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Nanopartículas de Quitosana: Produção, Características Físico-Químicas e Aplicações Nutracêuticas

Resumo: Moléculas biologicamente ativas como a quitosana e seus derivados apresentam grande potencial de aplicação na indústria de alimentos tendo em vista a necessidade da conservação de produtos alimentícios e as crescentes preocupações em relação ao impacto ambiental negativo dos materiais utilizados nas embalagens convencionais. Quitosana é um biopolímero derivado da quitina, obtido do rejeito das atividades da indústria pesqueira. Possui uma estrutura química única, um policátion linear com alta densidade de carga, com reativos como grupamentos hidroxila e amino, e possibilidade de ligações de hidrogênios, conferindo muitas aplicações a este biopolímero. Estas características físicas e químicas e a disponibilidade, associadas à biodegradabilidade, biocompatibilidade, além de atividades antibacterianas e antifúngicas oferecem grande potencial para uso visando a segurança e preservação de alimentos. A produção de nanopartículas de quitosana por tecnologia que preserva o meio ambiente e a aplicação como novos aditivos de alimentos foi apresentada e discutida. A aplicabilidade destas nanoparticulas de quitosana como um composto antimicrobiano natural e inovador tem sido testada e relacionada às variações no peso molecular e grau de desacetilação dos biopolímeros.

Palavras-chave: Nanopartículas de quitosana; atividade antimicrobiana; revestimento e embalagem de alimentos; qualidade alimentícia; segurança e preservação de alimentos.

Abstract

Biologically active molecules such as chitosan and its derivatives have significant potential in the food industry, in view of the necessity of food product conservation and the increasing concerns regarding the negative environmental impact of conventional packaging materials. Chitosan is a biopolymer derivative of chitin, obtained from the waste of industrial fishing activities. Its unique chemical structure, such as a linear polycation chain with high charge density, reactive hydroxyl and amino groups, as well as extensive hydrogen bonding, confer a wide range of applications to this compound. These particular physical and chemical characteristics and availability, along with a short biodegradability time, biocompatibility with human tissues and antibacterial and antifungal activities offer significant potential for applications in food safety and food preservation. The production of chitosan nanoparticles by environmentally-friendly technology and its use as new food ingredient is presented and discussed. The applicability of these chitosan nanoparticles as an innovative and natural antimicrobial compound was tested considering variations in the polymer molecular weight and degree of deacetylation.

Keywords: Chitosan nanoparticles; antimicrobial activity; food coating and packing; food quality; food safety and food preservation.

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Chitosan Nanoparticles: Production, Physicochemical Characteristics and Nutraceutical Applications

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1. Introduction

In recent years, the sustainable development concept has obtained important political and social attention, privileging the developing of "green technologies" and the use of "green products" over traditional ones, in order to contribute to sustainability through decreases in environmental degradation. The establishment of improved technologies to synthesize products is necessary for the progress of different areas, while at the same time reducing environmental impacts and satisfying consumer *commitment* to purchasing green products as a positive attitude of *consumers* towards *environmental protection.*[1]

Green chemistry explores chemistry techniques and methodologies that reduce or eliminate the use or generation of feedstock, products, by-products, solvents and reagents, hazardous to human health or to the environment.[2]

Two decades ago, however, a new approach to polymer synthesis was developed, employing enzymes as catalysts (enzymatic polymerization).[3] *In vitro* enzymatic catalysis has been extensively used in the biosynthesis of organic compounds as a convenient and powerful tool[4,5] which exhibits high efficiency, recyclability, the ability to operate under mild conditions and environmental friendliness.[6]

Natural biopolymers are attractive for use in different applications, such as improving human health, aiding in drug or vaccine production or even in the production of food preservatives and additives, since these compounds have inherent biocompatibility and biodegradable structures, are safe and also are more easily accepted by health surveillance regulatory and inspection institutions.

Natural biopolymers are produced in living cells by enzymatic catalysis. Research on discovering new enzymes and on the mechanisms of enzymatic reactions have been among the most important central topics in diverse fields such as organic chemistry, medicinal chemistry, biochemistry, polymer chemistry and pharmaceutical chemistry[7]. Currently, many thousands of enzymes are commercially available, and some have suffered modifications for industrial applications.

On the other hand, all *in vivo* enzymatic reactions have the following characteristics: high catalytic activity (high turnover number), reactions under mild conditions with regard to temperature, pressure, solvent, pH of the medium, among others, bringing about energetic efficiency, and high reaction selectivity of regio-, enantio-, chemo-, and stereo regulations, giving rise to perfectly structure-controlled products. If these *in vivo* characteristics could be obtained for *in vitro* enzymatic polymer synthesis, the following outcomes may be expected: perfect control of polymer structures, creation of polymers with new structures, clean and selective processes without forming byproducts and a low loading process that saves energy, in addition to biodegradable properties of polymer product, in many cases. These are indicative of the "green" nature of *in vitro* enzymatic catalysis for the development of new polymeric materials, and, in the polymer area, green chemistry has increasingly been deemed of interest.[8]

This review reports the production of chitosan (CS) and chitosan nanoparticles by green techniques and will conduct an overview of CS biological properties and its potential applications as a food additive and preservative.

2. Chitosan (CS)

Several sources have been used for the production of chitosan from chitin, but the most exploited sources of chitin are the processing waste of shellfish and marine crustaceans, especially shrimp, lobster, crab, oysters, krill and squid.[9] Generally, marine crustacean shells contain around 15–40 % chitin (dry weight), as well as proteins and calcium carbonate.[10]

Commercial chitin can be found with several average degrees of acetylation (DA) ranging from the fully acetylated to the totally deacetylated products. When displaying a high degree of acetylation, this polymer is soluble in very few solvents, which limits its application. The production of chitosan is usually performed in heterogeneous conditions, and, because of this, the residual acetyl substituent distribution depends on the source of chitin, on the deacetylation conditions and on the degree of residual acetylation. It is clear that the solubility of these polymers directly depends on the average DA, but also on the distribution of the acetyl groups along the polymer chains.[11]

Chitin shows severe limitation for large scale uses, since it is a water insoluble linear polysaccharide consisting of repeated *N*-acetyl-D-glucosamine (Glc*N*Ac) units linked by β-(1→4) glycosidic bonds (Figure 1), as explained above. However, water soluble derivatives can be produced after a chitin deacetylation process. The totally deacetylated chitin produces a CS molecule which contains an $-NH_2$ group and two –OH radicals in each glycoside residue, displaying a polycationic character formed by $-NH_3^+$

radicals when the pH of aqueous solutions is lower than its pKa.[12] The relative amount of these two monomers (2- amine and 2-acetamide) can be modified between CSs, depending on the extraction and production methods and the organisms used as the source for chitin extraction. The production of biopolymers with different physicochemical characteristics, due to distinct degrees of deacetylation (DD), is a consequence of the amount of deacetylated radicals (2-amine) present in the sample, varying between 60 and 95 % DD in CS polymers.

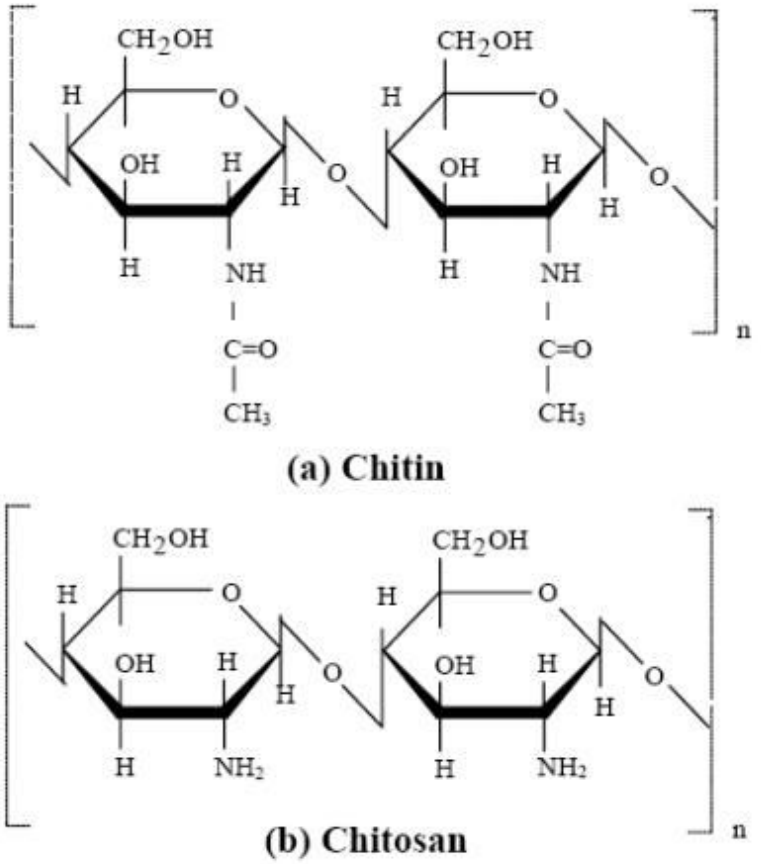

Figure 1. Molecular structures of (a) chitin and (b) chitosan[12]

The role of CS particles in foods can be viewed in broad categories where they can improve food quality, safety and preservation. CS and CS-particles can, thus, be categorized based on their functions, such as antimicrobial properties, as well as additive properties, including roles such as color stabilization, emulsification, antioxidant activities and dietary fiber-like properties, aiding water-holding and fat entrapment, thereby also imparting health benefits. Due to the multifunctional properties of chitosan, in the last decade the number of patents issued on the processing of chitosan and its derivatives related to use in food industry has increased.[13]

2.1. Physicochemical properties

CS is insoluble in both organic solvents and water. It can, however, be readily dissolved in weak acidic solutions, due to the presence of its amino groups. The solubility and acid-base behavior is directly dependent on the DD characteristics, defined as the glucosamine/N-acetyl glucosamine ratio.[14] Water-soluble chitosan derivatives can be obtained by the introduction of permanent positive charges in the polymer chains, resulting in a cationic polyelectrolyte, independent of the pH of the aqueous medium. According to Xie *et al.* (2002),[15] at neutral pH, the degree of protonation of NH_2 is very low, so NH_3^+ repulsion is weak. Solubilization occurs by protonation of the –NH_2 on the C-2 position of the D-glucosamine repeated unit at pH lower than 6.2 (the pKa of CS); thus the polysaccharide is converted to a polyelectrolyte in acidic media.[16] A soluble CS product is obtained when the DD reaches 60-85 % or higher.[17] The positive charge of the amine group (NH^{3+}) at lower pH values than the pKa (pH<6.2), is directly involved in the interaction of CS with negatively charged microbial cell membranes, a phenomenon which may cause leakage of intracellular constituents, leading to the microbial cell death.[18]

Molecular weight (MW) is also a fundamental characteristic; alongside DD it may contribute to the solubility of CS biopolymers, thus explaining seemingly controversial results. MW modifications alter the content of N-acethylglucosamine units in CS, which have both an intramolecular and intermolecular influence, resulting in CSs with different conformations. However, increasing CS solubility implies in control of the deacetylation of the residues, which is sometimes a low yield process.[19]

CS-derivatives can usually be obtained by chemical modifications of the amino or hydroxyl (especially at the C6 position in the CS backbone) groups, in order to improve their physicochemical properties.[20] Structural variations can be used to modify physicochemical characteristics and direct the use of the biopolymer, and are usually obtained by chemical modifications. Many CS-derivatives have been, described such as thiolated, carboxyalkyl, bile acid-modified, quaternized (N, N, N-trimethyl chitosan; TMC), sugar-bearing and cyclodextrin-linked modifications.[21]

2.2. CS manufacturing: enzymatic vs chemical process

Chitin purification and CS manufacturing can be carried out by different chemical or enzymatic methods. The most common method for CS synthesis is the deacetylation of chitin using NaOH, which requires chitin exposure to a combination of severe chemical and thermal conditions. This process results in a significant yield, but higher DDs require more severe treatment conditions. To obtain CS with a DD between 85 and 92 %, chitin should be exposed to harsh alkaline conditions, which may result in degradation. At the same time, there is high consumption of water and energy, as well as impacts on the environment due the large volume of discarded solvents and alkali[22]. Partial depolymerization is obtained by applying physical methods, such as irradiation with low-frequency ultrasound (20 kHz), reducing the average MW from 2000 kDa to 450 kDa or from 300 kDa to 50 kDa; however, this MW decrease is limited.[23]

The enzymatic process for chitin purification is very well explained by Hamed *et al.* (2016),[24] where CS can be manufactured by enzyme-catalyzed processes that replace alkaline or acid chitin treatments, saving energy, water and chemicals, which helps to improve product quality, and, furthermore, presents valuable environmental benefits. As mentioned previously, these protective environmental claims are becoming more important at a time of increasing awareness regarding sustainable development, green chemistry, climate change and organic production[25]. Different hydrolytic enzymes able to catalyze the cleavage of CS glycoside bonds have been isolated.[26,27] Cellulases, pectinases, pepsins, papains, neutral proteases, lipases and α-amylases show the ability to hydrolyze CS at comparable activity levels, but with different specificities.[28-30]

Our research group focused on the production of CS by a binary enzyme system, comprised by a hydrolytic step catalyzed by chitinase purified from grapes[31], followed by a subsequent deacetylation, where the removal of the acetate radical from the chitin D-glucosamine monomers is catalyzed by a recombinant chitin deacetylase enzyme from baker's yeast but cloned in *Pichia pastoris.*[32] The aforementioned enzymes can be obtained from different organisms and their use for chitin hydrolysis has been explored in order to improve the process efficiency and control over the physicochemical characteristics of the formed products. These enzymes are capable of reducing chitin crystallinity, and the assay conditions can be adjusted to produce chitosan macromolecules with distinct molecular weights (MW) between 4.0 - 10.0 kDa. Moreover, CS presenting distinct MWs and distinct DDs can be obtained if enzymes able to promote deacetylation of 2-acetamido monomers are associated to the hydrolytic process, according to the intended usage of the synthetized polymers.[28,32]

The production of large amounts of toxic waste and higher power consumption is observed in chemical methods in comparison to enzymatic methods and the formed products are heterogeneous, with variable MWs and DDs[33]. The homogeneity in size and DD of the formed CS is very important for the subsequent application of the generated product and higher uniformity will create better market value, since CS may be used in several different types of applications.[34] The homogeneity of the resultant CS polymers leads to the choice of the binary enzyme treatment of chitin over the alkaline alternative.

2.3. CS nanoparticle production

Nanotechnology has potential to produce new food ingredients and innovative products, with considerable benefits to human health[35] through the development of new structures to be used as nutraceutical polymers in fruits, seeds, vegetables and potable water, among others.[36] The consumption of foods providing extra health benefits as well as basic nutrition is very attractive to the consumer, and significant developments in the food industry have emerged in recent years.

Nanoparticles (NPs) prepared from either synthetic polymers or natural polymers have been involved in several applications. The potential application of a nanoparticle depends on certain factors, such as the type of material[37] and particle shape[38] and concentration.[39] The intrinsic properties of NPs are determined predominantly, by their size, composition, crystallinity and morphology.[40] The chemical composition of NPs, their surface shape, charge, hydrophobicity, besides size[41] and the presence or absence of functional groups or other chemical compounds[42] define the applications of these compounds.

Indeed, CS has been explored as a material of choice to produce NPs in the last years due to its biodegradability and biocompatibility[43]. The unique character of NPs, such as small size and quantum size effect could result in chitosan nanoparticles (CSNP) with many new application possibilities. They are simple and inexpensive to manufacture, their production process can be scaled-up, and they show unique sizes and large surface-to-volume ratios.[44]

Distinct methodologies have been used to prepare CSNP, and the selection of the preparative method depends on factors such as particle size requirement, thermal and chemical stability of the active agent, reproducibility of the release kinetic characteristics, stability of the final product and residual toxicity associated with the final product.[45] However, the selection of any of these methods depends on the nature of the active molecule, as well as the type of delivery device.

Even though chitosan nanoparticles (CSNP) appear to be safe in laboratory-scale studies, the knowledge of the risks involved in real-world applications leaves much to be desired.[46] The application of these particles on the macroscale is questionable, since nanomaterials exhibit novel properties due to their extremely small size, high surface area and reactivity.[47,48]

The early findings of the use of these nanoparticles in favor of human beings are promising signs for their possible safe environmental applications. However, some doubts have risen with regard to the use of nanoencapsulated food additives and nanocoated films in food packaging.[49] Their complete toxicity effects have not yet been studied, and additional exposure assessments are required in order to obtain a better picture of the relationships between nanoparticle applications and their health risks. Most of the time, the risks regarding nanoparticles are assessed by their chemical composition and, to date, no widely accepted or well-defined risk assessment methods or test strategies explicitly designed for NPs exist.[50] It is, thus, essential to gather more information regarding health and environmental risks associated with nanoparticle applications, in order to identify the proper risk assessment strategies and implement regulatory policies to ensure the safety of these nanomaterials.

2.4. Ultrasound applied to chitosan

One of the most promising technologies for the conversion of raw biomass material is ultrasonic irradiation, which offers the possibility to rupture polymeric carbohydrates and convert them into useful lower weight molecules.[25,28,51] Ultrasound application is one of the most economical and simple tools for the degradation of long polymeric macromolecules, breaking up aggregates and reducing the size and polydispersity of nanoparticles[52]. The application of ultrasound through temperature, frequency and intensity control and polymer concentrations, the extent of the degradation is mainly determined by the sonication time (*ts*).[6,53]

Recent studies have further investigated the effects that ultrasound treatments can cause in polymer macromolecules, and verified altered properties like chemical composition, size, shape, surface charge density, hydrophobicity,[41] polydispersity and the presence or absence of functional groups or other chemical agents[42] when subjected to different treatment conditions.[54]

Perhaps the most relevant evidence regarding the ultrasonic degradation of CS supports that scission occurs mainly through the β (1→4) linkage and that DD remains barely unaffected, even during long sonication times.[6,55] Consequently, the unaltered DD and the possibility of controlling molecular mass make ultrasonication a likely choice for preparing CS nanoparticles. However, the physical stability and *in vivo* distribution of nanoparticles are known to be affected by their mean size, polydispersity, and surface charge density[56] and should be tested before their use in biological systems.

3. CS applications and perspectives: food quality, food safety and food preservation

The growing number of scientific papers and patents regarding CS or CS nanoparticles and their applications demonstrates a surprisingly high level of research on this biopolymer. The number of publications related to CS applications in different technological areas in the last years is growing: in 2016, 4812 publications were available, more than two times the number of publications (2084) in 2010. Sixteen years ago, not even one thousand publications were available per year (928), demonstrating the increase in the study and importance of this biopolymer (Figure 2).

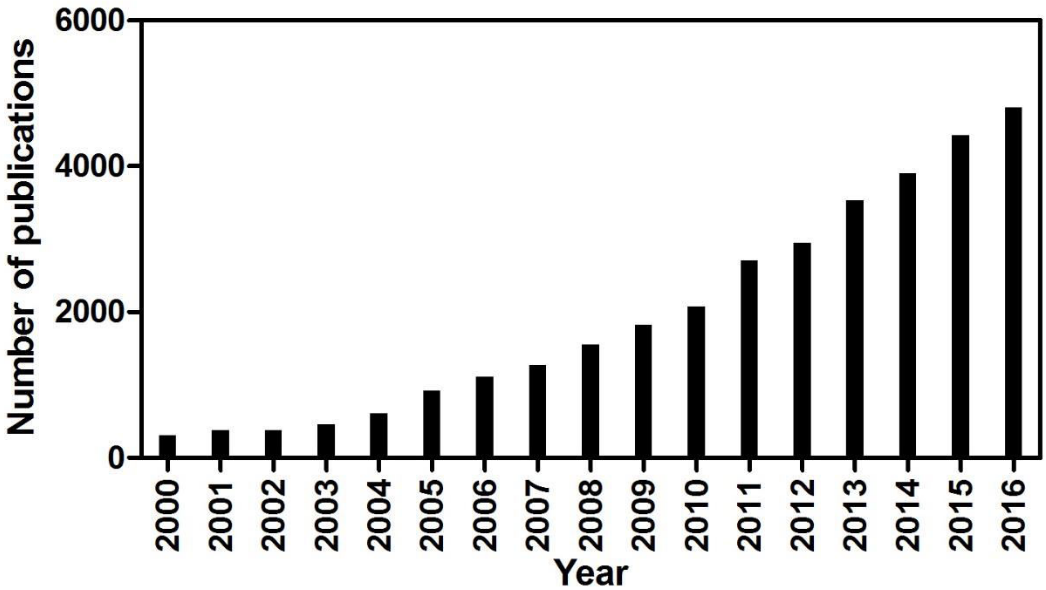

Figure 2. Data obtained from the website sciencedirect.com - August/2016-15:00 h, search using the following words: Chitosan and Application

Areas of particular CS or CNSP applications in the past 16 years are the pharmaceutical industry, including tissue engineering and drug transport, which correspond to 20 and 21 %, respectively. However, the versatility of CS applications can be demonstrated by the variety of uses in many areas, ranging from metal-contaminated water purification to the formation of nanotubes and use as an antimicrobial agent. This versatility directly contributes to the growth of studies conducted with this macromolecule, which is intrinsically related to new applications of this biopolymer (Figure 3). The Food and Drug Administration (FDA) agency in the U.S. approved CS as a food additive in 1983. Since it is considered a Generally Recognized as Safe (GRAS) compound, CS has, thus, been widely applied as a functional food, in environmental protection and as a safe biotechnology product to be used to promote health in human beings and animals.[57]

Many biological activities have been reported for CS, such as antimicrobial, anticancer, antioxidant, and immune stimulatory effects, that are dependent on its physicochemical properties (Table 1). Food applications have already been approved by the Regulatory Agencies regarding food consumption and drug administration in Japan, Italy and Finland, as well as the U.S.[58]

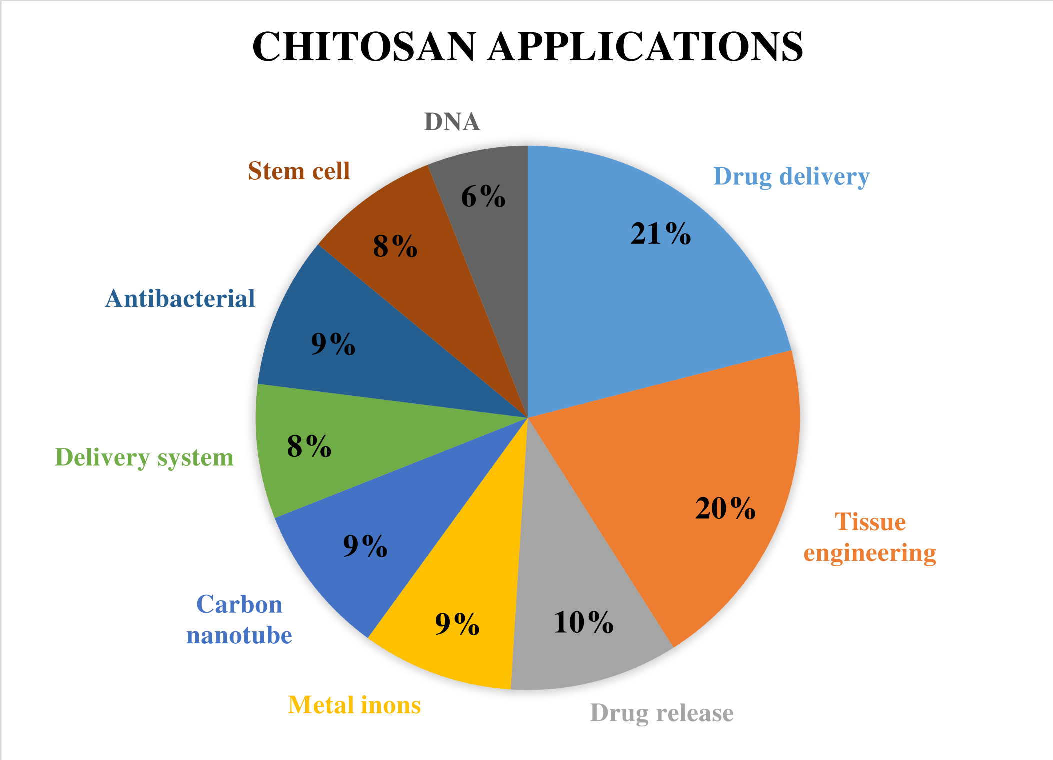

Figure 3. Major chitosan applications Source: sciencedirect.com – June/2015-21:00. Search using the following words: "Chitosan" and "Application", between 2000 and 2015

Table 1. Chitosan and chitosan-derivatives used as nutraceuticals for improving food quality, safety and preservation[78]

Use	Example	Ref.
Antimicrobial agent	Bactericidal, fungicidal; measure of mold contamination in agricultural commodities.	127, 128
Edible film industry	Controlled moisture transfer between food and the surrounding environment; controlled release of antimicrobial substances, antioxidants, nutrients; reduction of partial oxygen pressure; controlled rate of respiration: temperature control	129-132
Additives	Clarification and deacidification of fruits and beverages, natural flavor extender, texture controlling agent, emulsifying agent, colour stabilization	105, 133, 134
Nutritional quality	Dietary fiber; hypocholesterolemic effect; livestock and fish feed additive, reduction of lipid absorption; production of single cell proteins; antigastritic agent	80, 135, 136
Purification of potable water	Recovery of metal ions, pesticides phenols and PCB´s; removal of dyes	134, 138

3.1. Spoilage microbial growth control

Among the different CS bioactivities, perhaps the most applicable in the food chain production is its antimicrobial activity, enhancing food safety and preservation, while also impacting biosecurity, the food business and community health, since it can be effective in controlling foodborne pathogens without antibiotics. The antimicrobial activity of CS is influenced by several intrinsic and extrinsic factors, which can be classified into 4 categories (a) microbial factors related to the species of the target organism and cell age; (b) chemical properties of the CS molecule (density of positive charges, MW, concentration, hydrophilic/hydrophobic characteristics and chelating potential); (c) CS physical state and water solubility and (d) environmental factors (ionic strength of the medium, pH, temperature and pathogen exposure time pathogen).[59]

CS presents antimicrobial activity against a broad spectrum of microorganisms, including Gram-positive and Gram-negative bacteria, filamentous fungi and yeast[59].

CS exerts antifungal effects on different fungi developmental stages by suppressing sporulation, spore germination, mycelial growth, spore viability and the production of virulence factors.[60]

The observed antibacterial activity is a complicated process that differs between Gram-positive and Gram-negative bacteria, due to their distinct cell surface characteristics. Discrepancies exist however, since in several studies CS displays stronger antibacterial activity against Gram-negative when compared to Gram-positive bacteria[61], while in other studies Gram-positive bacteria were more susceptible.[62,63]

Some studies described the mechanism of action as a result of the interaction between the CS macromolecules, that are positively charged, and the membrane of the microbial cell, that is negatively charged, with subsequent breakage and, consequently, leakage of intracellular components, including proteins and nucleic acids.[64,65]

Besides causing alterations in cell membrane permeability,[66] CS antimicrobial activity is enhanced by its ability to act as a chelating agent, selectively binding to trace metals, thus inhibiting the toxin production and microbial growth. CS also activates various defense processes in the host tissue, acting as a binding agent to water and as an inhibitor to several enzymes.[67]

The alternative mechanism microbial growth inhibition by the interaction between the positively charged CS and intracellular fungi and bacteria DNA, which consequently inhibits the RNA and protein synthesis, is considered effective only for low molecular weight chitosans (LMW-CS), which can penetrate microorganism cells. Although this mechanism of action based on ionic interactions with DNA is still controversial, it could explain the inhibition of both Gram-positive and Gram-negative bacteria and fungi by CS, establishing a similar mechanism of action for all microorganisms, regardless of their cell membrane structure.[68]

CS definitely demonstrates a strong inhibitory effect on microorganisms growing in low pH media, confirmed by the fact that its antimicrobial activity is weakened with increasing pH values,[69] causing low protonation of amino groups, which in turn also influences the solubility of the biopolymer, causing decreases.[70,71]

Due to apparent discrepancies, the interactions of CS and its derivatives with the outer membrane barrier of bacteria should be better understood, but they certainly depend on the binomial combination of MW and DD.

LMW–CS of less than 10 kDa have greater antimicrobial activity compared to high molecular weight chitosan (HMW- CS), ranging between 10 and 500 kDa.[72] However, studies conducted with hydrochloride D-glucosamine, demonstrated that the CS monomer was not effective in inhibiting bacterial growth, suggesting that the antimicrobial activity of CS is related not only to the cationic nature of the deacetylated glucosamine, but also to the chain length of the biopolymer.[73]

Although some results regarding the bactericidal activity of LMW-CS are comparable, it has been reported that, depending on the bacteria strain, the conditions of the biological assays and CS physico-chemical characteristics (MW and DD), the results are not always in agreement with each other. Studies testing the 9.3 kDa CS have shown inhibition of *E. coli* growth, while the 2.2. kDa CS promotes the growth of the same bacteria.[74] On the other hand, the 4.6 kDa CS was most active against Gram positive bacteria, yeast and fungi.[75] Thus, results with LMW-CS are still somewhat controversial and unclear, indicating that additional experimental data are required to understand the antimicrobial mechanisms that take place.

Evaluating these studies, it seems that CS antimicrobial action depends on MW, but also on the different physical states of the polymer and its derivatives, that may, thus, provide distinct mechanisms of growth inhibition. Similar to the LMW-CS, water-soluble ultrafine nanoparticles can penetrate bacteria cell walls, interfering in the microorganism nuclei by binding to DNA and RNA, as well as inhibiting both the mRNA and protein synthesis.[68]

Despite the apparent discrepancies regarding CS effects, the natural antimicrobial properties of CS and its derivatives have resulted in their extensive use as commercial disinfectants, since some CS have an advantage over other disinfectants due to their high antimicrobial and broad spectrum of activity but low toxicity to mammalian cells, allowing them to be discarded with less damage to the environment.[76]

Recently, it has been demonstrated that bioplastic films composed by CS and its derivatives also display antimicrobial activity. The potential use of CS-based films and their derivatives may be directly dependent on particle size, film thickness and the structure of the matrix-forming fibers. In a previous study, two CS films with distinct structures and particle sizes were tested, where particles ranged between 74-500 µm (resembling a flake), and between 37-63 µm (resembling a sphere). The films exhibited superior antimicrobial activity against *S. aureus* when smaller-sized, spherical shaped particles were used, which provides greater specific surface contact.[77]

It is accepted that CS nanoparticle-based films can be effectively used in the food industry, as they provide various benefits, including good edibility, biocompatibility with human tissues, an aesthetically pleasing appearance, displaying barrier properties against pathogenic microorganisms, atoxicity, and are non-polluting and made from low cost material.[78]

Table 1 lists some applications of CS and its derivatives on food quality, food safety and food preservation.

3.2. Crop protection

CS can also be used primarily as a natural seed treatment and plant growth enhancer, since it is considered an ecologically friendly biopesticide substance that boosts the innate ability of plants to defend themselves against fungal infections.[79] CS applications in plants and crops are regulated by the EPA and USDA National Organic Program, which regulates its use on certified farms and crops as using organic production system[80]. EPA-approved, biodegradable CS products are allowed for use outdoors and indoors on plants and crops both grown commercially or home grown.[81]

CS has prevented numerous pre- and post- harvest diseases on various horticultural commodities. Microscopic observations indicate that CS had a direct effect on the morphology of CS-treated microorganisms reflecting its fungistatic or fungicidal potential. In addition to a direct antimicrobial activity, other studies have strongly suggested that CS induces a series of host defense reactions related to the enzymatic activities of the host organism.[57]

The foliar application of CS in pepper plants decreased transpiration and reduced water use by 26-43 %, while maintaining biomass production and yield. Hence, CS might be an effective antitranspirant product to conserve water use in agriculture.[82,83] Reports are also available indicating that coating seeds with depolymerized CS or its oligosaccharides typically increases chitinase activity in seedlings by 30-50 %, unless the seeds have a hard cuticle. A 5 kDa LMW-CS induced the accumulation of phytoalexins in plant tissue and decreased total content and changed the composition of free sterols, producing adverse effects on infesters, by the activation of chitinase and beta-glucanase enzymes, as well as lipoxygenase enzyme activity, by stimulating the generation of reactive oxygen species.[84]

In addition, CS can also induce structural barriers, for example, triggering the synthesis of a lignin-like material. For some horticultural and ornamental commodities, CS increased harvested yield, due to its ability to form a semipermeable coating. Cs also extended the shelf life of treated fruit and vegetables by minimizing the rate of respiration and reducing water loss. It was observed that CS at 0.1 or 0.5 % increased leaf area, leaf dry weight and leaf length of soybean, lettuce and rice, whereas CS at 0.1 % showed positive effects on leaf area, leaf length and dry weight of tomato.[85] As a nontoxic biodegradable material, as well as an elicitor, CS has the potential to become a new class of plant protectant, assisting towards the goal of sustainable agriculture.[60]

3.3. Preservation of fresh and processed food quality

Conventional food packaging systems are supposed to passively protect food, acting as a barrier between the packaged food and the surrounding environment. Antimicrobial food packaging systems have received considerable attention since they help control the growth of pathogenic and spoilage microorganisms on food surfaces, where microbial growth predominates[86]. Antimicrobial nanocomposite systems are particularly interesting, since materials in the nanoscale range have a higher surface-to-volume ratio when compared to their microscale counterparts. Nano-materials are thus more efficient, since they are able to attach themselves to more copies of microbial molecules and cells.[87]

CS films have shown potential to be used as a packaging material for the quality preservation of a variety of foods. CS has also been widely used in antimicrobial films to provide edible protective coating, and in the dipping and spraying of food products, due to its antimicrobial properties.[88]

Coatings based on CS have been used as an antifungal agent, which resulted in the enhancing of germination and quality of artichoke seeds. The effect of the formulation and thickness on seed germination (G%), fungi activity and vegetative growth were evaluated, and results indicated that significant differences between treatments regarding seed germination were observed, where all CS coatings reduced the number of fungi strains and increased plant growth.[89]

In another study, apples (*Malus domestica* Borkh. cv. Gala) were heat-treated at 38 °C for 4 days (heat treatment) before or after being coated by 1 % CS. The combination of the heat treatment plus CS fruit coating showed the lowest respiration rate, malondialdehyde levels, membrane leakage, ethylene evolution and the highest firmness and consumer acceptance among the treatments.[90]

When applied on wounded wheat leaves, CS induced lignification and, consequently, restricted the growth of nonpathogenic fungi in wheat. CS also inhibited the growth of *A. flavus* and aflatoxin production in liquid cultures, pre-harvest maize and groundnut, and enhanced phytoalexin production in germinating peanut plants[91]. In addition, CS also improved the microbiological quality of fresh cut broccoli.[92]

Edible coatings consisting solely of CS or a combination of CS with other biopolymers, such as sodium caseinate, were applied to carrots, cheese and salami.[93] The sodium caseinate/CS films inhibited bacteria and yeast growth and can be potentially applied to several food matrices. In other studies, acetic or propionic acid were incorporated into a CS matrix in Bologna ham, baked ham and fresh salmon,[94] with positive effects.

The application of high concentrations of CS is considered effective in the control of fruit colouring, decreasing fruit darkening and maintaining anthocyanin content, a pigment directly related to food freshness. Enzyme activity is influenced by the presence of O_2 concentrations inside the fruit. CS forms a physical barrier around the fruit, and, consequently, darkening is reduced. Furthermore, the positive charges present in the coating can stabilize anthocyanine pigments, aiding in maintaining fruit colour, sensory attributes and antioxidant features.[95]

Other studies were performed using CS biodegradable packaging and edible coatings for the preservation of fresh-cut fruits and vegetables.[96,97]

The use of CS-based edible films was also tested to preserve the quality of pork meat hamburgers. Their importance in the modulation of the oxygen permeability of films in order to avoid the undesirable effects of metmyoglobin (MtMb) formation was promoted by lower partial oxygen pressure in the surface of the coated hamburgers.[13]

The interest in edible coatings is on the rise, due to their ability to reduce fruit respiration and transpiration rates, and consequently increase storage time and consistency retention.[98-100]

The use of CS also decreased the respiration rate and production of ethylene in raspberries[95] and has a high selective permeability to respiratory gases, acting as a passing barrier for O_2.[101] This gas control between the fruit and the environment reduces respiration rates, as well as the enzymatic action of 1-carboxylic-1-aminocyclepropane oxidase and synthases, which are highly influenced by the presence of O_2.[102] The decrease of mass loss with CS applications has also been related to the formation of a selective barrier around the surface of the fruit, improving moisture loss and reducing respiration and the main metabolic processes that lead to loss of water.[103,104]

CS has also been used for juice clarification with good results for apple, carrot, grape, lemon, orange and pineapple juices[105].

CS antioxidative properties, especially in food products that contain high amounts of unsaturated fatty acids, which are sensitive to oxidation during storage, have also been reported.[106] CS scavenges free radicals or chelates metal ions from the donation of a hydrogen or lone pairs of electrons, increasing its antioxidant ability and free radical scavenging activity.[107]

3.4. Food nanotechnology

The United States Department of Agriculture implemented a project to develop green nanotechnology aimed at eliminating foodborne pathogens.[108] In this study, the USDA envisaged the development of a nanoparticle wash treatment with the capability of significantly reducing or eliminating pathogenic bacteria associated with fresh or fresh-cut fruits and vegetables, to be used with minimal processing. The specific tasks involve the design, synthesis and characterization of ultrapotent CS nanoparticles coated by antimicrobial peptides, the evaluation of peptide-enhanced nanoparticles as a lysis agent in realistic food processing environments and the development of a postharvest nanoparticle electric field treatment for decreasing the bacterial loads of fresh fruits and vegetables.[109]

Food-grade nanoparticles and microparticles can be fabricated from a range of different ingredients, including biopolymers, lipids, surfactants, and minerals. Biopolymer particles are often classified according to their structures, such as (filled) hydrogel particles, inclusion complexes, and polyelectrolyte complexes. However, the dimensions of the biopolymer particles alter their functional performance in foods.[110]

Nanoparticles composed of different materials (including silicates, silver, magnesium, and zinc oxide) have been incorporated into packaging materials[111], where they afford greater protection to foods due to several effects that include reduced gas and odor permeation, blocking of ultraviolet radiation, enhanced mechanical properties and thermal stability.[112] Studies of the health effects of these particles are especially important, because the packaging may have direct contact with the food.[111]

Functional bioactive ingredients have received much attention in recent years from the scientific community, consumers and food manufacturers. Potential functional bioactive ingredients include vitamins, probiotics, bioactive peptides, antioxidants, among others.

Micro/nanostructured CS can be used as bioactive ingredient carriers and have the potential for the development of novel encapsulation or immobilization carriers 113 (Zhao *et al.*, 2011). They also display mucoadhesive properties, which may prolong the contact time between bioactive and absorption sites, thereby increasing absorption.

CS particles are especially useful for the encapsulation of hydrophilic macromolecules, which are associated through electrostatic interactions or hydrogen bonding.[114] Encapsulation of bioactive compounds is a relatively old concept and was initially focused on protecting vitamins from oxidation.[115] Since then, many other types of active ingredients have been the focus of encapsulation technologies, and encapsulation is currently one of the most intensively studied application areas of microparticle and nanoparticle biopolymers. Generally, two types of active ingredients can be distinguished, bioactive molecules (nutraceuticals) and bioactive living cells (probiotics) .[116,117]

CS was successfully used in applications regarding the encapsulation of different bioactive compounds.[118] CS produces biopolymer particles to encapsulate proteins in combination with gellan gum[119] and colon-specific delivery systems for peptides and proteins[120-122] demonstrated that a CS/vitamin C nanoparticle system successfully increased the shelf life and delivery of vitamin C in rainbow trout during 20 days of storage.

Rajeshkumar *et al.* (2009)[123] demonstrated that CS nanoparticles could be used to encapsulate DNA, which was then beneficially incorporated into shrimp feed to protect them from white spot syndrome virus. Other additives encapsulated by CS described in the literature are shark liver oil in combination with calcium alginate beads[124] and tuna oil droplets.[125]

The use of CS nanoparticle-based edible films as food coating has been reported with respect to a variety of foodstuffs, including cheese and meat products, such as fermented sausages.[63] In another study, the possibility of producing food-grade stable nanoparticles with simple processing techniques was demonstrated, using lecithin and sodium caseinate, which could be further used as base systems for the production of nanocapsules.[126]

4. Conclusions

The application of biopolymer particles in maintaining food quality, enhancing food preservation and guaranteeing food safety is in the exploration phase, and food companies are seeking methodologies to create healthier products without compromising their appearance and sensory perception. Studies on nanoparticles biopolymers are demonstrating that those new compounds show the ability to protect and even target the delivery of bioactive ingredients, and/or to design foods with novel physicochemical attributes.

Chitosan is, thus, a versatile food biopolymer that has a variety of applications in all areas of food science. CS possesses promising broad-spectrum antimicrobial activities, and has, accordingly, been widely studied as a food preservative to improve food quality and extend the shelf life of perishable food products. The intrinsic properties of CS may be controlled by changing the MW and DD of the biopolymers using green processes, such as enzyme treatment and ultrasonication. The controlled synthetized CS shows versatile and promising activities that can preserve functional compounds in food, protect crops in pre- and post-harvest stages and/or maintain the quality of processed food.

Inherent antibacterial properties and its film-forming ability make CS an ideal choice for use as a biodegradable antimicrobial packaging material that can be used to improve the storability of perishable foods. As it has been convincingly proved that CS films exhibit good antimicrobial activity, which can aid in extending food shelf life, it is no surprise if a widespread use of CS films is witnessed in tomorrow's food packaging, replacing films made from petroleum-derived conventional polymers

It would be important to standardize the methods used for CS and CS-nanoparticle preparations, to validate the processes and produce CS at industrial levels with simple and low-cost manufacturing, increasing its applications in the food chain.

The chemical, physical and other qualities and characteristics of such CS derived materials should be adequately tested and analytically established. Academic research and industry support could accelerate the development of these products for human use, combining efforts to develop CS-derived products that would certainly support environmental sustainability for human health and the existence of other living systems.

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