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Comparison for the production of essential oil by conventional, novel and biotechnology methods

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ABSTRACT

Essential oils have many interesting applications in industry food, cosmetics, pharmaceutical, agriculture. Essential oils can be produced by various techniques, including conventional, novel and biotechnology methods. Novel extraction methods can be considered as a good alternative to conventional methods due to short extraction time, high efficiency and quality, non-decomposition of compounds due to heat and no pollution. Recently, due to the limitations of extraction methods, the attention of scientists has been focused on synthesizing aromatic compounds through biotechnological methods. In the biotechnology method, there is no concern about factors such as climate conditions, supply shortages, natural disasters, plant disease and a high-quality product is obtained. Biotechnology could provide an environmentally friendly alternative that does not require as much land and resources as traditional methods. This review covers up-to-date literature on extraction methods of essential oils, including conventional methods, novel methods and biotech methods, and a generally comparison between them.

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Essential oil; natural compounds; green extraction; conventional methods; novel methods; biotechnology

Introduction

Essential oils as colorless compounds are evaporated easily at normal temperatures and are known as secondary metabolisms (1–4). Produced compounds by plants in the nature can be classified into two main groups. The main or primary metabolites are the main elements of plant and animal life which are common to all, including carbohydrates, proteins, lipids and nucleic acids. In plants, there are other metabolic processes which the production of them by the organism is not fully understood. These compounds are referred to as secondary metabolites. Essential oils are secondary metabolites of plants (4–6) which can be extracted from various plant sources, for example flower (*Jasminum*, *Rose*, *Violet* and *Lavender*), leaves (*Thymes*, *Eucalyptus*, *Salvia*), bark (*Cinnamon*), buds (*Clove*), herbs, fruits (*Orange*, *Lemon*), twigs, seeds (*Cardamom*), wood (*Sandal*), rhizome and roots (*Ginger*) etc. (7–9).

Essential oils components are affected by factors such as genetics and the environment. Also, their compositions are dependent on the extraction methods and on how the plant grows (10,11). Some essential oils with their major constituents were randomly selected and are presented in Table 1 (12).

Two common and important techniques for the analysis of essential oils are chromatography and mass spectrometry. Gas chromatography – mass spectrometry (GC-MS) and high performance liquid chromatography

(HPLC) are the most widely used (4,13,14). In general, gas chromatography (GC) for the analysis of volatile compounds and liquid chromatography (LC) for an analysis of non-volatile compounds in essential oils is used (15).

In addition to essential oils, other aromatic compounds are extracted from plants including concrete (is obtained by extraction with non-polar solvents), absolute (is obtained by washing concretes in ethanol or methanol followed by evaporation and distillation of alcohol), pomade (is obtained by a process known as enfleurage (will be described in later sections)), resins (are extracted from the natural resin material by hydrocarbon solvent. Resins are usually derived from dry material) (16,17).

Essential oils can be extracted from different plants by various extraction methods. The essential oil extraction technology is dependent on used plants. Another factor for essential oil extraction is state and form of material. One of the important factors that has a great impact on the quality of essential oil is the extraction method. Inappropriate choice of extraction methods can damage the chemical structure of the essential oil. In fact, the extraction method is one of the prime factors that determine the quality of essential oil (7). generally, extraction methods can be divided into two groups: conventional methods (Distillation, Cold Pressing,

Table 1. Some essential oils with their major constituents.

Species	Popular Name	Major Components	The Main Organ Producing Oil
<i>Cinnamomum verum</i>	Cinnamon	Trans-cinnamaldehyde	Bark
<i>Santalum album</i>	Sandalwood	Santalol	Stem
<i>Mentha × piperita</i>	Peppermint	Menthone, Menthol, Isomenthone	Leaf
<i>Salvia officinalis</i>	Sage	Camphor, Thujone	Leaf
<i>Mentha spicata</i>	Spearmint	Carvone, Carveol	Leaf
<i>Origanum majorana</i>	Sweet marjoram	Terpenen-4-ol, Pinene	Leaf and flower
<i>Ocimum basilicum</i>	Common Basil	Chavicol, Linalool, Eugenol	Leaf
<i>Lavandula angustifolia</i>	Lavender	Geraniol, Linalool	Flower
<i>Artemisia dracunculus</i>	Wild Tarragon	Methyl chavicol	Leaf
<i>Thymus vulgaris</i>	Thyme	Thymol, Carvacrol, g-Terpinene, p-Cymene	Leaf
<i>Syzygium aromaticum</i>	Clove	Eugenol, Eugenyl acetate	Bud

Extraction Solvent, Enfleurage and Maceration Process) and novel methods (Supercritical Fluid, Ultrasound Assisted Extraction, Enzyme Assisted Extraction, Pulsed Electric Field Extraction, Pressurized Liquid Extraction, Microwave Assisted Extraction and High Voltage Electrical Discharge). Above-mentioned methods, such as distillation or supercritical fluid, can be useful in extracting volatile compounds and some technologies, such as solvent extraction, can be useful in extracting non-volatile compounds. In the last decades, investigation into novel technologies has led to more efficient extraction processes. Reduction of energy consumption and extraction time, high efficiency and improvement of essential oils quality are among the features of these methods.

Also, in addition to advantages, these methods include disadvantages, such as weather conditions, supply shortages, natural disasters, plant disease, etc. (18,19). So recently, scientists have been focused on biotechnological methods to produce a variety of plant compounds by microorganisms. In the new biotechnology method, which is a kind of green method, in addition to being environmentally friendly, there is no process pollution. In this method, scientists would not experience stress about problems, and a high-quality product is obtained. Biotechnology could provide an environmentally friendly alternative that does not require as much land and resources as traditional methods. Production of volatiles products by biotechnology can be an appropriate response to consumer demand in relation to the consumption of natural products. The aim of this review is to report production methods of

essential oils, including conventional, novel and biotechnology methods, and a generally comparison between them.

Conventional methods

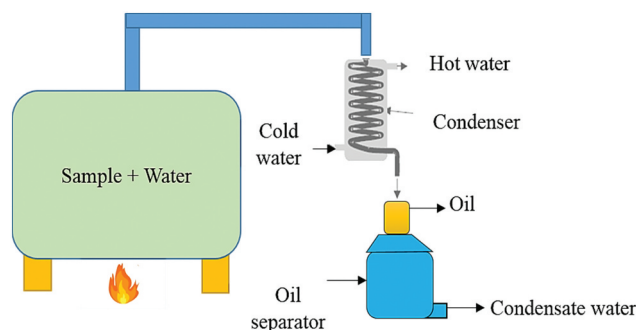
The conventional methods for the extraction include distillation, cold pressing, extraction solvent, enfleurage and maceration process.

Distillation

The most common method of essential oils extraction worldwide is distillation. During distillation, the essential oil of fragrant plants is released through evaporation. When the vapor pressure of liquids is equal to ambient pressure, it is converted to vapor (water + oil) followed by indirect cooling, vapor is condensed. Condensed mixture flows from the condenser to a separator, where oil is automatically separated from water (4,6,15,20). Types of distillation methods can be used, including hydro (water)-distillation, steam distillation, dry distillation and vacuum distillation.

Hydro-distillation

This method, as a standard method, is the simplest and cheapest method selected for essential oil extraction from plant material. In this method, the material is immersed in water and the mixture is boiled. The water and essential oil vapor are condensed to an aqueous fraction; the oil is separated from the water by a separator or decanter (Figure 1). Plant materials should always be immersed in water during the extraction process, so evaporated water should be replaced. This method is used to extract oil from dried flowers and powders. The main problem of this process is the loss of unstable and heat-sensitive compounds and their structural changes. Also, separation of water-soluble oils is difficult (4,7,21–23). Rose-scented geranium (*Pelargonium sp.*) (24), germander (*Teucrium orientale*)

**Figure 1.** Hydro-distillation of essential oils (21).

(25) and thyme (*Thymus vulgaris* L.) (26) were extracted by hydro-distillation and their efficiency was compared.

The turbo distillation process is the same as water distillation, the only difference in this method is stirring. The mixture is stirred continuously with a stainless steel stirrer at an appropriate speed. This method is suitable for coarse raw materials and hard-to-extract (spices, woods). In Turbo distillation, times and energy consumption are reduced and the degradation of compounds compared to aqueous distillation is prevented. In fact, it is a kind of green extraction of water distillation (Figure 2) (23,28). Among the essential oils that were extracted by turbo hydro-distillation method, the extraction of essential oils from *Kaempferia galangal* can be mentioned (27).

Water-steam distillation

The water-steam distillation system is similar to the water distillation design (Figure 3). The plant material is packed into the steel pot sitting on a grill above the boiling water. Due to the heat, the essential oil with water is converted to vapor and condensed. After that, the oil and water are separated from each other by a mechanical separator or decanter (21). It is used for dried and fresh plants that are not destroyed due to boiling. In this method, at first, the crushed dried plant material is mixed with water, then the flow of steam is passed through the wet material (29).

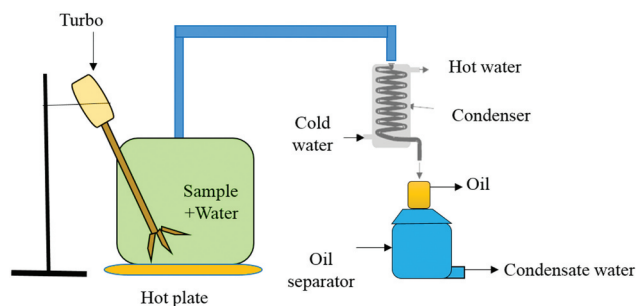


Figure 2. Turbo distillation of essential oils (27).

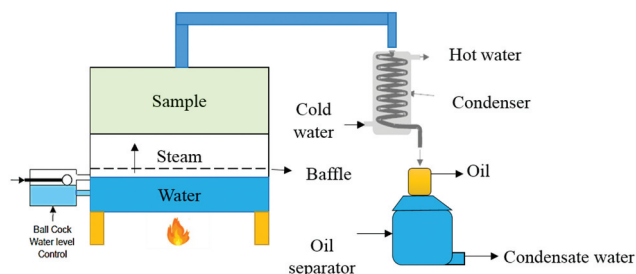


Figure 3. Water-steam distillation of essential oils (21).

Efficiency of essential oil extraction of *Cananga odorata* flowers by water-steam distillation was reported 43.25% (30). Also, production of citral oil from lemon grass (*Cymbopogon Cytratus*) was done by water-steam distillation with 40% efficiency (31).

Steam distillation

The most widely used method for extracting plant essential oil is steam distillation (7,32). Steam distillation is the process of distilling plant material with the generated steam in a boiler outside the still (Figure 4). As the steam-water distillation system, the plant material is placed on top of a perforated grid above the steam inlet (21). Either water and steam in this method are utilized, but the plant material is not in direct contact with water. Then generated steam in the boiler is flowed through a pipe into the bottom of the still. A mixture of water and oil is evaporated and condensed. Finally, the oil is separated by a separator. In this method, steam is always fully saturated, wet and never superheated (4,33). Also, the amount of steam is adjustable, and it is suitable for heat-sensitive compounds. Of course, this method is more expensive than the hydro-distillation and water-steam distillation. Extraction of *rosemary* essential oil by steam distillation was reported by Boutekedjiret (34). Also E. Cassel succeeded in extracting of *rosemary*, *basil* and *lavender* oils with 0.51%, 0.38% and 0.32% (w/w), respectively (35).

Hydro-diffusion extraction is a type of steam distillation where the only difference with the steam distillation is the inlet way of steam into the still. Steam is entered from the top of plant material, whereas in steam distillation, is entered from the bottom. This method is used when the plant material has been dried and is not damaged at boiling temperature (36). This process can be also worked under low pressure or vacuum and the steam temperature is reduced to below 100°C. This method is superior to steam distillation due to advantages such as shorter isolation times, higher efficiency and less used steam. Hydro-diffusion is also known as down hydro-diffusion or hydro-diffusion and gravity.

Dry distillation

Dry distillation is involved in heating in the absence of oxygen and heat is applied usually as a direct flame to the vessel. After releasing the essential oil at high temperature, the vapors of essential oil are condensed to produce essential oil. In extraction through dry distillation, many organic compounds may decompose and pyrolysis. This method is used to extract essential oils with a high boiling point from wood or coal.

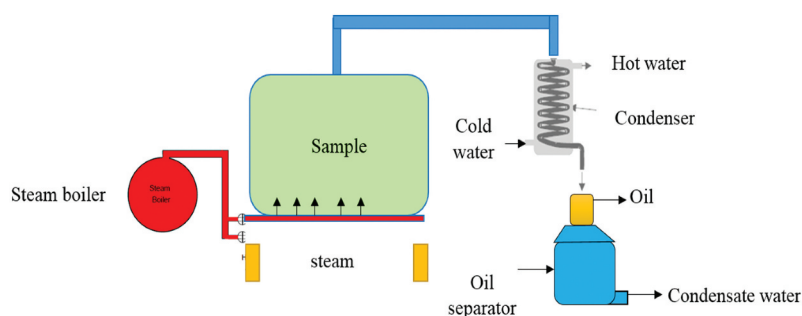


Figure 4. Steam Distillation of Essential Oils (21).

Birch tar and cade oils are produced by this method (20).

Vacuum distillation

The essential oil is obtained from plant materials under a vacuum varying in intensity depending on the material (37,38). The evaporation temperature of the material is lower under vacuum. As a result, materials can be separated by consuming less energy. Vacuum distillation is a suitable method for separating components with high boiling point and the destruction of plant tissue during extraction is prevented (16). This method is rarely used to distill oil directly from plant materials. For example, to extract volatile oil from oregano, the vacuum distillation method was used with an 8% increase in efficiency as compared to the conventional hydro-distillation (39).

Table 2 shows a generally comparison between conventional distillation methods (hydro-distillation, water-steam distillation, steam distillation). In this table, the construction, temperature, pressure, plant material suitability, use of Hydro-diffusion, hydrolysis condition and rate of distillation and yield are compared (16,21).

Cold pressing

Cold pressing is an extraction traditional method specific to citrus species (orange, bergamot, grapefruit, lemon, etc.) to produce essential oil. During extraction, oil glands are broken and volatile oils are released. The oil glands are located in the fruit rind or outside of the mesocarp. Figure 5(a) shows a cross section of a citrus fruit (Figure 5). This method is also called 'Expression' (6,23,40). In this physical method primarily, fruit peels are crushed and pressed between two rotating cylinders to express the volatile oils in the peels. In this section, a mixture of water and oil is produced simultaneously. Finally, the mixture is allowed to settle. Then the collected oil is washed with water and followed by

a centrifuge for separation (41). Figure 5(b) represents a schematic of the cold pressing (23). Today the systems of cold pressing can be classified into four categories: "Sfumatrici" 'machines and "Speciale Sfumatrici", 'Pellatrici' 'machines', 'FMC whole fruit process' "mand" 'Brown oil extractors (BOEs)' (6,42).

Soto et al. (43) used an enzymatic hydrolysis combined with cold pressing to obtain borage (*Borago officinalis*) seed oil for better yield. While, Anwar et al. (44) studied the effect of different enzymes on the yield of cold pressing and showed this system has higher yield in comparison to without the enzyme.

Extraction solvent

Solvent extraction method is used to extract non-volatile essential oils (45). The plant materials are placed in a fresh solvent and non-volatile essential oils of plants are removed. The solvent is more enriched with the oil compounds of plants during extraction, followed by filtration. Finally, the filtrate is concentrated by solvent evaporation and a thick residue that is called "concentrate" is prepared. Then the concentrate is mixed with alcohol to extract the oil compounds. oily compounds are absorbed by alcohol. Followed by distillation of alcohol at low temperatures, the concentrated compound called "absolute" is obtained for use in perfumery. Used solvents in this method should have a low boiling point, be free of odors and impurities, and should also be inert to oil compositions (7,46,47). Different solvents are used in this method, such as acetone, hexane, petroleum ether, methanol, or ethanol (48–50).

The most important advantage of this method is the appropriate and low temperature (50°C). Solvent extraction methods have disadvantages such as solvent toxicity in some cases, high solvent consumption, flammability of solvents and the most important factor is the remaining solvent in the final product (23). Improved concentration of citrus essential oil by solvent

Table 2. Comparison of conventional distillation methods.

Method	Construction	Plant Material Suitability	Pressure	Temperature	Hydrolysis	Hydro-diffusion	Rate-Yield
Hydro Distillation	Simple, based on ancient designs	Not suitable for finely powdered materials. Not suitable for materials that contain acidic material which can saponify, water soluble, or high boiling constituents. Materials must be covered by water.	Atmosphere	100°C	High rate of ester hydrolysis	Excellent	Low, due to hydrolysis and loss of water soluble constituents into water, high boiling constituents often un-distilled.
Water-Steam Distillation	Plant materials are placed on a grill over boiling water.	Can be used for the most herbs and leaves. Materials must be packed in a uniform way to avoid channeling steam.	Atmosphere	Approximately 100°C	Usually low, although excessive wetting of material through prolong distillation can promote hydrolysis.	Good	Moderate rate, Good yield
Steam Distillation	Using an external source of steam.	Most materials except fine powders. Suitable for high boiling materials. Materials must be packed in a uniform way to avoid channeling steam.	Both high and reduced pressures.	Can be increased or reduced according to pressures used.	Slight hydrolysis	Steam should be slightly wet to promote diffusion. Superheated or high press steam can dry out the plant and inhibit diffusion	Fast tare, good yield

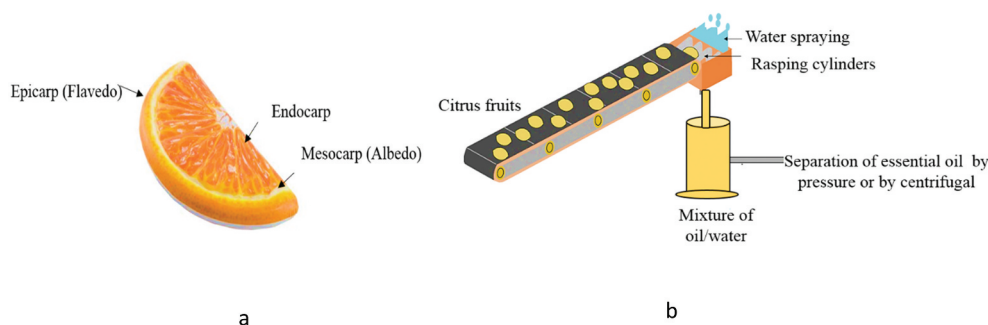


Figure 5. a) The parts of a citrus fruit (9), b) Schematic of cold pressing to obtain essential oil.

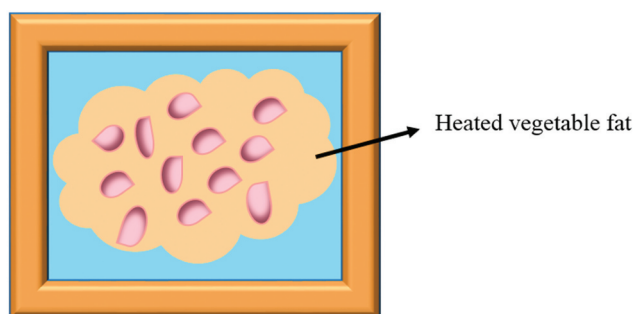


Figure 6. Hot enfleurage Process.

extraction with acetate ionic liquids was reported by Sara Lago (51). The results showed a great performance of these acetate-based ionic liquids. Extraction of essential oil from dried sage (*Salvia officinalis*) using ethanol – water mixtures was studied by Nicola E. Durling (52). The extract included 6.9% rosmarinic acid (55% recovery), 10.6% carnosic compounds (75% recovery) and 7.3% essential oil (42% recovery).

Enfleurage

This is an old method of extracting essential oils and is not commonly used today. Oil or animal fats (usually goat) are spread out over glass plates in a frame. Flowers or plant material are placed on top of the layer of fat and pressed in. This may take days or weeks, depending on the species. Followed the oil is filtered to leave a product called "Pomade". Then the absolute is obtained by mixing the pomade with alcohol and followed by distillation (16,53). The depleted material is then replaced by fresh ones until the fat is enriched with aromatic substances (54). The enfleurage process can be done either "hot" or "cold" way. The only difference is the heating of the fat during extraction in the hot process (Figure 6). Essential oil extraction from the double-flower variety of tuberose (*Polianthes tuberosa*L) by "hot" and "cold

effleurage was investigated. The results showed extracts including 0.3137%, 6.5808%, respectively (55).

Maceration process

Maceration process is used as a traditional and common method (56). Macerated oils are referred to as infused oils (15). Macerated oil has an advantage over distilled oil because it is produced more essential oil due to the absorption of larger molecules and most of the plant's valuable compounds are preserved.

Maceration is a variation on enfleurage. In this method, plant material should be dried because any moisture can deteriorate the oil and lead to the growth of germs. In the maceration process, plant material is finely crushed, or ground into coarse powder and is placed in a closed vessel. Solvent (Menstruum) is added. The mixture is allowed to stand for 1 week and is shaken occasionally (depending on the plant species). Liquid is pressed and finally, is clarified through filtration or subsidence. Maceration method is useful for flowers whose physiological properties are lost rapidly after harvest. For example, lily of valley. In the 'hot maceration' process, the plant material is crushed or ground into coarse powder and is immersed in molten fat at 45–60°C for 1 to 2 h depending on the plant species. Therefore, due to heat and immersion in fat, the long enfleurage time is reduced. After each immersion, the fat is filtered and separated from the plant material. The fat is separated from waste flowers after 10 to 20 immersions. Finally, absolute maceration is obtained through the process of extraction and concentration under reduced pressure (15,16,54,56,57). Figure 7 shows a schematic of the maceration process (Figure 7). Such as extraction of flavonoids from the garden (*Salvia officinalis* L.) by a classical maceration method was examined with two types of

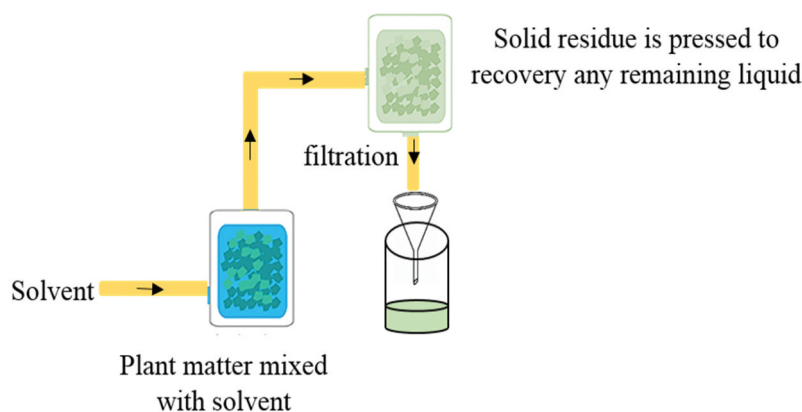


Figure 7. Schematic of maceration process.

polar and non-polar solvents. Higher yields of flavonoides were achieved using polar solvents (70% aqueous solution of ethanol and water) than the non-polar one (petroleum ether) (58).

Novel extraction methods

Novel extraction methods are also known as green methods. Green extraction can be defined as follows: Green extraction is based on extraction methods that reduce energy consumption, the use of alternative solvents and renewable natural products is allowed. Also, the process safety and product quality are ensured. In general, these types of extraction methods have the following characteristics (59).

- (1) Selection of varieties and renewable plant resources.
- (2) The use of alternative solvent.
- (3) Reducing energy consumption.
- (4) Production of co-products instead of waste.
- (5) Process control under safety aspects.

- (6) Production of biodegradable extracts without contamination.

These green methods have been considered as new methods including supercritical fluid extraction (SFE), subcritical extraction liquids, enzyme-assisted extraction, ultrasound-assisted extraction (UAE), microwave-assisted extraction (MAE), (PEF) pulsed electric field (PEF), pressurized liquid extraction (PLE) and high voltage electrical discharge (HVED) for the extraction of essential oil.

Supercritical fluid extraction (SFE)

One of the alternative methods for extracting essential oils is supercritical fluids Extraction. SFE is based on the use of solvents in their supercritical state. A supercritical fluid is any substance at a temperature and pressure above its critical point (the critical point is the highest temperature and pressure at which a pure material can exist in vapor/liquid equilibrium). This means that the temperature and pressure above which this phase exists

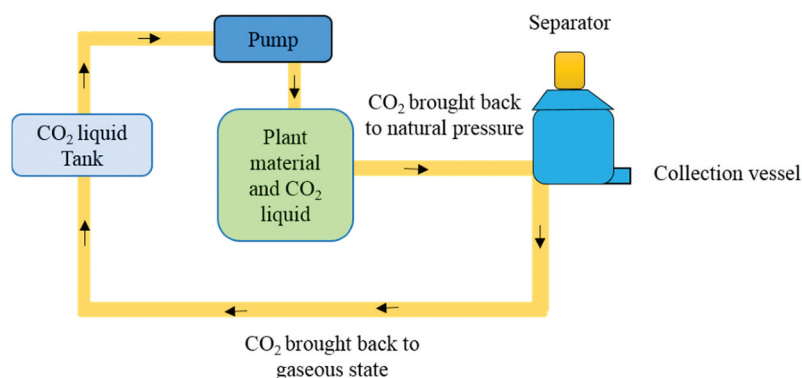


Figure 8. Supercritical fluid extraction (SFE).

is the critical point. SFE has a unique property. Supercritical fluid is between the liquid and gas phases depending on the pressure, temperature and composition of the liquids (7,9). In this method, the most widely used solvent is carbon dioxide, CO₂ (Figure 8). Due to moderate supercritical conditions (P_c : 72.9 atm, and T_c : 31.2 °C), it can be appropriate for heat-sensitive substances (60), it is not toxic and flammable; it is cheap and has high purity, it can be easily removed from the obtained extract (61), no solvent remains in the final product (62), etc.

SFE extracts show some properties of essential oils and absolutes. They have many beneficial therapeutic properties and, unlike absolutes; they are not extracted by chemical solvents; they are extracted using CO₂ gas under pressure and at room temperature.

Generally, extracts from SEF, have superior quality and better biological activities (63) compared to production of essential oil by hydro-distillation or liquid solvent (64,65). The high cost of capital and required skills in supercritical carbon dioxide extraction plant, also, the wide range of applications of the process, limits its use in industry. Rosemary, fennel and anise essential oils were obtained by supercritical fluid extraction. The results showed that extraction of supercritical fluids to obtain extracts is economically viable (66). Supercritical CO₂ extraction from *lavender* was performed by E. Reverchon (67). This compound showed 34.7% efficiency of the oil.

Subcritical extraction liquids (SEL)

Subcritical state is liquid under pressure at temperatures above usual boiling point. In fact, liquid is maintained in liquid form by applying pressure. Water and CO₂ are the most commonly used fluids in industrial applications. In the supercritical region, distinct liquid and gas phases do not exist. A supercritical fluid is similar to gas in terms of diffusion coefficient and is similar to liquid in terms of density (68,69). Process simplicity, low-cost and positive environmental impacts are advantages of this method. This technique has the advantages over other traditional extraction techniques including shorter extraction time, high-quality product, reducing extraction costs, an environmentally friendly method and low solvent consumption (60). When the temperature is between 31 and 55°C and the pressure is between 5.5 MPa and 4.7 MPa, the subcritical state of CO₂ is occurred. In this case, CO₂ behaves as a non-polar solvent (70). In the subcritical state of CO₂, the thermal degradation is prevented. K. Taraj reported extraction of essential oils of *chamomile* by subcritical CO₂ using high-pressure (65 bars). The extraction performed at 40°C and showed the high-yield (71).

Subcritical water extraction (SWE), also known as “pressurized hot water”, has dynamic conditions (under high-pressure to maintain the liquid state and temperature in the range of 100°C to 374°C) (4). Generally, the obtained extracts by subcritical carbon

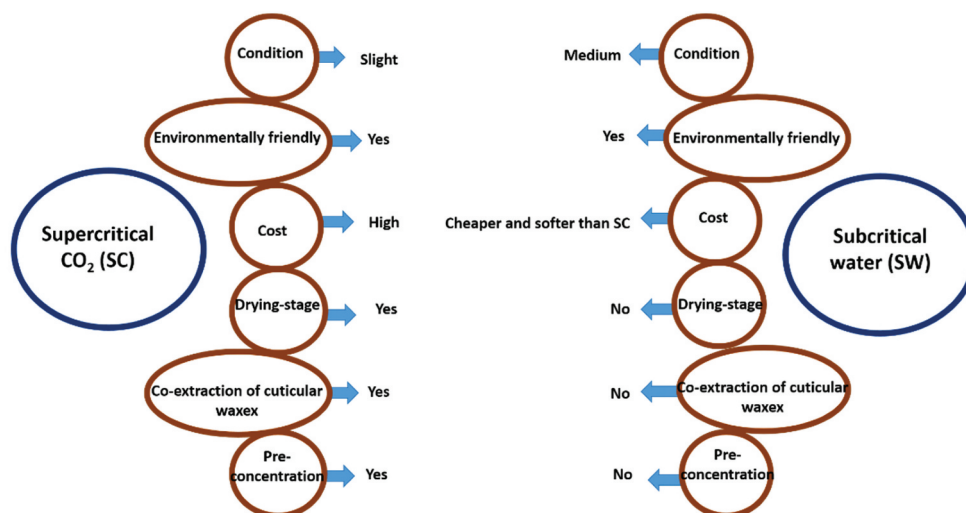


Figure 9. A comparison between supercritical CO₂ and subcritical.

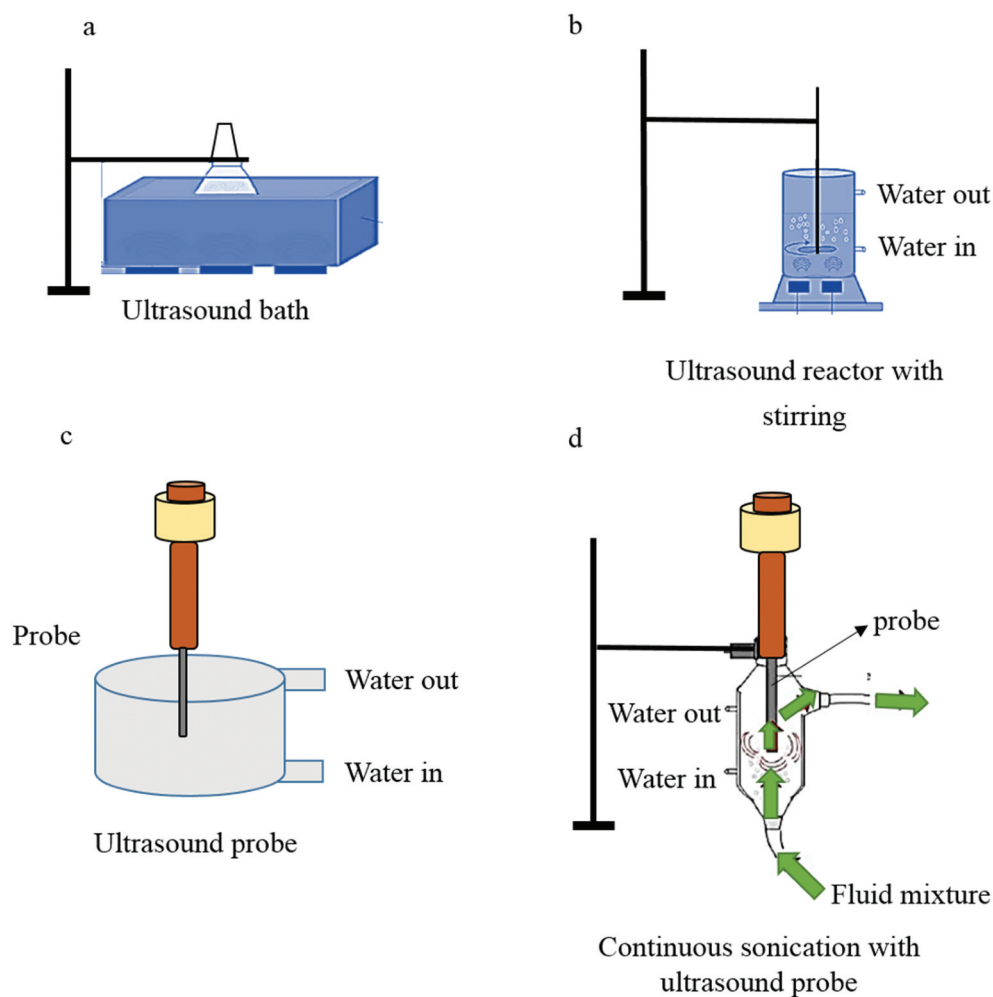


Figure 10. Ultrasonic systems (a: Ultrasound bath, b: Ultrasound reactor with stirring, c: Ultrasound probe, d: Continuous sonication with ultrasound probe) (78).

dioxide have high-quality and are better functional compared to SWE (72). According to studies, based on a comparison between supercritical CO₂ and SWE (73), it is concluded that although the SWE process is cheaper and softer than supercritical CO₂, but it is still expensive to operate due to the need for special equipment (74). The following schematic (Figure 9) compares supercritical CO₂ with subcritical water (60,74). For SWE, 130°C and 20 min extraction time were found optimal for obtaining high content of bioactive compounds. The results of this method were evaluated with other novel methods, such as microwave-assisted extraction, and showed lower efficiency (75). For example, Mustafa. Z. Ozel reported subcritical water extraction of essential oils from *Thymbra spiced* (76).

Ultrasound-assisted extraction (UAE)

In the ultrasound-assisted extraction (UAE) method, the plant material is immersed in water or solvent and

exposed to ultrasound. The ultrasonic waves have a frequency of 20 kHz – 1MHz depending on plant species. In this method, the created mechanical vibration by ultrasonic waves causes plant cell walls to rupture. As a result, essential oil droplets are released. In other words, the cell walls are diffused, and once the walls are broken, the essential droplets are washed out (9,77). The size of the plant material is an important factor. In the small-size material, the more cells are exposed to the waves (9). Figure 10 shows commonly used ultrasonic systems (78).

Compared to traditional methods, in the UAE, extraction time and temperature are improved and the range of solvent selection is increased. Also, high-quality products with higher efficiency are produced. This method is useful for heat-sensitive substances. Compared to other new techniques, the equipment is relatively simple and inexpensive (9,79). For example, citrus (lemon) were extracted by the UAE. According to

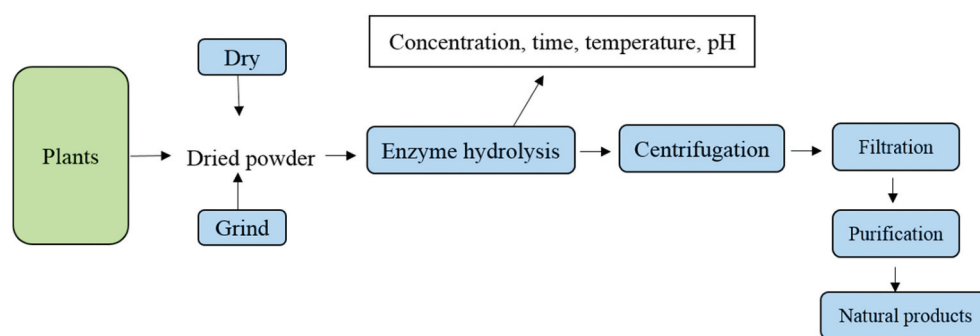


Figure 11. Enzyme-assisted extraction (81).

the result, the main component was Linalool with 11.3% efficiency (80).

Enzyme assisted extraction

Releasing essential oils from plant cells can be done by the green 'Enzyme Assisted Extraction' method. This method is based on the ability of enzymes to catalyze reactions under moderate conditions in aqueous solutions for releasing bioactive compounds. Enzyme-assisted extraction method is an environmentally friendly extraction technique (Figure 11). The used enzymes can be obtained from bacteria, fungi, animal organs and plant extracts. In this method, important factors such as efficiency, operating conditions such as time, temperature, pH, the ratio of enzyme to substrate and substrate particle size should be considered. The advantages of this method are: low solvent consumption, improving efficiency and quality. The limitations and problems of this method are: The relatively high-cost for large volume of raw materials, enzyme preparation and the problems of industrialization of process (due to the complex and different behavior of enzymes, it is depending on factors such as dissolved oxygen content, temperature and available materials) (81). There are two routes to enzyme-assisted extraction: enzyme-assisted aqueous extraction (EAAE) and enzyme-assisted cold pressing (EACP) (82). *Glycosidics*

essential oils (bitter almond and mustard essential oil) were obtained by enzyme-assisted extraction (17).

Pulsed electric field extraction (PEF)

PEF is a new and green technology for extracting valuable compounds from wastes and food-agricultural by-products. The pulsed electric field is applied to the material between two electrodes with a pulsed amplitude. In this system, the pulse amplitude is changed from 100–300 V/cm to 20–80 kV/cm with low energy 10–20 Kj/Kg. The process is performed at ambient temperature or slightly higher than ambient temperature in less than 1 second (microseconds or milliseconds). In this process, by exposing plant cells to pulsed electric field, cell membranes are damaged and temporary or permanent cavities are formed. The process of damaging cells and forming cavities with electricity is called electroporation (82–84). E. Bozinou succeeded in application of the pulsed electric field (PEF) technique to the production of extracts from *Moringa oleifera* plant material (freeze-dried leaves) (85).

Pressurized liquid extraction (PLE)

This green method was proposed by Richter et al. for the first time (1996) (86) as an environment-friendly method. The technology is now known by various names: Pressurized liquid extraction (PLE),

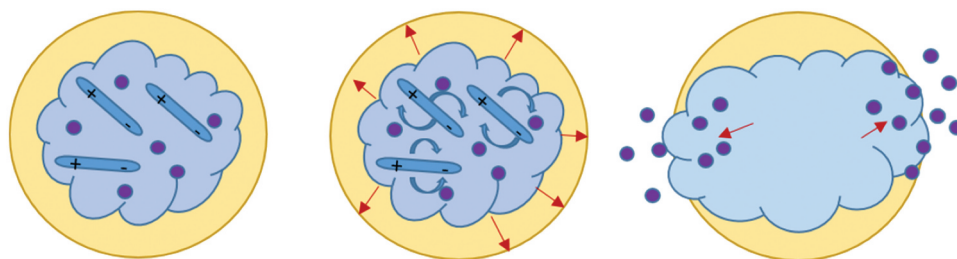


Figure 12. Mechanism of Microwave Extraction of Essential Oils Based on Dielectric Heating (23).

Accelerated solvent extraction (ASE), Enhanced solvent extraction, Subcritical water extraction (SWE) and Solvent extraction at high pressure (87,88). The use of high pressure (500–3000 psi) and temperature (50–200°C) results in a reduction of the extraction time and the solvent consumption, increase of extraction efficiency and process simplicity. This method is not suitable for heat-sensitive compounds due to high temperature (23,88,89). Water is considered as one of the used solvents in this method that, if maintained in a liquid phase under pressure, could show a variety of behaviors such as methanol or ethanol. In general, this technology due to the technological challenges is not considered as a valuable method on an industrial scale (88,90,91). Safflower oil extraction process using pressurized ethanol was reported successfully (92).

Microwave assisted extraction (MAE)

This green method was first proposed in 1986 (93). Microwaves radiations including, X-ray and infrared rays, are characterized by frequencies between 300 MHz to 300 GHz. In this method, which is also called “dielectric heating” or “high-frequency heating”, electromagnetic energy due to ionic conductivity and the molecular dipole rotation is converted to heat under the influence of the electric field (Figure 12). So, the only dielectric materials and solvents with permanent dipoles can be heated under a microwave.

In fact, electromagnetic waves lead to changes in cell structure, which, in turn, distinguishes MAE from other methods (94). In this method, due to the microwave energy, moisture inside the cells heats and evaporates, which, causes rupture of the cell wall. In fact, heat transfer occurs inversely from inside to outside in comparison with normal heating. The quality and efficiency of the final product is affected by parameters such as solvent, temperature and exposure time, pressure, sample viscosity, microwave power output and physico-chemical properties of materials (4,95–97). The advantages of this method over other common methods include reduction of the extraction time and solvent consumption, low temperature, energy saving and increasing efficiency (4).

Recent Advances in the microwave extraction method have led to the development of other methods of microwave extraction including microwave assisted solvent extraction (98), vacuum microwave hydro-distillation (99) microwave hydro-distillation (100), solvent-free microwave extraction (101), microwave accelerated steam distillation (102), microwave hydro-diffusion and gravity (MHG) (36) and microwave-assisted simultaneous distillation-solvent extraction

(103). Figure 13 gives a schematic description of microwave assisted extraction methods (20). Essential oil extraction by microwave steam diffusion (MSDF) was performed. 125 g raw material (*Lavandula Hybrida*) was soaked in water for 10 min, 500 W, t: 15 min, and the yield was obtained 5.4% (28). Essential oil of citrus *Camellia Sinensis* with yield of 5.43% was obtained by microwave steam distillation (MSD) (104).

High voltage electrical discharge (HVED)

HVED is a method of green extraction based on physical and chemical processes and interjects energy directly in an aqueous solution between immersed electrodes in water (105,106). In the HVED method, cellular tissues are disrupted and the valuable compounds are released. This extraction system can be divided into three different categories: discontinuous, continuous and eddy current systems. The basis of these systems is similar: discharge due to high intensity spatial electric field, cell destruction and increasing mass transfer due to various secondary phenomena. Their differences are related to the structure of the systems, especially the electrodes, and focus modes of the spatial electric field. The process of dielectric decomposition results from liquid ionization and occurs with high voltage (30–40 kV) and short pulse intensity approximately (10 kA) between two electrodes. The process mechanism is summarized by the following steps: electric pulse generation, electrostatic discharge, formation electric arc. Some major advantages of HVED technology over conventional methods are: higher extraction rate, reduction of processing time, higher mass transfer, lower temperature, reducing solvent consumption, reducing degradation of heat-sensitive compounds and environmental impacts, saving energy etc. (107). For example, extraction of bioactive compounds and aromas from Rosemary (*Salvia rosmarinus*) by means of high voltage electrical discharge (108).

The main difference between PEF and HVED is the geometry and composition of their electrodes (83).

Some essential oils with their major constituents were randomly selected and are presented in Table 3 with the different extraction technologies. Table 4 shows a generally comparison between bioactive compounds green extraction technologies, including flavors and fragrances (107,117).

Cohobation system

In the distillation method, distilled water (hydrosol (118)) after separation from the oil can be recycled and reused for another distillation process. This process

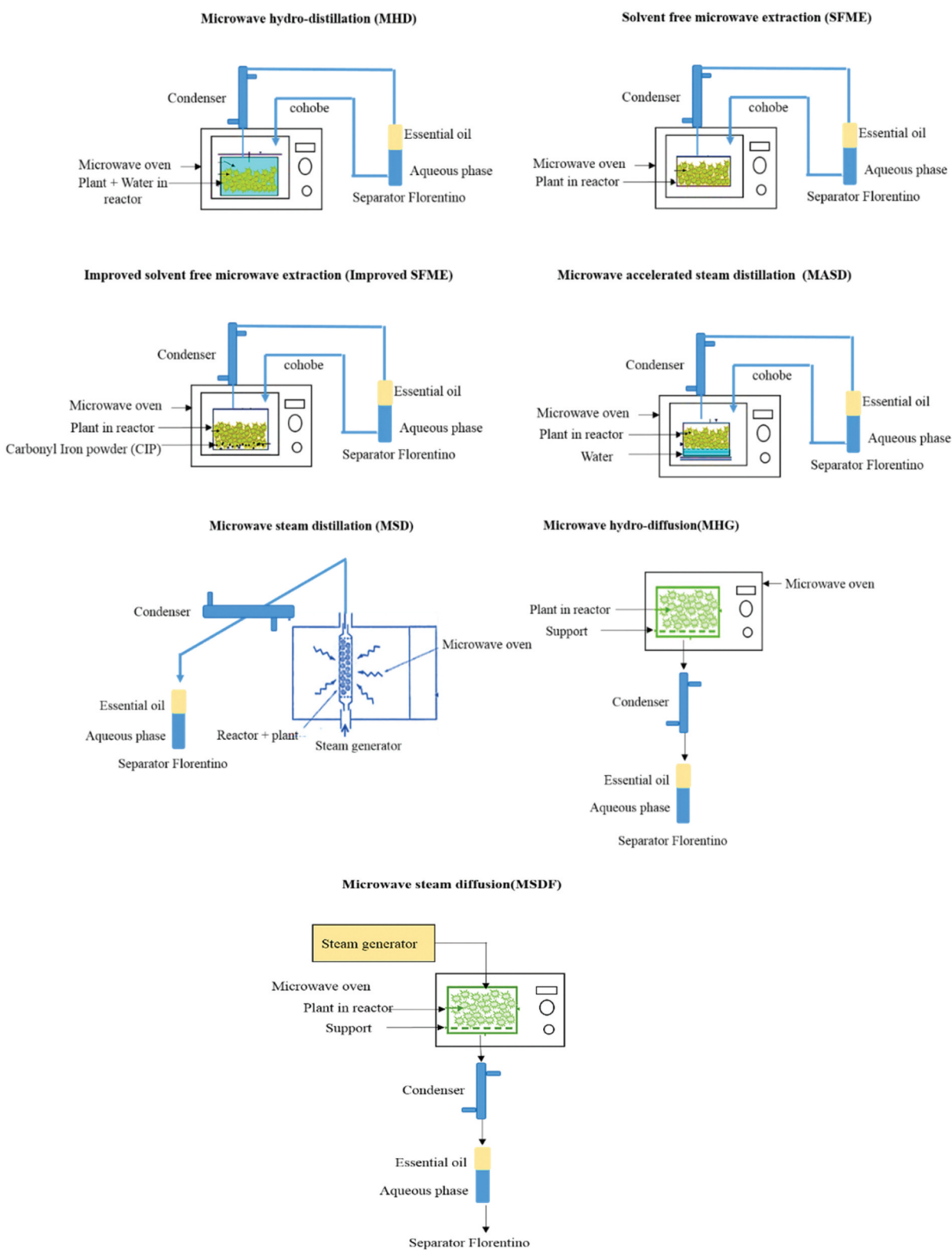


Figure 13. A schematic of microwave assisted extraction methods (20).

Table 3. Different extraction technologies of some essential oils and their major constituents.

Species	plant	Major Components	Technique Extraction	Ref
<i>Boswellia serrata</i>	Indian frankincense	(3E,5E)-2,6-Dimethyl-1,3,5,7-octatetraene	hydro-distillation	(109)
<i>Protium aracouchini</i>	Breu	<i>p</i> -Cymene	hydro-distillation	(110)
<i>Satureja hortensis</i>	savory	Carvacrol	microwave-assisted hydro-distillation	(111)
<i>Salvia rosmarinus</i>	Rosemary	1,8-Cineol	steam distillation	(34)
<i>Ocimum basilicum</i>	Basil	Linalool	hydro-distillation	(112)
<i>Citrus latifolia</i>	Tahiti lime	Limonene	steam distillation hydro-distillation	(113)
<i>Salvia officinalis</i>	sage	Linalyl acetate	Solvent extraction	(114)
<i>Syzygium aromaticum</i>	Clove	1,8-cineol	supercritical fluid hydro-distillation	(115)
<i>Aerva javanica</i>	Kapok bush	Eugenol	supercritical fluid steam distillation	(116)
<i>Citrus limon</i>	lemon (lemon peel)	Heptacosane	hydro-distillation	(80)
		Linalool	ultrasound Assisted Extraction	

is called 'Cohobation' (Figure 14 (119)). It is believed that will be controlled the loss of water-soluble oxygen compounds in hydrosol. This is due to the saturation of water with dissolved components which prevents further extraction. It is worth noting that the cohobation method is not recommended for high temperatures (above 100 °C), because in the case of continuous and direct contact of an oxygenated element with heat, the possibility of degradation and hydrolysis will be promoted (56,57,120). This type of system is a kind of continuous system and reduces extraction time and costs.

There are other types of continuous systems known as continuous systems. In the late twentieth century, essential oil producers had to load and unload still after each process regularly. So they tried to create a process that could produce essential oils continuously. In such a way, plant materials are slowly loaded into the upper still and are removed from the bottom of the still. This method is not suitable for inflorescences, fruits and roots (9).

Biotechnology for the production of essential oil

As mentioned in the previous sections, natural and volatile compounds have become commercially popular due to flavor and aroma properties. The concentration of the most desirable volatiles in essential oils is very low, about less than 10% (sometimes even less than 1%) (121). Essential oils are usually obtained through extraction of intact plants (18) such as distillation, solvents, carbon dioxide, etc. or by the methods of chemical synthesis. Each of them has drawbacks. Chemical synthesis often leads to the production of a mixture of

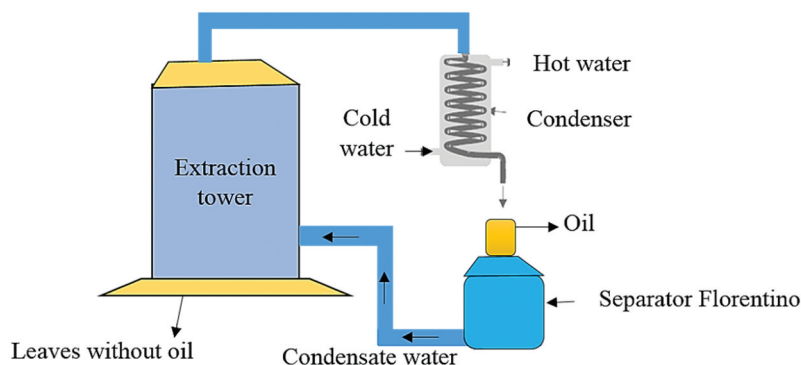
products with approximation quality. Also, this method is not the environmentally friendly method (121). Common extraction methods, in addition to being costly, have problems such as weather conditions including monsoon, drought, or even by volcano, supply shortages, natural disasters, plant diseases, low efficiency, etc (18,19). Therefore, due to the limitations and disadvantages of methods of extraction from intact plants or chemical synthesis, the attention of scientists has been focused on biotechnology methods that a wide variety of plant compounds are produced by microorganisms. In the new biotechnology green method, in addition to being environmentally friendly and no contamination of the process, there are no more problems mentioned, and a high-quality product with good efficiency is obtained. Production of natural and volatile products by biotechnology can be an appropriate response to consumer demand in relation to the consumption of natural products. One of the major applications of biotransformation is the production of biotechnology products that are usually synthesized by chemical methods. Bio-methods include volatile production through tissue and cell cultures, hairy roots cultures, culture of microorganisms, biotransformation, metabolic engineering. Various biotechnological methods for the production of volatiles are discussed below:

De novo production of volatile compounds through tissue and cell cultures

One of the most important techniques in the industrial production of volatile compounds is plant cultivation. The used techniques in this field are mainly: cell cultures, tissue cultures, hairy roots cultures, microorganism cultures.

Table 4. Comparison of green technologies in the extraction of natural compounds.

Disadvantages	Advantages	Technique
It is not suitable for the most medicines and herbal. Polar molecules are not soluble. Expensive	Compared to liquid extraction: Less viscosity, more penetration, better mass transfer, environmentally friendly, saving time, no solvent residue in product, better functional, room temperature, useful for compounds sensitive to heat.	SFE
Ultrasonic frequency, factors, nominal power, input power, system geometry and size of the plant materials are effective in increasing performance.	Reducing energy consumption, more extraction efficiency, saving time Reducing Consumption of chemical materials, useful for compounds sensitive to heat.	UAE
Relatively high cost for large volume. Not suitable for industrial scale due to complex conditions of the enzyme. Particle size, moisture, hydrolysis time, operating conditions should be controlled.	Environmentally friendly, Suitable for extracting bonded compounds, High extraction rate, low solvent consumption, More extraction efficiency and quality.	Enzyme Extraction
Process parameters are input energies, process temperature, field resistance.	Short extraction time and better efficiency. The refining processes are easy. Reducing environmental impacts. Reducing energy consumption. Ambient temperature or slightly higher than ambient temperature.	PEF
High equipment costs and is not suitable for samples weighing more than 100 grams. Not considered as a valuable method on an industrial scale due to the technological challenges	Suitable for solid samples. It is more suitable for polar compounds than SFE, Reducing extraction time and solvent consumption and increases extraction yield. Not suitable for compounds sensitive to heat	PLE
Equipment is expensive. Operation is more difficult than UAE. They are less environmentally friendly due to the use of organic solvents. Less extraction efficiency for non-polar compounds. Not suitable for compounds sensitive to heat. Parameters such as solvent, temperature and exposure time, pressure, viscosity, microwave output power and physicochemical properties of materials are important.	Better quality and higher selectivity, higher extraction efficiency, less extraction time and volume of solvent, energy saving. Compared to SFE, the operation is simpler and more economical.	MAE
Less selectivity than PEF, Their feasibility for industrial scale or pilot has not been studied yet.	Reducing energy consumption than new methods such as MAE and UAE. Saving time. Reducing solvent consumption and less penetration temperature	HVED

**Figure 14.** The water-steam distillation method by cohobation for extraction essential oil (119).

Cell cultures

It seems that each cultured cell has all the genetic information of the parent plant and, therefore; it is capable of producing chemicals in the plant (5). Through this culture, the desired volatile compound can be prepared. For reasons such as scarcity, the slow-growth plants, it is expected the *in vitro* under controlled conditions will be accelerated biomass propagation to produce essential oil (122). The yield of this method is affected by the factors of nutrients and growth regulators of the culture medium such as sugar, nitrogen, phosphate, growth regulators, precursors and suitable culture medium (123). But the yield of the obtained compounds is low (0.01–0.1 g/l day) (122), for reasons that are not yet clear (121). So

generally, this method does not seem to be a promising method, even with effective agents.

Tissue cultures

Due to the secondary metabolites synthesis in the differentiated tissues, volatile compounds production through cultures and differentiated organs such as root stems, etc. is preferred (124) Production of volatile compounds by hairy roots culture described below.

Hairy roots culture

Hairy root culture is a type of plant tissue culture for the production of volatile compounds (125). The formation of hairy roots in plants or hair-like root structures is

Table 5. A list of produced volatile compounds by the cell, hairy roots, and microorganism.

	Plant	Microorganism	Substrate	Product	Ref
Cell Culture	<i>Vanilla planifolia</i>	-	-	Vanillin	(129)
	<i>Smyrniun perfoliatum</i>	-	-	α -Pinene	(130)
	<i>Coleonema album</i>	-	-	Monoterpenes	(131)
	<i>Artemisia dracunculus</i>	-	-	phenylpropenes of the essential oil	(132)
	<i>Mentha piperita</i>	-	-	Mint oil components	(133)
Hairy Root Culture	<i>Cucumis melo</i>	-	-	2-Hexenal as main ingredient	(134)
	<i>Pimpinella anisum</i>	-	-	Yield of essential oil was comparable to that of normal plant.	(135)
	<i>Ambrosia trifida</i>	-	-	Essential oil of roots was similar to that of the normal roots.	(136)
	<i>Anethum graveolens</i>	-	-	Essential oil yield was only 0.02%, compared to 0.06%, 0.3% and 2% of normal plant root, leaf and fruits, respectively.	(137)
Microorganisms Culture	-	<i>Aspergillus oryzae</i>	castor oil	g-Decalactone, 0.86 g/l	(138)
	-	<i>Aspergillus nige</i>	Rice bran oil (4 g/l ferulic acid)	2.8 g/l Vanillin	(139)
	-	<i>Kluyveromyces marxianus</i>	Grape must + Phe	2 phenylethanol, 0.4 g/l	(127)
	-	<i>Sporobolomyces odoros</i>	Hydrolysed castor oil	γ -Decalactone, 5.5 g/l	(140)
	-	<i>Stigmatella aurantiaca</i>	Agar cultures	C ₁₀ volatile ketones	(141)

Table 6. Produced volatile compounds by biotransformation of plants or microorganisms.

	Plant/Microorganism	Substrate	Product	Ref
Plants or Microorganisms	<i>Capsicum annum</i>	Ferulic acid, 2.5 mM	Vanillin, 10 mg/l	(142)
	<i>Rosa (roses)</i>	Geraniol	Nerol, citronellol	(151)
	<i>Lasiodiplodia theobromae</i>	(+)-Valencene (sesquiterpene), 0.4 g/l	Nootkatone	(152)
	<i>Rhodococcus opacus</i>	Limonene	Carveol, carvone	(153)
	<i>Fungi and algae</i>	β -Ionone	Hydroxyl and oxo derivatives	(154)
	<i>Fragaria (strawberry)</i>	α -Ketovaleerat	Butanal, butanol	(155)

actually the result of a plant disease caused by a gram-negative bacterium called “*Agrobacterium rhizogenes*”. This roots are used as a source for the production of aromatic and volatile substances (126). Basically, the importance of hair roots is due to their rapid growth without the need for an external auxin (5) compared to conventional roots. They are produced volatile compounds at levels and patterns similar to natural roots, but secondary metabolites or volatile compounds are also produced in the aerial parts of plants. The efficiency of this method is higher than cell culture and, by the ingress of abiotic or biotic elicitors in the culture medium, efficiency can be increased (121).

Microorganisms culture

The culture of micrograms is another method of biotechnology. Microorganisms (such as bacteria, fungi, algae ...) are more potent than plant cells in bioreactor conditions (121). Also, the use of immediate precursor, increases the efficiency. In this case, the method is similar to biotransformation, which will be explained in the next section. For example, 0.4 g/l 2-phenylethanol with rose-like aroma can be produced by *Kluyveromyces marxianus* (127). Also, 2.8 g/l vanillin was achieved by

cultured *Aspergillus Niger* (128). Table 5 presents a list of produced volatile compounds by cell, hairy roots, and microorganism cultures.

Biotransformation

Biotransformation is a chemical modification and is performed by an organism on a chemical compound. In other words, microorganisms, through specific chemical reactions, can cause special changes in some compounds. This process is called ‘biotransformation’. In this method, either the whole-cell of the microorganism or the isolated enzymes of the microorganism are used. The advantages of biotransformation such as high purity product, minimal side reactions and balanced process, high efficiency, economical, environmentally friendly and production of compounds that cannot be produced by traditional methods have caused this method to be superior to other methods.

The used techniques in this field are mainly: biotransformation by plant cultures and microorganisms, biotransformation by isolated enzymes. Each of which is briefly explained in the next section:

Table 7. Produced volatile compounds by biotransformation of isolated enzymes.

	Isolated Enzyme	Source	Product	Ref
Isolated Enzymes	<i>Lipoxygenase/hydroperoxide lyase</i>	Apple pomace, crude mixture	Production of hexanal and 2,4-decadienal from unsaturated fatty acids.	(156)
	<i>100 kDa cut-off extract</i>	Tomato fruits	5 µg/min hexanal from 16 mM linoleic acid	(157)
	<i>Enzyme mix</i>	Citrus poonensis fruit peel	30% of limonene was converted to terpineol, linalool, and linalool oxide	(158)
	<i>Amine oxidase</i>	<i>Aspergillus niger</i>	Production of vanillin from vanillamine	(159)
	<i>Germacrene A hydroxylase</i>	<i>Cichorium intybus</i>	Production of nootkatone from valencene	(160)

Biotransformation by plant cultures and microorganisms

Biotransformation by plant cultures and microorganisms is a good method based on biotechnology for the preparation of volatile compounds. In biotransformation, an intermediate precursor is incorporated into the culture medium, which is bio-transformed or bio-converted to volatile compounds by enzymatic activities. The efficiency of this method is much higher than the methods in which no precursor is used. But it should be noted that cheap precursors should be searched and used to produce valuable products (121). For example, 10 mg/l vanillin was obtained on ferulic acid substrate by cultured *Capsicum annuum* (142). Citronellol can be obtained by biotransformation of citronellal by the *Peganum harmala* (143). Also, the biotransformation of ricinoleic acid by *Candida sorbophila* can be produced up to 40 g/l γ -decalactone (144).

This method, in addition to the positive aspects, also has the negative aspects. For example, volatiles are not dissolved easily in an aqueous medium. Dissolution facilitating agents can be used to solve this problem (cell growth is inhibited above a threshold concentration.). Even a high concentrations of the product itself can be toxic and an inhibitor of cell growth. As a result, continuous addition of substrate at non-harmful concentrations and removal of the product was developed. Among these methods, the pervaporation method (145)

and inserting a volatile binder compound are most commonly used. The issue of trapping and isolating produced volatile compounds in the culture medium were discussed by Georgiev et al. (146); and Ramachandra and Ravishankar (5). For example, β -cyclodextrins have an amphiphilic structure, which is composed of a hydrophilic exterior and a hydrophobic cavity that, with proper modification, the product can be trapped in the cavities.

Biotransformation by isolated enzymes

Isolated enzymes typically have higher biotransformation rates than cell culture-based methods. But the cost of enzyme separation is played a major role in using that enzyme to produce volatiles (147). Enzymes have a high selectivity and reduce the production of undesirable by-products (148). However, most studies have been done by whole-cells systems rather than isolated enzymes (7%) (139), although this method has been significant (149), but only in cases of biological transmission, volatile compounds are presented. The use of isolated enzymes for biotransformation is depended on the availability of the enzyme, pH, optimum temperature conditions, solvent and substrate. The nature of the enzyme, the type of biotransformation reaction and the need for a cofactor are some influencing factors in the method. In order to facilitate the separation of the product and the recycling catalyst, the use of immobilizing the enzyme is preferred (150).

Table 8. A generally comparison of conventional, novel and biotechnology methods.

Aspect	Conventional	Novel	Biotechnology
Environmentally Friendly	In Some methods such as solvent extraction: No	Yes	Yes
Quality and Yields	Low	More than conventional	In some method: Excellent
Thermal Decomposition	In some methods: Yes	No	No
Processing Time	Long	Short	Short
Energy Consumption	High	Low	Low
Harmful and Expensive Solvents	Some methods such as solvent extraction, Yes	No	No
Need to Raw Material	Yes	Yes	No
Affected by Environmental Conditions	Yes	Yes	No
Cost	Low	High	Low

Table 9. Extraction technologies some material from agricultural products.

Material	Extract	Extraction Techniques	Ref
Rapeseed	Pesticides	Pressurized Liquid Extraction	(169)
Paprika Powder	Pigments	Microwave Assisted Extraction	(169)
Capsicum Fruit	Capsaicinoids	Microwave Assisted Extraction	(169)
Oilseed Such as Almond	Oil	Solvent Extraction	(170)
Palm Fruit	Oil	Enzymatic Extraction Method (Pectinase/Cellulase/Tannase	(170)
Industrial Tobacco Waste	Solanesol	Supercritical Fluid Extraction (CO ₂)	(171)
Kalahari Melon and Roselle Seeds	Tocopherol	Supercritical Fluid Extraction (CO ₂)	(170)
Potato Peel	Carbohydrates and Phenolic	Subcritical Water	(171)

The Tables 6 and 7 present a list of produced volatile compounds by biotransformation of plant cultures, microorganisms and isolated enzymes.

Metabolic engineering

Metabolic engineering is the most promising and the newest path for volatile production. Metabolic engineering is the use of genetic engineering to modify the metabolism of an organism to increase the cells' production by a certain substance (121,161). Large amounts of the fragrance and flavors can be produced by metabolic engineering without need for the rare plants (18). The use of genetically engineered strains was first reported in 2010 when oil extraction from *Pogostemon cablin* (*Patchouli*) was severely deficient. Because it has reported that the rainy weather in Indonesia is caused the destruction of medicinal shrubs. Also, other natural problems, such as volcanic eruptions and earthquakes, are exacerbated supply problems. As a result, in order to solve these problems and supply shortages, scientists have been focused on engineering the genetics of the strains to produce the fragrances of the *Patchouli* plant. In addition, fragrances of bitter orange, grapefruit, rose and sandalwood were produced using genetically modified (19).

Genetic engineering of microorganisms

Microorganisms, such as bacteria and yeasts that are not normally unable to synthesize a particular compound, are genetically engineered to improve the synthesis of volatiles or to produce new compounds. The production of volatile substances in commercially valuable quantities by genetically modified microorganisms would be a major success in biotechnology. For example, using metabolic engineering, the scientists succeeded in producing a synthetic banana smell using the bacterium *E. coli* (18). By *Yarrowia Lipolytica* microorganism, Acyl-CoA oxidase genes *pox1*, *pox3* were disrupted, and γ -decalactone, 0.35 g/l from methyl ricinoleate was observed (162). Vanillin can be produced at 5 g/l by *E. coli* carrying the 3-deoxyarabino-heptulosonic acid 7-phosphate synthase gene (163).

Genetically engineered plants

In the genetically modified plant, the DNA has been modified using genetic engineering methods. Genetically modified plants have been engineered for scientific research to produce of special compounds, improvement of plant resistance, increase of plant production and advanced products creation (164). For example, Antisense suppression, 10–100-fold more methyl benzoate was observed by *Dianthus Caryophyllus* (*carnation*) with Flavanone-3-hydroxylase gene (165). *Arabidopsis thaliana* was genetically engineered by β -Farnesene synthase gene for Synthesis of (E)- β -farnesene (166).

Extraction of bioactive compounds in agriculture products

Some scientists have been focused on the transmutation of process conditions and development of capable models to explain observed phenomena during extraction (167), on the other hand, food production and processing in developing countries generate high levels of waste and by-products, causing a negative environmental impact and economics. But at the same time, many of these by-products are a source of valuable compounds such as proteins, starch, lipids, micronutrients, bioactive compounds and dietary fibers. As a result, by extracting bioactive compounds, they can be used in various innovative processes to produce beneficial products (168).

A generally comparison of conventional, novel and biotechnology methods as mentioned in Table 8. Modern extraction techniques of agriculture products are extraction solvent, supercritical fluid extraction, pressurized liquid extraction, pressurized hot water extraction, microwave assisted extraction, etc (169). Extraction technologies some material from agricultural products are mentioned in Table 9.

Conclusion

Plant essential oils are natural and aromatic compounds that are found in small amounts in plants with many

interesting applications as flavors and fragrances in perfumery, cosmetics, pharmaceutical, food, agriculture, industry, etc. Research on the methods of extracting essential oils has attracted the attention of many scientists. As is evidenced in this review, essential oils can be produced by various techniques, including conventional, novel and biotechnology methods. Novel or green methods can be a good alternative to conventional methods due to their advantages, such as good efficiency and quality, short extraction time, non-decomposition of compounds by heat, no pollution, etc. But due to the problems of the traditional technologies and green methods such as climate conditions, supply shortages, natural disasters, plant diseases, etc., recently scientists have chosen biotechnology methods for the production of natural compounds which, does not have the mentioned problems and a high-quality product is obtained. Production of natural and volatile products by a biotechnology green method can be an appropriate response to consumer demand in relation to the consumption of natural products.

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