, M. K. Khan. *Ultrasonics Sonochemistry* 18 (2011) 813.
- 841 [87] M. Vinatoru, *Ultrason. Sonochem.* 8 (2001) 301.
- 842 [88] E. Riera, A. Blanco, J. García, J. Benedito, A. Mulet, J. A. Gallego-Juárez, M. Blasco.
- 843 *Physics Procedia* 3 (2010) 141.
- 844 [89] S. Balachandran, S.E. Kentish, R. Mawson, M. Ashokkumar, *Ultrason. Sonochem.* 13
- 845 (2006) 471.
- 846 [90] K. Sugiyama, M., Saito, T. Hondo, M. Senda, *J. Chromatography A*, 32 (1985) 107.
- 847 [91] M. Saito, Y. Yamauchi, T. Okuyama, *Fractionation by packed column SFC and SFE.*
- 848 *Principals and applications.* VCH Publishers INC. New York, 1994.
- 849 [92] N. Sugihara, A. Kanda, T. Nakano, T. Nakamura, H. Igusa, S. Hara. *J. .Oleo Sci.* 59
- 850 (2010) 65.
- 851 [93] T. Bamba, E. Fukusaki, *J. Sep. Sci.* 32 (2009) 2699.
- 852 [94] Y. Yamauchi, M. Saito, *J. Chromatography A*, 505 (1990) 237.
- 853 [95] C. Desmortreux, M. Rothaupt, C. West, E. Lesellier, *J. Chromatography A* 1216
- 854 (2009) 7088.
- 855 [96] P. Ramírez, M. García-Risco, S. Santoyo, F. J. Señorans, E. Ibañez, G. Reglero, J.
- 856 *Pharmaceut. Biomed.* 41 (2006) 1606.
- 857 [97] P. Ramírez, T. Fornari, F. J. Señorans, E. Ibañez, G. Reglero, *J. Supercrit. Fluid.* 35
- 858 (2005) 128.
- 859 [98] M. R. García-Risco, G. Vicente, T. Fornari and G. Reglero. *J. Supercrit. Fluid.* 55
- 860 (2011) 949.
- 861 [99] E. E. Stashenko, B. E. Jaramillo, J. R Martinez, *J. Chromatography A*, 1025 (2004)
- 862 93.
- 863 [100] M. E. M. Braga, P. A. D. Ehlert, L. C. Ming, M. A. A. Meireles, *J. Supercrit. Fluid.*
- 864 34 (2005) 149.
- 865 [101] V. M. Rodrigues, P. T. V. Rosa, M. O. M. Marques, A. J. Petenate, M. A. A.
- 866 Meireles, *J. Agr. Food Chem.* 51 (2003) 1518.
- 867 [102] E. Ghasemi, Y. Yamini, N. Bahramifar, F. Sefidkon, *J. Food Eng.* 79 (2007) 306.
- 868 [103] R. N. Patel, S. Bandyopadhyay, A. Ganesh, *Biores. Technol.* 97 (2006) 847.
- 869 [104] P. Kotnik, M. Škerget, K Knez, *J. Supercrit. Fluid.* 43 (2007) 192.
- 870 [105] G. Wenqiang, L. Shufen, Y. Ruixiang, T. Shaokun, Q. Can, *Food Chem.* 101 (2007)
- 871 1558.
- 872 [106] J. Ivanovica, I. Zizovica, M. Ristic, M. Stamenica, D. Skalaa, *J. Supercrit. Fluid.* 55
- 873 (2011) 983.

874 [107] C. Grosso, V. Ferraro, A. C. Figueiredo, J. B. Barroso, J. A. Coelho, A. M. Palavra,
875 Food Chem. 111 (2008) 197.

876 [108] J. C. Francisco, E. P. Jarvenpaa, R. Huopalahti, B. Sivik, J. Agric. Food Chem. 49
877 (2001) 2339.

878 [109] H. Kazazi, K. Rezaei, S. Javad, G. Sharif, Z. Emam-Djomeh, Y. Yamini, Food Chem.
879 105 (2007) 805–811.

880 [110] A. Caredda, B. Marongiu, S. Porcedda, C. Soro. J. Agric. Food Chem. 50 (2002)
881 1492.

882 [111] C. Da Porto, D. Decorti, I. Kikic, Food Chem. 112 (2009) 1072.

883 [112] P. F. Leal, C. L. Queiroga, M. V. N. Rodrigues, I. Montanari, A.M.A. Meireles,
884 Pharmacognosy Magazine, 2 (2006) 153.

885 [113] E. Ghasemi, F. Raofie, N. M. Najafi. Food Chem. 126 (2011) 1449.

886 [114] L. Danielski , L. M.A.S. Campos, L. F.V. Bresciani , H. Hense , R. A. Yunes , S. R.S.
887 Ferreira. Chem. Eng. Process. 46 (2007) 99.

888 [115] Z. Zekovic, Z. Lepojevic, D. Adamovic, I. Mujic, S. Milic, Extraction rate constants
889 of menthe SFE by CO₂. In: Proceedings of the Eighth Conference on Supercritical
890 Fluids and Their Applications, Ischia, Italy (2006) 95.

891 [116] N. Aghel, Y. Yamini, A. Hadjiakhoondi, S.M. Pourmortazavi, Talanta 62 (2004) 407.

892 [117] S. R. S. Ferreira, Z. L. Nikolov, L. K. Doraiswamy, M. A. M. Meireles, A. J. Petenate,
893 J. Supercrit. Fluid. 14 (1999) 235.

894 [118] S. Glisic, J. Ivanovica, M. Ristic, D. Skalaa, J. Supercrit. Fluid. 52 (2010) 62.

895 [119] Y. Yamini, M. Khajeh, E. Ghasemi, M. Mirza, K. Javidnia, Food Chem. 108 (2008)
896 341–346

897 [120] G. Della Porta, R. Taddeo, E. D’Urso, E. Reverchon. Lebensm.- Wiss. U.-Technol. 31
898 (1998) 454.

899 [121] M. Moldao-Martins, A. Palavra, M.L. Beirao da Costa, M.G. Bernardo-Gil. J.
900 Supercrit. Fluid. 18 (2000) 25.

901 [122] I. Zizovic, M. Stamenic, J. Ivanovic, A. Orlovic, M. Ristic, S. Djordjevic, S.D.
902 Petrović, D. Skala, J. Supercrit. Fluid. 43 (2007) 249.

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906 **Table 1.** SFE of different plants and herbs to produce essential oils.

Raw material	Botanical name	Main constituents of essential oil	References
Anise verbena	<i>Lippia alba</i>	carvone, limonene, elemol, γ -muurolene, guaiol, bulnesol	[99, 100]
Aniseed	<i>Pimpinella anisum</i>	anethole, γ -himachalene, p-anisaldehyde, methylchavicol, cis-pseudoisoeugenyl 2-methylbutyrate, trans-pseudoisoeugenyl 2-methylbutyrate	[101]
Artemisa	<i>Artemisia sieberi</i>	camphene, 1,8 cineol, γ -terpinene, chrysanthenone, camphor, cis-chrysanthenone	[102]
Basil leaves	<i>Ocimum basilicum</i>	linalool, methyl-eugenol, 1,8 cineole, α -bergamotene, α -cadinene	[63]
Cashew	<i>Anacardium occidentale</i>	cardanol, cardol, dimethylanacardate	[103]
Chamomile	<i>Chamomilla recutita</i>	matricine, chamazulene, bisabolol	[104]
Clove	<i>Eugenia caryophyllata Thunb</i>	eugenol, caryophyllene, eugenol acetate	[105, 106]
Coriander	<i>Coriandrum sativum</i>	linalool, γ terpinene, camphor, geranyl acetate, α pinene, geraniol, limonene	[107]
Eucalyptus	<i>Eucalyptus camaldulensis Dehnh.</i>	1,8 cineole, a-pinene, β -pinene, terpinen-4-ol, allo-alomandrene, globulol	[108]
Fennel	<i>Foeniculum vulgare Mill.</i>	trans-anetole, methyl chavicol, fenchone	[72]
Hyssop	<i>Hyssopus officinallis</i>	sabibebem iso-pinocamphene, pinocamphene	[109]
Laurel leaves	<i>Laurus nobilis</i>	1,8 cineole, linalool, α -terpinylacetate, methyleugenol	[110]
Lavender	<i>Lavandula angustifolia</i>	linalool, camphor, borneol, terpinen-4-ol, linalyl acetate, oxygenated monoterpenes, oxygenated sesquiterpenes	[111]
Macela	<i>Achyrocline alata, A. satureioides</i>	trans-caryophyllene, α -humulene	[112]
Myrtus	<i>Myrtus communis</i>	α -pinene, Limonene, 1,8 cineole	[113]
Marigold	<i>Calendula officinalis</i>	acetyl eugenol, guaiol	[114]
Marjoram	<i>Origanum majorana</i>	4-terpineol, p-cymene, carvacrol, sabinene hydrate	[38]
Mint	<i>Mentha spicata insularis</i>	L-menthone, isomenthone, menthol, cis-b-terpineole, menthylacetate, trans β -caryophyllene, germacrene-D	[115]
Oregano	<i>Origanum vulgare</i>	carvacrol, tymol, sabinene hydrate, p-cypeme, linalool	[77, 106]
Pennyroyal	<i>Mentha pulegium</i>	menthone, pulegone, limonene.	[116]
Pepper black	<i>Piper nigrum</i>	3- γ -carene, limonene, β -caryophilene, sabinene	[117]
Rosmarinus	<i>Rosemary officianlis</i>	camphor, 1,8 cineole, borneol, linalool	[12, 55]
Sage	<i>Salvia officinalis</i>	1,8-cineole, camphor, β -thujone	[118]
	<i>Salvia mirzayanii</i>	linalyl acetate, 1,8 cineol, linalool, 8-acetoxy linalool	[119]
Star anise	<i>Illicium anisatum</i>	trans-anethole, limonene, chavicol, anisaldehyde	[120]
Thyme	<i>Thymus vulgaris</i>	thymol, carvacrol, camphor, linalool	[98]
	<i>Thymus Zygis</i>	thymol, carvacrol, linalool, borneol	[121]
Valerian	<i>Valeriana officinalis</i>	bornyl acetate, cis- α -copaene-8-ol, valerianol	[122]

908 **Table 2.** Comparison of the content of some common volatile oil compounds identified in oregano,
909 sage and thyme extracts produced with pure CO₂ at 30 MPa and 40°C [67].

910

Compound <i>i</i>	ratio between the content of compound <i>i</i> in the different matrixes	
	oregano/thyme	sage/thyme
1,8 Cineole	-	8.42
Sabinene hydrate	203.3	0.79
Linalool	0.91	0.07
Camphor	-	8.47
Borneol	-	0.43
α -terpineol	20.31	0.84
Linalyl acetate	-	-
Thymol	1.63	-
Carvacrol	7.58	-
E-caryophyllene	6.98	0.53

911

912 **Table 3.** Effect of cosolvent in the supercritical extraction of rosemary leaves.

913

	Extraction A	Extraction B	B / A
	30 MPa, 40°C, no cosolvent	15 MPa, 40°C and 5% ethanol	
	g compound / g leaves x 100		
1,8 Cineole	0.386	0.444	1.15
Camphor	0.132	0.227	1.72
Borneol	0.049	0.070	1.43
Bornyl Acetate	0.011	0.018	1.61
Carnosic acid	0.492	1.863	3.78
Carnosol	0.047	0.277	5.83

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915

916 **Table 4.** Supercritical extraction (30 MPa, 40°C, no cosolvent) and fractionation (S1: 10
917 MPa, S2: 5 MPa) of different plants from *Lamiaceae* family: extraction yield (mass extract /
918 mass plant matrix x 100) and percentage of essential oil recovered in S2 separator (total GC
919 area in S2 / total GC area in S1 + S2 x 100).

920

plant matrix	extraction yield		% essential oil in S2
	S1	S2	
oregano	3.18	1.59	88.4
sage	1.39	3.23	77.4
thyme	0.91	1.70	71.6
rosemary	1.77	1.75	71.2
basil	0.21	1.75	97.7
marjoram	0.30	1.73	77.9
marigold	2.35	2.20	100.0

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Table 5. Essential oil composition (% area of GC-MS analysis) of the S1 and S2 fractions obtained in the SFE (30 MPa and 40°C) of different plants from *Lamiaceae* family. NI: non-identified compound.

Tr	Compuesto	Marigold		Marjoran		Basil		Oregano		Thyme		Sage		Rosemary	
		S1	S2	S1	S2	S1	S2	S1	S2	S1	S2	S1	S2	S1	S2
6.28	α -Pinene	-	-	-	-	-	-	-	-	-	-	-	-	0.58	0.24
6.85	Camphene	-	-	-	-	-	-	-	-	-	-	0.06	-	0.26	0.14
8.3	1-octen-3-ol	-	-	-	-	-	-	-	0.06	0.23	0.03	-	-	0.04	0.11
8.85	β -Pinene	-	-	-	-	-	-	-	0.15	-	-	0.10	0.05	0.11	0.08
9.48	α -Phellandrene	-	-	-	-	-	-	-	-	-	-	-	-	-	0.05
10.54	M-Cymene	-	-	-	-	-	-	1.00	0.91	0.13	0.05	0.05	0.03	0.75	0.48
10.75	Limonene	-	-	-	-	-	-	-	0.25	-	-	0.25	0.13	0.37	0.28
10.88	1,8 Cineole	-	1.84	-	-	0.24	5.75	-	0.09	0.58	0.05	11.66	4.51	54.51	38.30
12.89	Sabinene hydrate trans	-	1.35	6.91	7.41	0.11	0.68	2.19	3.00	0.91	0.14	0.91	0.85	-	-
14.67	Sabinene hydrate cis	-	4.32	36.40	37.00	0.33	0.71	38.25	36.32	0.51	0.13	0.43	0.48	-	0.06
14.91	Linalool	-	10.73	2.76	2.49	4.78	27.81	1.95	1.74	3.25	0.54	1.34	1.47	1.06	1.24
17.25	Camphor	-	0.59	-	-	-	0.66	0.28	0.15	1.21	0.14	48.17	39.29	21.23	18.07
18.5	Borneol	-	-	-	-	0.77	0.44	0.61	0.25	3.26	0.96	9.10	12.78	4.86	10.00
19.29	1-terpinene-4-ol	-	5.17	13.33	12.81	0.57	1.62	2.16	4.66	0.64	0.14	0.73	0.95	1.21	1.71
19.85	P- Cymen-8-ol	-	-	-	-	-	-	-	-	0.16	-	0.11	0.24	0.11	0.19
20.1	α -Terpineol	-	4.42	8.86	8.10	2.98	3.03	2.32	2.61	0.43	-	1.45	2.44	5.40	9.85
21.12	Verbenone	-	-	0.93	0.89	-	0.06	-	0.17	-	-	-	0.20	-	-
23.84	Terpinene-4-acetate	-	-	15.85	16.20	-	-	0.83	1.32	-	-	-	-	-	-
25.6	Bornyl acetate	-	-	-	-	0.20	0.02	-	0.20	-	-	3.87	4.26	0.08	0.73
26.2	Myrtenyl acetate	-	-	-	-	-	-	-	-	-	-	6.57	7.94	-	-
26.31	thymol	-	-	-	-	-	-	35.73	30.27	73.58	69.62	-	-	-	0.12
26.46	Carvacrol	-	-	1.99	1.74	-	-	11.77	12.51	5.12	5.19	-	-	-	0.24
29.7	α -Terpineol acetate	-	-	-	-	-	-	-	-	-	-	4.45	5.89	-	-
30.3	Eugenol	-	12.11	0.99	0.88	41.28	24.76	-	-	-	-	-	-	-	0.33
31.12	Ylangene	-	-	-	-	-	-	-	-	-	-	-	-	-	0.19
31.4	Copaene	-	-	-	-	-	-	-	-	-	-	0.40	0.57	0.49	0.82
32.05	Acid Cinamic methyl ester	-	7.80	-	0.59	20.70	11.36	-	-	-	-	-	-	-	-
34.5	Caryophyllene	-	1.31	5.13	4.99	0.52	0.80	1.61	2.48	2.73	0.61	3.22	4.75	6.81	10.51
36.1	α -Bergamatone	-	6.63	1.24	1.10	9.38	12.27	-	-	-	-	-	-	-	0.03
36.83	NI	-	-	-	-	-	-	0.35	0.24	2.94	20.63	-	-	-	-
37.2	α -Caryophyllene	-	-	-	-	0.51	0.73	-	0.19	-	-	2.22	3.29	0.71	1.40
42.5	γ -cadinene	-	21.37	-	-	12.05	7.34	-	0.46	0.56	-	0.48	0.90	-	1.29
43.5	δ -Cadinene	-	22.36	-	-	-	-	-	0.14	0.58	0.33	0.88	2.19	1.18	2.53
48.12	Spathulenol	-	-	5.62	5.80	5.58	1.98	0.94	1.29	0.32	-	2.05	4.11	-	-
48.48	Caryophyllene Oxide	-	-	-	-	-	-	-	0.51	2.86	1.43	1.52	2.70	0.25	1.02

Figure caption

Figure 1. Isoprene (C₅H₈) chemical structure.

Figure 2. Chemical structure of some popular constituents of essential oil of plants and herbs: (a) limonene; (b) citral; (c) menthol; (d) linalool; (e) carvacrol; (f) α -pinene; (g) sabinene; (h) camphor; (i) valerenic acid.

Figure 3. Solubility in supercritical CO₂ of several constituents of plant matter. Essential oil compounds: (*) limonene, (-) α -pinene and (\diamond) linalool [18]; phenolic compounds: (O) protocatechuic acid [28], (Δ) methyl gallate [28] and (\square) *p*-cumaric acid [29]; pigments: (■) β -carotene [18]; waxes: (\blacktriangle) *n*-C₂₈H₅₈ [31]. Temperature range: 35-50°C.

Figure 4. Typical SFE scheme for the extraction of plant matrix. P1: CO₂ pump; P2: cosolvent pump; HE1, HE2, HE3: heat exchangers; EV: extraction vessel; S1, S2: separator cells; V, V1, V2: back pressure regulator valves; ST: CO₂ storage tank; F: filter.

Figure 5. Supercritical CO₂ extraction (30 MPa and 40°C) of oregano (\square), sage (■), thyme (Δ) and rosemary (\blacktriangle).

Figure 6. Scheme of a Supercritical Fluid Chromatography system.

Figure 7. SFC chromatogram of thyme supercritical extract produced by SFE at 15 MPa, 50°C and 3% ethanol co-solvent). (A) Injections carried out at 5 mg/ml; (B) Injections carried out at 20 mg/ml. F1, F2 and F3 indicate the intervals of time employed to collect the different fractions in the SFC semi-preparative system.

Figure 8. Chromatograms obtained by GC-MS analysis of basil supercritical extract produced by SFE at 30 MPa and 40°C: (a) S1 fraction; (b) S2 fraction.

Figure 9. Chromatograms obtained by GC-MS analysis of marigold supercritical extract produced by SFE at 30 MPa and 40°C (S2 fraction).

Figure 1.

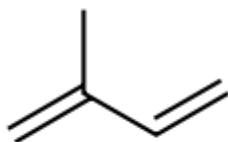
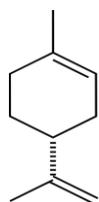
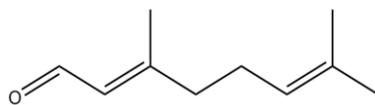


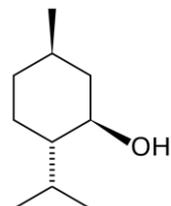
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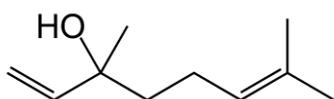
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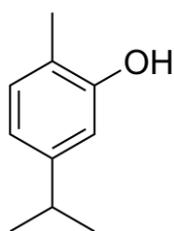
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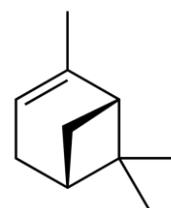
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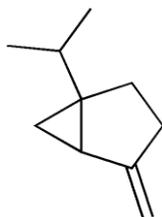
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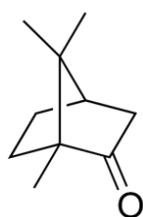
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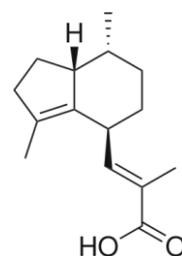
(f)



(g)



(h)



(i)

Figure 4.

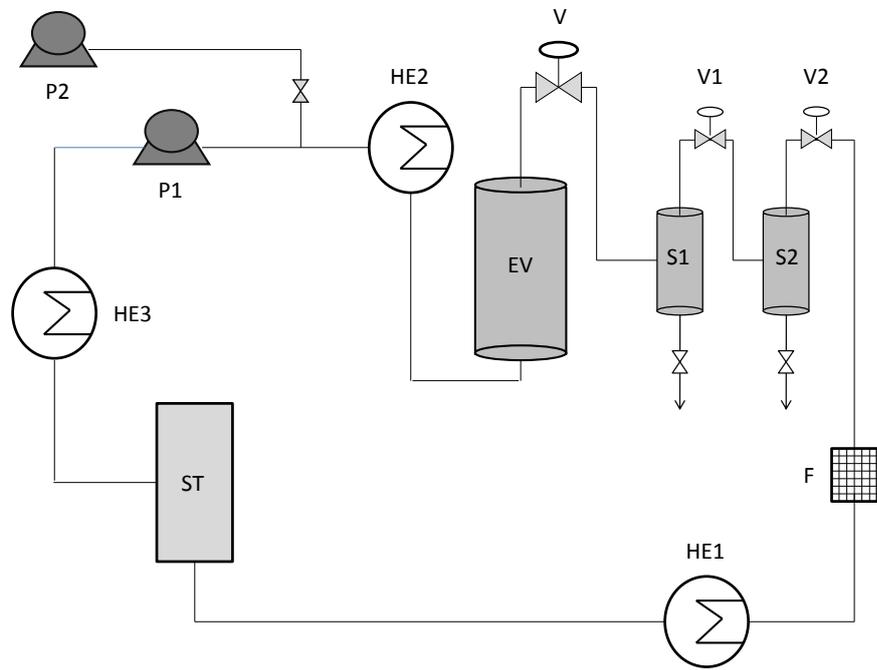


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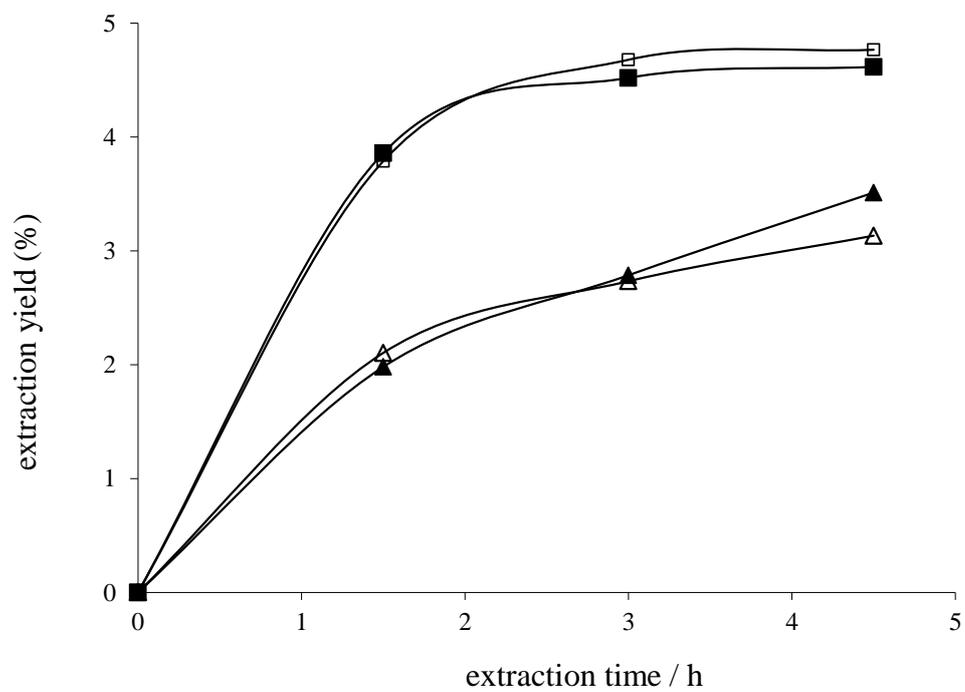


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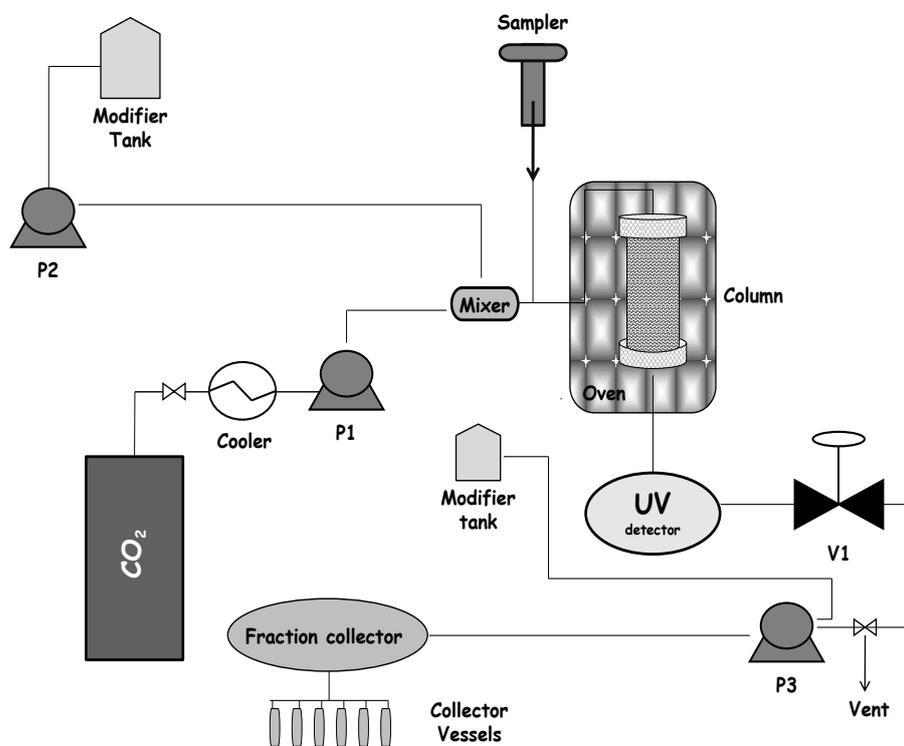


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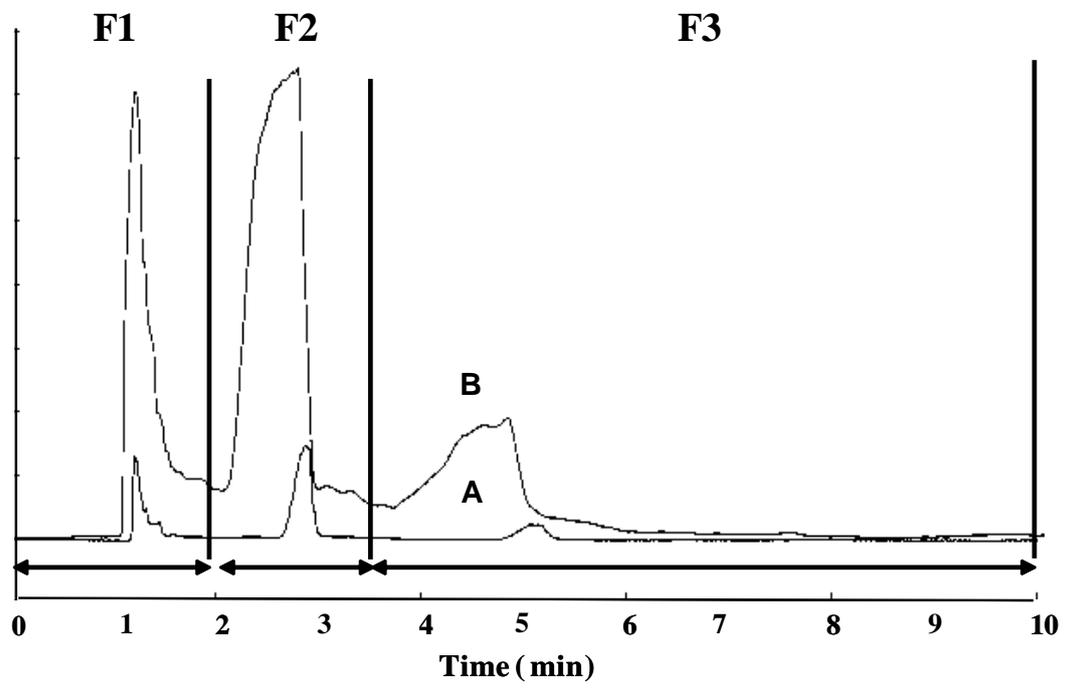


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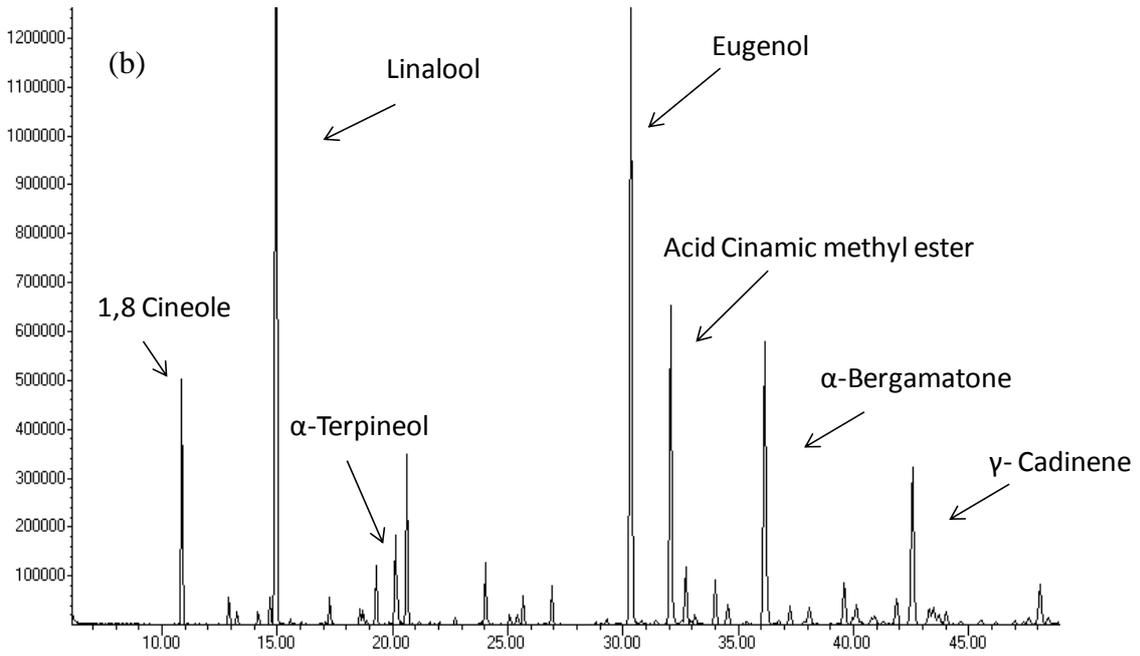
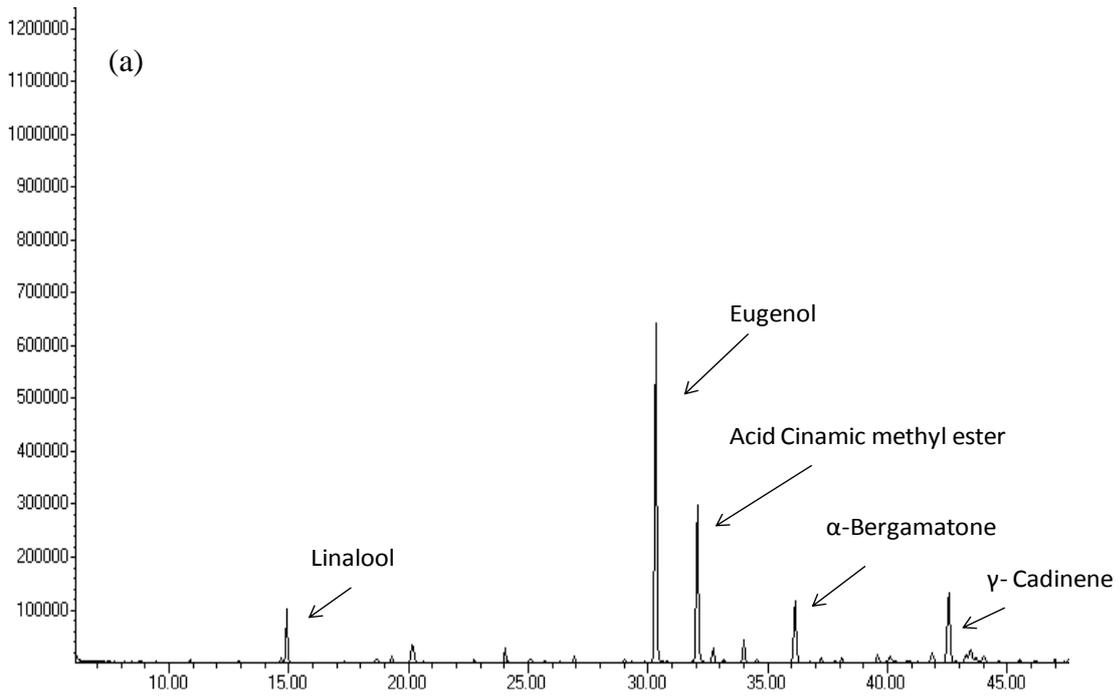


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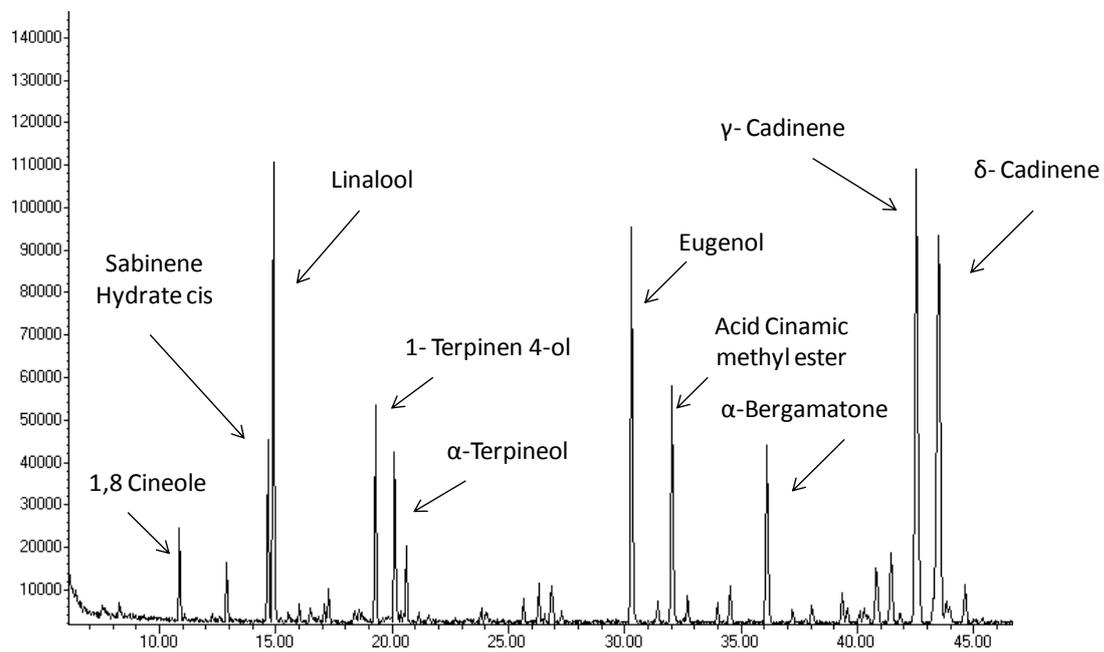


Figure 1

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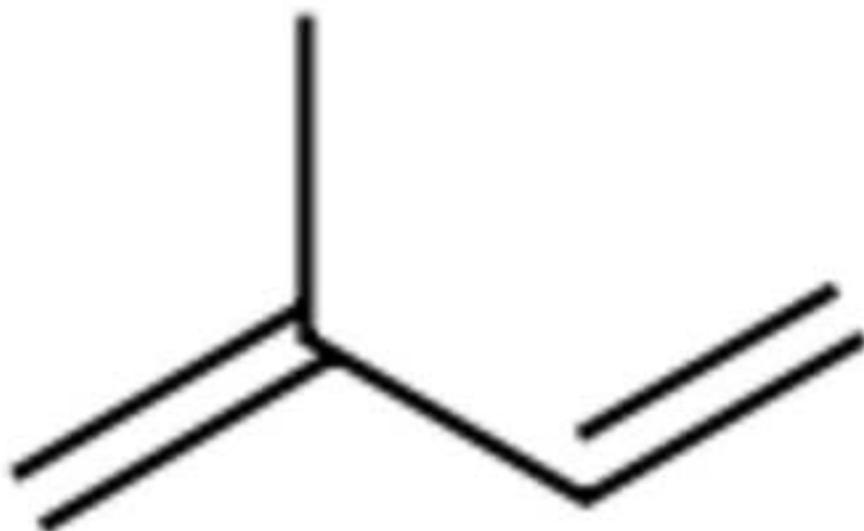
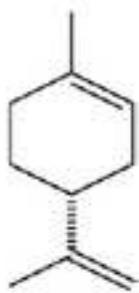
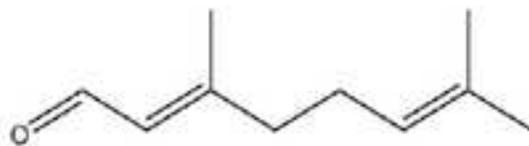


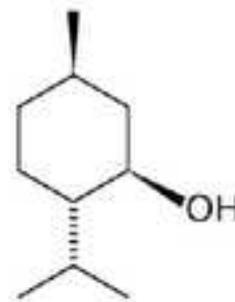
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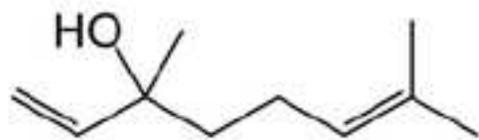
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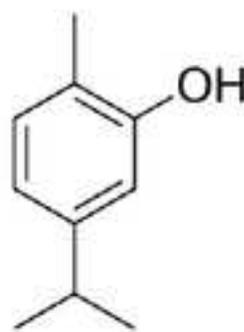
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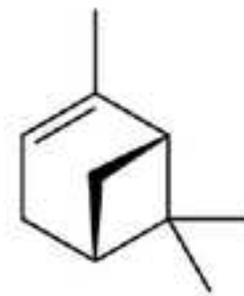
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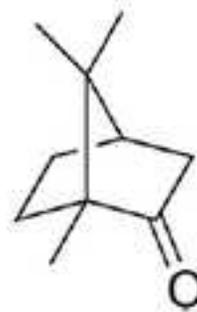
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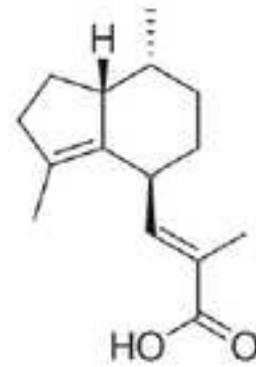
(f)



(g)



(h)



(i)

Figure 3
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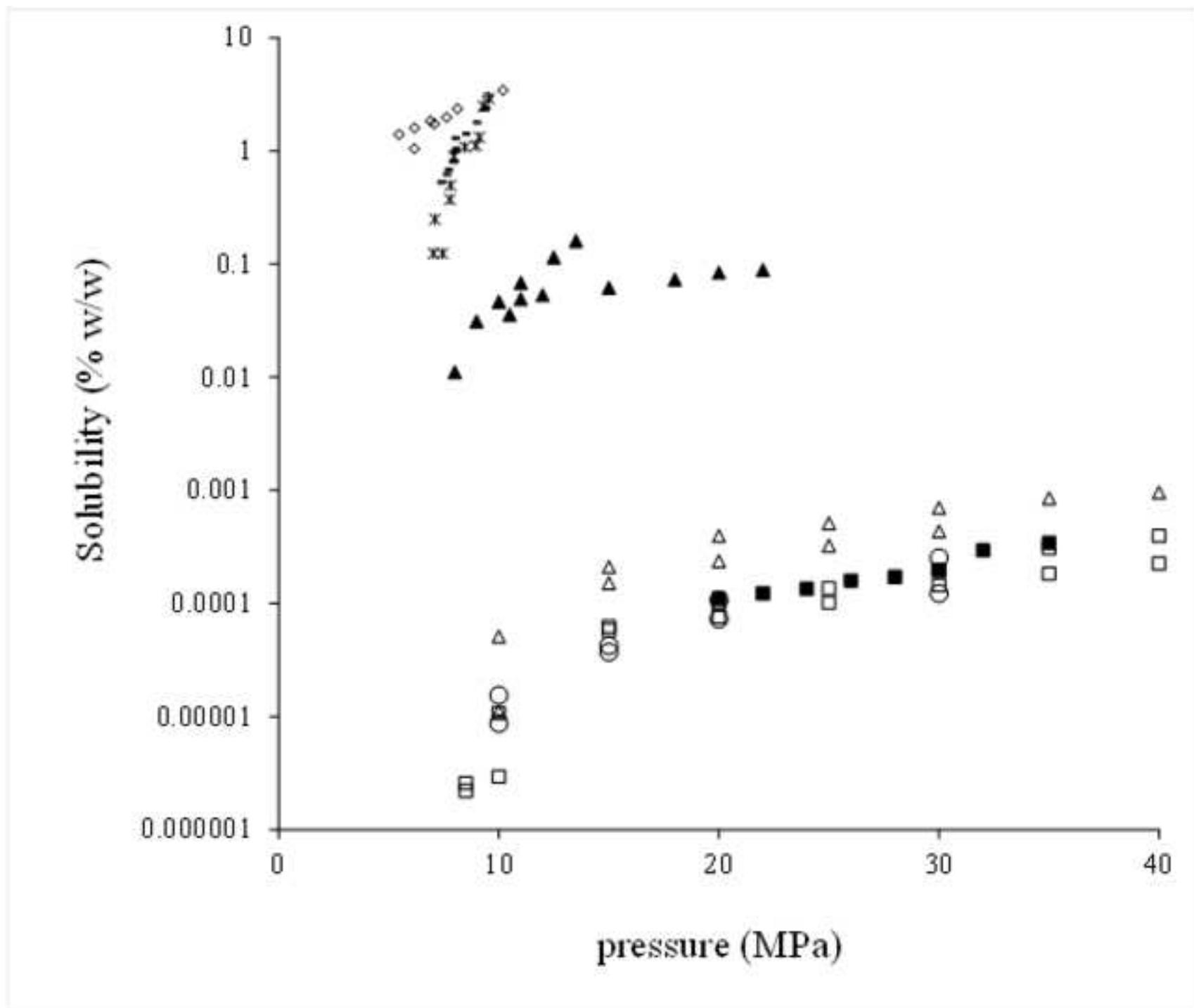


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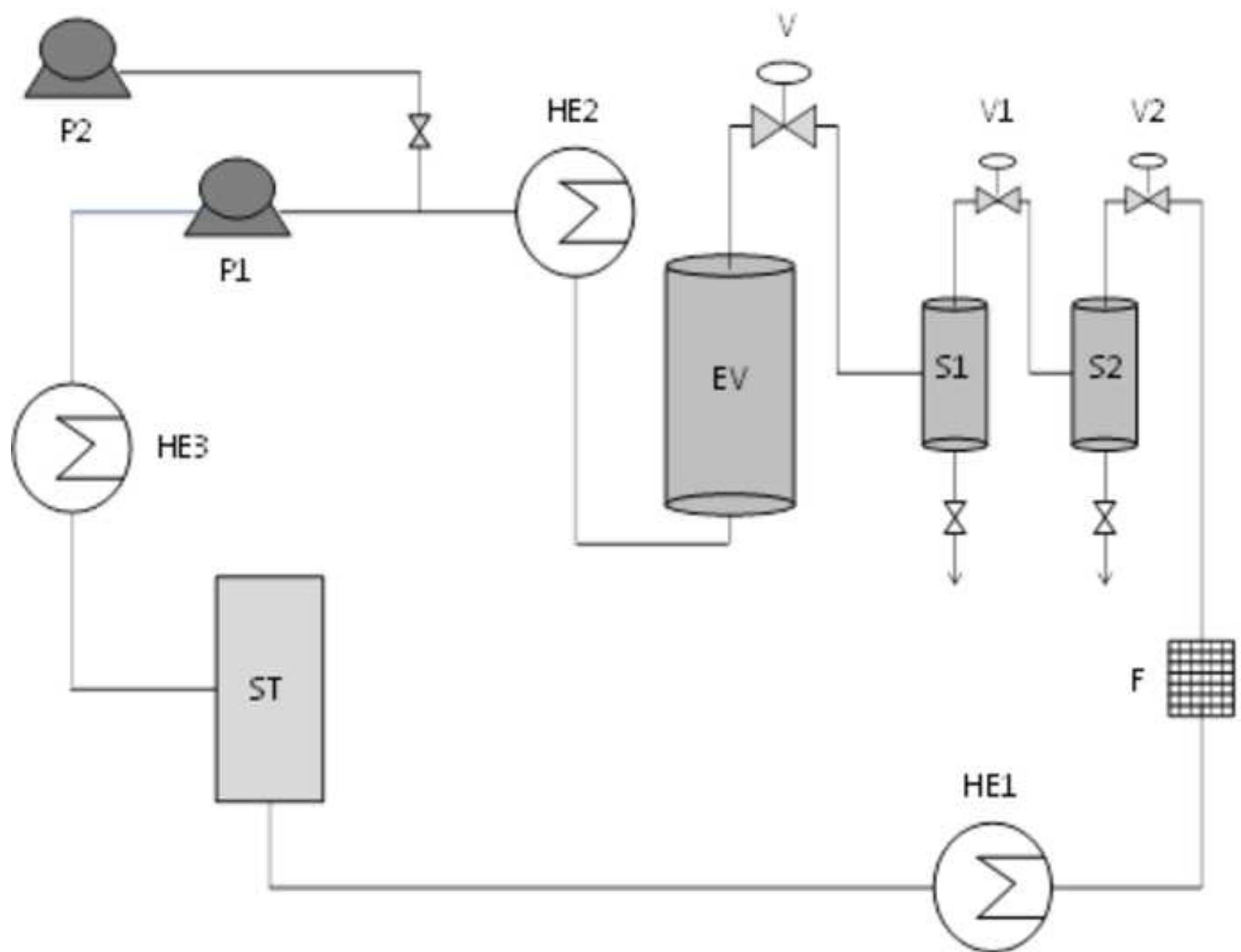


Figure 5
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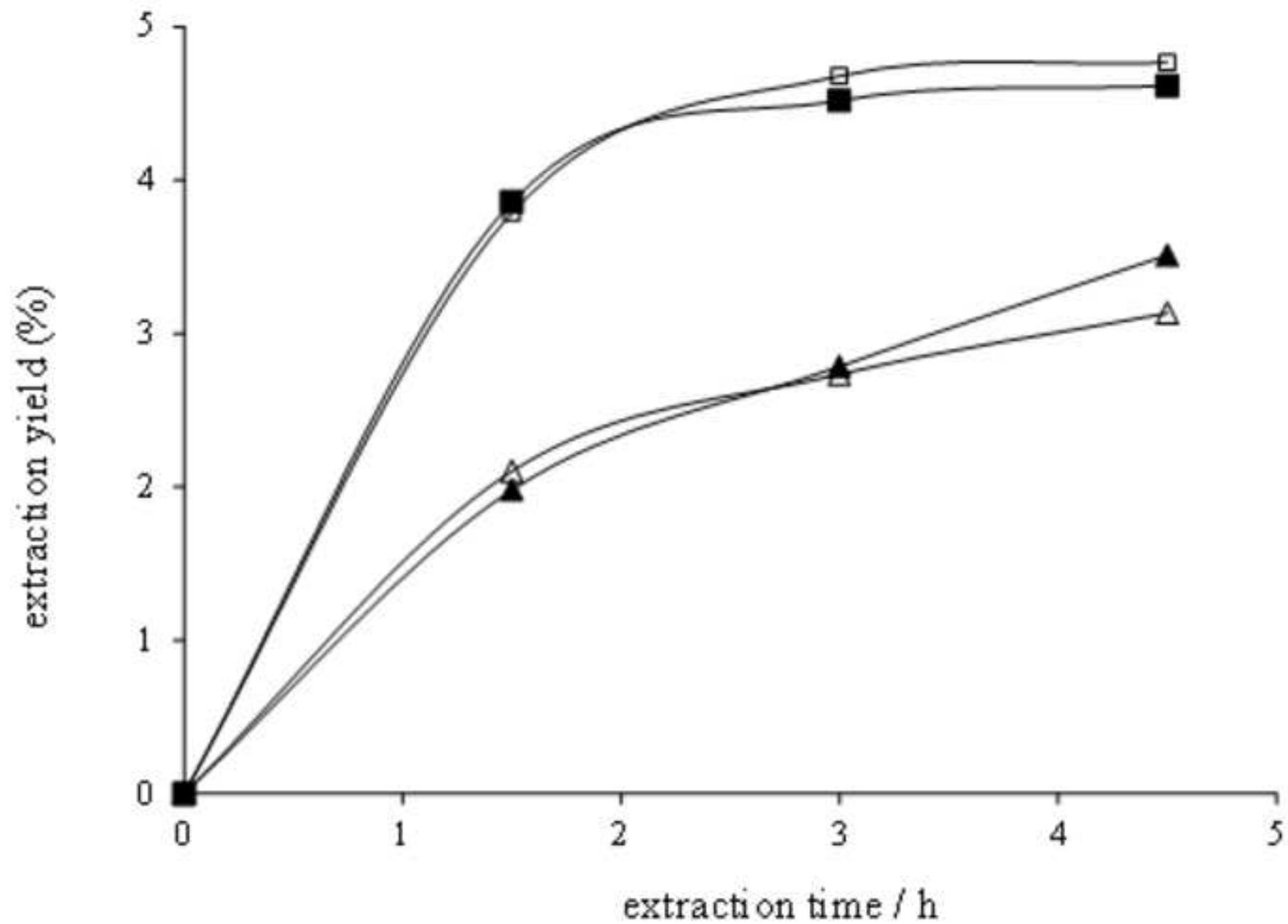


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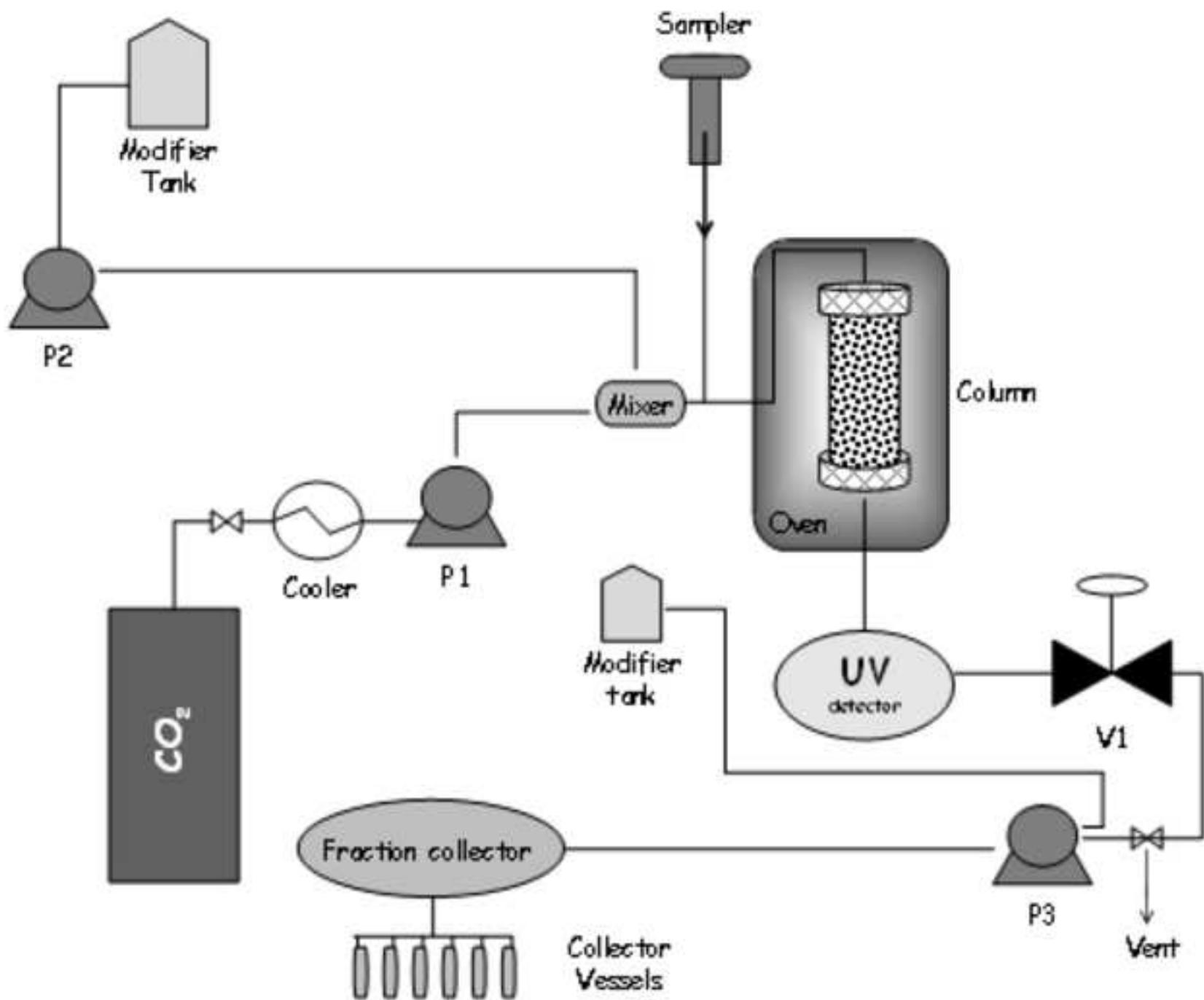


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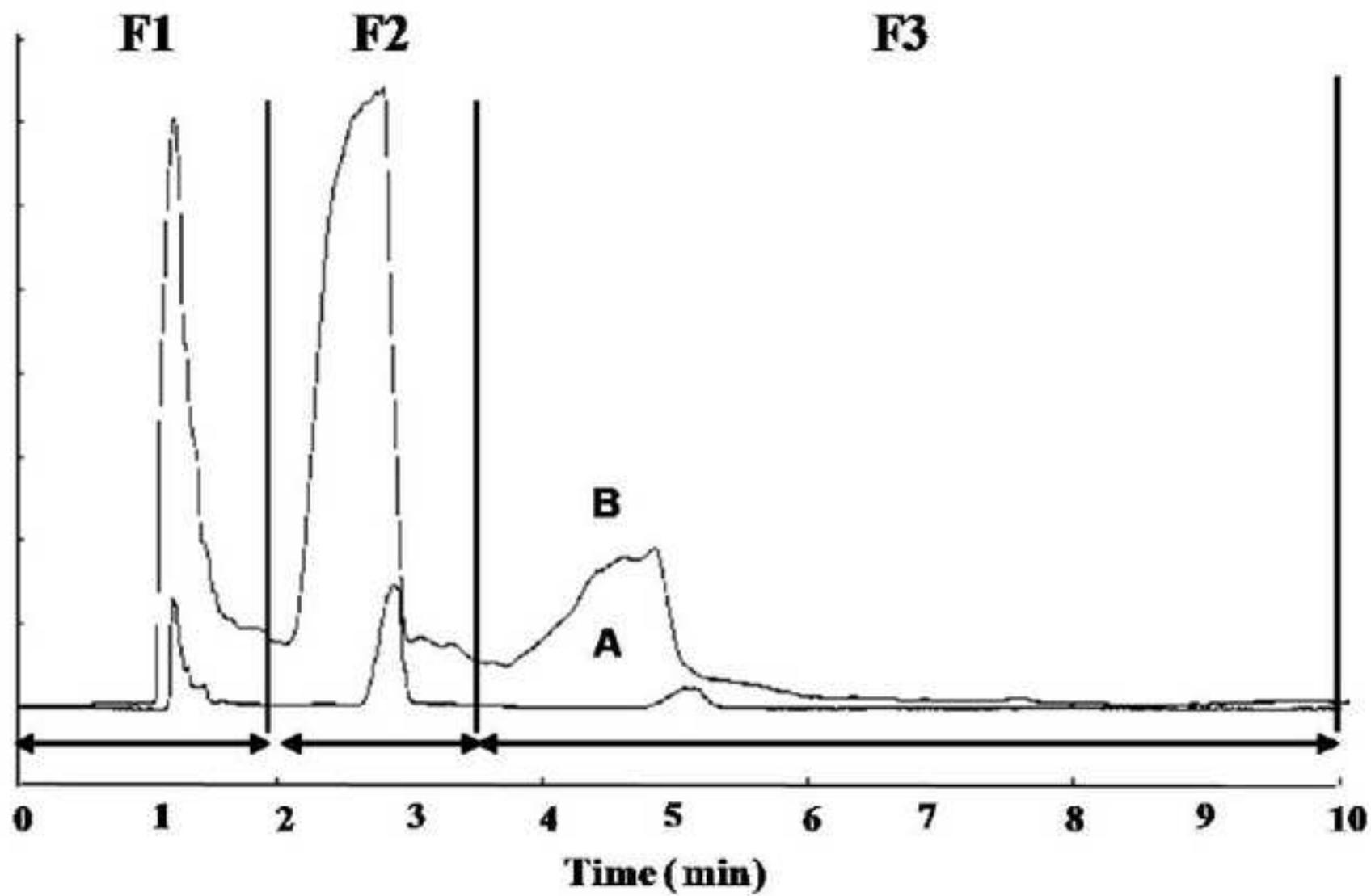


Figure 8

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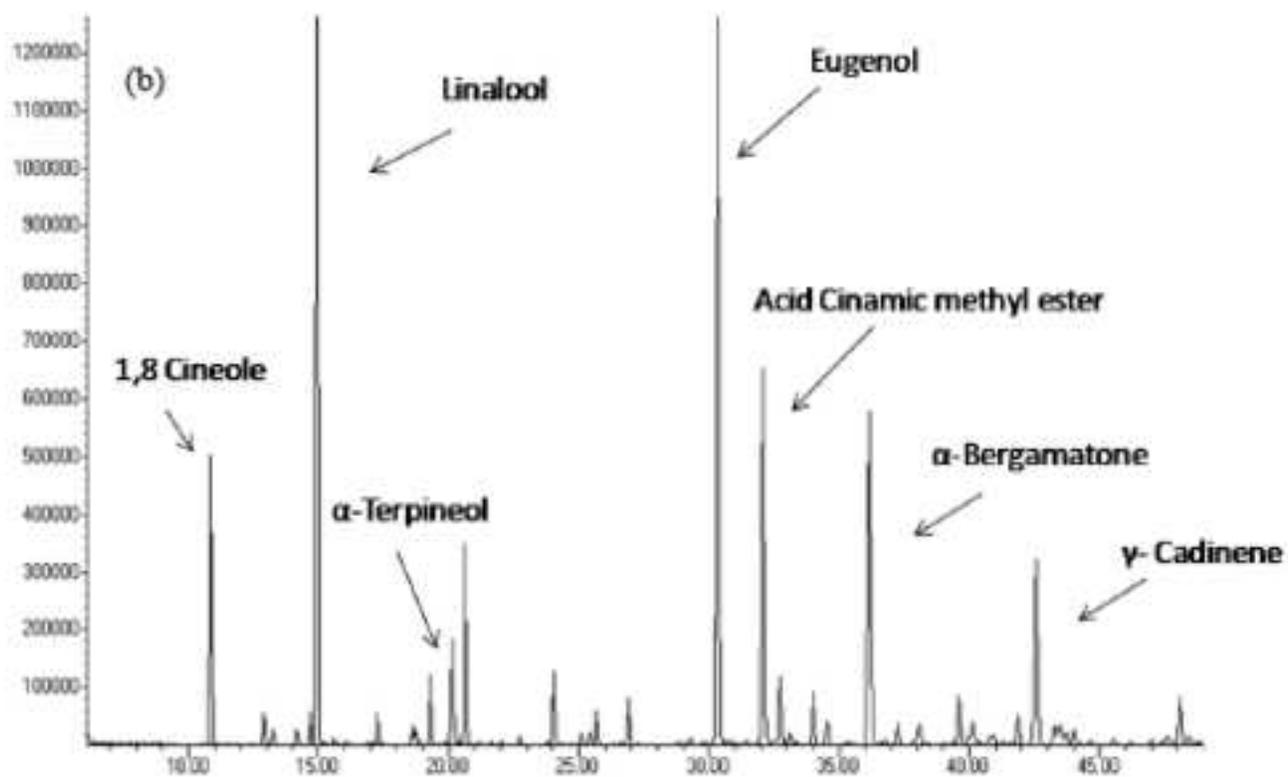
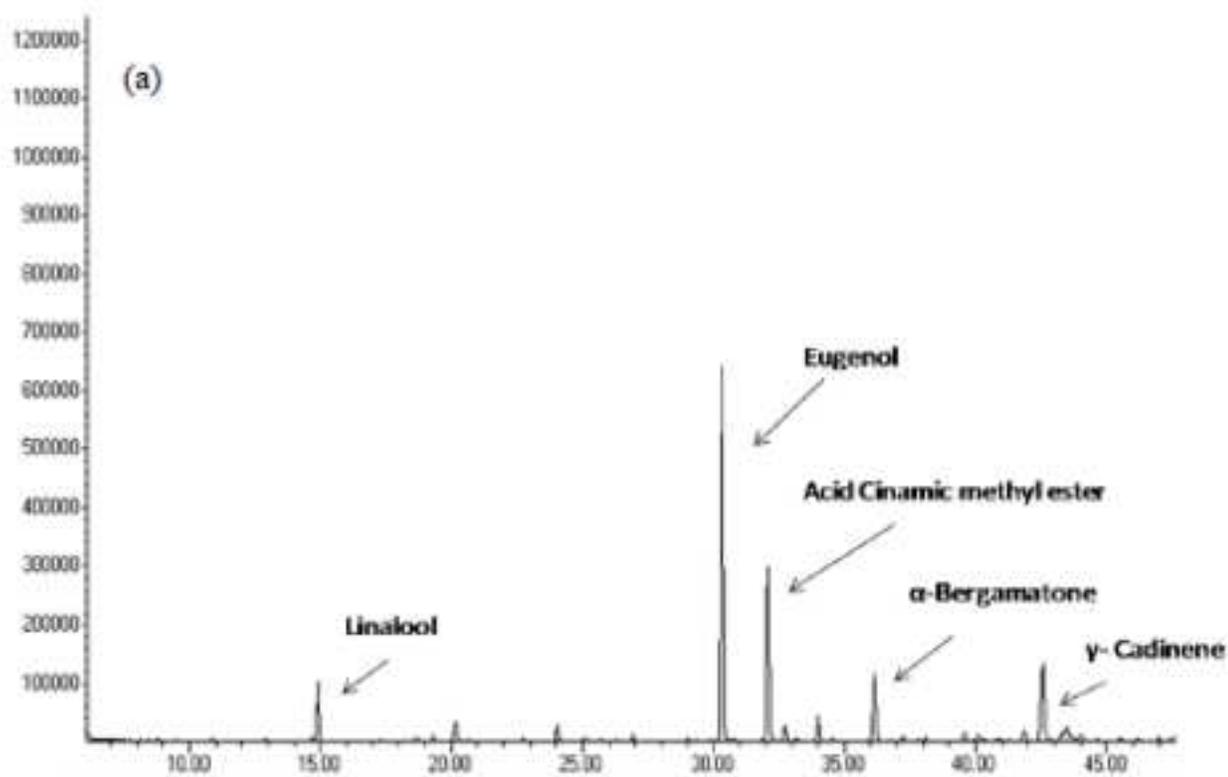


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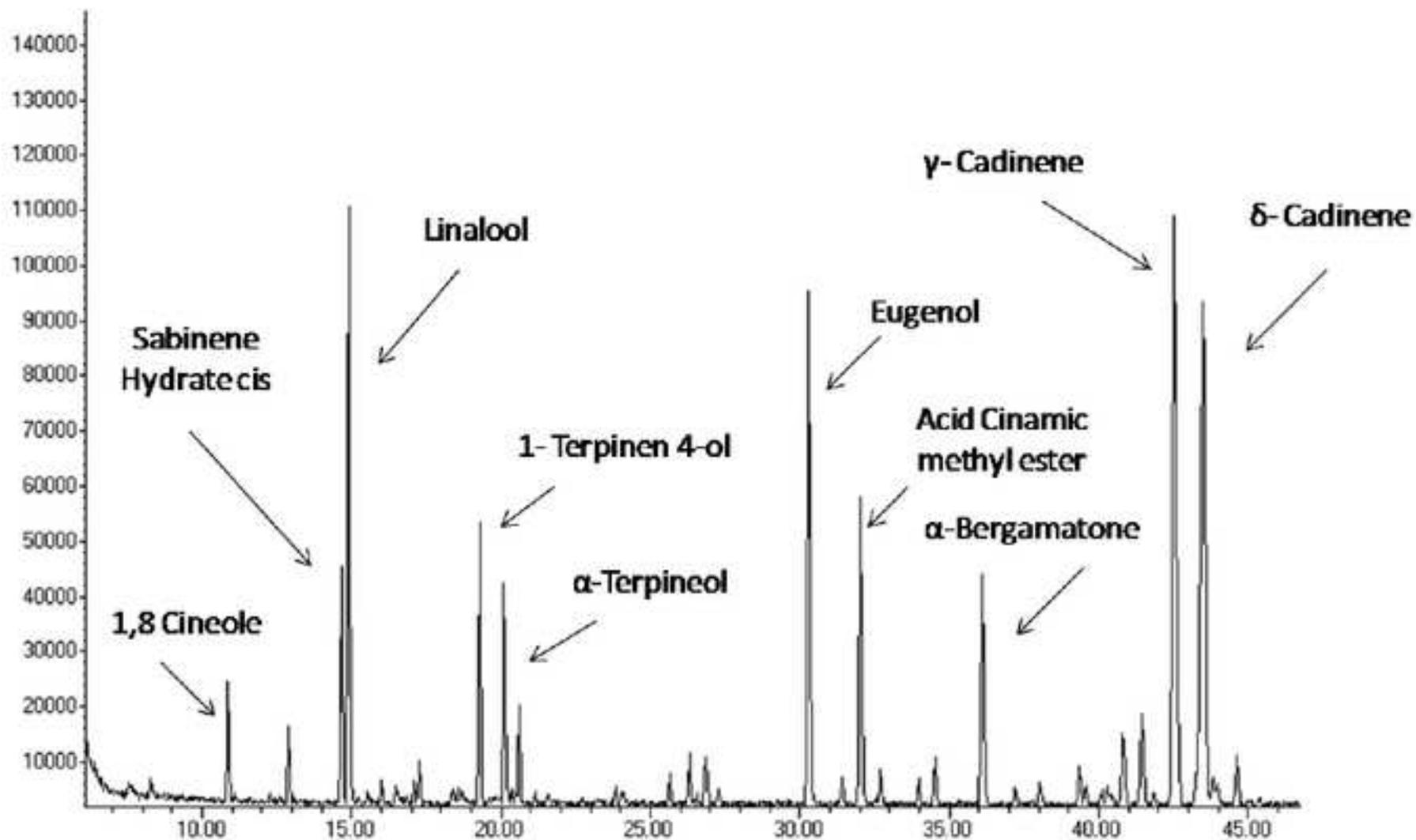


Table 1. SFE of different plants and herbs to produce essential oils.

Raw material	Botanical name	Main constituents of essential oil	References
Anise verbena	<i>Lippia alba</i>	carvone, limonene, elemol, γ -muurolene, guaiol, bulnesol	[99, 100]
Aniseed	<i>Pimpinella anisum</i>	anethole, γ -himachalene, p-anisaldehyde, methylchavicol, cis-pseudoisoeugenyl 2-methylbutyrate, trans-pseudoisoeugenyl 2-methylbutyrate	[101]
Artemisa	<i>Artemisia sieberi</i>	camphene, 1,8 cineol, γ -terpinene, chrysanthenone, camphor, cis-chrysanthenone	[102]
Basil leaves	<i>Ocimum basilicum</i>	linalool, methyl-eugenol, 1,8 cineole, α -bergamotene, α -cadinene	[63]
Cashew	<i>Anacardium occidentale</i>	cardanol, cardol, dimethylanacardate	[103]
Chamomile	<i>Chamomilla recutita</i>	matricine, chamazulene, bisabolol	[104]
Clove	<i>Eugenia caryophyllata Thunb</i>	eugenol, caryophyllene, eugenol acetate	[105, 106]
Coriander	<i>Coriandrum sativum</i>	linalool, γ terpinene, camphor, geranyl acetate, α pinene, geraniol, limonene	[107]
Eucalyptus	<i>Eucalyptus camaldulensis Dehnh.</i>	1,8 cineole, a-pinene, β -pinene, terpinen-4-ol, allo-alomandrene, globulol	[108]
Fennel	<i>Foeniculum vulgare Mill.</i>	trans-anetole, methyl chavicol, fenchone	[72]
Hyssop	<i>Hyssopus officinalis</i>	sabibebem iso-pinocamphene, pinocamphene	[109]
Laurel leaves	<i>Laurus nobilis</i>	1,8 cineole, linalool, α -terpinylacetate, methyleugenol	[110]
Lavender	<i>Lavandula angustifolia</i>	linalool, camphor, borneol, terpinen-4-ol, linalyl acetate, oxygenated monoterpenes, oxygenated sesquiterpenes	[111]
Macela	<i>Achyrocline alata, A. satureioides</i>	trans-caryophyllene, α -humulene	[112]
Myrtus	<i>Myrtus communis</i>	α -pinene, Limonene, 1,8 cineole	[113]
Marigold	<i>Calendula officinalis</i>	acetyl eugenol, guaiol	[114]
Marjoram	<i>Origanum majorana</i>	4-terpineol, ρ -cymene, carvacrol, sabinene hydrate	[38]
Mint	<i>Mentha spicata insularis</i>	L-menthone, isomenthone, menthol, cis-b-terpineole, menthylacetate, trans β -caryophyllene, germacrene-D	[115]
Oregano	<i>Origanum vulgare</i>	carvacrol, tymol, sabinene hydrate, p-cypeme, linalool	[77, 106]
Pennyroyal	<i>Mentha pulegium</i>	menthone, pulegone, limonene.	[116]
Pepper black	<i>Piper nigrum</i>	3- γ -carene, limonene, β -caryophilene, sabinene	[117]
Rosmarinus	<i>Rosemary officianlis</i>	camphor, 1,8 cineole, borneol, linalool	[12, 55]
Sage	<i>Salvia officinalis</i>	1,8-cineole, camphor, β -thujone	[118]
	<i>Salvia mirzayanii</i>	linalyl acetate, 1,8 cineol, linalool, 8-acetoxy linalool	[119]
Star anise	<i>Illicium anisatum</i>	trans-anethole, limonene, chavicol, anisaldehyde	[120]
Thyme	<i>Thymus vulgaris</i>	thymol, carvacrol, camphor, linalool	[98]
	<i>Thymus Zygis</i>	thymol, carvacrol, linalool, borneol	[121]
Valerian	<i>Valeriana officinalis</i>	bornyl acetate, cis- α -copaene-8-ol, valerianol	[122]

Table 2. Comparison of the content of some common volatile oil compounds identified in oregano, sage and thyme extracts produced with pure CO₂ at 30 MPa and 40°C [67].

Compound <i>i</i>	ratio between the content of compound <i>i</i> in the different matrixes	
	oregano/thyme	sage/thyme
1,8 Cineole	-	8.42
Sabinene hydrate	203.3	0.79
Linalool	0.91	0.07
Camphor	-	8.47
Borneol	-	0.43
α -terpineol	20.31	0.84
Linalyl acetate	-	-
Thymol	1.63	-
Carvacrol	7.58	-
E-caryophyllene	6.98	0.53

Table 3. Effect of cosolvent in the supercritical extraction of rosemary leaves.

	Extraction A 30 MPa, 40°C, no cosolvent	Extraction B 15 MPa, 40°C and 5% ethanol	B / A
	g compound / g leaves x 100		
1,8 Cineole	0.386	0.444	1.15
Camphor	0.132	0.227	1.72
Borneol	0.049	0.070	1.43
Bornyl Acetate	0.011	0.018	1.61
Carnosic acid	0.492	1.863	3.78
Carnosol	0.047	0.277	5.83

Table 4. Supercritical extraction (30 MPa, 40°C, no cosolvent) and fractionation (S1: 10 MPa, S2: 5 MPa) of different plants from *Lamiaceae* family: extraction yield (mass extract / mass plant matrix x 100) and percentage of essential oil recovered in S2 separator (total GC area in S2 / total GC area in S1 + S2 x 100).

plant matrix	extraction yield		% essential oil in S2
	S1	S2	
oregano	3.18	1.59	88.4
sage	1.39	3.23	77.4
thyme	0.91	1.70	71.6
rosemary	1.77	1.75	71.2
basil	0.21	1.75	97.7
marjoram	0.30	1.73	77.9
marigold	2.35	2.20	100.0

Table 5

Table 5. Essential oil composition (% area of GC-MS analysis) of the S1 and S2 fractions obtained in the SFE (30 MPa and 40°C) of different plants from *Lamiaceae* family. NI: non-identified compound.

Tr	Compuesto	Marigold		Marjoran		Basil		Oregano		Thyme		Sage		Rosemary	
		S1	S2	S1	S2	S1	S2	S1	S2	S1	S2	S1	S2	S1	S2
6.28	α -Pinene	-	-	-	-	-	-	-	-	-	-	-	-	0.58	0.24
6.85	Camphene	-	-	-	-	-	-	-	-	-	-	0.06	-	0.26	0.14
8.3	1-octen-3-ol	-	-	-	-	-	-	-	0.06	0.23	0.03	-	-	0.04	0.11
8.85	β -Pinene	-	-	-	-	-	-	-	0.15	-	-	0.10	0.05	0.11	0.08
9.48	α -Phellandrene	-	-	-	-	-	-	-	-	-	-	-	-	-	0.05
10.54	M-Cymene	-	-	-	-	-	-	1.00	0.91	0.13	0.05	0.05	0.03	0.75	0.48
10.75	Limonene	-	-	-	-	-	-	-	0.25	-	-	0.25	0.13	0.37	0.28
10.88	1,8 Cineole	-	1.84	-	-	0.24	5.75	-	0.09	0.58	0.05	11.66	4.51	54.51	38.30
12.89	Sabinene hydrate trans	-	1.35	6.91	7.41	0.11	0.68	2.19	3.00	0.91	0.14	0.91	0.85	-	-
14.67	Sabinene hydrate cis	-	4.32	36.40	37.00	0.33	0.71	38.25	36.32	0.51	0.13	0.43	0.48	-	0.06
14.91	Linalool	-	10.73	2.76	2.49	4.78	27.81	1.95	1.74	3.25	0.54	1.34	1.47	1.06	1.24
17.25	Camphor	-	0.59	-	-	-	0.66	0.28	0.15	1.21	0.14	48.17	39.29	21.23	18.07
18.5	Borneol	-	-	-	-	0.77	0.44	0.61	0.25	3.26	0.96	9.10	12.78	4.86	10.00
19.29	1-terpinene-4-ol	-	5.17	13.33	12.81	0.57	1.62	2.16	4.66	0.64	0.14	0.73	0.95	1.21	1.71
19.85	P- Cymen-8-ol	-	-	-	-	-	-	-	-	0.16	-	0.11	0.24	0.11	0.19
20.1	α -Terpineol	-	4.42	8.86	8.10	2.98	3.03	2.32	2.61	0.43	-	1.45	2.44	5.40	9.85
21.12	Verbenone	-	-	0.93	0.89	-	0.06	-	0.17	-	-	-	0.20	-	-
23.84	Terpinene-4-acetate	-	-	15.85	16.20	-	-	0.83	1.32	-	-	-	-	-	-
25.6	Bornyl acetate	-	-	-	-	0.20	0.02	-	0.20	-	-	3.87	4.26	0.08	0.73
26.2	Myrtenyl acetate	-	-	-	-	-	-	-	-	-	-	6.57	7.94	-	-
26.31	thymol	-	-	-	-	-	-	35.73	30.27	73.58	69.62	-	-	-	0.12
26.46	Carvacrol	-	-	1.99	1.74	-	-	11.77	12.51	5.12	5.19	-	-	-	0.24
29.7	α -Terpineol acetate	-	-	-	-	-	-	-	-	-	-	4.45	5.89	-	-
30.3	Eugenol	-	12.11	0.99	0.88	41.28	24.76	-	-	-	-	-	-	-	0.33
31.12	Ylangene	-	-	-	-	-	-	-	-	-	-	-	-	-	0.19
31.4	Copaene	-	-	-	-	-	-	-	-	-	-	0.40	0.57	0.49	0.82
32.05	Acid Cinamic methyl ester	-	7.80	-	0.59	20.70	11.36	-	-	-	-	-	-	-	-
34.5	Caryophyllene	-	1.31	5.13	4.99	0.52	0.80	1.61	2.48	2.73	0.61	3.22	4.75	6.81	10.51
36.1	α -Bergamatone	-	6.63	1.24	1.10	9.38	12.27	-	-	-	-	-	-	-	0.03
36.83	NI	-	-	-	-	-	-	0.35	0.24	2.94	20.63	-	-	-	-
37.2	α -Caryophyllene	-	-	-	-	0.51	0.73	-	0.19	-	-	2.22	3.29	0.71	1.40
42.5	γ -cadinene	-	21.37	-	-	12.05	7.34	-	0.46	0.56	-	0.48	0.90	-	1.29
43.5	δ -Cadinene	-	22.36	-	-	-	-	-	0.14	0.58	0.33	0.88	2.19	1.18	2.53
48.12	Spathulenol	-	-	5.62	5.80	5.58	1.98	0.94	1.29	0.32	-	2.05	4.11	-	-
48.48	Caryophyllene Oxide	-	-	-	-	-	-	-	0.51	2.86	1.43	1.52	2.70	0.25	1.02