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REVIEW ARTICLE

A review on antifungal activity and mode of action of essential oils and their delivery as nano-sized oil droplets in food system

Suradeep Basak[1] · **Proshanta Guha**[1]

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Abstract An escalated demand of minimally processed food and increased negative perception for synthetic preservative has led to a lookout for a natural preservative. Essential oils (EOs) are volatile and aromatic secondary metabolites of plants that have been tapped mainly for its flavour and fragrances and various biological properties such as antimicrobial and antioxidant. The constituents and antifungal potential of EOs have been reported widely in the present scientific literature. Moreover, the current scientific research dealing with the mode of action of EOs on fungal spores and mycelial cells are very scarce, unlike bacteria. The antimicrobial efficacy of EO in real food system may alter due to interaction with food matrix components. Besides, minimum alteration in sensory qualities while retaining its maximum activity is the most sought-after criteria for food preservation with EOs. If the oil is applied in excess to have better antimicrobial activity, it may end up having an unacceptable organoleptic impact on the food. Appropriate edible delivery systems of EOs as an emulsion is a probable approach to retain the maximum efficacy of EOs in the food system. Nano-emulsification of EO could increase its bioactivity due to increased bioavailability in the food matrix. The basis of this review is to provide an overview of current knowledge about the antifungal properties and antifungal mode of action of EOs, and to recognize the application of EO as nano-sized oil droplets in the food system.

✉ Suradeep Basak
suradeepbasak@gmail.com

Proshanta Guha
suradeepbasak@gmail.com

[1] Agricultural and Food Engineering Department, Indian Institute of Technology Kharagpur, Kharagpur, West Bengal 721302, India

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Introduction

Modernization of techniques used for food processing has substantially contributed to elevate food safety and quality. In spite of modern technological advancement, nearly 30% population in industrialized countries suffered from food-borne diseases each year and that 1.8 million people died from diarrhoeal diseases (WHO 2007). The demand for minimally processed fresh food products poses major challenges for food safety and quality. Microbial contamination is one of the main factors that determine loss of food quality and consequently shelf life of the product. Major food spoilage moulds are the opportunistic biological agents that are omnipresent in nature. About 25% of total annual production of various crops is affected by mycotoxins, especially those produced by *Aspergillus*, *Penicillium* and *Fusarium* (FAO 2004).

The demand of minimally processed food has promoted the use of naturally occurring antimicrobials like EOs (Juneja et al. 2012). Essential oils (EOs) are secondary metabolites of plants which can be extracted from herbs and spices. Many studies have reported antimicrobial activity of EO of different plant origin and the antimicrobial properties of EOs can be attributed to the presence of bioactive compounds (Burt 2004; Bakkali et al. 2008). These activities may be enhanced many folds if the EO is applied in the form of small droplets (preferably in nano-range).

Microemulsion and nanoemulsion are colloidal systems that consist of an oil phase dispersed in an aqueous continuous phase and a thin interfacial film of surfactant

molecule surrounding each oil droplet having size within nanometer range, which may preserve the appearance of the food products (McClements and Rao 2011). These appealing natures have encouraged preparation of nanoemulsions of EOs (Liang et al. 2012; Salvia-Trujillo et al. 2015). In food system, problem of substantial reduction in antimicrobial efficacy of EOs can be resolved by nano-emulsification of EO so as to enhance bioavailability, reduce ingredient interaction, and improve quality and safety of food (Weiss et al. 2009; Donsì et al. 2011).

The present review focuses on updated account on the antifungal activity, and antifungal mode of action of various plant based EOs, and to recognize the prospective application of EOs as nano-sized oil droplets in food products.

Essential oils

Essential oils or ethereal oils are natural, volatile secondary metabolites produced by aromatic plants (Guenther 1952). The term "essential oil" is supposedly derived from the term *quinta essentia* coined by the Swiss reformer of medicine Paracelsus von Hohenheim in the sixteenth century, which means effective component of a drug (Guenther 1952). Each EO is a complex mixture of terpenes (monoterpenes, sesquiterpenes, and their oxygenated derivatives, such as alcohols, aldehydes, esters, ethers, ketones, phenols, and oxides), and also phenolic and phenylpropanoid compounds derived from the acetate-mevalonic acid and shikimic acid pathways, respectively (Bakkali et al. 2008).

Sources of essential oil

About 3000 EOs from different plant species have been reported, but only 300 of them are economically important and used in the fragrance, food, pharmaceutical, agricultural, and sanitary industries (Burt 2004). EOs are stored in secretory cells, cavities, canals, epidermic cells, and glandular trichomes of plant organs (Bakkali et al. 2008). The composition of an EO is affected by harvesting season, geographical location, maturity, and part of the plant utilized, genetic variation, postharvest drying, and storage conditions (Hussain et al. 2008).

Chemistry of essential oil

Carbon, hydrogen, and oxygen are three elements which make up the basis of EO. They are the complex natural mixture of about 20–60 chemical components with major components at relatively high concentration (20–70%), and rest are minor components present in trace amounts (Burt 2004). Most common class of compound found in EOs is the terpenes. Terpenes are combinations of several 5-carbon-base (C5) units called isoprene (Guenther 1952). Terpenes form the building blocks of monoterpenes (C10), sesquiterpenes (C15), diterpenes (C20), triterpenes (C30) tetraterpenes (C40) and large sequences (Bakkali et al. 2008). A terpene containing oxygen is called a terpenoid. The monoterpenes formed by conjoining of two isoprene units in a head-to-tail configuration. They constitute 80–90% components of EO and allow a large variety of structures.

There are numerous EOs which are isolated and characterized throughout the world. Qualitative and quantitative estimation of EO is achieved using GC–MS (Daferera et al. 2000). The difference in composition results in different organoleptic properties of EOs. Likewise, the characteristic odor of betel leaf EO cv. Tamluk Mitha is due to the presence of low molecular weight complex chemical compounds mainly terpenes, terpenoid, and phenolic compounds (Basak and Guha 2015).

EOs are composed of major and minor bioactive components which are mostly responsible for its biological activities. The biological effects can be either due to major components or synergy between both major and minor chemical components of the EO. There are scientific articles which suggested a biological effect of a major component of various EOs (Bakkali et al. 2008). Characterization of EOs gives a clear overview of its spectrum of biological activity due to the presence of different bioactive chemical compounds. EOs can have varied biological activities such as antibacterial, antifungal, larvicidal etc. The present article document antifungal activity of various plant based EOs in nutrient medium as well as in real food system.

Antifungal activity of essential oil

In vitro antifungal activity

Antimicrobial activity of various EOs is recognized for a long time now. Presence of bioactive compounds provides antimicrobial properties to EOs (Burt 2004). Antimicrobial activity of EO can be due to individual effect of major components or due to a synergistic effect of its minor components (Daferera et al. 2000).

Among earlier studies on the antimicrobial activity of EO of betel leaf, Dubey and Tripathi (1987) have reported fungistatic nature of EO of *Piper betle* L. leaves against *Aspergillus flavus*. Nguefack et al. (2004) has shown complete inhibition of conidial germination and the mycelial growth of three fungi (*Fusarium moniliforme*, *A. flavus*, *A. fumigatus*) on corn meal agar using *Ocimum*

gratissimum (African basil), *Thymus vulgaris* (thyme) and *Cymbopogon citratus* (lemongrass) EO at 800, 1000, and 1200 ppm, respectively. Vági et al. (2005) investigated antifungal properties of *Origanum majorana* L. EO against three foodborne fungi (*A. niger*, *Trichoderma viride, Penicillium cyclopium*).

Soylu et al. (2006) showed growth inhibition of *Phytophthora infestans* with 6.4 µg/ml concentration of *Origanum syriacum* cv. Bevanii (oregano), *Thymbra spicata* subsp. *spicata* (thyme), *Foeniculum vulgare* (fennel) EOs, whereas *Rosmarinus officinal* (rosemary) and *Lavandula stoechas* subsp. *stoechas* (lavender) EOs were effective only at 12.8, and 25.6 µg/ml. Sharma and Tripathi (2006) have shown complete mycelial inhibition and spore germination of *P. chrysogenum* MPPLU 27 and *P. expansum* MPPLU 24 using *Citrus sinensis* L. Osbeck EO. Tzortzakis and Economakis (2007) have completely inhibited sporulation of *Colletotrichum coccodes*, *Botrytis cinerea*, *Cladosporium herbarum*, *Rhizopus stolonifer* and *A. niger* using 500 ppm of *C. citratus* L. EO. Viuda-Martos et al. (2008) demonstrated complete mycelial inhibition of *A. niger* and *A. flavus* with a concentration of 0.94% of lemon, orange, mandarin and grapefruit EOs. They also reported the maximum efficacy of grapefruit against *Penicillium verrucosum* and *P. chrysogenum*, orange EO against *A. niger* and mandarin EO against *A. flavus*. Complete inhibition of mycelial growth and H^+-ATPase activity of *A. flavus* using *Lippia rugosa* (Gossolhi) EO at a concentration of 1000 ppm was reported by Tatsadjieu et al. (2009).

Minimum inhibitory concentration (MIC) of *Ocimum sanctum* (holy basil) EO and its major component eugenol against *A. flavus* NKDHV8 was found to be 0.3 and 0.2 µl/ml, respectively (Kumar et al. 2010). Prakash et al. (2010) reported mycelial inhibition of *A. flavus* at 0.7 µl/ml of *Piper betle* L. cv. Magahi EO. Mycelial growth of five foodborne fungi such as *A. flavus*, *A. oryzae*, *A. niger*, *Alternaria alternata* was completely inhibited using 5 µl/ml concentration of *Cicuta virosa* L. var. Latisecta Celak EO (Tian et al. 2011a). Direct addition of 16,000 ppm orange peel EO inhibited mycelial growth of *A. flavus* (Velázquez-Nuñez et al. 2013). Growth inhibition of *P. expansum* using pomelo EO was shown to be most effective among four different EOs from citurs species such as orange, mandarin, pomelo, and lime (Van Hung et al. 2013). Tao et al. (2014a) have reported complete inhibition of *P. italicum* and *P. digitatum* at 2.5 and 40 µl/ml concentration of *Citrus reticulata* Blanco (ponkan) EO. Kohiyama et al. (2015) tested the antifungal efficacy of *Thymus vulgaris* EO against *A. flavus.* MIC and ergosterol biosynthesis of *A. flavus* was inhibited at 100 and 250 µg/ml of thyme EO. Antifungal activity of five EOs of *Pimpinella anisum*, *Thymus vulgaris*, *Pelargonium odoratissimum*, *Rosmarinus officinalis* and *Foeniculum vulgare* were tested against pathogenic fungus specific to cereals viz. *Oculimacula yallundae, Microdochium nivale, Zymoseptoria tritici, Pyrenophora teres* and *Fusarium culmorum* (Matusinsky et al. 2015). Li et al. (2016) reported 0.5 and 1.0 µl/ml as minimum inhibitiory concentration and minimum fungicidal concentration with fumigation of EO of *Litsea cubeba* against *A. flavus*. Božik et al. (2017) demonstrated antifungal activity of EO vapour of clove, thyme, oregano, and lemongrass against *A. flavus*, *A. parasiticus*, and *A. clavatus* isolated from oats. Some important findings of in vitro antifungal activity of EOs are summarized in Table 1.

Presence of volatiles compounds makes EO bioactive in vapour phase along with liquid phase, which makes them a potential natural fumigant for protection of stored products. Burt (2004) reported that antimicrobial activity of EOs in food could be achieved only at higher concentrations as compared to MIC in a nutrient medium. Preliminary tests used for measurement of the antifungal activity of EOs and plant-based components before more detailed studies are disc diffusion assay and disc volatilization assay (López et al. 2005; Rammanee and Hongpattarakere 2011). EOs may have static (inhibitory) effect or cidal (killing) effect on a range of microorganisms. MIC of an EO differs from one microbe to other, and its spectrum of antifungal activity depends on the range of microorganism it can act. Different experimental protocols were adopted and modified by various researchers to best suit their particular set of conditions. Many factors like methods used to extract EOs, inoculum volume, the concentration of inoculum, culture medium used, pH of the medium and incubation time affects the result of the experiments determining MIC (Burt 2004). In most of the cases, antifungal activity of EOs is reduced in food system as opposed to in vitro system due to interaction of EO components with food components (Basak 2017). This interaction results in lower availability of EO components to the microbial surface, which further leads to reduced antimicrobial activity of EOs.

Antifungal activity on food products

Apart from the flavour, fragrance, antiseptic and medicinal properties, EOs are also used as food preservatives, and as antimicrobial, analgesic, sedative, anti-inflammatory and spasmolytic remedies (Bakkali et al. 2008). The demand of minimally processed food has escalated with emergence of green consumerism, which in turn has promoted the use of naturally occurring antimicrobials (Juneja et al. 2012). Most of the chemical compounds in EOs have GRAS status as they originated from plants (Burt 2004; Hyldgaard et al. 2012). Spices and herbs, extracts and EOs of different plant families are a source of some major natural antimicrobials.

Table 1 Summary of in vitro antifungal activity of some EOs from different plants source

Target fungi	Essential oil	References
A. flavus	Betel leaf	Dubey and Tripathi (1987)
F. moniliforme, A. flavus, A. fumigatus	African basil, Thyme, Lemongrass	Nguefack et al. (2004)
A. niger, T. viride, P. cyclopium	Marjoram	Vági et al. (2005)
P. infestans	Oregano, Thyme, Fennel, Rosemary, Lavender	Soylu et al. (2006)
P. chrysogenum, P. expansum	Orange	Sharma and Tripathi (2006)
C. coccodes, B. cinerea, C. herbarum, R. stolonifer, A. niger	Lemongrass	Tzortzakis and Economakis (2007)
A. niger, A. flavus, P. verrucosum, P. chrysogenum	Lemon, Orange, Mandarin, Grapefruit	Viuda-Martos et al. (2008)
A. flavus	Gossolhi	Tatsadjieu et al. (2009)
A. flavus	Holy basil	Kumar et al. (2010)
A. flavus	Betel leaf	Prakash et al. (2010)
A. flavus, A. oryzae, A. niger, A. alternata	Shiluozi	Tian et al. (2011a)
A. flavus	Orange	Velázquez-Nuñez et al. (2013)
P. expansum	Pomelo	Van Hung et al. (2013)
P. italicum, P. digitatum	Ponkan	Tao et al. (2014a)
A. flavus	Thyme	Kohiyama et al. (2015)
O. yallundae, M. nivale, Z. tritici, P. teres, F. culmorum	Thyme, Rosemary, Anise, Fennel	Matusinsky et al. (2015)
A. flavus	Litsea	Li et al. (2016)
A. flavus, A. parasiticus, A. clavatus	Thyme, Clove, Oregano, Lemongrass	Božik et al. (2017)

Feng and Zheng (2007) have reported the 34.2% reduction of *Alternaria alternata* infected cherry tomatoes at 500 ppm of Cassia EO. Omidbeygi et al. (2007) reported maximum inhibitory concentrations of thyme (*Thymus vulgaris*) and summer savory (*Satureja hortensis*) against *Aspergillus flavus* in tomato paste were 350 and 500 ppm, respectively. Bluma and Etcheverry (2008) have studied antifungal activity of *Pimpinella anisum* L. (anise), *Peumus boldus* Mol (boldus), *Hedeoma multiflora* Benth (mountain thyme), *Syzygium aromaticum* L. (clove) and *Lippia turbinate* var. Integrifolia (griseb) (poleo) EOs against *Aspergillus* section *flavi* in maize grain under different water activity. Tzortzakis (2009) suggested that exposure of tomato fruit to cinnamon EO vapours (500 ppm) for 3 days before inoculation of *Colletotrichum coccodes* and *Botrytis cinerea* significantly reduced lesion development. Gutierrez et al. (2009) have evaluated the potential of oregano (*O. vulgare*) and thyme (*T. vulgaris*) EOs for the control of natural spoilage microflora on ready to eat lettuce and carrot. Soylu et al. (2010) on the other hand demonstrated the 77% curative activity and 33% protective activity at 75 ppm of origanum (*Origanum syriacum* L. var. Bevanii) EO against tomato grey mould disease agent *B. cinerea.*

Tian et al. (2011b) reported the reduction in percentage of decayed cherry tomatoes by 94.4% for *A. niger*, 88.9% for *A. flavus* and *A. oryzae*, and 83.3% for *A. alternata* using 120 µl/ml of dill (*Anethum graveolens* L.) EO. El-Mogy and Alsanius (2012) have also reduced the percentage of *B. cinerea* infected strawberries by 40% by using 800 ppm of cassia EO. Tyagi et al. (2014) showed the anti-yeast activity of lemongrass oil against *Saccharomyces cerevisiae* and in mixed fruit juices, respectively. Kedia et al. (2014) also reported the efficiency of cumin seed EO (*Cuminum cyminum*) against fungal deterioration of wheat and chickpea during storage. Inhibition of mycelial growth and aflatoxin B1 production of *A. flavus* in corn and soybean grains using mentrasto (*Ageratum conyzoides*) and oregano (*Origanum vulgare*) EOs was reported by Esper et al. (2014). Mishra et al. (2015) has demonstrated the efficacy of EO of *Caesulia axillaris*, *Cymbopogon khasans*, and *Cymbopogon martinii* against degradation of active component (andrographolide) of sun dried leaves of *Andrographis paniculata* by toxigenic *A. flavus* during storage. Kiran et al. (2016) demonstrated inhibition of *A. flavus* and *A. niger* on *Eleusine coracana* (finger millet) up to 60–83% when exposed to 0.5 µl/ml of *Cinnamomum zeylanicum* Blume EO. Boukaew et al. (2017) reported antifungal activity of EO of *Cinnamomum bejolghota*, *Syzygium aromaticum*, *Capsicum annuum* and *Vatica diospyroides* against 10 different isolates of *A. flavus* on maize seeds. They have showed complete inhibition of disease infection of *A. flavus* PSRDC-2 on maize seeds at 0.01 and 0.05 µl/ml of *S. aromaticum* and *V.*

diospyroides EO, respectively. Basak (2017) have described the efficacy of EO of *Piper betle* L. cv. Tamluk Mitha on growth and inhibition of *A. flavus* and *Penicillium expansum* on raw apple juice and tomato paste. Table 2 summarizes some significant findings on antifungal activity of EOs on grain, fruits, vegetables and their products. The antifungal activity of EOs can be understood with its mechanism of action on fungal strains.

Mode of action of essential oil on fungal strains

Most of the studies reported the cytotoxic nature of EOs and its constituents is due to their capability to disrupt cell wall and cell membrane, coagulate the cytoplasm, and hence damage of cellular organelles and escape of macromolecules (Burt 2004; Hyldgaard et al. 2012). The lipophilic nature of EOs allow them to pass through the cell wall and cytoplasmic membrane damage while disrupting various layers of polysaccharide, fatty acids and phospholipids eventually making them permeable (Helal et al. 2006; Rammanee and Hongpattarakere 2011; Dwivedy et al. 2016). Hydrophobic components present in EO could change the permeability of microbial cell membrane for cations such as H^+ and K^+, which change the flow of protons, modifying cellular pH and affecting the chemical composition of the cells and their activity (Hyldgaard et al. 2012; da Cruz Cabral et al. 2013). However, the magnitude of antimicrobial activity of EO or its active compounds depends on the differential permeability of the cell membrane (da Cruz Cabral et al. 2013). The loss in differential permeability results in an imbalance in intracellular osmotic pressure, which subsequently disrupt intracellular organelles, leakage of cytoplasmic contents and sometimes energy-storing molecules (ATP) and eventually cell death. EOs can depolarize mitochondrial membrane by decreasing the membrane potential that affects ionic Ca^{2+} cycling and other ionic channels and reduce the pH gradient of microbial cells (Bakkali et al. 2005). Dwivedy et al. (2017) revealed the increase in leakage of Ca^{2+}, K^+ and Mg^{2+} ions from *A. flavus* LHP-PV-1 cell membranes when treated with 1.25 and 2.5 µl/ml *Mentha cardiaca* L. EO. Some studies showed a change in the ultrastructure of fungal cells under the influence of EOs. Among them, Rasooli et al. (2006) have observed severe damage to the cell wall, cell membrane and cellular organelle mainly mitochondrial destruction of *A. niger* treated with an inhibitory concentration of *Thymus eriocalyx* and *Thymus x-porlock* EOs under transmission electron microscopy (TEM). On the other hand, Soylu et al. (2006) have investigated the effect of EOs obtained from an aerial part of several aromatic plants against light blight disease agent (*Phytophthora infestans*) of tomato under scanning electron microscopy (SEM). The images revealed considerable morphological alterations in the hyphae such as cytoplasmic coagulation, vacuolations, hyphal shriveling and protoplast leakage when exposed to volatile as well as contact phase of the EO. Soylu et al. (2010) have conducted similar experiments to observe the morphological alteration in the ultrastructure of hyphal cells of *B. cinerea* treated with an effective concentration of same EOs. Nogueira et al. (2010) have noted ultrastructure changes on endomembrane

Table 2 Summary of antifungal activity of EOs from different plants source on food products

Target fungi	Food matrix	Essential oil	References
A. alternata	Cherry tomato	Cassia	Feng and Zheng (2007)
A. flavus	Tomato paste	Thyme, Savory	Omidbeygi et al. (2007)
Aspergillus section *flavi*	Maize grains	Anise, Boldus, Mountain thyme, Clove, Poleo	Bluma and Etcheverry (2008)
C. coccodes, *B. cinerea*	Tomato fruit	Cinnamon	Tzortzakis (2009)
B. cinerea	Tomato leaves	Origanum	Soylu et al. (2010)
A. niger, A. flavus, A. oryzae, A. alternata	Cherry tomatoes	Dill	Tian et al. (2011b)
B. cinerea	Stawberries	Cassia	El-Mogy and Alsanius (2012)
S. cerevisiae	Real fruit juice	Lemongrass oil	Tyagi et al. (2014)
A. flavus	Soyabeans, Corn	Mentrasto, Oregano	Esper et al. (2014)
A. flavus	Sun dried *Kalmegh* leaves	Pink node flower, Cymbopogon species	Mishra et al. (2015)
A. flavus	Finger millet	Cinnamon	Kiran et al. (2016)
A. flavus	Maize seeds	Clove, Capsicum, Cinnamon, Vatica	Boukaew et al. (2017)

system, mainly plasma membrane and mitochondria of *A. flavus* cells treated with *Ageratum conyzoides* EO using TEM. They showed the plasma membrane with rough, villiform, invaginated vesicles and sometimes decoupled from the cell wall of the treated mycelial cells. They have also observed disrupted internal structure of mitochondria with a decreased ridge polarization in the cristae. Tolouee et al. (2010) revealed step-wise degradation of *A. niger* van Tieghem treated with different concentrations of *Matricaria chamomilla* L. flower EO which includes TEM images showing vacuolation of cytoplasm, early degradation of electron-dense granules and folding and detachment of plasma membrane from the cell wall at lowest concentration of the EO followed by disruption of plasma membrane accompanied by the formation of small vesicles (lomasomes) between plasma membrane and cell wall at concentration that inhibit fungal growth by approximately 41%. At the highest fungistatic concentration of EO, complete autolysis of cell occurred that involves disorganization and depletion of hyphal cytoplasm, and membranous organelles include nuclei, endoplasmic reticulum, and mitochondria. The group has also revealed SEM images of *A. niger* treated with the fungistatic concentration of the EO as collapsed and folded hypha with short branches, undifferentiated hyphal tips and lack of conidiation. SEM images of *A. niger* hypha treated with EO of *Anethum graveolens* was found to be disrupted, shriveled, collapsed, flattened and empty (Tian et al. 2011a). Manso et al. (2013) showed disrupted hyphae, and irregular cellular mass, shrunk conidia of *A. flavus* resulted after treatment with cinnamon EO. Tao et al. (2014b) have also shown disrupted plasmalemma and structurally disorganized cytoplasm of *P. italicum* treated with the highest concentration of citral using TEM and SEM images revealed distorted and collapsed hyphae.

Interaction of EO components with biomolecules of food components (such as carbohydrates, proteins, lipids) interfere with the antimicrobial activity of EOs (Hyldgaard et al. 2012; Perricone et al. 2015). Among four EOs viz. thyme, clove, oregano and lemongrass Božik et al. (2017) revealed that oats treated with moderate fungicidal concentration (250 µl/l of the container volume) of fumigated lemongrass EO was only acceptable by consumers. Mostly they confer inhibitory activity at a higher concentration in food as compared to in vitro system. This becomes a major hurdle to overcome while using EOs as a food preservative.

Challenges in application of essential oil in food matrix

There are a considerable amount of research findings that involved an external application of EOs on fresh produce to enhance its shelf stability. The technological challenges limited the direct incorporation of EOs in food. The hydrophobicity of EOs makes them barely water soluble, causing non-uniform distribution in food matrices. The hydrophobic binding of EO components with food components may minimize the antimicrobial efficacy and/or reduce the duration of effectiveness of EOs (Donsì et al. 2012; Hyldgaard et al. 2012; Perricone et al. 2015; Prakash et al. 2015). EOs may be added in higher concentration when applied in food matrices to attain similar antimicrobial activity as of nutrient medium (Burt 2004). Minimum alteration in sensory qualities while retaining its maximum activity is the most sought after criteria for food preservation with EOs (Gutierrez et al. 2009; Basak 2018).

Essential oil based edible delivery system

An appropriate edible delivery system of EOs is the probable approach to retain the maximum efficacy of EOs in the food system. According to Weiss et al. (2009), and Donsì et al. (2014), this strategy will provide following advantages over direct addition of EOs: (a) improve the stability of EO, (b) enhance uniform distribution in aqueous portion of the food, where proliferation of microorganism is high, (c) reduce impact of EOs in organoleptic properties, (d) enhance their biological activity through the promotion of mass transfer.

Important features of edible delivery system are: (a) use of food-grade ingredients for its formulation, (b) simple manufacturing process which is industrially scalable; (c) protection of encapsulated EOs from chemical degradation, (d) wide food matrix compatibility, (e) capability to cross biological membranes which enhance its bioavailability (McClements et al. 2007).

Emulsion

The emulsion is a colloidal dispersion of two immiscible liquids (usually oil and water, but not always), with one of the phase dispersed as small droplets in the other phase (McClements 2005). The emulsion can be classified based on its spatial arrangement of oil and aqueous phases into two categories: oil-in-water (O/W) emulsion and water-in-oil (W/O) emulsion. When oil droplets are dispersed in an aqueous phase, it is O/W emulsion, and when water

droplets are dispersed in the oil phase, it is W/O emulsion. The substance that constitutes the droplets in the emulsion is referred to as dispersed phase, whereas the substance that builds up the surrounding liquid is called continuous phase. Based on droplet diameter, the emulsion can be classified into three categories: macroemulsion, nanoemulsion, and microemulsion (McClements and Rao 2011).

A conventional emulsion, also known as macroemulsion is thermodynamically unstable because free energy of individual oil and water phases is lower than that of the emulsion. Consequently, they tend to break down over time. Macroemulsion scatter light strongly because the droplet size and the wavelength of light are in similar dimension and hence, they appear optically turbid or opaque. On the other hand, nanoemulsion and microemulsion have relatively small droplet size as compared to the wavelength of light that makes them optically transparent. Very small droplet size provides nanoemulsion a better stability to gravitational separation and aggregation than conventional emulsion. However, nanoemulsions are still thermodynamically unstable and will tend to break down over time due to the positive free energy associated with creating an interface between oil and aqueous phase due to hydrophobic nature of the former. On the contrary, thermodynamic stability of microemulsion is due to its lower free energy than of phase-separated components from which it was prepared. The microemulsion is stable only under certain conditions such as composition and temperature, and alteration in any of such conditions will make them thermodynamically unstable and will convert to another kind of system, e.g., phase separated, liquid crystalline, nanoemulsion, or macroemulsion. Again, if those conditions are put back to the original then it should revert to microemulsion, and the rate of conversion is dependent on kinetic energy barriers (McClements 2005; McClements and Rao 2011).

Nanoemulsion

The nanoemulsion is consists of an oil phase dispersed in an aqueous continuous phase, and a thin interfacial film of surfactant molecule surrounding each oil droplet of the size range of 10–100 nm (Tadros et al. 2004; McClements 2005; McClements et al. 2007; McClements and Rao 2011). Small droplet size causes Brownian motion that provides the nanoemulsion its stability against droplet aggregation and gravitational separation (McClements 2005). Nanoemulsions formulated using food grade ingredients can be used to encapsulate and deliver lipophilic functional components make them an ideal candidate as the carrier of EOs (McClements et al. 2007; McClements and Rao 2011). The oil phase can be formed of various non-polar sources such as monoacylglycerols, diacylglycerols, triacylglycerols, free fatty acids, EOs, flavour compounds, mineral oils, waxes, weighting agent, oil-soluble vitamins, and lipophilic compounds. The aqueous phase of nanoemulsion consists primarily of water, but it may contain various other polar compounds such as carbohydrates, proteins, minerals, acids, bases and co-solvents (e.g., simple alcohols, polyols). The formation, stability, and properties of nanoemulsion often depends on the bulk physicochemical characteristics of the oil and aqueous phase, e.g., polarity, water solubility, pH, interfacial tension, refractive index, rheology, density, phase behaviour, ionic strength and chemical stability (Tadros et al. 2004; McClements 2005).

Microemulsion

The microemulsion is a colloidal system, which is thermodynamically and kinetically stable under a particular set of environmental conditions (e.g., composition and temperature) and can be formed using oil, surfactant, and water with an input of low energy, such as stirring, mixing or heating. If the conditions are altered, then it will no longer remain thermodynamically stable and will convert to another kind of system, e.g., phase separated, liquid crystalline, bicontinuous, nanoemulsion, or conventional emulsion (McClements and Rao 2011). The thermodynamic stability is due to the low free energy of the microemulsion than that of phase separated components from which it was prepared. An input of low energy is sufficient to break the kinetic energy barrier between phase separated components and microemulsion (McClements and Rao 2011). Shelf-stability and low production cost make microemulsion advantageous over nanoemulsion.

Essential oil based emulsion having nano-sized oil droplets

The present scientific literature have mostly focussed on antibacterial activity of various EO based nanoemulsions in a nutrient medium as well as in food system (Liang et al. 2012; Salvia-Trujillo et al. 2015), whereas the activities of the same on fungi are scarce. Ribes et al. (2017) showed enhanced antifungal activity of encapsulated cinnamon leaf EO with emulsifiers such as whey protein isolate and polysorbate 80 against *A. niger*. Antimicrobial spectrum of EO based nanoemulsion varies with the composition of the emulsion. Donsì et al. (2011) have reported antimicrobial activity of encapsulated EO component D-limonene and a terpenes mixture in food-grade ingredients against *Lactobacillus delbrueckii* in pear and orange juices. In contrast, there are research findings by Terjung et al. (2012) that have reported higher antimicrobial activity of carvacrol and

eugenol macroemulsions than nanoemulsion [with a triglyceride (Miglyol 812 N) as oil phase] against *Escherichia coli* and *Listeria innocua*. They hypothesized that smaller the diameter of an oil droplet in the nanoemulsion, higher will be the droplet concentration that will result in a lower concentration of the selected EO compounds in an aqueous phase. The reduced availability of the compounds resulted in aqueous phase reduced the interaction with antimicrobial surfaces.

On the other hand, there is a limited number of studies in the current literature on the antimicrobial activity of EOs based microemulsions. Among them, Ghosh et al. (2013) have evaluated the antibacterial activity of cinnamon EO based microemulsion against *Staphylococcus aureus*. Similarly, Ma et al. (2016) have inhibited the cocktails of *Listeria monocytogenes*, *Salmonella enterica* or *E. coli* O157:H7 growth using microemulsions formulated with cinnamon bark oil, eugenol or thymol oil, Tween 80. Deng et al. (2015) have assessed antioxidant activity as well as the antibacterial activity of thymol microemulsions using different surfactants against *E. coli* and *S. aureus*. Finally, Basak and Guha (2017a) have also suggested the antifungal activity of microemulsified EO of betel leaves against *A. flavus* in tomato paste. They have also revealed similar activity using betel leaf EO based microemulsion against *P. expansum* in apple juice (Basak and Guha 2017b). Recently, Ribes et al. (2018) suggested the reduction in amount of cinnamon bark EO by 25% to achieve growth inhibition of *A. niger* when used in combination with antifungal agent (viz. zinc gluconate or *trans*-ferulic acid) in oil-in-water emulsion to control spoilage of strawberry jam.

Conclusion

Food safety is considered to be of prime concern for human health because a large population suffers from gastrointestinal or other types of diseases the world over due to unsafe food. Food spoilage fungi like *Aspergillus* spp. and *Penicillium* spp. infect and rapidly propagate in raw or processed food materials and produce mycotoxins, which is injurious to health causing various diseases. Therefore, it is necessary to preserve food by inactivating or inhibiting the harmful microbial growth in the food items. Food preservation is normally done with chemical preservatives that have been recognized by Food and Agriculture Organization of the United Nations. These chemical preservatives include lactic acid, citric acid, acetic acid, sodium benzoate, sodium diacetate, sodium propionate, potassium sorbate, methyl paraben, sulphur dioxide and sodium nitrite, etc. Some of these food preserving chemicals are known to be quite notorious for their adverse effect on human health if exceeds acceptable limits of daily intake. Therefore, search for innocuous botanical natural preservative started long back. As a result of this search, EOs of plant origin were found to be a much better alternative to the chemical-based synthetic food preservatives. The EOs have gained its niche as antimicrobials in food preservation with increasing trend of green-consumerism since most of the EO components have GRAS status as they originate from edible plants in contrast to synthetic chemicals. The volatile EOs not only prove to be useful antimicrobial and food preserving agents but also reported to have imparted additional advantage of better organoleptic properties in some preserved food items. Minimum alteration in sensory qualities while retaining its maximum activity is the most sought-after criteria for food preservation with EOs. EO extracted from various plant sources have been reported to be effective for food flavouring and preservation besides other uses. Some studies suggested the antifungal activity of EO against food spoilage moulds. These included inhibition of spore germination, inactivation, and mycelial growth. Further, EO, when added in the form of microemulsion or nanoemulsion may enhance its bioavailability and antifungal properties. Such application, instead of ordinary spray is also reported to reduce the required dose for satisfactory antifungal activity. This method of application is again supposed to help to preserve food with organoleptically acceptable dose, which does not interfere with the desired properties of the concerned foodstuff but at the same time extends its storability.

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