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

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Abstract

Supercritical fluid extraction (SFE) is an innovative, clean and environmental friendly technology with particular interest for the extraction of essential oil from plants and herbs. Supercritical CO_2 is selective, there is no associated waste treatment of a toxic solvent, and extraction times are moderate. Further supercritical extracts were often recognized of superior quality when compared with those produced by hydro-distillation or liquid-solid extraction.

This review provides a comprehensive and updated discussion of the developments and applications of SFE in the isolation of essential oils from plant matrices. SFE is normally performed with pure CO_2 or using a cosolvent; fractionation of the extract is commonly accomplished in order to isolate the volatile oil compounds from other co-extracted substances. In this review the effect of pressure, temperature and cosolvent on the extraction and fractionation procedure is discussed. Additionally, a comparison of the extraction yield and composition of the essential oil of several plants and herbs from *Lamiaceae* family, namely oregano, sage, thyme, rosemary, basil, marjoram and marigold, which were produced in our supercritical pilot-plant device, is presented and discussed.

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

Contents

1. Introduction

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

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

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

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

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

Among innovative process technologies, supercritical fluid extraction (SFE) is indeed the most widely studied application. In practice, SFE is performed generally using carbon dioxide (CO_2) for several practical reasons: CO_2 has moderately low critical pressure (74 bar) and temperature (32°C), is non-toxic, non-flammable, available in high purity at relatively low cost, and is easily removed from the extract. Supercritical CO_2 has a polarity similar to liquid pentane and thus, is suitable for extraction of lipophilic compounds. Thus, taking into account the lipophilic characteristic of plant essential oils, it is obvious that SFE using CO_2 emerged as a suitable environmentally benign alternative to the manufacture of essential oil products.

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

using supercritical CO_2 (50ºC and 45 MPa) and ethanol Soxhlet extraction. Extraction yields were, respectively, 3.8 and 9.1%. Nevertheless, the supercritical extract comprised 21% of essential oil, while the alcoholic extract contained only 9% of the volatile oil substances. Furthermore, studies related with the antibacterial and antifungal properties of the extract revealed better activity for the supercritical product. Another example of improved biological activity exhibit by supercritical extracts was reported by Glisic et al. [14], demonstrating that supercritical carrot essential oil was much more effective against *Bacillus cereus* than that obtained by hydro-distillation.

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

Knowledge of the solubility of essential oil compounds in supercritical CO_2 is of course necessary, in order to establish favorable extraction conditions. In this respect, several studies have been reported [15-18]. Nevertheless, when the initial solute concentration in the plant is low, as is the case of essential oils, mass transfer resistance can avoid that equilibrium conditions are attained. Therefore, pretreatment of the plant become crucial to break cells, enhancing solvent contact, and facilitating the extraction. In fact, moderate pressures (9-12 MPa) and temperatures (35-50°C) are sufficient to solubilize the essential oil compounds [15-18]. Yet, in some cases, higher pressures are applied to contribute to the rupture of the vegetal cells and the liberation of the essential oil. However, other substances such as cuticular waxes are co-extracted and thus, on-line fractionation can be applied to attain the separation of the essential oil from waxes and also other co-extracted substances.

In this review, on the basis of data reported in the literature and own experience, a detailed and thorough analysis of the supercritical extraction and fractionation of plants and herbs to produce essential oils is presented. Furthermore, the supercritical CO_2 extraction of several plants (oregano, sage, thyme, rosemary, basil, marjoram and marigold) from *Lamiaceae* family was accomplished in our supercritical pilot-plant at 30 MPa and 40°C. High CO_2 density was applied in order to ensure a complete extraction of the essential oil compounds. Then, on-line fractionation in a cascade decompression system comprising two separators was employed to isolate de essential oil fraction. Yield and essential oil composition was determined and compared.

2. The essential oil of plants and herbs

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

The main compounds of plant essential oils are terpenes, which are also called isoprenes since derived from isoprene (2-methyl-1,3-butadiene, chemical formula C_5H_8) (see Figure 1). Main hydrocarbon terpenes present in plant essential oil are monoterpenes (C10), which may constitute more than 80% of the essential oil, and sesquiterpenes (C15). They can present acyclic structures, so as mono-, bi- or tricyclic structures (see Figure 2). Terpenoids are derived from these hydrocarbons, for example by oxidation or just reorganization of the hydrocarbon skeleton. Terpenoids present in essential oils comprise a wide variety of chemical organic functions, such as alcohols, aldehydes, ketones, acids, phenols, ethers**,** esters, etc.

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

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

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

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

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

With respect to the solubility of essential oil compounds in supercritical CO_2, it could be stated in general that the solubility of hydrocarbon monoterpenes is higher than the solubility of monoterpenoids. For example, the reported solubility of limonene at 9.6 MPa and 50°C is 2.9 % w/w; at the same pressure and temperature conditions the solubility of thymol and camphor are, respectively, 0.9 and 1.6 % w/w [18]. Moreover, these values are considerably higher than the solubility of other extractable compounds present in plants and herbs, such as phenolic compounds, waxes, carotenoids and chlorophylls. As it is well-known phenolic compounds present in plans constitute a special class of bioactive substances due to their recognized antioxidant activity [27]. For example, Murga et al. [28, 29] reported that the solubility of protocatechuic acid, methyl gallate and protocatechualdehyde (phenolic compounds present in grapes) in pure supercritical CO_2 measured at different temperatures (40-60°C) and pressures up to 50 MPa were lower than 0.02 % w/w. Furthermore, also low solubilities were reported for carotenoids [30].

On the other side, the solubility of *n*-alkanes C24-C29 in supercritical CO_2 is in the range of 0.1-1 %w/w at rather low pressures (8-25 MPa) [31]. These values are quite close to the solubility values referred above for several monoterpene compounds and thus, waxes are in general the main substances co-extracted with essential oils. Thus, fractionation schemes are target towards an efficient separation of essential oil constituents from high molecular weight hydrocarbons and waxy esters.

Figure 3 compares the solubility in supercritical CO_2 of several substances, representing different family of compounds present in vegetal natural matter. Solubilities are represented as a function of pressure, for temperatures in the range 35-50°C. Particularly, the figure shows the solubility of main monoterpenes of grape essential oil, namely α-pinene, limonene and linalool; the solubility reported for some low molecular weight phenolic compounds (protocatechuic acid, methyl gallate and *p*-cumaric acid) also present in grapes; and the solubility of β-carotene and *n*-C28, as representatives, respectively, of pigments and waxy compounds. As can be observed in Figure 3, the solubility of main constituents of essential oil (monoterpenes) of grapes is considerably higher than the solubility of the phenolic

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

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

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

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

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

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

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

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

3. Supercritical fluid extraction (SFE) of essential oils

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

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

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

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

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

3.1 Effect of matrix pretreatment and packing

The particular characteristics of the plant species is, indeed, a decisive factor in the supercritical extraction kinetics. Recently, Fornari et al. [67] presented a comparison of the kinetics of the supercritical CO_2 extraction of essential oil from leaves of different plant matrix from *Lamiaceae* family. In their work, identical conditions of raw material pretreatment, particle size, packing and extraction conditions (30 MPa, 40°C and no co-solvent) were maintained. Figure 5 show a comparison between the global yields obtained for the different raw materials as a function of extraction time. As can be deduced from the figure, sage (*Salvia officinalis*) and oregano (*Origanum vulgare*) were completely extracted in less than 2 h, while rosemary (*Rosmarinus officinalis*) and thyme (*Thymus zygis*) were not completely exhausted after 4.5 h of extraction. Moreover, very similar kinetic behavior resulted for sage and oregano, so as for thyme and rosemary. Considering the first period of extraction (1.5 h) it was estimated a removal velocity of around 0.004 g extract / g CO_2 in the case of sage and oregano, and almost half of this value in the case of rosemary and thyme.

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

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

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

Despite the lipophilic character of essential oil compounds, the water present in the vegetable matrix may interfere in the solute-CO_2 interaction (particularly in the case of terpenoids which are most polar than terpenoids) and produce a decrease of extraction yield. For this reason, drying of the raw material is recommended.

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

Beyond the specific characteristics of the plant variety and the part of the plant employed for extraction, cell disruption is a crucial factor in solvent extraction processes and thus, in SFE. Essential oil compounds are found in intracellular spaces, more than on the surface of the vegetal cell. Thus, in order to attain an adequate contact with the solvent, a pretreatment to produce cell disruption (comminuting, grinding) is critical. Then, the efficiency of the extraction process is improved by a decreasing of mass transfer resistance. Indeed, particle size greatly affects process duration and both variables are interconnected with CO_2 flow rate. The selection of these parameters has the target of producing the exhaustion of the desired compounds in the shorter time.

Particle size plays an important role in SFE processes; if internal mass transfer resistances could be reduced, the extraction is controlled by equilibrium conditions and thus, short extraction times are required. For example, Aleksovsk and Sovová [49] proved that in the SFE of sage leaves ground in small particles, the essential oil was easily accessible to the supercritical CO_2 solvent at moderate conditions (9-13 MPa and 25-50°C) and the extraction was controlled by phase equilibrium. The same readily SFE of sage was observed by Fornari et al. [67] while a delayed kinetic (controlled by mass diffusion) was deduced for thyme and rosemary supercritical extraction [67, 71] although the same grinding method, particle size and packing procedure was applied for the three plants.

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

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

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

3.2 Effect of extraction conditions

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

Essential oils can be readily extracted using supercritical CO_2 at moderate pressures and temperatures. That is, from an equilibrium point of view rather low pressures are required to extract essential oils from plant matrix (9-12 MPa) (see Figure 3). Yet, higher pressures are also applied in order to take advance of the compression effect on the vegetal cell, what enhances mass transfer and liberation of the oil from the cell. High pressures produce the co-extraction of substances other than essential oil. The general rule is: the higher is the pressure, the larger is the solvent power and the smaller is the extraction selectivity. Thus, 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/m^3) 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 50ºC) main
457 compounds extracted are triterpenoid esters [77], while lower pressures (20 MPa and 40ºC)
458 produce extracts rich in aliphatic hydrocarbons, acetyl eugenol and guaiol [78].

459 Supercritical CO_2 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 CO_2 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 CO_2. 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 CO_2
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 CO_2 SFE of rosemary is observed on the recovery of its phenolic
482 antioxidant compounds but not in the extraction of essential oil substances.

483

3.3 Fractionation alternatives

Another technological alternative that can be very useful to improve the selectivity of SFE to produce essential oils is fractionation of the extract, what means the separation of the solutes extracted from the plant matrix in two or more fractions. This strategy can be used when it is produced the extraction of several compound families from the same matrix, and they show different solubilities in supercritical CO_2 (see Figure 3). Fractionation techniques take advantage of the fact that the supercritical solvent power can be sensitively varied with pressure and temperature.

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

In the case of multi-step fractionation, the conditions applied in the extraction vessel are varied step by step, increasing CO_2 density in order to obtain the fractional extraction of the soluble compounds contained in the organic matrix. Thus, the most soluble solutes are recovered in the first fraction, while substances with decreasing solubility in the supercritical solvent are extracted in the successive steps. Essential oils generally constitute the first fraction of a multi-step fractionation scheme due to their good solubility in supercritical CO_2.

For example, multi-step fractionation arrangement may consist in performing a first extraction step at low CO_2 density (≈ 300 kg/m^3) followed by a second extraction step at high CO_2 density (≈ 900 kg/m^3). Then, the most soluble compounds are extracted during the first step (for example, essential oils) and the less soluble in the second one (e.g. antioxidants). Fractionation of rosemary extract was first reported by Oca et al. [70]: two successive extraction steps resulted in a low-antioxidant but essential oil rich fraction in the first step (10 MPa and 40°C, CO_2 density = 630 kg/m^3) and a high-antioxidant fraction in the second step (40 MPa and 60°C, CO_2 density = 891 kg/m^3).

Multi-step fractionation was also employed by the authors (data non published) to produce the complete exhaustion of rosemary essential oil using pure CO_2 in a first step, and a fraction with high antioxidants content using CO_2 and ethanol as co-solvent in the second step. But in this case, high CO_2 density was applied first (30 MPa and 40°C, CO_2 density = 911 kg/m^3) in order to produce the complete deodorization of plant matrix. Despite the fact that some antioxidants were also co-extracted in this step, the high pressures applied ensured the complete exhaustion of essential oil substances from plant matrix. Then, a step using

ethanol cosolvent was applied at lower CO_2 densities (15 MPa and 40°C, CO_2 density = 781 kg/m^3). This second step produced an extract (5% yield) containing 33 %w/w of antioxidants (carnosic acid plus carnosol) and less than 2.5 %w/w of volatile oil compounds.

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

A different on-line fractionation alternative to improve the isolation of antioxidant compounds from rosemary has been recently presented by the authors [55]. The experimental device employed in the study is similar to the one schematized in Figure 4, comprising two separators (S1 and S2) in a cascade decompression system. The SFE temperature and pressure were kept constant (30 MPa and 40°C) but the depressurization procedure adopted to fractionate the material extracted was varied with respect to time. At the beginning (first period) on-line fractionation of the extract was accomplished; due to the lower solubility of the antioxidant compounds in comparison to the essential oil substances it is apparent that the antioxidants would precipitate in S1, while the essential oil would mainly be recovered in S2. Nevertheless, when the amount of volatile oil remained in the plant matrix is significantly reduced, no further fractionation is necessary. Then, during the rest of the extraction (second period) S1 pressure is lowered down to CO_2 recirculation pressure and all the substances extracted were precipitated in S1, and mixed with the material that had been recovered in this separator during the first period of extraction. The authors varied the extend of the first extraction period and determine the optimum in order to maximize antioxidant content and yield in the product collected in S1. In this way, a fraction was produced with a 2-fold increase of antioxidants in comparison with a scheme with no fractionation, and with a yield almost five times higher than that obtained when on-line fractionation is accomplished during the whole extraction time. With respect to rosemary volatile oil a 2.5-4.5 fold increase was 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 CO_2 flow rates. At
571 pressures around 20 MPa the improvement in the yield was low (≈ 15%) probably because
572 the solubility of almond oil in supercritical CO_2 is rather low. However, at higher extraction
573 pressures larger improvements between extraction curves with and without ultrasounds where
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 CO_2. 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

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

4. Supercritical chromatography fractionation of essential oils

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

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

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

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

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

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

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

Supercritical CO_2 extraction of several plants from *Lamiaceae* family were extracted and fractionated in a supercritical pilot-plant comprising an extraction cell of 2 l of capacity. The SFE system (Thar Technology, Pittsburgh, PA, USA, model SF2000) is similar to that schematized in Figure 4. Plant matrix consisted in dried leaves of oregano (*Origanum vulgare*), thyme (*Thymus vulgaris*), sage (*Salvia officinalis*), rosemary (*Rosmarinus officinalis*), basil (*Ocimum basilicum*) and marjoram (*Origanum majorana*), while dried petals were employed in the case of marigold (*Calendula officinalis*) extraction. All plant matrixes were ground in a cooled mill and were sieving to 200-600 μm of particle size.

The extraction cell was loaded with 0.50-0.55 kg of vegetal matter. The extractor pressure was 30 MPa and temperature of the extraction cell and separators was maintained at 40ºC. CO_2 flow rate was 60 g/min and extraction was carried out for 5 h. Fractionation of the extracted material was accomplished by setting the pressure of the first separator (S1) to 10 MPa, while the second separator (S2) was maintained at the recirculation system pressure (5 MPa). The same extraction conditions were applied for all plant varieties. A comparison of the extraction yield, fractionation behavior and essential oil composition was established.

The essential oil compounds of samples were determined by GC-MS-FID using 7890A System (Agilent Technologies, U.S.A.), as described previously [67]. The essential oil 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 (≈ 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

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

Conclusion

Essential oils of plants and herbs are important natural sources of bioactive substances and SFE is an innovative, clean and efficient technology to produce them. The lipophilic character of the substances comprising essential oils guarantees high solubility in CO_2 at moderate temperatures and pressures. Further, the use of polar cosolvents is not necessary and the subsequent processing for solvent elimination is not required. The low processing temperatures result in non-damaged products, with superior quality and better biological functionality. Higher extraction pressures produce the co-extraction of substances with lower solubilities and fractionation alternatives allow the recovery of different products with different composition and biological properties. More recent studies revealed the ultrasound assisted supercritical extraction may increase both extraction rate and yield.

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

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

907

908 **Table 2.** Comparison of the content of some common volatile oil compounds identified in oregano,
909 sage and thyme extracts produced with pure CO_2 at 30 MPa and 40°C [67].

910

Compound *i*	ratio between the content of compound *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 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

914

915

916 **Table 4. S**upercritical 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

921

922

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 (C_5H_8) 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 CO_2 of several constituents of plant matter. Essential oil compounds: (∗) limonene, (-) α-pinene and (◇) linalool [18]; phenolic compounds: (○) protocatehuic acid [28], (△) methyl gallate [28] and (□) *p*-cumaric acid [29]; pigments: (■) β-carotene [18]; waxes: (▲) *n*-$C_{28}H_{58}$ [31]. Temperature range: 35-50°C.

Figure 4. Typical SFE scheme for the extraction of plant matrix. P1: CO_2 pump; P2: cosolvent pump; HE1, HE2, HE3: heat exchangers; EV: extraction vessel; S1, S2: separator cells; V, V1, V2: back pressure regulator valves; ST: CO_2 storage tank; F: filter.

Figure 5. Supercritical CO_2 extraction (30 MPa and 40°C) of oregano (□), sage (■), thyme (△) and rosemary (▲).

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.

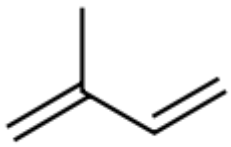
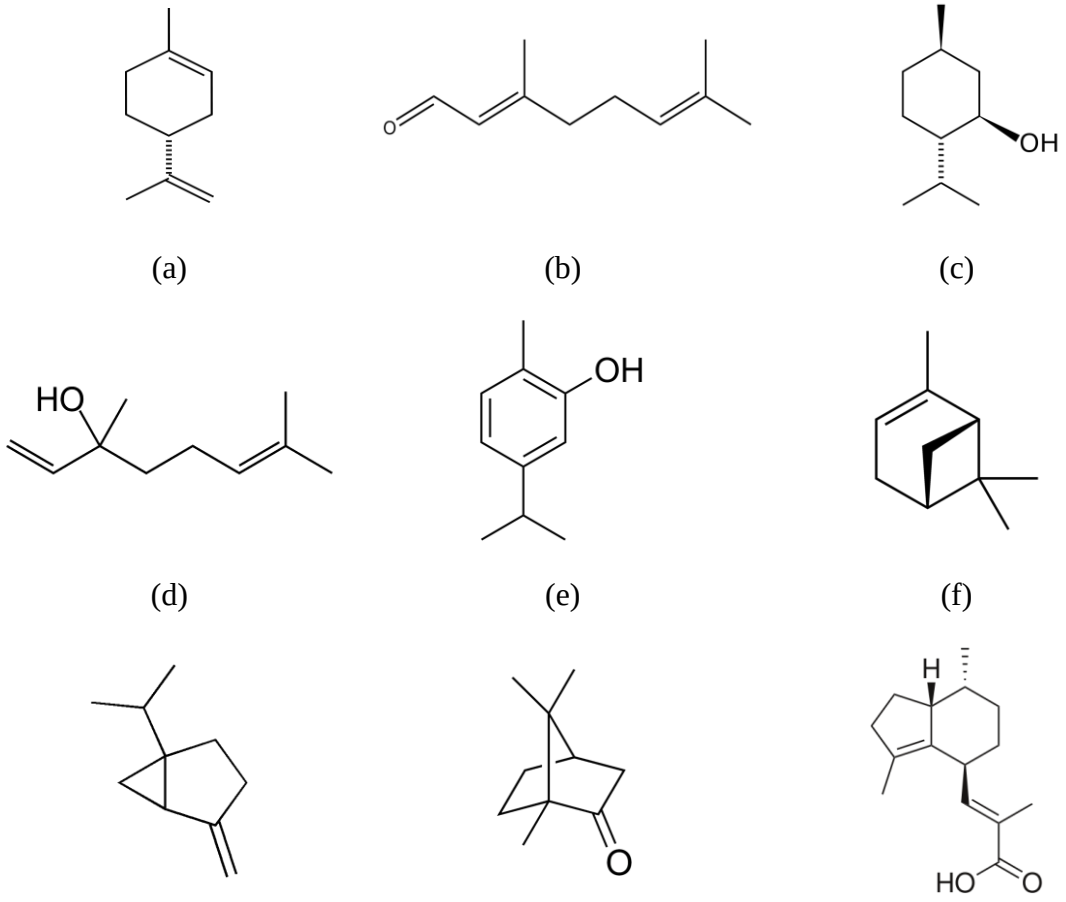


Figure 2.

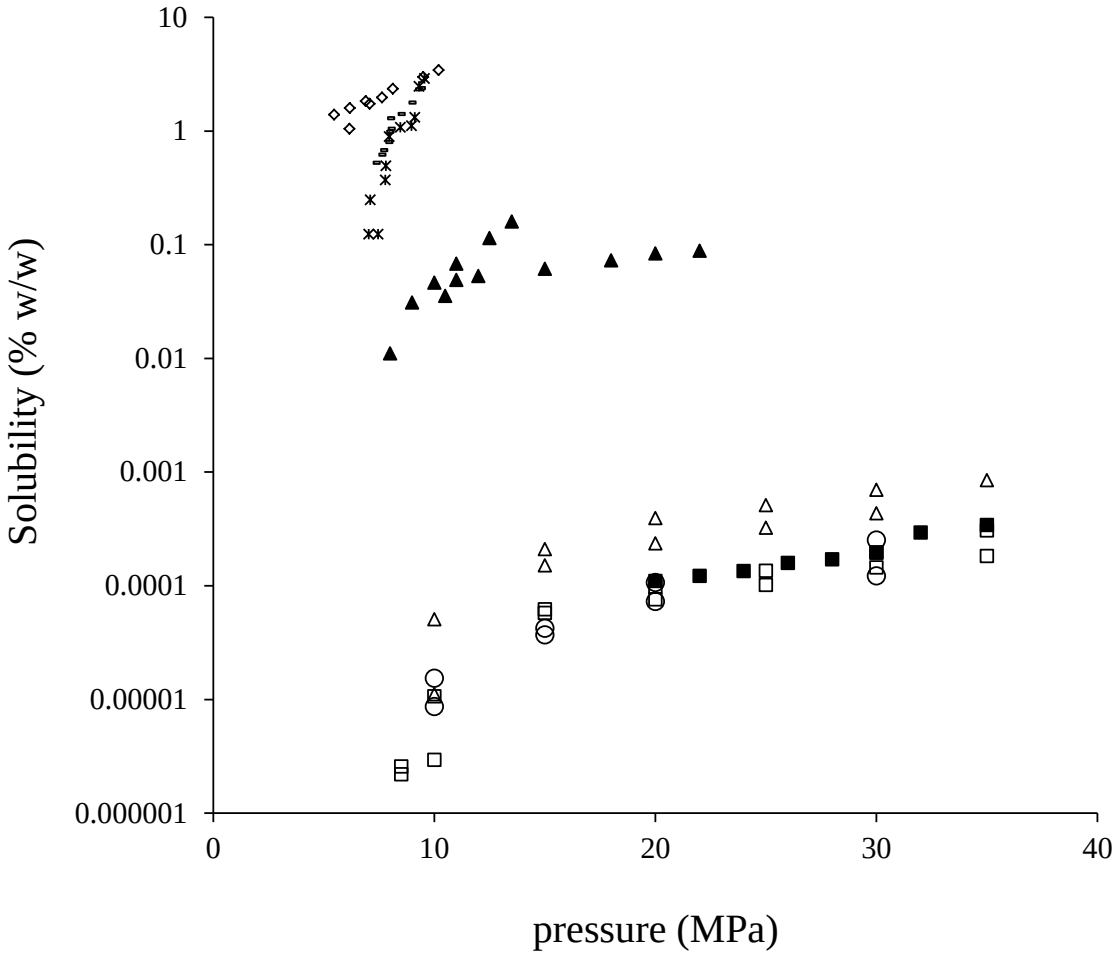


(a) (b) (c)

(d) (e) (f)

(g) (h) (i)

Figure 3.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30
40
pressure (MPa)

Figure 4.

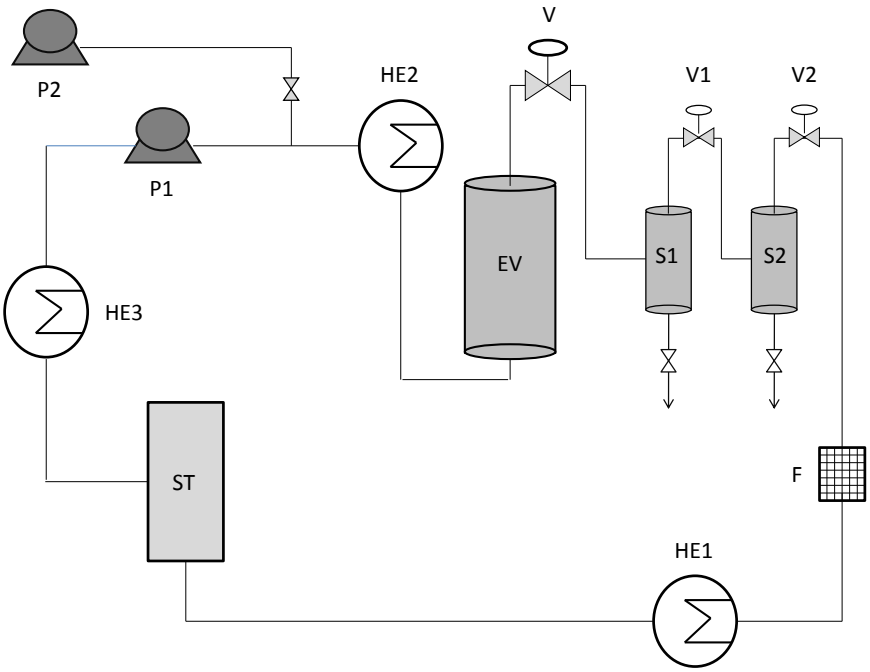
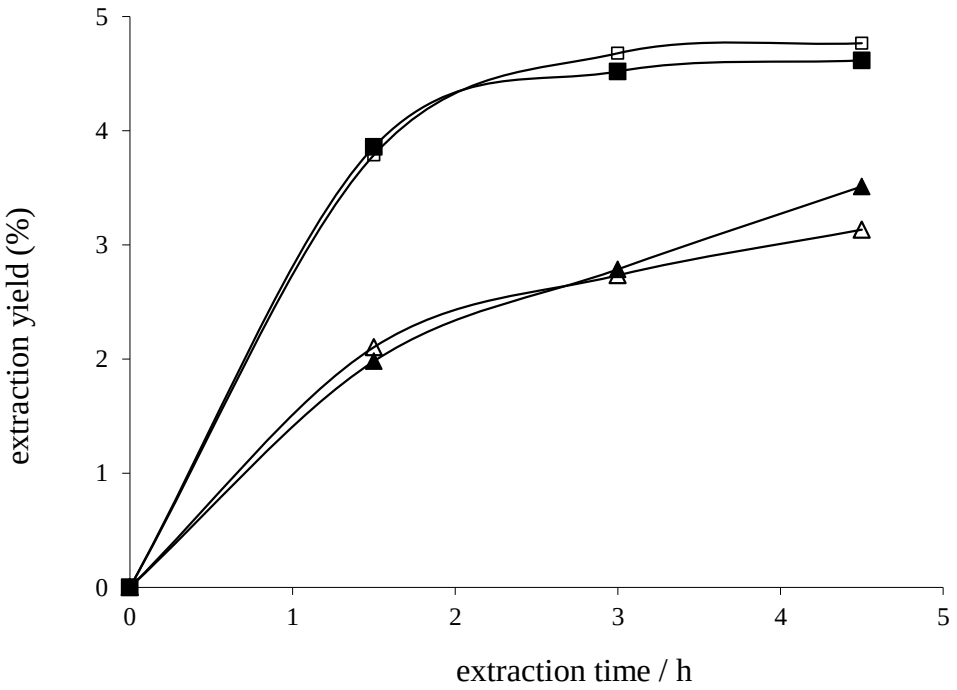


Figure 5.



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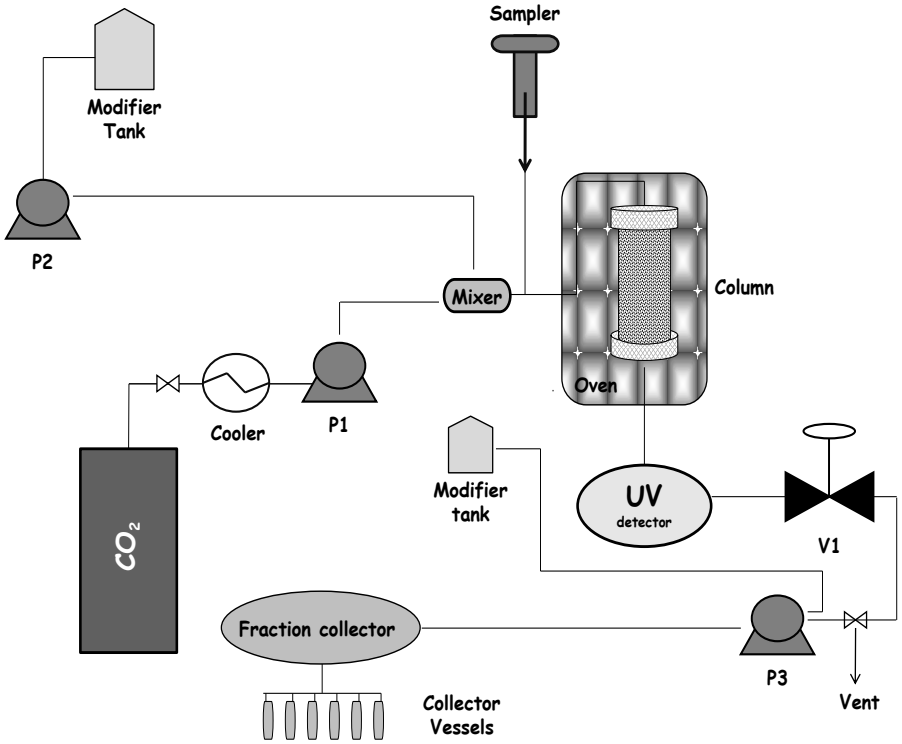
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Modifier Tank
P2
Mixer
Column
Oven
Cooler
P1
CO_2
Modifier tank
UV detector
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Fraction collector
P3
Vent
Collector Vessels

Figure 7.

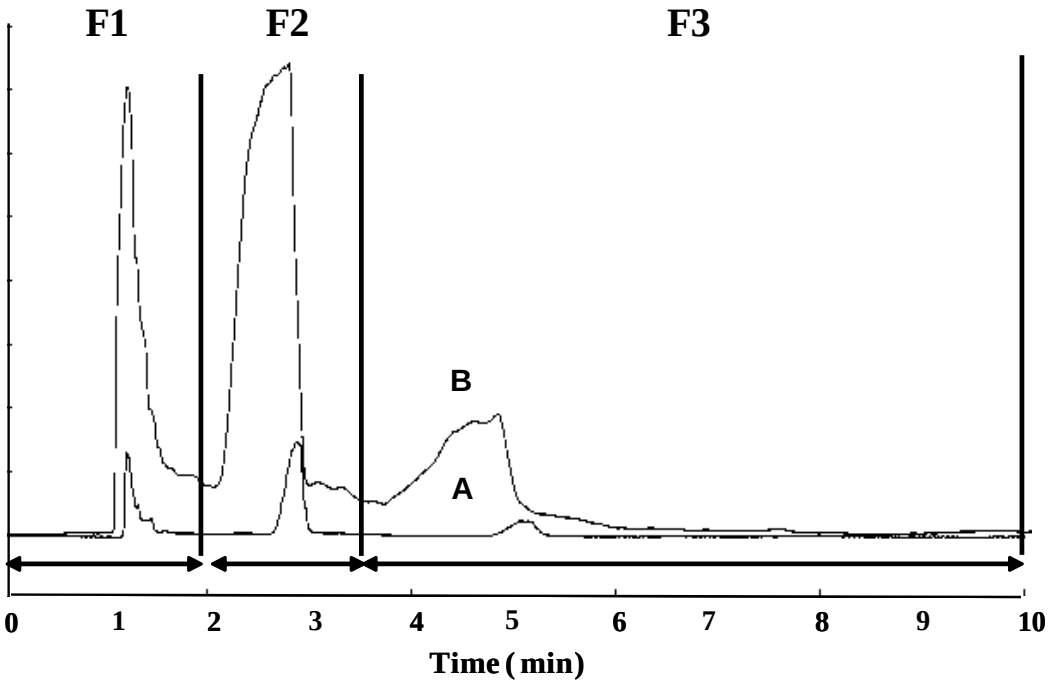
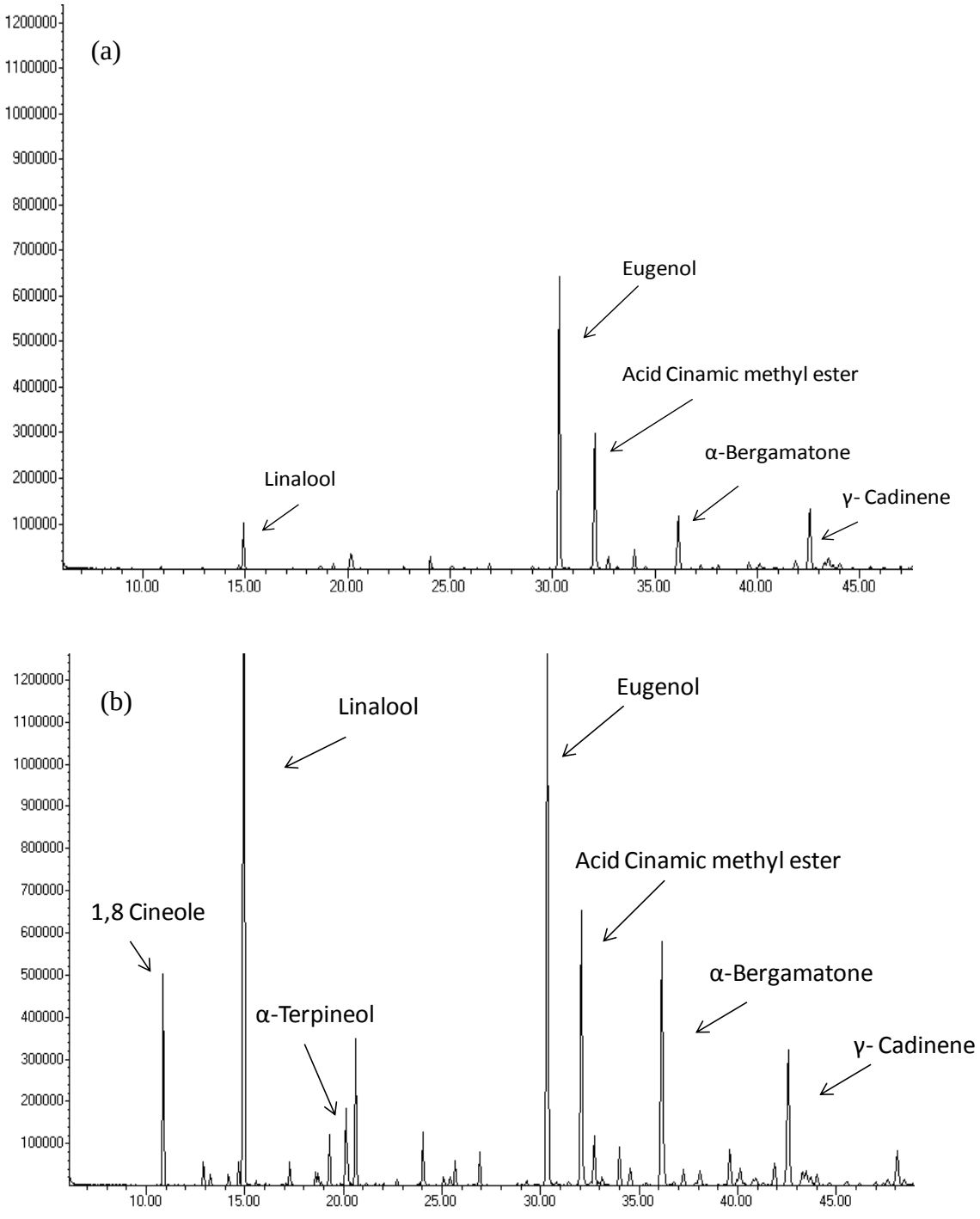


Figure 8.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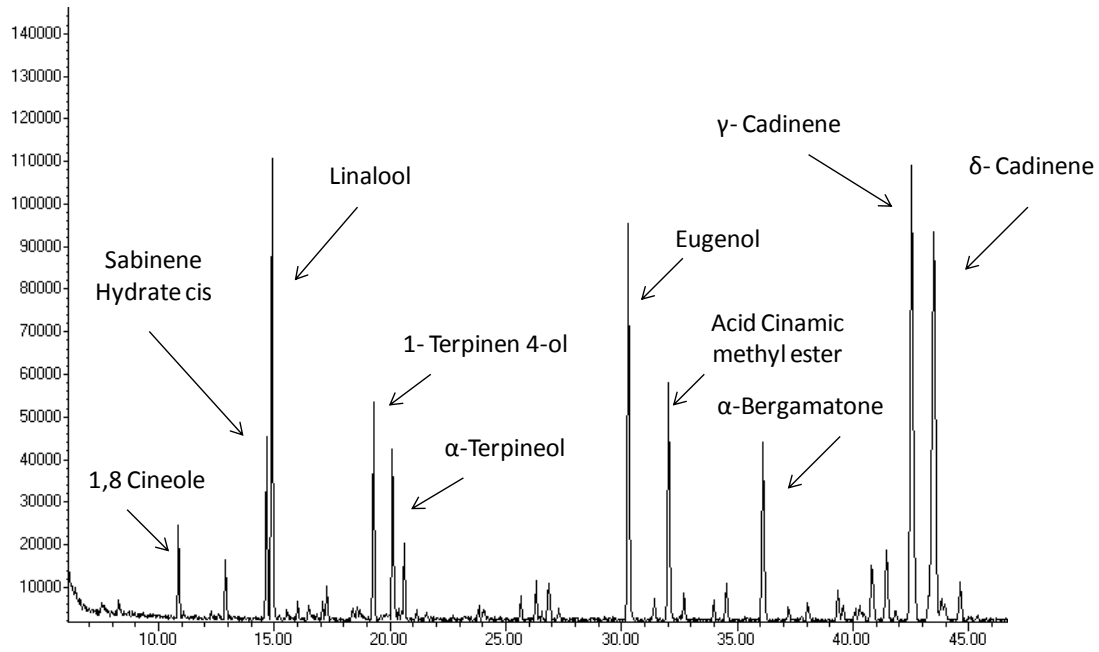
Acid Cinamic methyl ester
α-Bergamatone
Linalool
γ- Cadinene
(b)
Linalool
Eugenol
Acid Cinamic methyl ester
1,8 Cineole
α-Bergamatone
α-Terpineol
γ- Cadinene

Figure 9.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γ- Cadinene
δ- Cadinene
Linalool
Eugenol
Sabinene
Hydrate cis
Acid Cinamic
methyl ester
1- Terpinen 4-ol
α-Bergamatone
α-Terpineol
1,8 Cineole

Figure 1

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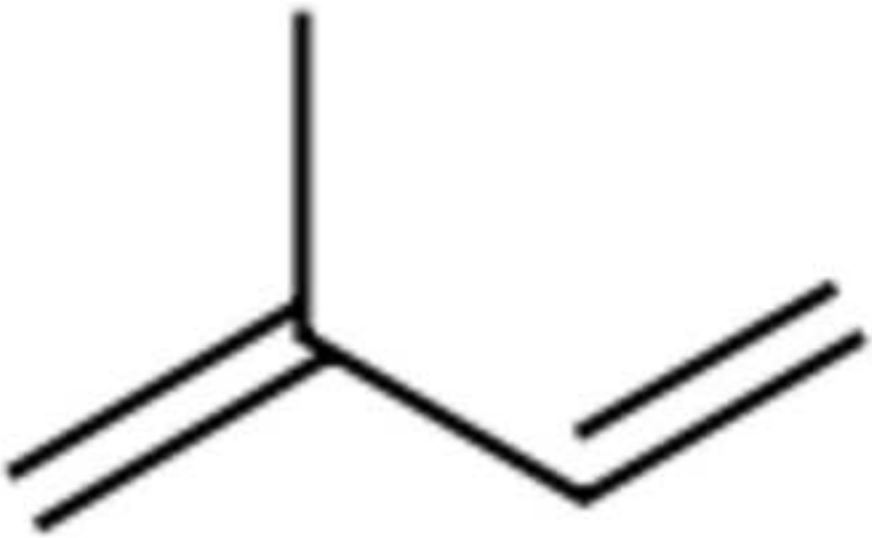

Figure 2

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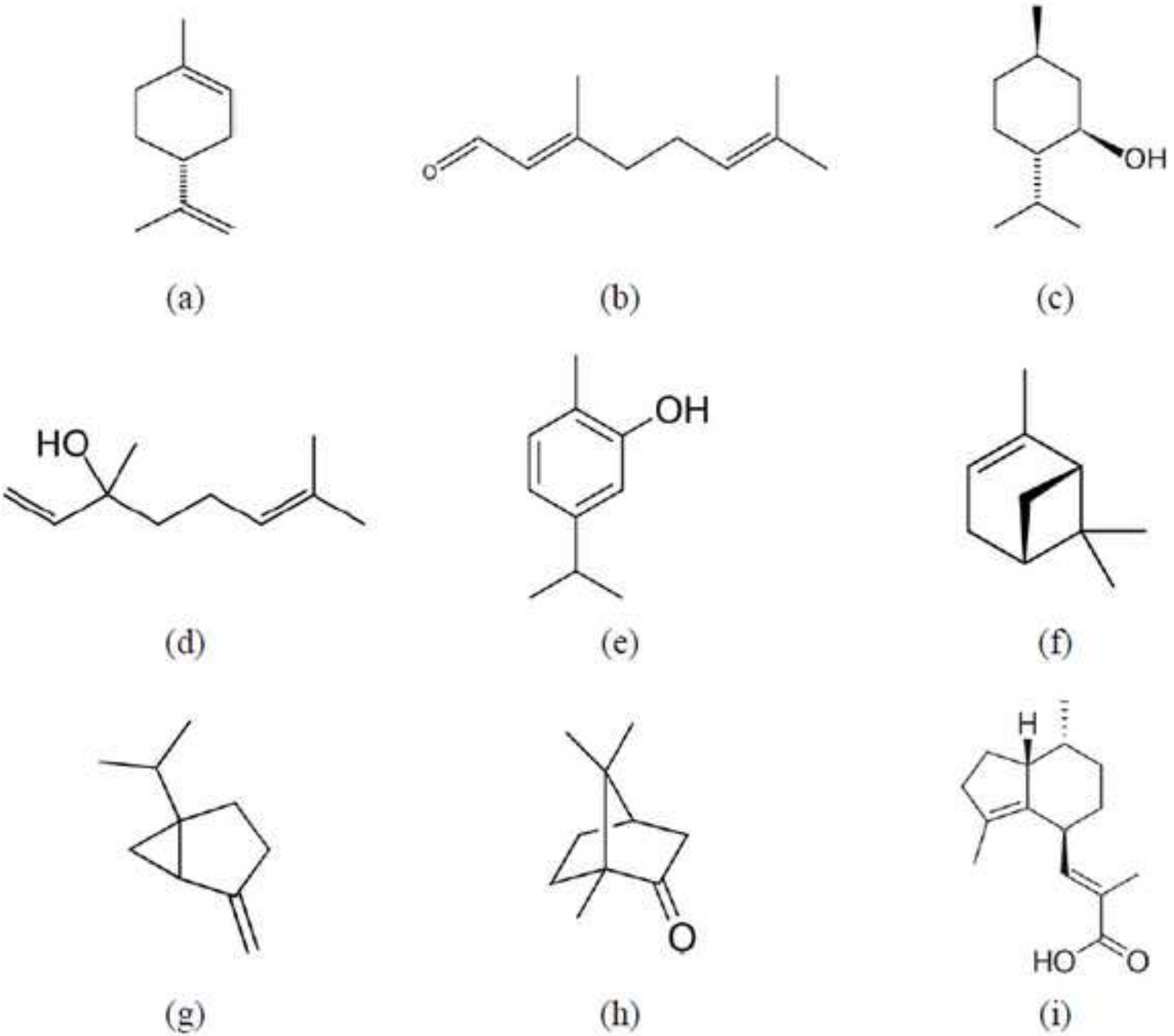

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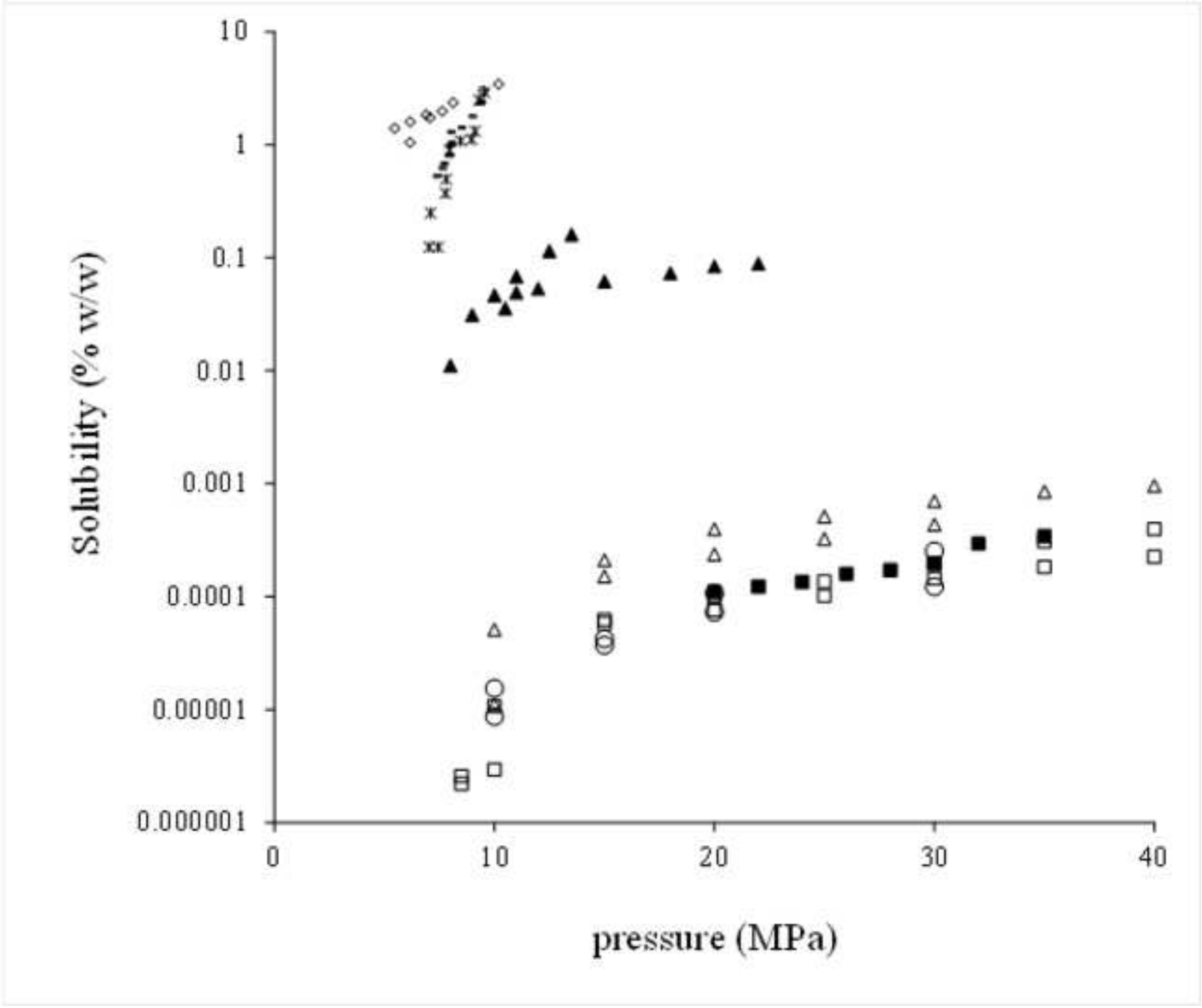

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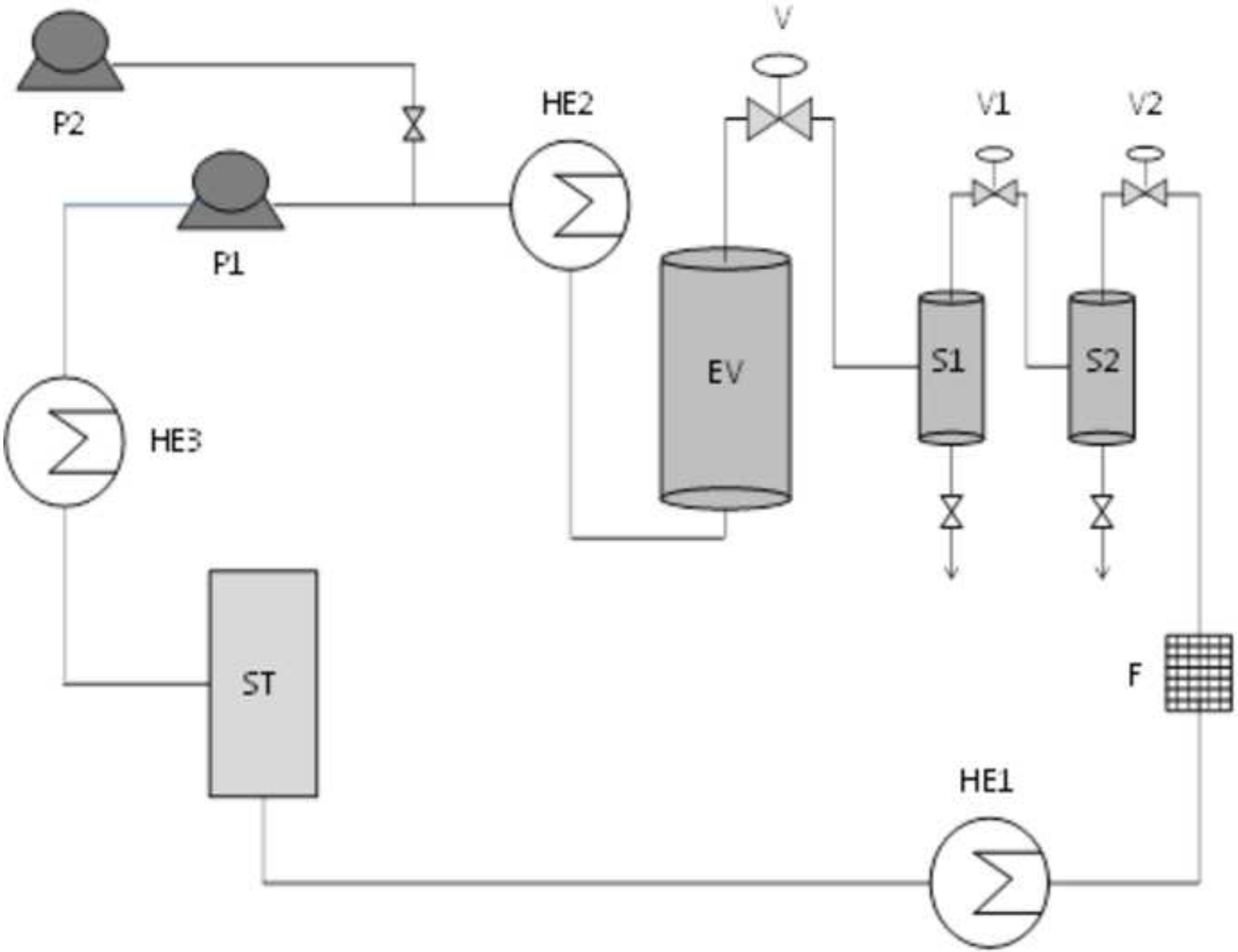

Figure 5

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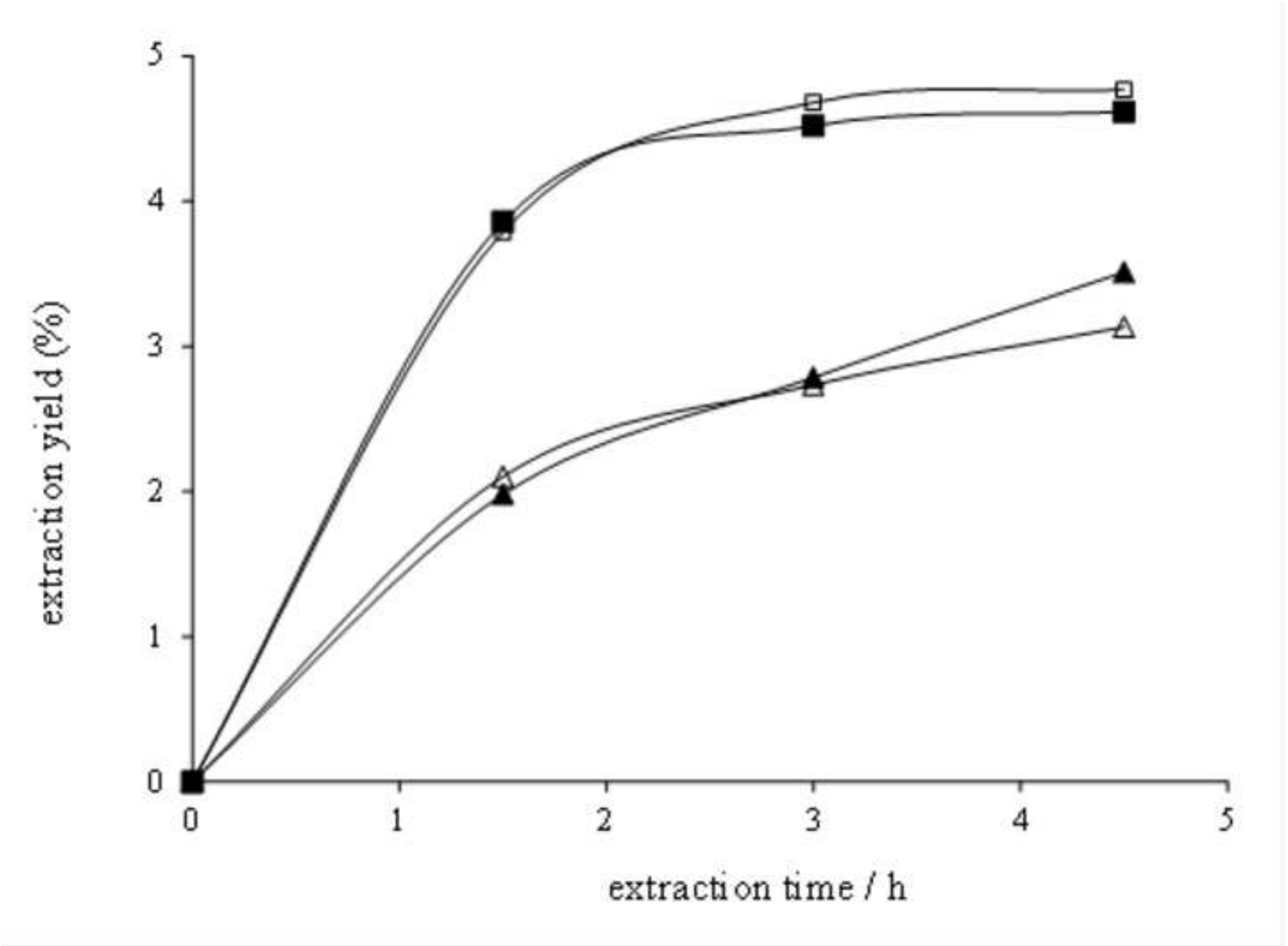

Figure 6

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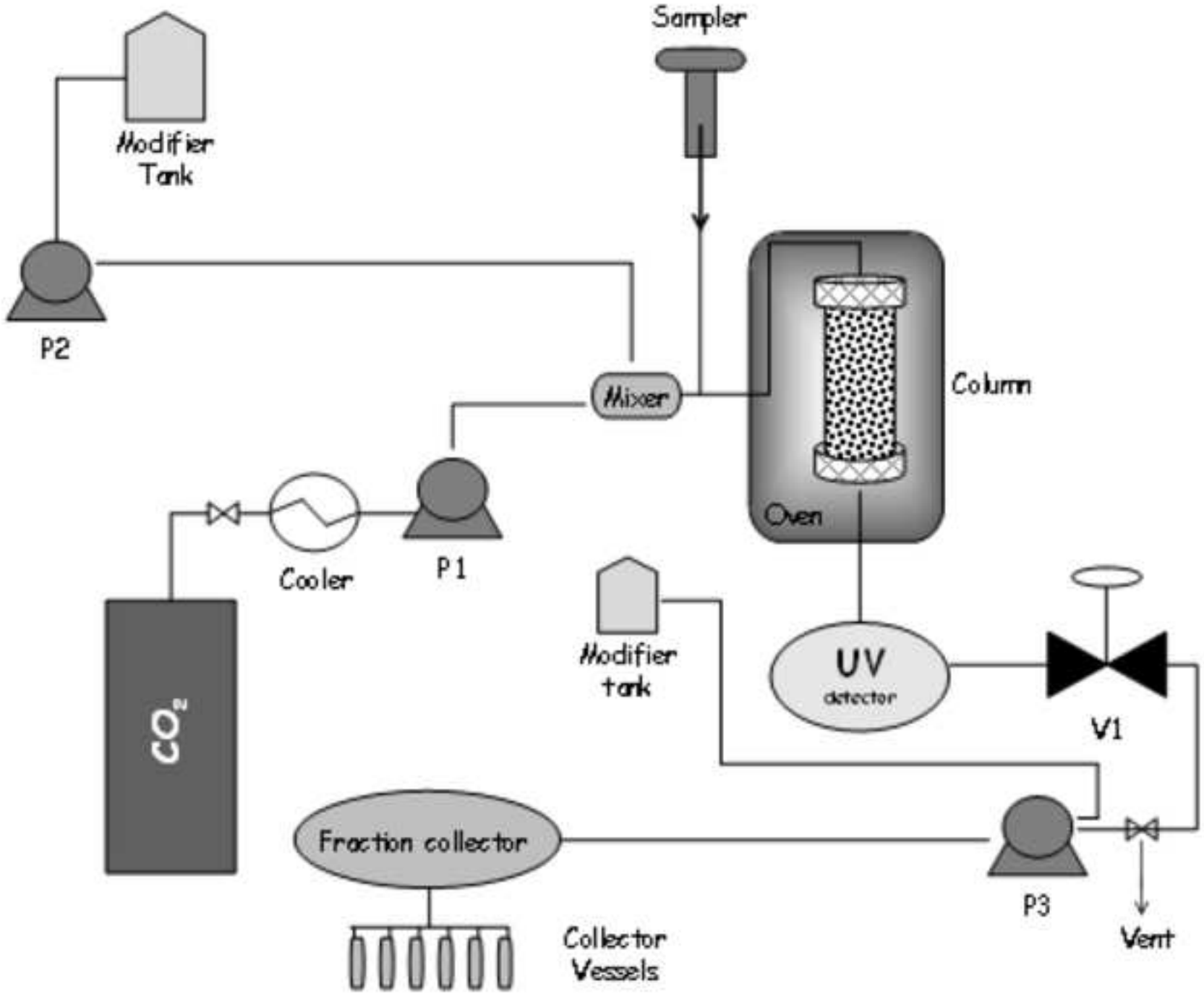

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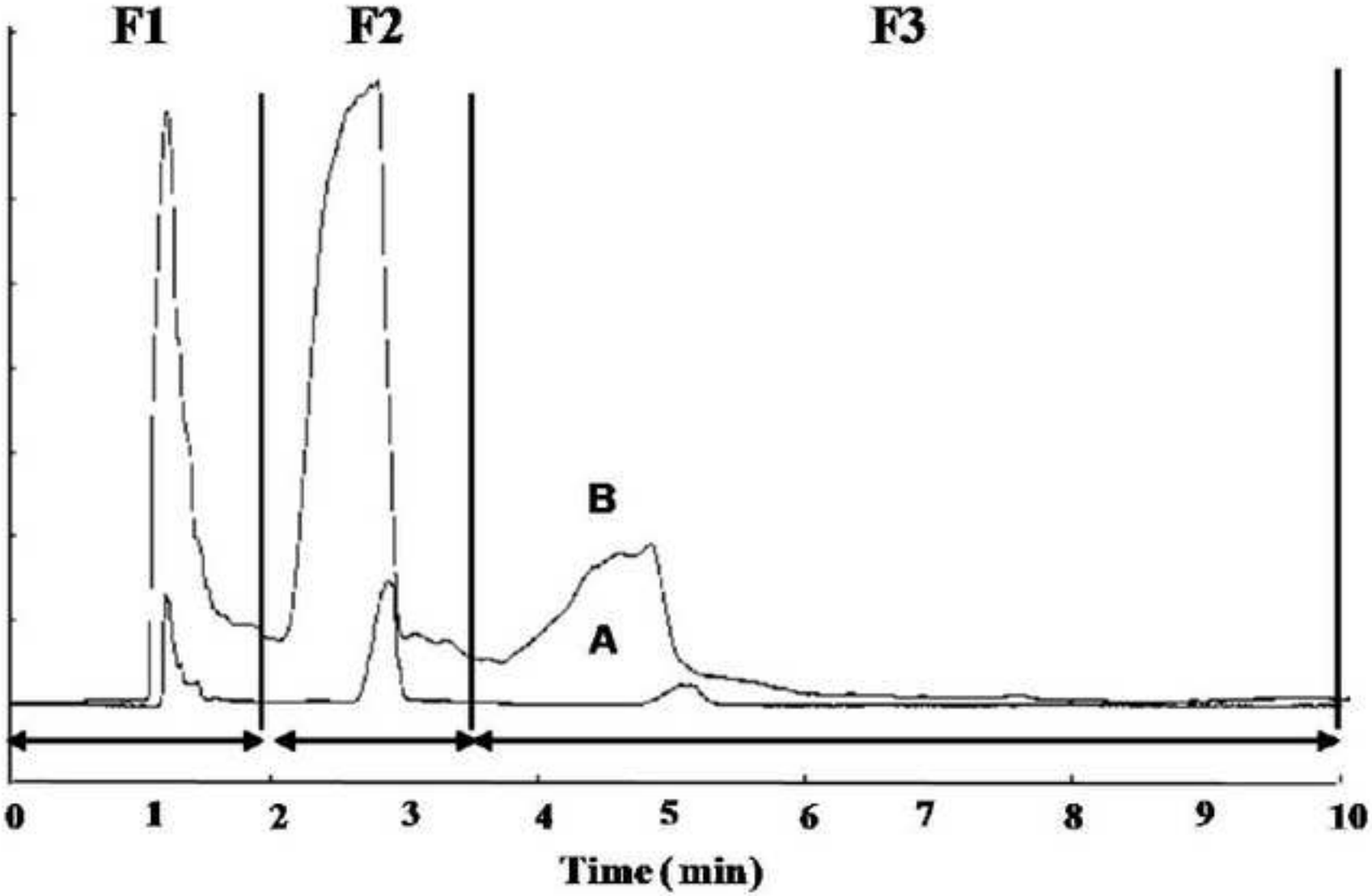

Figure 8

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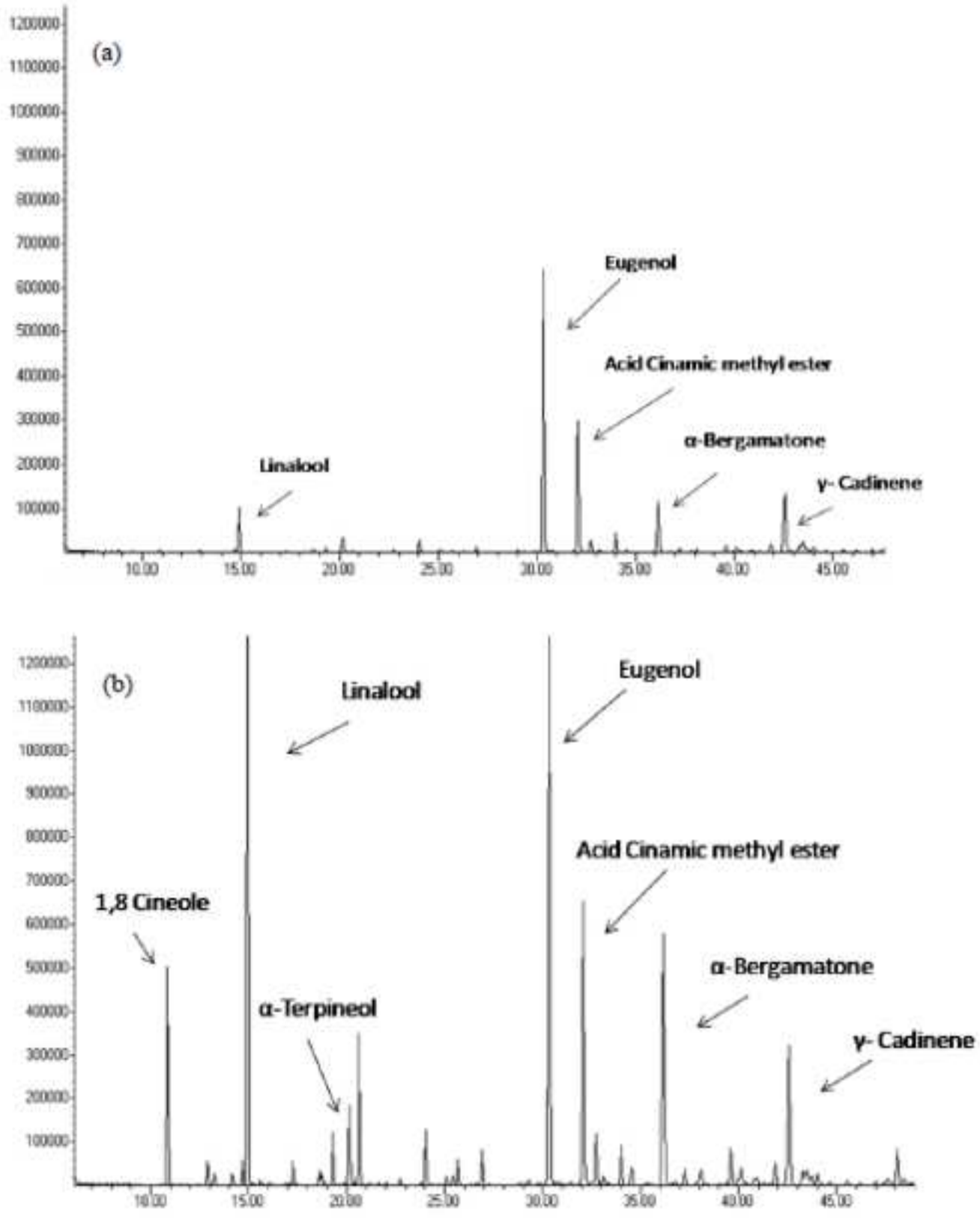

Figure 9

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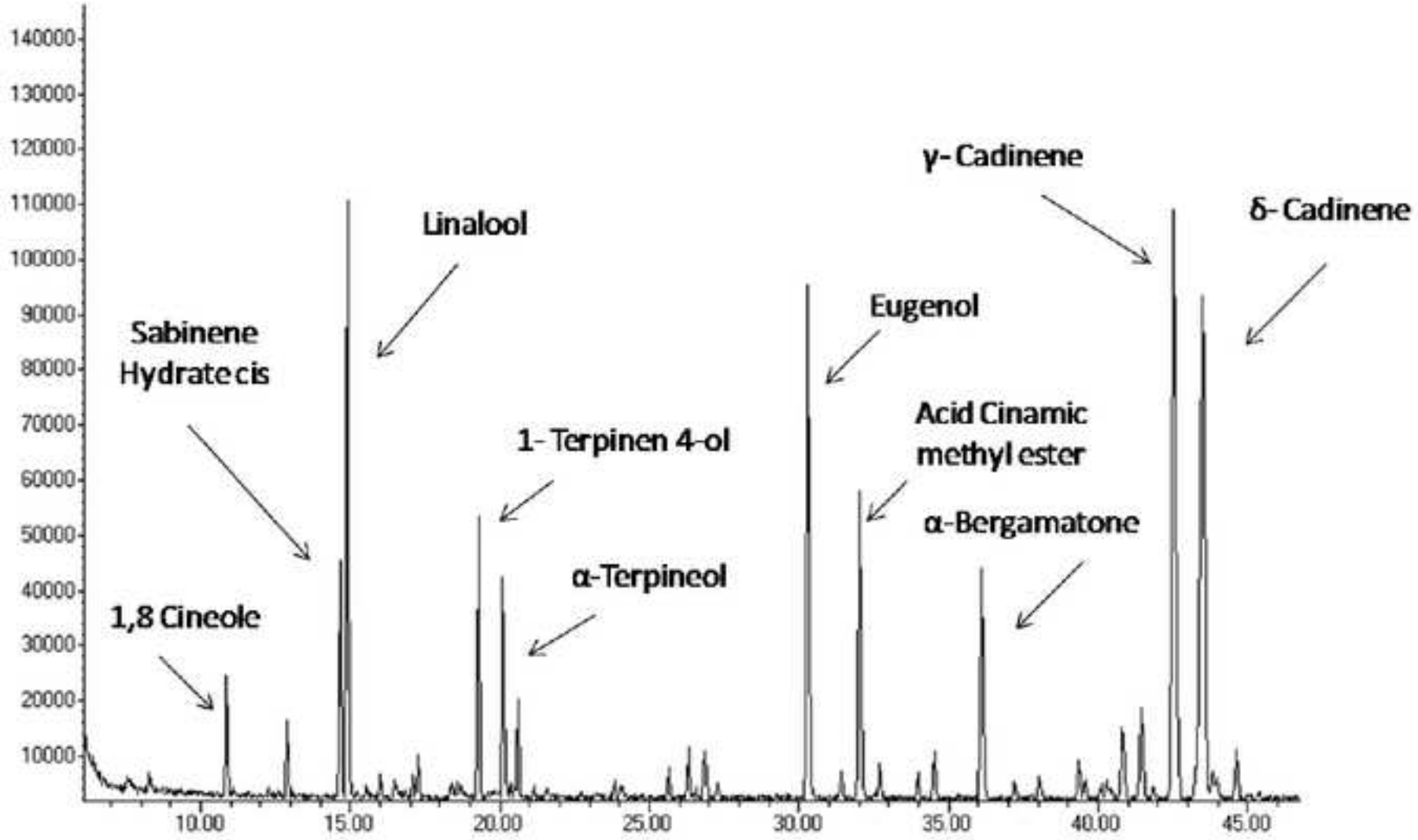

Table 1. SFE of different plants and herbs to produce essential oils.

Raw material	Botanical name	Main constituents of essential oil	References
Anise verbena	*Lippia alba*	carvone, limonene, elemol, γ-muurolene, guiaol, bulnesol	[99, 100]
Aniseed	*Pimipinella anisum*	anethole, γ-himachalene, p-anisaldehyde, methylchavicol, cis-pseudoisoeugenyl 2-methylbutyrate, trans-pseudoisoeugenyl 2-methylbutyrate	[101]
Artemisa	*Artemisia sieberi*	camphene, 1,8 cineol, γ-terpinene, chrysanthenone, camphor, cis-chrysanthenone	[102]
Basil leaves	*Ocimum basilicum*	linalool, methyl-eugenol, 1,8 cineole, α-bergamotene, α-cadinene	[63]
Cashew	*Anacardium occidentale*	cardanol, cardol, dimethylanacardate	[103]
Chamomile	*Chamomilla recutita*	matricine, chamazulene, bisabolol	[104]
Clove	*Eugenia caryophyllata Thunb*	eugenol, caryophyllene, eugenol acetate	[105, 106]
Coriander	*Coriandrum sativum*	linalool, γ terpinene, camphor, geranyl acetate, α pinene, geraniol, limonene	[107]
Eucalyptus	*Eucalyptus camaldulensis Dehnh.*	1,8 cineole, a-pinene, β-pinene, terpinen-4-ol, allo-alomandrene, globulol	[108]
Fennel	*Foeniculum vulgare Mill.*	trans-anetole, methyl chavicol, fenchone	[72]
Hyssop	*Hyssopus officinallis*	sabibebem iso-pinocamphene, pinocamphene	[109]
Laurel leaves	*Laurus nobilis*	1,8 cineole, linalool, α-terpinylacetate, methyleugenol	[110]
Lavender	*Lavandula angustifolia*	linalool, camphor, borneol, terpinen-4-ol, linalyl acetate, oxygenated monoterpenes, oxygenated sesquiterpenes	[111]
Macela	*Achyrocline alata, A. satureioides*	trans-caryophyllene, α-humulene	[112]
Myrtus	*Myrtus communis*	α-pinene, Limonene, 1,8 cineole	[113]
Marigold	*Calendula officinalis*	acetyl eugenol, guaiol	[114]
Marjoram	*Origanum majorana*	4-terpineol, ρ-cymene, carvacrol, sabinene hydrate	[38]
Mint	*Mentha spicata insularis*	L-menthone, isomenthone, menthol, cis-b-terpineole, menthylacetate, trans β-caryophyllene, germacrene-D	[115]
Oregano	*Origanum vulgare*	carvacrol, tymol, sabinene hydrate, p-cypeme, linalool	[77, 106]
Pennyroyal	*Mentha pulegium*	menthone, pulegone, limonene.	[116]
Pepper black	*Piper nigrum*	3-γ-carene, limonene, β-caryophilene, sabinene	[117]
Rosmarinus	*Rosemary officianlis*	camphor, 1,8 cineole, borneol, linalool	[12, 55]
Sage	*Salvia officinalis*	1,8-cineole, camphor, β-thujone	[118]
	Salvia mirzayanii	linalyl acetate, 1,8 cineol, linalool, 8-acetoxy linalool	[119]
Star anise	*Illicium anisatum*	trans-anethole , limonene, chavicol , anisaldehyde	[120]
Thyme	*Thymus vulgaris*	thymol, carvacrol, camphor, linalool	[98]
	Thymus Zygis	thymol, carvacrol, linalool, borneol	[121]
Valerian	*Valeriana officinalis*	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 CO_2 at 30 MPa and 40°C [67].

Compound *i*	ratio between the content of compound *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