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IMPORTANCE & APPLICATIONS OF NANOTECHNOLOGY

Novel Encapsulation of Bioactives: Use of Electrohydrodynamic Processing and Applications

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Published Online: Feb 27, 2021

eBook: Importance & Applications of Nanotechnology

Publisher: MedDocs Publishers LLC

Online edition: http://meddocsonline.org/

Introduction

Over the last decades, nanotechnology has received great attention as one of the most prominent tools in scientific research. This technology has revolutionized the entire food industry by the production and application of materials with sizes <1000 nm, thanks to which new and innovative food products with tailorable properties (such as sensory characteristics, process ability, shelf life, etc.) have been designed.

Those novel food products are based on food bioactives, which are physiologically active compounds found in plant or animal, and provide health benefits beyond basic nutrition and reduce the risk of disease. The interest of the food industry in these compounds has encouraged researchers to develop this new generation of food products based on bioactive ingredients having potential health benefits [1]. Currently, there are two main concerns in food industry: First, to improve the health-related characteristics of food; second, to develop intelligent packaging that prevents the deterioration of food.

The application of bioactives in food products is a challenging process since most of these compounds are very unstable and easily degraded under the exposure of oxygen, light and/or heat during processing and storage. In addition to these, their poor solubility, uncontrolled release, low bioavailability properties are considered as further drawbacks of their applications. Thus, an appropriate protection is required without losing the sensory properties of the food, to maintain bioactivity and consequently health benefits. Encapsulation is a prominent method that protects bioactive compounds by covering them with a resistant layer. The main objective of encapsulation is to improve the stability of labile compounds by hindering the adverse environmental conditions (light, moisture, oxygen, etc.). This technique provides many advantages that meet the needs of food industry, such as enhancing the nutritional quality of food, increasing solubility or dispersibility of the compounds, providing controlled release, concealing undesired flavors, as well as increasing the stability and the shelf life of the product.

Citation: Basar AO, Prieto C, Lagarón JM, (2021). Novel Encapsulation of Bioactives: Use of Electrohydrodynamic Processing and Applications. Importance & Applications of Nanotechnology, MedDocs Publishers. Vol. 6, Chapter 4, pp. 24-46.

In the nanotechnology and food industry, encapsulation of bioactive compounds and their incorporation into new and innovative functional food products is of great interest for the scientific community and for the food industry. Therefore, this chapter aims to discuss the characteristics of bioactive components and encapsulation techniques with their advantages, disadvantages, and applications.

Encapsulation of bioactive compounds

Encapsulation is defined as a technology that entraps bioactive compounds (core material) in micro- and nano- sized structures of biopolymer material (carrier or wall) [2]. Encapsulation of bioactives can typically be achieved in nano- micro- size range through either bottom-up or top-down approaches (Figure 1). A bottom-up approach involves the material construction into a larger structure by self-assembly and self-organization of molecules, where the common techniques for this are nanoprecipitation, inclusion complexation, supercritical fluid technique [3]. The other approach, top-down, refers to the miniaturizing of the bulk materials into nanosized structures or particles, where emulsification and solvent evaporation are the most common techniques [4].

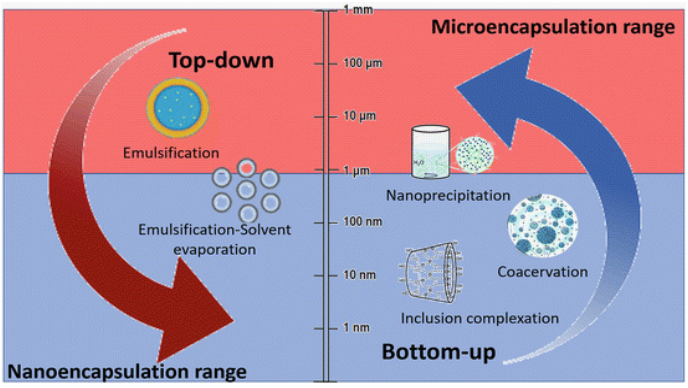

Figure 1: Top-down and bottom-up approaches in encapsulation technology (Adapted from [3]).

The use of microencapsulation dates back to the 1950s [5,6]. Micro- refers to the structural size in the range of 1-1000 µm [7]. In the food industry, microencapsulation has been traditionally used in the past to mask the undesired taste of particular ingredients and also to convert liquids to solids [6]. As technology advances, microencapsulation has become a versatile tool that can meet the needs of the food industry, such as protection of unstable bioactives against environmental conditions, controlled/sustained/targeted release, and better processability. This is carried out by various physical methods, such as spray drying, fluid bed coating, (co-)extrusion, molecular encapsulation, and also other chemical processes [8].

On the other hand, the introduction of nanoencapsulation technology has completely revolutionized the food industry. Nanoencapsulation is expressed as the encapsulation of substances in the range of 1 nm-1 µm; and thus, enables the production of particles with a greater surface-to-volume ratio, which in turn, enhances the solubility comparing with microparticles [9,10]. Particle size has a direct influence on the delivery of bioactive substances to various targets within the body. Nano-sized particles possess the ability to penetrate deeply into tissues due to their smaller size [11]. Hence, along with the microencapsulation benefits (protection against adverse conditions, masking undesired flavors, increased shelf-life, etc.), nanoencapsulation offers further improvements on bioavailability, solubility, release profile, and precision targeting of the bioactives [10,12]. A brief comparison of micro- and nano- encapsulation functionalities is given in Figure 2.

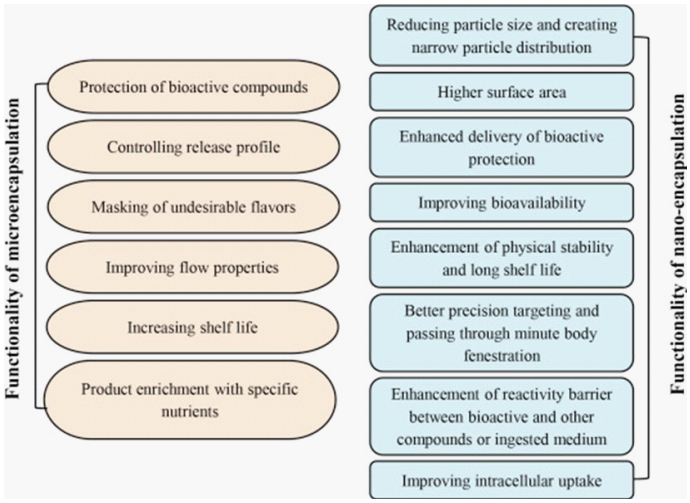

Figure 2: Functionality of micro and nano-encapsulation (Adapted from [10]).

Bioactive compounds and parameters

From past to present, bioactive compounds have been studied extensively by many different research areas for their beneficial physiological, behavioral, and immunological effects. To date, a large number bioactive substances have been discovered, that differ widely in chemical structure and function and grouped accordingly, such as polyphenolds, carotenoids, vitamins, minerals, essential oils, polyunsaturated fatty acids, peptides, probiotics, prebiotics or enzimes.

As discussed earlier, encapsulation is a very potent method of enabling the efficient use of bioactives. However, encapsulation of bioactives is not a straightforward process. There are many parameters that must be considered beforehand. Among them, the solubility, stability, and bioavailability of bioactives can be considered as the most challenging ones. In the following paragraphs, these aspects are going to be discussed for each type of bioactive compound.

Solubility of bioactives

In encapsulation of bioactives practises, solubility is one of the key parameters that must be considered. Classification of the solubilities are often as oil-soluble (e.g., lipophilic vitamins and minerals, carotenoids, polyunsaturated fatty acids, essential oils), water-soluble (e.g., hydrophilic vitamins and minerals, protein, peptides), or water/oil dispersible components (e.g. probiotics). The solubility of bioactive substances should be matching for the matrix of the food to be added. More particularly, the incorporation of lipophilic bioactives in food is limited due to their low water solubility. In recent years, encapsulation has been a prominent method to overcome such drawback, not only for enabling the delivery of lipid-soluble bioactives into foods, but also protecting them from adverse environmental conditions [13,14].

Stability of bioactives

Majority of the bioactive compounds exhibit poor stability. These instabilities compromise their conceived health-promoting effects, thus, limiting their applications. There are many intrinsic and extrinsic factors that can influence the stability of bioactive compounds; examples for the first one are water activity, pH, oxygen, preservatives, natural microflora, formula-

tion/composition of food, etc., whereas the examples for the second are temperature, pressure, relative humidity, environmental microflora, UV/IR radiation, etc [15].

Bioavailability of bioactives

In encapsulation practises, bioavailability is a key step for enabling the health-beneficial effects of bioactive compounds. In order to wield a health benefit, bioactive compounds, whether hydrophilic or lipohilic, need to endure food processing, be released from the food matrix and be bioaccessible. Several different process stages involve in bioavailability, such as Liberation, Absorption, Distribution, Metabolism and Elimination phases (LADME) [16]. As the first step of bioavailability, bioaccessibility should be considered beforehand. Bioaccessibility is defined as the fraction of a compound that is released from the food matrix in the gastrointestinal lumen, and thereafter becomes available for absorption at the luminal surface, and is affected by several parameters, such as the composition of the food matrix, pH, temperature, interactions with different components, etc. [17]. Overall, bioavailability includes gastrointestinal digestion, absorption, metabolism, tissue distribution, and bioactivity, and is usually defined as the fraction of a substance that reaches the systemic circulation [18]. In terms of bioavailability, intestinal permeability is the most important factor. When the challenges of bioaccessibility are overcomed, bioactive compounds can be absorbed in gastrointestinal lumen, thus incorporated into metabolic circulation. Bioavailability of the food components can be affected and altered by many factors, such as solubility, interaction with other food components, molecular transformations, different cellular transporters, metabolism and the interaction with the microbiota [17].

Bioactive compounds

Polyphenols

Polyphenols are aromatic molecules consisting of phenol as structural units, which are the most notable compounds in encapsulation of food bioactive area [19]. These compounds are an indispensable part of the human diet and abundantly found in herbs, fruits, vegetables, and categorized by the variation in the number and properties of phenol structures that consist of aromatic benzene ring having at least one hydroxyl group attached on it [20]. Polyphenols are known to have health-promoting effects by preventing the deterioration caused by oxidative stress, which can slow the progression of certain cancers, neurodegenerative diseases, diabetes, and can reduce the risks of cardiovascular disease [21]. However, the incorporation of these active substances into the human body is challenging due to their low bioavailabilities that are affected by many factors, such as gastric residence time, permeability, and/or solubility [19]. Furthermore, their stabilities are highly influenced by the conditions of processing/storage (temperature, oxygen, light) and gastrointestinal tract (pH, enzymes, other nutrients). Primarily, there are over 8000 types of polyphenols from simple molecules (phenolic acids) to highly polymerized compounds (tannin). Polyphenols can be categorized either based on the number of carbons (phenolic acids, flavonoids, tannins, etc.) or the number of phenolic rings in the structure [22]. Flavonoids are considered as the most important type of polyphenols having over 5000 compounds, which have also six main subcategories including flavanones, flavonols, flavones, isoflavones, flavanols, and anthocyanidins [23]. In these subdivisions flavanols (flavan-3-ols) are one of the most widely studied component in nano-encapsulation area due to their well-known health benefits.

Carotenoids

Carotenoids (or tetraterpenoids) are colourful liposoluble pigments that are found in plants, fungi, bacteria, algae and many foods (fruit, vegetables, fish, etc.) [24]. These pigments can be stored in fatty tissues of animals (exclusively, carnivorous). Only around 40 out of 600 carotenoids are present in a typical human diet and about 20 carotenoids have been observed in human blood and tissues, such as β-carotene, α-carotene, lycopene, lutein and cryptoxanthin [24]. Carotenoids have wide range of health-promoting effects on numerous diseases, such as cancers, cardiovascular diseases, age-related macular degenerations, and immune system disorders [25]. However, their practical application is often limited due to their extremely low water solubility and high sensitivity to several factors, such as heat, light, oxygen, metal ions and other processing conditions [26].

In general, carotenoids are divided into two subdivisions as xanthophylls and carotenes where xanthophylls contains oxygen as a functional group and carotenes consist of purely hydrocarbons but no oxygen [27]. Additionally, carotenoids having unsubstituted β-ionone ring (e.g. β-carotene, α-carotene and β-cryptoxanthin) present provitamin A activity [24]. Generally, carotenoids are hydrophobic substances that involve in hydrophobic areas of the cell. Also, they have very low water solubility, yet the polarity of carotenoids can be changed by the attachment of polar functional groups to the molecular chain [24].

Vitamins

Vitamins are organic and essential micronutrient compounds that cannot be synthesized in sufficient quantities by humans, therefore it is necessary to be obtained through the diet or supplements [28]. Thus far, 15 vitamins have been identified for human nutrition. Vitamins are often categorized depending on their solubility; vitamins C and B (as 8 vitamins group) as water soluble, vitamins A, D, E, and K as fat soluble vitamins [29]. Water-soluble vitamins cannot be stored in the body at high quantities due to their high dissolution rate and excretion through urine. On the other hand, fat-soluble vitamins can be stored and accumulated in the body by the absorption through the intestinal tract.

However, as the other bioactives mentioned, vitamins are very sensitive molecules against various physical and chemical conditions, such as temperature, light, pressure, pH, and humidity [29]. Therefore, it is important to protect them against these conditions, so that their structural break-down (degradation) can be hindered while their bioavailability is increased.

Minerals

Minerals are essential micronutrients that are required in larger or fewer quantities for different functions of the human body, such as building bones and teeth, controlling intra- /inter- cellular body fluids, and conversion of the nutrients into chemical energy [30]. As vitamins, minerals cannot be synthesized by human body, hence it is necessary to obtain them mainly through plants. Similar to the other bioactives, the main concern of mineral incorporation to the human body is their bioavailability, which can be affected by several factors, such as water solubility, physicochemical stability, chemical form, chelating agents, redox activity, mineral-mineral interactions, and the physiological state of consumers [31]. Minerals are classified into two subdivision, namely, major (or macro) minerals including calcium, phosphorus, potassium, sodium, and magne-

sium; and minor (or trace) minerals including sulfur, iron, manganese, chlorine, copper, cobalt, zinc, molybdenum, iodine, and selenium [31].

Polyunsaturated fatty acids

Polyunsaturated Fatty Acids (PUFAs) are abundantly present in marine fish, algae and some plant seeds [32]. PUFAs are divided into two families according to the location of the first carbon-carbon double bond, as omega-3 (ω-3) and omega-6 (ω-6). In the case of Omega-3, the first double bond presents at the third carbon from the methyl end of the chain; and for omega-6, the first double bond is at the sixth carbon from the methyl end of the chain [33].

The most important PUFAs in human diet are Linoleic Acid (LA, ω-6), Alpha-Linolenic Acid (ALA, ω-3), Eicosapentaenoic Acid (EPA, ω-3), and Docosahexaenoic Acid (DHA, ω-3) [14]. As most of the bioactives, PUFAs cannot be synthesised by the human body in required quantity; hence, they are often known as the Essential Fatty Acids (EFAs) and have to be taken through diet or dietary supplements [32]. Besides, Linoleic Acid (LA) and Alpha-Linolenic Acid (ALA) are considered as "true essential" fatty acids since they are precursors of the long-chain PUFA (e.g. DHA, EPA) synthesis [34].

In the literature, PUFAs have shown to be a highly beneficial substance for human heath in terms of cardiovascular diseases, insulin resistance, colorectal cancer, inflammation and autoimmune diseases, and cognitive function [35]. However, despite these benefits, their use in pharmaceutical and food applications is often challenging due to their susceptibility to lipid oxidation, which results in the development of off-flavors and reduced shelf-life [36].

Essential oils

Essential oils are organic compounds produced by plants and can contain about 20-60 volatile components in various concentrations [37]. They often consist of molecules that rich in functional groups (alkanes, alcohols, aldehydes, ketones, esters and acids) [38]. Essential oils are generally obtained by the common extraction processes such as distillation, solvent extraction, cold pressing, expression, absolute oil extraction, and resin wax embedding. Also, the majority of the common essential oils are recognized as safe by the FDA (Food and Drug Administration).

About 3000 essential oils are identified so far, and 10% of them are used in the cosmetic, food, pharmaceutical and agriculture industries and they are often categorized by these fields of use. In terms of structure, various essential oils may contain complex chemical structures, such as monoterpenes, sesquiterpenes, and phenylpropanes [38].

Over the centuries, essential oils have been used as medicine due to their effects on infections, chronic, and acute diseases. Also today, they have been utilized extensively in both pharmaceutical and food industries for their antioxidant, anticarcinogenic, antimicrobial, antiinflammatory, and antitumoral properties [39]. However, the use of essential oils is always confronted by their high volatility, and chemical instabilities upon exposure to air, light, moisture and heat [40].

Peptides

Bioactive peptides have been defined as specific protein fragments that are inactive within the sequence of the parent protein, yet may provide health-promoting effects after release during digestion [41]. Although their use may be hindered by several challenges such as bitter taste, hydrophobicity, reaction with the food matrix, incompatibility, limited bioavailability, and biostability; they have been increasingly used in functional foods due to their antioxidant and antimicrobial properties [42]. Thus far, more than 1500 different bioactive peptides have been identified in a database named "Biopep" [43].

In recent years, bovine milk, cheese, and other dairy products are considered as the greatest sources of bioactive peptides that need to be isolated from these complex matrices prior to encapsulation [44]. There are several ways to isolate bioactive peptides from precursor proteins, such as enzymatic hydrolysis, microbial fermentation, and enzymatic proteolysis [45].

Enzymes

Enzymes are a group of protein-based molecules that are biocatalyst responsible for conversion of substrate to product at varied biological conditions. These bioactive materials are often utilized in food industry due to their abilities of decreasing the concentration of a non-desired food constituent, and/or improving flavor, texture, and nutritional properties [46]. As examples of these bioactive enzymes, catalase, glucose oxidase, laccases, α-amylase, amyloglucosidase, β-galactosidase, flavourzyme, lipase, and trypsin can be given [47].

However, enzymes exhibit short-time stability under stressed environmental conditions, such as temperature and pH. In order enzymes to demonstrate maximum stability and performance, an optimal temperature and pH range is necessary [48].

Probiotics and prebiotics

Probiotics are living microorganisms that, when correctly administered in appropriate amounts (e.g. 10^6 CFU/g), can provide health benefits for the consumer [49]. Currently, probiotics are widely utilized in the production of functional foods, more particularly in dairy products. In food industry, *Lactobacillus* and *Bifidobacterium* are the main probiotic microorganisms which produce gram-positive lactic acid and constitute a major part of the intestinal microflora in animals and humans [50]. The major concern of the application probiotics in food applications is that probiotics may lose their viability before consumption and/or during the digestion process. The harsh gastric conditions of the human stomach have a significant negative effect on the viability of probiotics; thus, their encapsulation may be the solution to this difficulty [51].

Prebiotics (e.g. fructo-oligosaccharides, inulin, galacto-oligosaccharides, lactulose) are also bioactive agents that are not digested in the upper part of the gastrointestinal tract and have a health-promoting effects through their selective stimulation of growth and activity of intestinal bacteria (e.g. *Lactobacillus* spp and *Bifidobacterium* spp) [52]. These agents have potential to increase the bioavailability of nutrients and minerals (particularly calcium) [53]. Any dietary ingredient that reaches the colon has the potential of being a prebiotic. However for this, candidate prebiotics must fulfill these criteria: Non-digestibility (resistance to gastric acid, enzymatic digestion, etc.), selective fermentation by the intestinal microbiota, and selective stimulation of growth and activity of intestinal bacteria [54]. As with probiotics and other bioactive components, encapsulation of these structures facilitates their use in functional foods.

Carrier materials used for encapsulation of bioactive compounds

The selection of a proper encapsulating material is very important for designing a successful encapsulation process. A wide range of materials can be used as encapsulating material (carrier). However for the food applications, they must be approved as "Generally Recognized As Safe" (GRAS) materials due to the health and safety issues [55]. Main objective of the carrier materials is to protect chemically unstable bioactive compounds against light, oxygen, pH, heat, degradation or any other extreme conditions during the food processing, storage or packaging. In terms of functionality, carrier materials should be a good emulsifier, low viscous at high concentration, and possess good dissolution and network-forming characteristics [10]. Also, they should be chemically inert with bioactive compounds and overcome the acidic or enzymatic reactions in undesired areas of gastrointestinal tract (e.g. stomach), thus enabling the bioavailability through intestinal absorption. Addition to these, they should possess the capability of residence in the target sites of the gastrointestinal tract [10]. Selection of encapsulating carriers requires a good understanding of the physicochemical and rheological behavior of these substances and as well as the compounds to be encapsulated. There are wide range of food-grade encapsulating carrier materials, which are mainly polysaccharides, proteins, and lipids, with high availability and reasonable price. As more specific examples; starch [56], dextrins [57], cellulose [58], pectin [59], chitosan [60], alginate [61] gum [62] are for the polysaccharide-based carriers; whey protein [63], casein [64], gelatin [65], soy protein [66], zein [67] are for the protein-based carriers; polar lipids (e.g. monoglycerides [68], phospholipids [69]) and nonpolar lipids (e.g. triacylglycerol [70], cholesterol [71]) are for the lipid-based carriers.

Micro-and nano-encapsulation methods

The selection of encapsulation method is a crucial step for the encapsulation of bioactives. As discussed previously, encapsulated systems can be developed by two approaches, either top-down or bottom-up approaches (Figure 1). The top-down approach includes emulsification, solvent evaporation, and extrusion techniques, where the bottom-up includes spray drying, supercritical fluid, inclusion complexation, coacervation, nanoprecipitation [72]. The top-down approach possesses less control over particle size and structure but are suitable for the encapsulation of both hydrophilic and hydrophobic compounds [72]. On the other hand, the low energy consumption and greater control over particle size, distribution and structural morphology are the remarkable highlights for the bottom-up approach [73].

In the literature, a large diversity of techniques is available for the production of micro- and nano- encapsulation of bioactives. The encapsulation technique that will be utilized should provide high loading capacity, high encapsulation efficiency, improved stability of the compound with high shelf-life, high bioavailability, etc [10]. Some examples of the recent research (2015-2020) on various encapsulation techniques, as well as their principles, advantages, and disadvantages are presented in Table 1. Electrohydrodynamic processes are to be discussed exhaustively afterward.

Spray drying

Spray drying is a simple, rapid, and low-cost process that utilized largely for the microencapsulation of bioactives with the ability of converting bio-based suspensions into a powder. This technique is based on the atomization of liquid feed (which contains bioactive compounds) into the drying chamber, rapid drying of the chamber with hot gas, formation of microcapsules in the drying chamber, finally separation and collection of microcapsules through cyclone recovery [74]. One major advantage of spray drying is that high product yield with the maximum encapsulation efficiency can be achieved by means of selecting the optimum feed formulation [75]. Also, depending on the materials, temperatures between 150 and 300 °C can be operated, and generally, the mean size of the generated particles is in between 10-100 µm. However, the application of this technique is limited for the volatile or thermo-sensitive bioactive compounds. This technique is available at industrial scale, due to its simplicity and low cost. It is the most used technology in the food industry.

As an example, Shamaei *et al.* (2017) demonstrated the ability of spray drying microencapsulation in terms of high encapsulation efficiencies [76]. In this study, walnut oil encapsulated in three different wall materials including Skim Milk Powder (SMP), SMP + Tween 80, and SMP + maltodextrin. They reported that the combination SMP and Tween 80 as wall material exhibited highest microencapsulation efficiency (91.01%). Similarly, *Chew et al.* (2018) investigated the potential of β-cyclodextrin with different wall materials (gum arabic and sodium caseinate) in the microencapsulation of refined kenaf seed oil by spray drying [77]. According to the results, particle size distribution was 25.4 to 37.3 µm. More importantly, more than 90% encapsulation efficiencies were achieved for all model combinations of wall materials. In another study by Busch *et al.* (2017) encapsulated propolis extracts into different maltodextrins (with or without added gums) by spray drying [78]. They reported that the addition of gums to the matrix formulation improved the encapsulation of some polyphenols (particularly quercetin) during spray-drying. In addition, the extract encapsulation improved physical stability against humidification, as well as increased antioxidant activity.

Freeze drying

Freeze drying is another widely used technique to encapsulate thermo-sensitive compounds (essential oils, protein, pigments, etc.), which basically consists in drying the sample by sublimating its water content. It can retain the particle size in micro- or nano-scale. However, the product quality is affected by the operating conditions. For example, highly porous structures may be obtained depending on the ice crystal size and primary drying rate [79].

This technique involves three main steps, such as freezing, primary drying (sublimation), and secondary drying (desorption) [80]. In the freezing stage, compounds are cooled down until a frozen state is reached. Primary drying stage involves the removal of ice by sublimation at a very low temperature and pressure. Finally, at the time which primary stage ends, the secondary drying stage starts and the non-frozen water is removed by desorption through the pores of the dried material as water vapor. However, although freeze drying is an efficient method in terms of the stability of bioactive compounds and encapsulation efficiency, it is a highly expensive method with high time and energy consumption [81].

Ravichai *et al.* (2019) encapsulated bioactive compounds present in Concentrated Fermented Miang Wastewater (CFMW) by freeze drying using various coating materials (maltodextrin,

gum arabic, and modified starch) and CFMW-to-coating material ratios [82]. They reported that highest encapsulation efficiency was found for gum arabic coating in 10:1 CFMW-to-coating ratio (98.05%). It was also reported that the freeze-dried encapsulation efficiency of this best formulation was higher than that by spray drying (85.18%). In another study by Celli *et al.* (2016), anthocyanins compounds from blowbush blueberries were encapsulated by freeze drying in maltodextrin with different dextrose equivalents [83]. According to the results of accelerated shelf life studies (storage temperature of 70, 80, and 90 °C), the half life ($t_{1/2}$) varied from 0.96 h (freeze-dried extract) to 108 days (encapsulated extract). It was shown that freeze-dried encapsulation effectively retards anthocyanin degradation during storage. In another interesting study, Tan *et al.* (2020) encapsulated two model probiotics, *Lactobacillus rhamnosus* GG and *Escherichia coli* Nissle 1917, within alginate using freeze drying method to investigate the factors affecting freeze-drying survivability of encapsulated probiotics [84]. They reported that higher probiotic survivability was observed at a higher freezing rate. In addition to that divalent cations, which are used to cross-link alginates, were found to have antagonistic effects on encapsulated probiotics.

Spray-Freeze-Drying (SFD) is an unconventional freeze drying technique that overcomes the limitations of freeze drying. SFD consists of three main steps which are atomization of a liquid or solution into droplets, solidification by contact with a cold fluid or liquid nitrogen, and sublimation of moisture at low temperature and pressure [85]. This technique is unique by means of being the combination of spray drying and freeze drying. With this technique, it is possible to encapsulate low water soluble compounds; and since it is operated under sub-ambient conditions, it is highly suitable for the encapsulation of thermo-sensitive compounds comparing to spray drying [85]. Its total processing time is 6-8 hours, which is 4 times less than the freeze drying time consumption [86].

As an example of Spray-Freeze-Drying (SFD) method , Rajam *et al.* (2015) produced microcapsules containing probiotics (*Lactobacillus Plantarum*) using whey proteins with Sodium Alginate (SA)/Fructooligosaccharide (FOS) as wall materials [87]. They reported that SFD microcapsules were successfully produced with high encapsulation efficiency (87.98 - 94.86%) and, exhibited spherical particles with fine porous structure while, freeze-dried microcapsules were irregular in shape and exhibited porous, spongy, flake-like structure. Additionally in this study, SFD method showed significant lower processing time (8h) comparing to conventional freeze drying method (20h) with high encapsulation efficiency. In another study by Parthasarathi *et al.* (2016), vitamin E encapsulated into whey protein isolate by using spray drying, freeze drying, and spray freeze drying methods [86]. High encapsulation efficiencies were achieved for all three techniques (86.1-89.6%). Importantly, it was reported that the spray freeze-dried vitamin E microcapsules enhanced the oral bioavailability by 1.13 and 1.19 fold compared to spray dried, and freeze-dried microcapules, respectively. Thus, this study demonstrated the ability of spray freeze drying method on the potential encapsulation of poorly water-soluble bioactive compounds.

Complex coacervation

Coacervation is defined as the separation of colloidal systems into two liquid phases, and classified as simple (single biopolymer) and complex coacervation (two or more biopolymer) [88]. Complex coacervation is more preferred in food and pharmaceutical industries than simple coacervation as it provides better functionalities [89]. Complex coacervation involves the electrostatic attraction between two biopolymers having opposite charges. It consists in four steps, (1) emulsification of two immiscible solutions (e.g. oil in aqueous solution) containing 2 different polymers, (2) initial coacervation of polymers, (3) formation of encapsulating layer around the active compound, and (4) wall hardening with heating, desolvation, or cross-linking techniques [89]. By complex coacervation, particles can be produced in nano- or micro- range and exhibit good controlled release characteristics and thermo-resistant properties [88]. In addition to that, other advantages of this technique are that it does not require high temperatures, and has a very high payload (up to 99%) [90]; yet, it suffers from several drawbacks, such as high production cost, dependence on many factors (e.g. pH, ionic strength), limited stability in aqueous solutions, difficulty to control particle size and morphology due to being highly affected by the processing conditions and capsule agglomeration [10,91].

In a complex coacervation encapsulation study, Jain *et al.* (2016) produced microcapsules of β-carotene using casein and gum tragacanth as wall materials, and investigated several condition parameters on coacervation, such as pH, temperature, initial protein (Pr) to polysaccharide (Ps) mixing ratio (Pr:Ps), and electrostatic interactions [92]. It was reported that an optimum pH of complex coacervation was found 4.35 at Pr:Ps = 2:1, at which the intensity of electrostatic interactions was maximum. At these conditions, particle size, coacervation yield, and encapsulation efficiency was found as 159.71 µm, 82.51%, and 79.36%, respectively. It was also mentioned that intrapolymeric aggregation was occurred on further acidification. Importantly, thermal stability and long residual antioxidant activity of β-carotene were effectively improved after being encapsulated in microcapsules. As another example, da Silva Soares *et al.* (2019) encapsulated sacha inchi oil (rich in omega-3) via complex coacervation of ovalbumin and sodium alginate [93]. They reported a high encapsulation efficiency (94.12%) and increased thermal resistance at 189.89 °C. Furthermore, produced microencapsules presented low omeg-3 release during gastric simulation. In another study by Li *et al.* (2018), complex coacervation of zein and chitosan was utilized to encapsulate curcumin [94]. At the optimal pH of 4.0, developed nanoparticles containing curcumin exhibited smaller particle size (162.07 nm), higher encapsulation efficiency (94.67%), and prolonged release profile.

Extrusion

Extrusion can be defined as a process that consists in forcing a material to flow under certain conditions (such as high and low temperatures, high and low moisture and high and low speed) through a hole at a predetermined rate to achieve different products [95]. There are different types of extrusion modes based on their instrumentation and extrusion conditions, which the most common are (i) hot-melt extrusion, (ii) melt injection (ram extrusion), and (iii) centrifugal/co-extrusion. The principle of operation, which is feeding of raw material into the barrel, and the pushing out the material toward die and cutter, are similar for all extruder modes [96]. The first type of extrusion utilizes screw(s) to push material toward die and cutter. The hot-melt extrusion is similar to melt-injection method. The major differences of these two are that melt injection is a vertical screwless process with surface washed particles while hot-melt extrusion is a horizontal screw(s) process using temperature input without surface washed particles. However, melt injection

method can be considered as "out-dated" model compared to the production efficiency and commercial scalability of hot melt extrusion technique [97]. On the other hand, centrifugal/co-extrusion utilizes rotating extrusion head to push out bioactive compounds (in inner nozzle) into the wall materials (in outer nozzle) [89].

Extrusion is a versatile technique and all the modes mentioned have been widely used for the encapsulation of bioactives due to various advantages, such as the suitability for the encapsulation of wide range of compounds including hydrophilic or hydrophobic compounds, high productivity, adaptability, energy efficiency, and longer shelf-life [72,96]. However, one of the major drawbacks of these methods is that the production of relatively large particle size which reduces the bioavailability, release properties and solubility of the active components due to low surface-to-volume area and longer diffusion [96]. In addition, more specifically, hot-melt extrusion processes involve high temperature (80-150 °C); and thus, its application with thermo-sensitive bioactive compounds is often limited [96]. Moreover, a centrifugal/co-extrusion exclusive disadvantage is that the wall material non-uniformity which can cause ineffective encapsulation and instabilities [96]. Finally, depending on the instrumentation, these techniques can be highly expensive.

As an example, Chang *et al.* (2019) encapsulated ascorbic acid in different glassy carbohydrate matrices (maltodextrin, maltodextrin-gum arabic, and maltodextrin-trehalose) via hot-melt extrusion technique [98]. According to the results, ascorbic acid yield was above 97% for all encapsulation matrices. Storage tests revealed that no recrystallization occurs during the storage period of 3 months at 40 °C, thus, indicating a good physical stability. In addition to these, extruded structures exhibited significantly reduced dissolution rate comparing to non-encapsulated ascorbic acid. In another, study by Chew *et al.* (2016), kenaf (*Hibiscus cannabinus* L.) seed oil was encapsulated within alginate and high methoxyl pectin using co-extrusion method [99]. By optimizing the processing conditions and shell formulations, a successful encapsulation with an encapsulation efficiency of 74.25% and an average particle size of 300 µm was achieved. It was also highlighted that microencapsulation of kenaf seed oil by co-extrusion is possible in a stable and reproducible manner.

Other techniques

As discussed before, there is no single method for the encapsulation of bioactives**.** Table 1 summarizes the principles, advantages, and disadvantages of the aforementioned encapsulation techniques with recent literature examples (2015-2020). Besides the discussed ones in this chapter, there are several other techniques for the micro- and nanoencapsulation, such as fluid bed coating, anti-solvent precipitation, layer by layer deposition. Basically, fluid bed coating involves the suspension of bioactive compounds in hot air and the spraying of a coating material to form the encapsulation structures [90]. This technique possesses some advantages, such as low energy, consumption, good reproducibility, and reduced operation time and cost. As an example of this, Coronel-Aguilera *et al.* (2015) encapsulated β-carotene with a coating solution of hydroxypropyl cellulose using fluid bed coating method and the effect of temperature was investigated [100]. It was reported that particles were developed in a narrow size range of 48.6- 69.5 µm for all operating temperatures (60, 70, and 80 °C) and lower coating temperatures exhibited greater β-carotene encapsulation comparing to 80 °C. In an interesting comparative study by Pellicer *et al.* (2019), strawberry flavour was encapsulated by spray drying, freeze drying and fluid bed coating techniques [101]. It was reported that fluid bed method exhibited the homogeneity in terms of particle size distribution. However, compared to spray drying and freeze drying, fluid bed technique showed the worst drying yield and storage stability (at 4 and 25 °C for 5 months) with highest moisture content.

Anti-solvent precipitation is dealing with the dissolution of the bioactives in a binary solvent (e.g. water and organic solvent), and the formation of supersaturated solution leading to the development of nanoparticles and possesses several advantages, such as easy scalability, being cheap, ability to produce small-sized particles, etc [102]. In addition to that, supercritical anti-solvent precipitation technique, which uses supercritical carbon dioxide as an antisolvent, is an advanced anti-solvent precipitation method that provides better processing conditions for particle encapsulation with several advantages of being nontoxic, nonflammable, and chemically stable [103]. For example, Wang *et al.* (2018) encapsulated β-carotene within zein, Carboxymethyl Chitosan (CMCS) and Tea Polyphenols (TP) using anti-solvent precipitation methods [104]. Results showed that by means of optimized encapsulation formulations, nanoparticles within the range of 70-130 nm were produced with high encapsulation efficiency of >90%. Additionally, β-carotene-loaded nanocapsules with CMCS and CMCS-TP showed excellent stability and slow release under simulated gastrointestinal conditions. In another study by Arango-Ruiz *et al.* (2018), curcumin was encapsulated using supercritical anti-solvent method to improve its stability and solubility in water [105]. It was reported that average particle diameter of 5.67 µm was achieved; capsules exhibited largest aqueous stability and solubility at pH 4; and during 15 days of stability tests, only 3-10% degradation occurred during several conditions, such as pH, temperature, light.

Layer by layer deposition is a common encapsulation technique that consists in the alternate deposition of oppositely charged materials onto a charged template through electrostatic interactions and the removal of the template by chemical dissolution to form capsules [106]. This technique is simple, versatile, reproducible and also allows tailoring the physicochemical properties of the assembled materials with the precision in nanometer scale. As an example, Wang *et al.* (2019) encapsulated probiotics, *Lactobacillus pentosus* into chitosan and sodium phytate using layer-by-layer deposition method; and also investigated the probiotic survivability under several conditions, such as gastrointestinal environment, high temperature (37, 45, 55, and 65 °C), and long storage duration (30 days) [107]. According to the results layer-by-layer encapsulation significantly improved the survival rate of probiotics during the storage, gastrointestinal cultivation and heat treatments compared to plain-probiotics. More importantly, it was found that double-layer coated probiotics showed further survivability compared to mono-layer encapsulation. In another example, Li *et al.* (2019) developed curcumin-loadeed kafirin solid and hollow nanoparticles through electrostatic layer-by-layer deposition of dextran sulfate/chitosan components [108]. A successful encapsulation of curcumin was achieved with high encapsulation efficiency of >90%; and the size of curcumin-loaded hollow and solid particles was found as around 60 nm and 100 nm, respectively. Additionally, a slower kinetic release of hollow nanoparticles (compared to solid); as well as, an improved dissolution profile in gastrointestinal tract (for both) was reported.

Table 1: Principles, advantages, disadvantages [72-108] and recent studies (2015-2020) of different encapsulation techniques.

Encapsulation technique	Principles	Advantages	Disadvantages	Recent examples with highlights	
Spray Drying (SD)	Entrapment of core substance in wall material through atomization and rapid drying with hot gas	✓ Simple, low process cost ✓ Good encapsulation efficiency ✓ Possibility of large-scale production ✓ Short process time	× Not suitable for thermo- and/or air sensitive compounds × Lower survivability of living bioactives (e.g. probiotics) × Higher oxidation of oils	• Encapsulation of walnut oil into skim milk powder, Tween 80, and maltodextrin • High encapsulation efficiency (EE) of (91.91%) • Improved shelf-life	[76]
				• Encapsulation of kenaf seed oil into β-cyclodextrin, gum arabic, and sodium caseinate • High EE (90%) • Low moisture and improved aqueous solubility	[77]
				• Encapsulation of propolis extract into maltodextrins and gums • Addition of gum improved overall encapsulation properties of polyphenols • Higher physical stability and anxtioxidant activity	[78]
Freeze Drying (FD)	Entrapment by lyophilization of an core-coating material containing emulsion solution through freezing, primary drying (sublimation), secondary drying (desorption of non-frozen water)	✓ Suitable for thermo-sensitive compounds (High temperature not required) ✓ Suitable for air sensitive compounds ✓ High encapsulation efficiency ✓ Provides good stability ✓ Homogenous dispersion	× Highly expensive × Long processing time × High energy consumption × Relatively complex process × May yield highly porous structures	• Encapsulation of phenolic compounds from CFMW into maltodextrin, gum arabic, and modified starch • High EE (98.05%) • Freeze drying exhibited better EE than spray drying (85.18%) • Improved antioxidant activity	[82]
				• Encapsulation of anthocyanins into maltodextrins • Exceptional shelf-life • Improved thermal stability	[83]
				• Encapsulation of probiotics into alginate • Enhanced survivability of probiotics • Antagonistic effects of cross-linkers	[84]
Spray-Freeze-Drying (SFD)	Entrapment through the atomization of the core-in-carrier liquid, solidification by contact with cold fluid, and sublimation	✓ Suitable for thermo-sensitive compounds ✓ Fine pores comparing to freeze drying ✓ Faster processing time comparing to freeze-drying	× High cost × Relatively complex process × Inconvenient (require liquid N_2)	• Encapsulation of probiotics into whey protein, sodium alginate, and fructooligosaccharide • High EE (87.98 – 94.86%) • Spherical particles with fine porous structure • Lower processing time (8 h) compared to freeze drying (20 h)	[85]
				• Encapsulation of Vitamin E into whey protein isolate • High EE (86.1 – 89.6%) • Enhanced bioavailability compared to SD and FD	[86]
Complex Coacervation	The entrapment occurring by the deposition of a liquid coating materials around a core materials by electrostatic attractions	✓ Controllable release characteristics ✓ Good thermo-resistant properties ✓ Suitable for thermo-labile compounds (performing at room temperature) ✓ High encapsulation efficiency and payload ✓ Ability to encapsulate in nanoscale	× High production cost × Dependent on many factor (pH, ionic strength) × Limited stability in aqueous solutions × Difficult to control particle size and morphology	• Encapsulation of β-carotene into casein and gum tragacanth • High EE (79.36%) • Improved thermal stability and antioxidant activity	[92]
				• Encapsulation of sacha inchi oil into ovalbumin and sodium alginate • High EE (94.12%) • Improved thermal resistance • Enhanced release profile	[93]
				• Encapsulation of curcumin into zein and chitosan • High EE (94.67%) • Nanoparticles (162.07 nm) • Prolonged release profile	[94]

Extrusion	Forcing core material to flow in wall material mass through a die. Coating material hardens on contacting liquids, entrapping the active substances.	✓ Suitable for wide range of bioactives ✓ High productivity ✓ Adaptability ✓ Energy efficiency ✓ Longer shelf-life	× Production of large particle size × Relatively high temperature (80 – 150 °C) × May be highly expensive	• Encapsulation of ascorbic acid into maltodextrin, gum arabic, and trehalose • Hot-melt extrusion encapsulation of ascorbic acid with high yield (>97%) • Improved physical stability • Improved shelf-life • Improved solubility	[98]
				• Encapsulation of kenaf seed oil into alginate and high methoxyl pectin • Co-extrusion encapsulation with high EE (74.25%) • Stable and reproducible process	[99]
Fluid Bed Coating	Nozzle spraying the coating material into a fluidized bed of core material in hot environment	✓ Low cost and energy consumption ✓ Specific capsule size distribution and low porosities ✓ Reduced operation time	× Not suitable for thermosensitive compounds	• Encapsulation of β-carotene into hydroxypropyl cellulose • Narrow particle size range (48. – 69.5 μm) • Improved color stability	[100]
				• Encapsulation of strawberry flavours into maltodextrin, modified starch, and cyclodextrins • Comparative strawberry flavour encapsulation • Better size homogeneity compared to SD and FD • Worse drying yield and storage stability compared to SD and FD • Highest moisture content	[101]
Anti-solvent Precipitation	Dissolution of core material in a solvent, followed by the mixing with anti-solvent (in which core material is insoluble), and precipitation of solute due to the formation of supersaturated solution	✓ No need for specialized equipment (low cost) ✓ Simple process ✓ Ability to encapsulate in nanoscale ✓ Easy to scale-up	× Difficult to control particle size distribution × Particle growth by agglomeration and coagulation	• Encapsulation of β-carotene into zein, carboxymethyl chitosan, and tea polyphenols • High EE (>90%) • Nanoparticles (70 – 130 nm) • Improved stability and slow release under gastrointestinal conditions	[104]
				• Encapsulation of curcumin into PVP, Eudragit L100, and Pluronic F127 • Supercritical antisolvent precipitation encapsulation • Improved pH and photo-stability • Improved solubility in water	[105]
Layer by Layer Deposition	Deposition of oppositely charged materials onto a charged template through electrostatic interactions and removal of the template by chemical dissolution to form entrapment	✓ Low cost ✓ Simple and easily adaptable ✓ Ability to encapsulate in nanoscale ✓ Multilayer encapsulation provide better protection	× Stability is highly affected by molecular interactions, such as electrostatic, hydrogen bonding, charge-transfer	• Encapsulation of probiotics into chitosan and sodium phytate • Improved survivability during storage, gastrointestinal cultivation, and heat treatment • Double-layer coating provided further survivability	[107]
				• Encapsulation of curcumin into kafirin, dextran sulfate, and chitosan • High EE (>90%) • Hollow and solid nanoparticles produced (60 nm and 100 nm, respectively) • Enhanced release and dissolution profile in gastrointestinal tract	[108]

Electrohydrodynamic techniques and principles

Electrohydrodynamic processes, electrospinning and electrospraying, are cost-effective and scalable technologies that can produce high-performance micro-and nano-sized fiber and capsule carriers for bioactives with high surface-area-to-volume ratio [109].

Electrodynamic processes involve the evaporation of polymeric solutions by high electric voltage to produce ultrathin polymeric structures with variable morphologies [110]. This technique is divided into two; firstly, electrospinning as consisting of the production of ultrathin continuous fibres, and secondly, electrospraying as the production of capsules. The typical electrospinning and electrospraying setups are composed of: (1) a high voltage supply (often 1-30 kV), (2) a stainless steel needle or capillary, (3) a syringe pump, and (4) a ground collec-

tor which can be a flat plate or a rotating drum (Figure 3). During both electrospinning and electrospraying processes, a polymer solution is held at the end of capillary needle tip by surface tension. By means of the applied high voltage, the polymer solution becomes charged by the strong electrostatic forces that oppose its surface tension. As the electrostatic field reaches at a critical point, the shape of a pendant polymer solution drop changes from spherical to conical, which is known as "Taylor cone". When the electrostatic field overcomes the surface tension of the droplet, a charged jet emerges from the tip of Taylor cone, and as the solvent evaporates, the polymeric fibers or capsules are captured on the collector. Although electrospinning and electrospraying are very similar techniques, they differ each other in terms of the polymer concentration and solution viscosity and structures generated [111].

This technology solves the mentioned limitations of the previous technologies with numerous advantages, such as ease of use, low-cost, high encapsulation efficiency, no temperature requirement, greater thermal, light and storage stability, controllable particle/fiber size and morphology, tailorable release mechanics, potential use of non-toxic solvents, and scalability [110,112]. These aspects will be discussed later with recent examples in terms of electrospinning and electrospraying.

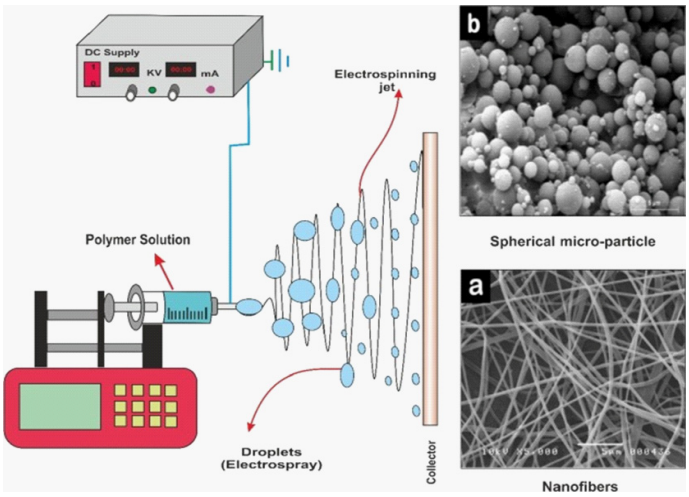

Figure 3: Schematic representation of (a) Electrospinning and (b) Electrospraying processess (Adapted from [110]).

Parameters affecting electrospinning and electrospraying

In electrohydrodynamic processes, the structure, morphology, and functionality of the resultant product depend on several factors, which are classified as solution, process, and environmental parameters [110]. The solution parameters include viscosity, surface tension, and conductivity. The process parameters include applied voltage, flow rate, distance between the needle and collector. Finally, the environmental parameters include relative humidity and temperature. All of these parameters have a direct effect on generating fibers and capsules.

Concentration and viscosity

Electrospinning and electrospraying processes are highly dependent to viscoelastic properties of the solution, which are determined by the polymer molecular weight, polymer-solvent interactions, and concentration. Viscosity of the solution is directly related to polymer concentration, molecular weight and solvent type [113,114]. As stated before, solution viscosity determines the transition from electrospraying to electrospinning. A higher viscosity provides higher cohesion and entanglements between polymeric chains. In the case of electrospinning, these promoted cohesion and molecular entanglements prevents the jet from breaking into droplets and thereby, a stable elongated jet can be obtained resulting in ultrathin fiber formation. On the other hand in the case of electrospraying, the jet becomes unstable due to low viscosity and fine highly charged droplets are formed as a result of the fragmentation of jet. In other words, continuous fibers will be formed when solution viscosity or polymer concentration is high, whereas the low viscosity or concentration will lead to formation of particles [113]. However, for the electrospining, on the extreme conditions where the viscosity of the solution is beyond the critical point (at which beadless continuous fibers are formed), the flow of the solution could be impeded, which results in defective nanofiber [114].

Surface tension

Surface tension plays a critical role in the electrohydrodynamic processes since it affects the initial drop formation on the tip of the nozzle. Since surface tension is the primary force that opposes the electrostatic forces generated by the applied voltage; and therefore, it influences the general electrospinnability and electrosprayability of the liquid polymeric solutions [115]. For electrospinning, the formation of droplets, beads and fibers is dependent to surface tension of the solution; and usually, beaded structures are formed when the surface tension of the solution is dominant over the electrical stress [115]. Also, lower surface tension of the solution enables the electrohydrodynamic processes to be carried out at lower electric fields [116]; yet, it is worth mentioning that this is not always linearly.

Conductivity

The electrical conductivity of solution is a critical element for electrohydrodynamic processes. In order to charge the surface of the liquid, the solution has to be conductive, otherwise electro-spinning/spraying will not occur. In the case of electrospinning, a higher electrical conductivity of the solution will lead to higher elongation of the jet, which results in smaller fiber diameter [117]. However, the conductivities beyond a critical level will hamper the stable Taylor cone formation and thus, leads to insufficient elongation and bead formation. For the electrospraying process, the higher electrical conductivity of the solution results in smaller electrosprayed particle size due to the further disentanglements in the polymer chain network; yet excessive high conductivity will lead to elongated particles and Coulomb fission [118]. Additionally, the electrical conductivity of the solution can be improved by the addition of ionic salts (e.g. NaCl, NaH_2PO_4, KH_2PO_4, etc.) [119].

Applied voltage

Applied voltage is an electrohydrodynamic process parameter that affects the resultant product size. For the electrospinning, an increasing applied voltage results in the formation of smaller-diameter fibers due to the stretching of the polymer solution by the increased repulsion forces within the polymer jet [120]. However, increasing the voltage beyond a certain level may draw more material out of the syringe; and as a consequence, formation of larger fiber diameter, as well as the beaded fibers, occurs [121]. In terms of electrospraying, morphological changes of the particles are more noticeable. With the increased voltage, spherical particles can transform into an elongated shape due to the stretching of highly charged particles; and also, these spherical particles could even form bead fibers in the case of high polymer concentration [118].

Flow rate

Flow rate is the another important process parameter as it determines the jet velocity and the materials transfer rate. In order to keep Taylor cone stable during the process, flow rate should be maintained at the end of the needle. In electrospinning, lower flow rates generally yield fibers with smaller diameters [122]. Similarly in the electrospraying method, small particle size can be achieved by using low flow rates, and vice versa [118]. For both cases, too high flow rates cause the solution not to dry before reaching the collector.

Tip to collector distance

The distance between the needle tip and collector has a great influence on the morphology of electrospinning and electrospraying products. The larger tip-to-collector distance corresponds the longer flight time of the electrospinning or electrospraying jet; and thereby, the jet finds more time to stretch and elongate before reaching to the collector. As the distance increases, fiber diameter and the average particle size decreases for the electrospinning and electrospraying processes, respectively [118,123]. It should also be pointed that a shorter distance may lead to the coalescence or aggregation of the structures due to the insufficient solvent evaporation time, while a long distance could result in the weakening of the electrical field [124].

Humidity and temperature

The relative humidity and temperature are the environmental parameters that can influence the sizes and morphologies of electrohydrodynamic process products. The evaporation rate of the fluid jet is decreased at higher relative humidity, so the charged jet continues to elongate [125]. However, the effects of relative humidity on the morphology are dependent on the chemical nature of the polymer [126]. Temperature on the other hand, causes two opposing effects: (i) it increases the rate of evaporation of the solvent, and (ii) it decreases the viscosity of the solution [126].

Encapsulation of bioactives through electrospinning

Over the last few years, electrospinning process has recently attained significant attention and found wide range of applications in the food industry from functional foods to active packaging for its ability of production of micro- and nano-sized fibers. It is a simple, straightforward, and versatile technology that allows the production of fibers with high surface-to-volume ratio and porosity [127]. Comparing to other common encapsulation techniques (e.g. spray drying, freeze drying, etc), the biggest advantages of electrospinning processes in the encapsulation of bioactives are that (i) it is very cost effective method, (ii) the incorporation of bioactive compounds into micro-, nano-fibers is easy, (iii) high encapsulation efficiencies (generally above 80%) can be achieved, (iv) it provides greater thermal, light and storage stabilities with enhanced protection against chemical degradation, (v) size and surface morphology of the electrospun fibers can be adjustable by changing the process parameter, (v) release mechanisms are adjustable via tuning the porosity, alignment, and size of electrospun fiber, (vi) potential use of aqueous solutions which are for toxicity concerns, and (vii) it does not require high temperatures, which is a key factor for preserving the thermo-sensitive compounds during processing, and (viii) fibers can alter the texture and mouthfeel of the food; however, they could be used in products such as chocolates, or cereal bars) [112,127-130].

Recently in the literature, electrospinning has been widely used for the encapsulation of bioactive substances due these mentioned advantages. It is a well-known fact that electrospinning process provides significant resistance to entrapped bioactives against heat and light, and also can improve the solubility of bioactives. For example, de Freitas Zômpero *et. al.* (2015) demonstrated that the bioactive entrapment through electrospinning may provide significant photo-resistance/stability. In this study, a hydrophobic carotenoid, β-carotene, was incorporated into water-based food formulations by the encapsulation through electrospinning method [131]. For this, hybrid encapsulation structures were developed, which consisting of the β-carotene loading into nanoliposomes, and then the encapsulation of β-carotene-loaded nanoliposomes within PVOH and PEO electrospun fibers. They reported that β-carotene-loaded nanoliposomes were successfully incorporated within PVOH and PEO electrospun fibers and the developed hybrid encapsulation structures exhibited significant UV photo-stability of β-carotene. After 6 h of UV exposure, 86.8% and 80.3% of initial β-carotene remained intact inside the PEO and PVOH nanofibers, respectively; while only 32.9% remained in β-carotene-in-hexane solution. Additionally, it was highlighted in this study that the developed hybrid structure enables the incorporation of β-carotene into water-based food formulations, overcoming the existing limitations associated with its hydrophobic character. Besides, no-heat-required electrospinning is also a very powerful tool for encapsulation of thermo-labile compounds. In this regard, Fahami *et al.* (2018) encapsulated Vitamin A into Cress Seed Mucilage (CSM)/Poly Vinyl Alcohol (PVA) matrices using electrospinning with a very high encapsulation efficiency of 97% and fiber diameter range of 90.25-169.95 nm [132]. It was reported that thermal stability of Vitamin A has been increased by encapsulation, so that it could be used in high temperature food processes. Also, a lower release rate of Vitamin A in gastric fluids was achieved. An another example, Moreira *et al.* (2019) encapsulated a heat sensitive substance, phycocyanin, into protein concentrate from Spirulina sp. LEB 18 and PEO electrospun fibers in nanometer scale [133]. According to the results, electrospinning encapsulation delayed the thermal degradation of phycocyanin (at 213.2 °C) by 30 °C. Also, developed phycocyanin-loaded electrospun structures exhibited good antioxidant properties. As discussed earlier, the health benefits of bioactives are highly dependent on their water solubility, which is directly linked to their bioavailability. Horuz *et al.* (2018) encapsulated low-water soluble carotenoid extracts into gelatin nanofibers by using electrospinning technique [134]. It was shown that nanofibers (around 230 nm in diameter) were obtained with a high encapsulation efficiency of >90% and the water solubility of the carotenoid extract was enhanced highly through nanoencapsulation method. The percentages of dissolution in water were 0.89% and 99.08 for the non-encapsulated and gelatin-encapsulated carotenoids, respectively. Additionally, the thermal stability as well as the antioxidant activity of the bioactive was improved. Also, as an encapsulation example of living microorganisms, which is often a very challenging task and it is crucial to maintain their viability during the process, Feng *et al*. (2018) encapsulated the probiotic, *Lactobacillus plantarum,* into Fructooligosaccharides (FOS) and PVA fibers to enhance their viability and thermal stability [135].Results showed that comparing to non-encapsulated ones, the survivability of probiotics encapsulated in FOS/PVA nanofibers was remarkably enhanced under heat treatments (60 and 70 °C).

In many applications, long-term or controlled release systems are required to enable the efficient use of bioactives, and this can be achieved with additional multiple functions by electrospinning. In this regard, Wang *et al.* (2020) fabricated a electrospun multilayer film with ethycellulose nanofibers as the outer layer and curcumin-loaded (polyphenol) gelatin nanofibers as the inner layer [136]. It was reported that multilayer film exhibited a sustained release of the encapsulated curcumin for 96 h, comparing to the burst release of the gelatin films (30 min). In addition, the developed multifilm showed enhanced antioxidant activity up to 96 h, as well as significantly improved thermal stability, owing to the hydrogen bonds between two adjacent layers. As another example by Ghitescu *et al*. (2018), a natural polyphenolic antioxidant, catechin, encapsulated in Poly(L-Lactide-Co-Glycolide) (PLGA) by emulsion electrospinning to form bicomponent fibers consisting of catechin within the core, and PLGA within the sheath [137]. According to in vitro catechin release studies, developed electrospun structures exhibited moderate burst release kinetics in the first phase, and followed by a linear sustained release at long term which is driven by a diffusion-controlled mechanism. It was also highlighted that electrospinning techniques are an effective tool for encapsulation and controlled release of functional bioactive substances. Also in electrospinning process, it is possible to tailor the release mechanism of the relevant bioactive compounds by changing the process parameters. In this sense, Altan *et al.* (2018) developed fibrous carvacrol-loaded zein and PLA composite films using electrospinning technique [138].According to the results, carvacrol-loaded zein and PLA fibers exhibited good antioxidant activity; and inhibited the growth of mold and yeast by 99.6% and 91.3%, respectively. More importantly, they reported that the release mechanism of bioactive-encapsulated electrospun structures has been influenced significantly by the surface morphology of the produced fibers which can be tunable with electrospinning parameters. Furthermore, along with the process parameters, the addition of chemical agents into the electrospinning solution can enhance and/or alter the release profile of bioactives. In an interesting study by Deng *et al.* (2017), the effects of surfactants on the morphology electrospun fibers, and on the release behaviour of encapsulated bioactive were investigated [139]. For this, electrospinning technique was utilized to encapsulate curcumin in gelatin fibers with the corporation of different surfactants (Tween-80, SDS, and CTAB). It was reported that the addition of SDS surfactant increased the fibre diameter and inhibited the release curcumin from the nanofibers. However, the surfactants Tween-80 and CTAB both greatly improved the release of curcumin. It was highlighted that the production of gelatin nanofibers with food-grade surfactants using electrospinning technique could offer a new way for the controlled release of curcumin in food applications. Additionally, electrospinning not only enables the prolonged delivery of bioactives, but can also provide burst release for the purposes of different food applications such as flavoring. For example, Rezaeinia *et al.* (2020) developed fast-dissolving electrospun mats that consist of Bergamot Essential Oil (BEO) loaded balangu seed gum and Polyvinyl Alcohol (PVA) fibers [140]. According to in vitro release studies, approximately 90% of BEO was released in simulated black tea beverage media within a short period of time (90s). These results were suggested as a novel strategy for enhancing the flavor in the food system, and also for overcoming the limitations of low-water soluble components, particularly volatile compounds and flavoring agents.

Many efforts have been made to preserve foods from deterioration and nutritional value loss. In this context, one of the most important aspects to consider is the antioxidant and antimicrobial properties of functional foods. In 2018, Li *et al.* encapsulated butylated hydroxyanisole (antioxidant) in gelatin fibers via electrospinning technique and investigated the strawberry preservation performance [141]. , They reported that butylated hydroxyanisole was successfully encapsulated in gelatin fibers with high encapsulation efficiency (above 90%) and the developed electropun structure exhibited enhanced antibacterial and antifungal activity against *Staphylococcus aureus* and four mould genera (*Rhizopus sp., Mucor sp., Aspergillus sp. and Penicillium sp*.), respectively. More interestingly, the "application to strawberries" test revealed that the developed fibrous structure improved the shelf-life of strawberries effectively up to 10 days, comparing to the shelf-life of control group (only strawberries) decaying in 4 days. Thus, the electrospun structures developed in this study has shown its great potential in active food packaging. In another study by Figueroa-Lopez *et al.* (2019), Oregano Essential Oil (OEO), Rosemary Extract (RE), and Green Tea Extract (GTE) were encapsulated in PHBV ultrathin fibers using electrospinning technique [142]. The antibacterial and antioxidant tests showed that OEO-containing PHBV films exhibited the highest antimicrobial activity against two strains of food-borne bacteria (*Staphylococcus aureus* and *Escherichia coli*), which was attributed to the presence of carvacrol and thymol bioactive substances in OEO. Additionally, PHBV (wall material) provided enhanced the thermal stability up to 200 °C. The developed structure has been suggested as an active food packaging material which provides the prolonged shelf-life in biopackaging applications. In another interesting study, Melendez-Rodriguez *et al.* (2019) developed electrospun antimicrobial films [143]. For this, eugenol (essential oil) was firstly encapsulated in the pores of mesoporous silica nanoparticles by vapor adsorption; then this was followed by the encapsulation of eugenol-containing nanoparticles into Poly(3-Hydroxybutyrate-Co-3-Hydroxyvalerate) (PHBV) fibers using electrospinning technique. Results showed that electrospun PHBV films filled with eugenol-loaded nanoparticles were developed with a fiber diameter of around 650 nm, and exhibited improved thermal resistance, mechanical strength, as well as enhanced water vapor barrier performance. Additionally, the antimicrobial assays showed that the developed structures inhibited the bacterial growth of *Staphylococcus aureus* and *Escherichia coli,* while sustaining the antibacterial activity even after 15 days. Solaberrieta *et al.* (2020) encapsulated different concentrations (5, 10, and 20 wt%) of *Aloe Vera* Extracts (AVE) from agrowastes in electrospun Poly(Ethylene Oxide) (PEO) nanofibers and investigated the antioxidant activity with three different assays (DPPH, FRAP and ABTS) [144]. High encapsulation efficiencies for 5 wt% AVE content were reported as 92%, 76%, and 105% according to DPPH, FRAP, and ABTS assays. More importantly, the total antioxidant activity of nanofibers were found to be increased significantly with increasing AVE loading, and maintained during the electrospinning process. Also, the capability of electrospinning in encapsulating thermolabile bioactive compounds and preserving their antioxidant activities during the process was emphasized. Another interesting study by Figueroa-Lopez *et al.* (2018), a high oxygen barrier Gelatin films (GEL) were coated with black pepper Oleoresin (OR) containing electrospun Polycaprolactone (PCL) mats with the aim of enhancing the water resistance and antimicrobial activity of gelatin films [145]. Results showed that the developed multilayer system increased thermal resistance

of GEL films, and significantly diminished its permeance to water vapor. Additionally, the multilayer exhibited a strong antimicrobial activity against *Staphylococcus aureus* by providing a controlled release of the active components for 10 days after processing. Thus, as it can be seen from these studies, one can expect that these materials produced with electrospinning can be applied as active/intelligent food packaging systems with fortified abilities to prolong the shelf-life and delay the proliferation of microorganisms and oxidation of foods.

Since the encapsulation via electrospinning is gaining significant attention from researchers, these examples are quite abundant in the recent literature. To give more examples, Aceituno-Medina *et al.* (2015) enhanced photo-stability of Vitamin B9 by entrapping it into Amaranth Protein Isolate (API): Pullulan using electrospinning [146]. No degradation of the encapsulated compounds was reported after 2 hours of UV exposure. Furthermore, Isik *et al.* (2018) encapsulated polyphenols from Sour Cherry Concentrate (SCC) into gelatin-lactalbumin using electrospinning technique [147]. They reported a significant improvement in bioaccessibility of SCC. According to *in vitro* digestion studies, electrospinning encapsulation exhibited 8 times better protection of SCC compared to the non-encapsulated ones. In 2016, Yilmaz *et al*. reported a potential use of curcumin-loaded zein nanofibers as edible and biodegradable antifungal surface-coating [148]. The antifungal activity tests revealed that developed nanofibers efficiently inhibited *Botrytis cinerea* and *Penicillium expansum* on the coated apple. Yang *et al.* (2017), investigated the influence of coaxial and single electrospinning on the encapsulation of fish oil [149]. They reported that comparing to single electrospinning, coaxial electrospinning encapsulation showed significantly better thermal and oxidative stability; and also, shelf life of coaxially encapsulated fish oil was 65 days longer. Maria Leena *et al.* (2020) developed an effective nanofibrous oral delivery system for resveratrol as an edible coating on processed foods [150]. An improved bioaccessibility, as well as controlled release of encapsulated resveratrol was reported. Table 2 summarizes given recent (2015-2018) examples of the electrospinning applications for the encapsulation of bioactive compounds.

Table 2: Recent (2015-2020) examples of bioactive encapsulation by electrospinning technique.

Bioactive ingredient(s)	Wall material(s)	Solvent(s)	Structure diameter, nm	EE, %	Highlights	Reference
β-carotene	PVOH, PEO	Water	Not specified	Not specified	• Enhanced UV stability • Enhanced aqueous solubility	[131]
Vitamin A	Cress seed mucilage (CSM), PVA	Water: Acetone (70:30), water	90 – 1700	97	• Improved thermal stability • Increased acidic stability • Lower release rate (diffusion driven) in gastric fluids	[132]
Phycocyanin	Protein concentrate from Spirulina sp. LEB 18, PEO	Acetic acid: Water (3:1)	269 ± 55 – 542 ± 126	Not specified	• Increased thermal stability • Higher antioxidant activity	[133]
Lycopene, β-carotene	Gelatin	Aqueous acetic acid	129 ± 26 – 246 ± 37	>90	• Improved thermal and storage stability • Enhanced antioxidant activity • Excellent water solubility	[134]
Probiotic (*Lactobacillus plantarum*)	Fructooligosaccharides (FOS), PVA	Water	410 ± 150	Not specified	• Enhanced probiotic survivability • Improved thermal stability	[135]
Curcumin	Ethylcellulose, gelatin	Acetic acid (50%), acetic acid/ethanol/water (7:2:1)	184 ± 51 – 649 ± 318	89.05 – 92.57	• Decreased water vapor permeability and increased water contact resistance • Enhanced thermal stability • Sustained release • Maintained antioxidant activities	[136]
Catechin	PLGA	Water, chloroform	634 ± 203 – 708 ± 367	Not specified	• Tailorable release profile • Initial burst release followed by linear long-term release (diffusion driven) • Good antioxidant activity	[137]
Carvacrol	Zein, PLA	Ethanol (80%), Chloroform/DMF (9:1)	539 ± 103 – 647 ± 113, 1822 ± 500 – 2268 ± 395	Not specified	• Good antioxidant activity • Inhibited growth of mold and yeast • Enhanced sustained release • Influence of surface morphology on release mechanisms	[138]
Curcumin	Gelatin	Acetic Acid	205 ± 64 – 368 ±39	Around 95	• Influence of added surfactants on morphology, release mechanisms, antioxidant/microbial properties • Enhanced release profile • High antioxidant and microbial activities	[139]
Bergamot essential oil (BEO)	Balangu seed gum (BSG), PVA	Water	218 ± 14 – 486 ± 38	95.23	• Fast-dissolving structures were developed for flavoring purposes • Burst release (Fickian diffusion) achieved	[140]
Butylated hydroxyanisole (BHA)	Gelatin	acetic acid: Ethanol: Water (3:2:3)	655 ± 6 – 848 ± 5	>90	• Enhanced antibacterial and antifungal activities • Greatly improved shelf-life of strawberries	[141]

Oregano Essential Oil (OEO), Rosemary and Green Tea Extracts (RE, GTE)	PHBV	Chloroform: 1-butanol (75:25)	800 – 1000	Not Specified	• Enhanced antimicrobial and antioxidant activities (highest was found for OEO) • Improved thermal stability	[142]
Eugenol essential oil	PHBV, mesoporous silica	TFE	630 ± 180 – 670 ± 240	Not Specified	• Improved thermal resistance, mechanical strength • Enhanced water vapor barrier performance • Improved and maintained antibacterial activity	[143]
Aloe Vera Extract (AVE)	PEO	Water	185 ± 25 – 250 ± 30	92, 76, 105	• Improved and maintained antioxidant activity	[144]
Oleoresin extract (OR)	PCL, gelatin	Chloroform: Butanol (75:25)	~700	Not Specified	• Gelatin films coated with OR-loaded PCL fibers • Improved thermal resistance • Decreased water vapor permeability • Strong antimicrobial activity • Provided controlled release	[145]
Vitamin B9	Amaranth protein isolate (API), pullulan	Formic acid (95%)	305 ± 71 – 377 ± 101	>95	• Enhanced thermal stability • Excellent photostability	[146]
Polyphenols	Gelatin (uniaxial), gelatin/lactalbumin (coaxial)	Acetic acid (50%)	Not Specified	70.3 – 91.3	• Great bioaccessibility in coaxial fibers	[147]
Curcumin	Zein	Ethanol (85%)	<350	82.4 – 86.7	• Great antifungal activity	[148]
Fish oil	Zein (core), PVP (shell)	Ethanol (80-100%)	560	96.9	• Great thermal and oxidative stability in coaxial nanofibers • Improved shelf-life	[149]
Resveratrol	Zein	Ethanol (80%)	404	96.9	• Improved bioaccessibility • Controlled release (diffusion driven)	[150]

Encapsulation of bioactives through electrospraying

Similar with the electrospinning, electrospraying is a promising technique for production of polymeric micro- and nanoparticles (spheres or capsules) for the encapsulation of bioactives. Electrospraying process possesses similar advantages with electrospinning over the other encapsulation technologies in terms of ease of use, cost-effectiveness, high encapsulation efficiencies, greater thermal, light, and storage stabilities, adjustable size, morphology and release profiles, potential use of water as solvent, non-requirement of high temperatures, etc. Along with these similar benefits, electrospraying offers the following additional advantages, such as (i) the production of small-sized particles with submicron diameters, (ii) narrow size distribution, (iii) ability of non-agglomeration of charged droplets due to repulsion forces, (iv) controllability of the particle shape [112,118,151].

Over the last years, electrospraying-based encapsulation methods have been utilized widely in many industries, since it provides the important properties sought in the encapsulation of bioactive area, such as high physicochemical/thermal/photo-stability, controlled release, high bioavailability, targeting precision of bioactive compounds, high loading efficiency, masking of undesired taste/odor, high particle deposition rate, and improved shelf-life [112,118]. In the context of preserving bioactives from different adverse conditions, Alehosseini *et al.* (2019) utilized electrospinning and electrospraying techniques to encapsulate bioactive compounds of a saffron extract (picrocrocin, safranal, and crocin) using zein as a wall material, and investigated the stability of active compounds under the exposure of UV light, pH, and temperatures [152]. According to the results, a successful encapsulation with high encapsulation efficiencies (74-97%) was achieved for all cases. The photostability of crocin and safranal was significantly increased with encapsulation through both electrospinning and electrospraying. Additionally, only 67.23% of the encapsulated crocin was degraded after 15 h of exposure to acetic acid at 75 °C, while non-encapsulated compound was almost lost under the same conditions. Overall, electrosprayed and electrospun zein structures were suggested as capable of improving the stability of saffron-derived bioactive compounds at different pH values (pH 2 and 7.4), different storage temperatures (20 and 75 °C), and upon UV exposure. Similarly electrospraying, as the "sister" method of electrospinning, does not require high temperatures for processing and hence, it allows successful encapsulation and protection of thermo-labile compounds. In this sense, many thermo-labile bioactive compounds have been encapsulated using electrospraying technique. For example, Schmatza *et al*. (2020) performed electrospraying technique to encapsulate a thermo-labile compound, phycocyanin, into PVA polymeric matrix [153]. The results showed that the encapsulation of phycocyanin was successful with high encapsulation efficiency (75%), and particles with a mean diameter of 395 ± 71 nm were developed. They also reported that the developed ultrafine particles provided greater thermal protection of phycocyanin up to 216 °C, while maintaining its antioxidant activity.Thus, this study highlights the efficiency, unreported in the literature previously, of using electrospraying technique as an alternative method to achieve phycocyanin encapsulation. Also, in the recent study of López de Dicastillo *et al.* (2019), a temperature sensitive polyphenol, anthocyanin (flavonoid), was encapsulated into zein particles through electrospraying technique [154]. It was shown that the thermal stability of anthocyanin was enhanced significantly and the thermal degradation starting at 100 °C was delayed up to 270 °C. In another study, D-limonene, which is highly susceptible to oxidation and volatilization under normal storage conditions, encapsulated through electrospraying in AHSG nanocapsules by Khoshakhlagh *et al.* (2018) [155]. It was showed that the nanocapsules provided a good storage stability

and maintained 91.2 and 82.4% of the loaded D-limonene during 90 days of storage at 4 °C and 25 °C, respectively.

As discussed before, antioxidant and antimicrobial properties of bioactive compounds are key parameters that must be considered. A well-established antioxidant and antimicrobial activities in the functional food products may enable many important sought-after features in the food industry, such as storage stability, active packaging, etc. In this context, Gomez-Estaca *et al.* (2017) encapsulated a water-insoluble bioactive compound, curcumin, into gelatin through electrospraying technique [156]. According to the results, curcumin-loaded spherical gelatin particles were produced with a very high encapsulation efficiency (~100%). Comparing to the raw curcumin, the water solubility of encapsulated curcumin increased 38.6-fold. Along with the improved antioxidant activity, gelatin-encapsulated curcumin exhibited outstanding antimicrobial activity against *L. monocytogenes*, *S. enterica*, *S. aureus*, and *E. Coli*, by reducing the microbial population by 2.08, 1.67, 2.70, and 2.18 log counts (CFU/ml), respectively. Another study by Gomez-Mascaraque *et al*. (2015), an antioxidant molecule, (-)-Epigallocatechin Gallate (EGCG), was encapsulated in food-grade gelatin with a very high encapsulation efficiency (around 100%) via electrospraying technique [157]. More importantly, during the 100 h of storage study, it was shown that the antioxidant activity of encapsulated EGCG retained completely, while free EGCG lost 30% of its activity. Additionally, encapsulated EGCG exhibited a delayed release in aqueous solutions. In 2020, Asadi *et al.* encapsulated curcumin into Walnut Protein Isolate (WPI) nanoparticles using electrospraying [158]. They reported that the developed nanoparticles exhibited remarkably high antioxidant activity in intestine digestion process compared to gastric digestion. As another example, Stoleru *et al.* (2016) developed a dual-bioactive layer based on electrospraying coating of Vitamin E-loaded chitosan onto surface of PE films [159]. According to results chitosan/Vitamin E modified PE multilayer system inhibited the growth of Gram-negative and Gram-positive bacterial strains and presented good antioxidative properties.

Bioavailability is the key phenomena in ensuring bioefficacy of bioactive substances, which is directly linked with the permeability and solubility. Recently, electrospraying technique has shown its potential in improving the bioavailability and bioaccessibility of the encapsulated bioactive ingredients. For example in the study of Jayan *et al*. (2019), resveratrol (polyphenol) was encapsulated in zein particles [160]. They reported that nanoencapsulation in zein protein protected resveratrol in high acidic conditions of stomach and enabled its release in the intestinal region indicating improved bioaccessibility of resveratrol. Additionally in the intestinal permeability studies, encapsulated resveratrol compounds showed improved effective permeability (1.15-fold) compared to unencapsulated resveratrol, which also indicates the improved bioavailability. In another study, Gomez-Estaca *et al*. (2015) developed curcumin-loaded gelatin microparticles using electrospraying technique [161]. They reported that comparing to commercial curcumin, the water solubility and bioaccessibility of encapsulated curcumin was significantly enhanced for 38.6-fold and 11.3-fold, respectively. Also, microencapsulation improved the coloring capacity of curcumin and provided a better dispersion in a gellified fish product. Furthermore in another study, Gomez-Mascaraque *et al.* (2017) demonstrated that the bioaccessibility of β-carotene can be improved by its encapsulation within zein and Whey Protein Concentrate (WPC) microstructures using electrospinning technique [162].

By looking these studies, it is evident that electrospraying technique has a great potential in bioavailability improvement purposes. Also, compared to other encapsulation techniques, it is a known fact that electrospraying can provide a very high surface area meaning better permeability and bioavailability. In this context, Mahalakshmi *et al*. (2020) conducted a comparative study consisting of encapsulation of β-carotene with zein in micro and nano levels by spray drying and electrospraying techniques, respectively [163].Results showed that higher encapsulation efficiency of 81% was achieved in the nanoencapsulation form of β-carotene through electrospraying. More importantly, nanocapsules produced with electrospraying exhibited faster release under simulated in-vitro gastrointestinal conditions, as well as 1.7-fold increased permeability in ex-vivo everted gut sac than the microparticles produced with spray drying. Hence, the nanoparticles produced with electrospraying provided enhanced permeability and bioavailability of β-carotene.

Similar to electrospinning, electrospraying technique enables the design of active-releasing particles with tailorable release profile, as well as precision targeting. As an example of this, Zhao *et al.* (2016) encapsulated *Ganoderma lucidum* Spores (GLS), which is a functional food susceptible to oxidation and acidic degradation, within alginate polysaccharides through electrospraying method in order to preserve the bioactivity of GLS and provide its controlled/targeted release [164]. Their results showed that alginate encapsulation system prevented the release of GLS components under simulated gastric conditions and provided sustained release behaviour in simulated intestinal environment. They also reported that the release mechanism of GLS can be further adjusted by changing the sizes of GLS-alginate particles with electrospraying process parameters. In another study, Nguyen *et al.* (2018) produced electrosprayed PLGA microspheres with a narrow size distribution (1-4 μm) for prolonged release of quercetin from the developed structures [165].The encapsulation efficiency and loading capacity of quercetin was found as 81.84% and 7.77%, respectively. Importantly, they reported that a long-term release of quercetin was achieved for 30 days *in vitro* with little evidence of burst release. Yuan *et al.* (2015) utilized a coaxial electrospraying approach to encapsulate curcumin (core) in PLGA (shell) for sustained release in pharmaceutical applications [166]. It was reported that single electrosprayed structures released about 60% and 100% of initial curcumin content in 5 and 30 days, respectively; whereas coaxially electrosprayed structures released only about 30% and 70% in 5 and 40 days respectively. This study was highlighted as an example of the effect of electrospraying method on the release profile with its flexibility to be modified.

A different encapsulation strategy, named Electrospraying Coating (EC), and patented by Lagaron *et al.* [167], was also proved itself to be highly efficient in terms of encapsulation of bioactives. In this regard, Ramos-Hernandez *et al.* (2018) encapsulated β-carotene into high degree of polymerization agave fructans (HDPAF) by direct electrospraying and EC [168]. The results showed that the EC method, which is a three-step process, yielded nanoparticles with the mean particle diameter of 650-760 nm. More importantly, the encapsulation in HDPAF by the EC method improved the photostability of β-carotene dramatically; β-carotene remained stable for up to 50 h of UV light exposure. It was emphasized that the EC method improved bioactive/polysaccharide ratios along with the photostability. As another interesting example of encapsulation by electrospraying coating technique, Libran *et al.* (2017) encapsulated probiotics (*Bifidobacteria longum* subsp. *infantis*) into different poly-

mers (maltodextrin, Fibersol, Whey Protein Concentrate (WPC), PVP, zein) to investigate the influence of EC method on the long-term survivability of probiotics [169]. The results showed that EC technology provided significantly longer survivability of the encapsulated probiotics that reached more than 1 year at room temperature and 23% relative humidity, especially for WPC, Fibersol and PVP.

Another very promising electrospraying modification approach, an innovative process termed high- throughput Electrospraying Assisted By Pressurized Gas (EAPG) has been developed recently, which is based on the combination of electrospraying with the pneumatic atomization [170]. In this context, Busolo *et. al* (2019) encapsulated DHA-enriched fish oil into zein by using, for the first time, EAPG technology [67]. Results showed that successful encapsulation of DHA-enriched fish oil was achieved with high encapsulation efficiency of 84% and mean capsule diameter of 1.4 µm. Also, developed microcapsules exhibited significantly high stability during 45 days of different conditioned storage time. As an another example, Prieto *et al.* (2020) encapsulated docosahexaenoic acid-enriched algae oil into Whey Protein Concentrate (WPC) and maltodextrin, and comparatively investigated the EAPG-produced particle characteristics in terms of morphology, encapsulation efficiency, oxidative stability and organoleptic properties [171]. According to the results, spherical encapsulating particles were obtained with sizes around 5 µm and >65% encapsulation efficiency. Furthermore, oil-loaded WPC microparticles exhibited the best antioxidant stability (especially at low oil loading ratios), whereas the maltodextrin microparticles were more susceptible to oxidation. Organoleptic test results also showed that as a result of oxidative stability, the lowest sensory difference was found for WPC microcapsules. As it can be seen, both electrospinning and electrospraying are very versatile techniques that make available a lot of strategies of interest in a wide range of food applications. These examples are quite numerous and diverse in the recent literature of encapsulation bioactives. Table 3 summarizes given recent (2015-2020) examples of the electrospraying applications for the encapsulation of bioactive compounds.

Table 3: Some examples of bioactive encapsulation by electrospraying technique.

Bioactive ingredient(s)	Wall material(s)	Solvent(s)	Structure diameter, nm	EE, %	Highlights	Reference
Picrocrocin, Safranal, Crocin	Zein	Ethanol (80%)	590 ± 298 – 330 ± 217 (capsule) 290 ± 128 – 210 ± 87 (fiber)	82 – 94 (capsule) 74 – 97 (fiber)	• Enhanced termo-, photo-, and heat stabilities • Great pH stability • Improved release profile (diffusion driven)	[152]
Phycocyanin	PVA	Water	395 ± 71	75.1	• Improved thermal resistance • Antioxidant activity maintained	[153]
Açaí Fruit extract (Polyphenols)	Zein	Ethanol (80%)	924	70	• Great thermal stability • Improved protection during *in vitro* digestion process	[154]
D-limonene	*Alyssum Homolocarpum* Seed Gum (AHSG)	Water	65.7 ± 9	73.4	• Improved storage stability (at 4, 25 °C)	[155]
Curcumin	Gelatin	Ethanol (96%), acetic acid (50%)	200 – 1000	Around 100	• Significantly improved aqueous solubility • Great antimicrobial activity	[156]
(-)-Epigallocatechin Gallate (EGCG)	Gelatin	Acetic acid (20%)	230 – 490	Around 100	• Improved and maintained antioxidant activity • Delayed release in aqueous media	[157]
Curcumin	Walnut Protein Isolate (WPI)	Water	142 ± 38	61.5	• Improved antioxidant activity • Enhanced solubility and bioavailability	[158]
Vitamin E	Chitosan, PE	Acetic acid (70%)	Not Specified	Not Specified	• Improved antibacterial, antioxidant, and pH responsive activities	[159]
Resveratrol	Zein	Ethanol (80%)	237 – 334	68.49 – 38.93	• Improved permeability and bioaccessibility • Protection and sustained release in gastric and intestinal conditions, respectively	[160]
Curcumin	Gelatin	Ethanol (96%), acetic acid (50%)	150 – 1200	100	• Improved water solubility • Greatly enhanced bioaccessibility • Enhanced antioxidant activity	[161]
β-carotene	Zein, Whey Protein Concentrate (WPC)	Water, ethanol (80%)	750 ± 300 – 1340 ± 770	26 – 74	• Enhanced bioaccessibility	[162]

β-carotene	Zein	Ethanol (80%)	599 – 906	67.37 – 81.76	• Better bioaccessibility, permeability, solubility and release profile comparing to spray drying	[163]
Ganoderma Lucidum Spores (GLS)	Alginate	Water	400000 - 2400000	Not Specified	• Prevented and sustained release in gastric and intestinal media, respectively. • Tailorable release profile with different process parameters	[164]
Quercetin	PLGA	Acetone (100%), acetone:DMF (9:1)	1000 – 4000	81.84	• Excellent prolonged release with little burst release • No toxic effect to INS-1 cells	[165]
Curcumin	PLGA	Ethyl acetate, acetone	2250 – 3940	80.48 – 95.42	• Controlled and prolonged release with coxial electrospinning • Higher encapsulation efficiency with coaxial electrospraying	[166]
β-carotene	High Degree Of Polymerization Agave Fructans (HDPAF)	Water:ethanol (9:1)	650 – 760	Not Specified	• A novel electrospraying coating (EC) technique utilized • Excellent photostability in EC nanoparticles • Improved thermal stability and bioactive/polymer ratio	[168]
Probiotic strain (*Bifidobacteria longum* subsp. *infantis*)	Maltodextrin, Fibersol, Whey Protein Concentrate (WPC), PVP, zein	Water, skimmed milk, ethanol (85%)	1950 – 2870 (WPC, Fibersol, maltodextrin) 150 ± 1 (PVP)	Not Specified	• Significantly improved long-term survivability with electrospraying coating (EC) technique	[169]
DHA-enriched fish oil	Zein	Ethanol (85%)	1400 ± 800	84	• High throughput process (EAPG) • Significantly high storage stability with different temperatures and relative humidity	[67]
DHA-enriched algae oil	Whey Protein Concentrate (WPC), maltodextrin	Water	5600 ± 2600 (WPC) 3800 ± 1800 (Maltrodextrin)	>65	• High throughput process (EAPG) • Improved oxidative stability • Masked sensory properties	[171]

Conclusions and future prospects

A new generation of food products with health benefits is increasingly being required by the food manufacturers to satisfy the demand of the consumers. However, the application of highly-labile bioactive compounds in food products is a very challenging process, and thus, it is crucial to protect them in order to maintain and deliver their health-promoting benefits.

Encapsulation is a very prominent way to protect and deliver bioactive compounds in micro- or nano-scale during the development of functional foods. A successful application of micro- and nano- encapsulation ultimately depends on the selection of carrier materials, formulation of the encapsulated delivery system, and proper encapsulation techniques. It is also crucial to consider the desired properties of the final products in parallel with the nature of bioactive compound to be encapsulated. As discussed before, there are many methods for the encapsulation of bioactives. Among them, this chapter addressed the role of electrohydrodynamic methods in the encapsulation applications, with special focus on the studies carried out in recent literature from 2015.

The emerging electrohydrodynamic processes, namely electrospinning and electrospraying, are gaining popularity in encapsulation of bioactives applications. As demonstrated in the recent studies, these techniques provide substantial advantages over other encapsulating techniques, such as simplicity, cost-effectiveness, high encapsulation efficiency, excellent release profile, enhanced thermal, light and oxidative stability, ability to produce micro-/nano- sized products, etc. However despite these advantages, one major limitation of electrohydrodynamic techniques is that it has a low processing throughput due to the use of single needle producing typically a few milliliters per hour. Still, the developments in instrumentation and process design (e.g. multi-needle or multiaxial arrangements) could offer the possibility of scale-up. In this sense, an innovative high-throughput Electrospraying Assisted by Pressurized Gas (EAPG) has been developed recently by Lagaron *et. al.* (2017) [170]. This technology is based on the atomization of the polymer solution by pneumatic injector using compressed air/gas that nebulizes within a high electric field. During this process, the solvent is evaporated at room temperature in a drying chamber and the encapsulated material is then collected as a free-flowing powder. Thus, EAPG enables common electrospraying technique in terms of mass production and makes it feasible for the industrial purposes. This technology, which can produce 1-3 kg of dry powder per hour, is currently available under the brand name of Capsultek in the company called Bioinicia S.L., Paterna, Spain (www.bioinicia.com). Although electrohydrodynamic techniques are still being developed on a lab-scale, such

further industry-laboratory collaborations will bring this technology to the market in near future.

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