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Strategies to Improve Stability of Essential Oils

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Abstract:
The review includes components included in essential oil, factors affecting quality of oil and their mechanisms and various techniques, strategies which are useful to determine and improve the stability of various vegetable oil. The methods like nano encapsulation, physical, chemical, physicochemical methods are summarized in this article.

Keyword: Essential oils, quality of essential oil, nano encapsulation, stability strategies,

INTRODUCTION:

Essential oils (EO) /Burt/ethereal or volatile oils are natural volatile compounds that large amount of them are produced from plant raw materials or plants organs like flowers, seeds, bud, leaves, fruits, wood, roots, barks and twigs. They give a different flavour, odour or fragrance to an aromatic plant [1], [2], [3]. EOs are obtained from more than 17,500 aromatic flora or plants bearing many angiosperm families such as Rutaceae, Lamiaceae, Myrtaceae, Asteraceae and Zingiberaceae [4]. They are byproducts observed from plant metabolism are referred to as secondary metabolites of volatile plant. EOs can be applied in various cases including pharmaceutical and health industries (as antiseptic, antibiotic, antiparasitic and anti-inflammatory agents), chemical industries, food sectors (as preserving and flavorings agents), the cosmetics and perfume industries (as fragrances, antibacterials and aromatherapeutic agents), and also, agriculture pathogenic agents such as phytopesticides, agricultural pests and weeds or bioinsecticide and biofungicide. Due to requirement for fresh natural ingredient their applications is increasing, which caused these materials be inseparable part of our life. An increasing demand for them has been observed in the field of adulteration. [1], [5], [6], [7]

Essential oils are obtained by steam or hydro-distillation first developed in Middle Ages by Arabs [8]. Essential oils have been largely employed for their properties already observed in nature, like for their antibacterial, antifungal and insecticidal activities. Presently, almost 3000 essential oils are known, 300 of which are important specifically for the pharmaceutical, agronomic, food, sanitary, cosmetic and perfume industries. And some of their components are used in perfumes, in sanitary products, make-up products, and in agriculture, in dentistry, as food preservers and additives, and as natural remedies. For example, d-limonene, geranyl acetate or d-carvone are occupied in soap, creams, perfumes, as flavour additives for food, as fragrances for household cleaning products and as industrial solvents. In addition, essential oils are used in massages as mixtures with vegetal oil or in baths but generally in aromatherapy. Some essential oils show particular medicinal properties that have been claimed to treat one or another organ dysfunction or systemic disorder. [9], [10], [11]

Owing to the new interest for natural products like essential oils, although their wide use and being familiar to us as fragrances, it is important and essential to develop a better understanding of their mode of biological action for new applications and uses in human health, environment and agriculture. Some of them create effective alternatives to synthetic compounds of the chemical industry, without showing the same secondary effects. [12]

Other applications show medical and technical textiles. In this case, encapsulation is preferred technique in industries process as a means of imparting finishes and properties on textiles that were not possible or cost-effective using other technologies. In textiles, the major uses and application of encapsulation is permanent fragrances and skin softeners. Other applications like dyes, vitamins, insect repellents, antimicrobial agents, phase-change materials and medical applications, such as antibiotics, hormones and other drugs. Essential oils are unstable and fragile volatile compounds. Therefore, they could be degraded easily (by light, temperature, oxidation, volatilization) if they are not protected from external factors. These protections could increase their action duration and provide a controlled release. Essential oils stability can be increased by encapsulation [13]. Encapsulation also shows to improve the antibacterial activity of different antibiotics [14]. The aim of this review is to study different strategies to improve stability of volatile oils.

Essential Oil

Essential oils are odorous, high volatile substances present in plants [15]. Volatile oils get evaporate when exposed to

air at ordinary temperature, they are also called as ethereal oils or essentials oils [16]. Essential oils are composed of lipophilic and highly volatile secondary plant metabolites [17], [18], [19], [20].

CLASSIFICATION [21]**:** The volatile oils are classified in different classes. The classes with examples are summarized in table a. The EOs are containing various chemical classes as mentioned in fig. no. 1

Table a: Classification of volatile oils

Class	**Examples**
Hydrocarbon volatile oil	Bitter orange, turpentine, jupiner, cade etc.
Alcoholic volatile oils	Mentha, coriander, geranium, rose, sandalwood etc.
Ester volatile oils	Rosemery, sweet orange, lavender, neroli, etc
Aldehyde volatile oils	Cinnamon bark, cassia bark, lemon, lemongrass etc.
Ketonic volatile oils	Caraway, dill, spearmint, etc.
Phenolic volatile oils	Cinnamon leaf ,clove, thyme, ajowan, horsement, etc.
Esters	Anise, star anise, fennel, parsley, nutmeg, etc.
Oxides and peroxides	Eucalyptus, cajuput, chenopodium, etc
Non-tepenoids and derived from glycoside	Mustard, wintergreen, bitter almond, etc

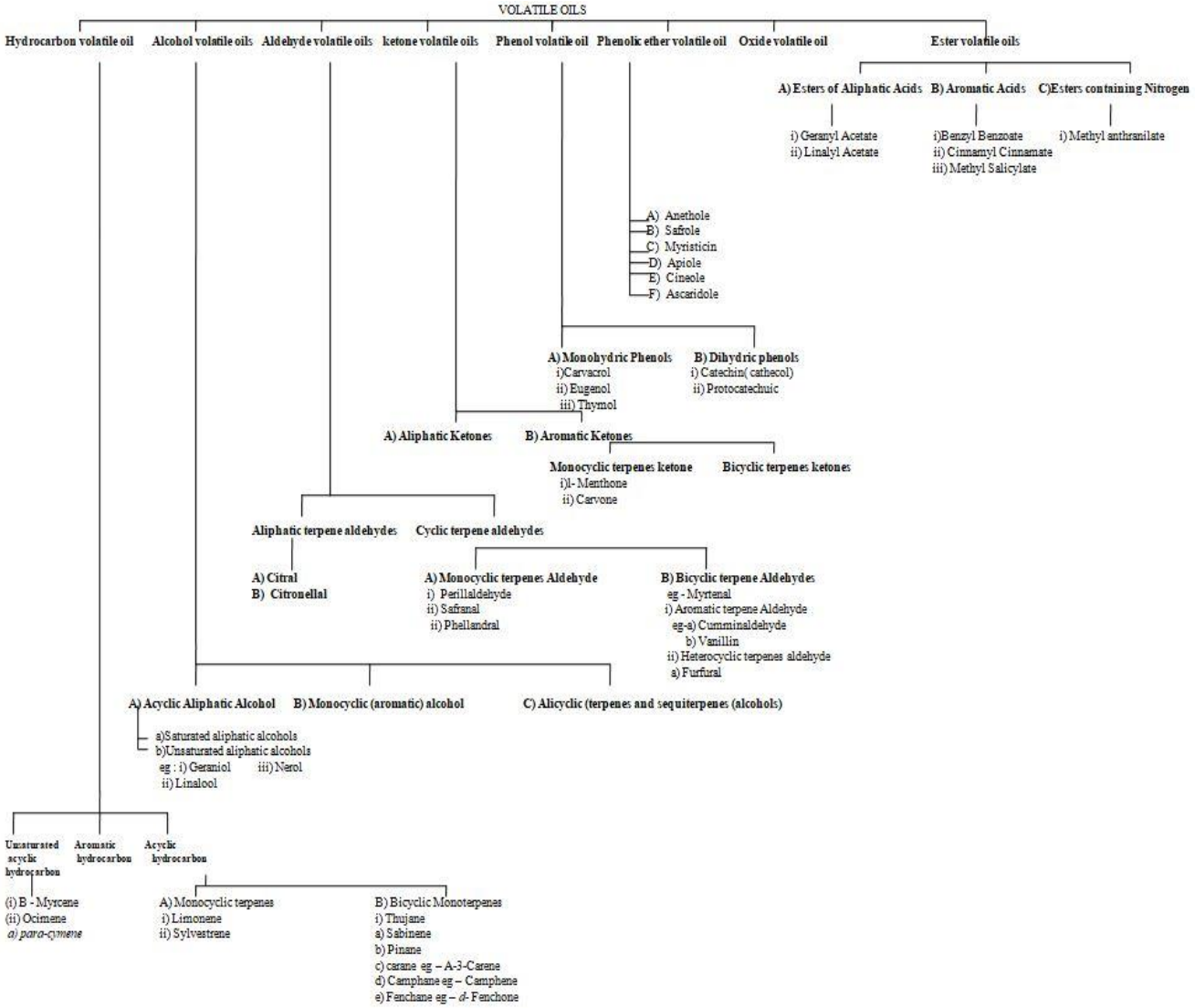

Figure 1: Chemical Classes of Eos. [22]

FACTORS AFFECTING QUALITY OF OIL:

External factors like light, temperature, and access to atmospheric oxygen need to be thoroughly considered.

1) **Light**: Ultraviolet (UV) light and visible (Vis) light are considered to accelerate autoxidation processes by triggering the hydrogen abstraction that results in the formation of alkyl radicals [23]. Also in lemon oil in which decreasing amounts of geranial, terpinolene, and γ - terpinene together with a rise in p-cymene have been observed [24].

2) **Temperature**: Ambient temperature crucially influences essential oil stability in several respects: Generally, chemical reactions accelerate with increasing heat due to the temperature-dependence of the reaction rate by Arhenius equation [25], and as per Vant's Hoff law temperature rise of 10°C doubles chemical reaction rates ,a relation that conclude stability at different temperatures[26],both autoxidation as well as decomposition of hydroperoxides advances with increasing temperature, even more so since heat is likely to contribute to the initial formation of free radicals[23].

3) **Oxygen availability**: As oxidation reactions are among the main causes for spoilage of essential oils, if not even the most frequent ones, it is obvious that oxygen access plays a decisive role in essential oil stability. As per Henry's law oxygen solubility is high at low temperature and highly reduces with an increase in degree Celsius, so peroxyl radical and hydroperoxide have various compounds at low temperature upon oxidation of edible oil [27]. Differently alkyl or hydroxyl radical and their reactions become more important at high temperature as oxygen time was limited [28]. However depending upon appropriate essential oil and temperature, oxidation will not compulsorily interrupt by prevention of container headspace, rather essential oil should be treated with argon gas to display remaining air cautiously flushed to avoid formation of peroxides accurately [29].

4) **Metal contaminants**: Upon distillation in primitive stills or during storage in metallic containers, impurities of metals can be released into essential oils [30]. Similar to light and heat, heavy metals, specially copper and ferrous ions, are considered to promote autoxidation, in certain if hydroperoxides are already present [31], [23].

5) **Water content**: Moisture has been considered as a possible reason for essential oil spoilage [31].

Possible conversion reactions in EO as shown in figure no 2

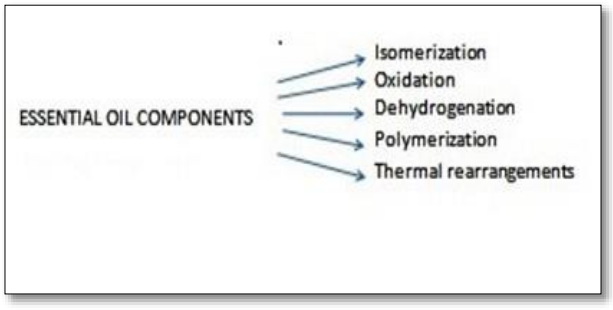

Figure 2: Possible conversion reactions in EO [27]

MECHANISMS OF DEGRADATION OF OIL:

Mechanism of volatile oils it is important factors which affects stability of oil, essential oils depends on several chemical and edaphic factors that influence both the possibility of the essential oil to oxidize as well as the course of the reaction, so here are some external factors which affects the stability of essential oils are given below:

1) Light
2) Temperature
3) Oxygen availability
4) Metal contaminants
5) Water content

1) **Light** : Ultraviolet (UV) light and visible (Vis) light are considered to accelerate autoxidation processes by triggering the hydrogen abstraction that results in the formation of alkyl radicals [23].All the same , compositional changes continued highly faster when illumination was involved. Specially monoterpenes have been shown to degrade rapidly under the influence of light [32]. The similar study also reported on transformation reactions taken place in marjoram oil during storage under light that led to the formation of several unknown minor components. This showed that changes in several essential oils were promoted under the impact of light, however, oils from varying plant species responded differently: While essential oil from thyme did not alter much, rosemary oil turned out to be very susceptible to imitated daylight that readily led to a changing chemical composition. This was especially showed by a considerable increase in p-cymene, camphor, and caryophyllene oxide concomitant to the degradation of β-caryophyllene and the monoterpenes β-myrcene, α-terpinene, as well as α-phellandrene [33]. Futhermore, one minor compound in lavender oil but not further identified totally broke down when illuminated, while another unidentified substance was initially built up during the 1st month stored under light but degraded again upon advanced storage [34]. Such photo-artifacts induced by sunlight or by UV irradiation with a distinct spectral distribution were identified in stored essential oils from anise (photoanethole) and lemon (photocitral) [35]. For the former, the light-induced photoanethole, identified as 4, 4'-dimethoxystilbene (Fig 3) , was suggested to result from photocycloaddition between anethole and anisaldehyde [35]. Moreover, in sweet fennel oil, trans-anethole had completely oxidized to anisaldehyde or isomerized to cis-anethole (Fig 4) after 2 mo of storage at room temperature under light [36]. trans-anethole, the main component in sweet and bitter fennel oil, was reported to be converted into cis-anethole when treated with UV rays or high temperatures, the cis-isomer being 10 to 12 times extra harmful than the trans-form [37]. In keeping with these data, a range of photochemically catalyzed intramolecular isomerization reactions such as cycloaddition or trans-cis conversions of several monoterpenoids [38].

Furthermore, illumination plays a crucial part in a 2nd oxidative pathway named photooxidation in which aerial triplet oxygen is converted into its excited singlet state in

the presence of an organic sensitizer. Hydroperoxides derived from this so-called "ene" reaction decompose in the same manner as those formed by autoxidation [39].

2) **Temperature**: Ambient temperature crucially influences essential oil stability in several respects: Generally, chemical reactions accelerate with increasing heat due to the temperature-dependence of the reaction rate as expressed by the Arrhenius equation [25]. Based thereon, Van't Hoff law states that a temperature rise of 10°C approximately doubles chemical reaction rates, a relation that can be consulted to predict stability at different temperatures [26]. Hence, both autoxidation as well as decomposition of hydroperoxides advances with increasing temperature, even more so since heat is likely to contribute to the initial formation of free radicals [23]. Conversly, lower temperatures favor the solubility of oxygen in liquids, which in turn may oppositely affect essential oil stability [40].

3) **Oxygen availability**: Oil oxidation accelerates with the concentration of dissolved oxygen, which in turn depends largely on oxygen partial pressure in the headspace as well as ambient temperature. Without stimulating, oxygen diffusion into the sample takes place slowly over time [23]. As per Henry's law, oxygen solubility is high at low temperature and radically decreases with an augmentation in degrees Celsius. Therefore, peroxyl radicals as well as hydroperoxides have been reported to be the most numerous compounds upon oxidation of edible oils at lower temperatures. Compounds formed through termination reactions like polymers were only built up at later oxidation stages and at the end of the induction time, when either the amount of oxygen or oxidizable substrate was exhausted. On the other side, alkyl or hydroxyl radicals and reactions thereby became more essential at elevated temperature as oxygen availability was limited [28]. A recent study revealed the individual character of essential oils regarding their liability toward oxidation: While peroxide formation in essential oils from rosemary and pine was promoted at room temperature, oxygen solubility appeared to play a more important role for the peroxide level present in lavender oil and thyme oil stored at 5 ◦C. In these oils, highest POVs were found upon storage in the refrigerator [41]. These outcome make clear that conclusion obtained for one type of essential oil cannot be simply transferred to additional one.

4) **Metal contaminants**: Upon distillation in primitive stills or during storage in metallic containers, impurities of metals can be released into essential oils [30]. Equal to light and heat, heavy metals, especially copper and ferrous ions, are considered to promote autoxidation, in particular if hydroperoxides are already present [31], [23] . By catalyzing hydroperoxide decomposition, Fe^{2+} or Cu^{+} as well as Fe^{3+} or Cu^{2+} give rise to alkoxy and peroxyl radicals, respectively,which in the formation of singlet oxygen by ferrous ions and thus the initiation of photooxidation have likewise been reported [23].

5) **Water content**: Moisture has been considered as a possible reason for essential oil spoilage [31]. For instance, water distillation procedures at approximately 100 ◦C were shown to distort compound spectra. Citral is known to have acid-catalyzed reactions in aqueous solutions into p-cymene, p-cymene-8-ol, α p-dimethylstyrene, methylacetophenone, and cresols [42]. On the other hand, could not display significant changes in different oils stored in the presence of water, even at a water content of 20% (v/v) [43]. This level is above the concentration that might possibly be dispensed in essential oils after hydrodistillation when done properly. Then to dry essential oils subsequent to distillation by addition of water-binding substances, such as reduced contents of linalool and geraniol content as well as an increase in citronellol and geranyl formate in geranium oil which had been kept at room temperature for 1 yr and which contained an undefined amount of water. However, observed chemical shifts might have equally been triggered by the presence of 50% air space in the container [44].

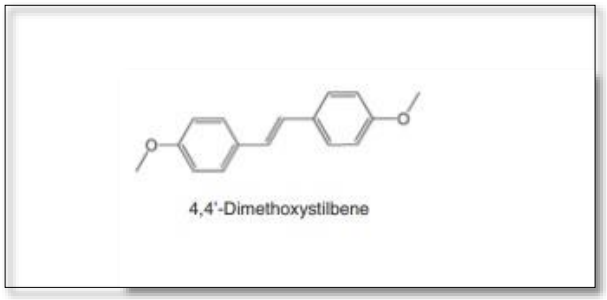

Figure 3- 4,4'-dimethoxystilbene identified as photoartifact in stored anise oil

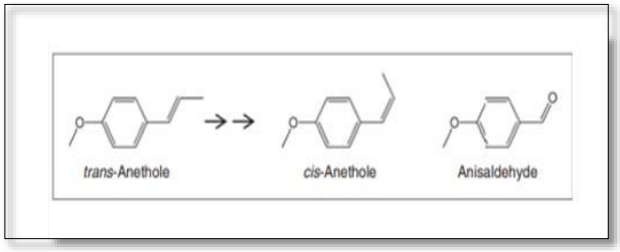

Figure 4 - Isomerization and oxidation products of trans-anethole detected in fennel oil

STRATEGIES TO PREVENT DEGRADATION

1) Nanoencapsulation Technique: Encapsulation of bioactive compounds shows a feasible and effective approach to modulate drug release, increase the physical stability of the active substances, protect them from the connection with the environment, decrease their volatility, improve their bioactivity, lower toxicity, and improve patient compliance and suitability [45]. A substantially huge part of current literature on the encapsulation of Eos shares with micrometric size capsules, which are used for the protection of the active compounds against environmental factors (e.g, oxygen, light, moisture, and pH), to reduce oil volatility and to transform the oil into a powder. Encapsulation in nanometric particles is an alternative for conquering this difficulty but moreover, due

to the subcellular size, may increase the cellular absorption mechanisms and increasing bioefficacy [46].

Schematic illustration of nanosystem platforms for essential oils is shown in Fig 5 [46].

EO loaded nano-delivery system [46]:

1) Polymer-based Nanocarrier
2) Lipid-based Nanocarrier
a) Micro and nano-emulsion
b) Liposomes
c) SLN(solid lipid nanoparticle)
3) Molecular complexes:

In these molecular complexes,complexation of essential oil is done by CD-complexation (cyclodextrin). It is natural macro oligosaccharides with rigid lipophilic cavity and outer surface hydrophilic. Inside the hydrophobic cavity which consist of molecular encapsulation, CD can enclosed higly hydrophobic molecules [47]. It gives protection of active ingredient against oxidation,light reaction, temperature,reduce microbial contamination etc ,also to protect essential oil or to convert oil into microcrystalline powder [48].

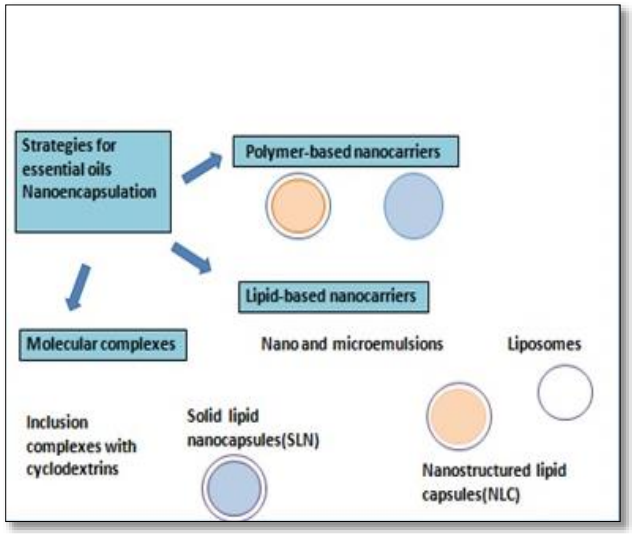

Figure 5 - Schematic illustration of nanosystem platforms for essential oils. [46]

2) Chemical Methods: Among chemical approaches liposomes have been widely used for the encapsulation of EOs. Liposomes are normally prepared by mixing lipids in organic solvents and subsequent drying either by rotary evaporator, spray drying or by lyophilization. Phospholipids have typically been used for the preparation of liposomes [49].

3) Physicochemical Methods: Coacervation is a physico-chemical process that involves phase separation of one or more hydrocolloids from solution and subsequent deposition of newly formed coacervate phase around the active ingredient suspended in the same reaction media [50].

4) Mechanical Methods: Among these spray drying is the low cost, commercial process which is mostly used for the encapsulation of EOs. In spray drying, core material is dispersed in polymer solution, and sprayed into a hot air chamber.spray deried OEO emulsion prepared using β-cyclodextrin [51]. Similarly, various researchers used high energy emulsification approach for the generation of EOs loaded nanoemulsions has been carried out by many researchers [52].

5) Other encapsulation methods: Eugenol and carvacrol grafted chitosan nanoparticles were prepared via Schiff base reaction. Briefly, chitosan nanoparticles (CH-NPs) were prepared using ionic gelation method [46].

a) Co-crystallization: Method is used for encapsulation of orange peel oil.

6) Emulsifiers/wall materials used for essential oils: A variety of carbohydrate, proteins, and gums based emulsifiers or wall materials have been used for the encapsulation of EOs. Carbohydrate, β-CD is used for emulsion, these were spray dried to obtained stable microcapsules also modified starch shows better stability of oil in spray dried powder [51], [53].

7) Rapid expansion of supercritical solutions (RESS):

Conventional methods have disadvantages like, the use of large amounts of organic solvents, large particle size distributions, and solvent residues. To conquered these disadvantages, supercritical fluids based processes have been used. The latter process has become an interesting substitute to encapsulate natural substances due to the use of environmentally friendly solvents [54]. Among the spectrum of supercritical fluids, supercritical CO_2 is widely used in both the process for designing particles of organic and pharmaceutical compounds due to its environmentally benign nature and low cost. Supercritical CO_2 is often used thanks to its low critical temperature (31.1 °C) which is very useful thermally sensitive materials precipitation [55]. In the RESS, the solutes are dissolved in supercritical CO_2 at high pressures (up to 250 bar) and temperatures (up to 80 °C), and then the solutions are expanded. The solubility of the solute decreases at lower pressures and as a result they precipitate. And also both the solutes and the used active molecule should be soluble in supercritical CO_2 for encapsulation [56].

8) Encapsulation in liposomes:

Liposomes are systems formed by one or various phospholipids bilayers defining one or several aqueous compartments. Phospholipids are amphiphilic molecules that are able to self-organize immediately in aqueous media. Liposomes can be classified depending on their size and lamellarity to: (1) multilamellar vesicles (MLV) size greater than 0.5mm, (2) small unilamellar vesicles (SUV) size in between 20 nm and 100 nm and (3) large unilamellar vesicles (LUV) size greater than 100 nm [57]. They are broadly used as carriers of both hydrophilic molecules in aqueous compartments and lipophilic ones in the bilayers, but also amphiphilic molecules[58] . In addition, the use of liposomes for encapsulation of EOs is an beautiful approach to overcome their physicochemical stability concerns (sensibility to oxygen, light, temperature, and volatility) and their decrease bioavailability which is due to low solubility in water[59] . Different methods have been used to encapsulate Essential oils, from most conventional Bangham method to those employing supercritical fluids[60].

a) Thin film hydration method : Thin film method,called as the Bangham classical method[61] , is used to form multilamellar vesicles (MLV) with a size up to less micometers. This both that is phospholipids and essential oils are dissolved in an organic phase. A thin phospholipid film of stacked bilayers is produced at the bottom of the flask after rotative evaporation of the organic solvent under pressure. This dry film is hydrated with an aqueous phase under agitation which allows simple or spontaneous formation of MLV. So, this method gives large vesicles with heterogeneous size distribution and lamellarity. Different approaches are used to produce liposomes suspension with homogenous and decrease size. The main principle is the conversion of MLVs into SUVs (small unilamellar vesicles) or LUVs (large unilamellar vesicles). Sonication and extrusion are foremost common methods (Fig 6) [62]. So, sonication was the most often used final step to encapsulate essential oils in liposomes by thin film hydration method[63],[64],[59] . Ultrasonic wave application provides more energy to disrupt MLVs. Even if this technique is simple to implement, many disadvantages has been raised. Phospholipids and other materials may be degraded. The resulting liposomes also show low encapsulation efficiency [62] . The thin film hydration method has been also used by to [65] encapsulate lavandin essential oil. They altered the classical method by trying three different procedures. In the first , the thin film was heated above the lipid transition temperature (60 °C for soybean lecithin) 20 min and placed in an ultrasound bath for 30 min in sonication. It is proven that this condition transforms lipids in gel state, which favors continuous closed bilayered structures formation [66]. In the second, the lipid film hydrated in the aqueous phase was agitated in a vortex mixer at 1700 rpm for 15 min, then hydration of lipid film was carried out in 2 h in the dark at room temperature. In the last procedure, the lipid film was heated at 60°C for 20 min, and then, shaken in a vortex mixer at 1700 rpm for 15 min. The results show that liposome size is ranged between 0.42mm to 1.29mm, and were greater when the lavandin oil/lipid ratio increased. Generally, cholesterol is added in the liposome preparation to improve stability and enhance membrane permeability observed an impact on the liposome size: a decrease of the amount of cholesterol reduced the liposome size. [65]. Vortex mixing gave smaller vesicles than sonication. The incorporation efficiency was better with the second procedure until about 60% with a lavandin/ lipid ratio of 3:5. But the highest stability after 50 days is obtained with the third method.

9) Extrusion :

Extrusion which is a most common method and is used to reduce size and lamellarity of MLVs produced by thin film hydration. The passage through a track-etched polycarbonate membrane with pores of different diameters is performed various times. The size of membrane pore is the very important parameter to take into account from it affects the final liposome size and size distribution mainly depends on it. Therefore, the pressure applied on the membrane has also an impact [62]. Bergamot essential oil with this method is also encapsulated. These results showed the formation of small liposomes (less than 200 nm) with an encapsulation efficiency of 75%. So, it has been proven and reported that the presence of the essential oil in the formulation leads to polydispersity [67].

10) Freeze-thaw:

Freeze-thaw technique is another mean to homogenize and reduce liposome size formed by thin film hydration method, generally MLVs. It was reported that this system would permit to get LUVs from MLVs. The main advantage is the higher encapsulation efficiency because of the increase interactions between the lipid film and the EO to incorporate during freeze thaw cycles to prepare liposomes entrapping essential oil of Eucalyptus camaldulensis Leaf. Phospholipids and cholesterol were dissolved during a cosolvent (chloroform/methanol) which was then removed by rotatory evaporation under vacuum. The lipid film was hydrated with a phosphate buffer saline (pH 7.4) containing the volatile oils and vortexed during 5 min. Then, 3 freeze-thaw cycles were performed. The freeze step was carried out in ice-ethanol or acetone during 5–10 min and therefore thaw step was made at room temperature succeeded to possess stable liposomes during 3 months with an encapsulation efficiency of 95%. They highlighted that to make small liposomes, short freezing time with an honest homogenization were essential [68].

11) Reverse phase evaporation method :

The reverse phase evaporation may be a conventional method capable to make LUV (large unilamellar vesicles). The preparation of an oil-in-water emulsion by mixing a phospholipids organic phase, contains generally the lipophilic active substances, in an aqueous phase. Then the organic solvent is evaporated, that gives LUVs. However, formation of MLV showed that MLV proportion may be reduced with lower concentrations of phospholipids. It is interesting to note that only few works are dedicated to EOs encapsulation in liposomes using this this method. incorporated into liposomes three different EOs distilled from *Artemisia afra*, blue gum and *Melaleuca alternifolia*. The method of preparation employed was the traditional one except that sonication with a search was applied to succeed in nanosize dispersions. After the removal of the organic phase, a 3–5 freeze-thaw cycles last step was performed to rework the eventual MLV to unilamellar vesicles. The size of liposomes ranged from 8mm to 10mm. These large vesicles gave good encapsulation efficiency, respectively 69.2% for *E. globulus* and 47.1% for *M. alternifolia* but results decline with encapsulation of A. afra with an encapsulation efficiency of 18.7%. Therefore, it's going to be considered that each one EOs aren't adapted for entrapment in liposomes. Few of them could exhibit destructive effects on phospholipid bilayers. Modified reverse phase evaporation method is used to capture in liposomes tea tree oil (TTO), an EO from *M. alternifolia*. Indeed, the TTO was directly dispersed into the aqueous phase leading to an emulsion on which is applied sonication. This emulsion is stabilized by polvinylalcohol (PVA). The phospholipids organic phase was added slowly into this earlier phase. Then, the organic solvent was removed as lastely described [69].

12) Supercritical fluid technology:

Conventional supercritical fluid based methods may require some modifications. For encapsulation of EOs or their components, two methods are used: rapid expansion of supercritical solutions (RESS) and particles from gas saturated solutions (PGSS)-drying of emulsion.

(a) Modified rapid expansion of supercritical solution technique (RESS) :

In the conventional RESS process, solutes dissolved in the supercritical solvent and the solution is fastly expanded into atmosphere to precipitate the solutes as microparticles see (Fig.7). So, phospholipids are dissolved hardly in the pure supercritical CO_2. Therefore, phospholipids can only assemble itself into liposomes in an aqueous medium. As a result, conventional RESS process is not applicable for liposomes formation.Also adapted conventional method for liposome formation to encapsulate volatile oils or their components. For self-assembly of phospholipids in liposomes, an aqueous phase is required. The modified RESS technique is to predissolve phospholipids, cholesterol and the essential oil in ethanol and not directly in supercritical CO_2 because of their low poor solubility. Ethanol is then used as a cosolvent to increase phospholipids solubility. This organic phase is fixed into a reactor. Supercritical CO_2, which is formed from liquefaction of CO_2 gas in a refrigerating system, is introduced via syringe pump into the reactor. After 1 hr of equilibrium at desired temperature and pressure, all components are dissolved in the supercritical carbon dioxide (SC- CO_2)/ethanol mixture. Then, this phase is diffused in an aqueous phase and sprayed into a collector allowing rapid elimination of CO_2. Finally, liposomal suspension is freeze-dried. Preparation of liposomes entrapping volatile oil as modified RESS technique, but this technique is not effecient for micronizing soy lecithin and revisited his modified RESS technique for liposomal encapsulation of other essential oils components that is rose oil, atractylone, hinesol. This method is newly called rapid expansion from supercritical to surfactant solution (RESSS). In fact, volatile oils components and other liposomal materials were dissolved in a SC- CO_2/ethanol phase, as previously described, and then the mixture was sprayed into a surfactant solution. Here, 2 hr of equilibration is required. When the dissolved phospholipids and essential oil components reach desired preexpansion pressure and temperature, they precipitate together. Then latter phase is sprayed into a collector by releasing CO_2 rapidly with a nozzle. This collector contains a surfactant solution where essential oil components or phospholipid coprecipitates are hydrated. These results to the self-assembly of phospholipids in liposomes with incorporation of essential oil components. The SC- CO_2 flow is maintained for 1 h to eliminate residual ethanol in the liposomal suspension before its expansion in the atmosphere. So the role of the surfactant is to provide a more stability of the prepared liposomes, by reducing particle growth and reducing agglomeration. More bubble formation, related to SC CO_2 depressurization and phase conversion into a gas, is also avoided. It has been shown that poloxamer 188 was the good surfactant. It allows a steric stabilization, a narrow size distribution and high entrapment efficiency [70].

(b) Particles from gas saturated solution (PGSS)-drying process:

This is another supercritical fluid precipitation method which is used for encapsulation of essential oil components. It permits to incorporate essential oils in different polymeric particles (PEG, starches) for agricultural purposes. Also work on the liposomes encapsulation of essential oils by PGSS-drying of emulsion, especially with lavandula oil. This process requires in prior the preparation of an essential oil-in-water emulsion. Lecithins are diffused in deionized water at 50°C under magnetic stirring. Then, essential oil is constantly incorporated in the suspension while keeping agitation. The obtained coarse emulsion is passed under a rotor-stator machine to filtered clean droplets. Then, the emulsion is saturated with CO_2 at a convenient pressure and temperature in order to low the viscosity. However, this saturated CO_2 emulsion is easily pumped into supercritical CO2 at high pressure and temperature. Only a few second connections is required to attain an intimate mixing. Then, the vaporization and expansion of CO_2 is triggered by a return to atmospheric pressure with a nozzle. Appropriately, a very fine and dried powder is formed. The liposome encapsulating the lavandula oil shows only after hydration of the previously dried powder. But during the spray step, it is important to work at temperature conditions above the dew line of the temperature-composition phase equilibrium diagram of $CO_2$and water in order to generate dry powder. The obtained liposome size range in between 1.39mm to 24.84mm. The encapsulation efficiency reached to 14.5%. The effectiveness of this method depends on various guidelines or a parameter. Certainly, the liposomes become small when the gas to product ratio (GPR) is higher or when the pre-expansion temperature and pressure is reduced. This is explained by an increased CO_2 concentration in the emulsion, which implies a good atomization. Again, particle size increased when phospholipids concentration increased because it makes the emulsion more viscous which generates an opposition to atomization. The encapsulation efficiency is also changed by the GPR. When GPR increased, essential oil evaporates, that reduces entrapment efficiency. When pre-expansion temperature and pressure is increased, then encapsulation efficiency also increased .Volatile oils or their components are also used as penetration enhancers for skin drug delivery [65].

13) Encapsulation in solid lipid nanoparticles (SLN) [71]: The method is described in Fig 8

14) Other Encapsulation techniques [72]:

- Coacervation (Phase Separation)
- Spray drying, spray cooling , spray chilling
- Fluid –bed spray coating
- Emulsification solvent evaporation/extraction
- Interfacial and in situ polymerization

The methods for improving stability of essential oils are described in Table b.

Table b - Methods to improve stability of EOs

Technique	Application	Reference
Nanoencapsulation technology	Protection of the active compounds against environmental factors (e.g., oxygen, light, moisture, and pH	[45], [46]
Molecular complexes	Cyclodextrin complexation	[48]
Chemical Procedures	Used for preparation of liposomes	[49], [50]
Physicochemical procedure	Protection of lipophilic compounds in inclusion complexes	[50]
Mechanical Procedures	Used for encapsulation of essential oils	[51], [52]
Co-crystallization	Used for the encapsulation of orange peel oil.	[46]
Emulsifiers/wall materials used for essential oils	Used for encapsulation of essential oils	[51], [53]
Rapid expansion of supercritical solutions(RESS)	To encapsulate natural substance with use of environmentally friendly solvents	[54], [55], [56]
Thin film hydration method	To encapsulate lavendin essential oil	[59], [61], [62], [63], [64], 65],[66]
Extrusion	Used to reduce size and lamellarity of MLVs produced by thin film hydration	[62],[67]
Freeze-thaw	To homogenize and reduce liposome size	[68]
Reverse phase evaporation method	Capable to form LUV (Large unilamellar vesicles)	[69]
Modified rapid expansion of supercritical solution technique (RESS)	Used for encapsulation of essential oils	[70]
Particles from gas saturated solution (PGSS)-drying process	Used for encapsulation of essential oils	[65]
Encapsulation in solid lipid nano-particle	Used to prepare SLNs	[71]
Simple coacervation	High encapsulation efficiency	[72]
Complex coacervation	Efficient control of particle size	[72]
Spray chilling	Suitable for water soluble material	[72]
Fluid bed spray coating	High thermal efficiency process	[72]
Emulsification	Suitable for biodegradable and non-biodegradable polymeric micro-particles, and a wide range of liquid and solid core materials	[72]
Interfacial polymerization	High encapsulation efficiency	[72]

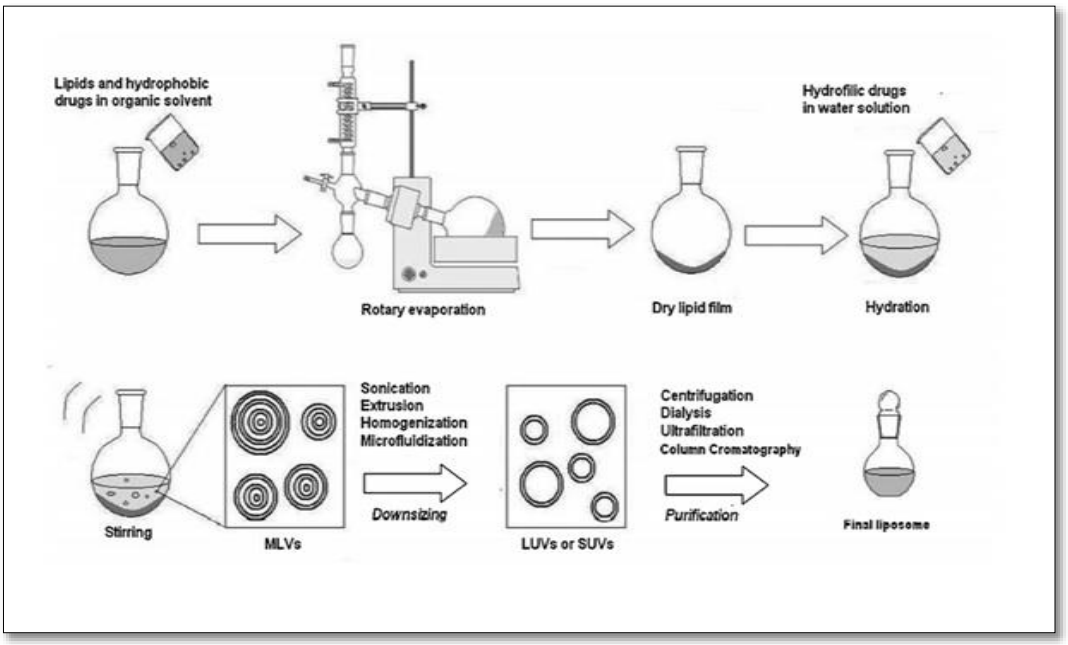

Figure 6 - Thin film hydration method and methods of size reduction. [62]

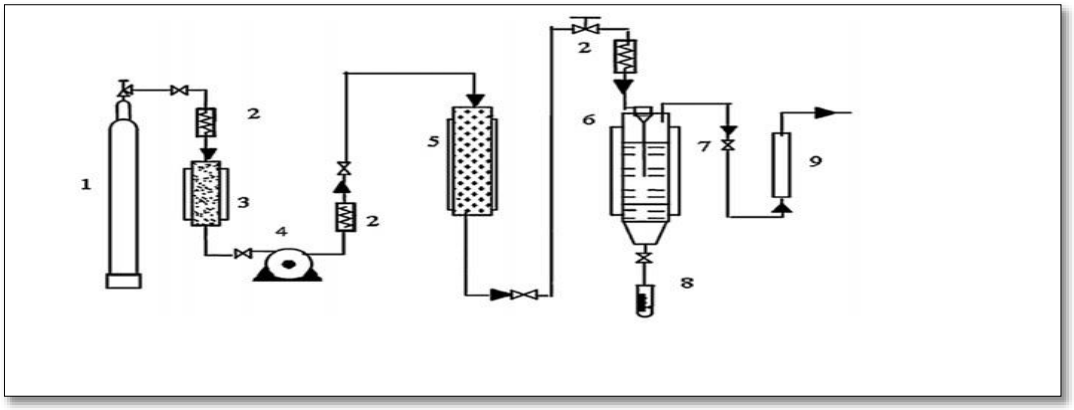

Figure 7 - Schematic diagram of the RESS (1, Cylinder; 2, heat exchanger; 3, refrigerating machine; 4, syringe pump; 5, reactor; 6, nozzle; 7, collector, 8, volumetric cylinder; 9, rotameter). [70]

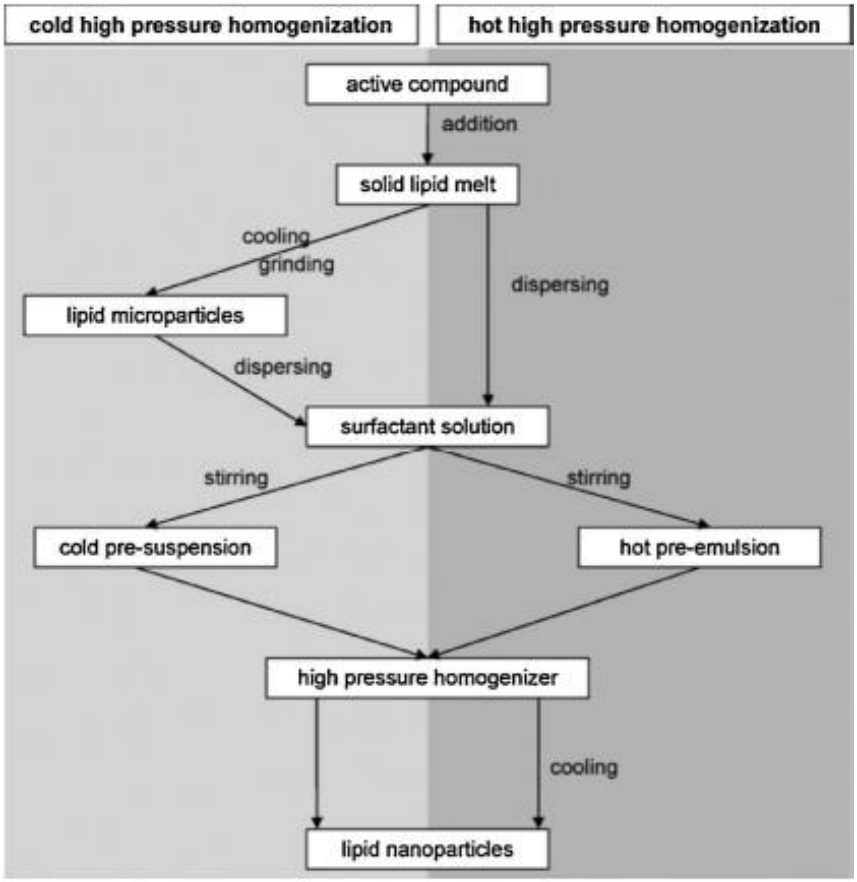

Figure 8 - Production process of lipid nanoparticles using cold (light gray background) and hot (dark gray background) high pressure homogenization technique [71]

CONCLUSION

Consumption of EOs depends on shelf life. It is well-known that the oils considered as medicinal agents are often highly sensitive to light, temperature, oxygen availability, metal contaminants, water contents, oxidation, hydrolysis, thermal decomposition, enzymatic oxidation. There are permanent efforts to develop methods in order to improve the stability of volatile oils using natural antioxidants, nanoencapsulation, chemical, physicochemical, mechanical methods.

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