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Recent Advances in Nanotechnological Approaches to Enhance the Industrial Application of Essential Oils and Their Application in Food Packaging

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Abstract

Essential oils (EOs) are lipophilic volatile compounds synthesized by plants and used for centuries in several areas, including medicine and cosmetic. Currently, they have gained great visibility in the food industry, including their application in food packaging development. However, EOs present inherent characteristics that may limit their application in food products/packaging, especially triggered by the nature of their constituents (e.g., strong sensorial aspects, high sensibility to external factors: temperature, and oxygen). Thus, efforts have been made in the search for technologies to help circumvent these drawbacks. Among these alternatives, nanotechnology has been highlighted as an important one. Studies have shown that there are some promising nanotechnological approaches to

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improve the application of EOs in food and food packaging. This chapter describes the main aspects circumventing EOs' hole in the food industry, especially when used as an active additive in food packaging, and how nanotechnology can be an important tool to increase their application in this area. Some examples of nanotechniques are presented and discussed in order to present an overview of this important and current topic.

Keywords

Essential oils · Volatile compounds · Nanotechnology · Food packaging · Food industry

14.1 Introduction

Essential oils (EOs) are secondary metabolites synthesized by plants, mainly composed of lipophilic volatile compounds. They have been employed over centuries mainly for pharmacological, medicinal, aromatic, or cosmetic purposes (Carpena et al. 2021). Nevertheless, since the nineteenth century, their application has been extended, attending great areas in the nutrition field. Several studies have already covered the bioactive properties of EOs, such as antibacterial, antifungal, antioxidant, antiviral, antiparasitic, and insecticidal, broadening the application spectrum of these complex mixtures (Viuda-Martos et al. 2011; Galié et al. 2018; Marsin et al. 2020; Sharma et al. 2022).

Triggered by the current consume prospects that seek healthier diets and natural consumption, EOs have been widely applied by the food industry not only as a flavoring ingredient (i.e., due to their strong sensorial characteristics) but also as a food preservative (Pisoschi et al. 2018; Zanetti et al. 2018; El-Sayed and El-Sayed 2021). Furthermore, EOs have evidenced great results in active food packaging (e.g., antioxidant and antimicrobial activities along with the enhancement of water vapor barrier of the active films) (de Andrade et al. 2020; Scaffaro et al. 2020).

However, some limitations of EOs, discussed in the following sections of this chapter, may restrict their application in food packaging systems, especially considering the large-scale produce. Hence, some strategies have been adopted to improve the applicability of EOs by circumventing these drawbacks and, in some cases, enhancing their properties and bioactivity. One great example of this strategy is nanotechnology. Some EO nano-systems are already well established, while others still need further investigation (Zanetti et al. 2018; Das et al. 2021).

This chapter aims to provide a comprehensive approach to EOs background in the food industry and present the aspects surrounding how nanotechnology has been applied to EOs to improve their applicability in food packaging. The main nanosystems (nanoemulsions, nanocapsules, nanoliposomes, nanocubosomes, and others) are listed and discussed along with examples of successful application in food packaging materials. Safety and toxicological concerns are also assessed in order to provide an entire overview of the topic.

14.2 Essential Oils

Records have revealed that EOs play a remarkable role in traditional medicine (Prakash et al. 2018a). In food industry, as a food additive, the application of EOs as a natural, safe, and effective alternative to the use of commercial synthetic chemicals has increased (Reyes-Jurado et al. 2019, Ni et al. 2021; Rehman et al. 2020; El Khetabi et al. 2022), due to their antimicrobial, coloring, seasoning, and antioxidant properties (Calo et al. 2015; Prakash et al. 2018a). Furthermore, EOs reduce spoilage of crops and foods with shelf-life extension, as well as control weed growth (Abd-ElGawad et al. 2020; Senthil-Nathan 2020; Walia and Kumar 2021; El Khetabi et al. 2022). Figure 14.1 illustrates the main applications of EOs in food processing and packaging.

In general, EOs are natural volatile liquids composed of a complex mixture of various secondary metabolites such as terpenes, terpenoids, phenylpropenes, aldehydes, ketones, ethers, alcohols, and phenolic compounds (Prakash et al. 2018a, b; Ni et al. 2021; Vianna et al. 2021). Most of these substances are simple lipophilic compounds characterized by a strong aroma that can be obtained from plant materials such as flowers, buds, leaves, seeds, bark, and stems (Prakash et al. 2018a; Reyes-Jurado et al. 2019; Doost et al. 2020; Ni et al. 2021; Arruda et al. 2022; Teixeira et al. 2022).

As a complex mixture, these metabolites have been explored concerning their biological properties such as antimicrobial and antioxidant activity. In this context, EOs can inactivate pathogenic microorganisms without promoting the acquisition of resistance (Prakash et al. 2014; Prakash et al. 2018a). In addition, most EOs have low mammalian toxicity, which makes them relatively safe and due to their relatively low cost, they play an important role in the food, pharmaceutical, and cosmetic industries (Calo et al. 2015; Prakash and Kiran 2016; Prakash et al. 2018a; Vianna et al. 2021).

Some examples of EOs categorized as Generally Recognised as Safe (GRAS) by the U.S. Food and Drug Administration (U.S. Code of Federal Regulations 2016) include jasmine (*Jasminum officinale* L.), cumin (*Cuminum cyminum* L.), coriander (*Coriandrum sativum* L.), ylang-ylang (*Cananga odorata* Hook. f. and Thoms.), basil (*Ocimum basilicum* L.), origanum (*Origanum* spp.), winter savory (*Satureia montana* L.), chamomile flowers (*Matricaria chamomilla* L.), peppermint (*Mentha piperita* L.), and pimenta leaf (*Pimenta officinalis* Lindl.); in which as main active components are highlighted linalool, benzyl acetate, linalyl acetate, thymol, germacrene, carvone, cinnamaldehyde, eugenol, menthol, vanillin, citral, limonene, carvacrol, among others (Prakash et al. 2018a; Vianna et al. 2021; El Khetabi et al. 2022).

EOs can be extracted by hydrodistillation, steam and water distillation, solvent extraction, or supercritical fluid extraction (Shukla et al. 2019; Kaya et al. 2020; Ni et al. 2021; Teixeira et al. 2022). The composition and quality of EOs can be affected by numerous factors inherent to the plant, such as botanical source, geographical location, stage of development, climatic conditions, growing season, stage of ripeness, and harvest (Sabo and Knezevic 2019; Arruda et al. 2022). In addition, the

Fig. 14.1 Applications of essential oils in food processing and packaging

extraction method and storage conditions can strongly affect the stability of EOs (Vianna et al. 2021).

In this sense, the variability in the composition of EOs can confer different biological properties and directly affect their antimicrobial and antioxidant potential (Arruda et al. 2022; Teixeira et al. 2022). Therefore, knowing the mechanism of action of each EO is essential to define which compound will be used depending on the target microorganism and the food matrix used (Arruda et al. 2022; Teixeira et al. 2022).

14.2.1 Bioactivity of EOs: Mechanism of Antimicrobial and Antioxidant Activities

The main compounds associated with EOs' antimicrobial activity are described as low molecular weight substances, most of them are aldehydes, terpenes, and terpenoids, with aromatic or aliphatic carbon chains (Ju et al. 2019; Arruda et al. 2022; Teixeira et al. 2022). The antimicrobial activity of these complex mixtures has already been verified in a wide spectrum of microorganisms, including Gram-positive and Gram-negative bacteria, yeasts, and molds (Nionelli et al. 2018; Arruda et al. 2022).

The mechanisms of action of EOs are directly associated with the chemical structure of their components (Ju et al. 2019). In general, these compounds can damage the structure and affect the function of the bacterial cell membrane (Ni et al. 2021). In addition, EOs may have other pathways of action, such as cell wall damage, inhibition of peptidoglycan and ATP synthesis, mitochondrial disruption, ribosomal dysfunction, DNA damage, and inhibition of gene expression (Ju et al. 2019; Ni et al. 2021; Arruda et al. 2022). Furthermore, EOs may exert interesting effects on virulence, motility, sporulation, and biofilm formation factors of microorganisms (Sabo and Knezevic 2019; Cáceres et al. 2020; Moradi et al. 2020; Arruda et al. 2022).

In parallel, EOs are excellent sources of natural antioxidants (Simionato et al. 2019; Bastos et al. 2020). The antioxidant mechanism of each fraction strongly depends on its chemical composition (Arruda et al. 2022). Mainly, the antioxidant activity of EOs is associated with biologically active compounds such as terpenoids and phenolic acids (Thokchom et al. 2020), acting by donating hydrogen to highly reactive compounds, preventing the formation of radicals (Arruda et al. 2022).

14.2.2 Essential Oils and their Prospects in Food Packaging

EOs present some challenges, such as low water solubility, strong lipophilicity, and high volatility (Prakash and Kiran 2016; Prakash et al. 2018a; Ju et al. 2019). Furthermore, they lack photothermal resistance and are prone to oxidative decomposition, which limits their direct application in food systems (Ju et al. 2019). Furthermore, EOs have several intrinsic challenges that make their application difficult as food preservatives. For example, scarcity of raw materials, chemotypic variation, low solubility in water, low stability during storage, and the threat of biodiversity loss are some of the main challenges to producing EOs on an industrial scale (Prakash and Kiran 2016; Prakash et al. 2018a). In parallel, the characteristics of the food matrix together with extrinsic factors such as temperature, gaseous composition, and microbial species can reduce the bioactivity of EOs (Calo et al. 2015; Prakash et al. 2018a). Therefore, improving the stability and durability of EOs has become essential to maintain biological activity, reduce volatility, and minimize adverse effects on aroma and flavor (Ju et al. 2019).

For this, EOs are being incorporated into food packaging to overcome these barriers and enhance their performance by increasing the contact area and dispersibility of these components on the food surface (where there is a higher incidence of microorganisms) (Donsì and Ferrari 2016; Ni et al. 2021). As a result, there is an increase in the shelf life of perishable foods, protecting against oxidation, vitamin loss, enzymatic browning, and microbial growth (Mir et al. 2018; Vianna et al. 2021; Arruda et al. 2022).

In recent years, there have been reports of food preservation based on EOs (directly applied into food matrix and as active additive in food packaging) obtained from fennel (*Pimpinella anisum*), cloves (*Syzygium aromaticum*), peppermint (*Mentha piperita*), oregano (*Origanum vulgare*), cinnamon (*Cinnamomum zeylanicum*), lemongrass (*Cymbopogon citratus*), rosemary (*Salvia rosmarinus*), thyme (*Thymus vulgaris*), ginger (*Zingiber officinale Roscoe*), and other plants (Tu et al. 2018; Ju et al. 2019; Satyal and Setzer 2020).

Strategically, the incorporation of EOs in packages modifies the characteristics of the packaging material and can increase its durability along with the improvement of the packaged food's shelf life (Mir et al. 2018; Alizadeh-Sani et al. 2020; Arruda et al. 2022). In addition, the combination of different EOs is an interesting strategy to obtain a synergistic effect (Arruda et al. 2022).

However, several aspects must be considered to guarantee the desired effects, including the polymer used, the composition and concentration of the EO, the desired activity, and the characteristics of the food to be packaged (Arruda et al. 2022). In specific situations, the sensorial aspects of EOs can carry a positive interpretation for consumers due to their harmony with packaged food (Moraczewski et al. 2020; Jafarzadeh et al. 2021). Nevertheless, in other cases, the presence of EOs directly incorporated into films can negatively affect consumer perception (Noori et al. 2018; Arruda et al. 2022), requiring other incorporation strategies.

In this sense, nanotechnology has been extensively studied to improve the application of EOs in food packaging to overcome limitations such as low solubility, high instability, and degradability (Bora et al. 2020; Alfei et al. 2020). In the following section, this topic is discussed in more detail.

14.3 Carrier Systems: Nanotechnological Approaches Versus Industrial Application of Essential Oils

The field of nanochemistry research has shown great progress in the development of novel nanocarriers as potential active compound delivery systems. As mentioned, nanomaterials have at least one dimension in the range between about 0.1 to 100 nm, which differs from the bulk material, resulting in novel characteristics. Nanotechnological materials have been prepared by the top-down approach, which involves the breakdown of materials, or through "bottom-up" forms in which nanostructures and nanomaterials are synthetized through the utilization of supramolecular and biomimetic materials. Both approaches have interfaces with biology and biomimetic chemistry, engendering the field of nanobiology (Steed et al. 2007).

The nanocarrier materials offer many advantages such as (1) improving pharmacokinetics and biodistribution of active agents due to a higher ratio of surface area to volume; (2) diminishing toxicity by their preferential accumulation at the target site, facilitating intracellular delivery; and (3) improving compound stabilization (Steed et al. 2007; Pardakhty and Moazeni 2013; Moghassemi and Hadjizadeh

2014). Therefore, the use of nanotechnology to develop EOs-based preservatives may increase their effectiveness in food packaging (Abdalkarim et al. 2021; Ahmed et al. 2022; Arruda et al. 2022).

There are distinct systems that can be used as carriers and their forms are important for the application as followed by the discussion.

14.3.1 Nanoemulsions

Despite the huge interest in the use of EOs by the food industry, some issues still need to be solved. To overcome those disadvantages mentioned in previous sections, the nanoemulsions (NEs) can be explored. NEs are emulsions with droplet sizes around 100 nm, in which the dispersed phase is oil, and the continuous phase is water (O/W). These two immiscible phases are kinetically stabilized by a surfactant. Some characteristics of NEs, such as high surface area per unit volume, higher kinetic stability, optically transparency, and increase of bioavailability of EOs (Safaya and Rotliwala 2020) make these nanomaterials a great choice for a lot of applications in food production, especially in food packing.

NEs can be prepared by high-energy methods, including high-pressure homogenization, ultrasound emulsification and microfluidization, or low-energy methods, such as spontaneous emulsification, phase inversion temperature, phase inversion composition, and emulsion inversion point. Each method has advantages and disadvantages. In general, low-energy methods are easy to scale up to industrial production, however, requiring high concentrations of surfactants. On the other hand, the high-energy methods are less suitable for industrial scale compared to low-energy methods and, due to their higher energy requirements, are more expensive (Singh and Pulikkal 2022).

Although some issues still need to be improved, several studies have shown great potential for the application of NEs in food packaging. Functional gelatin-based composite films incorporated with oil-in-water lavender essential oil nanoemulsion were effective for cherry tomatoes preservation. The films exhibited strong antibacterial activity against *Staphylococcus aureus*, *Escherichia coli*, and *Listeria monogytogenes*. Additionally, the films present good UV light barrier and heat-sealing properties (Sun et al. 2021).

Another study, with *Trachyspermum ammi* essential oil nanoemulsion (TOEN) loaded in alginate-based edible coating, evidenced that TOEN prevented the growth of *L. monocytogenes* in turkey fillets even after 12 days of cold storage. The study comparatively investigated TOEN in the forms of emulsion and NE against inoculated *L. monocytogenes* in turkey fillets for 12 days of storage at 4 ± 1 °C, with NE being very effective (Kazemeini et al. 2021).

Studies also have proved that NEs can be successfully applied in food coating. Carnauba NE-based coatings improved shelf life and maintained papaya fruit quality during storage by retarding firmness loss, and color changes, also reducing respiration rates, resulting in delayed ripening. Furthermore, the incorporation of ginger essential oil in the coating presented some positive effects on fungal disease control (Miranda et al. 2022).

The influence of cumin EO NE (COEN) used as a brined solution on the quality of white soft cheese was reported (El-Sayed and El-Sayed 2021). *S. aureus, Bacillus cereus, Pseudomonas aeruginosa, E. coli, L. monocytogenes, Salmonella* Typhimirum*, Yersinia enterocolitica, Aspergillus niger,* and *Aspergillus flavus* were significantly inhibited by COEN. In cheese preserved with 1% COEN, the counts of psychrotrophic, yeasts and molds were not detected after 60 days of storage.

The antimicrobial mechanism of action of EOs NE is related to the cell membrane. The high surface area of NE facilitates the interaction of cell membranes and EOs. Thyme EO NE (TEON) prepared by ultrasonication promoted membrane disintegration with massive leakage of cytoplasmatic inclusion on *E. coli* 0157:H7 and *S. aureus*. The bacterial death was associated with modifications in fatty acid composition after TEON exposure (He et al. 2022).

Frank et al. (2018) developed alginate-based films incorporated with cinnamon EO NE (CEO-NE) to assess antimicrobial potential. These films based on CEO-NE/alginate showed antibacterial activity against Gram-positive and Gram-negative bacteria and may be useful in obtaining active packaging against the proliferation of bacteria and preservation of fresh foods.

In another example, Souza et al. (2021) developed thermoplastic starch (TPS) films containing Pickering emulsions stabilized with nanocellulose of different EOs (ho wood, cardamom, and cinnamon). All obtained films showed lower crystallinity than pure TPS, high thermal stability, and lower water vapor transmission rate.

14.3.2 Polymeric Nanomaterials

14.3.2.1 Nanoparticles

Polymeric nanoparticles (PNPs) are formed by polymers, which can be of natural, semisynthetic, or synthetic origin, nontoxic, biodegradable, biocompatible, and responsible for carrying bioactive compounds. PNPs are particles with a size ranging from 1 to 1000 nm. Some of these PNPs can adsorb bioadditive compounds on the surface of the polymeric core or can carry these compounds through entrapment. The term “nanoparticle” refers to nanocapsules and nanospheres, which differ in structural organization and composition, as shown in Fig. 14.2 (Zielińska et al. 2020; Lima et al. 2022).

Nanocapsules are reservoir-type systems that have a polymeric shell arranged around an oil core. The EO (or another bioactive compound) may be contained in the core and/or adsorbed to the polymeric wall. The nanospheres are of the matrix type and do not have oil in their formulations. Despite being formed by a polymeric matrix, it is not possible to identify a differentiated nucleus. In this case, the bioactive constituents can be homogeneously dispersed or solubilized within the polymeric matrix (Singh and Lillard 2009; Jawahar and Meyyanathan 2012; Ivanova et al. 2020).

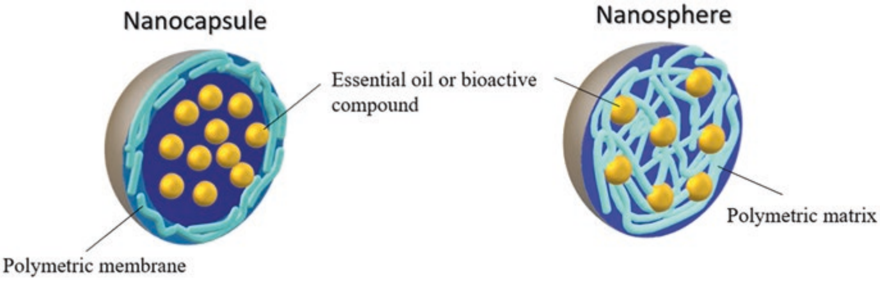

Fig. 14.2 The two main types of polymeric nanoparticles: nanocapsule and nanosphere

PNPs are used as carriers of proteins, antigens, and drugs for topical, oral, injectable administration, and bioadhesive systems, being the main component of controlled release systems in the form of implants (Pudlarz and Szemraj 2018; Lima et al. 2022). One of the main advantages of PNPs encompasses the carrying of bioactive compounds that can have controlled release. In addition, PNPs can protect bioactive constituents from the environment, resulting in better biological activity, therapeutic index, and bioavailability (Begines et al. 2020; Zielińska et al. 2020).

PNPs can be used in the area of pollution control and environmental technology, in conductive materials, sensors, biotechnology, medicine, the food industry, and others. One of the highlights of the use of PNPs is the application in active materials for food packaging, which can provide a barrier to gases, humidity, temperature, and stability (Kumar et al. 2021a; Gupta et al. 2022).

14.3.2.2 Nanofibers

Along with PNPs, nanofibers (i.e., fibers with nanometric diameter) have been widely used to provide a suitable vehicle system to deliver EOs. As well as PNPs, the nanofibers can supply naturally derived chemical compounds, such as EOs, in a controlled manner and prevent their degradation. However, the main characteristic of these 1-D structured nanomaterials is their large surface-to-volume ratio compared to other nanostructures (Partheniadis et al. 2022).

Despite the large number of procedures that can be applied to nanofibers manufacturing (e.g., freeze-drying, self-assembly, phase separation, template synthesis, and spinneret-based engineered parameters), so far, the most widely applied to bioactives' delivery is electrospinning. This technique is based on the application of a strong electric field, with high voltages being used during the nanofibers produce (c.a.,1–30 kV) (Hu et al. 2014). The core material (EO) is located inner the nanofiber, covered by the shell material (polymer), but the dispersion of EO on the nanofiber depends on which generation method is employed during the fibers' manufacturing: nanoemulsion, conventional, and coaxial electrospinning (Partheniadis et al. 2022) (Fig. 14.3).

Furthermore, considering nanofibers produced from electrospinning, some advantages may be acquired, such as (1) it can produce very thin fibers (of few nanometers in diameter) with large specific surface areas (high surface-to-volume

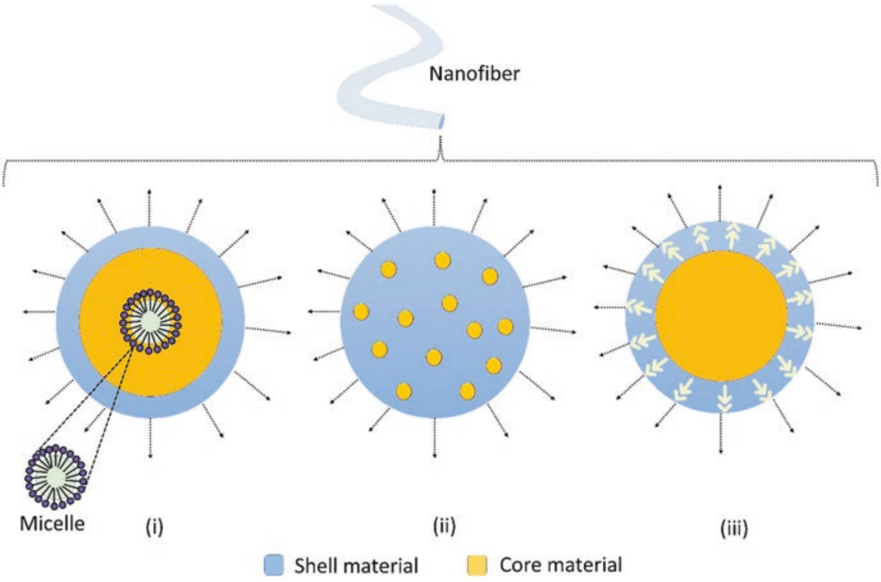

Fig. 14.3 Schematic illustration of shell and core materials' disposition into nanofibers, depending on electrospinning procedure: (1) nanoemulsion, (2) conventional and (3) coaxial

ratio) and large interstitial spaces (voids), (2) it can provide ease of functionalization, (3) the produced electrospun nanofibers exhibit superior mechanical properties, (4) it is a versatile process, and (5) it is amenable for scale-up (Partheniadis et al. 2020); thus, several studies have been covering the EOs encapsulation by electrospun nanofibers (Rather et al. 2021).

14.3.2.3 Natural Biopolymers Used in Polymeric Nanomaterials Development

Polimeric materials can be employed as the package material perself or as the suport material in nanocarriers. The use of synthetic polymers in food packaging is very practical and cost-effective. The most used are polyethylene, polypropylene, polyvinyl chloride, polyethylene terephthalate, polyurethane, polyamides, etc. These materials have excellent properties concerning chemical and physical resistance, sealing qualities, mechanical properties, lightness, and durability. However, most of the plastic is discarded and can already be found in a widespread way: in the soil, in the oceans, in drinking water, in the air, and in the bodies of animals and human beings (Nielsen et al. 2020). Worldwide, only 18% of synthetic polymers are recycled, 24% are incinerated and the rest, 58%, enter the natural environment or are landfilled, potentially accumulating with long persistence (Geyer et al. 2017; Chamas et al. 2020). Thus, employing natural materials in the development of polymeric nanocarrier systems may act as a great strategy, especially concerning food packaging development.

The main biodegradable polymers of natural origin that are currently applied at nanozised carriers to EOs are cellulose, chitosan, alginate, carrageenans, agar, starch, proteins, and pectins. We can also cite other biopolymers that have been currently studied on the production of PNPs, such as PLA (polylactic acid), PHA (polyhydroxyalkanoate), PCL (poly-ε-caprolactone), PGA (polyglycolide), PVA (poly vinyl alcohol), and PBS (polybutylene succinate). The advantages of these polymers are that they are easily hydrolyzed by enzymes and are environmentally friendly (Teixeira-Costa and Andrade 2022; Zheng et al. 2022).

Cellulose and Derivatives

Cellulose is the most abundant and renewable biopolymer found in nature, which plays a structural role in the cell walls of higher plants, aquatic species, and microorganisms (e.g., bacteria, fungi, and algae) (Favier et al. 1995; Andriani et al. 2020). Cellulose can be extracted from materials such as wood, cotton, straw, and hemp. This polymer is a polysaccharide formed by linear D-glucose chains joined by β(1–4) bonds, which do not exist in the form of molecules in nature but crystallize due to intermolecular hydrogen bonds (Klemm et al. 2005).

Cellulose-based packaging presents a barrier to water vapor, air, and oxygen. Hydrogen bonds within and between cellulose fibers are considered to be an important factor in determining their chemical and physical properties. Furthermore, some derivatives with greater solubility and processability can be obtained from cellulose (Liu et al. 2021). For example, cellulose acetate is one of the most widely used cellulose derivatives, being obtained from the reaction of cellulose with acetic acid or acetic anhydride, catalyzed by sulfuric or perchloric acid, resulting in a molecule with one or more -OH groups of glucose replaced by an acetate group (Fig. 14.4).

Cellulose acetate can be dissolved in acetone and has been used as a food packaging material, although it has a relatively high permeability to water and oxygen. In addition to forming films, cellulose can also be processed into nanocrystals and nanofibrils, which are widely used as fillers in other polymers. Cellulose nanocrystals are obtained by acid or enzymatic hydrolysis and cellulose nanofibers are obtained by mechanical homogenization of pure cellulose fibers, which have previously been chemically or enzymatically treated. Cellulose nanofibers have properties of some fluids and gels, being considered a pseudo-plastic (Romão et al. 2022).

Bacterial nanocellulose is a β-D-glucopyranose-type biopolymer produced by specific types of bacteria *(Agrobacterium, Rhodobacter, Komagataeibacter, Alcaligenes, Achromobacter, Aerobacter, Pseudomonas, Rhizobium, Sarcina*, and

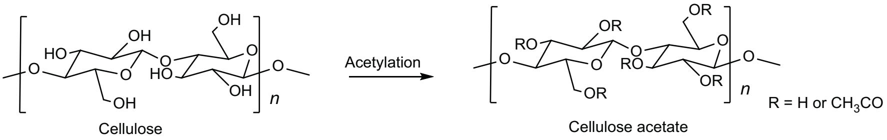

Fig. 14.4 Scheme of the cellulose acetylation reaction to obtain cellulose acetate

Dickeya). The material is secreted extracellularly as a network of randomly distributed nanofibers, linked in a highly cross-linked three-dimensional fashion with a unique, porous matrix. Compared to plant cellulose, the advantages of bacterial nanocellulose are: they are physically and mechanically more resistant, purity, they are produced free of hemicelluloses and lignin, they can be shaped during cultivation into three-dimensional structures, they have a high degree of crystallinity, high porosity, excellent water retention capacity and biocompatibility. Thus, it is a material of interest not only to the food industry but also to several other sectors (e.g., electronics, pharmacy, medicine, cosmetics, and textiles) (Sharma and Bhardwaj 2019; Ludwicka et al. 2020).

Cellulose has great sensorial advantages (e.g., non-pronounced sensorial aspects, such as aroma and color, good appearance) that reflect on their applicability to food packaging development, being also a great option to carry antimicrobials and antioxidants, for example, by nanoencapsulating bioactive compounds for their controlled release in the matrix (Gupta et al. 2022; Silva et al. 2020).

Razavi et al. (2022) used cinnamon EO (CEO) nanocapsules at concentrations of 0.03–0.48% (v/w), using fish gelatin as the main polymeric phase (3% w/w), stabilized using bacterial cellulose nanocrystals at 0.06% (w/w). The obtained film effectively provided a great barrier against water vapor, oxygen, and carbon dioxide, also showing interesting potential for increasing the shelf life of mollusks, crustaceans, vegetables, and fruits.

Chitosan

Chitosan is one of the most studied biopolymers with antimicrobial properties for food packaging, due to its ability to form films and coatings. Chitosan exhibits antimicrobial activity against foodborne bacteria, yeasts, and a wide variety of filamentous fungi, being more active against yeasts (Díaz-Montes and Castro-Muñoz 2021).

Chitosan is obtained from chitin by a partial deacetylation reaction (Fig. 14.5). Chitin, the second most abundant polysaccharide in nature after cellulose, is found mainly in the exoskeleton of crustaceans, crabs, shrimp shells, and insects. The structure of chitin is similar to that of cellulose, with the difference being the presence of the acetamide group (-$NHCOCH_3$), located on carbon 2, meanwhile, in cellulose, there is a hydroxyl (-OH) in this position. Chitin is composed of (1,4)-β-2-acetamido-2-deoxy-D-glucopyranose units and small amounts of (1,4)-β-2-amino-2-deoxy-D-glucopyranose units, and chitosan is composed of

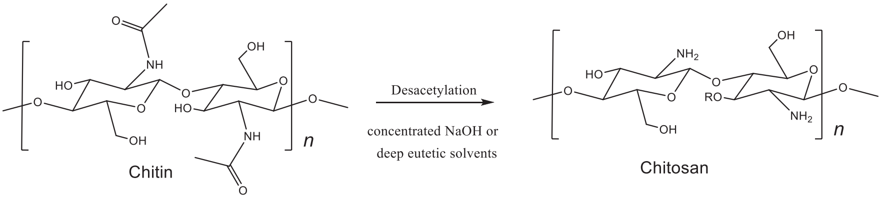

Fig. 14.5 Scheme of the chitin deacetylation reaction to obtain chitosan

N-acetyl-D-glucosamine and deacetylated D-glucosamine units (Jin et al. 2021; Kozma et al. 2022).

Chitin is insoluble in many organic and inorganic solvents, while chitosan is soluble in slightly acidic solutions, such as formic, acetic, malic, citric, tartaric, or lactic acid, among others, due to the presence of the amino group (-NH_2) located in the C-2 from D-glucosamine (Melro et al. 2020). This NH_2 group can be protonated in an acid medium, promoting the polycationic behavior of chitosan chains, which can influence its physical-chemical, mechanical, and biological properties. Furthermore, the polycationic chain of chitosan can electrostatically interact with polyanionic biopolymers, such as sodium alginate, and amphiphilic proteins. The protonation of amino groups provides the interaction of chitosan with microorganisms' cell membranes that are mainly negatively charged, altering their permeability and metabolism, chelating trace metals and spores of microorganisms, thus leading to cell death and inhibiting microbial growth (Li et al. 2020).

Chitosan can be chemically modified, due to the presence of -OH and -NH2 groups, with the introduction of other functional groups that aim to enhance its solubility and antimicrobial activity (Aljawish et al. 2015).

Gasti et al. (2022) developed chitosan/pullulan (CS/PL) nanocomposite films loaded with clove EO (CEO):chitosan-ZnO hybrid PNPs. Incorporation of the active PNPs led to higher film hydrophobicity, higher oxygen barrier, higher UV light blocking ability, higher tensile strength, and higher water vapor barrier compared to pure CS/PL film by the inclusion of PNPs. There was an increase in antioxidant activity and relevant antibacterial activity against *P. aeruginosa*, *S. aureus* and *E. coli*. Additionally, the obtained films showed potential to poultry meat's shelf life extension by up to 5 days when stored at 8 ± 2 °C.

Ferreira et al. (2021) prepared thermoplastic starch (TPS) films filled with chitosan PNPs containing ho wood and cinnamon EOs. The films containing 1 and 3% w/w of active PNPs showed lower permeability to water. At a concentration of 3% w/w of PNPs, there was inhibition of the bacterial growth of *E. coli* and *Bacillus subtillis*. Applying the active films on the shelf life extension of strawberries, films containing 1 or 3% w/w loaded-PNPs were responsible to inhibit fungal growth, also preventing fruits' weight loss.

Alginates

Alginate is a biopolymer extracted from brown algae such as *Ascophyllum nodosum*, *Laminaria digitata*, and *Macrocystis pyrifera*. In the food industry, alginate is often used as a gelling agent, thickener, and stabilizer (Abka-Khajouei et al. 2022). Alginate is a polysaccharide composed of molecules of β-D-mannuronic acid and α-L-guluronic acid linked by 1,4-bonds, which can be in the form of β-D-mannuronate (M) and α-L- guluronate (G) (Abasalizadeh et al. 2020). These units are randomly distributed in a linear chain, formed by links between M-G, M-M and G-G (Fig. 14.6).

The properties of alginate are influenced by changes in pH, due to the presence of carboxylic groups in the structure at pHs lower than 3.4, that is, below the pKas of M (3.38) and G (3.65) the carboxylic groups become non-ionized (-COOH), so

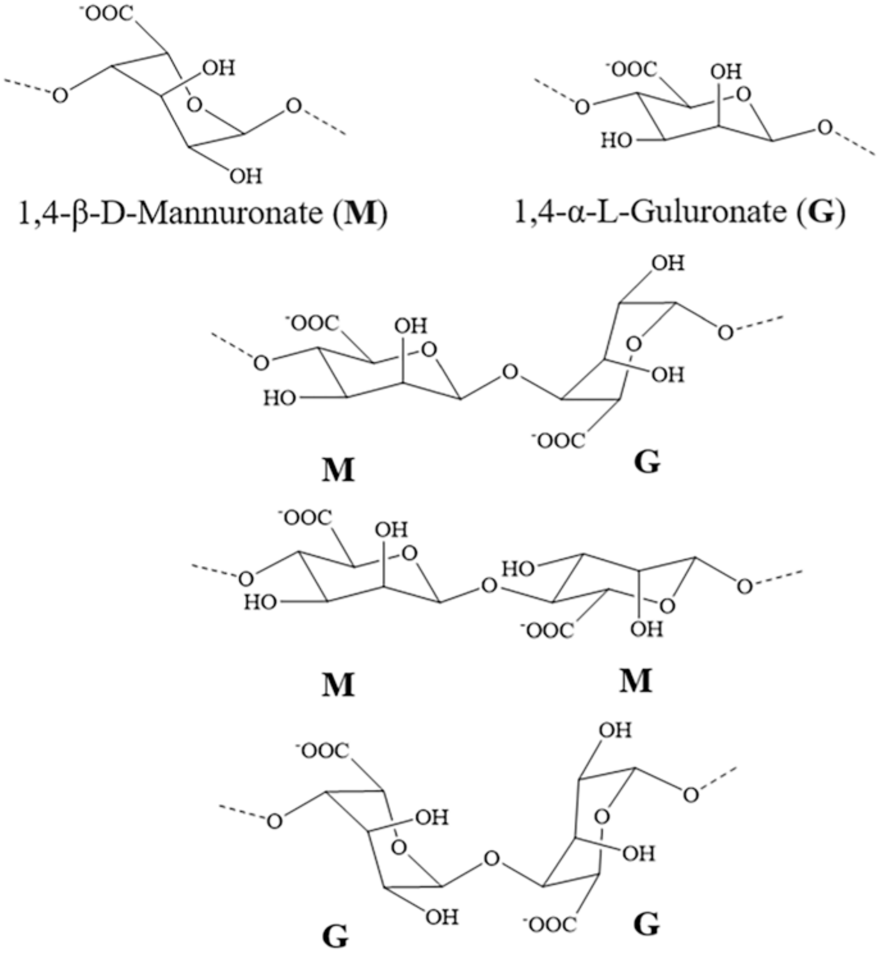

Fig. 14.6 Structures of alginates monomers: 1,4-β-D-Mannuronate (M) and 1,4-α-L-Guluronate (G) and the possible bonds between them forming M-G, M-M and G-G

the alginate is insoluble. In pH conditions greater than 4.4, the carboxylic groups become ionized (-COO⁻) and the expansion of the alginate chain occurs due to the electrostatic repulsion of negative charges, with the apex at higher pH values, in this case, pH above 7.4 (Agüero et al. 2017; Teixeira-Costa and Andrade 2022).

Alginate can absorb cationic ions, due to its anionic nature, and can therefore be used to remove pollutants in an aqueous solution. In addition, alginate can absorb dyes and can be useful in the food packaging industry, which can prevent food contact with dyes (Zhang et al. 2022b; ALSamman and Sánchez 2022). This interesting property also allows alginate to be a potential material in the development of PNPs, especially along with other polymeric material. Some examples of alginate-based PNPs are alginate/cashew gum nanoparticles for *Lippia sidoides* EO encapsulation (de Oliveira et al. 2014) and chitosan/alginate nanocapsules as carrier materials for turmeric (*Curcuma longa*) and lemongrass (*Cymbopogon citratus*) EOs (Natrajan et al. 2015).

Carrageenans and Agar

Carrageenans are anionic sulfated linear polysaccharides obtained from red marine algae of the Rhodophyceae family. Carrageenans contain alternating units of 1,3-β-D-galactopyranose and 1,4-α-D-galactopyranose (Chauhan and Saxena 2016; Lee 2020). The most used types in the industry are the κ-(kappa), ι-(iota), and λ-(lambda) carrageenans (Fig. 14.7), this classification is according to their molecular structures, such as the sulphation arrangements, as well as the presence or absence of 3,6-anhydro-α-galactopyranose into the 1,4-linked α-galactopyranose unit (Lin et al. 2022a; Rupert et al. 2022).

The κ-(kappa) and ι-(iota) carrageenans present 25–30% and 28–30% of sulfate content, respectively. These carrageenans can be used as a design for edible films and coatings, due to their high hydrophilicity, water solubility, and structural ability to form a helical assembly (Jancikova et al. 2020; Teixeira-Costa and Andrade 2022), but also present great prospects on the development of natural PNPs.

Fani et al. (2022) designed κ-carrageenan (κC) PNPs containing the main constituent of citrus EO, D-limonene, performing one-step electrospray synthesis. The D-limonene-κC PNPs of spherical morphology showed excellent levels of thermostability and photostability. Toxicological studies of the material must be carried out

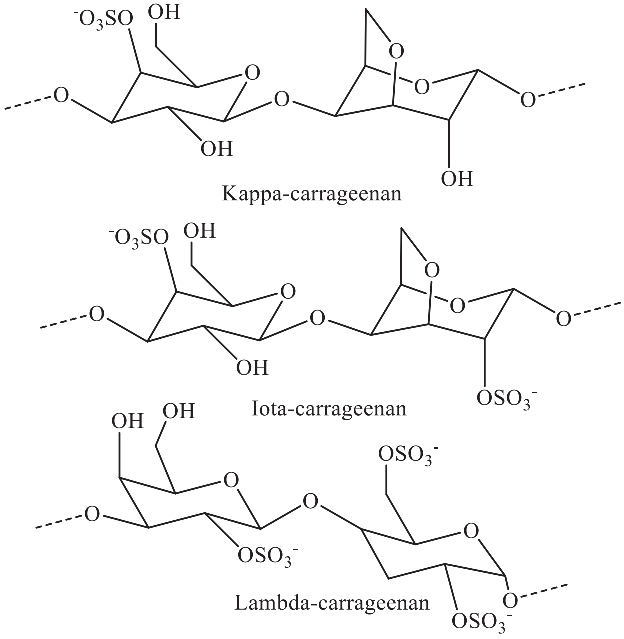

Fig. 14.7 Structures of κ-(kappa), ι-(iota) and λ-(lambda) carrageenans

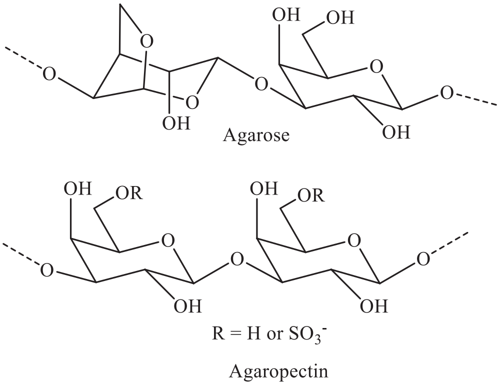

Fig. 14.8 Structures of agar components

to verify its potential application in the release of bioactive molecules for drugs or foods.

On the other hand, agar is a gelatinous polysaccharide derived from red algae, of the class Rhodophyceae, the main sources of this biopolymer are *Gracilaria* sp. and *Gelidium* sp. Agar has two components in its composition: agarose and agaropectin, which structures are shown in Fig. 14.8.

Agarose is the gelling portion of agar, which is neutral and is made up of disaccharide units formed by galactose and 3,6-anhydro-α-L-galactopyranose. Agaropectin is the non-gelling portion, which contains a sulfate group, D-glucuronic acid and pyruvic acid in its structure (Martínez-Sanz et al. 2019; Mostafavi and Zaeim 2020).

Carrageenans and agar have been used to make biodegradable packaging films (i.e., together, these materials can improve their negative aspects, such as the low mechanical properties of agar and high water solubility of carrageenan). Their properties can be also successfully used to develop EOs' PNPs, helping to overcome EOs limitations on food packaging applications.

Starch

Starch is a natural polysaccharide used as a carbohydrate reserve in various plants, such as rice, potatoes, soybeans, oats, corn, sweet potatoes, yams, barley, sago, wheat, and vegetables (Horstmann et al. 2017). The different origins of starch and environmental factors during raw material cultivation can lead to differences in its crystallinity, which will significantly influence its processing properties (Dos Santos et al. 2016; Cornejo-Ramírez et al. 2018).

Starch is composed of two types of polysaccharides: amylopectin (75–80%) and amylose (20–25%) (Fig. 14.9). Amylopectin has α-1,4-glycosidic linkages, and α-1,6-glycosidic linkages, which form highly branched polymers with 24 to 30 glucose residues. Amylose is composed of end-to-end connected α-1,4-glycosidic bonds, which is an unbranched helical structure (Alcázar-Alay and Meireles 2015). This biopolymer is of great importance in the biotechnological and food industries, having as its great advantages: great abundance, low cost, worldwide availability,

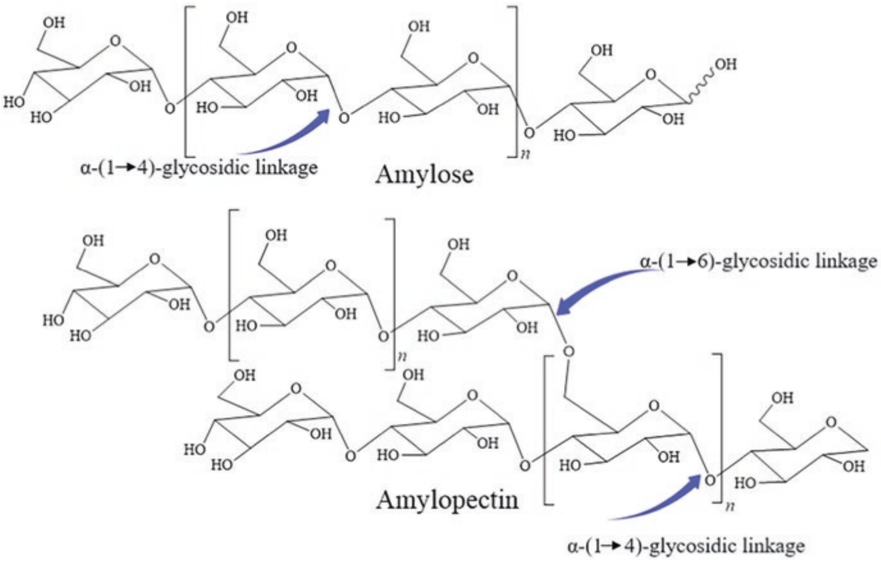

Fig. 14.9 Structures of starch components

biodegradability, good processability in conventional plastic processing equipment, and edibility (García-Guzmán et al. 2022).

The high amylose content attributes better properties to starches in terms of strength, elongation capacity, and flow properties. One of the disadvantages of films made from starch is that they are generally brittle due to retrogradation, a phenomenon that occurs when amylose in the solution can crystallize forming two left-handed helices, which are packaged into amorphous types A and B, in this case, on cooling the starch reorganizes into a more crystalline structure (Liu et al. 2022).

To circumvent the problem of retrogradation, mixing starch with water-soluble polymers such as PVA, or modifying and cross-linking the –OH groups can effectively suppress retrogradation, resulting in starch-based polymers that are useful for packaging applications (García-Guzmán et al. 2022).

Starch molecules are highly hydrophilic as they contain many hydroxyl groups, which results in poor water resistance and poor mechanical properties in humid environments. In this case, it is necessary to strengthen the mechanical and barrier properties of starch-based packaging materials (Liu et al. 2022).

One of the strategies is to convert natural starch into thermoplastic starch (TPS) by treatment with plasticizers (glucose, amino acids, urea, glycerol, etc.) under heat and/or shear. Still, TPS has lower mechanical and barrier properties than conventional petroleum-based plastics (Lopez-Gil et al. 2014). The TPS has to be reinforced to obtain homogeneous properties for packaging, which can be done by mixing the surface, modifying the presentation, cross-linking, acetylation, organic and inorganic fillers, and mixing with other polymers. (PVA, PLA, PE, PP, PS, and others) (Mohammadi Nafchi et al. 2013; Tarique et al. 2021).

Wang et al. (2022) developed a film obtained from starch/polyvinyl alcohol (STA/PVA) loaded with oregano essential oil (OEO) adsorbed on microporous starch (MS) to be used in sea bass packaging. The films with the incorporation of OEO-MS obtained better tensile strength, lower permeability to water vapor and oxygen, and exhibited antimicrobial and antioxidant activities. Using such film extended the shelf life and better preserved the freshness of the sea bass.

Proteins

Natural proteins can be used in food packaging development. Protein-based biopolymers have low oxygen permeability and good mechanical properties, which may be superior to those presented by biopolymers based on polysaccharides and lipid compounds (Hammam 2019; Kumar et al. 2021a). Along with this usage as the polymer matrix in food packaging manufacturing, proteins also have been studied as shell material in PNPs carriers for EOs.

Proteins naturally exist as fibrous or globular, the former serves as a structural material for animal tissues and are insoluble in water. Globular proteins are present in living systems, being soluble in water or aqueous solutions of acids, bases, and salts. Fibrous proteins are shown to be extended and with chains associated with each other by hydrogen bonding to form fibers. Globular proteins fold into complex spherical structures held together by hydrogen bonds, ionic, hydrophobic, and covalent forces (disulfide bonds) (Basiak et al. 2017; Senthilkumaran et al. 2022)

The physical and chemical properties of these proteins depend on the location and amount of amino acid residues along the polypeptide chain. Collagen is a fibrous protein that can be used in the production of edible films. Among the globular proteins, soy protein, whey protein, mung bean protein, wheat gluten, and corn zein have been studied for use in food packaging (Kumar et al. 2022).

Proteins are not completely hydrophobic, due to the predominantly hydrophilic amino acid residues, there is a limitation in their moisture barrier property. For example, to obtain edible films of proteins with low permeability to water vapor, it is necessary to add hydrophobic constituents (Huo et al. 2018; Mihalca et al. 2021; Chaudhary et al. 2022).

Gelatin is a high molecular weight polypeptide (composed of 19 amino acids) obtained from the hydrolysis of collagen. Gelatin has a high content of proline, hydroxyproline, and glycine, having a mixture of unfolded single and double chains with a hydrophilic character (Fig. 14.10). Gelatin has excellent film-forming properties, reasonable barrier and mechanical properties, and high flexibility. One of the great advantages of film gelatine is that it intrinsically has antimicrobial and antioxidant activity (Ramos et al. 2016; Ma et al. 2018; Lu et al. 2022).

The limitation of the use of gelatin as a film is the issue of its high sensitivity to humidity, in contact with water, they swell, crack and dissolve. To improve the water barrier of gelatin-based materials, it is common to mix them with other materials, such as fibers and other polymers (Said and Sarbon 2022).

For example, Angourani et al. (2022) successfully developed plant-protein-based PNPs containing Rosemary (Rosmarinus officinalis) EO, which can be applied not only in food packaging but also in other industry sectors, such as the

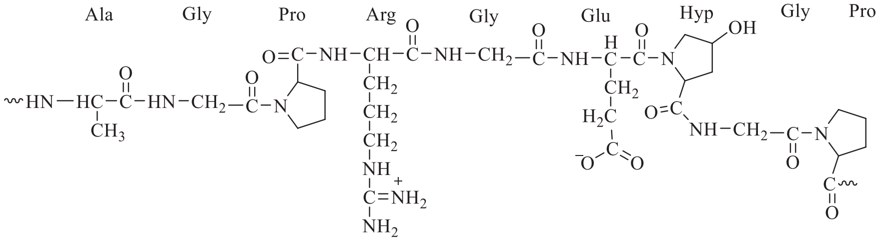

Fig. 14.10 Gelatin structure

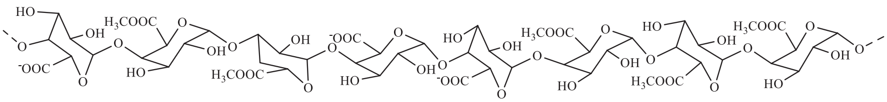

Fig. 14.11 Structure of a pectin molecule. Residues of rhamnose, galactose, arabinose, and xylose are not included. Adapted from Alkorta et al. (1998)

pharmaceutical. Furthermore, proteins can also be applied in nanofibers. Using the electrospinning technique, a study was carried out with gelatin nanofibers with encapsulated angelica essential oil (AEO) in order to obtain a material with the potential to be used in active packaging. With the addition of AEO, there was an improvement in hydrophobicity, an increase in antioxidant activity and an antibacterial effect against Gram-positive and Gram-negative bacteria. In addition, there was no effect of cytotoxicity (Zhou et al. 2020).

Pectin

Pectin is a natural and abundant heteropolysaccharide, present in plant cell walls, composed of β-(1–4)-D-galacturonic acid, galactose, arabinose, and rhamnose (Fig. 14.11). The pectin chain can have different degrees of methyl esterification, an important factor in its film-forming and gelling properties (Ravishankar et al. 2012; Chandel et al. 2022).

Pectin has excellent gelling ability in acidic solutions, which is why it is widely used as an additive in the beverage and food industry. Pectin is used in ice cream, dairy drinks, and jellies as a thickening agent or colloidal stabilizer. Pectin can be used in the production of films for food packaging, as it is edible, can form gels and films, and is biocompatible and biodegradable (Jiang et al. 2021; Freitas et al. 2021).

The use of pectin in packaging has major limitations, as this biopolymer is highly hydrophilic, fragile, and has low tensile strength. To overcome these obstacles, other biopolymers must be added to the pectin matrix to improve its mechanical properties and reduce its flexibility (Almasi et al. 2020; Huang et al. 2021). For example, D-limonene (i.e., an important EO's component) was successfully nanoencapsulated by pectin and whey protein concentrate (Ghasemi et al. 2018; Rehman et al. 2019). The authors investigated different pectin (0.5, 0.75, 1%) and whey protein concentration (4, 6, 8%), in 3 distinct pH values (3, 6, 9), determining the

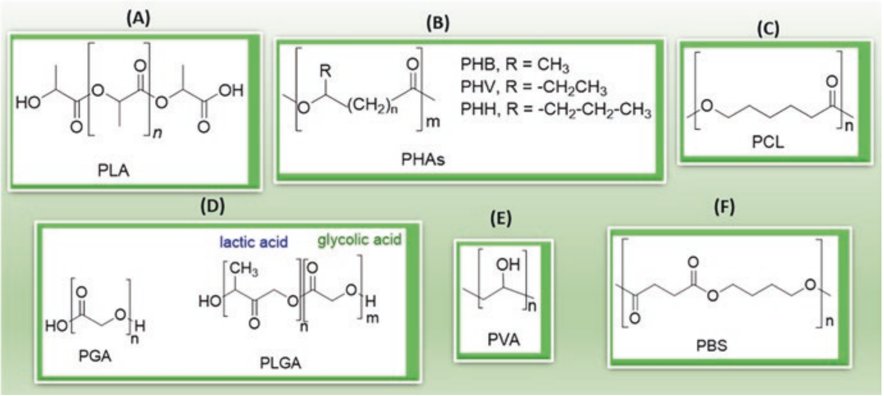

Fig. 14.12 Structures of biopolymers. (**a**): PLA (polylactic acid); (**b**): PHAs (polyhydroxyalkanoates) - poly(3-hydroxybutyrate) (PHB), polyhydroxyvalerate (PHV) and polyhydroxyhexanoate (PHH); (**c**): PCL (polycaprolactone); (**d**): polyglycolic acid (PGA) and poly(lactic acid-co-glycolic acid) (PLGA); (**e**): polyvinyl alcohol (PVA); and (**f**): poly(butylene succinate) (PBS)

stability of the product. The results indicated that optimum formulation regarding stability, color, and viscosity was obtained with 1% pectin and 4% WPC, with pH 3.

Other Biopolimers

Some relatively novel bioplastics have been used in food packaging development. These materials have been developed to obtain sustainable packages and replace synthetic polymers. Examples of these polymers include polylactic acid (PLA), polyhydroxyalkanoate (PHA), polycaprolactone (PCL), polyglycolic acid (PGA), polyvinyl alcohol (PVA), poly lactic acid-co-glycolic acid (PLGA), and poly butylene succinate (PBS). The structures of these polymers are shown in Fig. 14.12.

PLA

Polylactic acid (PLA) is an aliphatic polyester synthesized from lactic acid monomers, which can be obtained from the fermentation of renewable materials such as corn and waste cellulosic materials. PLA has important properties that make it attractive to be used as packaging, such as low permeability to gases and water, high transparency, and moderate tensile strength (Balla et al. 2021).

Some studies have already investiaged PLA as nanocapsules material for EO. Antonioli et al. (2020) nanoencapsulated lemongrass EO in PLA nanocapsules and evaluated their antifungal activity in vitro and in vivo. They have shown in vitro antifungal activity against *Colletotrichum acutatum* and *Colletotrichum gloeosporioides* with a MIC dosage of 0.1% (v/v) for both phytopathogens. The in vivo were performed with postharvest apples, and the ones treated with nanoencapsulated EO showed bitter rot lesions three times smaller than the ones treated with non-encapsulated EO, or in comparison to the apples in positive control.

PHAs

Polyhydroxyalkanoates (PHAs) are thermoplastic, biodegradable polymers produced by microorganisms. Currently, more than 100 types of PHA are known, the most widespread being polyhydroxybutyrate (PHB), followed by poly(3-hydroxybutyrate) (PHB), polyhydroxyvalerate (PHV), and polyhydroxyhexanoate (PHH). The use of encapsulation in PNPs based on polyhydroxyalkanoate (PHA) is being explored in obtaining biodegradable material for obtaining active packaging (Gadgil et al. 2017; Kumar et al. 2021b).

Zheng et al. (2022) encapsulated Mexican oregano EO in polyhydroxybutyrate (PHB) and poly-3-hydroxybutyrate-co-hydroxyhexanoate (PHB-HHx) and studied in vitro release in simulated food media. Both nanosystems containing EO showed antimicrobial activity against *Micrococcus luteus*, PNPs based on PHB-HHx were more efficient than pure EO. The use of PLA/PHA containing oregano EO was efficient in active packaging for puffer fish fillets.

PCL.

PCL (poli-ε-caprolactona) is obtained by ring-opening polymerization of ε-caprolactone or polycondensation of 6-hydroxyhexanoic acid. PCL has lower tensile strength than PLA and has greater permeability to water vapor and oxygen than other biopolymers. To be used in active packaging, PCL is usually blended with other biopolymers (Shaikh et al. 2021).

Concerning its application as a nanocarrier material, PCL was successfully used for the development of nanofibers aiming active packaging (Ferreira et al. 2021). *Lavandula luisieri* EO was successfuly incorporated in the form of a nonwoven substrate for museological packaging, but we can state that it can be potentially extended for further studies as food packaging.

PGA and PLGA

PGA (polyglycolide acid) is obtained by direct polycondensation of glycolic acid, ring-opening polymerization of glycolide (ROP), and solid-state polycondensation of halogen acetates. This polymer has high biodegradability, similar to cellulose. The greatest utility of PGA is as copolymers, such as poly(lactic-co-glycolic acid) (PLGA) obtained in 88:12, which means that the polymer is made of 88% lactic acid and 12% glycolic acid (Ayyoob et al. 2017).

Zhu et al. (2019) encapsulated thymol (a constituent of oregano and rosemary essential oil) using PLGA microparticles, obtaining spherical and smooth microparticles, with optimal efficiency when encapsulating 20% (w/w) of thymol. These thymol-loaded microparticles showed relevant antibacterial activities against *S. aureus* and *E. coli*. Thus, PLGA microparticles loaded with thymol have the potential for use as preservation additives in foods.

PVA

PVA (polyvinyl alcohol) is a synthetic biopolymer. It is synthesized by the polymerization of polyvinyl acetate and then the subsequent hydrolysis of the acetate group. PVA has high polarity and hydrophilicity, thus, it is usually used in mixtures with more hydrophobic constituents to obtain material to be used in active packaging (Moulay 2015).

Lamarra et al. (2020) produced PVA electrospun nanofibers and chitosan-based emulsions functionalized with cabreuva EO extracted from the wood of *Myrocarpus fastigiatus*. To overcome the low solubility of the EO and to protect it, the authors proposed a two-step process, emulsion formation compound by chitosan and PVA, and subsequent ionic cross-linking with sodium citrate. Nanofibers were also manufactured through the electrospinning method. The electrospun nanofibers exhibited the ability to be an effective carrier of the cabreuva EO and the capacity of controlling the compound release that proved an effective activity against broad spectra of microorganisms (*Candida albicans*, *E. coli*, *S. aureus*, and *S. epidermidis*).

PBS

PBS is a biodegradable polymer that can be obtained by the polycondensation of succinic acid (or dimethyl succinate) and 1,4-butanediol, these monomers can be obtained from renewable or fossil resources (Rafiqah et al. 2021).

Using the PBS/geraniol mixture, solid and porous plaques with antimicrobial activity were obtained, which were inserted into bread packages. The bread preservation period was extended by 5 and 10 days with the insertion of these solid and porous antimicrobial plates, containing 8% by weight of geraniol in the PBS matrix. (Petchwattana et al. 2021).

There is a long way to go in terms of spreading the use of bioplastics. Globally, less than 1% of plastics used are of biological origin. These materials are hydrobiodegradable and despite some of them are still poorly adressed as nanocarriers for essential oils aiming food packaging application, there is a remarkable increase on the interest on this topic (Atta et al. 2022).

14.3.3 Inclusion Complexation: Cyclodextrins Cases

Within the area of supramolecular chemistry, molecular inclusion or inclusion complexes (ICs) are studied, which refer to the confinement of a guest molecule within the cavity of a host molecule, also known as a cage compound, molecular capsule, or molecular container. The interactions between the trusting molecule and the host molecule are noncovalent, they occur through intermolecular forces (Huang and Anslyn 2015). This way, the interaction between the guest and host molecules differ to the encapsulation descibed before.

Cyclodextrins (CDs) have been widely used in supramolecular encapsulation, mainly in the formation of inclusion complexes with volatile compounds, such as EOs (Kfoury et al. 2018). CDs are cyclic oligosaccharides composed of at least six D-glucose units linked by α-1,4 bonds. The production of CDs occurs from starch, by the action of cyclodextrin-α-glucosyltransferase, a transglycosidase responsible for cleaving an α-1,4 bond in amylose and forming the cycle (Wüpper et al. 2021).

The most studied CDs are called α-, β-, and γ-cyclodextrins, which are composed of six, seven, and eight D-glucose residues, respectively. The shape of CDs molecules is similar to a truncated cone, where the outer surface is hydrophilic due to the presence of hydroxyl groups (-OH) and the inner cavity has lipophilic/hydrophobic characteristics. In this way, compounds with hydrophobic characteristics can remain inside the internal hydrophobic cavity of CDs, leading to the formation of inclusion complexes with several hydrophobic substances (Fig. 14.13).

One of the applications of the entrapment of EOs in CDs is to enable and modulate the controlled release of these volatile constituents when incorporated into active food packaging. ICs with basil EO (BEO) and *Pimenta dioica* (PDEO) in β-cyclodextrin (β-CD) were prepared in order to obtain sachets with antimicrobial activity for food preservation. The prepared ICs showed great potential for antimicrobial application and the sachets can be used in packaging for food preservation (Marques et al. 2019b).

Marques et al. (2022b) used garlic essential oil (GEO) complexed or not with β-cyclodextrin, incorporated into a blend formed by cellulose acetate and zein, and added by glycerol or tributyrin as plasticizers, aiming to obtain films for active packaging. The IC addition into films, however, did not ensure antibacterial action, albeit that the IC with GEO, when tested alone, showed activity against both bacteria.

14.3.4 Nanoliposomes

The Greek roots of the word liposome mean “fat body”; however, we can describe them as hollow structures made of phospholipids (Lasič 1992). Liposomes are well-defined by Laouini et al. (2012) as microscopic spherical-shaped vesicles, which consist of an internal aqueous compartment, entrapped by one or multiple

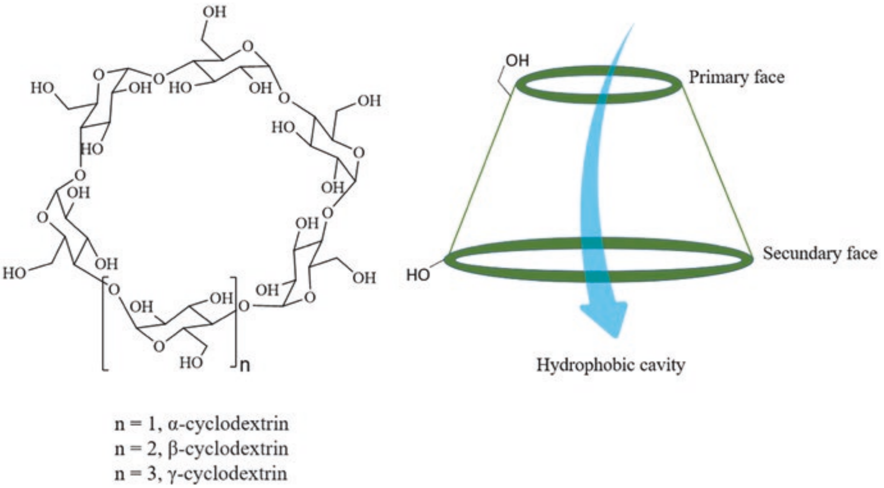

Fig. 14.13 Structures of α-, β-, and γ-cyclodextrins and the truncated cone shape of CDs molecules

concentric lipid bilayers. Because of their characteristics, hydrophilic substances can be encapsulated in the interior aqueous compartments, while lipophilic compounds can be mainly entrapped within lipid bilayers. The similarity to the structure of biological membranes allows liposomes to mingle with the cells and tissues of human bodies (Lasič 1992).

Liposomes were discovered in the early 1960s by the British scientist Alec Bangham, who soon recognized the encapsulated capacity of phospholipids with spherical forms (Lasič 1992). From 1970 to 1980, the potential applications of liposomes in the pharmaceutical and medical industries produced an increasing interest as potential drug carriers (Lasič 1992, Laouini et al. 2012). The versatility of liposomes leads them to a large number of applications including pharmaceutical, cosmetics, and food industrial fields (Lin et al. 2022b; Muñoz-Shugulí et al. 2021; Ohishi et al. 2022).

The phospholipid reorganization is mainly driven by the hydrophobic effect to minimize entropically unfavorable interactions between hydrophobic acyl-chains and surrounding aqueous medium (Lasič 1998, Lasič and Papadjopoulos 1995, Laouini et al. 2012). This effect is further settled by various intermolecular forces such as electrostatic interactions, hydrogen bonding, Van der Waals, and dispersion forces (Israelachvili et al. 1980; Laouini et al. 2012).

The liposomes enhance the compounds' performance through the increase of compounds' solubility and stability, the delivery of encapsulated substances to specific target sites, the sustained compounds release, the high encapsulation efficiency, due to the low toxicity of the system, the substance protection against degradations factors such as pH and light, and the reduction of tissue irritation (Laouini et al. 2012; Guimarães et al. 2021).

To ensure proper liposome performance, batch-to-batch reproducibility and stability of the liposome dispersions have to be established (Laouini et al. 2012).

14.3.4.1 Classical Liposomes Procedures

The liposome is manufactured mainly with a phospholipid molecule, consisting of a polar hydrophilic "head," typically a phosphate group, attached to a long nonpolar hydrophobic "tail" made of two long hydrocarbon chains (Lasič 1992). Generally, liposome composition includes natural and, or synthetic phospholipids (phosphatidylserine, phosphaticylethanolamine, phosphatidylinositol, phosphatidylcholine, also known as lecithin, and phosphatidylethanolamine) (Laouini et al. 2012). Liposome bilayers may also contain other constituents such as cholesterol, hydrophilic polymer conjugated lipids, and water to improve the membrane fluidity and bilayer stability, and reduces the permeability of water-soluble molecules through the membrane (Mozafari 2005; Laouini et al. 2012).

Three attributes interest a scientist preparing a batch of liposomes, which are (1) the chemical composition of the lipid bilayer, (2) the size distribution of the liposomes, and (3) the number of layers in each liposome (Lasič 1992).

There are four classical methods of liposome manufacture. The difference between those methods is the step of drying the liposomes in the organic solvents, followed by their redispersion in aqueous media (Mozafari 2005; Laouini et al. 2012).

Briefly, the classical methods are (1) the Bangham method or hydration of a thin lipid film, in which the phospholipid and cholesterol were dispersed in an organic solvent, removed by evaporation, and then, hydrated with water under agitation (Laouini et al. 2012; Bangham et al. 1962); (2) the reverse-phase evaporation technique, a lipid film is prepared by evaporating organic solvent under reduced pressure with nitrogen and the lipids are redissolved in a second organic phase (diethyl ether and/or isopropyl ether); (3) the solvent (ether or ethanol) injection technique involves the dissolution of the lipid into an organic phase (ethanol or ether), followed by the injection of it into warmed aqueous media; (4) the detergent dialysis technique that produces liposome size ranging of 40–180 nm through lipids solubilization with detergent which is removed by controlled dialysis, forming homogeneous unilamellar vesicles (Laouini et al. 2012).

Bangham method is used to produce multilamellar heterogeneous liposomes with different sizes and shapes such as single bilayer shells around 10 nm diameter (Johnson et al. 1971), or multivesicular liposomal, when incorporated with lavender essential oil, sizing around 0.4–1.3 μm (Varoma et al. 2011). The reverse-phase evaporation technique is used to produce large unilamellar and oligolamellar vesicles (Laouini et al. 2012). While the solvent injection technique forms small liposomes, with narrow distribution, without extrusion or sonification. The ether is removed from the liposomal product through heating while ethanol remains in the product (Laouini et al. 2012, Kryeziu et al. 2022).

Classical techniques require large amounts of organic solvents, which are harmful to the environment and human health, and consume a large amount of energy (Laouini et al. 2012). Therefore, new methods have been developed.

14.3.4.2 New Large-Scale Liposome Technique

An important point in the development of a suitable liposomal system is whether it is possible to prepare, isolate, and characterize a stable particular liposomal formulation on an industrial scale, with clearly defined and reproducible properties, and accessible cost. The manufacturing of liposomes for human and animal use needs a sterilization step such as filtration; however, this technique cannot remove viruses (Brandl et al. 1993, Mozafari 2005).

Heating is a method for the fast production of liposomes that involves the hydration of liposome components and glycerol (3 wt.) in an aqueous medium, followed by heating up to 120 °C (Kikuchi et al. 1991). Glycerol is a water-soluble and physiologically acceptable chemical with the ability to increase the stability of lipid vesicles and does not need to be removed from the product (Laouini et al. 2012). No degradation of the lipids occurred at the above-mentioned temperatures, and no further sterilization procedures are necessary, reducing the time and cost of liposome production (Mozafari et al. 2004).

Another technique is the spray-drying process which is considered to be a fast single-step procedure applied in the nanoparticles' formulation (Chougule et al. 2008). Hence, liposomes were prepared by suspending lecithin and mannitol in chloroform, sonicated, and subjected to spray-drying equipment. The conditions established were inlet and outlet temperatures, airflow rate, and flow rate (Laouini et al. 2012). Liposomal powder formulations are more stable and more appropriate for long-term storage compared with liposomal dispersions (Ingvarsson et al. 2011). Spray drying is less expensive, and less time and energy-consuming compared with freeze drying, making it a suitable strategy to dry liposomal dispersions (Sepúlveda et al. 2021). However, one of the challenges of using liposome spray drying is to avoid membrane bilayer instability caused by heat-induced phase transitions (Van Den Hoven et al. 2012). Furthermore, both heat and high shear forces involved in the process may also induce degradation of the liposomal bilayer structure (Ingvarsson et al. 2011).

Freeze drying process converts a liquid into a solid through three primary steps: (1) freezing, (2) primary drying (sublimation of ice), (3) and secondary drying (desorption of the remaining water under vacuum) (Franks 1998). This process, alone, generates various stresses on liposomes, induced by the dehydration and crystallization of ice. The stresses often lead to phase separation, aggregation, and drug leakage due to liposome disruption (Habib et al. 2022). The lyophilized product spontaneously forms homogenous liposome preparation on the readdition of water. According to Laouini et al. (2012), the lipid/carrier ratio is the key factor that affects the size and the polydispersity of the liposome preparation.

Alternatively, supercritical reverse-phase evaporation allowed aqueous dispersions of liposomes to be obtained through emulsion formation. A given amount of water is introduced into a homogeneous mixture of supercritical carbon dioxide and phospholipids, with sufficient stirring and subsequent pressure reduction (Otake et al. 2001). The vesicles produced by this method are large unilamellar, with diameters ranging from 0.1 μm to 1.2 μm (Laouini et al. 2012). Varoma et al. (2011) produced a gas-saturated solution with particle size between 1.4 μm and 24.8 μm, and poor incorporation efficiency of EOs (3–14.5%). However, Otake et al. (2001) indicate the method as an excellent technique that permits the one-step preparation of large unilamellar liposomes with a high trapping efficiency for both water-soluble and oil-soluble substances.

Another technique is the crossflow injection which has been recently used as a promising novel scalable approach. The ethanol injection method is one of the preferred reported techniques used to produce small unilamellar liposomes, simply and rapidly. The method involves the injection of an ethanolic solution of lipids into a large volume of an aqueous phase, which leads to the rapid formation of vesicles without passing through any intermediate process (Gouda et al. 2021). Upon injection, the unfavorable exposure of lipids to the aqueous medium results in the arrangement and precipitation of lipids at the boundary phase between ethanol and water in the form of bilayer phospholipid fragments (Lasič 1982, 1995). The main parameters influencing the process are injection velocity, stirring rate, injection hole diameter, lipid concentration, osmolality/pH/viscosity of the aqueous phase, the volume of utilized ethanol and its residual amount, dimensions of the reaction vessel, and type of drug to be loaded (Gouda et al. 2021).

Other methods to produce liposomes are the extrusion method, high shear homogenization, sonication, dual asymmetric centrifugation, supercritical antisolvent, rapid expansion of the supercritical solution, calcium-induced fusion, nanoprecipitation, and emulsion techniques. All methods can be checked in Andra et al. (2022)) review and the works of Papahadjopoulos et al. (1990), Holland et al. (1996), Cauchetier et al. (1999), Piacentini et al. (2022), and Sun et al. (2022).

14.3.4.3 Classification

Liposomes can be classified in terms of composition and mechanism of intercellular delivery into five types (Sharma and Sharma 1997; Laouini et al. 2012) such as (1) conventional liposomes, (2) pH-sensitive liposomes; (3) cationic liposomes; (4) immunoliposomes; and (5) long-circulating liposomes.

Otherwise, liposomes were typically classified based on their size and number of bilayers into (1) small unilamellar vesicles or nanovesicles, 20–100 nm; (2) large unilamellar vesicles, >100 nm; (3) giant unilamellar vesicles, >1000 nm; (4) oligolamellar vesicle: 100-–00 nm; and (5) multilamellar vesicles, > 500 nm (Laouini et al. 2012)

New developed types of liposomes, designated as double liposomes and multissecular vesicles were reported (Laouini et al. 2012).

14.3.4.4 Nanosized Liposomes

Nanoliposomes (NLs) are a category of liposomes whose structures form vesicles composed of one or more lipid bilayers and the size does not exceed 100 nm. NLs are stabilized by Brownian motion, which explains the lack of need for surfactants to stabilize these preparations. The methods to produce NLs are almost the same used to produce liposomes; however, the parameters should be well-established to obtain the system at the size required, nanosized (Bondu and Yen 2022).

In reality, NLs suspensions are not perfectly stable and they are susceptible to self-aggregation or fusing into larger vesicles. This so-called coalescence phenomenon should be avoided to preserve the encapsulated active substances and the functional size of the vesicles (Bondu and Yen 2022). Factors can be controlled favoring the stability of NLs such as temperature, size, and the superficial charge of the system.

Transition temperature (TT) is the temperature at which a lipid bilayer loses its organization, becoming initially more fluid and then unstructured (Mozafari et al. 2008). The control of the temperature occurs at two levels. First, during the preparation of the NLs, the temperature must be higher than the TT in order to mix with the active substances to be encapsulated. Secondly, to maintain the integrity of the structure of NLs, the temperature of conservation must be lower than the TT (Bondu and Yen 2022). The TT depends on the type of lipids of the bilayer, including the polarity of the phospholipid heads, the fatty acid chain lengths, the degree of unsaturation, and the ionic strength of the suspension medium (Szoka Jr and Papahadjopoulos 1980; Leserman et al. 1994; Maherani et al. 2011).

Through electrophoretic mobility measures, the surface charge of the lipid droplets can be determined which considerably influences NLs' stability and their ability to repel each other well enough to avoid precipitation during storage. The charge density that determines this electrophoretic mobility and the binding affinity of the different ions is provided by the zeta potential (Bondu and Yen 2022). The composition of phospholipids is therefore important since they are not all charged in the same way. Some phospholipids are neutral such as phosphatidylcholine or phosphatidylethanolamine, and others negative such as phosphatidylserine, phosphatidylglycerol, phosphatidic acid, or diacetylphosphate. Other molecules besides phospholipids can be added such as dioleoyl trimethyl ammonium propane or stearylamine, which are positively charged (Maherani et al. 2011). The combinations in different proportions of these phospholipids can, therefore, significantly change the value of the NLs' zeta potential, influenced by the pH of the system.

Stable NLs have become a subject of growing interest in the field of nanotechnology, particularly given their ability to encapsulate bioactive substances, which makes them interesting as vectors or carriers. Because of their biocompatibility and biodegradability, along with their nano size, nanoliposomes have potential applications in a vast range of fields, including nanotherapy (e.g., diagnosis, cancer therapy, and gene delivery), cosmetics, food technology, and agriculture (Mozafari et al. 2008).

14.3.5 Nanoniosomes

The difference between liposomes and niosomes is slight. The liposomes were very well elucidated until here. Otherwise, niosomes are vesicles composed of non-ionic surfactants, amphipathic compounds with an overall neutral charge, in many cases, cholesterol, or its derivatives. These non-ionic surfactants are cheap and safe for use in biomedicine, for example, as niosomal drug carriers for both hydrophilic and hydrophobic drugs, similar to liposomes. They could be coated by various types of agents such as polyethylene glycol (Van den Bogaart et al. 2007), hyaluronic acid (Lee 2003), antibodies (Allen and Cleland 1980), for specific applications.

While most niosomes are in the nano or sub-micron (colloidal) size range, not many authors used the "nano-niosome" or "nanovesicle" term in their published articles.

Bartelds et al. (2018) approach a complete characterization of the functional properties of niosomes composed of ternary surfactant mixtures compared to liposomes made of saturated or unsaturated phosphatidylcholine and phosphatidylethanolamine-based lipids plus cholesterol. Similar to liposomes, niosomes composed of saturated amphiphiles compounds are more stable and less leaky when tested below the phase TT than vesicles composed of unsaturated components. Taken together, niosomes behave in many aspects similar to phospholipid-based vesicles but they may not provide the right lipid head-group composition for functional membrane transport, that is, the requirement of many transporters for a fraction of anionic headgroups (Lee 2003).

The membrane packing of niosomes is somewhat less dense than that of liposomes. The authors concluded that niosomes exhibited physical chemical properties similar to those of liposomes, albeit that permeability for small ions/solutes is higher, which makes them potentially attractive as drug carriers or delivery systems for all sorts of molecules (Bartelds et al. 2018).

Drug incorporation into niosomes has been accomplished and the authors show that for retention of cargo, the vesicles should not be frozen and thawed. The same factors e.g., size, charge, and stability, should be controlled to use niosomes as encapsulated systems or compound delivery (Bartelds et al. 2018). The niosomes system can be produced through the same techniques than liposomes.

14.3.6 Nano-Cubosomes and Others

Depending on the physicochemical properties of the compounds, different characteristics of the carriers are required. Therefore, there is no perfect delivery system that meets the demands of all kinds of compounds. A wide range of carriers is available, from liquid NE, polymer conjugates, mixed micelles, liposomes, niosomes, and cubosomes (Weiss et al. 2012). The most complex of the liquid crystalline phases formed in lipid/aqueous systems is the cubic phase. One general type of cubic phase is both lipid- and aqueous-continuous, where the lipids form curved, non-intersecting bilayers. The degree of swelling and the curvature of the interfaces as well as the type of cubic phase will be important factors in the entrapment of compounds (Nylander et al. 1996)

Recently, nano-structured cubosomes (NCs) have been used as novel drug nanocarriers owing to their great potential as a substitute delivery system for liposomes (Farag et al. 2022). NCs, particularly composed of binary systems of water and monoolein, are the most investigated systems (Larsson 1983). They can be considered hydrophilic surfactant systems that can self-assemble in the form of a bicontinuous cubic liquid crystalline phase (Bei et al. 2009). They are characterized by their viscous isotropic nature and their large internal surface area (Nylander et al. 1996).

NCs can incorporate lipophilic, hydrophilic, and amphiphilic drugs. Additionally, their lipidic contents are biocompatible, bioadhesive, and digestible (Barauskas et al. 2005a, b). Also, nano-structured cubosomal systems have been explored for diverse pharmaceutical purposes (delivery of enzymes, peptides, analgesics, and antibiotics) (Drummond and Fong 1999; Garg et al. 2007; Almoshari et al. 2022; Farag et al. 2022).

In this context, the aqueous phase behavior of unsaturated monoglycerides, such as glycerol monooleate and glycerol monolinoleate, has been thoroughly investigated due to their extensive polymorphism and widespread use in industrial products, e.g., food (Clogston et al. 2000). Depending on various molecular and ambient conditions, unsaturated monoglycerides can form four liquid crystalline mesophases, lamellar, reversed hexagonal, and two reversed bicontinuous cubic phases (Lutton 1965; Hyde et al. 1984; Qiu and Caffrey 2000).

Unsaturated monoglycerides have a unique property to form the cubic phase in equilibrium with the excess aqueous solution, expanding the application. Moreover, the unsaturated monoglycerides cubic phases have also been suggested for use in practical/technical applications such as bioelectrodes (Rowinski et al. 2004), biosensor construction (Barauskas et al. 2003), and protein (Katona et al. 2003) and metal nanoparticle (Norling et al. 1992) crystallization.

The simplest method of producing cubic phase particles is through the agitation of the glycerol monooleate with a magnetic stirrer in the presence of poly(ethylene oxide)-based stabilizers, resulting in a coarse dispersion with a particle size in the range of 1–100 μm. Further size reduction can be achieved by the use of high-shear energy input techniques such as ultrasonication, homogenization, and emulsification of the coarse dispersion (Farag et al. 2022).

Another method for producing cubic phase dispersions is based on mixing the glycerol monooleate with ethanol in miscible proportions and diluting the system with an aqueous solution containing a stabilizer. If the dilution trajectory falls into a cubic phase region, the cubic phase particles are formed spontaneously due to molecular diffusion difference at the liquid/liquid interface. However, also with this preparation method, a substantial amount of vesicular material was obtained and typically the particle size distributions were rather broad (Barauskas et al. 2005a, b).

Although several methods have been developed for the manufacturing of cubic phase dispersion, there is still no established process available for controlling the properties and quality of cubic phase particle dispersions. Importantly the particle dispersions of the method exhibit excellent colloidal stability during storage and dilution.

According to Barauskas et al. (2005a), a combination of high-shear energy homogenization and heat treatment provides a powerful and scalable way of producing glycerol monooleate-based cubic phase nanoparticles. The heat treatment of the homogenized dispersions consisting of predominantly vesicular-like particles results in their effective and reproducible conversion to cubic phase particles with narrow particle size distribution and well-defined inner morphology. The process is simple and results in cubosome nanoparticles containing minimal amounts of lamellar aggregates and possessing good colloidal stability. In addition, the amphiphile concentration, the amount of charged species, and salt content provide an elegant way of further controlling dispersion particle size and nanostructure (Almoshari et al. 2022).

14.4 Nanotechnological Approaches in Essential Oil-Based Food Packaging Systems

The natural appeal of EOs, combined with their bioactive properties and GRAS status, greatly interests the food industry. Nowadays, it is no secret that consumers' eating habits are changing, and a growing number of individuals are seeking more natural and health diets, as well as showing concerns for the environment. As a result, recent trends such as "clean label" products, sustainable packaging, and nanotechnology are thriving (Delgado-Pando et al. 2021; Ibrahim et al. 2022; Nile et al. 2020). The incorporation of EOs into the packaging to manufacture active packaging for the preservation of different food systems is another trend that deserves to be highlighted (Marques et al. 2022a; Sayadi et al. 2022; Tavares et al. 2021). An advantage of active packaging over conventional ones is the inclusion of the preservative agent in the packaging material, which ensures longer shelf life for the packaged products and promotes greater microbiological safety.

However, we must not ignore that there are several characteristics of the EOs that hinders their application as a preservative for foods, as well as potential additives for active packaging. They are prone to oxidative damage, possibly will degrade when exposed to light, and are extremely volatile and thermolabile. They are also hydrophobic compounds, and this feature may result in incompatibility with the polymer matrix, especially when working with bio-based polymers since the majority of them have a hydrophilic nature. In addition, their strong odor may compromise the food sensory attributes, such as aroma and flavor, and therefore affect negatively consumer acceptance, as reported by Ghabraie et al. (2016) and Marques et al. (2019a). In this sense, nanotechnology may be the key to bypassing these obstacles and ensuring the successful application of these compounds into active packaging (Arruda et al. 2022; Nile et al. 2020). Several approaches have been studied over the years, and a few recent examples are summed up in Table 14.1.

Nano-encapsulation of EOs, or their major compounds, is one of the most studied strategies to overcome EOs' drawbacks due to the great versatility regarding techniques, materials, and advantages provided, such as thermal protection, higher stability, a more sustained release, which ensure a longer bioactive of the compound, and more bioavailability and solubility (Delshadi et al. 2020; Liao et al. 2021; Surendhiran et al. 2022; Tavares et al. 2021). Moreover, the wall materials used to compose the nanocapsules varies widely according to the desired encapsulation system: cholesterol and phospholipids to produce nanoliposomes (Fattahian et al. 2022; Tavares et al. 2021); several oils, proteins (whey protein, for example) and carbohydrates (alginates, for example) for the obtainment of nanoemulsions (Almasi et al. 2021; Sun et al. 2021); distinct biopolymers (casein, zein, gelatin, chitosan, alginate, among others) to produce nanoparticles and nanofibers (Liao et al. 2021). The necessary equipment will also depend on the desired particles: ultrasonicators, homogenizers, spray dryers, and/or freeze dryers are commonly used for the manufacturing of liposomes, emulsions, and nanoparticles; while electrospinning and electrospray are required for the elaboration of nanofibers.

Tavares et al. (2021) used cholesterol and lecithin to produce liposomes for carvacrol nanoencapsulation through the lipid film hydration technique. The resulting vesicles provided effective thermal protection to the volatile compound and showed activity against *E. coli* and *S. aureus*. Later, the authors incorporated the vesicles into poly(vinyl) alcohol films to produce active packaging.

Ghoshal and Shivani (2022), in turn, used an ultrasonicator to incorporate thyme EO nanoemulsion into edible films composed of tamarind starch and whey protein. The films were evaluated on tomatoes and displayed the potential to be used as active edible packaging. At last, Zhang et al. (2022a) manufactured a multifunctional cellulose acetate membrane by electrospinning to act as both intelligent and active packaging. The authors incorporated a natural pigment (anthocyanin) as the freshness indicator and chamomile EO as the bioactive agent. The resulting nanofiber membrane was able to monitor freshness in pork, changing color as a pH

Table 14.1 Nanotechnological strategies to allow the use of EOs as preservative agents in active packaging aiming food preservation

Essential oil	Packaging material	Nanotechnological approach	Outcomes	Reference
Allyl isothiocyanate	Cellulose acetate	Nano-composite with carbon nanotubes and β-cyclodextrin	Controlled release of antimicrobials in the food model	Dias et al. (2018)
Rosemary and ginger	Chitosan	Development of montmorillonite nanobiocomposites	Extension of poultry shelf life	Pires et al. (2018)
Pimenta dioica and basil	Cellulose sachets	Complexation with β-cyclodextrin	Greater thermal stability; In vitro antimicrobial activity against *listeria monocytogenes* and spoilage mold *Byssochlamys nivea*	Marques et al. (2019b)
Peppermint and chamomile	Gelatin	Nanofibers manufactured by electrospinning	In vitro antimicrobial activity against *Escherichia coli* and *Staphylococcus aureus*; Antioxidant activity; UV barrier property; Not cytotoxic	Tang et al. (2019)
Coriander	–	Cyclodextrin nanosponges	In vitro antimicrobial activity against *L. monocytogenes*, verotoxigenic *E. coli*, *campylobacter* strains, and other pathogenic bacteria	Silva et al. (2019)
Carvacrol	Poly(vinyl) alcohol	Nanoencapsulation of carvacrol into liposomes	Greater thermal stability	Tavares et al. (2021)
Lavander	Gelatin	Nanoemulsion	Antimicrobial and antioxidant activity UV barrier; Heat-sealing property; Extension of cherry tomato shelf life	Sun et al. (2021)
Garlic	Chitosan	Nano-liposome	Extension of chicken fillet shelf life	Kamkar et al. (2021)
Cuminum cyminum	Chitosan	Nano-encapsulation of the EO into liposomes	Reduce microbial counts in meat fillets; Delay lipid oxidation in samples	Fattahian et al. (2022)

(continued)

Table 14.1 (continued)

Essential oil	Packaging material	Nanotechnological approach	Outcomes	Reference
Green tea extract and ginger EO	Starch	Development of nanocomposites with nanofibrillated cellulose	Extension of strawberry shelf life	Rodrigues et al. (2021)
Cumin	Gelatin	Development of nanocomposites with TiO_2	Delay lipid oxidation in fresh chicken meat	Sayadi et al. (2022)
Garlic	Cellulose acetate and zein blend	Complexation with β-cyclodextrin	Greater thermal stability	Marques et al. (2022b)
Thyme	Tamarind starch/whey protein	Nano-emulsion	Antimicrobial activity against *E. coli* and *S. aureus*; Extension of tomato shelf life	Ghoshal and Shivani (2022)
Clove	Poly(lactic acid)	Development of nanocomposite films with alkali-treated halloysite nanotubes	Better water vapor barrier, surface hydrophobicity, mechanical properties, and thermal stability; Delay weight loss of fresh-cut apples	Boro et al. (2022)
Turmeric	Chitosan	Development of magnetic-silica nanocomposite	Sustained release of the EO; Extension of surimi shelf life	Surendhiran et al. (2022)
Chamomile	Cellulose acetate	Fabrication of nanofiber membrane by electrospinning	Extension of pork shelf life	Zhang et al. (2022a)

response. Also, the membrane was able to extend the food shelf life by displaying both antimicrobial and antioxidant properties.

In another work, the cinnamon EO was also used in the preparation of new biodegradable material based on poly(butylene adipate-co-terephthalate) (PBAT) containing cellulose nanofibers (CNF) for application in food packaging. To prepare the films, CNFs were impregnated with cinnamon essential oil in a 2:1 ratio. The modified CNF (M-CNF) was then mixed with the PBAT solution at different concentrations of 0.5%, 1%, and 3%. The results were promising, as the films showed good thermal stability, evidencing a decrease in water vapor permeability values and relevant antimicrobial activity against *Salmonella* and *Listeria monocytogenes*. The films were tested as packaging for strawberries, the results indicated that in films with 0.5% by weight of modified CNF, the fruits had no fungal attack, less mass loss in 15 days of storage and greater preservation of freshness (De Matos Costa et al. 2020).

Roy and Rhim (2021) obtained a carrageenan/agar-based film containing Pickering tea tree oil emulsion (PET) and zinc sulfide nanoparticles (ZnSNP). PET was obtained with tea tree essential oil stabilized with nanocellulose fibers. PET and ZnSNPs were uniformly dispersed in the binary polymeric matrix and formed compatible films. There was an improvement in thermal stability, water vapor barrier, water resistance, and the composite membrane based on carrageenan/agar showed antibacterial and antioxidant activity. Thus, the developed film could be useful to be applied in active packaging for food.

The complexation of EOs with CDs is also an interesting nanotechnological strategy to enhance the EO stability and enable their application in packaging. The obtained EO/cyclodextrin inclusion complex usually is available in solid form, making it easier to handle and allowing its use in different ways. For example, it could be contained in sachets, as a powder (Marques et al. 2019a, 2019b) or dispersed in a polymer matrix (Dias et al. 2018; Marques et al. 2022b). The EOs could also be loaded in cyclodextrin-based nanosponges, a highly cross-linked polymeric network (Simionato et al. 2019; Silva et al. 2019), or nanofibers films prepared by electrospinning (Qin et al. 2022; Shi et al. 2022).

Regardless of the strategy chosen, EO/cyclodextrin inclusion complexes are known to enhance EO stability, mainly concerning thermal stability. Since the methods used to produce packaging usually involve high temperatures, it is of utmost importance to ensure thermal protection for the extremely thermolabile EO compounds, which can be successfully achieved by complexation with cyclodextrins (Arruda et al. 2021, 2022).

However, the properties of both CDs and the main polymer must be carefully studied before choosing the working materials. There are several cyclodextrins available on the market, usually classified into two groups: native cyclodextrins (α-, β-, and γ-cyclodextrins) and derivative cyclodextrins (e.g., hydroxyl-propyl-β-cyclodextrin) (Arruda et al. 2021). Each CD has particular features, such as higher or lower solubility in water and/or different organic solvents. β-cyclodextrin, for example, is not soluble in acetone, the main solvent used for cellulose acetate films manufactured by the casting technique. This characteristic may have been the main issue faced by Marques et al. (2021) during the elaboration of cellulose acetate films incorporated with β-cyclodextrin and allyl isothiocyanate, the major component of mustard essential oil. The authors verified that the films incorporated with β-cyclodextrin were more heterogeneous, brittle, and fragile than films without the compound. A similar result was verified by Dias et al. (2018), who described the films produced with cellulose acetate and β-cyclodextrin as less homogeneous, porous, and cracked when compared to the other elaborated films.

The choice of the technique could be a strategy to overcome drawbacks like that. In this sense, electrospinning has been successfully used to manufacture cyclodextrin nanofibers with different polymers, cellulose acetate among them. For example, Nthunya et al. (2017) synthesized β-cyclodextrin/cellulose acetate nanofibers via electrospinning to produce an active material aiming for bacteria removal from water. Although the antimicrobial agent of choice is not an EO, but silver/iron nanoparticles, the technique allowed the manufacture of nanocomposite fibers with strong antibacterial activity. Ghorani et al. (2019) also used the electrospinning technique to fabricate β-cyclodextrin/cellulose acetate nanofibrous webs aiming adsorption of undesirable volatile molecules. The authors used binary solvent systems composed of acetone and N,N-dimethylacetamide or acetone and dimethylformamide to synthesize uniform webs with adsorbent properties.

In fact, the electrospinning technique can be employed to produce EO/cyclodextrin-loaded nanofibers with several polymers. Figueroa-Lopez et al. (2020) used the technology to incorporate oregano EO/γ- or α-cyclodextrin inclusion complex into poly(3-hydroxybutyrate-*co*-3-hydroxyvalerate) fibers to act as active packaging. The films elaborated with 25% (w/w) of γ-CD inclusion complex displayed a homogeneous and continuous surface, and transparency, ensuring thermal protection for the EO, as well as antimicrobial and antioxidant activity when evaluated in vitro. Along the same line, Shi et al. (2022) developed electrospun nanofibers with polylactic acid (PLA) and polycaprolactone (PCL) incorporated with oregano EO loaded in β-cyclodextrin. The material acted as an active packaging when tested on blackberry, extending the shelf life of the fruit and maintaining its postharvest quality.

At last, the development of nanocomposites or nanobiocomposites formed by a blend of EO-polymer-nanostructure is also worth mentioning. Nanocomposites are materials that have at least one component at nano dimensions and are widely studied as potential active packaging. They could involve the incorporation of pure or encapsulated EOs in the polymer matrix aiming an antimicrobial and/or antioxidant activity, and a nanostructure as a filler to enhance certain features of the packaging. Carbon nanotubes, nanocrystalline cellulose and inorganic materials (such as nanoclay) are examples of nanoparticles studied as nanocomposites components to improve mechanical, optical, thermal, and/or barrier properties of the developed packaging, or even contribute to the active property, stability, or the controlled release of the EOs (Pola et al. 2016; Pires et al. 2018; Marques et al. 2021; Boro et al. 2022; Mahmud et al. 2022).

A potential concern that could guide future research in the active packaging field is the possibility of the acquisition of resistance by the bacteria in contact with the antimicrobial films. As discussed by Marques et al. (2022a), several studies showed that subinhibitory concentrations of EOs could lead to more resistant bacterial strains, which could, in turn, become a serious public health issue. In this sense, it is also necessary to evaluate the risk of the developed active packaging to induce microbial resistance acquisition.

14.5 Safety Issues and Toxicological Aspects

In general, EOs are considered safer than synthetic preservatives because they are naturally derived from plants; however, being aware of the complexity of the components of these aromatic substances and the conclusions of toxicological assessments, comprehensive safety concerns and regulatory issues must be considered before applying to food/packaging systems, especially when it comes through nanotechnology (Prakash et al. 2018b). Unfortunately, until the moment, there is still a lack of regulation of EOs and nanotechnological-based EOs in food matrices and packaging.

In a broad aspect, Regulation 450/2009/EC established the regulatory aspects surrounding the application of active packaging for food contact in Europe, claiming other guidelines such as that nanotechnology could not be used without further evaluation, even when direct contact with packaged food is not considered because of a functional barrier (European Commission 2009; Arruda et al. 2022). Also, the released substance must be listed on the European country's Positive List of Additives, and its use must characterize a technological need (Regulation 1333/2008/EC, European Parliament and the Concil of the European Union 2008).

The use of EOs can have negative health consequences (i.e., especially considering oils containing phenol and aldehyde groups), including the irritation of some tissues such as mucous membranes, eyes, and skin (Sharma et al. 2022). The Federal Food Drugs and Cosmetics Act (FFDCA) describes that a different safety standard has to be considered for naturally occurring substances in foods, other than that applied to intentionally added food ingredients. However, as EOs are mostly employed as flavoring agents, and many of which are purposefully added to foods as separate chemicals, a current standard cannot be easily applied to the safety evaluation of these complex substances; they are neither a direct food additive nor food themselves, falling somewhere in the middle (Reis et al. 2022).

EU No. 10/2011 establish guidelines concerning plastic materials and articles intended to come into contact with food, specifying that nanosized particles must be assessed case by case before their incorporation into active food packaging materials (European Union 2011).

The interaction of nanosized EOs substances with the food system raises a concern about human and animal health. In other words, these components on the surface of the packaging material are not detrimental to human health, but their translocation and integration into food may be. Their toxicity is stimulated by dynamic, kinetic, and catalytic properties and functionalization, net particle reactivity, agglomeration, and functional environment (Mahmud et al. 2022). Toxicologic issues caused by the nanotechnological EOs are mainly because of their persistent, non-dissolvable, and nondegradable behavior (Nile et al. 2020).

During in vitro and (the few conducted) in vivo studies, the main toxicity mechanisms that have been investigated by observing changes in diverse biomarkers are, for example, levels of glutathione (GSH), inflammation response, DNA damage, cell death, and ROS generation, with special attention to the last one, being one of the most significant factors (Nile et al. 2020; Mahmud et al. 2022). However, despite the advances already evidenced, so far, there is no standard protocol for the toxicity testing of nanomaterials that assesses their excretion mechanisms after digestion (Guidotti-Takeuchi et al. 2022).

It is important to state that, although these systems have the potential to be implemented in the recent future by the food industry, some nanostructures, such as carbon nanotubes, raise concerns due to safety hazards and toxic effects caused by their possible migration/release from the packaging to the food. The impact on the environment due to the disposal of the nanocomposite packaging is also a concern (Kotsilkov et al. 2018; Emamhadi et al. 2020). The lack of information regarding exposure, availability, and toxicity to humans can be an important drawback to the industrial application of nano-EOs in food packaging. Thus, continuous research and developments along with comprehensive government regulations are required, especially on the migration/toxicity of these nanosubstances from the nanocomposite to packaged food and their potential impacts on human health and to the environment.

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