Chapter 2
Nanoencapsulation Technology: Boon to Food Packaging Industries

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2.1 Introduction

Global food security is one of the biggest issues nowadays. Various food-borne pathogens like bacteria, fungi, and insects not only invade the food but also alter their nutritional profile by producing toxic metabolites (Scallan et al. 2011; Marin et al. 2013; Kim et al. 2003; Park et al. 2003; in't Veld 1996). Fungi, most importantly belonging to the species of *Aspergillus*, *Penicillium*, and *Fusarium*, are the major contributors for food and feedstuff spoilage. They produce mycotoxins, viz., aflatoxins, fumonisins, ochratoxins, deoxynivalenol, and zearalenone. Among these mycotoxins, aflatoxins in particular aflatoxin B_1 (AFB_1) produced mainly by different species of *Aspergillus* are a severe concern as it pose carcinogenic, teratogenic, mutagenic, and immunosuppressive potential. Based on the toxicity, it has also been recognized as class I human carcinogen (IARC 1993). The consumption of these contaminated products may have a direct effect on the consumer's health in the form of chronic and acute toxicity. It is estimated that annually around 200 deaths of adults and 40% of children's death up to 5 years are due to illness caused by consumption of contaminated foods (WHO 2017). In order to reduce these harmful contaminants from food, several physical methods like heating, UV or ionizing radiation, membrane filtrations (Farkas 2007), and use of synthetic chemical preservatives, viz., potassium bromate, butylated hydroxyanisole (BHA), and butylated hydroxytoluene (BHT) (Inetianbor et al. 2015), are widely used; however their irrational use may cause pest resistance and adverse effect on humans as well as on the environment, which has enforced the scientists to look toward natural food preservatives in order to meet sustainable food security (Damalas and Eleftherohorinos 2011)

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Since antiquity, plant products in different forms like powder or extracts have been used as preferred alternatives for the protection of food commodities from storage contaminants; however plant products in the form of essential oils are of great concerns as they are volatile, biodegradable, with no residual toxicity, safe to mammalian system, and eco-friendly (Isman 2000; Burt 2004). Many literatures also claim the importance of essential oils as the source of natural preservatives in view of their strong antimicrobial and antioxidant potential (Tripathi et al. 2009; Sánchez-González et al. 2011). Although, essential oils have merit over chemical preservatives, they often fail to exert their full preservative potential, due to oxidation and instability in presence of heat, light and other abiotic factors (Prakash et al. 2018).

In view of these obstacles, introduction of nanotechnology has revolutionized the food industry for the progress of nano based formulation of essential oils and other bioactive components, to achieve major goal of sustainable food preservation and security. Nanoparticles enclosing active principles are claimed to enhance the efficacy of bioactive components, as well as the stability, texture, taste and flavor of food products.

Based on the above background, the present chapter provides an overview of currently employed nanotechnologies and their application in food sectors to develop an approach for the preservation of food during storage in order to achieve sustainable food security.

2.2 Nanomaterials Used for Food Packaging

Recent development in EO formulations based on nanoencapsulation technology has gained much attention in food sectors. These formulations can take display several advantages over conventional methods of food preservation. They confer the controlled release of essential oils and protect them from thermal and photodegradation which assures their improved stability, organoleptic impacts, flavor, and function, consequently extending the final product shelf life (Liang et al. 2012; Madene et al. 2006; Lakkis 2016). There are several methods which have been used for the preparation of nanoparticles using various coating materials. Some of the important methods including different types of polymer used for encapsulation of essential oils or plant bioactive components are discussed and listed in Table 2.1.

2.2.1 Lipid-Based Encapsulation of Essential Oils

Lipids are an important group of natural molecules supporting vital life processes. Lipids give desired qualities like texture, aroma, color, and flavor and mouth-filling properties to food products (Bourne 2002). Currently, due to their nontoxicity and

Table 2.1 List of key nanoparticle types enclosing active materials and their biological properties

S no.	Nanoparticle type	EO/bioactive compounds	Inference	References
1.	Nanoemulsion	*Eugenia brejoensis*	Antimicrobial	Mendes et al. (2018)
		Pimpinella anisum	Insecticidal	Hashem et al. (2018)
		Lemongrass	Antimicrobial	Guerra-Rosas et al. (2017)
		Thyme oil	Antimicrobial	Ryu et al. (2018)
		Lemon myrtle	Antimicrobial	Buranasuksombat et al. (2011)
		Cinnamomum zeylanicum	Antimicrobial	Tian et al. (2016)
2.	Solid lipid NPs	*Eugenia caryophyllata*	Antimicrobial	Fazly Bazzaz et al. (2018)
		Melaleuca alternifolia	Insecticidal/ repellent	Clerici et al. 2018
		Frankincense and myrrh	Anticancer	Shi et al. (2012)
		Miconazole (drug)	Antifungal	Aljaeid and Hosny (2016)
3.	Liposome-mediated NPs	*Geranium maculatum*	Insecticidal	González et al. (2017)
		Nigella sativa	Anticancer	Shanmugam et al. (2017)
		Torreya grandis	Antibacterial	Wu et al. (2018)
4.	Micelles	Tea tree	Antimicrobial	Ganguly et al. (2018)
		Carvacrol and eugenol	Antibacterial	Gaysinsky et al. (2005)
5.	Chitosan-based NPs	*Gaultheria procumbens*	Antifungal	Kujur et al. (2017)
		Zataria multiflora	Antifungal	Mohammadi et al. (2015)
		Garlic	Antifungal	Yang et al. (2009)
6.	Strach-based NPs	Menthone, citral, and lavender oil	Antimicrobial	Qiu et al. (2017)
		Oregano oil	Antimicrobial	Pelissari et al. (2009)
		Cinnamon oil	Antimicrobial	Souza et al. (2013)
7.	Gum-based NPs	Carvacrol and thymol	Antibacterial	Guarda et al. (2011)
		Lemongrass and cinnamon oil	Antifungal	Maqbool et al. (2011)

high acceptability in food products, lipid-based encapsulation of EOs is gaining high attention among scientists worldwide for their use in the food system (Aditya and Ko 2015). Generally lipid-based encapsulation includes emulsions (nano- and micro-emulsions), solid lipid-based nanoparticles (SLBNs), liposome-mediated nanoparticles, micelles, etc.

2.2.1.1 Emulsions

The emulsions can be grouped into three major categories, viz., macro-, micro- and nanoemulsions, which are of major focus for food industries employing bioactives exhibiting low water solubility including essential oils (Xue 2015). Generally, surfactants are used for the preparation of emulsions; however, proteins and lipids have also been used. Preparation and characterization of essential oil loaded nanoemulsion incorporates different modern technologies such as homogenization, sonication, X-ray diffraction and scanning and transmission electron microscopy (Gupta et al. 2016; Solans et al. 2005).

2.2.1.2 Solid Lipid Nanoparticle (SLNs)

These are novel colloidal carriers developed as an alternative material to the polymers, where the solid lipids were involved. Solid-phase lipid nanoparticles are one of the most popular approaches in the field of food sciences for improvement of essential oils biocompatibility (Mukherjee et al. 2009; Saupe and Rades 2006). So far, several methods have been used for the preparation of SLNs, among which high-pressure homogenization, ultrasonication, solvent evaporations, spray drying, and emulsion-based preparations are the most common (Ekambaram et al. 2012).

2.2.1.3 Liposome as Nanocarriers of Bioactive Molecules

Liposome is one of the important systems formed by one or more phospholipids bilayer with an internal aqueous compartment. Due to their amphipathic nature, phospholipids are able to self-organize in the aqueous phase. On account of these properties, liposome-mediated encapsulation is useful for both the hydrophobic and hydrophilic components (El Asbahani et al. 2015). They are efficient carrier molecules for incorporating EOs and components, by improving their physicochemical properties, bioavailability, and solubility (Coimbra et al. 2011). Thin film evaporation, extrusions, sonication, freeze thaw, and saturation of gas-phase solution were the most commonly used methods in the preparation of liposome-mediated encapsulation (Sherry et al. 2013).

2.2.1.4 Micelles

Micelles are the supramolecular complexes consisting of polar and nonpolar materials containing different interwoven gelling agents (Rodríguez et al. 2016). Incorporation of essential oils with anticancerous activities into micelles matrix may be an important strategy to combat the rising incidence of cancers and other untreated diseases. Micelle sizes vary in the dimension from 2 to 20 nanometers based on experimental procedures. Commonly used techniques for micelle preparation are suspension, solvent removal through appropriate methods, dispersion, and dialysis (Hoar and Schulman 1943; Jones and Leroux 1999). An outline of preparation of different lipid-based nanoparticle has been presented in Fig. 2.1.

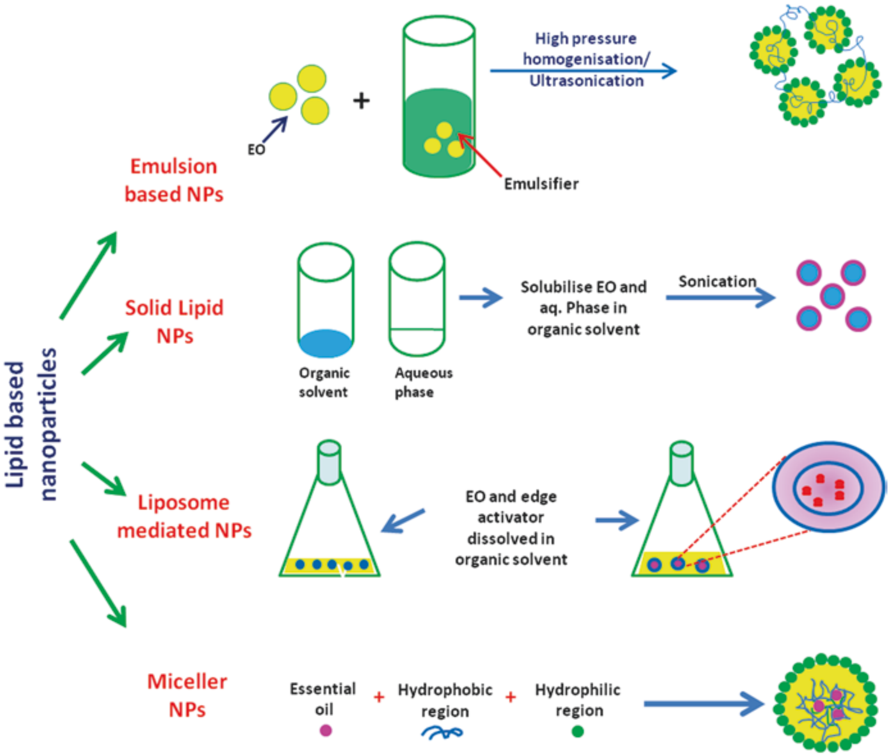

Fig. 2.1 An illustrated diagram showing methodology for the preparation of lipid-based nanoparticles

2.2.2 Polymer-Based Encapsulation of Essential Oils

Polymers including chitosan, starch, cellulose, plant gums, dextrin, and alginates are some of the main wall materials used to entrap essential oils into micro- or nano-sized matrix to prevent them from physical degradation and to enhance their antimicrobial efficiencies.

Starch is a natural and biodegradable polymer used as energy source by many plants. It is also an important polymer in nature. Starch and its modified forms maltodextrins, cyclodextrins, and dextrin whites are being used in the formation of essential oil loaded nanoparticle owing to their abundance, emulsifier property, high stability, and biocompatibility. To date, several starch-based nanoparticles have been developed to enhance the efficacy of bioactive components as evidenced by recent literatures (Shao et al. 2018; Hasani et al. 2018).

Chitosan is an important biopolymer mostly used for active encapsulation of essential oils and their components to enhance activity toward a wide range of pathogens and storage pests (Hosseini et al. 2013). In general, chitosan is isolated

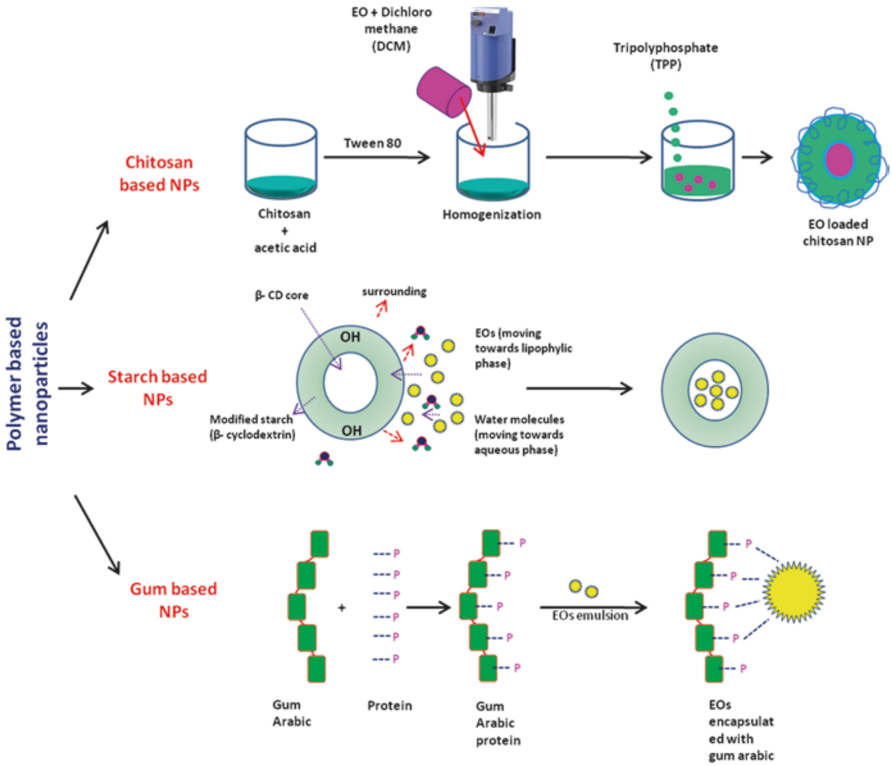

Fig. 2.2 Important polymer based nanoencapsluation methods for bioactive materials

from outer skeletal part of crustacean members, which contains different amines along with primary and secondary alcoholic groups for active binding with essential oil (Vishwakarma et al. 2016).

Gums are natural plant products serving as excellent wall materials for the encapsulation of important plant-derived bioactive components. The semipermeable character of gums helps in stabilization during the encapsulation process (Sarkar et al. 2012; Hamid Akash et al. 2015). In addition, some other polymers like cellulose, wax, gluten, alginate, xanthan, whey, etc. have also been utilized for essential oil encapsulation. A systemic representation on polymer-based nanoencapsulation process for bioactive materials is presented in Fig. 2.2.

2.3 Active Packaging of EO Nanoparticles as Food Protectant

Active packaging of EOs and their bioactive components is one of the important approaches used by food industries to protect food items from microbial and toxic metabolites contamination thereby maintaining their nutritional status (Coles et al.

2003). Utilization of essential oils through packaging provides protection against three major classes of external factors, viz., chemical, physical, and biological. Therefore, current researchers are focussing on modern delivery systems for encapsulation and release of essential oil vapors in a controlled manner. Since, food industries are facing numerous challenges in developing healthy, safe, and environmentally acceptable foods (Neethirajan and Jayas 2011), nanotechnology may be exploited successfully as one of the important tools to improve production and preservation of food, for extending shelf life (Roco 2002). Nanoencapsulation deals with the reduction in size of the coating materials up to 1 μm having the bioactive components enclosed in a tight matrix. The involvement of essential oil based nanotechnology for efficient food packaging supports the preservative potential in terms of controlled release, greater surface area to volume ratio and optimized interaction with food components. Another important point of food packaging is the selection of suitable active packaging polymers, which do not interact with the organoleptic properties of food viz. flavor, color, taste and texture. The most commonly used matrix materials for edible thin film packaging of food products are chitosan, gelatin, carrageenan, polylactic acid, and alginate. These edible films are commonly developed by carbohydrates, proteins, or lipids acting as a barrier of oxygen, carbon dioxide, and other soluble gases providing off-flavors and odors. Among different polymeric matrices, montmorillonite (MMT) has been used by some workers due to associated benefits of potential release of antimicrobial compounds and controlled permeability of the gas and water vapor inside the packaging matrix (Fabra et al. 2009).

Polymeric particles are generally of two types, i.e., nanocapsules and nanospheres. Incorporation of essential oils into the polymeric matrix provides significant antimicrobial and antioxidant properties for control of microbial contamination in the food products. Essential oils get conjugated with these polymeric materials and constitute a biocompatible nanoparticle. The antimicrobial property of developed packaging film greatly rely over the ratio of essential oil and polymeric matrix utilized. Encapsulation of essential oil enhances effective concentration of bioactive components in the food system especially in water-rich phases or solid-liquid interphases where microbial contamination occurs. For instance, nanoencapsulated terpenes and D-limonene showed significant efficacy against *Lactobacillus delbrueckii* and *Escherichia coli* (Donsì et al. 2011). Gaysinsky et al. (2005) performed nanoencapsulation of two important essential oil components eugenol and carvacrol with different nano-based surfactant for enhancing antimicrobial efficacy against pathogenic microbes. Citronellal, one of the major components of the *Eucalyptus citriodora* essential oil encapsulated in chitosan matrix, has shown better antifungal, antioxidant, and insect repellant activity with significant distribution and polydispersity (Ribeiro et al. 2014). Encapsulation of *Zataria multiflora* essential oil within chitosan nanoparticle significantly inhibited the growth and sporulation of *Botrytis cinerea* in the storage condition at 4 °C (Mohammadi et al. 2015). Essential oil loaded nanoemulsion delivery system is widely acceptable for practical application being stable, transparent and colloidal in nature. The use of oregano essential oil nanoemulsion with active preservative potential against

Listeria monocytogenes, *Salmonella typhimurium*, and *Escherichia coli* on fresh lettuce has recently been reported by Bhargava et al. (2015). Recent findings of mandarin essential oil enclosed within modified chitosan-based coating for beans have been investigated for large-scale future application (Severino et al. 2014; Donsì et al. 2015). The schematic representation of preparation and role of nanoparticle-based delivery system in food industries is represented in Fig. 2.3.

There are different delivery systems used to achieve maximum oil concentration and efficient antimicrobial activity in food system. The most common delivery systems are emulsions, suspensions, and liposomes which are colloidal particles associated with essential oils as internal core materials, acting as good delivery system with potential sustained release properties and significant activity against fungi and other microbes (Gomes et al. 2011). Essential oil loading within different biopolymeric matrices following a variety of fabrication techniques, viz., interfacial polymerization, solvent evaporation, and emulsification, has been demonstrated to maintain good quality and stability of nanoparticles in storage practices (Akrachalanont 2008).

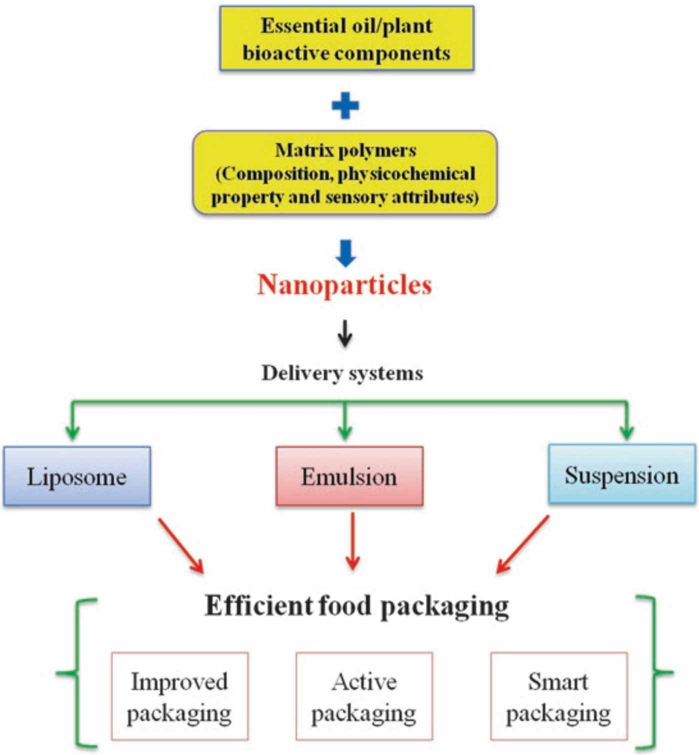

Fig. 2.3 Brief outline of synthesis and application of essential oil-based nanoparticles in food sector

2.4 Mode of Action of Nanoparticles

Recently, there has been an increasing interest for the use of plant essential oils as fungicide, bactericide, insecticides, and rodenticides to control fungal, bacterial, and insect- and rodent-mediated deterioration of food items. However, volatile nature of essential oils and physico-chemical degradation by abiotic factors limits their long term application. Therefore, use of nano based delivery system may provide the efficient way to control the pests by involving different mode of action on cells leading to cell lysis and subsequent death. The smaller size of nanoparticles allows them to traverse within the cells culminating into loss of cellular activity. Furthermore, the lipophilic chemical components of essential oils can also easily cross the plasma membrane of fungi and insects and cause different enzymatic alterations and biochemical dysfunction inside their body (Lee et al. 2004). Inhibition of fungal membrane ergosterol by different essential oils has been actively reported by Tian et al. (2012). The active ingredients of essential oils can be used as potent repellant, fumigant, antifeedant, and insecticidal agent. In general, the terpenoids and phenolic components of essential oils target nervous system of insect by interfering with functions of enzymes such as acetyl choline esterase (AChE) or neurotransmitters such as octopamine and γ-aminobutyric acid (GABA). In insects, the toxic effects on octopamine neurotransmitter, GABA gated chloride channel and cytochrome P_{450} monooxygenase may ultimately cause hypersensitivity, convulsions and paralysis of organs (Enan 2001). Besides their lipophilic nature, the bioactivity of essential oil also depends on different functional groups (nature and position), volatility, and molecular weight of compounds. Acetate-derived methyl groups and double-bonded benzene ring of eugenol, safrole, and limonene actively take part in knockdown of different enzymes and fumigant toxicity of *Spodoptera litura*, *Callosobruchus chinensis*, *Tribolium castaneum*, and other storage insects. Lower solubility, higher volatility and oxidation tendency, are the major problem in practical application of essential oil for post harvest pest management. These problems may be effectively resolved to a greater extent by nanoencapsulated essential oil-based pesticides (nanopesticides) or nanoformulated essential oil. Christofoli et al. (2015) reported on *Zanthoxylum rhombifolium* essential oil loaded nanospheres against *Bemisia tabaci*. Silver nanoparticles loaded with bioactive principles of *Aristolochia indica* showed maximum larvicidal, repellant, and cytotoxic effect on *Helicoverpa armigera* (Siva and Kumar 2015). Lead and silver nanoparticle of *Avicennia marina* plant extract exhibited prominent toxicity against *Sitophilus oryzae* (Sankar and Abideen 2015). The list of chemical and biological nanoparticles used for broad management of insect pests is described in Table 2.2.

Table 2.2 Important chemical and biological nanoparticles against fungi, bacteria, insects, and rodents

Types of nanoparticles	Targeted pests	References
Silver nanoparticles	*Spodoptera litura* and *Achaea janata*	Yasur and Rani (2015)
Zinc nanoparticle	*Aphis nerii*	Rouhani et al. (2012)
Silver nanoparticle	*Pseudomonas aeruginosa*	Salomoni et al. (2017)
Chitosan nanoparticle	*Spodoptera litura*	Chandra et al. (2013)
Silver nanoparticle with *Aristolochia indica*	*Helicoverpa armigera*	Siva and Kumar (2015)
PEG-based garlic essential oil nanoparticle	*Tribolium castaneum*	Yang et al. (2009)
Chitosan nanoparticle	*Callosobruchus maculatus*	Sahab et al. (2015)
Silver nanoparticle	*Sitophilus oryzae*	Zahir et al. (2012)
Illicium verum essential oil loaded chitosan nanoparticle	*Aspergillus flavus* and other storage fungi and aflatoxin B_1	Dwivedy et al. (2018)
Mentha piperita essential oil loaded in chitosan-cinnamic acid nanogel	*Aspergillus flavus*	Beyki et al. (2014)
Pectin/papaya puree/ cinnamaldehyde nanoemulsion	*Escherichia coli*, *Staphylococcus aureus*, *Listeria monocytogenes*, and *Salmonella enterica*	Otoni et al. (2014)
Clove/cinnamon loaded nanoemulsion	*Bacillus subtilis*, *Salmonella typhimurium*, and *Staphylococcus aureus*	Zhang et al. (2017)
Mustard oil microemulsion	*Escherichia coli*	Gaysinsky et al. (2008)
Eugenol loaded nanoemulsion	*Staphylococcus aureus*	Ghosh et al. (2014)
Encapsulated terpene mixture and D-limonene nanoemulsion	*Lactobacillus delbrueckii*, *Saccharomyces cerevisiae*, *Escherichia coli*	Donsì et al. (2011)
Santolina insularis essential oil encapsulated with liposome	Herpes simplex virus type-1	Valenti et al. (2001)

2.5 Factors Controlling the Stability of Nanoparticles in Food System

The rising disciplines of nanotechnology and their applications in food and agricultural industries are of major focus in the current generation. Varied applications of nanotechnology are expanding rapidly with a multitude of potential factor for nanoparticle stability and enhancement of food shelf life by maintaining the flavor properties and ultimately improved nutritional value of food products during storage (Chellaram et al. 2014). Nanoencapsulation provides protection to bioactive food ingredients by eradication of incompatibilities and masking the unpleasant taste or odor. Besides having effective applications, there are some controlling factors which affect their physical and chemical stability.

2.5.1 Free Energy of Different Phases

Nonpolar interaction of lipophilic oil with hydrophilic component increases the thermodynamic instability of nanoemulsion depending upon the free energy status (Wooster et al. 2016). Therefore, changes in physical characteristics of nanoemulsions ultimately result in phase alteration (Chung and McClements 2018). Thus, nanoemulsion characterization is a major aspect not only in the early stage but also during long-term storage. Generally, storage practices occur in varied environmental conditions that may be quite useful to enhance the stability and activity of the nanoemulsions. Furthermore, under certain conditions, nanoemulsions may become unstable due to gravitational separations resulting primarily in the differences in densities as observed by densitometric studies (Chung and McClements 2018).

2.5.2 Droplet Aggregation and Particle Size

Nano-sized droplets in emulsions are always in constant collision reaction because of gravitational force, Brownian motion, and other mechanical forces (McClements 2015), favoring the aggregations of nano-droplets. Droplets may get associated with each other without interfering their original size, and this process is known as flocculation. In addition, a number of nano-droplets may come across each other and form a large droplet by the process known as coalescence.

However, for effective commercial applications, it is necessary to avoid nano-droplet aggregation as much as possible. For this, attractive forces, viz., hydrophobic and van der Waals forces, may be compensated by the repulsive forces, viz., electrostatic and steric actions between the nano-droplets. In addition, the decreased nanoparticle size is correlated with reduction of nano-droplet aggregation (Degner et al. 2014). According to McClements (2004), along homogenization, differences in soluble phases and emulsifier concentration and types are the governing factors regulating the size of the droplets. The stability of nanoencapsule suspension can also be achieved by drying the nano-suspension (Nakagawa et al. 2011).

2.5.3 Emulsifier Type

The stability and size of nanoemulsion suspension formed after homogenization are greatly influenced by the nature of emulsifier used. McClements (2004) studied protein and lipid droplet interaction which are very much susceptible to changes in ionic strength and pH as their major stabilization by repulsing forces. In contrast to this, Qian et al. (2011) have described the coating of lipid droplets into polysaccharide and non-ionic surfactants that are not much susceptible to these parameters such as steric repulsion.

2.5.4 Ionic Strength and pH

Ionic strength and pH of aqueous phase are two other key factors which have leading impact over stability as well as properties of nanoemulsion by influencing their electrostatic interactions. Ionic concentration and its type present in the aqueous phase can influence the strength of electrostatic interactions by influencing the effect of ion binding, ion bridging, and electrostatic screening. Besides ionic concentration, pH of nano-suspension also has considerable impact over strength of electrostatic interactions depending on charge potential on nanoparticle surface (McClements 2004).

Nanoparticles consisting of protein-coated fat droplets have strong affinity toward flocculation when ionic strength of nano-suspension exceeds beyond a certain level because of electrostatic screening and ion binding effects (De La Fuente et al. 1998). Moreover, addition of salt to the nanoemulsion prior to or after homogenization may influence the stability and aggregation of nanoparticles (Kim et al. 2005). Similar effects are also observed when pH value of nanoemulsion is near to their isoelectric point (Demetriades and McClements 1998). As mentioned above, ionic strength and pH value of nano-suspension influence the aggregation and stability of nano-droplets. Besides this, some other properties like texture of emulsion and their optical properties may also get affected.

2.5.5 Thermal Processing

Thermal processing is another factor that governs nanoemulsion stability by influencing the droplet aggregation over and above visual and textural properties of nano-suspension. Croguennec et al. (2004) have reported that adsorbed globular proteins may experience an irreversible change in their structure and chemical reactivity on heating beyond their thermal denaturation. Moreover, Donato et al. (2007) have reported the formation of protein aggregates after heat treatments.

2.6 Nanoparticles as Active Biosensor for Detection of Food Contaminants (Chemicals and Food-Borne Pathogens)

A sensor is a measurement system consisting of specific probe to detect particular species or element at trace levels. Biosensor have been used for detection of different biological organisms such as viruses, bacteria, proteins, nucleic acids and pathogens (Pérez-López and Merkoçi 2011). Biosensors contain a bioreceptor and transducer which interact with specific analyte molecule, generating an electrical signal. Bioreceptor consists of living systems viz. cells, tissue, enzymes, proteins, nucleic acids and antibodies which are utilized for the biochemical adhesion property. Nowadays, nanobiosensing technology is being utilized in food safety analysis to detect various sensitive contaminants in food materials. A nanobiosensor is also called as a second-generation biosensor due to the involvement of ultrasensitive nanoparticles and their effective transducers (Sagadevan and Periasamy 2014). The major advantages of using nanomaterials in biosensing are the combined effect on biological receptor molecules and higher surface to volume ratio (Kumar et al. 2012). Among different synthesized nanoparticles, metal nanoparticles, carbon nanoparticles, semiconductor quantum nanoparticles, and magnetic nanoparticles have been used for detection of food allergens. Metal nanoparticles, basically colloidal gold, have been successfully used in DNA biosensing based on their amount of immobilized biomolecules in an active sensor (Cai et al. 2002). There are different types of nanotubes, viz., one-dimensional nanostructures (1-D), nanotubes with single or multichannel carbon tape, and carbon nanowires, based on the conducting polymer and biochemical reaction routes. The organic nanosensors have been actively applied for detection of organophosphate pesticide contamination in fruits and other stored products as well as their solubility and residual toxicity in different food products. Interestingly these nanobiosensors have marked advancement for detection of mycotoxins and different mycotoxigenic fungi in different food products (Li et al. 2012). Aflatoxin contamination can be measured on pre-harvested and post-harvested food products at a very low concentration, i.e., 0.3 pg/mL by different nanocomposites (Gan et al. 2013). Yotova et al. (2013) have developed a nanosensor with modified tyrosinase enzyme for detection of toxigenic fungi. Gold nanorods (GNRs) have active sensing capacity for AFB_1 detection up to 0.04 ppb in food samples (Xu et al. 2013). Modified gold nanoparticles with electrochemiluminescent aptamers have been used for detection of ochratoxin A. AF-oxidase enzyme linked with modified carbon nanotubes and poly-o-phenylenediamine integrated with gold electrode has the ability to detect sterigmatocystin concentration up to 3 ng/mL (Chen et al. 2009). Similarly, immuno-chromatographic biosensors with attached monoclonal antibodies have been used for rapid detection of zearalenone in corn samples (Shim et al. 2009). Maragos (2012) has developed colloidal gold nanoparticles with interferometry principle for detection of deoxynivalenol in wheat.

2.7 Application of Nanoparticles in Different Food Sectors

In today’s world, the applications of nanoscience and nanotechnology in food materials have contributed a major status for nutrition and health of consumers. Most of the nanoparticles are colloidal in nature, and stability is achieved by van der Waals forces and steric stabilization provided by core materials and adsorbing matrix polymers. In the food industry, nanotechnology have both “top-down” and “bottom-up” approaches with smart delivery of nutrients and proteins, rapid identification of contaminants, and encapsulation of essential oil with adsorbing matrices (Verma et al. 2009). Globally, the overall sales of nanotechnology products have been observed to increase from US$ 150 million to US$ 860 million in between 2002 and

2004. Application of essential oil in food matrices as biopreservatives and natural antioxidant is one of the most important aspects in the current generation. This rapidly developing encapsulation technology have an ultimate focus on food processing, packaging, transportation, shelf life enhancement, and bioavailability of nutrient compounds. The microencapsulated essential oils having antimicrobial agents are used in efficient smart packaging, an effective approach in food industries (de Barros Fernandes et al. 2014). Use of nanoparticles for the improvement of food processing involves different biological aspects such as effective preservative potential, targeted and controlled release of bioactive components, high absorption rate related to greater surface area, high availability, and improved organoleptic properties. Active antimicrobial effect, improved barrier properties and biosensing properties have been applied in nanopackaging of food materials. The active use of nanosensors for pathogenic microbes and toxin detection in stored foods is an important aspect of nanotechnology during transportation. Nanoparticles are efficient due to selective binding and easy removal of pathogens from food surface (Duncan 2011). Industrial application of encapsulated essential oil in food systems is a very limited approach until now. Wu et al. (2012) have reported efficient antimicrobial property of zein nanoparticle loaded with thymol and carvacrol without affecting organoleptic properties of food commodities. Nano-formulation of *Artemisia arborescens* essential oil with solid lipid nanoparticles allowed controlled release of vapor and served as an efficient ecological pesticide (Lai et al. 2006). A number of essential oils and their bioactive components have been incorporated into edible film exhibiting better efficacy against food-borne pests and pathogens. Khalili et al. (2015) confirmed potential efficacy of thyme essential oil encapsulated within chitosan-benzoic acid nanogel against growth and aflatoxin secretion by *Aspergillus flavus* in stored foods. Hence efficient food packaging, formulation, and delivery can be assisted by several compatible nanomaterials. A comprehensive diagram representing the application of nanoparticles in different food sector is shown in Fig. 2.4.

2.8 Safety Issues Associated with Application of Nanotechnology in Food Packaging/Food Preservation

Before discussing the possible consequences of nanomaterial ingestion via food, it is very important to recall that our food naturally comprises of several nanorange components. Several forms of biopolymers such as proteins, carbohydrates, and fats are present in the nanorange in variety of food items (Magnuson et al. 2011; Cockburn et al. 2012). Even the digestive processes (mechanical/chemical) performed in the gastrointestinal tract leads to the conversion of complex food structures into simple forms of mono−/disaccharides, fatty acids, or amino acids/peptides, which can be included under nano−/micro-material range (Martirosyan and Schneider 2014). Such nanomaterials formed in the gastrointestinal tract are subjected to absorption or translocation into the blood/lymph through the gut epi-

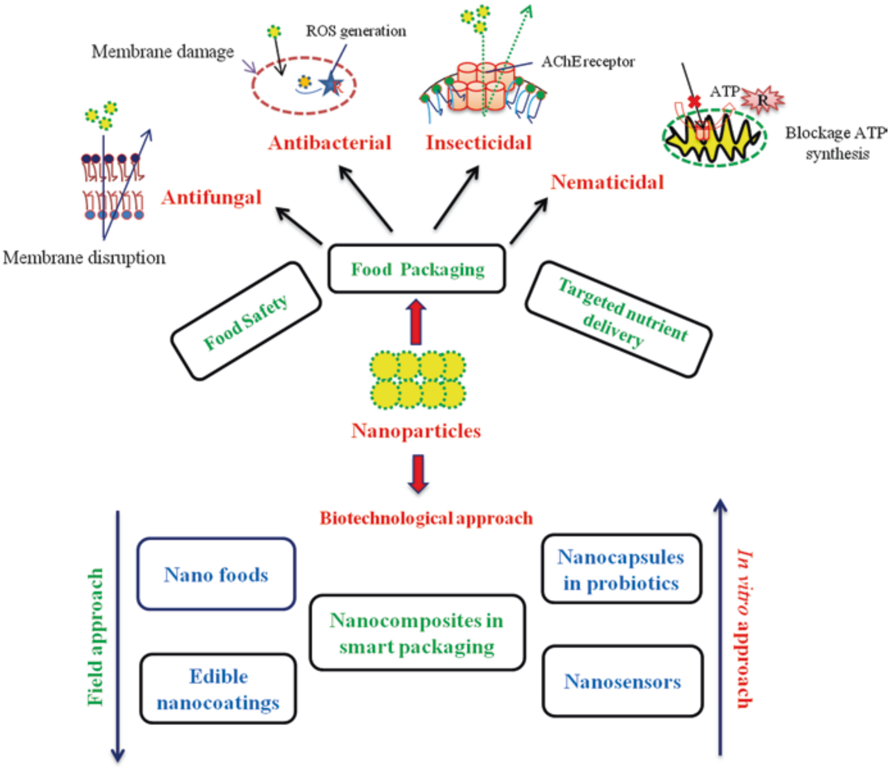

Fig. 2.4 Application of nanoparticles in different food sectors

thelia (Powell et al. 2010). Thus, it should be made clear that the gut lumen and epithelia are well exposed to various nanomaterials ingested orally via food.

The major concern of nanomaterial usage in food packaging is "leaching out" of active ingredients from the packaging materials into the food items. The incorporation of nanomaterials in the food as stabilizers, anticaking agents, in order to improve the shelf life, odour, taste and aroma, will also increase the chances of oral entrance (Laloux et al. 2017). In this context, there are several issues, most importantly the very small size of nanomaterials favoring the greater absorption through the intestinal/gut epithelia (Martirosyan and Schneider 2014). If there is a possibility of internalization and retention in cells or tissues, the nanomaterials could enter into the food chain. After entry, the nanomaterials may get coated with lipids/proteins as such forming a "corona" which facilitates the absorption, thus making the nanomaterial biofunctional (Bellmann et al. 2015). This corona stage is not stable and is subjected to alterations under changing macromolecules and environmental conditions it encounters. This property complicates the expected behavior of nanomaterials as well as the absorption/metabolic profile of food components inside the gastrointestinal tract. Such unknown effects associated with the nanomaterial behavior inside the gut have been termed as the "Trojan horse effect" (Park et al.

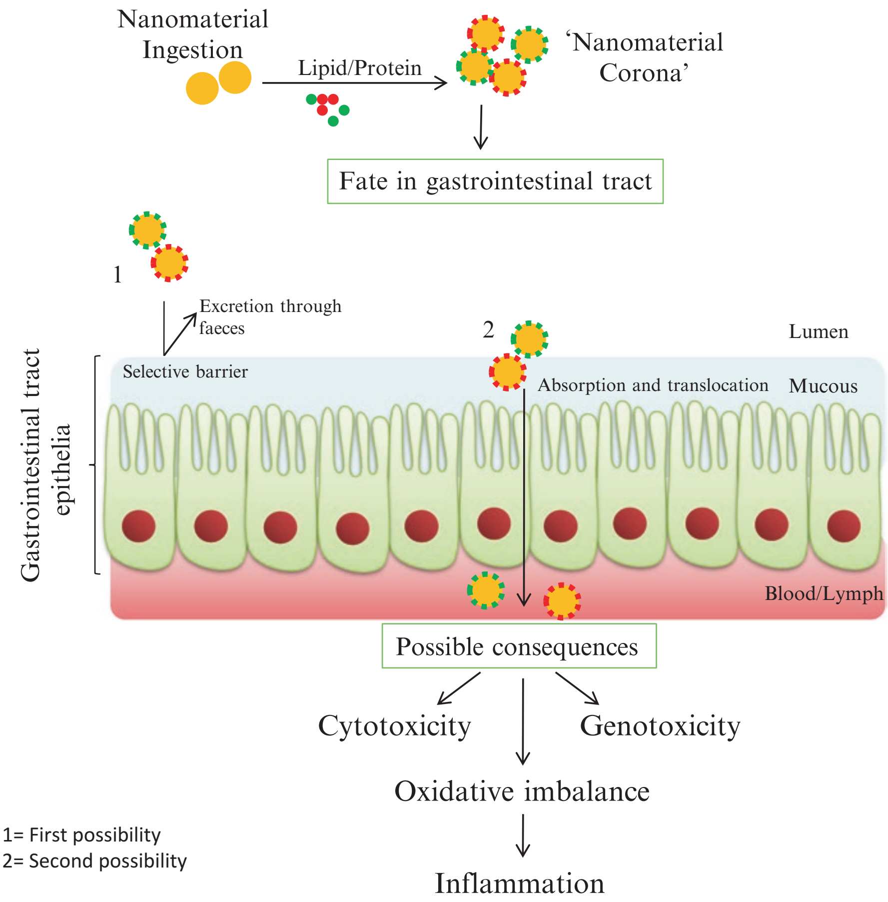

Fig. 2.5 The fate and possible effects of nanomaterial ingestion

2010; Souza et al. 2018). The fate and the potential consequences of nanomaterial ingestion via food have been represented in Fig. 2.5.

The gastrointestinal tract has a mucous layering which functions as mechanical barrier to foreign particles entering the tract. The nanomaterial may get excreted via feces if not allowed to pass through the epithelia. However, if it gets absorbed/translocated into the blood/lymph, it has the passage to stream through different compartments of the body (Bellmann et al. 2015). The consequences of nanomaterial acquisition in different body parts have been demonstrated in vitro (Owens III and Peppas 2006; Corbalan-Penas 2010; Athinarayanan et al. 2014). Factors governing the fate of nanomaterials inside the body include dose, particle size, shape, charge, solubility, reactivity, and surface coating. The lipid bilayer of the cell membrane has been speculated as the primary target of nanorange materials. Pores formed in the membrane due to nanomaterials lead to cytotoxicity. The nanomaterials have been shown to induce ROS (reactive oxygen species) and RNS (reactive nitrogen species; nitrosative stress) production (Athinarayanan et al. 2014). The oxidative imbalance inside the cell has also been shown to activate the pro-inflammatory cytokines and inflammasome-mediated inflammation (Dankovic et al. 2007; Moos et al. 2011). Genotoxicity induced by nanomaterial interaction could result in direct interaction with genomic DNA or indirectly through ROS generation (Singh et al. 2017).

In conclusion, it is essential to perform safety assessment of nanomaterials before their application in food packaging or processing. Cockburn et al. (2012) have provided a five-step systematic approach in this connection. Therefore, definite description as well as the comprehensive characterization of the nanomaterials is vital, followed by risk evaluation through utilization of “decision tree,” whether the properties favor the need of detailed investigations on toxicological testing or not.

2.9 Future Prospective

In the recent past, there is an increasing interest toward the use and incorporation of nanometric structures in food industries for active packaging, processing, or preservation of quality. Therefore, the scientific community has focused greatly on the development of nanoscale materials that could fulfill changing world food demands. Nanobiotechnology in food packaging has immense potential to preserve food quality and nutritional profile by serving as antimicrobials and barriers against UV rays, gas, and moisture. To date, various methods have been proposed to encapsulate the bioactive compounds in an array of nano-vehicles to facilitate the stability as well as efficacy. Although research toward nanopackaging has gained momentum, still a lot of questions remained unanswered regarding their fate in the biological systems, environmental impacts, consumer acceptance, effects over sensory profile, and the cost to benefit ratio. Moreover, consumers face hesitation in accepting the incorporation of nanotechnology in the food system. Nanosensors incorporated in plastic packaging could be very much effective against detection of food contamination and spoilage (Thiruvengadam et al. 2018). Rigorous research over safety evaluation together with efforts for increasing the consumer’s acceptance should be in priority to further explore the application of nanotechnology in food industries.

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