RSC Advances

ROYAL SOCIETY OF CHEMISTRY

www.rsc.org/advances

Essential Oil Encapsulations: Uses, Procedures, and Trends

Hamid Majeed[a], Yuan-Yuan Bian[a], Barkat Ali[a], Anjum Jamil[b], Usman Majeed[b], Qaiser Farid Khan[c], Khalid Javed Iqbal[d], Charles F Shoemaker[e] and Zhong Fang[a]

[a]Key Laboratory of Food Colloids and Biotechnology, Ministry of Education, School of Food Science and Technology, Jiangnan University, Wuxi 214122, P.R. China.

[b]Department of veterinary sciences, University of Agriculture Faisalabad, Pakistan

[c]Microbial Electro-chemistry Research Group, Swette Centre for Environmental Biotechnology Biodesign Institute Arizona State University, Tempe, AZ.

[d]Department of Life Sciences, The Islamia University of Bahawalpur, Pakistan

[e]Department of Food Science and Technology, University of California, Davis, CA 95616, USA

[a] Corresponding author:

Hamid Majeed

Key Laboratory of Food Colloids and Biotechnology,
Ministry of Education, School of Food Science and
Technology, Jiangnan University,
Wuxi 214122, P.R. China.
Tel. +8618351570870.

E-mail address: hamid.majeedju@yahoo.com

Essential Oil Encapsulations: Uses, Procedures, and Trends

Hamid Majeed, Yuan-Yuan Bian, Barkat Ali, Anjum Jamil, Usman Majeed, Qaiser Farid Khan, Khalid Javed Iqbal, Charles F Shoemaker and Zhong Fang

Abstract:

Recently there has been an increased interest towards the biological activities of essential oils (EOs). However, EOs are unstable and susceptible to degradation when exposed to environmental stresses like oxygen, temperature, and light. Therefore, attempts have been made to preserve them through encapsulation in various colloidal systems such as microcapsules, nanospheres, nanoemulsions, liposomes, and molecular inclusion complexes. This review focuses on various techniques used for the encapsulation of EOs, potential applications in food, and their behaviours/trends after encapsulation. The encapsulation efficiency, particle size, and physical stability of EOs encapsulated in colloidal systems is dependent on the kind of technique and the type and concentration/ratio of emulsifier/wall material used. Moreover, the benefits associated after encapsulation, namely bioavailability, controlled release, and protection of EOs against environmental stresses are discussed. The applications of encapsulated EOs are also summarized in this review. Encapsulated EOs are promising agents that can be used to increase the anti-microbial, antifungal, antiviral, and pesticidal activities of EOs in real food systems, to study their action mechanism, and to provide nonlethal therapeutic agents to treat several diseases.

Keywords: Essential oils, Antimicrobial, Bioavailability, Therapeutic agents and Colloidal systems.

1. Introduction
2. Essential oil and their properties
3. Encapsulation of essential oils: Techniques/strategies
    3.1 Chemical Procedures
    3.2 Physicochemical Procedures
    3.3 Mechanical Procedures
    3.4 Other Encapsulation Methods
    3.5 Emulsifiers/wall materials used for essential oils
    3.6 Encapsulation and its benefits
        3.6.1 Encapsulation of essential oils for controlled release
        3.6.2 Encapsulation of essential oils for controlled release
        3.6.3 Encapsulation of essential oils for increased stability
4. Encapsulated essential oils applications
5. Encapsulated essential oils behaviours/trends
6. Conclusions and Future Perspectives

1. Introduction

EOs are diverse group of natural aromatic compounds isolated mostly from non-woody plant materials by hydro-distillation, solvent-solvent extraction, and liquid CO2 extraction[1]. They contain terpenoids, especially monoterpenes (C10), sesquiterpenes (C15), and diterpenes (C20), along with a variety of aliphatic hydrocarbons (low molecular weight), acids, alcohols, aldehydes, and esters.[2,3,4] They are characterized by main constituents present in higher concentrations rather than components in trace quantities. For example, EOs of clove contains 85% eugenol, 10-12% eugenol acetate, and these determine its biological activity.[5] Due to these versatile compounds EOs possess bactericidal, fungicidal, antioxidant, virucidal, and anticarcinogenic properties. EOs have already been utilized to control bacterial

and fungal contaminations.[6,7,8] Fu and others (2007)[6] evaluated the bactericidal and fungicidal potential of clove and rosemary EOs alone and in combination. They found significant inhibition of *S.epidermidis*, *E.coli,* and *C.albicans* at MIC values of 0.062- 0.500% (v/v), 0.125-1.00% (v/v) for clove and rosemary EOs, respectively. Similarly, other researchers have also used EOs as antibacterial and antifungal agents.[7,8,9,10,11,12,13] Gortzi and others (2008)[14] used *Myrtus communis* extract to inhibit the oxidation of sunflower oil. The results showed considerable reduction in oxidation at 160 ppm, and the antioxidant activity of extracts further improved when encapsulated in liposome. EOs possess volatile constituents which are sensitive to oxygen, light, humidity, and heat. To increase their stability and functional performance EOs can be encapsulated.

Encapsulation has been widely used for protection, target delivery, and enhanced biological functions of bioactive compounds.[15,16,17] Wang and others (2009)[17] prepared carvacrol loaded microcapsules, designed to target the intestine for enhanced antimicrobial activity and increased bioavailability. They found < 20% oil released in stomach and rest was completely released in intestine. Similarly, variety of researchers reported sustained release characteristics of EOs after encapsulation in different matrices[15,16,18,19,20]. Encapsulation not only provides controlled release, but also increased the bioavailability of bioactive compounds/drugs. The same trend was observed, when different EOs constituents (peppermint oil, eugenol, carvacrol and thymol) were nanoencapsulated, which resulted in enhanced antimicrobial activity compared to bulk oil.[21,22] To achieve these benefits, EOs have been encapsulated by using various chemical, physicochemical and mechanical procedures. Among these liposomes,[14] molecular inclusion,[23,24] coacervation and complex coacervation,[25,26] spray drying,[27] emulsification,[21,28] ionic gelation[29] and emulsion extrusion[17] have been used by many researchers to encapsulate EOs. Encapsulation consists of two important things namely core (bioactive) & wall material/emulsifier (that protects bioactive).

The latter is of great importance because the stability, release behavior of core (bioactive) depends on its physiochemical nature, and further on type, and parameters of encapsulation technique. Different types of protein,[26,30] polysaccharide[21,31] and synthetic [22,32] emulsifiers/wall materials have been used to encapsulate the EOs/constituents. This article will focus on various procedures employed for EOs encapsulation, benefits after entrapment, uses in food, and their trends.

2. Essential oils and their properties

EOs are complex natural, volatile aromatic compounds, characterized by two or three major components at fairly high concentrations (20–70%) compared to other components present in trace amounts. Biological activities of EOs are mainly due to main components that are present in high concentration. For example, *Artemesia alba* EO posses camphor 24%, while *Mentha piperita* contain menthol and menthone 59% and 19%, respectively. Because of these components, EOs have been largely employed for their well-known antibacterial, antifungal, antioxidant, and anticarcinogenic applications. Walsh and others (2003)[8] reported antimicrobial properties of natural bactericidal compounds eugenol, thymol, triclocarbon (TCC), and didecyldimethylammonium chloride (DDDMAC) against *E.coli*, *S. aureus* and *P. aeruginosa*. Eugenol, thymol, and alkyl dimethyl amine oxides (ADMAO) were effective against *E.coli, S. aureus,* while TCC showed activity only against *S.aureus*. The observed minimum inhibitory concentration values (MIC) of eugenol, and thymol against *E.coli, S.aureus* and *P.aeruginosa* were 0.05, 0.1 and > 0.1% (v/v), respectively. Strong inhibitory action of eugenol, carvacrol, thymol, diacetyl, and cinnamic acid against *E. sakazakii* has also been confirmed.[33] Fu and others (2007)[34] suggested inhibition of *S. epidermidis, S.aureus, B. Subtilis, E.coli, P. Vulgaris, P.aeruginosa, C.albicans* and *A.niger* by clove and rosemary EOs alone and in combination. The MIC values for clove & rosemary EOs were in the range

of 0.062% to 0.5% (v/v) and 0.125% - 1% (v/v). Bactericidal action of EOs have also been confirmed by many studies.[9,10,11,13]

EOs possess promising antifungal activity, and have potential to replace synthetic preservatives as revealed by many researchers.[35,36,37,38,39,40,41] Bansod and Rai (2008)[42], suggested fungicidal action of *Cymbopogon martini*, *Eucalyptus globules,* and *Cinnamomun zylenicum* EOs against *A. niger, and A. fumigatus*. The MIC values were 0.06, 0.12, and 0.12% (v/v), respectively. Amiri and others (2008)[43], reported eugenol (2 mg/ml) induced mycelial growth inhibition of *P.vagabunda, P. expansum, Bortrytiscinerea, and M. fructigena*. The average growth inhibition varied between 88.6- 90% at 4˚C and 72.5-84.4% at 20˚C, respectively. EOs also possess antioxidant activity as evidenced by many studies.[44,45,46,47,48,49,50] Viuda-martos and others (2010)[51] used EOs for in vitro evaluation. Among five spice EOs oregano, thyme, rosemary, sage, and clove, clove EO showed strong antioxidant potential as it inhibited (98.74%) DPPH radical. Antioxidant potential of cumin (Cuminum Cyminum L.) stem, leaves, and flowers EOs have also been reported. They found that cumin flower acetone extract was more effective antioxidant interms of DPPH radical scavenger, lipid peroxidation inhibitor, and reducing agent with IC50 value 4, 32 and 8 µg/ml, respectively.[52]

EOs have been also utilized as potential source of anticarcinogenic agents.[53,54,55,56,57,58,59,60] The EOs from lemon grass (*Cymbopogon flexuosus*), sage (*Salvia officinalis*), katafa (Cedrelopsis grevei), and bugleweed (*Lycopus lucidus*) have been reported to be cytotoxic to human cancer cell lines.[61,62,63] Sylvestre and others (2006)[64], found significant tumor growth reduction of human lung carcinoma cell line A-549 and human colon adenocarcinoma cell line DLD-1, when treated with *Croton flavens* leaf EO. The GI50 values were 27±4 µg/ml for A-549 and 28±3 µg/ml for DLD-1, respectively. Similarly, Ashour (2008)[65] reported anticarcinogenic potential of *Eucalyptus sideroxylon* and

Eucalyptus torquata leaf, stem, and flower EOs against human hepatocellular carcinoma cell line (HEPG2), and human breast adenocarcinoma cell line (MCF7). Inspite of all these characteristics, EOs have certain limitations such as low water solubility, high volatility, and strong odor that limit their applications in food and pharmaceutical industry. To overcome such barriers, EOs can be encapsulated to retain their stability, flavor retention, and functional properties.

3. Encapsulation of essential oils (EOs): Techniques/strategies

Encapsulation of EOs has been carried out by variety of chemical,[15] physicochemical[24] and mechanical procedures.[21,22,28,30] Commonly used procedures to encapsulate EOs are summarized in Figure: I.

3.1 Chemical Procedures

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. For thymol and carvacrol egg L-α phosphatidylcholine & cholesterol were mixed in methanol, solvent removed at 35˚C under nitrogen stream & the lipid film obtained was hydrated to prepare multilamellar vesicles (MLV) from unilamellar vesicle. A total of 1.07 mg carvacrol was added in liposomes and the encapsulated percentage was 4.16% (0.045mg). The liposomes incorporated carvacrol & thymol showed enhanced antimicrobial activity and its long term retention favored its stability in liposomes as evident from stability study.[71] In another study *Myrtus communis* extract was incorporated in liposomes prepared by L-α phosphatidylcholine & cholesterol mixed in chloroform-methanol solution to determine the antioxidant activity. Whereas, Phosphatidylglycerol was replaced with L-α

phosphatidylcholine for antimicrobial activity determination. The liposomes prepared were spherical in shape and the size was in the range of 270-300 nm.[14] Similarly, Sinico and others (2005)[32] prepared MLV of *A.aboresscence* EO (2.5 mg/ml) using soya phosphatidylcholine in chloroform. They reported less incorporation of EO in Brij30 based vesicles, while soya phospholipid vesicles entrap 60-74% of *A.aborescence* EO with size in the range of 70-150 nm. The liposomes incorporated *A.aborescence* oil significantly reduced herpes simplex virus-1 (HSP-1) at 100 ug/ml while free/unencapsulated oil at similar dose showed poor activity. This result also confirmed the stability of oil in liposomes with increased bioactivity. In addition to chemical procedures various researchers used physicochemical procedures to encapsulate EOs[25,26].

3.2 Physicochemical Procedures

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. Rosmarinus and thymus EOs were dispersed in 10% gelatin solution prepared at 40˚C, mixture was emulsified using high shear mixer with subsequent addition of sodium sulphate (20% wt/wt) to obtain coacervate phase at 5˚C under continuous stirring for 1 hour. Further, glutaraldehyde (1mmol/g gelatin) was added at pH 8 under stirring at 750 rpm at 5˚C for 3 hours and finally microparticles were filtered and freeze dried. The microcapsules prepared were spherical with an average diameter of 60µm and retained 75% of oil in microcapsules. However, significant increase in morality of Indian meal moth (*P. interpunctella*) was recorded as concentration of microcapsules increased in the diet.[26] Similarly, citronella oil was also encapsulated by simple coacervation as described earlier except formaldehyde solution (37% v/v) that was used to rigidize citronella oil entrapped gelatin microcapsules. The microcapsules showed slow release of citronella oil (CO) and after 10 hours 70% was released. The results of this

study showed sustained release and protection of oil from environmental factors.[31] In another study, lavender oil microcapsules were prepared using complex coacervation of collagen hydrolysate (CH), chitosan (C) crosslinked with glutaraldehyde. Briefly, both CH (5 g) and C (2.5 g) were dissolved separately in distilled water (100 ml), heated until transparency achieved. Both CH-C solution (1/0.0, 0.75/0.25, 0.5/0.5 & 0.25/0.75) were mixed under stirring (800 rpm) at 30˚C. After that temperature increased to 42˚C, lavender oil (2-10ml) was added drop wise and coacervate formed by the addition of sodium sulphate (10% wt/v) at pH 7. Further microcapsules were cross-linked using glutaraldehyde (0.1-0.3 mmol/g) under stirring at 42˚C for 6-8 hours, temperature reduced to 30˚C and microcapsules washed with 0.1% Tween80 solution to remove surface oil & finally freeze dried. The microcapsules prepared were spherical and encapsulation efficiency was 36.84-73.73%.[67]Complex coacervation was also used to prepare camphor oil loaded microcapsules of gelatin blended with gum arabic and further fabricated with polystyrene. The microcapsules showed encapsulation efficiency between 80-100% and particle sizes were 85.7, 167.2 & 294.7µm respectively.[25]

Ayala-Zavala and others (2008)[23] prepared cinnamon and garlic oil loaded microcapsules using molecular inclusion/β-cyclodextrin inclusion complex method. Briefly, β-cyclodextrin (50 g) was dissolved in ethanol-water mixture (1:2) at 55 ˚C, after that each EO dissolved in ethanol (10% w/v) was slowly added to warm β-cyclodextrin solution in variable weight ratios (0:100, 4:96, 8:92, 12:88 & 16:84). Finally, resultant mixture was stirred without heating for 4 h and precipitated oil- β-cyclodextrin microcapsules were filtered and dried in convection oven (50˚C). The microcapsules of both cinnamon & garlic oil showed encapsulation efficiency of 94.82, 93.76%, respectively and were effective against *A.alternata* fungus. Choi and others (2009)[24] prepared eugenol loaded microcapsules using β-cyclodextrin & 2-hydroxyl propyl (2HP) β-cyclodextrin by molecular inclusion method. They

reported higher encapsulation efficiency of eugenol- β-cyclodextrin (90.9%) than eugenol-2HP β-cyclodextrin (89.1%) and confirmed that hydrophilic chain of 2- hydroxyl propyl group in 2HP β-cyclodextrin was not efficient for the inclusion of lipophilic compounds like eugenol. Another research group encapsulated flax seed oil in β-cyclodextrin inclusion complexes in variable ratios (5:95-20:80 wt/wt). They obtained maximum load (95.8 mg of oil/g β-cyclodextrin) of flaxseed oil at 20:80 ratio and further no change in the composition of oil was observed after inclusion in β-cyclodextrin as evidenced from gas chromatography results. These results showed protection of eugenol and favored its encapsulation in inclusion complexes as suitable approach.[81] PO,[19] lemon oil[82] in β-cyclodextrin inclusion complexes have also been encapsulated as described earlier. Hill and others (2013)[83] prepared β-cyclodextrin inclusion complexes of eugenol, trans-cinnamaldehyde, clove extract, cinnamon bark extract and eugenol: trans-cinnamaldehyde via freeze drying. The particle sizes were in the range of 0.86 – 2.00 μm, showed spherical morphology with entrapment efficiency of 41.7-84.7%. They confirmed inhibitory action of microcapsules against *S.enterica, Typhimurium* & *L.innocua* but unencapsulated/free oil and extracts showed poor inhibition at same concentrations. These results showed better retention and protection of lipophilic compounds in inclusion complexes.

3.3 Mechanical Procedures

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. Arana-Sanchez and others[74] spray dried OEO emulsion prepared using β cyclodextrin, fed at room temperature with an inlet air temperature of 105˚C, and pump flow rate was 1.1 ml/min. Microcapules showed spherical to ovoid shape with size in the range of 0.71-20μm, 1.42- 28.14μm, and 1.07-38μm, respectively. The formulation with bigger size showed increased encapsulation efficiency (81.03%) compared

to smaller sized capsules (0.71-20 μm) with 53.90%. Thymol loaded emulsion prepared with whey protein-maltodextrin conjugate was also spray dried with an inlet air temperature of 150˚C, compressed air pressure of 600 kpa, air flow rate of 35 m^3 /h, and feed rate was 6.67 ml/min. The encapsulation efficiency was 73.8% - 82.8% at 10% volume fraction of oil phase, but reduced to 67.6% when oil phase fraction increased to 30%. They also reported loss of thymol due to inlet temperature that was equivalent to its vapor pressure (8.0 kpa), and concomitant loss of capsule shape i.e, ruptured wall that happened during spray drying as evident from AFM images[75,76]. Another research group prepared powdered eugenol nano-dispersions by spray drying at 6.67 ml/min feed rate and outlet temperature was 80-90˚C. They reported stable and transparent nano-dispersions having small particle size.[75,76] Basil oil emulsion emulsified with gum arabic was spray dried using emulsion feeding rate of 0.7 L/h, drying temperature of 180˚C, and vapor pressure of 0.4 MPa.[77] The droplets mean diameter was 1.17 – 2.87μm, and oil retention varied from 56.43- 88.28%. Baranauskiene and others[30] spray dried Peppermint oil (15.25% wt/wt) dispersed in wall material solutions of n-octenyl succinic anhydride modified starches. The dispersions were dried in Buchi mini spray drier at inlet air temperature of 200 ± 10˚C, outlet temperature of 120 ± 10˚C, and compressor pressure of 400 mm/H2O. They also reported increased retention of oil (39.2-97.4%) was associated with small size droplets compared to bigger ones. On the other hand, more oil evaporated/lost during atomization as modified starches take longer time for film formation around droplets during spray drying. Clove extract (2.5g) emulsion prepared with maltodextrin (12 g) and gum arabic (6 g) was spray dried at inlet & outlet temperature of 150˚C & 86˚C, respectively. The powder particles showed (1-15μm) shriveled morphology with yield percentage of 62%, when air flow, emulsion feed rate, and atomization pressure were kept at 40 mm, 6.67 ml/min, -45mbar, respectively. Rosemary EO emulsified with whey protein isolate, and inulin was fed into spray dryer at 0.9 L/h with an inlet temperature of

170˚C to prepare encapsulated powders. The microencapsulation efficiency was 28.97-38.34%, and the retention of oil was more when whey protein, and inulin concentration was 3:1 and 1:1. However, no increase in oil retention was observed with higher inulin concentration. They reported increased efficiency of EO during spray drying that was dependent on wall material type and its emulsifying capability.[78] In another study rosemary EO dispersed in gum arabic solution (1:4 oil/wall) was spray dried to prepare encapsulated powder.[79] Spray drying was done at an inlet temperature of 171˚C, feed rate of 0.92 L/h, and atomizing air pressure was kept at 40 L/h. They reported efficient drying at higher inlet temperature, and increased wall material concentration, which resulted in more powder recovery. They observed loss of oil content from particles due to volatilization during drying by atomization because of delay in the formation of semi permeable membrane when carrier concentration was low. However, maximum oil retention was 36.95% when wall material (gum arabic) concentration was 19.3%. Similarly, Beirao-da-costa and others (2013)[18] spray dried OEO emulsion, the capsules prepared were in the range of 3-4.5 μm and some capsules represent ruptured wall due to high inlet temperature. Najafi and others (2011)[80] compared spray and freeze drying method for the encapsulation of cardamom oil using HI CAP 100 and skim milk powder as wall materials. The spray drying retained more volatiles (91-94%) than freeze drying (84-86%) and microcapsules prepared were of high quality compared to freeze dried microcapsules. However, in case of skim milk powder, microcapsules particle size (13.97-19.91 μm) was smaller than HI CAP 100 (15.41-21.87 μm) but, former released volatile contents much faster during drying than later one. Therefore, spray drying was recommended as suitable method for the encapsulation of EOs.

Similarly, various researchers used high energy emulsification approach for the generation of EOs loaded nanoemulsions has been carried out by many researchers. For example, Liang and others (2012)[21] for the preparation of PO loaded nanoemulsion, blended

PO with medium chain triglyceride (MCT), and mixed with purity gum 2000 solution 12% (w/w) to prepare coarse emulsion using Ultra Turax (a high speed blender). Finally, coarse emulsions passed through high pressure homogenizer at 150Mpa, and 5 processing cycles, showed particle size <200 nm and were effective against *S.aureus* & *L.monocytogenes* till 24 hours at 0.25% (v/v) concentration. They confirmed similar composition of PO components before and after encapsulation. The long term inhibition of bacterial growth by PO nanoemulsions and their better protection promoted high energy emulsification a suitable method for encapsulation of EOs. Similarly, another research group prepared D-limonene, Cinnamaldehyde and carvacrol loaded nanoemulsions (130-293 nm) using soy lecithin (3%), pea proteins (3%), sugar ester (1%) and Glycerol monooleate with Tween 20 (0.5:0.5%) as emulsifying agents. The formulations with glycerol monooleate with Tween 20 showed prolonged bactericidal action against *S.cerevisiae*, *E.coli* & *L.delbrueckii* because of more availability of antimicrobial compound by this emulsifier compared to other emulsifiers. D-limonene & terpenes mixture from *Melaleuca alternifolia* based nanoemulsions (74-365 nm) were also prepared using Tween 20 & glycerol monooleate, soy lecithin and cleargum individually as emulsifiers. Soy lecithin based emulsions formulations of D-limonene & terpene mixture were efficient and delayed growth of *L.delbrueckii* for 5 days in orange & 2 days in pear juice compared to control when added at concentration of 1 g/L.[28] Terjung and others (2012)[22] also reported preparation of eugenol and carvacrol loaded nanoemulsions using high pressure homogenization technique. They observed antimicrobial activity of eugenol and carvacrol loaded variable particle sized emulsions (80, 200, 1000 and 3000nm) against *E.coli* & *L.innocua*. They reported complete killing of bacterial cells when treated with bigger particle size (3000 nm) due to more concentration of antimicrobial compound and vice versa happens with smaller particle size (80 nm). Rosemary EO loaded emulsions prepared by high pressure homogenization was stable for 50 days at ambient conditions.[84]

Chang and others (2012)[85] prepared thyme oil (10% v/v) loaded nanoemulsions with average particle size of (160, 170 nm) using high pressure homogenization. They reported decreased antimicrobial efficacy because of increased concentration of ripening inhibitors type (corn oil or medium chain triglyceride MCT oil) in lipid phase and when 70% ripening inhibitor was added in lipid phase the minimum inhibitory concentration against Zygosaccharomyces bailii containing corn & MCT oil were 750 and 3000 µg/ml, respectively. These results showed physical stability of thyme oil in nanoemulsions droplets but decreased antimicrobial activity was due to partition coefficient. Vegetable,[86] soybean,[87] corn or octadecane,[88] castor,[89] citronella, basal & vetiver oil[90] based nanoemulsions have also been prepared by using high pressure homogenization technique.

3.4 Other encapsulation methods

Eugenol and carvacrol grafted chitosan nanoparticles were prepared via Schiff base reaction.[29] Briefly, chitosan nanoparticles (CH-NPs) were prepared using ionic gelation method. For this 0.5% w/v chitosan in 1% v/v acetic acid at pH 4.1, TTP solution (2.5 mg/ml) was added drop wise in chitosan solution under stirring at 10000 rpm, separated by centrifugation and were freeze dried. Further, eugenol and carvacrol aldehydes were grafted on CH-NPs via Schiff base reaction. A 30ml methanol, 100 mg CH-NPs with excess of eugenol & carvacrol aldehydes and reaction mixture was refluxed for 48 hours. Finally, eugenol and carvacrol grafted nanoparticles were separated by centrifugation and vacuum dried at 42˚C for 12 hours. Among eugenol (235 nm) & carvacrol (260 nm) grafted CH-NPs, eugenol grafted nanoparticles showed antioxidant activity at lower EC50 (2.6 mg/ml) value than carvacrol grafted nanoparticles (>4.0 mg/ml). On the other hand, eugenol grafted CH-NPs showed higher antimicrobial efficacy than carvacrol grafted CH-NPs. In another research, OEO was encapsulated in chitosan nanoparticles by two step process i.e, oil in water o/w emulsion and ionic gelation of chitosan with sodium tripolyphosphate (TPP). In

this chitosan (1% w/v) in acetic acid (1% v/v) solution was stirred and Tween 80 was added to get homogenous mixture under stirring at 42˚C for 2 hours. After that OEO (0.04, 0.08, 0.16 & 0.32 g) was dissolved in CH_2Cl_2 (4ml) and dropped in chitosan solution during homogenization to prepare oil in water emulsion. TTP solution was then added drop wise into agitated emulsions till 40 minutes. Finally, nanoparticles were centrifuged (9000 g), suspension was sonicated in ice bath (0.7 s working & 0.3 s rest) and freeze dried at -35˚C for 72 hours. The prepared nanoparticles were spherical (40-80 nm) and the encapsulation efficiency was 21- 47%.[20] In the orifice method citronella oil loaded microcapsules were prepared as follows, 0.5 wt.% yellow dye was added in citronella oil, citronella oil (2 ml) was added in chitosan solution (0.2, 0.5, 1 & 1.5%) under stirring. After that 0.1-1.5wt% NaOH was added slowly and the prepared microcapsules were centrifuged to remove excessive material and kept in 5 wt. % natural coconut oil for 10 days. Finally microcapsules were vacuum oven dried at 30˚C for overnight. They concluded that encapsulation efficiency affected by chitosan concentration and when these were 0.5, 1 & 1.5 % the encapsulation efficiencies were 98.2, 95.8 and 94.7%, respectively.[15] Carvacrol loaded microcapsules were also prepared by using Ca-alginate hydrogel using emulsion extrusion method. For this alginate (20 g l^{-1}) in deionized water, carvacrol & Tween 80 were added to a final concentration of 200 and 0.5 g l^{-1} respectively. The mixture was emulsified using blender, emulsion was extruded into collecting water bath using 20 g l^{-1} $CaCl_2$ in encapsulator with 500 µm nozzle. The microcapsules kept for 30 minutes in $CaCl_2$ for hardening, washed and air dried for 30 h at 22˚C. The microcapsules showed strong antimicrobial activity against *E.coli* in vitro and during in vitro digestion only 20% carvacrol released in gastric fluid while remaining in intestinal fluid after 6 hours of incubation.[17]

Co-crystallization is another method used for the encapsulation of orange peel oil. In this process crystal structure of sucrose is modified from a perfect crystal to conglomerate.

The resultant structure has porous configuration which can accept the addition of second ingredient. For orange peel oil sucrose syrup of 70^0 Brix was concentrated to 95^o Brix under hot magnetic stirring, orange peel oil was added at variable ratios (100, 150, 170, 200 and 250 g oil/kg sucrose) using shear mixer till crystallization achieved. When crystallization started, heating was stopped and heat of crystallization eliminated water to make granular product. The encapsulation efficiency of sucrose syrup was >90% and oxidized flavor were observed in formulations that have antioxidants prior to co-crystallization. This method is rarely used for the encapsulation of food ingredients due to lack of versatility and health concerns related to sucrose.[90] In the emulsion evaporation method.[91] poly (DL-lactide-co-glycolide) PLGA (50 mg) in dichloromethane (2 ml) with trans-cinnamaldehyde or eugenol (16% w/w) was prepared. After that aqueous phase containing poly vinyl alcohol (PVA: 0.3% w/v) mixed with organic phase and oil in water (O/W) emulsion was prepared using homogenization. Further, emulsions were sonicated at 2˚C for 10 minutes at 70w energy output and organic phase evaporated using rotary evaporator. Finally, nanoparticles loaded with eugenol and trans-cinnamaldehyde were purified through ultrafiltration and freeze dried. The nanoparticles were in the size range of 200 nm with encapsulation efficiency of both compounds ranging from 92-98% and were effective against *salmonella* and *Listeria* spp. These results favored encapsulation of EO using this approach because of its ability to protect and increased the shelf stability of encapsulated compound. Zein nanospheres loaded with thyme, cassia & oregano EO were prepared using phase separation method. For this each oil (250 mg) with zein (1 g) dissolved in ethanol (85%), solution was rapidly mixed with 0.01% silicone fluid till single phase formed and the opaque solution containing oil was lyophilized overnight. The nanospheres prepared were of irregular shape and the encapsulation efficiencies of oils were in the range of 65-75%. This technique is rapid, enabled protection of EOs and controlled its release in stomach, small intestine & large intestine.[92]

Emulsification external gelation method was used for the preparation of turmeric oil loaded nanocapsules.[93] Briefly, turmeric oil solution in ethanol (20mg/ml) was mixed with alginate solution (0.6 mg/ml) containing Tween 20 (1% /v) to prepare o/w emulsions. For gelification of oil droplets emulsions were combined with 0.67 mg/ml $CaCl_2$ solution under stirring and another polymer chitosan (0-0.6 mg/ml acetic acid) was subsequently added, oil loaded alginate-chitosan nanocapsules were equilibrated overnight and solvent was removed using rotary evaporator. The nanocapsules prepared were in the size range of 162-667 nm and were stable for 120 days under room temperature and 4˚C. Yeast cell has been successfully used for the encapsulation of limonene. To carry out this, yeast cells cytoplasmic material was removed by plasmolyser, cells were washed with water, centrifuged and spray dried. Finally, yeast cells (80 g) were infused with limonene solution (9.1% w/w). The oil was successfully encapsulated as evident from transmission electron microscope (TEM) images and proved to be a suitable approach for the encapsulation of water soluble bioactives.[68]

3.5 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 (Table 1). Among carbohydrates, β-cyclodextrin was used to prepare OEO loaded emulsions that were spray dried to obtain stable microcapsules having size in the range of 1.07-38 μm.[74] Similarly a variety of researchers used β-cyclodextrin to prepare inclusion complex around hydrophobic EOs that protect them against oxidation, heat damage and increased their antibacterial efficacy for a longer time period.[24,81,83,82] Beirao-da-costa and others (2013)[18] used inulin as wall material for the encapsulation of OEO, microcapsules prepared were spherical (3-4.5 μm) and enabled sustained release of oil till 200 minutes. N-octenyl succinic anhydride modified (HI CAP 100, Purity Gum 2000, Capsul & N-lock) and hydrolyzed starches (EnCapsul 855) used for the encapsulation of PO. Modified starches showed increased retention, better stability of oil

in spray dried powder[30] and nanoemulsions[21] (< 200 nm). Eugenol and Carvacrol were encapsulated by chitosan and Tripoly phosphate (TPP) ionic cross linking and the nanoparticles produced (217, 235 & 260 nm) showed better retention of compounds incorporated. Further, nanoparticles were stable for extended time period before and after incorporation of EO components.[29] Another research group encapsulated orange peel oil using sucrose syrup, the granular co-crystallizate showed 90% encapsulation efficiency and showed better protection against oxidation.[90] In case of proteins, gelatin was used to encapsulate citronella, thyme and rosemary oil, microcapsules (60 μm) prepared were spherical and exhibited controlled release of oil (10 hours).[26,31] Parris and others (2005)[92] encapsulated thyme, oregano and cassia oil using zein, nanoparticles prepared had an average diameter of 100 nm and showed oil yield from 65-75%. They reported less release of oil from zein nanospheres during in vitro digestion in stomach, slow release in small intestine and rapid release in large intestine. Soy lecithin (3%) and pea proteins (3%) have been used as emulsifiers for the encapsulation of Limonene, trans-cinnamaldehyde and *Malaleuca alternifolia* terpenes. The nanoemulsions prepared were stable in terms of particle size and had particle size in the range of 184-239 nm.[28] Corn oil nanoemulsions have also been prepared using sodium caseinate and β-lactoglobulin and were physically stable having particle diameter 150 nm.[89] Among gums acacia, gum arabic has been used for the encapsulation of ginger, basil and rosemary EO.[77,94] Gum arabic (1:4 w/w oil to wall ratio) emulsified emulsions of both oils exhibited low viscosity and particles prepared after spray drying showed retention of basil and rosemary oil (56.43-90.6%:7.15-47.57%), respectively. They also reported more loss of EO from larger droplets compared to smaller ones during spray drying due to longer time of film formation around droplets. Najafi and others (2011)[80] compared protein (skim milk powder) and carbohydrate (HI CAP 100) based wall material for the encapsulation of cardamom oil. They reported narrow droplets size (13.97-19.91 μm)

prepared from HI CAP 100 compared to skim milk powder (15.41-21.87 μm) and subsequently more loss of volatiles occurred in skim milk encapsulated powders.

Inspite of individual use of protein/carbohydrate based emulsifiers or wall materials, mixtures of carbohydrate: protein, carbohydrate: gums and protein: gums have also been used to encapsulate EOs for better retention and protection. Fernandes and others (2014)[79] used whey protein isolate (WPI): Inulin blends for the encapsulation of rosemary EO. They reported better protection of oil particles in powder against oxidation due to low moisture content as WPI concentration in blend increased. WPI: Inulin blends (1:1 & 3:1) were the effective carriers for the entrapment of rosemary oil and had particle size in the range of 11.5-11.9 μm. Similarly, WPI: maltodextrin maillard conjugates (1% /v) were used for the encapsulation of thymol oil,[75] microcapsules prepared were spherical having size in the range of 1-5 μm and oil retention increased as concentration of maillard conjugates increased from 1-11.1% w/v. Chatterjee and Bhattacharjee (2013)[27] used maltodextrin: gum arabic (12:6 g) mixture for the encapsulation of eugenol rich clove extract, microcapsules (1-15 μm) showed shrivelled morphology and maximum retention of eugenol was 65%. They reported increased percentage of carbohydrate (4.8) in relation to gum (2.4) that was effective in increasing the retention of oil.

In addition to above mentioned food grade emulsifiers/wall materials, variety of synthetic emulsifiers have also been used for the encapsulation of EOs. Sinico and others (2005)[32] used Brij30 (5.75 mg/ml) as surfactant for the synthesis of *A.aborescens* oil loaded liposomes. They reported lower retention of oil (66.09%) in niosomal bilayers prepared with Brij30 compared to phospholipid based liposome (74.15%). However, liposomes and niosomes were stable for one year when stored at 4 -5˚C. The agglomeration of lemon grass oil microcapsules was overcome by the addition of 0.4wt% sodium dodecyl sulphate (SDS) due to repulsion mechanism as it is ionic surfactant.[55] Donsi and others (2011)[28] prepared D-

limonene & terpenes mixture from *Melaleuca alternifolia* based nanoemulsions using natural (soy lecithin, cleargum) and synthetic emulsifiers (glycerol monooleate with Tween 20). Nanoemulsions of terpenes, D-limonene prepared with soy lecithin had particle size (74 & 240 nm) while tween20/glycerol monooleate based emulsions droplet diameter was (130-155nm). Moreover, all emulsions formulations were physically stable for a period of 4 weeks, showed no visible creaming and consistent particle diameter. Corn oil nanoemulsions prepared with synthetic emulsifiers (Tween 20 & SDS) showed smaller droplet size 60 nm while in case of sodium caseinate & β-lactoglobulin the particle size raised to 150 nm. However, later nanoemulsions were physically more stable than former ones.[88] Similarly Span 60,[84] Tween 80[84,22] & Tween 20, Montanov 82,[89] Triton x100[87] have been used to encapsulate EOs.

3.6 **Encapsulation and its benefits**

3.6.1. Encapsulation of essential oils (EOs) for controlled release

Encapsulation represents a viable and efficient approach to increase the physical stability of EOs, protection from evaporation, and because of narrow size range enables controlled release & enhanced bioactivity. Chang and Daobashi (2003)[66] prepared eucalyptus oil loaded alginate, and calcium chloride complex capsules of 1- 2.5 mm, using interfacial insolubilization reaction. They showed controlled, slow release of eucalyptus oil after encapsulation as analyzed by using incubation and finger crash force technique. Briefly, capsules were placed in between the fingers and crashed; the crashing force was standardized by taking 10 volunteers (Chinese students) index finger readings. The force was measured, and determined as 5.4×10^6 dyne. In another study, camphor oil was encapsulated to attain its sustained release, using complex coacervation method. Microcapsules of variable sizes (294.7±14.2, 167.2±11.2, 85.7±8.7 μm) at different homogenization speed 500, 1000 & 2000 (rpm) were prepared. They observed that microcapsules prepared at 500 rpm & 0.75 oil/wall

ratio showed 99.6% (wt/wt) encapsulation efficiency. Moreover, they reported that camphor oil sustained release properties were directly dependent on cross linking agent called polystyrene.[25] Hosseini and others (2013)[20] reported controlled release pattern of oregano essential oil (OEO) loaded nanoparticles, prepared by using two step method that involves oil in water emulsion, and then ionic gelation of chitosan and tripolyphosphate (TPP). They observed rapid release of oil from smaller nanoparticles, and 82% of OEO released during 3 hours even though at low concentration of OEO (0.1% w/w chitosan), but its release was slowed down at higher chitosan concentration (0.8% w/w), and reduced from 82% to 12% during 3 hours. Similarly, contrasting results were obtained when citronella oil was microencapsulated using chitosan, NaOH, and coconut oil as cosurfactant. They observed slow, and sustained release in microcapsules (225± 24 μm) that have larger size, and higher concentration of chitosan than smaller (131±20, 11±3μm) ones.[15] Moreover, slow release of neem oil (azadirachtin) loaded nanoemulsions based beads coated with gum arabic, and polyethylene glycol (PEG), having particle size in the range of 1.28±0.006 – 1.49±0.004mm have also been reported. They showed increased release of azadirachtin (44.2, 66.8, 79.4 & 100%) from nanoemulsions after 6, 12, 18 and 24 hours. However, significant decrease in azadirachtin release from nanoemulsions (31.6, 40.45, 51.6 & 70.6% and 41.6, 54.2, 66.8, 80.6%, respectively) was attained after being coated with gum arabic, and PEG. They concluded that gum arabic coating on nanoemulsions was better for controlled and slow release of azadirachtin than PEG.[67] Beirao-Da-Costa and others (2013)[18] also reported controlled release of OEO when encapsulated using inulin. The microcapsules prepared (3-4.5 μm) showed increased release of Oil during first 75 min but, later it became slow till 200 min and after that lag phase appeared. Release of lavender oil was also controlled by encapsulating it in collagen hydrolysate, chitosan, and glutaraldehyde as cross linker. They observed slow release of lavender oil from microcapsules by increasing concentration of

chitosan, and cross linker. Protection of aroma compounds, and controlled release, and their increased bioavailability have also been confirmed by other researchers.[19,68,69]

3.6.2. Encapsulation of essential oils (EOs) for increased bioavailability

In addition to controlled release characteristics, various researchers have also reported increased bioavailability of EOs after encapsulation in variable matrices. For example, Liang and others (2012)[21] encapsulated peppermint oil (PO) in starch based nanoemulsions to increase its stability, and bioavailability. The nanoemulsions with particle size of 200 nm showed enhanced bactericidal activity against *L.monocytogenes,* and *S.aureus* compared to bulk PO. This was attributed to greater solubility, and more availability due to subcellular size of particles. Similarly, increased bactericidal action due to more absorption of D.limonene, carvacrol, eugenol, and cinnamaldehyde by *L.delbrueckii, S.cerevisiae,* and *E.coli* cells from nanoemulsion has also been reported.[28, 22,70] Increased absorption of EOs after encapsulation lowered the amount of oil required to kill microorganisms. Meanwhile, increased bactericidal action of OEO attained at 25.0×10^{-8} g/ml, when incorporated in liposome and this quantity was equivalent to 6.0×10^{-3} g/ml unencapsulated OEO. These results showed enhanced batericidal activity of OEO in liposomes against human pathogenic bacteria (*S.aureus, S.epidermidid, S.mutans, S.viridans, P.aeruginosa, E.coli, E.cloacae, K.pneumonia*), fungi (*C.albicans, C.tropicalis, C.glabrata*) and food borne pathogen (*L.monocytogenes*) because of increased uptake/bioavailability by living cells.[71] Increased absorption of EOs in nanoemulsions by bacterial cells *S.aureus, B.cereus, E.coli* and *P.mirabilis* has also been reported by other researchers.[72,73]

In addition to increased absorption of EOs by bacterial cells, *A. aborescens* EO loaded unilamellar (78±11, 104±19, 123±21nm) and multilamellar liposomes (232±25, 252±29,304±21nm) also showed increased antiviral potential against herpes simplex 1 (HSP)

534 virus due to increased absorption after encapsulation. They confirmed poor antiviral activity
535 of unencapsulated, and unilamellar liposomes incorporated *A.aborescens* EO at 100 µg/ml.
536 However, antiviral activity significantly increased when it was incorporated in multilamellar
537 liposomes and EC50 value reduced to 18.5 µg/ml.[32]

538 3.6.3. Encapsulation of essential oils (EOs) for increased stability

539 Encapsulation not only provides controlled release, and improved bioaccesibility of
540 EOs but, also increased their stability[21,22,28,70] as they are susceptible to conversion and
541 degradation after exposure to environmental stresses[97]as shown in Figure II. Various
542 researchers reported increased bioactivities (antimicrobial & antiproliferative) of EOs in
543 encapsulation matrix compared to free oil even at same or lower concentration, that suggests
544 their resistance against conversion (oxidation, isomerization, polymerization, thermal
545 rearrangements etc.) and degradation[17,22,70]. For example, Liang and others[21] prepared
546 peppermint oil (PO) loaded nanoemulsions, and observed decrease (5 %) in main constituent
547 of PO (menthol) after quantification using GC-MS compared to PO. Inspite, of decrease in
548 main constituent of PO, the nanoemulsions showed enhanced and long term bactericidal
549 growth inhibition against *L. monocytogenes* and *S. aureus* compared to free PO even at same
550 MIC value. These results suggest the better stability of EOs after encapsulation. Similarly,
551 other researchers[28,98] also reported greater bactericidal activities of EOs in nanoemulsions
552 based encapsulation system, even after minimal losses of their constituents during processing.
553 On the other hand, carvacrol loaded calcium alginate microcapsules showed better stability,
554 when passed through gastrointestinal digestion model. Further, retention of carvacrol
555 antimicrobial activity against *E.coli* after intestinal digestion of microcapsules suggests
556 encapsulation being a suitable approach to prevent EOs from degradation and conversion.[17]
557 Similarly, *Ocimum basicilicum* and *Oreganum vulgare* EOs showed better stability against
558 degradative action of oxygen and temperature, when encapsulated in β-cyclodextrin inclusion

559 complex and microparticles[99,100]. Moreover, the better retention, and increased protection of
560 EOs in colloidal matrix depends on the type, and parameters of technique/procedure used that
561 ultimately affect the bioavailability, and controlled release of active compound.

562 **4. Encapsulated essential oils applications**

563 Encapsulated EOs have been used in vitro and in vivo applications for food by many
564 researchers. Liang and others (2012)[21] used PO loaded nanoemulsions (0.25% v/v) to inhibit
565 the growth of food borne pathogens *L.monocytogenes* and *S.aureus*. They observed long term
566 inhibition of bacterial growth when treated with PO nanoemulsions even though MIC values
567 of both bulk oil and PO nanoemulsions were same. Similarly, eugenol & carvacrol loaded
568 nanoemulsions (800 ppm) were also used to inhibit the growth of *E.coli* & *L.innocua* and
569 strong inhibition occurred with emulsions having droplet size 3000 nm. Improved
570 bactericidal action (*E.coli, L.delbrueckii* & *S.cerevisiae*) of carvacrol, cinnamaldehyde and
571 D-limonene nanoemulsions has also been reported.[28] In vitro application of OEO loaded
572 microcapsules against *E.coli* (0.20-0.05)*, S.aureus* (0.10-0.05) and *P.areuginosa* (0.20-0.10)
573 decreased minimum bactericidal concentration of OEO two to fourfold compare to pure oil.
574 They also reported four-eightfold increase in antiradical activity after encapsulation.[74]
575 Similarly, increased antibacterial potential was also observed when eugenol and carvacrol
576 grafted nanoparticles were used against *E.coli & S.aureus*.[29] Growth of food borne pathogens
577 (Salmonella & Listeria spp) was also reduced to a greater extent when exposed to eugenol
578 and trans-cinnamaldehyde loaded nanoparticles (10-20 mg/ml).[91] OEO loaded liposomes
579 caused growth inhibition of gram positive (*S.aureus*, *epidermidis, mutans & viridians*), gram
580 negative (*E.coli, E.cloacae, k.pneumoniae & P.aeruginosa*)*,* three human fungal pathogens
581 (*C.albicans, glabrata & tropicalis*) and *L.monocytogene* at concentaration 25.0×10^{-8} g/ml
582 that was equivalent to unencapsulated 6×10^{-3} g/ml.[71] The results of this study showed
583 encapsulated EOs are better to use in food and other applications to overcome the challenge

584 of sensory attributes variation that happened due to the use of higher EO concentrations. In
585 addition to above mention in vitro applications encapsulated EOs have also been used in vivo
586 as natural preservative to increase the shelf life. Donsi and others (2011)[28] used *Melaleuca*
587 *alternifolia* loaded nanoemulsions in orange and pear juices to extend the shelf life of juices.
588 The nanoemulsions with variable terpenes concentrations of 5 g/l, 10 g/l completely inhibited
589 the initial microbial (*L.delbrueckii*) load 10^3 CFU/ml whereas, 1 g/l only delayed bacterial
590 growth till 5 days in orange juice and 2 days in pear juice compared to control. Similarly,
591 nano-dispersed eugenol was also used to increase the shelf life of milk (4% fat, 2% fat &
592 skimmed milk <0.5% fat). Nanodispersed eugenol completely inhibited bacterial growth of
593 *E.coli* at 3.5 g/l, while 4.5 g/l was not effective to inhibit the bacterial growth in full fat milk.
594 However, 5.5 g/l completely inhibited bacterial growth of *E.coli* in all milk types. In case of
595 *L.monocytogenes* nano-dispersed eugenol at 5.5 g/l concentration was not effective as against
596 *E.coli* but, showed better efficacy than free eugenol and reduced bacterial growth in full fat
597 milk to 2.6 log CFU/ml and 5.5 log CFU/ml in case of free eugenol.[76]

598 In order to control herpes simplex virus1 (HSP1), *Artemesia arborescens* EO loaded
599 liposomes showed excellent alternative to drugs. Liposomes inhibited HSP1 growth at EC50
600 dose of 5.95 µg/ml that was equivalent to 100 µg/ml of free EO.[32] Nuchuchua and others
601 (2009)[89] reported prolonged action of citronella, basil and vetiver oil nanoemulsions against
602 *Aedes aegypti* both in vivo and in vitro. Nanoemulsions applied to 3×10 cm^2 area of human
603 skin, well spreaded due to small droplet size (50-220 nm) and increased the protection time to
604 4.7 hours (basil:vetiver:citronella 5:5:10 w/w%). In another study, carvacrol loaded
605 microcapsules (calcium alginate) used to control the enteric diseases in pig. In vitro release
606 study of microencapsulated carvacrol showed limited release in stomach, slow release in
607 small intestine and more release in large intestine. However, in vitro antibacterial test showed
608 similar minimum bactericidal concentration (200 µl/l) of both free and encapsulated carvacrol

against *E.coli* with K88 pili.[17] Moretti and others (2002)[96] used thymus and rosemary oil loaded nanoemulsions against *Limantria dispar*, a cork oak forest pest. They reported 100% mortality rate after emulsions treatment till 7 hours.

Improved bioactivity of liposomes encapsulated *Myrtus communis* extract was reported by[14] when incorporated in sunflower oil at concentration of (160 ppm), the encapsulated extract showed 25% higher oxidation protection factor (1.5 after incubation of 29.5h) compared to free extract (1.2 after incubation of 23.5h). However, liposomes encapsulation concentration was not equivalent to free extract. They also confirmed antioxidant potential of liposomes encapsulated *Myrtus communis* in terms of onset of thermo oxidation process using differential scanning calorimetry (DSC) and the temperature ranges for control, free extract & liposome encapsulated extract were 218, 237 and 272˚C, respectively. In case of soybean oil the microencapsulated eugenol rich clove extract powder showed similar antioxidant protection values (0.085± 0.006) as observed in BHT and unencapsulated clove extract. On the other hand both BHT and unencapsulated clove extract showed pro-oxidant activity due to excessive antioxidants in soybean oil that degrade linoleic acid and ultimately forms free radicals by the decomposition of hydrogen peroxide. However, encapsulated clove extract incorporated soybean oil showed no pro-oxidant activity and therefore, recommended to use as natural antioxidant in food rather synthetic ones.[27]

5. Encapsulated essential oils behaviours/trends

The composition of PO in purity gum 2000 based nanoemulsions before and after encapsulation using high pressure homogenization was quantified to interpret the loss of oil constituents during processing. They reported quite similar composition of pure and encapsulated PO.[21] Sweet orange oil microencapsulated by complex coacervation (soybean protein isolate: gum Arabic mixture) method also showed complete retention of flavour

compounds in microcapsules as in pure oil (D-limonene 89.5% in pure & 90.97% in microcapsules.[95] Similarly, Arana-sanchez and others (2010)[74] also reported no degradation of OEO components when emulsions were spray dried and analysed using GC-MS. The percentage composition of OEO constituents pure & extracted from microcapsules were quite similar (P-cymene 34.68- 34.66%, Thymol 9.42-19.52% & carvacrol 7.34-7.36%, respectively. Moretti and others (2002)[96] also confirmed similar profile of thymus and rosemary EO constituents before and after encapsulation. However, terpenes mixture of *Melaleuca alternifolia* was quantified using GC-MS after processing through high shear and high pressure homogenizer.[70] They observed degradation of active compounds (α-fellandrene 1.50-0.36, terpinolene 10.03-1.21, carvacrol 4.31-0.50 g/kg). In another study, changes in the composition of PO constituents were observed when liquid emulsified and spray dried products were analysed using GC & GC-MS. β-pinene was decreased 2-3 times compared to pure EO and percentage of oxygenated terpenol menthol increased in processed products from 47.5-50.1%.[30] Gaysinsky and others (2005)[97], reported temperature stability of micellar encapsulated eugenol & carvacrol. They observed with increased eugenol concentration in micelle, the temperature stability decreased i.e, at 0.1% eugenol the micelles were stable at 90˚C but at 0.9% eugenol the micelles were stable at 60˚C. Eugenol encapsulated in β-cyclodextrin, 2HP- β-cyclodextrin & PCL using molecular inclusion and emulsion diffusion method showed irradiation induced stability as evident by TGA after 60 days of storage under light and without light in desiccator at 25˚C. Both β-cyclodextrin-eugenol, 2HP- β-cyclodextrin-eugenol complexes after O_2 injection during TGA analysis showed significant weight gain (7.9 & 15.2%) at 20-150˚C and it was attributed to free oxidation reaction occurred from free eugenol with oxygen that injected as purged gas.[24] In the emulsion ionic gelation of OEO (carvacrol), improved thermal stability was confirmed after encapsulation at

elevated temperature of 340.6˚C.[20] The result from this study favours the use of encapsulated EOs in various food applications even at elevated temperature during processing.

6. Conclusions and Future Perspectives

Encapsulation is therefore an efficient approach to protect the EOs from light, air and humidity, because these interactions lead to oxidation or volatilization and reduced biological activities. Moreover, encapsulation increases the solubility of oil, provides controlled release and makes it more bioavailable. Spray drying and emulsification are the most versatile and commercially available techniques that had been used widely for EOs encapsulation. The encapsulated EOs showed enhanced antimicrobial, antifungal, antioxidant, antiviral and pesticidal activities. The use of encapsulated EOs in food, cosmetic and pharmaceutics could be an economic benefit and also fulfill the consumer concern regarding safety. The use of encapsulated EOs in cosmetics and pharmaceutics is lacking. Further, research is required to underpin recent analytical approaches in order to gain deeper understanding of oxidation, isomerization and thermal rearrangements processes and strategies to avoid them. Moreover, identification of products generated from these processes appears to be a valuable future objective. Further, encapsulated EOs can be used to increase the bioactivities of EOs in real food systems, to study their action mechanism on cell membranes, and to provide non-lethal therapeutic agents to treat several diseases.

Acknowledgements:

This work was financially supported by National 863 Program 2011BAD23B02, 2013AA102207, NSFC 31171686, 30901000, 111 project-B07029 and PCSIRT0627.

References:

1 A.E. Edris, Phytotherapy Research, 2007, 21, 308−323.

2 H.J.D. Dorman and S.G. Deans, Journal of Applied Microbiology, 2000, 88, 308-316.

3 S.F.A. Jones, European Journal of Gastroenterology and Hepatology, 1996, 8, 1227−1231.

4 M. LisBalchin and SG. Deans, Journal of Applied Microbiology, 1997, 82, 759−762.

5 S. Moon, H. Kim and J. Cha, Archives of Oral Biology, 2011, 56, 907-916.

6 Y. Fu, Y. Zu, L. Chen, X. Shi, Z. Wang and S. Sun, Phytotherapy Research, 2007, 21, 989-994.

7 B. Joseph and S. Sujatha, Asian Journal of Biological Sciences, 2011, 4, 35-43.

8 E.S. Walsh, Y.J. Maillard, D.A. Russell, E.C. Catrenich, L.D. Charbonneau and G.R. Bartolo, Journal of Applied Microbiology, 2003, 94, 240-247.

9 M.L. Al-hadi, Journal of Bagh College Dentistry, 2011, 23, 146-50.

10 K. Fisher and A.C. Phillips, Journal of Applied Microbiology, 2006, 101, 1232-1240.

11 W.J.R. Lambert, P.N. Skandamis, P.J. Coote and G.J.E. Nychas, Journal of Applied Microbiology, 2001, 91, 453-462.

12 Y.S. Lee and HH. Jin, Letters in Applied Microbiology, 2008, 47, 315-321.

13 V.F.S. Vuuren, S. Suliman and M.A. Viljoen, Letters in Applied Microbiology, 2009, 48, 440-446.

14 O. Gortzi, S. Lalas, L. Chinou and J. Tsaknis, European Food Research Technology, 2008, 226, 583-590.

15 W. Hsieh, C. Chang and Y. Gao, Colloids and surface B: Biointerfaces, 2006, 53, 209-214.

16 B. Ocak, Journal of Environmental Management, 2012, 100, 22-28.

17 Q. Wang, J. Gong, X. Huang, H. Yu and F. Xue, Journal of Applied Microbiology, 2009, 107, 1781-1788.

18 S. Beirao-da-costa, C. Duarte, A.I. Bourbon, A.C. Pinheiro, M.I.N. Januario, A.A. Vicente, M.L. Beirao-da-costa and I.D. Dillo, Food hydrocolloids, 2013, 33, 199-206.

19 A. Ciobanu, I. Mallard, D. Landy, G. Barbie, D. Nistor and S. Fourmentin, Food Chemistry 2013, 138, 291-297.

20 F.S. Hosseini, M. Zandi, M. Rezaei and Farahmndghavi, Carbohydrate Polymers, 2013, 95, 50-56.

21 R. Liang, S. Xu, C.F. Shoemaker, Y. Li, F. Zhong and Q. Huang, Journal of Agriculture and Food Chemistry, 2012, 60, 7548-7555.

22 N. Terjung, M. Loeffler, M. Gibis, J. Hinrichs and J. Weiss, Food Function, 2012, 3, 290−301.

23 F.J. Ayala Zavala, H. Soto-valdez, G.A. Lez-leo, A.E. Ivarez-parrilla, O. Maartin-Belloso and G.A.G. Lez-agular, Journal of inclusion phenomenon Macrocyclic chemistr, 2008, 60, 359-368.

24 M. Choi, A. soottitantawat, O. Nuchuchua, S. Min and U. Ruktanonchai, Food research international, 2009, 42:148-156.

25 C. Chang, T. Leung, S. Lin and C. Hsu, Colloids and Surfaces B: Biointerfaces, 2006, 50, 136-140.

26 S.G. Passino, E. Bazzoni and L.D.M. Moretti, Veg. Plagas, 2004, 30, 125-132.26.

27 D. Chatterjee and P. Bhattacharjee, Journal of food engineering, 2013, 117:545-550.27.

28 F. Donsì, M. Annunziata, M Sessa and G. Ferrari, LWT−Food Science and Technology, 2011, 44, 1908−1914.

29 F. Chen, Z. Shi, G.K. Neoh and T.E. Kang, Biotechnology and Bioengineering, 2009, 104:30-39.

30 R. Baranauskiene, E. Bylaite, J.Z. Ukauskaite and R.P. Venskutonis, Journal of agricultural food chemistry, 2007, 55, 3027-3036.

31 B. Solomon, F.F. Sahle, T. Gebre-Mariam, K. Asres and H.H.R. Neubert, European journal of Pharmaceutics and Biopharmaceutics, 2012, 80, 61-66.

32 C. Sinico, D.A. Logu, F. Lai, D. Valenti, M. Manconi, G. Loy, L. Bonsignore and M.A. Fadda, European journal of Pharmaceutics and Biopharmaceutics, 2005, 59, 161-168.

[33] Y.S. Lee and HH. Jin, Letters in Applied Microbiology, 2008, 47, 315-321.

[34] Y. Fu, Y. Zu, L. Chen, X. Shi, Z. Wang and S. Sun, Phytotherapy Research, 2007, 21, 989-994.

35 P. Chuang, C. Lee, J. Chou, M. Murugan, B. Shieh and H. Chen, Bioresource technology, 2007, 98, 232-236.

36 M.L. Grbic, M. Stupar, J. Vukojevic, M. Sokovic, D. Misic, D. Grubisic and M. Ristic, Journal of Serbian chemical society, 2008, 73, 961-965.

37 E.N. Pinto, C.L. Pinavaz, L.G. Salgueiro, M.J. Goncalves, S. Costadeoliveira, C. Cavaleiro, A. Palmeira, A.C. Rodrigues and J. Martinezdeoliveira, Journal of medical microbiology, 2006, 55, 1367-1373.

38 S. Siripornvisal, W. Rungprom and S. Sawatdikarn, Asian journal of food and agro industry, 2009, 5, 229-233.

39 S.R. Sridhar, R.V. Rajagopal, R. Rajavel, S. Masilamani and S. Narasimhan, Journal of agricultural food chemistry, 2003, 51, 7596-7599.

40 M. Viudamartos, Y. Ruiznavajas, J. Fernandezlopez and J.A. Perezalvarez, Journal of food safety, 2006, 27, 91-101.

41 S. Wang, P. Chen and S. Chang, Bioresource Technology, 2005, 96, 813-818.

42 S. Bansod and M. Rai, World journal of Medical Sciences, 2008, 3, 81-88.

753 43 A. Amiri, R. Dugas, L.A. Pichot and G. Bompeix, International Journal of Food
754 Microbiology, 2008, 126, 13-9.
755 44 H. Amiri, Evidence based Complementary and Alternative Medicine, 2012, DOI 10.
756 1155/2012/728065.
757 45 I. Goze, A. Alim, A.S. Tepe, M. Sokmen, K. Sevgi and B. Tepe, Journal of medicinal
758 plants research, 2009, 3, 246-254.
759 46 M. Ennajar, J. Bouajila, A. Lebrihi, F. Mathieu, M. Abderraba, A. Raies and M.
760 Romdhane, Journal of food science, 2009, 74.
761 47 I. Hammami, M.A. Triki and A. Rebai, Archives of applied science research, 2011, 3,
762 135-144.
763 48 G. Miguel, M. Simoes, A.C. Figueiredo, J.G. Barroso, L.G. Pedro and L. Carvalho,
764 Food Chemistry, 2004, 86, 183-188.
765 49 M.A. Saleh, S. Clark, B. Woodard, and S.A. Deolu-sobogun, Ethnicity and disease,
766 2010, 20, 78- 82.
767 50 M. Vishnupriya, S. Nishaa, J.M. Sasikumar, P.D.D. Teepica, C.P. Hephzibah and
768 V.K. Gopalakrishnan, International research journal of pharmacy 2012, 3, 99-103.
769 51 M. Viuda-martos, Y.R. Navajas, E.S. Zapata, J. Fernandez-lopez and A. Perez-
770 alvarez, Flavour and fragrance journal, 2010, 25, 13-19.
771 52 I. Bettaieb, S. Bourgou, W.A. Wannes, I. Hamrouni, F. Limam and B. Marzouk,
772 Journal of Agricultural food chemistry, 2010, 58, 10410-10418.
773 53 V. Dwivedi, R. Shrivastava, S. Hussain, C. Ganguly and M. Bharadwaj, Asian pacific
774 journal of cancer prevention, 2011, 12, 1989-1993.
775 54 S.K. Jaganathan, A. Mazumdar, D. Mondhe and M. Mandal, Cell biology
776 international, 2011, 35, 607-615.

55 H.P.A. Mary, T.A. Vargheese, K.J.J. Jeeja, M.R. Abiramy, N. Sajina and S.S. jaya, International journal of current pharmaceutical review and research, 2012, 2.

56 X. Ni, M.M. Suhail, Q. Yang, A. Cao, K. Fung, R.G. Postier, C. Woolley, G. Young, J. zhang and H. Lin, Complementary and alternative medicine, 2012, 12, 53.

57 A. Ozkan and A. Erdogan, Turk journal of biology, 2011, 35, 735-742.

58 A. Salazar, J. Hoheisel, M. Youns and M. Wink, International journal of clinical pharmacology and therapeutics, 2011, 49:93-95.

59 P.R. Sharma, D.M. Mondhe, S. Muthiah, H.C. Pal, A.K. Shahi, A.K. Saxena and G.N. Qazi, Chemico-Biological Interactions, 2009, 179, 160-168.

60 Y. Zu, H. Yu, L. Liang, Y. Fu, T. Efferth, X. Liu and N. Wu, Molecules, 2010, 15, Doi:10.3390.

61 S. Afoulous, H. Ferhout, E.G. Raoelison, A. Valentin, B. Moukarzel, Couderc, C Francois and J. Bouajila, Food and chemical toxicology, 2013, 56, 352-62.

62 A. Russo, C. Formisano, D. Rigano, F. Senatore, S. Delfine, V. Cardile, S. Rosselli and M. Bruno, Food and chemical toxicology, 2013, 55, 42-47.

63 J. Yu, J. Lei, X. Zhang, H. Yu, D. Tian, Z. Liao and G. Zou, Food chemistry, 2011, 12, 1593-1598.

64 M. Sylvestre, A. Pichette, A. Longtin, F. Nagaub and J. Legault, Journal of Pharmacology, 2006, 103, 99-102.

65 H.M. Ashour, Cancer Biology and Therapy, 2008, 7, 399-403.

66 C.P. Chang and T. Dobashi, Colloids and Surfaces B: Biointerfaces, 2003, 32, 257-262.

67 J. Jerobin, R.S. Sureshkumar, H.C. Anjali, A. Mukherjee and C. Natarajan, Carbohydrate polymers, 2012, 9, 1750-1756.

68 G. Dardelle, V. Normand, M. Steenhoudt, B. Pierre-Etienne, M. Chevalier and B. Pauline, Food Hydrocolloids, 2007, 21, 953-960.

69 V.D. Ramesh, Trends Biomaterial and artificial organs, 2009, 23, 21-33.

70 F. Donsi, M. Annunziata, M. Vincensi and G. Ferrari, Journal of Biotechnology, 2012, 159, 324-350.

71 C.C. Liolis, O. Gortzi, S. Lalas, J. Tsaknis and I. Chinou, Food Chemistry 2009, 112, 77-83.

72 S. Saryana, N. Chandrasekaran and A. Mukherjee, International journal of pharmacy and pharmaceutical sciences, 2012, 4, 668-671.

73 S. Sugumar, V. Ghosh and M.J. Nirmala, Ultrasonic Sonochemistry, 2014, 21, 1044-1049.

74 A. Aranaa-Sanchez, E.M. Espinosa, N.E. Obledo-Vazquez, E. Padilla-Camberos, R. Silva-Vazquez and E. Lugo-Cervantes, Letters in Applied Microbiology, 2010, 50, 585-90.

75 B. Shah, M.P. Davidson and Q. Zhong, International journal of food microbiology, 2012, 161, 53-59.

76 B. Shah, S. Ikeda, M.P. Davidson and Q. Zhong, Journal of food engineering, 2012, 113, 79-85.

77 B.V.R. Fernandes, V.S. Borges, A.D. Botrel, K.E. Silva, G.M.J. Costa and F. Queiroz, Drying Technology, 2013, 31, 1245-1254.

78 B.V.R. Fernandes, V.S. Borges and A.D. Botrel, Carbohydrate polymers, 2014, 101, 524-532.

79 N.M. Najafi, R. Kadkhodaee and A.S. Mortazavi, Food Biophysics, 2011, 6, 68-76.

80 A.E.A. El-kader and S.F. Aggor, Journal of Applied sciences Research, 2013, 9, 2951-2958.

826 81 I. Padukka, B. Bhandari and B. Darcy, Journal of food composition and analysis,
827 2000, 13, 59-70.
828 82 E.L. Hill, C. Gomes and M.T. Taylor, LWT- Food Science and Technology, 2013, 51,
829 86-93.
830 83 S. Rodriguez, S. Varona, M. Nunez and J.M. Cocero, Industrial crops and products,
831 2012, 37, 137-140.
832 84 Y. Chang, L. McLandsborough and J.D. McClements, Journal of Agriculture Food
833 Chemistry, 2012, 60, 12056-12063.
834 85 A. Achouri, Y. Zamani and I.J Boye, Journal of Food Research, 2012, 1, 254-67.
835 86 T. Hamouda and R.J. Baker, Journal of Applied Microbiology, 2000, 89, 397-403.
836 87 C. Qian and J.D. McClements, Food Hydrocolloids, 2011, 25, 1000-1008.
837 88 O. Nuchuchua, U. Sakulku, N. Uawwongyart, S. Puttipipatkhachorn, A.
838 Soottitantawat and U. Ruktanonchai, American Asssociation of Pharmaceutical
839 scientists, 2009, 10, 1234-1242.
840 89 I.C. Beristain, A. Vazquenz, S.H. Garcia and J.E. Vernon-carter, Lebensm-wiss
841 Technology, 1996, 29, 645-647.
842 90 C. Gomes, G.R. Moreira and E. Castell-Perez, Journal of food science, 2011, 76, 16-
843 24.
844 91 N. Parris, H.P. Cooke and B.K. Hicks, Journal of Agricultural and Food Chemistry,
845 2005, 53, 4788-4792.
846 92 P. Lertsutthiwong, P. Rojisitthisak and U. Nimmannit, Materials Science and
847 Engineering, 2009, 29, 856-860.
848 93 M.L. Kadam, S.I. Hashmin and R.V. Kale, journal of environmental, agricultural and
849 food chemistry, 2011, 10, 2382-2390.
850 94 X. Jun-xia, Y. Hai-yan and Y. Jian, Food Chemistry 2011, 125, 11267-1272.

95 L.D.M. Moretti, G. Sanna-Passino, S. Demontis and E. Bazzoni, Pharmaceutical science and technology, 2002, 3, 1-11.

96 P. M. Gaysinsky, B. D. Davidson and J. B. Weiss, Journal of food protection, 2005, 68, 2559-2566.

97 C. Turek and F. C. Stintzing, Comprehensive reviews in food science and safety. 2013, 12, 40 – 53.

98 H. Majeed, J. Antoniou, C. F. Shoemaker, and Z. Fang, Archives of Microbiology. 2014, 197, 35 – 45.

99 A. A. C. T. Hijo, J. M. G. D. Costa, E. K. Silva, V. M. Azevedo, M. I. Yosnida and S. V. Borges, Journal of Food Process Engineering. 2014, 38, 1-10.

100 D. I. Hadaruga, N. G. Hadaruga, C. I. Costescu, I. David and A. T. Grucia, Beilstein journal of organic chemistry. 204, 10, 2809 – 2820.