

Review

The Functionalization of Nanostructures and Their Potential Applications in Edible Coatings

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Abstract: Nowadays, edible coatings incorporated with nanostructures as systems of controlled release of flavors, colorants and/or antioxidants and antimicrobial substances, also used for thermal and environmental protection of active compounds, represent a gap of opportunity to increase the shelf life of food highly perishable, as well as for the development of new products. These functionalized nanostructures have the benefit of incorporating natural substances obtained from the food industry that are rich in polyphenols, dietary fibers, and antimicrobial substances. In addition, the polymers employed on its preparation, such as polysaccharides, solid lipids and proteins that are low cost and developed through sustainable processes, are friendly to the environment. The objective of this review is to present the materials commonly used in the preparation of edible coatings, as well as the advances that these structures have represented when used as controlled release systems, increasing the shelf life and promoting the development of new products that meet the characteristics of functionality for fresh foods ready to eat.

Keywords: nanotechnology; antimicrobials; antioxidants; colorants; prebiotics; controlled release

1. Introduction

Current global consumer trends demand minimally-processed products with characteristics such as "freshly-made" and "microbiologically-safe". One key goal of minimally-processed products is maintaining and delivering fresh products in a convenient way without losing nutritional quality but ensuring sufficient shelf life to allow their distribution to potential consumers [1]. Technologies available for obtaining minimally-processed products include edible coatings, which are systems that provide safety and functionality while also increasing shelf life and food quality [2]. Today, edible coatings are strongly impacting food processing due to their advantages over synthetic films. Edible coatings can be used to physically protect food, prevent drain of liquid, and control the physical, chemical and microbiological activities of products [3]. In addition to being edible, they constitute a barrier to gases and water vapor, and can function as carriers of bioactive substances with antioxidant and antimicrobial properties, as well as dyes, flavorings, prebiotics and probiotics, among other important compounds.

One classification of edible coatings is based on the material used to prepare them. The principle materials used in this technology are polysaccharides, proteins and lipids [4]. Hence, the functionality



of edible coatings depends largely on the components of the film and on the interaction with the product to which they are applied. The choice of film-forming material and/or active substance depends on the desired objective, the nature of the food product, and the specific application [2].

Currently, interest is growing in the formulation, study and application of nanostructures in various fields of knowledge, including food science, due primarily to their excellent properties as vectors for delivering bioactive substances and systems for bioimaging and biodetection [5]. Nanotechnology is defined as the production, processing and application of nanometer-scale materials [6]. Nanostructured materials have applications in the food industry that include acting as nanosensors and incorporation into new packaging materials with better mechanical and barrier properties, but these materials also improve solubility and bioavailability, facilitate controlled release and protect bioactive substances during manufacturing and storage [7]. This indicates that adding functionalized nanostructured systems to edible coatings has a great impact, since they offer improved properties—including those just described—that may lead to new applications apart from those already reported in the literature.

Regulation of the use of nanostructures in foods is a controversial issue. In the United States, FDA issued three final guidance documents related to the use of nanotechnology in regulated products, including cosmetics and food substances. Currently, there is a lack of accepted regulations on the response to public health and general occupational risks associated with the manufacture, use and elimination of nanomaterials, with uncertainty regarding the risk characteristics [8,9].

Food nanotechnology can affect the bioavailability and nutritional value of food based on its functions. It is recognized that the biological properties (including toxicological effects) of nanomaterials are largely dependent on their physicochemical parameters [8].

Our research group has developed nanostructuring methodologies of various bioactive substances, which have been used on the conservation of food as edible coatings. It is for this reason that the main objective of this review, then, is to highlight the applications of functionalized nanostructured systems and their potential uses in edible coatings by presenting a general overview of the matrices that have been used and of the active substances that have potential for use in food processing. The article concludes with comments on future perspectives for food science.

2. Nanostructured Matrices

Some components of interest in food that serve as a matrix of certain nanostructures are found naturally on the submicron scale, which facilitates the process of nanostructuring them in individual or conjugated forms. These components are numerous and diverse, so we will deal only with proteins, carbohydrates and lipids, which can be combined to form complex colloidal mixtures with important physical and chemical properties, as described below.

2.1. Polysaccharides

Among the matrices that have been widely-used to form nanostructures in food science, we find polysaccharides. These biopolymers are made up of multiple saccharides linked by glycosidic bonds. There are different types of polysaccharides, which differ in terms of molecular weight, polydispersity, solubility, structure (linear or branched), and whether they are monofunctional or polyfunctional, among other factors. The various structures and properties of polysaccharides offer molecular and biological advantages when used to prepare nanostructures [10]. Depending on their specific properties, polysaccharides have important functionalities in the formation of nanostructures that impact food science, especially in the preparation of edible coatings. Nanostructures based on effective support matrices that make them functional for use with bioactive substances offer such properties as controlled release as a function of pH [11].

There is now abundant research on the synthesis of polysaccharide-based nanostructures [12], much of it focused on the functionality of polysaccharides and/or the controlled release of natural ingredients. One clear example is starch, which can be nanostructured and functionalized with

bioactive substances for targeted administration. These bioactive substances may be hydrophilic or lipophilic in nature. Studies show that starch-based nanosystems have higher encapsulation efficiency and offer better protection of bioactive substances [13]. In addition, the use of cellulose nanocrystals (CNC) has significant advantages for the formulation of biofilms, since adding certain amounts of them can positively modify optical and gas barrier properties [14].

Studies by [15] with chitosan nanostructures have shown that these compounds can form effective delivery systems for bioactive substances—such as polyphenols—that can be incorporated into functional foods, since they have properties appropriate for this purpose, among which particle size (300–600 nm) stands out. Regarding the preparation of nanosystems, methodologies for the nanostructuring of enzymes such as lipase in guar gum matrices using dialysis have been developed [16] and present great potential for applications in functional foods, including edible coatings. Coatings made with nanochitosan applied to apples showed positive results on changes in fruit respiration by decreasing maximum ethylene production (33%), while also controlling the enzymatic activity of polyphenol oxidase and peroxidase [17]. In addition, novel developments have been achieved in the field of edible coatings with a nanocomposite of silver/titanium dioxide/chitosan adipate (particle size = 50–100 nm) by photochemical reduction [10], which exhibits high zeta potential (from 30.1 to 33.0 mV) during 60 days of storage. In addition, this nanocomposite six-log reduced the population of *Escherichia coli* after 24 h of incubation, thus revealing its potential antibacterial protective power for fruit storage.

2.2. Lipids

Lipid-based nanostructures are innovative administration systems similar to emulsions, but that differ in size and structure. Their water-insoluble core is dispersed in a combination of solid and liquid lipids stabilized by surfactants. Important properties of nanostructured lipid matrices include high encapsulation efficiency, controlled release, and directed effect, among others [18,19]. Solid lipid nanoparticles and nanolipid carriers play significant roles in lipid-based nanostructured systems. Solid lipid nanoparticles (SLN) are submicron-size colloidal lipid systems that are fully crystallized and have an organized structure in which the bioactive components is housed inside the lipid matrix. They have been developed to encapsulate and administer functional lipophilic components [20,21]. Nanolipid carriers (NLC), meanwhile, are prepared by dispersing a mixture of solid and liquid lipids with bioactive ingredients in water together with emulsifiers. The mixture of lipid substances in NLC promotes a slow polymorphic transition and low crystallinity index [19]. The composition of the internal phase of NLC provides high encapsulation efficiency with greater bioavailability of the nanoencapsulated systems [22].

Several projects have been carried out on the development of nanostructured systems with a lipid base. Compritol 888 ATO, for example, has been used to form SLN with approximate sizes of 241–333 nm with ultrasound as a complementary technique to ultrahigh agitation [23]. Glycerol monostearate has been incorporated as a lipid nanomatrix using high pressure homogenization techniques that have shown great effectiveness in the efficiency of citral encapsulation (above 50%) [24]. Palmitic acid and corn oil have also been employed to form SLN. In this case, crystals of palmitic acid enveloped the oily surface of the encapsulated β -carotene, while corn oil decreased the exclusion of β -carotene from the matrix to the surface [25]. SLN prepared with Candeuba[®] wax (Multiceras S.A. de C.V., Monterrey, Mexico), meanwhile, present values of zeta potential (ζ) = -25.7 mV, after three cycles of ultrahigh homogenization, and have shown excellent results on increasing the shelf life of guava [21]. Since ζ is a measure of the degree of repulsion between similarly-charged particles in the dispersion, colloids with a high *z* (either positive or negative) are electrically-stabilized [6].

Turning to NLC, formulations elaborated with different concentrations of solid lipids (lauric acid, stearic acid, and cacao butter), oils (glycerol, Miglyol[®] 812, corn oil, and oleic acid), and surfactants (Poloxamer 407, Tween 80, and Tween 20) were used, highlighting their great potential for use in food

applications thanks to their ability to maintain the chemical stability (NLC was successfully protected the chemical structure of t-resveratrol from decomposition phenomena). In addition, they all have average particle sizes below 120 nm [18]. Cocoa butter as an NLC has shown significant advantages in the encapsulation of active substances, such as the essential oil of cardamom, where it has generated encapsulation efficiencies above 90%, accompanied by particle sizes below 150 nm [26].

2.3. Proteins

In recent years, research has shown that proteins derived from corn, wheat, soy, peanuts, milk or gelatin are excellent options for the formation of edible coatings, because they present hydrophilic surfaces that provide resistance to the diffusion of water vapor and constitute a barrier to the exchange of oxygen and carbon dioxide [4]. Moreover, these proteins are naturally of submicron size and some of them can self-assemble into complexes or larger nanostructures with the potential to play vital biological roles. Such nanostructuring of proteins holds promise for uses in foods given their gelling, thickening and emulsifying properties [27], which makes them candidates for incorporating specific matrices in food preservation, such as edible coatings, because they modify the mechanical and rheological properties of food [28].

The nanostructuring of proteins has stimulated great interest in the scientific community due to the properties it offers, including biodegradability and availability [29]. In addition, protein nanostructures present greater stability in biological fluids and so allow the controlled release of bioactive substances of interest in foods [30]. One clear example is that numerous studies of the nanostructuring of zein are currently being conducted and showing great potential for applications in foods. Nanostructured zein systems are advanced submicron support systems that show improved properties in terms of stability, release of bioactive substances and supply efficiency [31].

Studies have also been conducted on the nanostructuring of such proteins as gelatin and bovine serum albumin, and they show promise for the effective delivery of resveratrol and curcumin by obtaining average particle sizes of 315 nm [29]. Various nanohydrogels have been formed using proteins of distinct origin, such as milk and soy protein. When used as coatings, results show that they are efficient vehicles for entrapping, protecting and delivering nutraceuticals and bioactive components [32].

Another area of research on the nanostructuring of proteins as edible coatings involves the formation of nanofibers based on whey protein. In this case, in vitro nanosystem increased the transparency of the films while decreasing moisture content and solubility in water. In addition, when applied to freshly-cut apple they exhibited the best protective action in terms of retaining total phenolic content and inhibiting darkening and weight loss [33].

3. Active Substances in the Functionalization of Nanostructures

Edible coatings can be effective carriers of functional ingredients, which can perform various functionalities, such as increasing the shelf life of products and delaying undesirable changes in fresh products [4]. This section discusses different active substances that have the ability to be nanostructured and, moreover, present potential for applications in edible coatings.

3.1. Antioxidants

Antioxidants are substances that, when present in foods at low concentrations compared to that of an oxidative substrate, markedly delay or prevent oxidation [34–36]. According to the US Food and Drug Administration (FDA), antioxidants are defined as substances used to preserve food by retarding deterioration, discoloration and rancidity due to oxidation [36]. Food manufacturers have used food-grade antioxidants to prevent quality deterioration of products and maintain their nutritional value [34]. Antioxidant compounds are classified into natural and synthetic types. Of the latter category, butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT) and propyl gallate (PG) are the ones most widely-used in food preservation. However, due to the potential carcinogenic properties of synthetic antioxidants, many researchers are striving to replace them with natural substances [37]. In addition, growing consumer preference for natural food additives is shifting emphasis to natural materials as sources of novel antioxidants. Phenolic compounds (phenolic acids and flavonoids), carotenoids (β-carotene and lycopene), tocopherols (vitamin E) and ascorbic acid (vitamin C) are the most extensively-studied and oft-used natural antioxidants in the food industry.

The reaction mechanism of antioxidant compounds depends mainly on their structure. In this regard, five antioxidant routes have been identified: (i) scavenging species that initiate peroxidation; (ii) chelating metal ions so that they are unable to generate reactive species or decompose lipid peroxides; (iii) quenching O_2^- to prevent the formation of peroxides; (iv) breaking the autoxidative chain reaction; and (v) reducing localized O₂⁻ concentrations [35]. Phenolic acids (gallic acid) are characterized by a C_6 - C_1 structure [38] and generally act as antioxidants by trapping free radicals [39]. Flavonoids (quercetin, hesperetin, catechins, epicatechins, and proanthocyanidins) constitute the largest group of plant phenolics [40]. They are low-molecular weight compounds made up of 15 carbon atoms arranged in a $C_6-C_3-C_6$ configuration [38]. Their free radical-scavenging potential appears to depend on the pattern (both number and location) of free -OH groups on the flavonoid skeleton [41], since they have the ability to donate a hydroxyl hydrogen group and stabilize the resonance of the resulting antioxidant radical [42]. Carotenoids, meanwhile, are lipid soluble C_{40} tetraterpenoids. These compounds are divided into xanthophylls, which contain oxygen (astaxanthin), and carotenes, which are pure hydrocarbons with no oxygen in their structure (α -carotene, β -carotene and lycopene) [38]. Carotenoids have the capacity to scavenge peroxyl radicals (ROO) more efficiently than any other reactive oxygen species (O_2 , O_2^- , OH, RO, H_2O_2 and LOOH), which allows them to deactivate ROO by reacting with them to form resonance-stabilized, carbon-centered radical adducts [42].

Finally, vitamins are the best-known antioxidant compounds, with C and E being the ones most frequently used in the food industry. Vitamin C is a water-soluble free radical scavenger that changes into an ascorbate radical by donating an electron to the lipid radical to terminate the lipid peroxidation chain reaction. Vitamin E, meanwhile, performs a chain-breaking function during lipid peroxidation and exerts its antioxidant effect by scavenging lipid peroxyl radicals [42]. Other natural antioxidants, such as curcumin, also show chain-breaking antioxidant activity, as their free radical scavenging correlates to the phenolic –OH group and the CH₂ group of the β -diketone moiety. It is important to note, however, that the application of antioxidants is limited by factors that include the low solubility of hydrophobic types, poor stability, degree of bioavailability, and targeted specificity [43–45]. Functionalizing nanostructures is currently being explored as a possible means of resolving these problems (Table 1), since submicronic size particles have been shown to enhance both bioavailability and stability, and to provide controlled release of the compounds involved [46], while also providing excellent results with respect to parameters such as particle size and encapsulation efficiency.

Antioxidant Compound	Nanostructure Functionalized	Findings
Curcumin	Alginate-pectine/Zein core-shell NPs, (A-P/Z-NPs)	A-P/Z-NPs shown to have superior antioxidant and radical scavenging activities than curcumin solubilized in ethanol [47]
Fisetin	PCL-NPs	NPs can be proposed as an attractive delivery system to control the release of antioxidant fisetin for nutraceutical application [48]
Gallic acid	Zein ultrafine fibers	Gallic acid had retained its antioxidant activity after incorporation in zein electrospun fibers [49]

Table 1. Various antioxidants used in the functionalization of nanostructures.

Antioxidant Compound	Nanostructure Functionalized	Findings
Quercetin and Ferulic acid	Amaranth protein isolated/Pullulan nanofibers	Both bioactives showed a sustained release, keeping a greater extent their antioxidant capacity in comparison with non-functionalized compounds [51]
Catechin and Epicatechin	Bovine serum albumin NPs	NPs showed satisfactory sustained release, maintained antioxidant potential and found improved efficacy [52]
Catechins from white tea extract	PCL/Alginate NPs	NPs protected tea polyphenols from degradation thus opening new perspectives for the exploitation of white tea extract-loaded NPs for nutraceutical applications [53]
Savory essential oil	CS-NPs	Encapsulation enables stronger antioxidant activity to phenolics as compared to their pure forms by entrapping them into capsules and protecting from negative effects of environmental conditions [54]
<i>Lippia sidoides</i> essential oil	CG/CS-NGs	In vitro release profiles revealed a prolonged. These results showed that the CG/CS nanogels were designed and present sustained release features [55]
Retynil palmitate (Vitamin A)	Palmityol/CS-NPs	The submicron particles can be used as antioxidant systems to improve biodisponibility of vitamins [56]
α-Tocopherol (Vitamin E)	Alginate/α-tocopherol NPs	The authors reported an improvement of biodisponibility of α -tocopherol through the encapsulation of the oily antioxidant compound [43]
Folic acid (Vitamin B9)	Soy protein/Soy polysaccharide NGs	The protein and polysaccharide can inhibit the reactions between dissolved oxygen and folic acid during UV irradiation. The NGs are a suitable delivery system of folic acid in food and beverages [57]
Astaxanthin	Astaxanthin NLC	NLC containing nutraceuticals have potential to be used for functional beverages/food development [58]
β-carotene	Cocoa butter SLN	β-carotene degradation was observed during storage. SLN showed an increase in particle size (35%) and color change ($\Delta E = 20$) after 8 days of storage. The authors mentioned that blending other kinds of fats in the SLN's production will avoid the partial coalescence of lipid crystals and expulsion of carotenoids, leading in physical and chemical stability improved [59]

Table 1. Cont.

3.2. Antimicrobials

Antimicrobial agents have long been studied for their effectiveness in killing or inhibiting the growth of microorganisms in and on foods [60,61]. These efforts have been directed towards increasing food safety for consumers and the shelf life of food products by reducing or eliminating pathogens and microorganisms that cause spoilage. Antimicrobials widely-used in food preservation include those of non-natural and natural origin. In the first category, fungicides and triclosan are highlighted, while those called "natural" can be classified into animal source antimicrobials as proteins, enzymes and polysaccharides (lactoferrine, lysozyme and chitosan); microbial products such as bacteriocins (nisin and pediocin); plant delivered compounds as spices, essential oils and plant extracts; and metals (ZnO, Au and Ag) [62]. Nevertheless, another natural antimicrobial is not included within this classification. Electrolyzed water (EW) is one of the alternative storage technologies for fresh meat products and has attracted increasing attention because of its good antimicrobial effect [63]. All of them have been shown to present specific action mechanisms. Phenolic compounds (essential oils and plant extracts), metals, enzymes, bacteriocins and EW are known to damage the cell wall of microorganisms, while inorganic compounds such as triclosan inhibit the synthesis of fatty acids. As is the case of most

Table 2 identifies some studies on the functionalization of nanostructures with antimicrobial compounds such as NPs, nanovesicles, nanofibers and carbon nanotubes that seek to improve the physical stability of the structures, monitored mainly through the particle size, as well as its chemical stability, determined through the degradation of the antimicrobials and its bioavailability. In a similar vein, studies of the potential effect of these systems on the inactivation of pathogenic microorganisms (*Salmonella* spp., *L. monocytogenes*, *C. jejuni*, *E. coli*, and *S. aureus*) and prevention of food spoilage have also been conducted. On this point [64], and [65] focused their work—with some success—on developing nanostructures that are capable of interacting with polymeric matrices, such as polylactic acid or thermoplastic flour, in packaging production. However, according to the literature reviewed, many nanostructures have both the potential to be functionalized with antimicrobial agents and the properties required to interact with natural or synthetic materials that could be incorporated into new antimicrobial packaging systems.

Antimicro-Bial Compound	Nanostructure	Applica-Tion	Targeted and Inhibition Microorganism	Inhibition or Reduction
ZnO	ZnO NPs (10–17 nm)	in vitro	E. coli, P. aeruginosa, S. aureus, B. subtilis	14, 18, 13, 14 mm of zone inhibition/1 cm sample [66]
ZnO	ZnO NPs	Chitosan edible coating	E. coli	Total inhibition [67]
Nisin and EDTA	NSs (130–270 nm)	in vitro	E. coli, S. aureus	8 log (CFU/mL) after 24 h 2.3 log (CFU/mL) after 24 h [68]
Nisin	SLN (159–167 nm)	in vitro	L. monocytogenes, L. plantarum	8.5 log (CFU/mL) after 24 h 8 log (CFU/mL) after 24 h [69]
Lactoferrin	L-NVs (100–200 nm)	in vitro	S. aureus, Salmonella sp., E. coli, P. fluorescens, L. innocua, B. cereus C. albicans	Minimum inhibitory concentration (MIC): 2000 μg/mL (<i>S. aureus</i> , <i>L. innocua</i> , <i>B. cereus</i>); 200 μg/mL (<i>C.</i> <i>albicans</i>) Not antimicrobial activity observed for <i>Salmonella</i> sp., <i>E. coli</i> , <i>P.</i> <i>fluorescens</i> [70]
Eugenol Trans-cinnamaldehyde	PLGA-NPs (174–317 nm)	in vitro	Salmonella spp., Lysteria spp.	MIC: 800 μg/mL 1600 μg/mL [71]
Oregano essential oil (Carvacol, Thymol) (6:1)	Liposomes (263–287 nm)	in vitro	S. aureus, S. aeroginosa, E. cloacae, K. pneumoniae, E. coli, S. mutans, S. viridans, C. albicans, C. tropicalis, C. glabrata, L. monocytogenes	17, 13.3, 12, 13, 14, 17, 17, 12, 14.1, 14.1, 17 mm of zone inhibition [72]
Carvacol, p-cymene	Pullulan	Edible coating for Turkey deli meat	S. aureus, L. monocytogenes	28 and 25.5 mm of zone inhibition after 7 weeks of storage at 25 °C [73]
Garlic essential oil	PEG-NPs (<240 nm)	in vitro	T. castaneum	>80% mortality at dose of 8000 mg/kg [74]

Table 2. Recent studies of the functionalization of nanostructures with antimicrobials.

Antimicro-Bial Compound

Cardamom essential oil

Peppermint oil

Electrolyzed

water-chitosan (EW-C)

TG-NCs (22 nm)

Obscure puffer fish

rinsed with EW

and coated with

chitosan

	Table 2. Con	ι.	
Nanostructure	Applica-Tion	Targeted and Inhibition Microorganism	Inhibition or Reduction
CS-NPs (50–100 nm)	in vitro	E. coli S. aureus	CS-NP inhibited the growth of pathogens till first 48 h (amount not mentioned) [75]

E. coli, S. aureus, C.

albicans

Aerobic bacteria

Table 2. Cont.

in vitro

Edible coating

3.3. Probiotic and Pre-Biotic

The science of food conservation is constantly on the lookout for new ingredients that, aside from helping preserve quality, are important for the ingestion of so-called functional foods; that is, foods which, in addition to nutrients, contain components that produce a positive impact on health. These include prebiotics and probiotics. A probiotic is a live microbial food supplement that has beneficial exerts for the host by improving its microbiological balance in the intestine [77]. The most commonly-used probiotics are lactic acid excretors such as lactobacilli and bifidobacterial [78]. Probiotics have been incorporated into several food products and supplements, mostly dairy products such as cheeses, dairy desserts and ice cream, though fermented milks such as yogurts are the most popular matrices [79]. The global market for probiotics has been expanding in recent years due to growing consumer demand for healthy diets and wellness [79].

A prebiotic, in contrast, is a non-digestible substance that provides a beneficial physiological effect on the host by selectively stimulating growth or activity of a limited number of indigenous bacteria [80]. Prebiotics are non-active food constituents that migrate to the colon where they are selectively fermented [81]. This category includes both natural and chemically-produced substances [82]. Lactulose, galactooligosaccharides, fructooligosaccharides, inulin and its hydrolysates, maltooligosaccharides, and resistant starch are all prebiotics commonly used in human diets [81]. Naturally-occurring prebiotics can be found in various foods, including asparagus, chicory, tomatoes and wheat, and they are natural constituents of breast milk. Prebiotics and probiotics have been associated with several health benefits, such as increasing the bioavailability of minerals—especially calcium—modulating the immune system, preventing the incidence or reducing the severity and duration of gastrointestinal infections such as acute diarrhea, and modifying inflammatory conditions, including irritable bowel syndrome, ulcerative colitis and inflammatory bowel disease, among others [83].

Currently, mixtures of probiotics and prebiotics are often used to take advantage of their synergic effects in applications with food products. Mixtures of this kind are called synbiotics [81]. They improve the survival, implantation and growth of newly-added probiotic strains, or promote growth of existing strains of beneficial bacteria in the colon [84]. These compounds are very important for the body, since, to exert their positive health effects, these bacteria must reach their site of action alive and establish themselves in certain quantities [85]. However, the acidic conditions of the stomach, together with the bile salts secreted into the duodenum, are formidable obstacles to the survival of ingested bacteria [86]. For this reason, it is necessary to protect them with a physical barrier that prevents exposure to adverse environmental conditions. Several options have been explored to provide the protection that these compounds require. One recent discovery is that microencapsulation is a useful technique for stabilizing probiotics in functional food applications [87]. Reviews of some other works reveal deeper probes into the science of the encapsulation of probiotics and research on different

Antibacterial/antifungal

h [76]

storage [63]

activities was 100% after 12

EW-C treatment retarded the

increase in the total viable

counts reaching 4.69 log

(CFU/g) after 6 days of

methods [88–92]. Alternatively, probiotics may be carried inside edible polymer matrices similar to the ones used in the food packaging industry. Probiotics and many other active compounds [93] have been incorporated into biopolymeric matrices to develop active/bioactive food packaging materials as an alternative method for controlling pathogenic microorganisms and improving food safety, coupled with the potential to favor consumer health. Incorporating probiotic cultures into edible coatings was first proposed in 2007 by [94], but several later studies have successfully microencapsulated bacteria using various materials and methods. In addition to showing their use in edible coatings with different matrices, Table 3 also presents the most recent studies that have encapsulated probiotics and prebiotics in distinct matrices that help protect them from adverse environmental conditions and so keep them alive.

Microorganism	Matrix	Application	Findings
Lactobacillus casei Lactobacillus brevis Lactobacillus plantarum	Resistant starch from rice	Microencapsulation	The viability of (> 7 log CFU/g) for 2 months at 4 $^{\circ}$ C [86]
Lactobacillus acidophilus L. casei, L. rhamnosus, Bifi-dobacterium bifidum	СМС	Edible films	Viability of 10 ⁷ CFU/g [95]
Bifidobacterium animalis Bb12 [®] Lactobacillus casei-01	Whey protein isolates	Edible coatings on sliced ham preservation	Viability of 108 CFU/g and inhibited detectable growth of <i>Staphylococcus</i> spp., <i>Pseudomonas</i> spp., <i>Enterobacteriaceae</i> [96]
Lactobacillus bulgaricus	Whey protein isolate Alginate coating	Microencapsulation	Microencapsulated cells exhibited much better retainability of cell survival during storage, especially under low temperatures [97]
Nanofibers of chitin, lignocellulose and bacterial cellulose	Pectin	Nanofibers biocomposites	The optimal biocomposite exhibited the highest survival of the entrapped probiotic bacteria under simulated gastric (97.7%) and intestinal (95.8%) conditions [98]
Bacillus subtilis HFC103	Candelilla wax	Edible coatings in strawberry quality during the shelf life	Effective to control R. stolonifer [99]
Lactobacillus plantarum	Pectin Starch	Hydrogel particles by extrusion method	The numbers of surviving cells were 5.15 and 6.67 Log CFU/g for pectin and pectin/starch hydrogel, respectively [100]
Bacillus coagulans	Bacterial nanocellulose, Pectin Schizophyllum commune extract	Bionanocomposites	Survivability of probiotic under drying process and gastrointestinal condition. During storage period at ambient temperature, 4 °C and -20 °C performed viability reduction: 1.3, 1.7 and 1.8 log CFU/g [101]
Lactobacillus plantarum,	Whey protein	Electrospraying conditions for the microencapsula-tion	Viability losses lower than 1 \log_{10} CFU and the bacterial counts of the final products exceeded 8.5 \log_{10} CFU/g [102]
Lactobacillus casei	Inulin incorporated into alginate and chitosan coated alginate beads	Microencapsula-tion	Using inulin and chitosan-coating, the survival of co-encapsulated cells in simulated gastro-intestinal condition was improved with only 2.7–2.9 log [103]
Fructooligosaccharides	Cassava starch	Edible films	The addition of FOSs resulted in higher solubility and elongation, a decreased water vapor permeability of the films [104]
Bifidobacterium animalis Bb12 [®] Lactobacillus casei-01	Whey protein isolate	Edible films	Viability of 106 CFU/g film until Day 60 at 23 and 4 °C [105]

Table 3. Recent studies that containing probiotic and pre-biotic in different matrix.

Microorganism	Matrix	Application	Findings
Lactobacillus rhamnosus GG	Native rice, corn starch, bovine skin gelatin, sodium caseinate and soy protein concentrate	Edible films	Loss of <i>L. rhamnosus</i> in presence of proteins (0.91–1.07 log CFU/g) and starch based systems (1.71 log CFU/g) [106]
Fructooligosaccharides Lactobacillus plantarum CIDCA 83114	Methylcellulose	Green apple baked snacks functionalized with edible coatings	Bacteria were still alive after the simulated gastric ($5.5 \times 106 \pm 0.7 \times 106$ CFU/g) and intestinal ($1.0 \times 106 \pm 0.4 \times 106$ CFU/g) [107]
Lactobacillus plantarum CIDCA Kluyveromyces marxianus	Kefiran	Edible film	<i>L. plantarum</i> viability decreased less than 1.3 logarithmic cycles and <i>K. marxianus</i> 0.7 logarithmic cycles [108]
Lactobacillus rhamnosus GG Pre-biotic: inulin, polydextrose, glucose-oligosaccharides and wheat dextrin	Gelatine	Edible films	The supplementation of edible films helps to maintain the viability of <i>L.</i> <i>rhamnosus GG</i> and ameliorated the storage microbiological stability of <i>L.</i> <i>rhamnosus GG</i> at different temperatures [109]
Lactobacillus rhamnosus GG	Sodium alginate-WPC	Edible films for bread	A lower viability percentage was determined in the case of the breads coated with sodium alginate (15%) compared to those containing WPC (76.3%) [110]
Lactobacillus delbrueckii subsp. bulgaricus CIDCA333 Lactobacillus plantarum CIDCA 83114	Methylcellulose and fructo-oligosaccharides	Edible films	Viability of $1.1 \times 1010 \pm 1.6 \times 109$ CFU/mL for <i>L. delbrueckii subsp.</i> <i>bulgaricus</i> and $2.7 \times 1012 \pm 8.7 \times 1011$ CFU/mL for <i>L. plantarum</i> [111]

Table 3. Cont.

3.4. Colorants and Flavors

Colorants and flavors can be synthetics or naturals. Natural flavors and colorants are preferred in food preparation and development of new products. Products that present wavelengths within 380–770 nm allow the perception of different colors through the human eye [112–114]. Naturals colors and flavors have advantages on the health and safety such as antioxidant and antimicrobial effects, which give the characteristic of functional food when they are applied in food process. Flavors have numerous volatile substances that are susceptible to loss, thus the formation of nanostructures to protect these flavors is an alternative in the food process. Nanostructures have been formed by ionic gelation using chitosan, or by emulsification-diffusion using poly- ε -caprolactone [115]. Table 4 shows same colorants nanostructured.

Table 5 presents nanostructured flavors, showing that nanoencapsulation is the principal form of protection. The retention of flavor in submicronic systems depends largely on the physicochemical properties of aromatic compounds and components of the food matrix [124].

Colorant	Matrix	Findings
β-carotene	PCL	NCs provided a little aggregation between NPs of β -carotene during storage at 25 °C for 28 days [116]
Anthocyanins from purple sweet potato	Konjac glucomannan/Chitosan	NCs of anthocyanins revealed that the compounds were stable up to pH 3 with controlled release of the active ingredient [117]

Table 4. Recent studies of nanostructured matrix containing colorants.

Colorant	Matrix	Findings
Anthocyanins from red cabbage	Palmitic acid	The SLN containing anthocyanins was successfully achieved, showing high entrapment efficiency (>90%), decreasing degradation of these compounds against pH and temperature [118]
Lycopene	PCL	NPs provides a protecting effect that impedes the quick degradation of lycopene under light, oxygen and temperature, which makes it a potential colorant for edible coatings [119]
β-carotene	PCL/Xanthan gum	Nanocoatings with carotene/PCL NCs in a xanthan gum matrix help preserve the color of fresh cut melon for 21 days at $4 ^{\circ}$ C [120]
Anthocyanins from black carrot	WPC	Formation of nanogels of anthocyanins have greater physical stability (the composite gel microcapsules could protect the color) in dairy products [121]
Tea polyphenols/β-carotene	CMC-CS/Zein	The powders obtained have a high dissolution rate and better solubility properties. The percentage of rehydration was greater than 90% [122]
Anthocyanins from black carrot	Gelatin/CS	It was found that the release of the anthocyanins was a function of the concentration of chitosan, the encapsulation efficiency was relatively high (greater in all cases than 75%) [123]

Table 4. Cont.

 Table 5. Recent studies of nanostructured matrix containing flavors for food applications.

Flavor	Matrix	Food	Findings
Cinnamon and oregano	BCD/CS	in vitro	The encapsulation favored the retention of volatile compounds in the formation of nanofibers [125]
Carvacrol	Starch/PCL	in vitro	Nanostructure presented better cohesiveness and adhesiveness in the electrospun material, which also contribute to the coating performance [126]
Curcumin	Gelatin	Gellified fish product	Curcumin encapsulation increase water solubility and improving its dispersion/solubility in the aqueous food matrix used as a food model [126]
Cardamom	Cocoa butter	in vitro	NLC had fine size (<150 nm) and high entrapment efficiency (>90%) [26]
Rosemary	PCL or Ethyl cellulose	in vitro	NCs prepared by ultrasound had sizes smaller than 200 nm with high entrapment efficiency (>70%) with applications in food conservation [127]
Clove	CS	Beef cutlets	The application of nanogels was effective to preserve the natural color of the product, in addition to preventing the volatility and instability of the active substance [128]
Black pepper	BCD	in vitro	Nanostructures with BCD can maintain the bioactive properties of the essential oil of black pepper [115]
Vanillin	BCD	in vitro	Inclusion nanostructures of vanillin, enhancing its antioxidant capacity, improving its functionality as a flavoring agent [129]

4. Effect of the Functionalization of Nanostructures in Edible Coatings

The preceding paragraphs reviewed important aspects of the materials, active compounds and substances involved in the preparation of nanostructures. Today, commercial interest in foods with functional characteristics that increase shelf life during storage is growing rapidly. However, many cases require a specific functionality at the time of consumption, such as digestive enhancers and anticarcinogenic and anti-cholesterol properties, among others. In addition, these materials must be effective in combatting microorganisms to improve consumer safety [130]. Several different natural polymers have been used in the preparation of edible coatings. Some preparation methods include crosslinking polymer molecules with a crosslinker substance, while others require temperature control and the addition of inorganic substances such as mechanical resistance reinforcers and gas permeability modifiers [79,131,132]. This explains why edible coatings options are increasing the potentialities of functionality and providing effective protection when added to submicron-sized systems that can incorporate bioactive substances with antimicrobial and/or antioxidant properties, such as essential oils (oregano, rosemary, cinnamon, lavender, citrus, mint, peppermint and curcumin, among many others). All these substances have been functionalized by small nanostructures that are incorporated into edible coatings using different preparation methods. It is important to note that it is preferable to use natural polymers such as alginates, carboxymethylcellulose, pectin, zein, whey, casein, etc. [133]. Incorporating these nanostructures helps improve the barrier and mechanical properties of edible coatings, especially nano- clays, zinc oxides, titanium and zeolites, to mention just a few. It is possible to incorporate active substances by adsorption methods that modify their functionality to preserve foods and the nutrimental components of edible coatings [134,135].

The functionalization of nanostructures allows them to reduce the volatility and sensitivity of antioxidant compounds to temperature, light and oxygen exposition, and increase solubility and affinity for the components of edible coatings, thus increasing their compatibility with the surface of the food and favoring preservation. These systems are able to persist during storage, distribution and commercialization of food as they are gradually released from the nanostructure towards the components of the edible coating [120,136]. Another important objective in pursuing the functionalization of nanostructures is to protect the flavors of foods that have such characteristics as sweetness and floral, spicy and menthol smells, among others. Here, challenges include the volatility of these properties, their short endurance, and their vulnerability to changes in sensory perception due to oxidation and exposure to temperature and chemical interactions. For these reasons, developing systems of submicron size can improve the entrapment and decrease the degradation of many essential oils, phenols and plant extracts with antimicrobial and antioxidant activity [137].

The possibilities for developing edible coatings are now innumerable, due to the existence of different composition matrices for coatings, different structures and compositions of nanostructures, and the functionalization of these materials with different antioxidants, antimicrobials, permeability modifiers and mechanical properties, to mention just a few. The benefits for food preservation will be further enhanced with the development of controlled release systems and others with special characteristics designed to control respiration, enzymatic activity in the maturation of cheeses, and microbial control in meat, bread and other food products. Table 6 shows some of the main applications of edible coatings based on different matrices that incorporate functionalized nanostructures with different components, all with a focus on the benefits potentially obtained for commercializing products with minimal processing or with functional and special sensorial characteristics that are attractive to consumers.

Bioactive Substance	Nanostructure/Wall Material	Matrix of Edible Coating	Ingredient Type	Food	Findings
Cinnamon bark extract (CBE)	NCs: PLGA/Poly- <i>N</i> -isopropyl acril amide (PNIPAAM)	CS	Natural, plant derived compounds	Fresh-cut lettuce	NCs with PLGA were prepared by emulsion-evaporation method. NCs were most efficient for Antimicrobial effect for <i>L. monocytogenes</i> , that those prepared with PNIPAAM [138]
D-limonene	Liposomes	CS	Others (pure ingredient)	Blueberries	The limonene encapsulated in liposomes had good functionality and inhibit the growth of <i>Botrytis cinirea</i> , <i>E. coli</i> and <i>L. monocytogenes</i> . Moreover, kept the quality for nine weeks of storage at 4 °C [139]
Tarbush (phyto molecules)	Candelilla wax/CS-NPs	Arabic gum	Natural, plant derived compounds	Apples	Nanocoatings were prepared by hot homogenization method and applied on surface of apples- shown that use of Phyto molecules reduce the color changes and the action of polyphenol oxidases increase the shelf life by 8 weeks [140]
Thyme oil	CS-NPS	Canola oil/glycerol	Natural extract	Avocado	Thyme oil concentration had beneficial effect over the shelf life of avocado. The high concentration was more effective for inhibition of <i>C. gloesporiodies</i> [141]
Acerola puree	Nanocomposites Montmorillonite (MMT), Cellulose whiskers (CWAA)	alginate/acelora puree	By-product vegetal origin	Acerola	MMT and CWAA were used as enforcement in edible coating of acerola puree and employed in acerola fruit preservation contribute to the film forming dispersions, improved the ascorbic acid retention [142]
Mexican oregano essential oil	NCs Modified starch	Modified Starch	Natural extract	Pork meat	Inhibition of microbial growth on meat previously inoculated with <i>Brochothrix thermosphacta</i> , <i>Micrococcus</i> <i>luteus</i> , <i>Lactobacillus plantarum</i> , <i>Pseudomonas fragi</i> , and <i>Salmonella Infantis</i> was tested [143]
Nano-ZnO	Nanoreinforced	СМС	Inorganic compounds	Pomegranate	Edible coatings based on 0.5% of CMC and 0.2% of nano-ZnO, contributed to maintaining the quality parameters of pomegranate [144]
Extract of Urtica dioica L.	Nanofibers PCL	WPI	Natural ingredient Extrac plant and animal protein	Rainbow trout	Coatings were applied on rainbow trout fillets, showing that nanofibers inhibit the growth of bacteria's (lactic acid and mesophilic) and reduce the total volatile basic nitrogen and thiobarbituric acid [145]

Table 6. Some nanostructures employees in edible coatings for food preservation.

Table 6 shows that most coatings are used for fruit preservation, because these food items are usually consumed directly without treatment and, therefore, are among the foods most often purchased by consumers, while other foods—e.g., meat, fish, cheese, chocolate, etc.—can be packaged in film coatings with different nanostructures, classified as active food packaging because the coatings are prepared in ways similar to edible coatings that are applied directly to the food.

5. Release of the Active Substances in Edible Coatings

Different strategies and methods of functionalization of nanostructures are carried out to increase the encapsulation efficiency (usually 10%–75%). They depend on the type of encapsulating polymer—alginate, chitosan, zein, pectin, starch, poly- ε -caprolactone, poly-lactic acid, etc.—and the properties of the bioactive substance used [146]. The selection of the wall material, the substance to be encapsulated, and the support matrix for developing an edible coating are directly related to the kinetics of the release of the active ingredient contained in the functionalized nanostructures and embedded in a matrix usually formed by edible polymers. Other important considerations are the different barriers that the bioactive substance must cross to interact with the food surface and fulfill the primary function of increasing the commercialization time while maintaining freshness, characteristics and/or functional properties. Therefore, the establishment and study of the kinetics of release of analyze kinetics and relate this to increased shelf-life [147].

One of most attractive effects of the use of edible coatings is the ability to control the release of bioactive compounds, considering parameters such as humidity, temperature, pH, and swelling of the matrix, among other conditions that may affect the diffusivity of substances with antimicrobial or nutraceutical effect, at different times during food storage or ingestion [148]. Several different mechanisms may be involved in the release of an active compound through the components of edible coatings. These include melting, degradation, swelling or rupture of nanostructures, or diffusion release as a function of the initial solubility of the bioactive compound in the nanostructure and the permeability of the bioactive compound across the core of the polymer and, later, in the matrix of the edible coating. Different release models can be used to explain this, so the release kinetics can be of distinct types since they depend on both time and the way in which the matrix is modified. Thus, the non-time-dependent zero-order release kinetic $M_t = k_0 t + M_0$, which has a constant release of the active compound (k_0) , helps maintain its concentration and thus preserve food quality. However, they have the disadvantage that they can be removed quickly from the coating surface, causing them to lose their food-conservation properties. First-order kinetic release is expressed by the equation: $\log M_t = \log M_0 - k_1 t/2.303$, where M_0 is the initial active concentration, k_1 is the first-order rate constant, and t is time. This type of kinetic release is time-dependent and takes into account variations in the concentration of the bioactive compound as a function of storage time and its release into the edible coating to increase release during storage [120,149].

Figure 1 presents the different forms of release of a bioactive substance when functionalized in a nanostructure, and its potential incorporation into the polymer matrix employed in the edible coating formulation. This indicates that the ways of modifying the polymeric wall of functionalized nanostructures and the matrix polymeric employed in the formulation of the edible coating can provide other release kinetics that may explain the behavior of the bioactive substance used in food preservation, such as the release kinetics of a bioactive ingredient transferred through different functionalized nanostructures to the edible coatings [58,150]. The following is a brief description of some of the most commonly-used models to describe release kinetics, including the Korsmeyer–Peppas approach, which considers the cumulative release of the component with respect to time, is represented by the equation: $M_t/M_1 = k't^{\eta}$, where M_t/M_1 is a fraction of the active substance released at time t, k' is the release rate constant, and η is the release exponent [149,151]. The value of " η " predicts the release mechanism of the active substance such that $\eta = 0.45$ corresponds to the Fickian diffusion mechanism; $0.45 < \eta < 0.89$ to non-Fickian transport; $\eta = 0.89$ to Case II transport; and h > 0.89 to super case II transport [152]. Other models of polymer matrices consider the dissolution of the bioactive compound, as in the case of Higuchi, who presented an empirical model for water soluble and poorly water-soluble active compounds, described as follows: $Q_t = k_H t^{1/2}$, where Q_t is the amount of the active substance released at time t, and k_H is the release rate constant for Higuchi's model [153,154]. Finally, a model used to describe the release of the bioactive substance across an edible coating is the Weibull model, which has been described for different dissolution processes. It considers the relation $M_t = M_0[1 - e^{(t - T)b/a}]$, where a denotes a scale parameter that describes the time dependence, and b describes the shape of the curve which corresponds exactly to the shape of an exponential profile [149].



Figure 1. Release process in edible coatings incorporated with functionalized nanostructures.

The release of a bioactive substance with low molecular weight from a homogeneous, swelling polymeric network can produce the diffusion of water into the polymeric matrix, followed by matrix relaxation with the subsequent diffusion of the active compound out of the swollen polymeric network onto the food surface in edible coatings [155]. Table 7 shows some studies that have explored controlled release in nanostructures functionalized with different bioactive compounds and incorporated into an edible coating. Many of these works have developed the release of the active substance only from the edible coating "in vitro", and only a few have analyzed applications in foods [156]. Finally, to highlight the release behavior of bioactive compounds in edible coatings, some studies have incorporated microcapsules or essentials oil directly into the coating matrix.

Active Component	Nanostructuro Typo	Model of Palazza	Palaaca Typa
Active Component	Nanostructure Type	widdel of Kelease	Release Type
Limon essential oil	NCs: Sodium caseinate	Exponential, Higuchi, and Weilbull Model	Correlations between films with microcapsules of lemongrass essential oil and this oil without microcapsules in films of alginate matrix. Peppas model, showed values of the η exponent in both cases was in the zone indicative of a Fickian release mechanism: $n = 0.205 < 0.43$ for microcapsule spheres and $n = 0.311 < 0.5$ for thin films [157]
Cooper cations	Microcapsules: Alginate	Kinetic model	The release constant k increases with initial copper cation concentration. Relatively high η values ($n > 0.43$) indicate copper cation release following anomalous kinetics (diffusion and polymer relaxation). <i>T. viride</i> released in the surrounding solution [158]
Ethylvanillin	NPs: Ethylcellulose	Ritger–Peppas and first order release model	The values of η for all concentrations of polymers in the NPs prepared were found to be >0.48 and <0.89, indicating that release occurred through a non-Fickian diffusion mechanism [159]
α-tocopherol NCs: Methylcellulose Fickian release		Fickian release	The release profiles of all NCs films exhibited an initial burst effect (first hour), followed by a sustained release over 10 days, with a typical Fick's curve [160]

Table 7. Recent studies on deliver	y and kinetics of nanostructures in edil	ole coatings.
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6. Conclusions and Future Trends

Currently, nanoengineered structures containing bioactive substances have a fundamental role in food processes, particularly for food preservation. Thus, the use of submicron systems represents great advantages in contrast to the systems of conventional size, since these improve the initial properties of the materials increasing the interaction of the active components with food surface. These systems can adapt to different conditions such as ionic strength, thermal resistance, pH, food composition, etc., keeping their capacity to release bioactives and, in several cases, spatial ubication. The diversity of nanostructures reviewed, solid lipid nanoparticles, polymeric nanospheres and nanocapsules, nanogels, liposomes, lipid carrier nanoparticles, etc., shows the possibility to adapt their architecture and composition to the functionalization with diverse substances such as pre-biotics, pro-biotics, antimicrobial, antioxidants, colorants, flavor, etc. Their application is diverse and still need further study, in particular their interactions with food cellular structures or tissues. Edible coatings are the best way to apply these functionalized nanostructures on the food in a homogenous and safe way with minimal processing steps and using conventional equipment. The edible coatings prepared with functionalized nanostructures can be considered a versatile tool for long-term food preservation complying with safety aspects. Future trends include the development of composite edible coatings, containing two or more nanostructured systems, which have improved gas barrier properties, greater firmness to the products, enhance or add colors and flavors unique to defined products and increase the meaningful nutritional value.

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Abbreviation

- CG Cashew gum
- CMC Carboxymethyl cellulose
- CNC Cellulose nanocrystals
- CS Chitosan
- L-NVs Lipid-based nanovesicles
- NCs Nanocapsules
- NGs Nanogels
- NLC Nanolipid carrier
- NPs Nanoparticles
- NSs Niosomes
- PCL Poly-ε-caprolactone
- PEG Polyethylene glycol
- PLGA Polylactide-co-glycolide
- SLN Solid Lipid Nanoparticles
- TG Tragacanth gum
- IG Iragacanth gum

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