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Chitosan-based electrospun nanofibers for encapsulating food bioactive ingredients: A review

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Abstract

Today, society has been more aware of healthy food products and related items containing bioactive compounds, which potentially contribute to human health. Unfortunately, the long-term stability and bioactivity of biologically active compounds against environmental factors compromise their target and effective action. In this way, lab-designed vehicles, such as nanoparticles and nanofibers, provide enough properties for their preservation and suitable delivery. Here, the electrospinning technique acts as an effective pathway for fabricating and designing nanofibers for the entrapments of biomolecules, in which several biopolymers such as proteins, polysaccharides (e.g., maltodextrin, agarose, chitosan), silk, among others, can be used as a wall material. It is likely that chitosan is one of the most employed biomaterials in this field. Therefore, in this review, we reveal the latest advances (over the last 2-3 years) in designing chitosan-based electrospun nanofibers and nanocarriers for encapsulation of bioactive compounds, along with the key applications in smart food packaging as well. Key findings and relevant breakthroughs are a priority in this review to provide a cutting-edge analysis of the literature. Finally, particular attention has been paid to the most promising developments.

Keywords: Polysaccharides; delivery systems; nutraceuticals.

Abbreviations:

AOP: antioxidant peptide

CGA: chlorogenic acid

CS: chitosan

DES: Deep eutectic solvent

EE: encapsulation efficiency

ENs: electrospun nanofibers

ES: electrospinning

FXM: flaxseed mucilage

GA: gum Arabic

HNTs: halloysite nanotubes

HPβCD: hydroxypropyl-β-cyclode

NFs: Nanofibers

PCL: poly(ε-caprolactone)

PEO: poly(ethylene oxide)

PLA: poly lactic acid

PVA: polyvinyl alcohol

WVP: water vapor permeability

XG: xanthan gum

1. Introduction

The production of healthy products and related items enriched with bioactive compounds (bioactives) is a primary concern for the food industries to meet the healthy diet of customers, who desire to improve their habits and health. However, most of the bioactives (phenolic compounds, terpenoids, phytosterols, peptides, capsaicinoids, carotenoids, alkaloids, among many others) present fast and easy reactivity with external agents [1–3], such as humidity, temperature, among other environmental conditions, compromising their long-term stability, bioactivity, and bioavailability; thus, less effectiveness in the target action. Scientists seek for new methods and strategies to preserve such compounds for a longer time and maintain their bioactivity. Today, lab-designed vehicles, such as nanoparticles and nanofibers, confer a suitable environment for their preservation once such molecules are encapsulated and thus providing efficient delivery as well [4].

Dealing with the different methods of encapsulating, electrospinning (ES), as any electrodynamic technique, stands out as one of the most promising for protecting bioactives since does not use high temperatures [5,6]. As part of its mechanism of operation, it uses a high-voltage electric field on the jet of a polymer solution[7]. In this way, the resulting electrospun fibers may present either nano or microstructure with high porosity, large area and interconnected pore structure, and enhanced thermal stability [8,9]. Thanks to all these latter properties, electrospun nanofibers (ENs) offer high encapsulation efficiency (EE) with outperforming protection for highly sensitive bioactives [8]. Additionally, the high surface area favours fluid absorption while the high porosity allows, for instance, the facilitated exchange of oxygen, water, and nutrients, and minimal penetration of microorganisms.

Proteins (zein, gelatine, whey) and polysaccharides (starch, maltodextrin, agarose, chitosan) are among the main biomaterials used for the fabrication of ENs aimed at the encapsulation of bioactives for food and nutraceutical interest [5,10,11]. Particularly, chitosan (CS) presents good film-forming and nanostructured properties, together with biodegradability, non-toxicity, and biological properties (i.e., antimicrobial, antifungal, and antiviral) [12]. Considering its plenty of functional groups (e.g.,

amino and hydroxyl), CS can be easily modified and can react with additives and species for tailoring desired self-assembled structures [13], making it suitable for the fabrication of ENs as a wall material for encapsulating biomolecules [14,15]. In this review, we collect the recent pieces of evidence for applying CS in the fabrication of ENs; herein, we analyse the latest developments over the last 2-3 years. A particular emphasis has been focused on the utmost ideas in designing CS-based ENs and their important findings and results.

2. Electrospinning and chitosan: an overview of factors affecting electrospun fabrication

CS is an animal-origin biopolymer, produced from the deacetylation reaction of chitin (poly(β-(1-4)-N-acetyl-D-glucosamine). This latter biomaterial is extracted from the outer part of crustaceans' shells. Regarding its availability, CS is reported to be produced around 1011 tons yearly, in which the global market has grown from USD 553 million in 2017 to 1,088 by 2022 [16]. This opens a window on its availability and further applications in several sectors such as flocculants, ion exchangers, chelating agents, coating and film-forming materials, drug carriers, membranes, and scaffolds for tissue engineering, to mention just a few [16–19]. CS is a water-soluble material in an acidic environment (at pH < 6) because of the strong hydrogen bonding between its acetamide and hydroxy groups. It also presents exceptional biodegradability and null toxicity. According to its chemical functionalities in structure (e.g., hydroxyl groups), CS displays hydrophilic properties with facilitated transport of polar substances thanks to hydrogen bonding interactions. The polar groups (-OH) are available for chemical modifications, including sulfonation, carboxymethylation, phosphorylation, or hydroxymethylation [15,20].

CS can form hydrogels thanks to its all-chemical functional groups, which could also interact with each other by distinct pathways, e.g., ionic and hydrophobic interactions, molecular entanglements, or hydrogel bonds, resulting in the production of physical hydrogels [21]. Additionally, high-molecular-weight molecules of CS can generate self-assembled micro and nanostructures based on hydrogen-bond networks. Certainly, the conformational types of these CS structures depend on several factors, such as pH, temperature, and types of additives (salt, acids, cross-linking agents),

while their properties depend on their molecular weight and nanostructures. CS can form 3D polymeric networks or complexes designed by non-covalent strategies based on electrostatic, hydrophobic and/or hydrogen bonding forces between the polymeric chains rather than chemical bonds. **Figure 1** illustrates the different ways of using structures based on CS for food and drug delivery systems.

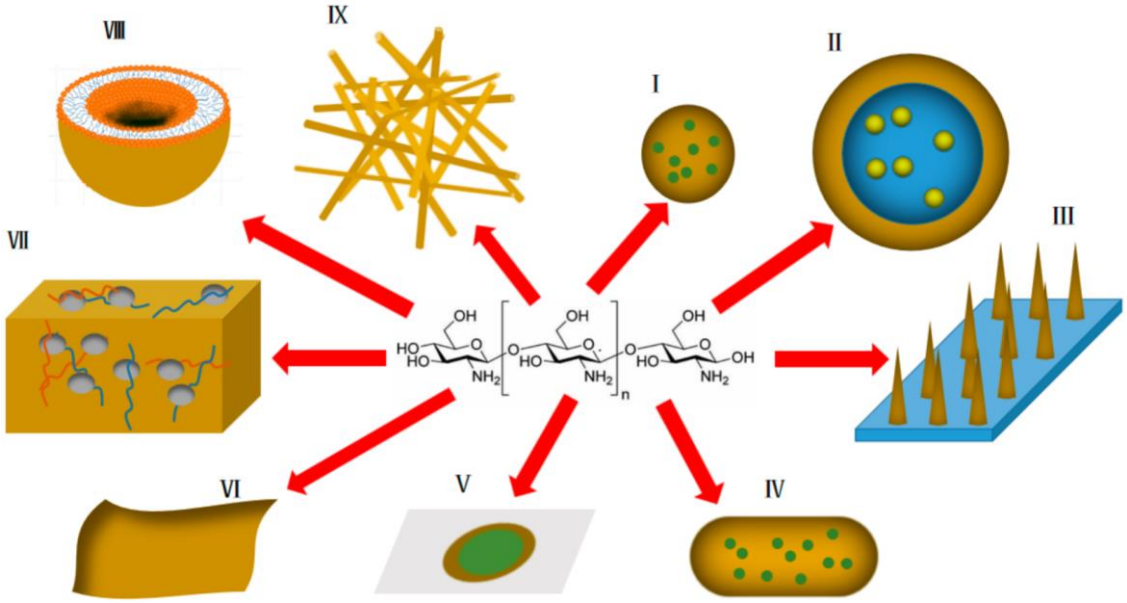


Figure 1. Distinct structures based on chitosan for food and drug delivery systems (yellow colour represents chitosan, including nanoparticles (I); emulsions (II); transdermal microneedles (III); nanocapsules (IV); transdermal patches (V); transdermal membranes (VI); hydrogels (VII); liposomes (VIII); nano-scaffolds (IX).

Importantly, even if CS presents great potential as carrier material in bioactive delivery systems [22], it still displays a capacity limitation for modulating/controlling the release of bioactives due to its fast dissolution in systems with high water content, e.g., in the stomach [23]. To face this drawback, typical chemical modifications, such as co-polymerization or derivatization, have been proposed. While specific chemical modifications are applied to improve other CS properties, including solubility, biodegradability, good biocompatibility, drug-carrying capacity, as follows:

- Carboxylation modification: It involves the introduction of carboxyl groups into the molecule using mainly glyoxylic acid and chloroacetic acid. Nevertheless, when using chloroacetic acid, chloroacetic acid, they can react with C_2-NH_2 and C_6-NH_2 on CS producing N,O-carboxymethyl CS. This latter CS derivative molecule presents substantially improved water solubility, biocompatibility, and antibacterial properties [24].

- Alkylation Modification: In general, hydroxyl or amino groups of CS are subjected to alkylation reaction with halogenated hydrocarbons or sulfates, resulting in alkylated derivatives under alkaline conditions. As C_2-NH_2 on the sugar molecule chain exhibits strong nucleophilic lone pair of electrons, the alkylation of CS takes place preferentially on C_2-NH_2. Apart from the improvement of CS solubility, alkylated derivatives of CS also show exceptional biocompatibility, coagulability, and antibacterial and hemostatic properties [25]. For instance, N-alkylated CS derivative can be synthesized via halogenated alkanes. Here, the introduction of an alkyl group with suitable molecular weight to the CS molecular chain will also boost CS solubility, while the introduction of a large molecular weight alkyl group will result in worsened solubility[26].
- Acylation modification: This a quite complicated pathway of chemical modification of CS. Generally, organic acid derivatives (including acid anhydrides, or acid halides) are employed as acylating agents in a certain reaction medium, and subsequently aliphatic or aromatic acyl groups are introduced into the molecular chain of CS, resulting in acylated CS. Commonly, such CS derivatives present good biocompatibility, drug-carrying capacity, and drug release control capability, which are related to improved solubility [25].
- Esterification modification: In this chemical modification, hydroxyl or amino groups on CS are subjected to esterification reactions (such as sulfation and phosphorylation) with oxygen-containing mineral acids or acid derivatives, resulting in esterified CS. These CS derivatives exhibit improvements in terms of adsorption and antibacterial properties. Looking for improved CS solubility, phosphorylation via methanesulfonic acid stands out as the main esterification reaction for obtaining a CS derivative with good water solubility [27].
- Sulfonation modification: This involves the introduction of sulfonate groups into CS by reacting its hydroxyl or amino groups with concentrated sulfuric acid or sulphate, this reaction takes place at the C_2-NH_2 of CS [25].
- Quaternary Ammonium salt modification: This chemical modification involves the

introduction of quaternary ammonium groups into the amino groups (or even hydroxyl ones) of CS. According to the high activity of the amino functionalities, quaternary ammonium salt modification mostly takes place on the amino groups. Interestingly, quaternary ammonium salt groups feature large steric hindrances (such as strong hydrate-ability) greatly weakens the hydrogen bond between CS added to the quaternary ammonium salt group, and consequently improves its water solubility. In addition to this, N-quaternary ammonium CS derivatives also exhibit better biocompatibility, biodegradability, and good mucosal adhesion [28].

- Graft copolymerization modification: This modification technique is the most attractive pathway for improving both physicochemical and biological properties via physical and chemical modifications of CS. Oxidative coupling copolymerization, free radical graft copolymerization, and condensation copolymerization are among the most typical methods in this method. They comprise the introduction of chemical functionalities (such as hydroxyl, carboxyl, ester, and amide) into CS. The resultant properties of the CS after this chemical modification are mainly controlled by the molecular structure, length, and number of side chains. In general, this method mainly involves the graft copolymerization with alcohols, esters, acids, and amides into CS, which results in improving the antibacterial, antitumor, antioxidant, and anticoagulant properties of resultant CS derivatives [25].
- Schiff base reaction: It involves the condensation reaction of the amino groups of CS with carbonyl compounds, such as fatty aldehydes, aromatic aldehydes, and ketones, in a neutral medium in order to eliminate water molecules and thus synthesize the Schiff base with imine group. Acetic acid, ethanol, and methanol, or their mixtures are among the main solvent media for the Schiff base reaction. After this reaction, there is a change on the molecular structure of the CS molecular chain while increasing the number of positively charged ions, allowing to greatly strengthens the water solubility and antibacterial activity of the resultant CS derivative [25,29].

According to these previous modifications, the resulting CS structure may display different

properties in terms of solubility, water retention and emulsification capacity, and digestibility, along with structural and chemical compatibility with other molecules [30]. In this latter point, the carrier morphology, e.g., in ENs, can be potentially designed during the ES process and spinning dope [5]. **Figure 2** summarizes the main operating parameters and factors of ES influencing directly the ENs. To some extent, the fabrication of ENs greatly depends on the solution (polymer) viscosity and the chain entanglement degree of the wall material which belong to the solution parameters; however, some other factors, such as process variables and environmental parameters, may also play an important role. The right optimization of the aforementioned factors leads to the designing and fabrication of ENs with desired geometry, structure and architecture.

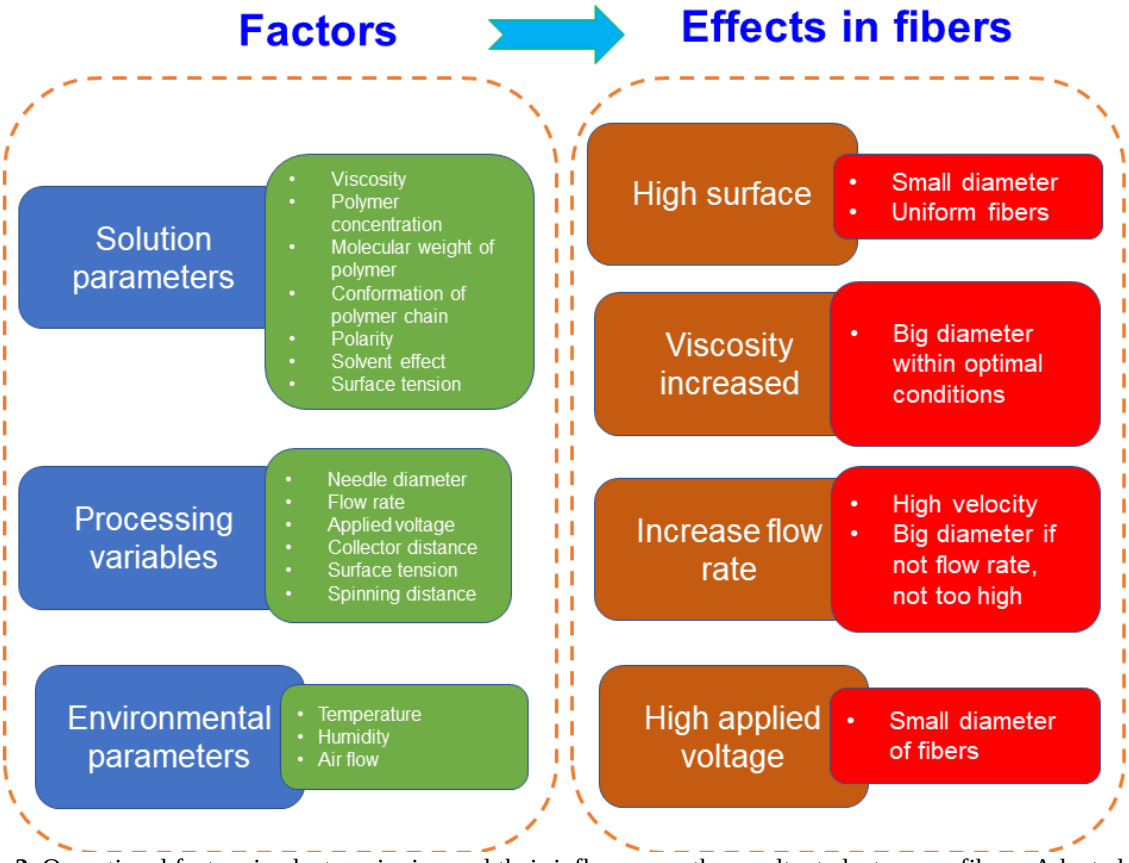


Figure 2. Operational factors in electrospinning and their influence on the resultant electrospun fibers. Adapted with permission from Coelho et al. [31].

As for the encapsulation of bioactives and their post-delivery, ENs offer structural advantages, such as a high surface area to volume ratio, tailored morphology and porosity. Furthermore, these ENs also confer functional advantages including improved stability and bioavailability, high EE, and controlled release. Even if the properties of the final fibers are a result of the ES process, wall

materials are also determinants in the final features of ENs. Here, distinct materials have been used as wall material, such as polylactic acid (PLA) [32], polyvinyl alcohol (PVA) [33], and polycaprolactone (PCL) [34], which are all categorized as biocompatible polymers. While hyaluronic acid [35,36], sodium alginate [37], silk [38], zein [10,39], xylan and guar gum [40,41], and CS, among many others are some examples of biopolymers. **Table 1** gives, for instance, some examples of using such materials for the encapsulation of bioactives for different purposes. These latter biopolymers, like CS, have somehow contributed to overcoming the challenges of ENs in terms of biodegradability and biocompatibility for the fabrication of innovative and safe products within the food and pharmaceutical field. In the following section, our attention has been emphasized to analyze and discuss only the most recent advances in designing and optimizing CS-based ENs for bioactives encapsulation.

Table 1. Examples of biopolymers for encapsulating food ingredients and biomolecules via electrospinning.

Biopolymer	Bioactive compound	Purpose of electrospinning	Ref.
Amaranth protein isolate	Folic acid	Enhanced preservation of the antioxidants from UV light exposure	[42]
Gelatin/zein	Curcumin	Enhanced preservation and release in food packaging	[43]
Poly(lactic-co-glycolic acid)	Curcumin	Enhanced release rate for delivery systems	[44]
Zein	Curcumin	Boosting storage and stability for coloring food systems	[45]
High-amylose starch	-	Studying the rheological properties for potential food and pharmaceutical application	[46]
Gliadin	Ferulic acid	Enhanced long-term stability and solubility for food packaging	[47]
Zein	Omega-3-rich fish oil	Evaluating *in vitro* gastrointestinal activity for nutraceutical applications	[48]
Poly(ethylene oxide)	*Spirulina* microalga	Improving thermal properties and release rate for food packaging applications	[49]

It is important to point out that CS has been intensively explored for nanofiber fabrication due to its ability in releasing satisfactorily the encapsulated food bioactives. For instance, in an acidic medium (with pH=1.2), bioactives release from the CS hydrogel is increased thanks to the higher swelling ratio of the hydrogel whose amine groups in CS chains become protonated, resulting in enhanced electrostatic repulsion between them and thus weakening the bonding interaction of the CS molecules. Hence, the CS hydrogel network is relaxed allowing the diffusion of the bioactives [50,51].

3. Recent breakthroughs in CS-based electrospun nanofiber design for food ingredients

3.1. Pristine CS-based electropun nanofibers

Over the last two years, great effort has been done on manufacturing ENs utilizing CS as a primary material. **Table 2** reports the recent state of the art of CS ENs and the main findings reported by the researchers in the field. According to its chemical structure and multiple chemical functionalities, CS presents suitable film-forming properties itself. For instance, in a series of studies aiming the protection of perishable foods, Ceylan and co-workers investigated the microbiological stability of sea bass fillets protected with smoke-doped CS NFs [52] and ENs containing a liquid smoke/thymol mixture as well [53]. Both electrospun mats presented a smooth, and ultrafine biopolymeric structure with a fiber diameter ranging from 72 to 132 nm. Regarding their applications in food packaging, the ENs presenting the mixture of smoke/thymol exhibited around 60% limitation of growth in terms of total mesophilic bacteria, while the fiber-containing smoke only showed a limitation between 40-50%. In both cases, the antimicrobial effects observed in fish fillets, opening a new window for extending the shelf-life of perishable foods. To date, CS has been used as a primary phase in fabrication of ENs; however, in a recent development, Zahiri and co-workers [54] utilized CS as a nanocarrier of curcumin within PCL and gelatin fibers. This concept and utilization of CS nanostructures in ENs fabrication allow extending the application of such materials, e.g., for wound healing and dermal reconstruction. The versatility of CS nanoparticles is widely open for a polymer blending with other polysaccharides, like sulfobutyl-β-cyclodextrin, in which the potential nanoparticles present the ability to protect phenolic compounds (eugenol) [55].

As for electrospinning process, it is important to mention that the different parameters, including voltage, flow rate, distance between electrodes, temperature and related humidity, along with electrospinning solution properties (e.g., pH, viscosity, molecular weight, and mixing ratios of polymers and solvents) are fundamental in the resultant properties of the nanofibers [56–58]. Considering the excellent antibacterial properties of CS-based ENs, the resultant nanofibers have been mainly investigated in packaging applications of several food products to extend the self-life of

fruits and vegetables, meat shelf-life prolongation [59]. To some extent, the main parameters influencing the antibacterial properties of CS are:

- pH: CS exhibits low solubility above pH 6.5, and thus meaning the antibacterial effect is only observable at an acidic pH, which is ascribed to the protonation of NH_2 groups. Herein, where CS becomes polycationic and interacts with negatively charged microbial cell membrane compounds, such as phospholipids, proteins, anionic polysaccharides, fatty acids, and bile acids [60].
- Concentration: It has been investigated that the antibacterial activity of CS increases with increasing concentration [75–78]. On the contrary, it has been also discovered that at low concentrations, CS is able to bind to the negatively charged cell surface, destroying the cell membrane and finally kill the cell [61].
- Molecular weight: As it is well known there are three distinguished types of CS, such as high-molecular-weight, low-molecular-weight, and oligochitosan (short-chain) [62]. Experimentally, it was reported that lowering molecular weight increases the antibacterial efficacy for Gram-negative bacteria, while a reverse impact has been noted for Gram-positive bacteria [63].
- Degree of deacetylation: the deacetylation degree (as the number of amino groups) of CS influences the solubility and charge formation of the chemical functionalities, as well as is dependent on the positive charge density. To some extent, the antibacterial effect of CS is more effective with a higher positive charge density [64].

Therefore, several parameters should be considered when fabricating ENs based on CS for targeted application. However, there are still some properties to be improved for food packaging (e.g., higher solubility, chemical, thermal and mechanical stability), and in some cases, higher capacities when encapsulating food ingredients; therefore, CS usage in nanofiber fabrication has been extended by combining this material with other biopolymers, proteins and some other compatible matrices as addressed in following section.

3.2. CS-based blend electrospun nanofibers with other polymer materials

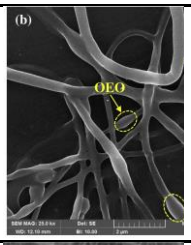
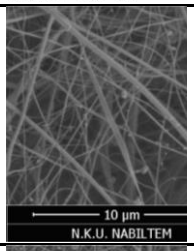
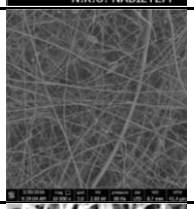
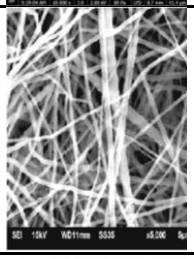
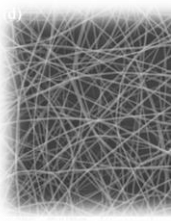
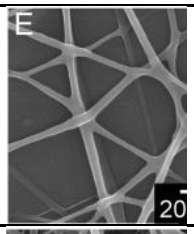
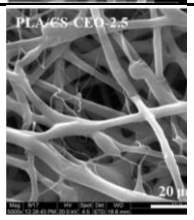
CS also presents the ability to be blended with other biocompatible wall materials resulting in exceptional polymer blends. To some extent, it is likely that CS has been mostly investigated in combination with other film materials proposing new materials for food packaging, Ardekani-Zadeh et al. [65], for instance, fabricated mat blends based on CS and PCL doped with oregano essential oils (EOs) via ES. This has been proposed according to the main hypotheses of the authors, i) two of the essential oil-doped CS/PCL fiber mats can offer similar mechanical and barrier properties to cast film counterparts that can be fabricated via ES method, ii) the new composite mat can effectively reduce the growth of model foodborne bacterial pathogenic. After characterization, the resultant ENs exhibited a dense structure, a diameter between 206-332 nm and a roughness between 62.9–84.6 nm; such dense morphology was a sign of exceptional blending thanks to hydrogen bonding between CS/PCL. Regarding the EE, it remained between 56.4 and 81.7% over 96 h. Interestingly, the fiber blend containing 5% EOs showed antibacterial activity against Gram-positive, such as *Staphylococcus aureus, Listeria monocytogenes*, and Gram-negative bacteria, such as *Salmonella enteritidis*, *Escherichia coli*. According to the results, the nanofibers (NFs) displayed high hydrophobicity in terms of water contact angle measurements, which is suitable in food packaging applications since it prevents the wettability of the structure and thus long-term integrity.

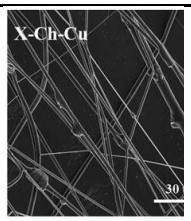
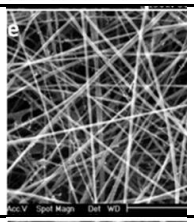
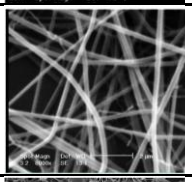
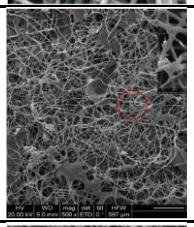
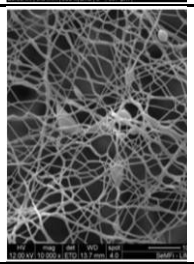
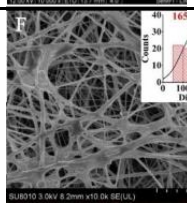
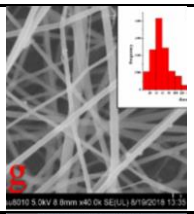
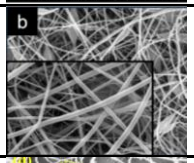
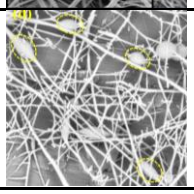
Pursuing hydrophobic surfaces, hordein, which presents several nonpolar and hydrophobic residues and low contents of charged amino acids, has been timely combined with CS to improve the hydrophobicity and its water resistance [66]. While hordein can form ultrathin fibers, it presents weak mechanical strength and low stability in aqueous environments. Therefore, both materials were intentionally merged to offset their drawbacks. Compared with Ardekani-Zadeh's study [65], Rostami et al. [67] reported higher EE ca. 86% when loading resveratrol in CS-gellan ENs, which achieved to deliver in the intestinal region approximately 43–51% of the amount presented in the initial fibers. It is important to mention that the hydrophobicity of hordein/CS NFs has been also improved by heat

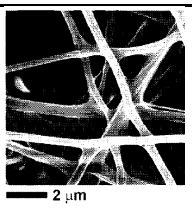
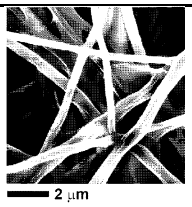
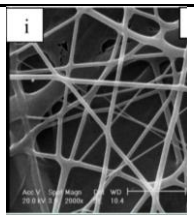
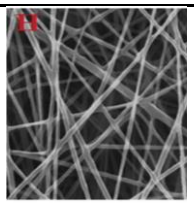
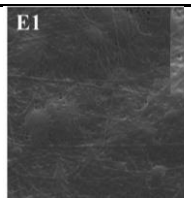
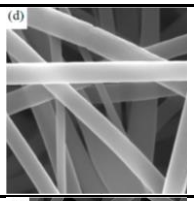
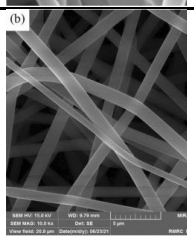
treatment. Another insight due to thermal treatment, the fiber diameter was slightly reduced from 393 to 379 nm. In a real application, the ENs were applied to apples with an incision, retaining the fresh color and inhibiting the enzymatic browning for 6 h. Similar effects were observed in potatoes with incisions in which their properties were maintained over 12 h [68].

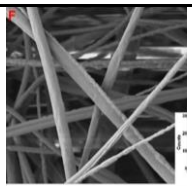
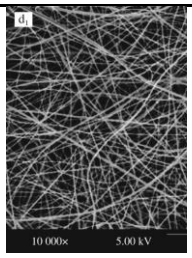
Polullan, which is produced from starch by the fungus *Aureobasidium pullulans*, is another polysaccharide blended with CS to tailor ENs [69]. The main justification of the authors for blending polullan with CS deals with common issue of fabricating pure CS, since this latter biopolymer displays a polycationic nature in solution, stiff structure and intra-molecular interactions making challenging the manufacture of nanofibers. In general, the electrospinning processing can be facilitated by using direct or indirect solvents, but they are undesirable in edible systems. Considering this, it has been reported that pullulan has been used to improve the electrospinnability of electrospinning solutions by raising viscosity and lowering conductivity and surface tension. Therefore, the purpose of the authors was to improve the limitations of CS in this process and concurrently develop fast-dissolving oral films keeping in mind that both polymers present edible properties. In this regard, polullan meets such criteria since several properties have been widely studied, including non-toxic, non-mutagenic, odorless, tasteless and low-caloric [70]. Apart from the good interaction translated to biocompatibility among the biopolymers, CS improved the thermal stability of pullulan. Interestingly, the authors concluded that the CS/pullulan ratio had an influence on solution property and nanofiber morphology, e.g., with the increase of CS, viscosity and conductivity of solutions increased, while diameter of nanofibers decreased initially and then increased. Finally, in such natural composite fibers, aspirin was selected as an active compound, demonstrating fast-dissolving properties which make it promising for oral mucosal release [69].

341 **Table 2.** Advances in chitosan-based nanofibers for embedding different food bioactives and their main findings.

Bioactives	Biopolymers	CS properties	SEM image	Application	Encapsulation efficiency	Highlights of the study	Ref.
Oregano essential oil (OEO)	CS/PCL	Medium molecular weight, DD: 75–85%		Food packaging	56.4–81.7%	Excellent antibacterial properties with outstanding EE. Excellent hydrophobicity	[65]
Smoke	CS	Low molecular weight: 70 kDa DD:75-85 %		Food packaging	-	Promising antibacterial properties (40-50% growth limitation)	[52]
Smoke/thymol mixture	CS	Low molecular weight: 70 kDa DD:75-85 %		Food packaging	-	Promising antibacterial properties (60% growth limitation)	[53]
α-tocopherol	Zein/CS	Medium molecular weight: 900 kDa DD: 90%		Delivery systems	-	Tocopherol did not affect ENs morphology The bioactive compound enhanced mucoadhesivity	[71]
Fish peptide	CS/PVA	Medium molecular weight: 300 kDa DD: 75-85%		Food packaging	94%	High hydrophobicity of ENs; Enhanced mechanical and thermal properties. High EE independent from the peptide level.	[72]
Cinnamaldehyde	CS/PEO	Molecular weight: 460 kDa		Delivery systems	-	Fast release of cinnamaldehyde, High inactivation efficiency toward *Escherichia coli* and *Pseudomonas aeruginosa*.	[73]
Cinnamon essential oil	PLA/CS	Molecular weight: 80,000 DD: 85%		Delivery systems and anti-microbial application	47-55%	Improved antimicrobial Activity, High long-term inactivation rates toward *Escherichia coli* and *Staphylococcus aureus*	[74]

Curcumin	XG/CS	Molecular weight: 28 kDa DD: 89% DP: 175		Delivery systems	69%	Excellent carriers for hydrophobic bioactives, ENs displayed long-term pH-stimulated release properties.	[75]
Resveratrol	Gellan/CS	Molecular weight: 50-190 kDa DD:75%		Delivery systems	86%	Resveratrol released in intestine was between 43-51%.	[67]
-	CS/Gelatin	Molecular weight: 60-120 kDa		Food packaging (edible films)	-	Resultant ENs displayed exceptional mechanical strength.	[76]
-	Hordein/CS	DD: 90-98%		Food packaging (edible films)	-	Excellent hydrophobicity allowed ENs to display water resistance.	[66]
Cabreuva essential oil	CS/PVA	Medium molecular weight: 300 kDa DD: 85%		Delivery systems	-	ENs showed ability to modulate oil release.	[77]
Chlorogenic acid	CS/PCL	Medium molecular weight:150-300 kDa DD: 95%		Active food packaging	70%	Long-term release of phenolic compounds. Enhanced antioxidant and antimicrobial activities.	[78]
Aspirin	Pollulan/CS	Molecular weight: 400,000		Fast dissolving oral films	-	Improved thermal properties, Fast dissolving oral properties	[69]
Quercetine	Hordein/CS	Molecular weight: 152,143 DD: 95%		Food packaging	-	Improved hydrophobicity of films using heat treatment.	[68]
Bifidobacterium animalis	CS/PVA	Medium molecular weight DD:75–85%		Functional food applications	-	Survivability of probiotic in fiber mat was substantially enhanced.	[79]

Black pepper essential oil	CS-coated PLA	Medium molecular weight		Biomedical applications	65%	Enhanced hydrophilicity, Acceptable antimicrobial properties, Improved cell adhesion compared with pristine fibers	[80]
Limonene	CS-coated PLA	Medium molecular weight		Biomedical applications	43%	Enhanced hydrophilicity, Acceptable antimicrobial properties, Improved cell adhesion compared with pristine fibers	[80]
Thyme essential oil	CS/Gelatin	Molecular mass: 60000–120000		Functional food applications for meat products	40%	Thyme essences exhibited antimicrobial activity against *Clostridium perfringens* ENs represented an alternative for nitrate substitute in sausages	[81]
Pomegranate peel extract	CS/PEO	Medium molecular weight:100-300 kDa		Active food packaging for meat products	-	The ENs displayed acceptable properties in terms of thermal and mechanical tests. The composite ENs preserved and enhanced the shelf life of beef.	[82]
3-Phenylacetic acid	CS/Gelatin	DD: 95%		Active food packaging	-	At optimized acid level, ENs displayed enhanced thermal and water stability, and water vapor permeability.	[83]
Ziziphora clinopodioides essential oil, Sesame oil	CS/FXM	Medium molecular weight:190-310 kDa DD: 75–85%		Active food packaging	>93%	Active CS/FXM ENs displayed acceptable antioxidant and antimicrobial activity.	[84]
Anthocyanins	CS/GA	Medium molecular weight:250 kDa DD: 75–85%		Active food packaging for chicken products	97%	The extract reduced the tensile strength and increased elongation at break. The extract improved the water barrier and thermal stability	[85]

						of the resultant ENs.	
Thymol/ HPβCD	CS/PCL	Medium molecular weight:100-200 kDa DD: 95%		Active food packaging for fruit products	-	Improved thermal stability of the resultant ENs. Long-term release of the thymol over 240 h Good antifungal activity in tomato samples.	[86]
Curcumin	CS/Gelatin	Medium molecular weight DD: 75–85%		Active food packaging	-	ENs displayed high sensitivity of colorimetric changes due to ammonia presence. Both mechanical and thermal properties in the ENs were improved by embedding curcumin.	[87]

*PEO: poly(ethylene oxide); PLA: Poly lactic acid; XG: xanthan gum; PCL: polycaprolactone; FXM: flaxseed mucilage; GA: gum Arabic; HPβCD: hydroxypropyl-β-cyclodextrin; DA: Degree of deacetylation, DP: Degree of polymerization.

Zou et al. [78] also reported the manufacture of ENs based on CS and PCL, in which this latter polymer has been intentionally blended to improve the spinnability limitations of CS, while halloysite nanotubes (HNTs), doped with chlorogenic acid (CGA), were incorporated in the polymer blend to improve the encapsulation efficiency of the composite ENs due to the hollow tubular structure of the aluminosilicate nanomaterial. **Figure 3a** illustrates the physical embedding of such bioactives in the composite fiber. To some extent, the usage of HNTs contributed to higher EE, which was noted to increase at higher ratio of such nanomaterials (**Figure 3b**). The maximum EE was found to be ca. 70%. Regarding the surface nature of the fiber, they resulted to be highly hydrophilic with a contact angle of approximately 30-50° (see **Figure 3c**) adding between 2-6% CGA@HNTs. While the water vapor permeability decreased significantly with the incorporation of CGA@HNTs, as illustrated in **Figure 3d**. This latter property was improved by adding such a complex biomolecule composite; in theory, the water vapor permeability is a relevant parameter for packaging materials since it correlates with the moisture transfer between the environment and food item. Additionally, these ENs displayed excellent antioxidant and antimicrobial properties, in which the resultant mats offered a long-term release of CGA governed by Fickian diffusion. After the complete study, the authors stipulated that such CGA@HNT/PCL/CS ENs could be used as an inner layer added to the packaging substrates and thus extend the self-life of perishable food products [78].

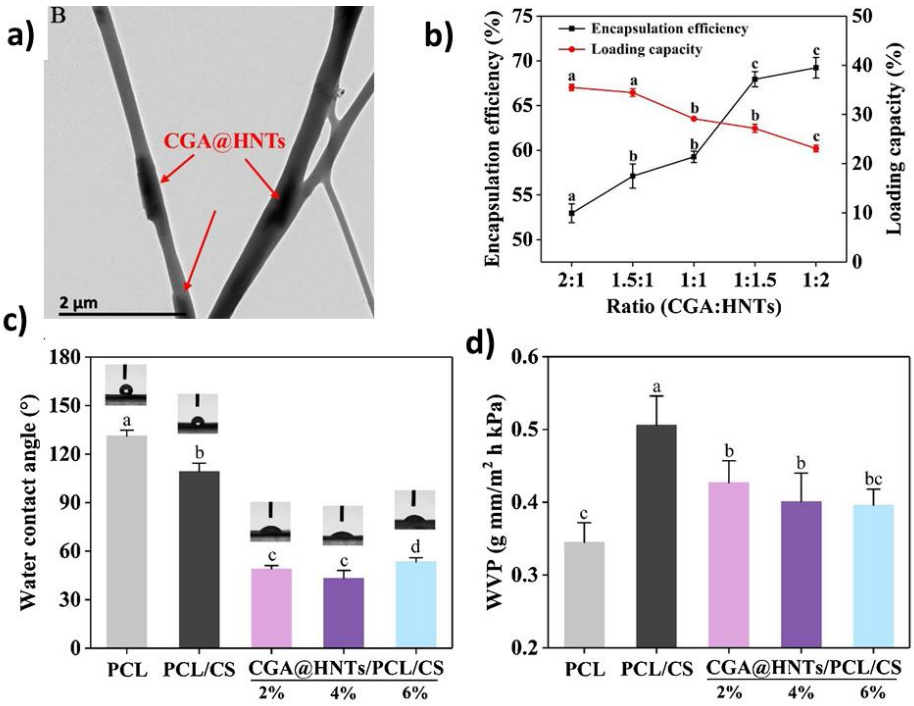


Figure 3. CS/PLA nanofibers loaded with chlorogenic acid for food packaging, a) Micrograph of the resulting ENs, b) their encapsulation efficiency and loading capacity, c) water contact angle measurements and d) water vapor permeability (WVP). Adapted with permission from Zou et al. [78].

In the same field of food packaging application, Hosseini et al. [72] blended CS with another highly polar polymer, like PVA, to further encapsulate fish-purified antioxidant peptide (AOP). In this study, PVA was introduced as a host easily electrospinnable polymer to counter the generated repulsive force between CS ionic groups. These new blend mats showed a homogenous and bead-free hydrophobic nanostructure (see **Figure 4a**) with a diameter varying from 157 to 195 nm, which supported the good miscibility among both polymer phases. This agrees with similar findings in terms of structure and fiber diameter (ca. 200 nm) reported by Lamarra et al. [77], who prepared CS/PVA ENs as well. Structurally, the blended fiber also showed improved thermal and mechanical properties due to the possible intermolecular hydrogen bonding among PVA and CS [72]. As shown in **Figure 4b**, the antioxidant activity increased as a function of AOP concentration; also, the loading capacity of ENs increased proportionally with the peptide concentration, reaching its maximum 50% at 5 mg/mL of peptide (**Figure 4c**), while the EE remained unchanged (~94%)[72]. As a perspective, these ENs still need to prove their preservation ability of any food system in real practice; or at least antimicrobial activity should be assessed, as proved by CS/PVA ENs containing Cabreuva EOs

against *Candida albicans*, *E. coli*, *S. aureus*, and *S. epidermidis* [77].

In agreement with Hosseini et al. [72], Shen et al. [86] also confirmed both improved thermal and mechanical properties thanks to hydrogen bond interaction among the polymer phases. In this case, the authors used CS/PCL to encapsulate thymol/2-hydroxypropyl-β-cyclodextrin complexes for the packaging of tomatoes. The bioactive compound was released over 240 h, exhibiting acceptable antifungal activity *in vitro* and *in vivo*. As for the incorporation of the complexes, the ENs benefited from increased average diameters 243.84 nm to 560.55 nm, improved water vapor permeability, crystallinity decrease and a hydrophilic surface.

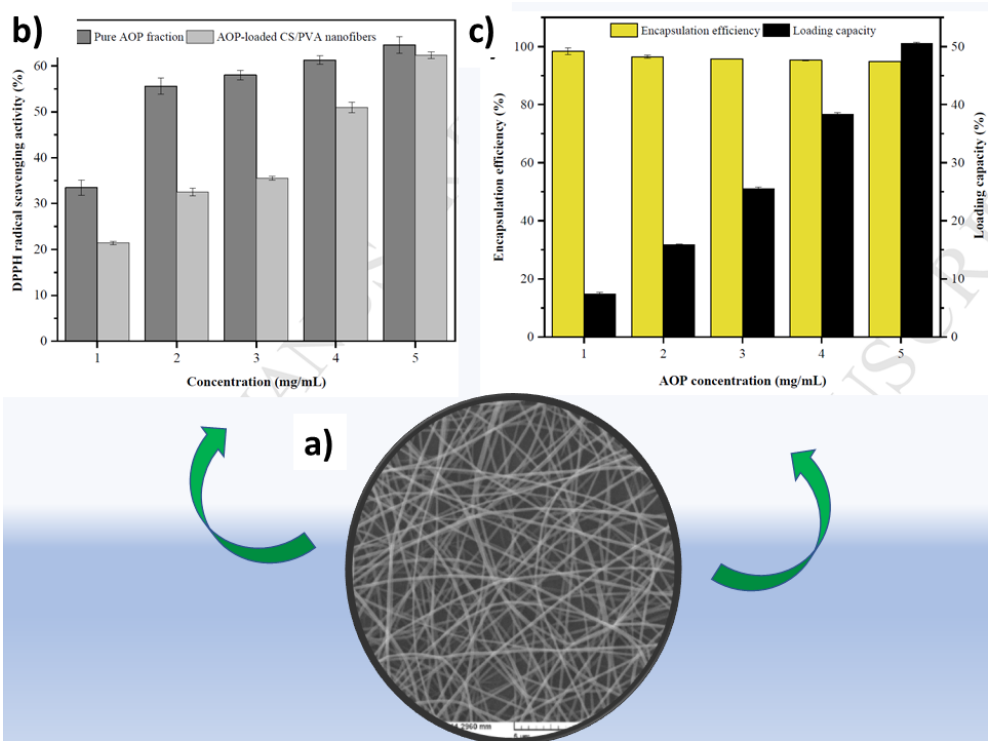


Figure 4. CS/PVA nanofibers loaded with bioactive peptide for food packaging, a) Micrograph of the resulting ENs, b) their antioxidant activity and c) encapsulation efficiency and loading capacity. Adapted from Hosseini et al. [72].

Differently from Hosseini et al. [72] and Lamarra et al. [77] who encapsulated bioactives, Mojaveri et al. [79] proposed CS/PVA electrospun matrix for the protection of probiotics, such as *Bifidobacterium animalis* Bb12. The CS/PVA system, which was also enriched with inulin as a prebiotic, evidenced the compelling thermal protection of the probiotic. The main target of simultaneously incorporated the prebiotic and probiotic in the ENs was to exhibit a synergistic effect (known as symbiotic) and thus improve probiotic proliferation in the intestine while helping the modifications of the gut

community. For instance, **Figure 5a** shows clear evidence of the attached bacteria, which were also observed in fluoresce microscopy (**Figure 5b**). The authors demonstrated the survival of probiotics in ENs, compared with free cells, which was substantially improved under simulated gastric and intestinal fluids, as evidencing probiotic presence after the exposure in such conditions (see **Figure 5c & d**). This research gives proof for symbiotic nutraceutical supplements. As a perspective, the authors highlighted the future evaluation of the cell viability at room and refrigerated conditions [79]. Similar to Mojaveri et al. [79], Xu et al. [88] recently encapsulated *Lactobacillus rhamnosus* in a PVA/pectin matrix maintaining the survival of the probiotics over 90%.

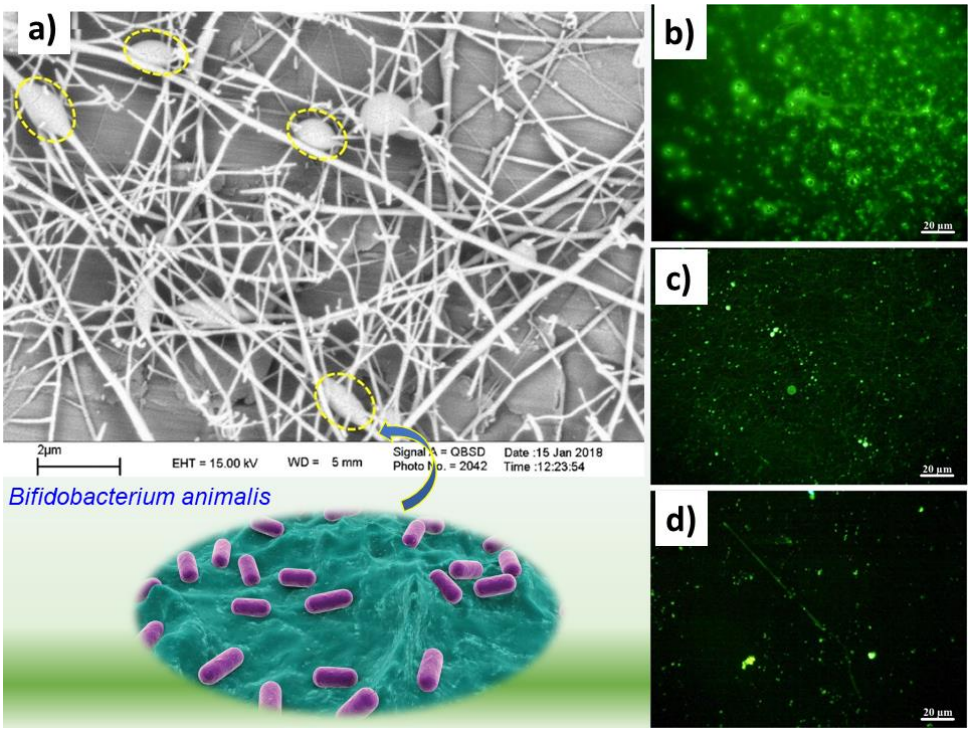


Figure 5. CS/PVA nanofibers enriched with inulin for probiotic encapsulation, a) SEM view of the resultant ENs, b) florescence micrograph of the probiotic embedded into the CS/PVA composite, and fluorescence micrograph of the ENs after exposure in simulated gastric (c) and intestinal fluids (d). Adapted with permission from Mojaveri et al. [79].

To date, several successful attempts have been achieved for the blending of CS with gelatine in electrospun mat fabrication [76,81]. Gelatine, which is well known as food grade and edible protein, composed of several amino acids, was merged into CS to prepare ENs loaded with thyme EOs [81]. In Vafania's study [81], it was documented that NFs based on CS/gelatine doped with thyme EOs exhibited antimicrobial activity against *Clostridium perfringens*. Particularly, NF mats containing 500 ppm thyme EOs were additionally added into sausages, showing similar organoleptic features compared with the ones containing nitrites. It is relevant to mention that nitrites are typically used in

the meat industry as a curing agent, providing better flavor, taste, and aroma while preserving the characteristic red-pinkish color of the meat; and prevent the risk of bacterial contamination; unfortunately, nitrites are considered harmful for consumers' health [89]. Therefore, the addition of thyme EOs-enriched CS/gelatin fibers into sausage formulation represents an alternative for healthier meat products. An important aspect in Varania´s study [81] relies on the non-interaction of any of the wall materials (gelatin or CS) with the ingredient, but it is likely that both materials may interact since it is reported that the incorporation of proteins-based compounds into chitosan is able to weaken the interactions between chitosan molecules and may lead to a possible disruption of the three-dimensional structure of the mats. This latter interaction may also result in improved solubility water solubility of the resultant composite and this easy release of the ingredient [90].

Within the field of meat product preservation, Surendhiran et al. [82] designed CS/PEO NFs enriched with pomegranate peel extract which substantially extended the self-life of beef. In this study, PEO, as a water-soluble and biodegradable synthetic polymer, was utilized to avoid the formation of CS gel via hydrogen bond at the tip of the needle and thus facilitate its spinnability. The resultant NFs were able to reduce the population of *E. coli* O157:H7 up to 2.96 and 5.80 log CFU/g at 4 and 25 °C, respectively.

To date, it seems that ENs can prolong the shelf-life and freshness of meat products; for instance, Shavisi et al. [85] observed that CS/gum Arabic mats enriched with anthocyanins acted as a protective barrier for chicken fillets. Apart from that, such NFs changed their colour tonality, from white to light khaki, as a response to ammonia vapor presence. Potentially, these composite mats could be used as a quality indicator of these products. Herein, the colour response to ammonia vapor needs to be further evaluated in terms of volatile nitrogen-based compounds, which are typically produced during perishable food spoilage [91]. In this regard, Duan et al. [87] recently proposed a hypothetical mechanism attributed to the colour change, as graphically shown in **Figure 6**. The gas state NH_3 mixed with water in the ENs produces forms of NH_4^+ and OH^-. After this, OH^- induced the generation of an alkaline environment on the ENs, inducing the phenolic hydroxyl group to be transformed into

a phenolic oxygen anion in the curcumin structure [92], and thus producing the visible colour shift. As a concluding remark, Duan et al. also proposed ENs based on CS/gelatin containing curcumin as smart packaging material for the preservation and quality indicator of the freshness of protein-rich food items. The authors selected these both polymer considering that both display good fiber-forming ability, non-toxicity and biocompatibility, which are crucial characteristics for food-grade materials in edible items. It is worth mentioning that the addition of bioactive compounds in composite mats results in the enhancements of specific fiber properties, e.g., the addition of curcumin can greatly influence diameter of nanofiber mats, varying from 160 to180 nm; however, the right concentration of the curcumin (ca. 0.2%, m/m) allowed to generate a stronger intermolecular interactions, resulting in improved the thermal stability and tensile strength of the resultant nanofibers [87].

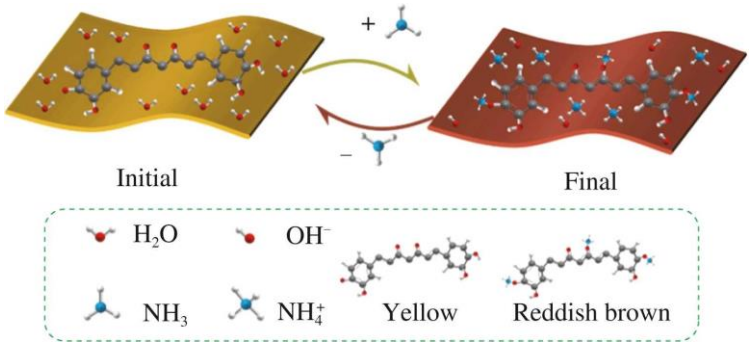


Figure 6. Hypothetical mechanism for the colour changes in CS/gelatine nanofiber loaded with curcumin. Taken with permission from Duan et al. [87].

To boost the antimicrobial properties of CS/gelatine ENs, Liu et al. [83] added 3-phenylacetic acid into such fiber blends. This latter carboxylic acid displays antimicrobial properties towards different fungi and bacteria. As for its production, the acid is produced in honey, various lactic acid bacterial species and fermented food. Experimentally, 3-phenylacetic acid coffered potential antibacterial features to the ENs, e.g., it reduced the presence of *Salmonella enterica* Enteritidis and *S. aureus* around 4 log CFU/mL in 30 min. As for the ENs loaded with 2% 3-phenylacetic acid, they exhibited a homogenous and smooth structure with fiber diameters ranging from 40 to 70 nm. Interestingly, CS and 3-phenylacetic acid positively interacted and formed hydrogen bonds under acidic conditions, conducting to decrease the crystallinity of ENs. Considering previous findings of Li et al. [68], such crystallinity decrease is favourable for the water resistance properties of the films, which was proven

by increasing the water contact angle from 82 to 88° [83].

Regarding novel biopolymers, Karami et al. [84] proposed a natural polysaccharide, like flaxseed mucilage, to be blended with CS and subsequently fabricated ENs. This mucilage was proposed due to its discovered nanofiber-forming property credited to its polysaccharide fractions, including a pectin-rich composed of rhamnogalacturonan I and hemicelluloses-rich comprising mainly of arabinoxylan. However, the resultant mucilage-based mats lack in mechanical stability; therefore, CS has been selected to offset mucilage properties. In these fibers, the authors added two different oils, including *Ziziphora clinopodioides* EOs and sesame oil, which display antimicrobial and antioxidant properties. Especially, *Ziziphora clinopodioides* EOs is composed of natural antioxidant elements, such as carvacrol, thymol, ɣ-terpinene, and p-cymene. During the ES, it was noticed that the fiber diameter increased when adding such EOs, from 323 to 451 nm. While doped ENs exhibited smoother structures compared with the pristine ones. As for the EE, the fibers efficiently encapsulated over 93% of both fragrances, proving their ability for hosting high molecular weight compounds and long-term release over 96 h.

4. Conclusion and perspectives

Over the last 2-3 years, CS has been mainly used as a base biopolymer for possible food packaging applications, in which its multiple chemical functionalities coffer to the polymer an exceptional ability to be blended with several biopolymers, including PCL, PLA, zein, xanthan and gellan gum, gelatin, hordein, among many others (see **Table 2**). Thanks to its ability to generate stable composites, CS is likely to be a target of study for fabricating sustainable packaging material together with new green materials. It is important to mention that the multiple advantages of CS in terms of biodegradation, biocompatibility, anti-microbial activities, low price, good transparency, high impact resistance and processing, non-toxicity, acceptable mechanical and film-forming properties will also support the application of this biopolymer in the manufacturing of ENs aimed for food packaging and as an encapsulation support of food ingredients [93]. However, the well-designed structure properties of CS nanofibers allow extending their implementation in other fields, such as water and air filtration,

wound dressings, tissue repair by controlled drug release, etc. [59,94,95]. Furthermore, new green solvents and additives, such as deep eutectic solvents, are also an alternative to tune specific physicochemical properties (e.g., transport, selective, mechanical, thermal and adsorption properties) of CS structures [15,20,96,97]. As an evidence, Wen et al. [98] used hydrophobic DES based on thymol and octanoic acid to boost the water barrier properties in CS/gelatin ENs, which displayed interesting antimicrobial properties as well. Therefore, the merging of different DESs and CS is also a latent perspective in the field.

The resultant composite ENs exhibit favourable properties and structures for hosting not only bioactives but also probiotic cells. However, the application of CS-based ENs has been extended to other approaches, e.g., within the formulation of meat products (like sausages) to replace some harmful ingredients [81]. The addition of bioactives not only provides nutraceutical, antimicrobial or antioxidant properties to the resultant CS-based mats; in this review, it has been found that specific bioactives (e.g., 3-phenylacetic) are able to decrease intrinsically the crystallinity of the fibers resulting in an enhanced water resistance with hydrophobic properties [68,83]. This latter property is pursued when dealing with the long-term integrity of the materials aimed at food packaging. For instance, CS-based mats tend to display rough surfaces translated to hydrophobic surface nature, resulting in water resistance and long-term stability.

Considering that these smart materials can interact with nitrogen-based compounds produced during food spoilage of meat products (like chicken fillets) and consequently change the colour properties of the material [85,87,92], CS nanofibers can be introduced as quality indicators in smart food packaging. Based on these findings, it seems that CS fibers and their blends with other biomaterials will be explored in the coming years since the food industries are looking for materials that not only protect the main food items but also meet the criteria of eco-sustainability, as well as indicate the freshness of the product. It is worth mentioning that all literature compiled in this review finds the successful fabrication of novel concepts of CS-mats with great advances, but they still remain at the lab scale. At this point, the researchers in the field should start to evaluate the feasibility of fabrication at a

larger scale conspiring also the possible scale-up of ES devices [95,99].

As a final perspective in the field, different chemical modification methods are applied to CS in order to improve specific physicochemical properties, such as solubility, biodegradability, biocompatibility, and drug-carrying capacity. In fact, such CS derivatives (such as quaternized CS) with improved properties have started to be used in electrospun nanofiber fabrication [100] but have not been exploited for encapsulating food ingredients. Therefore, it is likely that these CS derivatives will be explored in this field in near future.

Acknowledgments

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Conflict of Interest

The authors declare no conflict of interest.

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