



Nanoencapsulation- A Novel Strategy for Enhancing the Bioactivity of Essential Oils: A Review

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ABSTRACT

An upsurge in the global demand for safe and healthy food with minimal synthetic preservatives has raised the need for natural antimicrobial agents. Plant based products, especially essential oils (EOs) exhibit strong antimicrobial activities that could play a significant role as a novel source of food preservatives. However, hydrophobicity, high volatility, susceptibility to oxidation, low stability and solubility limit the uses of essential oils. Therefore, nanoencapsulation could be promising technique to address these limitations as it prevents the exposure and degradation of essential oils, by creating a physical barrier that protects the bioactive constituents. Furthermore, it also facilitates their controlled release, resulting into enhanced bioavailability as well as efficacy in the food system. Present review highlights the various encapsulating methods and provides insight about some encapsulated essential oils and their bioactive properties.

Key words: Antimicrobial, Essential oils (EOs), Nano-encapsulation, Preservatives.

Essential oils (EOs) are hydrophobic liquids containing various volatile compounds. In the 16th century, the word 'essential oil' was defined as 'Quinta Essential' by Paracelsus von Hoenheim (Pichersky *et al.*, 2006). EOs are produced in large quantities in oil sacs/oil glands of medicinal and aromatic plants and can be extracted from leaves, bark, seeds and flowers (Tongnuanchan and Benjakul, 2014; Mahato *et al.*, 2019). They are a complex mix of over 300 different compounds which are mostly organic and volatile in nature (Vainstein *et al.*, 2001; Pophof *et al.*, 2005; Sell, 2006). Since medieval ages essential oils had been used in various cultures, due to their medicinal properties. Their various promising properties, like being stimulants, anti-depressants and anti-bacterial have helped them in gaining popularity in recent years. They have found applications in the field of therapeutics, being natural, safe and cost effective (Herman *et al.*, 2019). Similarly their hydrophobic nature enable them to infuse into lipids of the cell membranes of microbes, resulting in disruption of the cell structure (Sikkema *et al.* 1994). Also different phenolic compounds present in them contribute to their anti-bacterial activity (Lambert *et al.*, 2001; Dorman *et al.*, 2000). In addition, EOs being lipophilic in nature, disrupt microbial cell homeostasis by making cells more permeable and less organized (Chaudhari *et al.*, 2020).

In spite of their several advantages, the use of EOs is limited due to various intrinsic factors like high volatility, photo-sensitivity, hydrophobicity and low stability (Kumar *et al.*, 2020). Therefore, in order to overcome these limitations associated with the use of essential oils there is a need for development of innovative technologies. Nanotechnology based techniques like nano-encapsulation has been developed as one such technology that has significant potential in addressing this problem. It involves the use of delivery vessels, also referred to as nano carriers to encapsulate

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bioactive molecules. This protect these molecules from various environmental factors such as oxygen, pH, light *etc.* It also offers different advantages to EOs such as protection from degradation, enhanced bioactivity targeted delivery and controlled release (Singh *et al.*, 2020; Bastos *et al.*, 2020). Currently, a wide range of coating materials are being extensively used namely starch, cellulose, chitosan, guar gum *etc.*, as encapsulating materials which enhance the bioactive potential of EOs (Kumar *et al.*, 2020). Moreover encapsulation of EOs improves the bioactive properties of essential oils and can be used in food and pharmaceutical industries (Kapustova *et al.*, 2021). Nano carriers can be utilised for encapsulating essential oils with enhanced antifungal activity (Kapustova *et al.*, 2021; Napoli *et al.*, 2020). Furthermore, nano based essential oil capsules show controlled mycotoxin and fungal contamination in agri based food systems (Chaudhari *et al.*, 2021). This review illustrates various techniques/methods for encapsulation of essential oils.

Chemical nature of essential oils

EOs are complex mixture of compounds which are mostly alcohols, ethers, aldehydes, ketones, esters, amines, phenols and terpenes (Dihifi *et al.*, 2016; Sell, 2006). The terpene family occupies the major composition of EOs. Thousands of compounds belonging to terpenes have been characterized in essential oils namely, alcohol derivatives (geraniol), ketones (menthone), aldehydes (citronellal) and phenols (thymol) (Modzelweska *et al.*, 2005). They are rich in mono-terpenes, which include geraniol, terpineol (lilacs), limonene (citrus), myrcene (hops), linalool (lavender) and pinene (pine) and sesquiterpenes like chamazulene (German chamomile) (Breitmaier, 2006). Non-terpene compounds such as eugenol, saffrole *etc.*, are also present in EOs. Some esters are also found in EOs such as linalyl acetate and geraniol acetate in lavender and sweet marjoram respectively (Safayhi *et al.*, 1994; Arumugam *et al.*, 2016). Essential oils containing ketones include rosemary, clary and sage (Nazzaro *et al.*, 2013). Some selected essential oils and their major chemical compounds are illustrated in (Table 1).

Various volatile components present in essential oils possess biocidal activity. These volatile compounds have a profound demand in wide spanning industries like food, pharmaceutical and pesticide. Chemical compounds present in EOs and their biocidal activities are depicted in Table 2.

Different techniques of essential oil nanoencapsulation

Essential oils can undergo chemical alterations because of their volatility and decomposition when exposed to light, heat and oxygen (Scott, 2005). Nanoencapsulation of EOs, involve two terminologies namely core materials and wall materials. The material which gets encapsulated is known as the core material, active agent or internal phase, whereas, the encapsulating substances are called as wall materials, matrices, carrier agent or external phase (Zuidam and Shimon, 2010; Pandit *et al.*, 2016). Encapsulation of essential oils leads to their sustained release in a controlled

manner, can penetrate deep inside the tissues and are readily taken up by the cells because of their miniscule size (Ravi Kumar, 2000). Thus, nanoencapsulation of essential oils leads to their increased bioavailability, enhanced controlled release and precision targeting of the bioactive compounds (Mozafari *et al.*, 2006).

Nanoencapsulation techniques

Nanoencapsulation of bioactive compounds either involve top-down or bottom-up approaches. In top-down approach, precise tools are applied to reduce the size and shape to achieve desirable applicability of the nanomaterials that are created. On the other hand, the bottom-up approach constructs materials through self-organization and self-assembly of molecules and is influenced by several factors such as temperature, pH, ionic strength and concentration (Augustin and Sanguansri, 2009).

Classical approaches of essential oil nano-encapsulation

Emulsification

This technique has been used to encapsulate bioactive compounds in aqueous solutions by producing nanoemulsions which are colloidal dispersions consisting of two immiscible liquids, one being dispersed into the other having droplet sizes in the range of 50 to 1,000 nm (Sanguansri and Augustin, 2006). Drying techniques like spray drying and freeze drying after emulsification can be used to produce nanoemulsions that can either be used directly in the liquid state or in a dried powder form. Nanoemulsions exhibit high kinetic stability because of their extremely small emulsion droplet size and this plays a critical role in the retention of surface oil content of the product (Solans *et al.*, 2005; Sonnevile-Aubrun *et al.*, 2004; Jafari *et al.*, 2008). Being a non-equilibrium system, spontaneous formation of nanoemulsions is not possible and consequently requires energy input. Hence production of nanoemulsions is generally attained

Table 1: Selected essential oils and their chemical composition.

Plants	Parts used	Chemical components	References
<i>Artimisiafrigid</i>	Aerial parts	1,8-cineole	Lopes-Lutz <i>et al.</i> , 2008
<i>Cuminum cyminum</i>	Leaves	γ -Terpin-7-al, γ -terpinene, β -pinene, cuminaldehyde	Bisht <i>et al.</i> , 2014
<i>Eugenia caryophyllata</i>	Flower buds	Thymol, eugenol, carvacrol, cinnamaldehyde	Santos <i>et al.</i> , 2015
<i>Ocimum basilicum</i>	Leaves, stems	Methylchavicol, γ -terpinene	Arumugam <i>et al.</i> , 2016
<i>Nigella sativa</i>	Seeds	Longifolene, thymoquinone thymohydroquinone, α -thujene, p-cymene	Beatovic <i>et al.</i> , 2015

Table 2: Selected active ingredients and the pathogens inhibited.

Major active ingredients (MAIs)	Pathogens inhibited	Reference
Santolina triene, α -pinene, camphene	<i>Escherichia coli</i> , <i>Staphylococcus aureus</i> ,	Lopes-Lutz <i>et al.</i> , 2008
1,8-cineole, methylchavicol, camphor	<i>E. coli</i> , <i>S. aureus</i> , <i>S. epidermidis</i>	Lopes-Lutz <i>et al.</i> , 2008
β -Himachalene, α -humulene, γ -himachalene	<i>S. aureus</i> , <i>Listeria monocytogenes</i> , <i>Enterococcus faecium</i>	Ait-Ouazzou <i>et al.</i> , 2012
γ -Terpin-7-al, γ -terpinene, β -pinene, cuminaldehyde	<i>Salmonella typhimurium</i> , <i>E. coli</i>	Bisht <i>et al.</i> , 2014
Thymol, eugenol, carvacrol, cinnamaldehyde	<i>S. epidermidis</i>	Santos <i>et al.</i> , 2015

through high-energy emulsification methods. Nanoemulsions provide significant possibility for the encapsulation of bioactive food supplements or oil-soluble nutraceuticals that can be utilized in food stuffs (Silva *et al.*, 2012).

Coacervation

The technique of coacervation involves phase separation of single or mixture of polyelectrolytes from a solution with later deposition of these around essential oil or bioactive components resulting in the formation of coacervates. When cross linking agents like glutaraldehyde and transglutaminase are added to coacervates, the coacervate becomes more robust (Tiwari *et al.*, 2020). Significant increase in the antimicrobial and antioxidant potential of *Pimentadioca* essential oil after encapsulation in chitosan/carrageenan using the method of complex coacervation had been reported (Dima *et al.*, 2014).

Inclusion complexation

This technique, in general refers to the encapsulation of a supra-molecular association of a ligand into a cavity-bearing substrate *i.e.*, shell material by Vander Waals force. This technique is chiefly utilized to encapsulate volatile organic molecules like essential oils and vitamins. Essential oils are nanoencapsulated by entrapping them inside a polymer cavity by utilizing hydrogen bond and Vander Waals forces. Cyclodextrins have been widely used polymers to nanoencapsulate essential oils using this technique (Tiwari *et al.*, 2020).

Trending approaches in essential oil nanoencapsulation

Electro spinning

It is a process of producing nanofibers utilizing high electric voltage. Its principle is based on the processing of biopolymers by exposing to high electric impulses. The resulting

materials are nano structures showing better performances over bulk materials (Rostamabadi *et al.*, 2020). Nanofibers prepared by electrospinning have excellent characteristics, such as controllable fiber diameter, high porosity and large specific surface area. Furthermore, various functional active substances can be added to the spinning solution to prepare nanofibers with a wide range of functional properties (Yao *et al.*, 2021). Various variables/parameters governing the production of nano fibres using this technique are:-

- Parameters governing the process (electric field intensity).
- Solution characteristics (viscosity, surface tension).
- Electrospinning environment (temperature) (Jaiturong *et al.*, 2018; Rostamabadi *et al.*, 2020; Ding *et al.*, 2019).

The process of electrospinning can be categorized into coaxial electrospinning, single nozzle electrospinning (Dev and Hemamalini, 2018) and emulsion electrospinning (Garia-Moreno *et al.*, 2016; Feng *et al.*, 2019). Electrospin based nano structures can be efficiently utilised for encapsulating bioactive molecules (Rostamabadi *et al.*, 2020). In addition to various advantages of electrospinning technique, some limitations also accompany this process. These include:

- Industrial up scaling in an eco-friendly way.
- Inadequate *in vivo* studies.
- Precise solvent evaporation control rate.

Therefore, further research in this approach is necessary in order to circumvent these limitations.

Electro-spraying

Electro-spraying is a promising approach as it is versatile and possesses properties like no use of organic solvents and high temperature (Wang *et al.*, 2020; Jawarok *et al.*, 2008; Zhu *et al.*, 2012). It is an emerging area of research

Table 3: Various nano-encapsulation techniques, the steps involved and their uses.

Nanoencapsulation techniques	Steps involved in technique	Uses	References
Nanoprecipitation	Precipitation of polymer from organic phase (comprised of organic solvent, polymer and bioactives) on addition of aqueous phase (comprised mixture of polymer non solvents along with surfactants).	Best suited for encapsulating hydrophobic substance (EOs) in comparison to hydrophilic core material.	Rosset <i>et al.</i> , 2012; Ladj-Minost, 2012
Inclusion complexation	Entrapment of sample inside polymer cavity through Van der Waals forces and hydrogen bonding. Cyclodextrins are the most commonly used polymer for encapsulating EO through inclusion complexation.	Formation of transparent solution and increment in water solubility, protection against degradation upto 44-fold leading to physical and chemical stability.	Kfoury <i>et al.</i> , 2019
Liposome	Formation of membranous structure of liposome colloid particle due to the dispersion of phospholipids in aqueous phase.	Suitable carrier for hydrophobic, hydrophilic and amphipathic compounds as well as increased bioavailability, stability and water solubility of encapsulated compound along with enhanced bio-efficacy.	Sherry <i>et al.</i> , 2013

Table 4: Various nanoencapsulated essential oils/compounds and their antimicrobial activity.

Essential oil	Method	Microbes	Results	References
<i>Zataria multiflora</i>	Ionic gelation	<i>Botrytis cinerea</i>	Complete inhibition of fungal growth at 1500 ppm.	Mohammadi <i>et al.</i> , 2015b
Clove (<i>Syzygium aromaticum</i>)	Oil in water emulsion	<i>Aspergillus niger</i>	Better results were observed at 1.5 mg/mL as compared to free oil.	Hasheminejad <i>et al.</i> , 2019
<i>Thymus vulgaris</i>	Nanogel	<i>Aspergillus flavus</i>	Completely inhibited the growth of <i>Aspergillus flavus</i> at 500 mg/L.	Khalili <i>et al.</i> , 2015
Lime essential oil	Nanoprecipitation	<i>Staphylococcus aureus</i> , <i>Listeria monocytogenes</i> , <i>Shigella dysenteriae</i> and <i>Escherichia coli</i>	For CSNPs-LEO, the minimum inhibitory volume (MIV) was 2.5 mL for <i>E. coli</i> and 1.25 mL for <i>S. aureus</i> , <i>L. monocytogenes</i> and <i>S. dysenteriae</i> . While for CSNCs-LEO (nanocapsules) it was 5 mL for <i>S. aureus</i> , <i>L. monocytogenes</i> and <i>S. dysenteriae</i> and 10 mL for <i>E. coli</i> .	Sotelo-Boyas <i>et al.</i> , 2017
Cardamom essential oil	Ionic gelation	<i>E. coli</i> and <i>Staphylococcus aureus</i>	MIC for <i>E. coli</i> was 25% (v/v) and for <i>S. aureus</i> 10% (v/v).	Jamil <i>et al.</i> , 2016
<i>Cinnamomum zeylanicum</i>	Ionic gelation	<i>Phytophthora drechsleri</i>	Significant reduction in the disease severity at 1.5 g/L as revealed through <i>in vivo</i> studies.	Mohammadi <i>et al.</i> , 2015
<i>Ocimum sanctum</i>	Nanoemulsion	<i>Aspergillus flavus</i>	The minimum inhibitory concentration was 60 µL/L plus minimum aflatoxin inhibitory concentration was 30 µL/L.	Singh <i>et al.</i> , 2019
<i>Mentha piperita</i>	Nanogel	<i>Aspergillus flavus</i>	Completely inhibited the growth of <i>Aspergillus flavus</i> at 500 ppm.	Singh <i>et al.</i> , 2019
Savory essential oil (SEO)	Nanocomposite films	<i>Listeria monocytogenes</i> , <i>Staphylococcus aureus</i> <i>Bacillus cereus</i> and <i>Escherichia coli</i> .	SEO can be used as active packaging for improving the safety and shelf-life of food stuff.	Atef <i>et al.</i> , 2015
Peppermint and green tea oil	Emulsification-ionic gelation	<i>Escherichia coli</i> and <i>Staphylococcus aureus</i>	Antibacterial activity of CS/GTO NP was more potent than CS/PO NP against <i>Staphylococcus aureus</i> with 9.4 folds improvement compared to pure GTO and 4.7 fold against <i>Escherichia coli</i> .	Shetta <i>et al.</i> , 2019
<i>Gaultheria procumbens</i>	Nanogel	<i>Aspergillus flavus</i>	Completely inhibited the growth of <i>A. flavus</i> and aflatoxin B1 production at 1.0 mL/mL.	Kujur <i>et al.</i> , 2017
<i>Cymbopogon martinii</i>	Emulsification technique	<i>F. graminearum</i>	Ce-CMEO-NPs were presented efficient with enhanced antifungal and anti-mycotoxin activities and it could be due to perseverance of antifungal activity by controlled release of antifungal constituents from Ce-CMEO-NPs.	Kalagatur <i>et al.</i> , 2018
<i>Myristica fragrans</i>	Nanogel	<i>Aspergillus flavus</i>	Minimum inhibitory concentration (MIC) and minimum aflatoxin inhibitory concentration was 1.25 µL/L.	Yadav <i>et al.</i> , 2019
<i>Satureja hortensis</i>	Ionic gelation	<i>Staphylococcus aureus</i> , <i>Listeria monocytogenes</i> and <i>Escherichia coli</i>	All EO loaded NP samples completely inhibited the growth of bacteria.	Feyzioglu <i>et al.</i> , 2016

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<i>Pelargonium</i> L.	Nanogel	<i>Aspergillus flavus</i>	Inhibition of the growth as well as aflatoxin B ₁ synthesis at 1.00 µLmL ⁻¹ , as compared to the free oil at 1.25 µLmL ⁻¹ .	Kujur <i>et al.</i> , 2020
<i>Origanum vulgare</i> , <i>Eucalyptus globulus</i>	Nanoemulsion	<i>Aeromonas hydrophila</i> , <i>Streptococcus iniae</i>	The best antibacterial potential was shown by the nanoemulsion of <i>Origanum vulgare</i> (oregano) having 3.12 µg/ml as minimal inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) against all strains.	Gholipouranani <i>et al.</i> , 2019
<i>Monarda citriodora</i>	Nanoparticle	<i>Aspergillus flavus</i> and other storage moulds	Broad spectrum fungitoxicity and completely inhibited the growth of all tested molds and aflatoxin B1 (AFB1) synthesis by AF-LHP-SH1 at 1.40 and 1.20 µL/ml respectively.	Deepika <i>et al.</i> , 2020

for encapsulating bio active compounds. Here polymer liquids are exposed to high electric field resulting in fine liquid droplets. Nano sized particles are the final processed products when the solvents (in liquid particles) are evaporated. Various challenges such as low throughput hinder its large scale commercialization. Moreover electrosprayed products are subjected to aggregation and need appropriate wall materials (Wang *et al.*, 2020).

Supercritical fluid technique

Conventional encapsulation techniques employ the use of high temperatures/evaporation which limits or deteriorates the structures of volatile oils. Techniques such as supercritical fluid encapsulation can be used as an alternative. It involves non-pre/post thermal processing (Akolade *et al.*, 2020). It is broadly utilized due to its low critical temperature requirement as well as minimal utilization of organic solvents (Ezhilarasi *et al.*, 2013). The SCF technique can be categorized according to the function of SCF in the encapsulation process, as solvent, antisolvent, solute or cosolvent, nebulization compound, extractor and antisolvent techniques (Keven *et al.* 2014). There are various techniques that involve supercritical fluids and these include supercritical anti-solvent process (SAS) and its various modifications, rapid expansion of supercritical solutions (RESS), gas antisolvent process (GAS), supercritical fluid extraction of emulsions (SFEE), aerosol solvent extraction system (ASES), precipitation with compressed fluid antisolvent (PCA) *etc.* However, the SAS has recently received an enormous attention more than other methods because of its feasibility of application (Nerome *et al.* 2013; Esfandiari and Ghoreishi 2015).

Nanoprecipitation/solvent displacement

Its principle relies on the precipitation of polymer from organic phase on addition of an aqueous phase (Singh *et al.*, 2020). It is an effective method to produce nanocapsules in the size range of 100 nm and below which exhibit properties like, good stability against degradation, sustained release, higher encapsulation efficiency and enhanced bioavailability during *in vivo* studies along with displaying enhanced uptake by cells (Ezhilarasi *et al.*, 2013). Appropriate solvent and

non solvent phase need to be selected, which may vary for each bioactive components and the polymer and solvent need to be of food grade. Since it is a fast and economic method, it has been found to be most suitable for encapsulating hydrophobic substance than hydrophilic core materials (Ladj-Minost, 2012). Table 3 summarizes the techniques used for the encapsulation of essential oils.

Augmented antimicrobial activities of essential oil

Essential oils are sensitive volatile liquids which can be readily degraded when exposed to environmental factors (Sebesan and Caraban, 2008). Therefore to protect them from these intrinsic factors, essential oil formulations came in light which involves dispersing them in special carrier materials such as nanogels and nanoemulsions which has high loading capacity, high stability and significant release properties (Rasoli *et al.*, 2008). Thus, encapsulation is one of the most efficient methods for the formulation of bioactive oils and various approaches have been developed in this direction. EOs exhibit potential antimicrobial activities against a wide spectrum of micro flora. The interest in the use of essential oils as natural antimicrobials and preservatives in the food industry has geared up in the last years due to growing consumer demand for natural and safe preservatives with good organoleptic properties. Various encapsulated oils and their antimicrobial activity with improved efficacy has been shown in (Table 4).

CONCLUSION

In conclusion, many essential oils exhibit antimicrobial activity against foodborne pathogens due to the synergism of their major and minor components. The precise molecular composition of essential oils plays an important role in determining their antimicrobial efficacy. Due to their low water solubility, strong organo-leptic properties and low stability together with the high volatility they find a little application in medicine. Most of these drawbacks can be overcome by nano-encapsulating EOs thereby, lowering their dose and increasing long-term stability. Nano-encapsulation of EOs in liposomes, solid lipid nanoparticles, nano and micro-emulsions

and polymeric nanoparticles represent a promising strategy for overcoming their limitations. Although a number of different types of delivery systems have been developed, there is still a relatively poor understanding of the major factors governing the rational design of these systems for particular applications.

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