CHITOSAN: A NATURAL POLYMER WITH POTENTIAL INDUSTRIAL APPLICATIONS

Inmaculada Aranaz, Marian Mengibar, Niuris Acosta, Angeles Heras†*

ABSTRACT

Chitosans are a family of natural polymers, composed by N-acetylglucosamine and glucosamine, commercially isolated from by-products of the fishing industry and aquaculture.

Chitosans have been promising biopolymers for almost fifty years and their use has been proposed in several fields, such as medicine, food industry, agriculture, cosmetics, biotechnology and so on. Unfortunately, the launch of chitosan-based products into the market has been irregular. Chitosan properties are strongly related to the polymer's physico-chemical characteristics and the first chitosan generation was unable to fulfill the requirements of most industrial applications since chitosan bioactivities claimed in the scientific literature were not reproducible.

The first generation of chitosans was poorly characterized and was followed up with a second generation of chitosans with well-defined characteristics. These samples allow producing reproducible chitosan-based products but are not suitable for very specific applications in biomedicine or pharmacology. Therefore, a third chitosan generation with defined monomer distribution, low polydispersity and clearly defined activities will open the door of new markets to these polymers.

Keywords: *Physico-chemical properties; biorenewable polymers; chitin, chitosan; oligomers*

1. INTRODUCTION

The manufacture of crustaceans from the fish industry and aquaculture produce tons of solid residues mainly composed of crustacean shells. This waste is a major source of surface pollution in coastal areas. The Food and Agriculture Organization (FAO) has estimated that the worldwide amount of crustaceans captured in 2014 in inland waters was around 530000 tons in marine fishing areas.

This implies that tons of crustacean waste (shells) is produced every year and that this huge volume of material needs to be properly managed. Each country has its own legislation regarding crustacean shells disposal but, in general, the tendency is a more restrictive legislation that increases the cost of waste management.

Traditionally, crustacean shells management includes the waste disposal in the ocean or landfills, their use as fertilizers after composting or their use as flour to feed animals in small amounts due to their high composition in inorganic materials. All these strategies have low or no added value. On the contrary, the isolation of the crustacean shells valuable components (chitin, pigments and calcium) reduces the amount of waste generated with a positive economic impact.

The proportion of inorganic salts (30-50%), proteins (30-40%) and pigments that can be found associated with chitin (20-30%) depends on several factors, such as the type of crustacean, the body part under consideration (head, torax, claws etc.), the molt stage, the season of harvesting and the location and environmental conditions (Naczk, et al., 1981; Yanar and Celik, 2005).

Chitosan can be easily produced from chitin after a multi-step process, in which the shells are demineralized and deproteinized to isolate chitin. When necessary, an extra step can be included to remove residual pigments depending on the final application. Chitin pigments are mainly astaxanthin and carotenoids that can be of interest for the food industry (for instance, as food coloring agents) and, therefore methods to pigment extraction has been specifically developed. Recently, an extensive review of chitin and chitosan isolation methods has been published (Younes and Rinaudo, 2015). During demineralization, inorganic salts (mainly $CaCO_3$ and phosphates) can be chemically removed by using strong basic media. Several conditions have been reported in the literature, none of them being able to avoid effects on the molecular weight and deacetylation degree of the polymers (Aranaz, et al., 2009). During the deproteinization step, proteins can be chemically removed using acid media or the process can be enzymatically carried out using proteolytic enzymes (tripsine, pepsine, papaine, etc). Complete deproteinization is very difficult due to the strong interactions among chitin and proteins. The chemical methods are more effective to remove the proteins than the enzymatic ones and, therefore, when human uses (food, biomedicine, pharmacy) are under consideration an extra chemical step with a sodium hydroxide solution (lower concentration and times that in a typical chemical process) is included (Aranaz, et al., 2014). The complete removal of proteins is necessary for human intended applications since a

* Biofunctional Studies Institute, Complutense University, Spain. Email: iaranaz@ucm.es †Email: aheras@farm.ucm.es

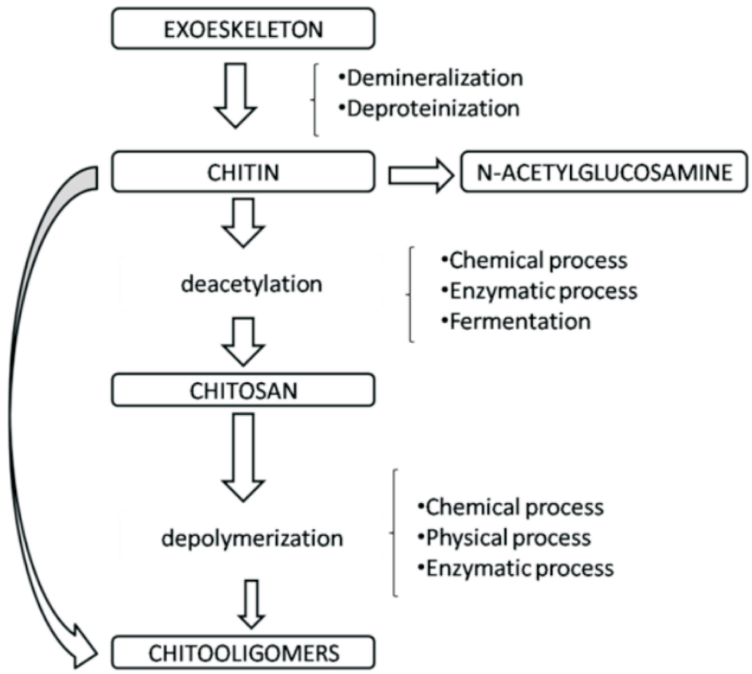

Figure-1: Production of Chitin, Chitosan and Chitooligomers

percentage of the human population is allergic to the protein components of the shellfish. The potential allergenic effect of chitosan is a controversial issue, eventhough some reports indicate the safety of chitosan (Muzarelli, 2010; Waibel, et al., 2011).

Chitin can also be isolated from microbial fermentation; in this case, both demineralization and deproteinization occur in one step. Although the biological methods (enzymatic or fermentation) produce chitin with a more preserved structure and have environmental advantages when compared with the chemical methods, these processes produce chitin at a higher cost and lower purity than the chemical ones (Kim and Park, 2015). It is worth mentioning that at this moment no standardized method to chitin isolation has been determined. In particular, further development is needed to find a cost-effective process that allows us to produce preserved chitin (samples with high molecular weight and deacetylation degree) having high purity.

Once chitin is produced, it can be submitted to a deacetylation process to obtain chitosan by alkali deacetylation. However, by this method, a partial degradation of the polymer occurs. The use of chitin deacetylases permits the production of well-defined chitosan samples avoiding polymer degradation. From chitin and chitosan, it is possible to produce oligomers and monomers (N-acetylglucosamine and glucosamine) by chemical, enzymatic or physical depolymerization (Harish Prashanth and Tharanathan, 2007; Kazami, et al., 2015; Kim and Rajapakse, 2005; Zou, et al., 2016). Depending on the composition of the polymers (chitin or chitosan with variable deacetylation degree) different oligosaccharides are produced. Homo-chitooligosaccharides are defined as low molecular weight polymers of variable length only composed by N-acetylglucosamine or glucosamine residues. Hetero-oligosaccharides are defined as copolymers of variable lengths composed of both types of monomeric units. Hetero-oligosaccharides differ in the degree of polymerization (DP), the degree of acetylation (DA) as well as in the location of monomers along the oligomer chain. The solubility in water of hetero-oligosaccharides depends on their DP, being soluble when the value is equal or minor to 10. The solubility of samples with higher DP depends on DA and pH of the solution. The interest of chitin and chitosan oligomers is due to their lower viscosity than the high molecular weight polymers, good solubility in aqueous media (in particular in biological environments), biocompatibility and their ability to penetrate biological membranes resulting in a high degree of adsorption. A general overview of the chitin, chitosan and oligomers isolation processes is shown in Figure-1.

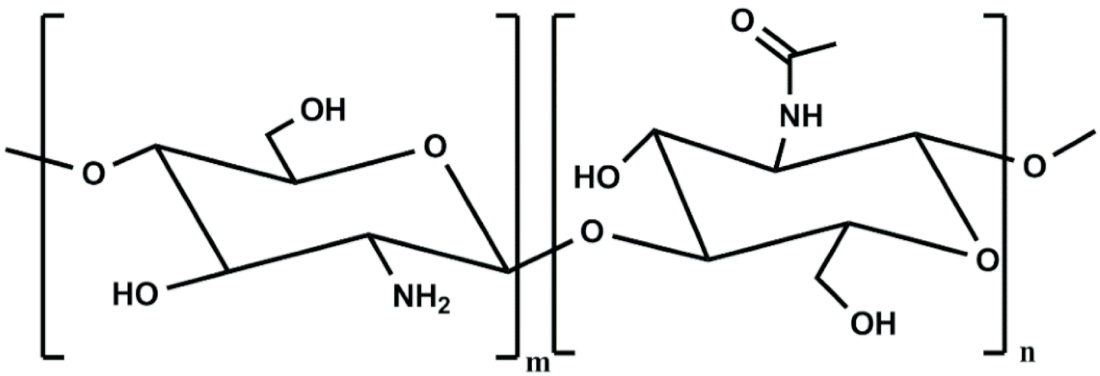

Figure-2: Chemical structure of Chitosan

2. TECHNOLOGICAL AND BIOLOGICAL PROPERTIES OF CHITOSAN

Chitosan is a biodegradable and biocompatible copolymer composed of randomly distributed monomers of β-(1→4)-N-acetyl-D-glucosamine β-(1→4)-D-glucosamine (Figure-2). The degree of acetylation (DA) of chitosan is defined by the molar fraction of N-acetylated units or as a percentage of acetylation (DA%).

As seen in Figure-2, chitosan contains primary amino groups, and primary and secondary hydroxyl groups on its structure. Chitosan is a polycation whose charge density depends on the degree of acetylation (amount of protonable glucosamine units along the polymer chain) and pH. Chitosan is not soluble in water but it can dissolve in diluted aqueous acidic solvents due to the protonation of the primary amine groups. Even highly deacetylated chitosan tends to form aggregates in acid aqueous solution due to strong interactions among polymeric chains. This hydrophobic behavior is based on the presence of both the main polysaccharide backbone and the N-acetyl groups of the N-acetylglucosamine units.

This chemical structure confers chitosan very interesting technological properties that are of great interest in several fields (Table-1). For instance, chitosan is able to produce and stabilize w/o/w emulsions without the addition of any surfactant due to the presence of N-acetylglucosamine residues that stabilize the water droplets inside the oil drops, while the glucosamine residues stabilize the oil drops in the multiple emulsion formed (Schultz, et al., 1998). Both molecular weight and deacetylation degree play a fundamental role in this process (Klinkerson, 2013). These properties are very valuable in the food industry where chitosan has been used as additive.

Chitosan has the ability to adsorb different types of molecules, such as dyes, metals and organic compounds among others. Chitosan is also involved in the coagulation-floculation process of different types of compounds. Interestingly, it seems that the accessibility of the primary amino groups is more relevant than chitosan deacetylation degree and, therefore the process is controlled by chitosan crystallinity (Guibal, et al., 2006). Due to this property chitosan has been used in waste water treatment and in the food industry for clarifying wines or juices (Li, et al., 2014; Wei, et al., 2013). The particular hydrophilic-hydrophobic balance in chitosan is related to its ability to stabilize carbon nanotubes suspensions in acid aqueous environments. The production of macroporous biocompatible carbon-chitosan nanocomposites has been reported with potential application as 3D electrodes in fuel cells and in tissue engineering (Aranaz, et al., 2014). The versatility of chitosan to produce films, scaffolds, fibers as well as micro and nanospheres is also very remarkable. Chitosan produces films by simple solvent casting methodology, scaffolds are easily produced by simple lyophilization process and micro and nano spheres are produced by several methodologies involving the use of multivalent anions, such as tripolyphosphate (Aranaz, et al., 2014; Aranaz, et al., 2016; Bugnicourt and Ladavière, 2016; Calderon, et al., 2013; Divya, et al., 2014). By interaction with polyanions, it is possible to produce chitosan-based polyelectrolyte complexes capsules or hydrogels (Luo and Wang, 2014). The production of chitosan fibers by electrospinning is not trivial. Chitosan has a limited solubility and chitosan solutions have large surface tension. That is why a polymeric mixture of chitosan along with other polymers is often used (Geng, Kwon and Jang, 2005).

Apart from these technological properties, some specific chitins and chitosans display biological activities that are strongly correlated with the polymer physico-chemical characteristics; mainly the molecular weight, the degree of acetylation and the monomer distribution along the polymer chain (Table-1).

Chitosan has antimicrobial and antifungal activities. These activities are of great interest in the food industry and cosmetics as preservatives and in the agriculture field in order to control post-harvest diseases (Acosta, et al., 2015; Fernández-Sáinz, et al., 2013; Palou, et al., 2016; Romanazzi, et al., 2017; Ruocco, Costantini and Guariniello, 2016). In the textile industry, chitosan is a valuable material not only due to its antimicrobial properties but also due to its anti-shrinking properties (Yang, Wang and Huang, 2010; Zhang, Chen and Ji, 2003). Chitosan exhibits excellent mucoadhesive performance and it has been described as a potent adsorption enhancer that increases the absorption of poorly permeable drugs such as peptides and protein drugs (Caramella, et al., 2010). The mucoadhesive behavior has been related

Table-1: Main Technological and Biological Applications of Chitin and Chitosan and Fields of Use

Field of Use	Technological Application	Biological Application
Biomedicine Pharmacy	Fiber formation (electrospinning) Film production Polyelectrolyte complex formation with polianions Stabilizer of carbon entities Gelification in the presence of multivalent ions Ability to produce polymeric micro and nano spheres Polyelectrolyte complex formation with polianions (alginates, chondroitin sulfate, heparin and so on)	Antimicrobial Antifungal Mucoadhesive Hemostatic Mucoadhesive Anticholesterolemic Antioxidant Neuroprotective Antitumoral Anti-inflammatory Anti-diabetic Anti-HIV Adsorption enhancer
Food industry and nutraceuticals	Emulsifying activity Emulsion stabilizer Film production Flocculant Gelification in the presence of multivalent ions	Antimicrobial Antifungal Anticholesterolemic Antioxidant Neuroprotective
Cosmetics and Toiletries	Emulsifying activity Emulsion stabilizer Film production Ability to produce polymeric micro and nano spheres	Antimicrobial Antifungal
Biotechnology	Gelification in the presence of multivalent ions Ability to produce polymeric micro and nano spheres	
Textile industry	Fiber formation (electrospinning)	Antimicrobial Antifungal
Biocatalysis	Polyelectrolyte complex formation with polianions Ability to produce polymeric micro and nano spheres	
Waste water treatment	Flocculant	
Agriculture	Polyelectrolyte complex formation with polianions Ability to produce polymeric micro and nano spheres	Antimicrobial Antifungal

to its ability to interact with mucin due to its positive charge. The extent of this union depends on the amount of sialic acid present in the mucin and on the Mw and DD of chitosan (Aranaz, et al., 2009). Other authors have proposed a more complex behavior and suggested that hydrogen bonding and hydrophobic effects may also play a certain role. These properties have been widely exploited to produce pharmaceutical formulation for controlled drug delivery through different administration routes such as oral, ocular, nasal, vaginal and so on (Khutoryanskiy, 2011).

Chitosan has shown a significant scavenging capacity against different radical species, this antioxidant activity is very useful in the food industry to avoid the oxidation of highly unsaturated food lipids that cause off-favors and rancidity. Moreover, several diseases, such as cancer, Parkinson's disease, cardiovascular disease, premature aging rheumatoid arthritis, and inflammation among others have been related to oxidative processes. Chitosan has been proposed as an ingredient for production of nutraceuticals, which may prevent age-related and diet-related diseases (Hamed, et al., 2016). The neuroprotective effect of chitosan is not only due to its antioxidant activity and other effects, such as suppression of β-amyloid formation, acetylcholinesterase inhibitory activity (both related to Alzheimer's disease) and anti-neuroinflammatory effect has been described (Pangestuti and Kim, 2016).

A reduction of cholesterol after oral intake has been observed without reducing the serum iron and hemoglobin (Muzarelli, 1996). Other less frequent explored biological properties of these polymers are their analgesic effect, anti-HIV activity and antidiabetic activity (Gogineni, et al., 2015; Ju, et al., 2010; Li, et al., 2016; Okamoto, et al., 2002).

As previously mentioned, the first generation of chitin and chitosan were based on polymeric mixtures poorly characterized with a variable purity degree. Therefore, the composition of the mixtures was not reproducible. These samples are appropriated for technical applications such as floculant or adsorbent in waste water treatment, fungicidal in agriculture, clarifying in the food industry and so on. All these applications have in common that the physico-chemical properties of the polymers have a low impact on the behavior of the polymer. This first generation of chitosan is still dominating the current market; however, it is not appropriate for applications where purity and controlled composition are required.

Therefore, a second chitosan generation emerged

Table-2: Comparison Between Chitosan Generations and their Main Uses

Chitosan generation	Advantages	Disadvantages	Main Uses
First	- Large amounts in the market - Cheap	- Poor characterization - No reproducibility between batches - May contain pollutants	- Waste water treatment - Technological applications
Second	- Better Sample characterization - Higher Purity - Regulatory perspectives	- No reproducibility between batches	- Agriculture - Cosmetics - Food - Nutraceuticals
Third	- Control on sample characteristics - Physico-chemical characterization - High reproducibility of batches - Traceability - High Purity - Regulatory perspectives	- Limited amount in the market - High cost	- Agriculture - Cosmetics - Food - Nutraceuticals

driven by the needs of the market. In these samples, the relationship between the structure and function of the polymers is well controlled and, therefore, are more suitable for the development of high valuable products. These chitosans are now increasingly appearing in the marketplace produced by companies (Novamatrix AS, Primex EHF, Ynsect, Heppe Medical Chitosan GmbH, InFiQuS S.L. among others) with expertise in the field of purified chitin and chitosan production. Thanks to this second chitosan generation, new products with application in different fields are emerging (see section 4). Moreover, as it will be explained in the next section, the regulatory framework has noticeably changed after their appearance in the market.

The chitin and chitosan research community is becoming more and more aware of the requirement of a third chitosan generation in which we will be able to produce exceptionally controlled samples, that is, chitosan with very low polydispersity or even monodisperse chitooligomers and with defined patters of acetylation. These samples will allow us to establish the cellular mechanisms that explain chitosan bioactivity and therefore they will be tremendously useful to develop specific chitosan based products in medicine and pharmacy. The advantages and disadvantage of each chitosan generation are shown in Table-2.

3. CHITIN AND CHITOSAN MARKETS

Global chitin and chitosan market is expected to witness significant growth in the following years due to the strong demand in various end-use industries, such as water treatment, pharmaceuticals, medical, food and beverage industry, and cosmetic industry. The market will be also driven by the increasing demand for natural based products in textile manufacturing and agricultural chemicals (Global Chitosan Market 2015-2019 report).

Chitin, chitosan and oligomers prices depend on the quality and physico-chemical properties of the polymers. In the low-end market, with products developed for waste water treatment, some agriculture uses and paper industry prices are around 9 USD/Kg. The prices can reach hundreds or even thousands of dollars per kilogram in the high-end market (chitosan for uses in pharmacy, biomedicine or cosmetics).

Depending on the final application chitin and chitosan are classify in three large groups according to the purity requirements: i) Technical grade chitosan to be used in agriculture and waste water treatment; ii) pure grade to be used in food and beverage, and cosmetics, and iii) ultra-pure grade to be used in pharmacy and biomedicine.

Ultra-pure chitosan is less common in the market than technical and pure grades since their production requires a strict control of the polymer isolation using high quality raw material (low contaminants in the exoskeleton) and a detailed sample characterization. From an economical point of view, this control is costly and the vast majority of the samples belong to low-end market since they are currently the most demanded ones.

Asian countries, headed by Japan and China, dominated the chitosan market over the past few years owing to the abundant availability of raw material. In the coming years, India and Korea are expected to witness high growth in terms of demand owing to rapid growth of end-use industries, including food and beverage, cosmetics, personal care and agrochemical. Europe and North America are expected to witness significant growth owing to increasing consumer demand for bio-based products.

4. INTRODUCTION OF CHITOSAN BASED PRODUCTS IN THE MARKET

The major impediment to launching chitosan-based products to the market is the legal regulation. This explains why in some markets the presence of chitosan based products are more prominent than in others.

For instance, the use of chitosan as a food additive was approved in Korea and Japan more than 20 years ago. Chitosan has been included as one of the thirty seven generic health/functional food ingredients in Korean Health/Functional Food Act and the Ministry of Health, Labour and Walfare of Japan has accepted chitosan as Foods of Specified Health Use (FOSHU). The requirements for FOSHU approval are summarized below:

- Effectiveness on the human body is clearly proven;
- Absence of any safety issues (animal toxicity tests, confirmation of effects in the cases of excess intake, etc.);
- Use of nutritionally appropriate ingredients (e.g. no excessive use of salt, etc.);
- Guarantee of compatibility with product

specifications by the time of consumption;
- Established quality control methods, such as specifications of products and ingredients, processes, and methods of analysis.

On the contrary, chitosan has not been included as a food additive in the general list of the Food and Drug Administration (FDA) or the European Food Safety Authority (EFSA). Currently, chitosan is included in the Codex Alimentarius as antifoam in juices and nectar.

As a dietetic supplement and taking advance of a more permissive legislation, there are several products in the European market, such as ChitoClear® (Primex ASA, Norway) that was recognized as grass in the USA in 2001. The use of glucosamine hydrochloride from Aspergillus niger and the use of a commercial product based on chitin-glucano (KiOnutrime-CG™) as a dietetic supplement have been positively evaluated by the EFSA in several scientific opinion documents of the Panel on Dietetic Products Nutrition and Allergies published in 2009 and 2010, respectively.

In agriculture, chitosan has been recognized as basic substance by the European Commission, which means that now chitosan products in agriculture can be used without the need of toxicity studies and registration processes (Spanish Official Bulletin, 2014). In the USA, the use of chitin and chitosan as pesticides is regulated by the United States Environmental Protection Agency (Bautista-Baños, et al., 2016).

Chitosan regulation in medicine and pharmacy depend on the final use as excipient, active ingredient

Table-3: Chitosan-based Products in the Market

Use	Comercial name	Company
Biomedicine	Reaxon® nerve guide Chitosan-FH02® Beschitin® ChitiPack® S Celox TM Chito-SAM TM HemCom® Chito-flex®	Medovent GmbH Medoderm GmbH Unitika Ltd Eisai Co Ltd MedTrade Products Ltd Sawyer Products, Inc Tricol Biomedical Inc Tricol Biomedical Inc
Textile	Chitoskin™ Diabetic chitosan socks Crabyon®	Tidal visión Inc +MD TM Swicofil AG
Water treatment	Storm-Klear Gel-Floc™	Natural Site Solutions Llc
Pharmacy	Betalfatrus	ISDIN Laboratories SA
Food industry	Chitoseen™-F QiUP ChitoFresto	SIVEELE B.V. Perdomini-IOC S.p.A PT Biotech Surindo
Nutraceutical	Chitosan gold Tea KiOcardio® Liposan Ultra ChitoClear® KiOnutrime-CG™)	KITTOLIFE Co Ltd. Kytozyme SA SAFIC ALCAN SA Primex EHF Kitozyme SA
Cosmetics	Hydamer™ CMF/CMFP HydamerTM DCMF HydamerTM HCFM CT-2 NYLON SP-500 Iden Bee propolis-body lotion Iden Bee propolis-body wash	Chitinor AS Chitinor AS Chitinor AS Kobo Products, Inc. Iden International Inc Iden International Inc
Agriculture	Biorend® ChiFarm Softguard	Idebio S.L. PT Biotech Surindo Leili-Canada Oceanic Inc.

or medical device. In 2002, the use of chitosan hydrochloride as excipient was approved by the European Pharmacopeia and more recently, in 2011, chitosan itself has been approved by the US Pharmacopeia. Unfortunately, eventhough some specific chitins, chitosans and oligomers have several biological activities as shown in Table-2, the use of chitin, chitosan or their oligomers as active ingredients is not yet approved. Regarding medical devices, the American Society of Testing Materials (ASTM) has launched several guides covering different aspects of the use of chitosan in biomedical and pharmaceutical applications (Guides F2103-11, Guide F2260-12 and Guide F2602-13). Although the legislation issues are being a bottle neck to the launching of chitosan-based products to the market in biomedicine and pharmacy some products are reaching the market as medical devices or as para-pharmaceutical products. For instance, hemostatic dressings (HemCom®, Chito-flex®, Celox TM, Chito-SAMTM) or a nail lacquer to nail psoriasis treatment based on chitosan have been successfully launched to the market. In Table-3, several examples of commercial chitosan-based products with applications in different fields are summarized.

5. CONCLUSIONS

Chitosan is a natural polymer with very interesting technological and biological properties. Over the past decades, it was predicted as a significant development of chitin and mainly chitosan-based products. However, the launching of chitin or chitosan-based products in the market has been very irregular due to issues at the commercial level and public sector.

Traditional uses of chitin and chitosan in the low end-market are well established, the low regulatory pressure over these products and the small effect of chitosan physico-chemical properties in the final performance of the product allow the use of large amounts of poorly characterized polymer with a competitive price.

In the last years, an extensive research has been carried out which has permitted a better knowledge of the relationship among physico-chemical characteristics and properties of these polymers. This so-called functional characterization of chitosan has driven the regulation of these polymers by several organizations in different countries.
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The more controlled production and the characterization of these samples increase the final price of the polymer and, therefore, its commercial use is limited to the high-end market products. These products developed for the food, biomedical, pharmaceutical or cosmetics industry need to fulfil strict regulatory aspects.

The better knowledge of the relationship among chitin, chitosan and oligomers physico-chemical properties and their biological properties is helping to boost their legal status. This improvement of the regulatory aspects allows us to predict a flattering future of the chitosan based products since one of the major impediments is the lack of regulation in high-end valuable applications, such as biomedicine, pharmacy or food industry.

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