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Chito-oligosaccharides: A mini-review on sources, production, and agricultural applications

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Abstract: A concise review of the main current natural sources used to produce chitin—the starting material to produce chitoooligosaccharides (COS)—is presented, including algae, arthropods, birds, fish, fungi, mollusks, and, possibly, plants. The principal approaches addressed to produce COSs, grouped as physical, chemical, and biological processes, are also outlined. Subsequently, the COS more relevant applications related to agriculture are briefly outlined, i.e., induction of innate immunity in plants, growth biostimulation, soil amending, biocidal activity, etc. Some interesting findings of this review are: (a) A clear relationship has been undoubtedly established between the low molecular weights (MWs) of these chitinous materials and their striking bioactivities (b) There is no universal consensus about the limit MW below which a substance can be considered a COS and some of the proposed limit values are supported in works that have not proposed them (c) The preparation and application of COS is an active field of research due to the accessibility of chitin sources anywhere and the variety of preparation methods available, as well as the multiple possibilities of modification that these materials offer for the preparation of bioactive derivatives (d) The chemical modification of the great number of existing COS, by a wide range of agents and approaches, including computer simulation studies, is a virgin field that could generate products with powerful elicitor proper-ties (e) Biocidal activities of COSs, advantaged with their greater water solubilities than chitin and chitosan, are remarkably attractive due to the possibility of replacing, partial or completely, injurious synthetic products currently in use. Similarly, this review makes it possible to appreciate that the preparation and separation of COS with well-defined structures could boost the discovery of the specific regulatory mechanisms that each oligomer species can activate (or repress), that is, defense mechanisms in plants.

Keywords: COS; depolymerization; chemical modification; enzymatic methods; plant growth

1. Introduction

Chitin, a biopolymer presents in a wide range of organisms such as insects, crustaceans, mollusks, fungi, fish, algae, etc. [1], has a wide range of biological properties that include antioxidant and antimicrobial activity, which have been used in agricultural applications, especially as a soil amendment agent [2]. Chitosan, its main derivative, which is usually obtained through its chemical or enzymatic deacetylation, has been more widely studied due to its greater processability because it is soluble in an acidic aqueous medium. In such a sense, its use has currently extended to many areas of science and technology, in applications ranging from the wound and burn healer [3], seed treatments [4], manufacturing of biofilms for food preservation [5], formulation of smart hydrogels for applications in medicine [6], preparation of nano-fertilizers [7], encapsulation of bioactive species, including live

microorganisms that promote better soil health and increase crop productivity [8], manufacturing of food nanosensors [9], just to name a few.

Likewise, it has been firmly demonstrated that there is a strong dependence on the properties of these biopolymers on their degree of polymerization and deacetylation degrees (DP and DD, respectively), and acetylation pattern (PA) [10–12]. Chitinous materials are homo- or hetero-copolymers made up of glucosamine (GlcN) and N-acetyl-glucosamine (GlcNAc) units, which are usually distributed randomly in the latter. Those with a fraction of glucosamine units greater than 0.50, that is, having a DD (**Figure 1a**) greater than 0.50, are considered chitosan because they usually solubilize in aqueous acid medium when $DD > 0.50$ [13,14].

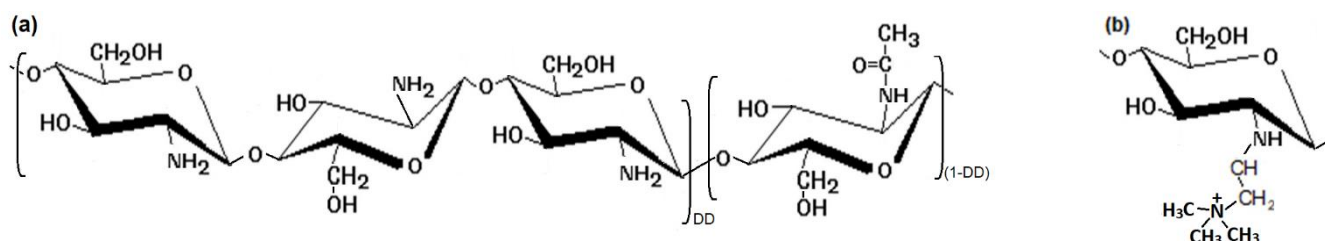


Figure 1. (a) General chemical structure of chitinous materials illustrating its composition as a function of the DD; (b) Partial chemical structure of the QCOS showing the quaternized moiety.

On the other hand, it is known that most of the properties of chitosan, such as those related to its biological activity, i.e., antibacterial, antifungal, plant bio-stimulant, antitumor, immuno-regulatory, antioxidant, and anti-inflammatory, etc., are enhanced when it contains appreciable amounts of oligomeric species [15–17], which are usually known as low molecular weight chitosan (LMWC). Furthermore, the success of some of these LMWCs has generated interest in the production and purification of a type of such materials, from both chitin and chitosan, which are known by the generic name of chito-oligosaccharides (COS) [17]. These materials can be defined, in a general way, as the degradation products of chitin and chitosan obtained under different approaches, i.e., by using physical, chemical, and biological procedures, or a combination of them. According to IUPAC-IUB Joint Commission on Biochemical Nomenclature, Recommendations [18], an oligosaccharide is a molecule containing a small number (2 to about 10) of monosaccharide residues, connected by glycosidic linkages; however, in the case of the COSs, there is no universal consensus on this matter and some limits have been advanced to define its maximum molecular weight, being 3900 Da and 10,000 Da the normally stated values. The molecular weight choice as a criterion for this definition does not seem the most appropriate because some oligomers with the same molecular weight could have a different number of repetitive units, depending on their DD, making the comparison of the structural effects of COS more complicated. Additionally, some works indicating the value of 3900 Da [19–21] are based on other works, which in turn establish that it was proposed by Muzzarelli [22], although no limits for the molecular weight of chito-oligomers are mentioned in the cited Muzzarelli's paper. Similarly, the mentioned 10,000 Da limit value [23] is also supported by an article that does not establish it [24]. Thus, greater, and more systematic work seems necessary so that the COSs can be better defined in this regard.

Concerning its use in areas related to agriculture, different studies are currently

being developed, including the computational simulation, to establish the relationships of their structural characteristics, i.e., DP, DD, PA, etc., on the mechanisms by which COS favors some processes directly related to the growth of plants as well as other related, such as soil remediation mechanisms, biocidal activity, among others. **Table 1** presents a few examples to illustrate the type of research already underway on this topic.

Table 1. Some illustrative studies on the effect of structure on the properties of COS.

Studied system	Findings
COS with DP ranging from 2 to 12 was prepared and five fractions were separated from the prepared COS by CM Sephadex C-25 column.	The COS with low DP showed a better effect of scavenging hydroxyl radicals and reducing power. The superoxide radical scavenging activity of all the tested COSs increased with DP [25]
Arabidopsis seeds treatment with a quaternized COS with glycidyl trimethylammonium (QCOS)	Obtained QCOS possess better elicitor properties than the original COS and QCOS stimulate plant protection against <i>B. cinerea</i> attack [26]
Treatment of <i>Arabidopsis</i> seedlings with chitin oligomers and a mixture of chitin oligomers (DP 2–4)	Oligomer with DP = 4 activated a transcriptional response in genes principally related to plant development [27]
COSs with determined DPs were applied to explore the relationship between the DP and the growth of wheat seedlings under salt stress	Chitohexaose, chitoheptaose, and chitooctaose exhibited stronger activity compared with other COS samples, suggesting a close relationship between their activities with the DP [28]
COSs with DP = 4–12 were tested for elicitor and priming activities in rice cells.	Both activities were influenced by DP. An apparent DA-dependent priming activity was also observed. The closer the acetyl group is to the non-reducing end, the higher the priming activity is. Neither fully nor partially deacetylated (D) tetramers with an acetyl group (A) at or close to the reducing end (DDDD, DDDA, DDAD, DAAA, ADAA, AADA) were active [29]
The growth-promoting effect of chitin oligomers, (GlcNAc) _{2–6} , was studied by measuring the content of inorganic elements and global gene expression in tomato plants grown hydroponically at ultra-low nutrient concentrations.	Significant increases in the biomass of aerial parts and concentration of chlorophyll following treatment with chitin nanofibers CNF or short-chain chitin oligomers were observed. Concentrations of nitrogen and carbon significantly increased [30]
The preparation of COS with different structural characteristics and findings on its antioxidant, anti-inflammatory, anti-obesity, bacteriostatic, and antitumor activity are summarized.	The correlation between the molecular structure and bioactivities of COSs is described, and new insights into their structure-activity relationship are provided [31]
Enzymatic preparation of single COS, spanning protein engineering, enzymatic membrane bioreactors, and transglycosylation reactions, is reviewed.	COS's bioactivities, i.e., anti-tumor, antioxidant, antibacterial, anti-inflammatory, and plant defense induction, exhibit close associations with DP values [32]

This work presents a concise review of the different sources and methodologies employed to produce COSs and their main agricultural applications. The general objectives of the work are to provide an overview of the main sources and methods for obtaining these materials, especially those with well-defined structural properties, which could help to establish more precisely the relationships with their striking bioactivities, as well as highlight their significant achievements in these applications.

2. Methodologies used in COS production

2.1. Main sources of chitinous materials

The production of COS is strongly influenced by its starting materials, i.e., chitin and chitosan. In this regard, natural sources containing chitin are very varied, including the exoskeleton of insects as abundant as cockroaches [33] and crickets [34], cell walls of fungi such as *Mucor rouxii* [35], algae [36], and finding in the shells of a variety of

crustaceans the traditional source for its current production [37]; furthermore, the scales of some fish [38], the chicken feet [39] and, presumably, the “*esponjilla*” plant (*Luffa cylindrica*) [40–42] have also been recently added to the extensive list of potential sources of chitin, which broaden the horizon of suitable materials to produce these biopolymers. Otherwise, the controlled cultivation of microalgae [43] and fungi [44] is also being intensively explored in search of chitinous materials whose properties do not show variations dependent on factors such as the stage of growth of the different species used for their traditional production, or the seasonality of their captures; furthermore, this type of material should have better qualities regarding allergen and metal contents [45]. **Figure 2** shows a diagram of the main stages involved in the production and application of COS in agriculture, including the main natural sources for obtaining chitin and chitosan.

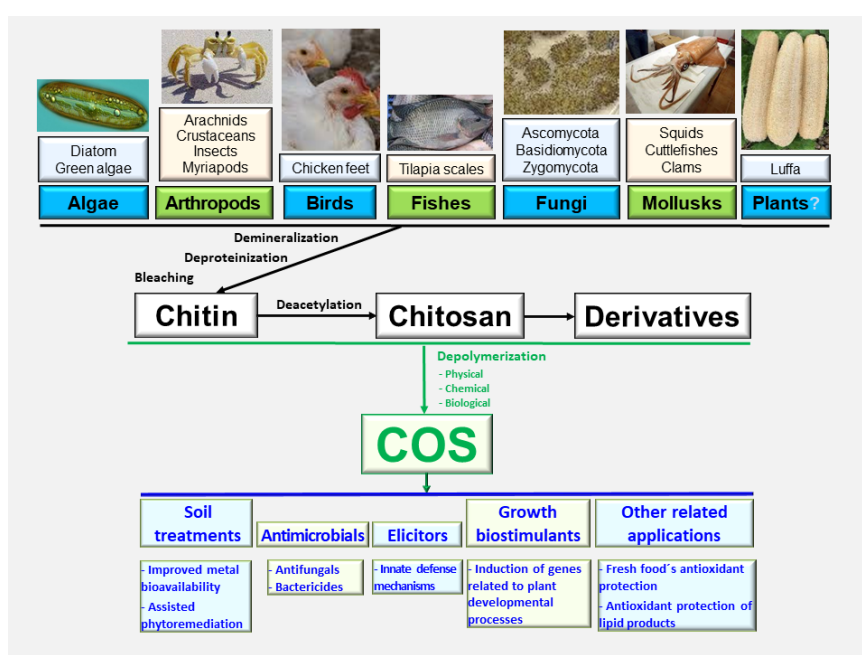


Figure 2. General sources and stages involved in COS production and its main applications in agriculture.

2.2. Methods for obtaining COS

The preparation of COS can be conducted in two ways: (i) through synthesis of oligomers, or oligomerization, starting from its structural units, GlcN and GlcNAc (ii) through the degradation or depolymerization of chitin and chitosan to obtain the desired values of molecular weight. Although the chemical oligomerization processes can generate COS highly pure and with well-defined chemical structures, it often involves different protection and deprotection steps of the intermediate products, including sometimes its purification, which increases the production costs of these materials, thereby discouraging their massive use. Furthermore, the COS biochemical synthesis starting from GlcN and GlcNAc is also possible by using a combination of enzymes, i.e., chitin synthases and chitin deacetylases [46]; however, the current availability and excessive costs of suitable enzymes may limit the COS produced by this way although this could change soon.

On the other hand, the situation of chitinous material degradation or depolymerization is completely different because COS preparation can be addressed by a wide range of approaches [15,46–48]. Below, the main procedures employed to obtain COS are briefly presented:

- 1) Physical: Among the methods that allow obtaining LMWC and COS with controlled properties through physical degradation/depolymerization of chitinous materials are the plasma treatment [49]; use of electromagnetic radiation, i.e., UV [50], gamma- and X-rays [51,52], microwave [53]; milling [54]; sonication [55]; etc.
- 2) Chemical: The main approaches for the chemical degradation of chitinous materials are based on the application of hydrolytic processes that usually lead to the split of the polymer chains into smaller species. Some of these processes allow largely to preserve the integrity of the internal GlcN and GlcNAc units during the formation of the smaller chains, i.e., X-ray irradiation [52]; there are also depolymerization processes where the chemical structures of these units are altered by the occurrence of uncontrolled deacetylation reactions of the GlcNAc units, the formation of undesirable reducing sugars impurities, like 5-hydroxymethylfurfural [56], among others. Thus, it often becomes difficult to obtain chitinous materials of different molecular weights and the same degree of acetylation, and vice versa, even starting from the same initial sample, a situation that can become more complex when considering the different PA that can occur. Among the simplest chemical hydrolytic agents used to obtain COS, oxidizing agents, such as hydrogen peroxide [57] and potassium persulfate [58], and strong acids, i.e., nitrous acid [59], and hydrochloric acids [60], and acid mixtures [61], can be mentioned.
- 3) Biological: Among the available methods, enzymatic preparation stands out as a viable and environmentally friendly approach for COS synthesis due to the better control that can be exercised in the generation of dangerous byproducts and for being carried out in moderate conditions [46]. Besides the chitinases [62] and chitosanases [63], different non-specific enzymes, i.e., glycosidases such as proteases [64], lipases [65], cellulases [66], etc., have been assessed to produce COS. Furthermore, the production of chitosan with controlled acetylation patterns, using N-acetylases, has also been recently reported [67], which seems to open new horizons for these materials, where the preparation of COS with more specific PA has a place.

Table 2 presents a few examples where the preparation of COS and its application in agriculture-related research are briefly described.

Table 2. A few works related to methods for preparing chitinous oligomers and the results of their application in research related to agriculture.

COS preparation method	Reported observations
Chemical depolymerization of chitosan using KIO ₄	A markedly superior effect was found only for the chitosan sample containing a higher fraction of oligomeric chains in the germination of zucchini seeds [14].
Combination of COS-C/secondary metabolites of <i>Streptomyces</i> spp. and COS-C/hydrolyzed gluten	Prepared combinations were effective in controlling powdery mildew during Grapevine field studies [68].

Table 2. (Continued).

COS preparation method	Reported observations
Chitosan depolymerization using a heterogeneously expressed chitosanase derived from <i>Aspergillus fumigato</i>	Obtained COS significantly inhibited the mycelium growth of phytopathogenic fungi such as <i>Botrytis cinerea</i> , <i>Fusarium graminearum</i> , <i>Alternaria alternate</i> , <i>Magnaporthe grisea</i> , <i>Erysiphe cichoracearum</i> , and <i>Alternaria solani</i> [69].
Chemical modification of N-chitosan oligosaccharide with dithiocarbamate	Modified COS possess multiple activities, including high nematocidal and egg-hatching inhibitory activities, plant growth regulating effects, low cell toxicities, and phytotoxicities [70].
Gamma-irradiation of chitosan	Chitosan and oligo-chitosan were applied on potato plants. Improvement in shoot height and number of nodes was observed after foliar spray at 50–75 mg/L [51].
Carboxymethyl chitosan's (CMC) degradation by gamma rays	CMCs with lower Mw had a good effect on delaying spoilage and decreasing the malondialdehyde (MDA) content of peach fruits [71].
Gamma Co-60 irradiation of chitosan in solution.	COS foliar spraying of the chili plant attained a noticeable increase in fresh weight (71.5%) and dry weight (184%) of the shoot [72].
COSs synthesized by gamma-ray irradiation were used to prepare nano-silica mixtures.	Foliar application of COS and COS-nano silica increased soybean seed yield increased 10.5 and 17.0%, respectively [73].
COS with well-defined DPs were separated from a mixture of fully deacetylated COS by CM Sepharose column	The results of the growth and photosynthesis parameters of wheat seedlings evidenced a close relationship between the DP of COS and its bioactivities. ADP > 3 is needed for significant promotion of growth and photosynthesis [74].
COS (MW < 2000 Da) were obtained using an enzymatic and ultrasonic combined treatment.	By using the conjugate complexes prepared with COS and bioactive compounds from <i>Streptomyces</i> spp. as coating, table grapes were found to maintain the turgor and delay the appearance of the pathogen (<i>Botrytis cinerea</i>) by 10–15 days [75].

3. Agricultural applications of COS

The bioactivity of COSs depends strongly on its chemical structures, i.e., DP, DD, and GlcN-GlcNAc distribution pattern, which define its physicochemical properties; furthermore, the dosage, application method, pH, and temperature of the medium, among other factors, would modify the bioactivity of the COSs molecules [74]. Diverse bioactivities of COSs have been exploited in agriculture-related applications [46,76–78], as briefly it is shown below.

3.1. Induction by COSs of innate immunity in plants

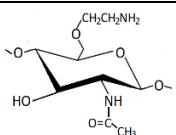
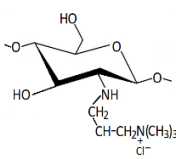
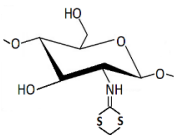
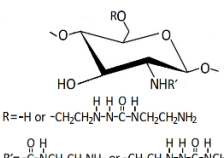
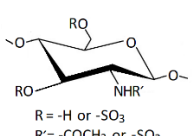
Plants can detect pathogen-associated molecular patterns, which are chemical species that have been conserved throughout evolution and are collectively called PAMPs. These act as general elicitors to activate immune responses, in a process known as PAMP-triggered immunity (PTI). Chitin oligomers are well-recognized PAMPs, and chitin oligosaccharides (COS-CHs) are the most frequently encountered PAMPs related to pathogen fungi [79]. Chitosan- and quaternized chitosan-oligomers (COS-C and QCOS, respectively) have also been reported as effective elicitors [26,80]. Thus, when transplanted blackberry plants were sprayed weekly with a soluble bioprocessed COS-C (cross-linked with ascorbic, 4 g/L⁻¹ of water) a significantly higher total soluble phenolic at 4, 6, and 7 weeks was obtained, which can be associated with a subsequent improved resilience against abiotic and biotic stresses after transplanting [81], as observed previously during creeping bent grass COS treatment [82].

Interestingly, the QCOS prepared by reacting COS-C with glycidyl-trimethyl ammonium chloride (**Figure 1b**) induces hydrogen peroxide accumulation and callose deposition in *Arabidopsis* seedlings and significantly increases the peptidyl-arginine deiminase 3 expression (more than 5-fold by QCOS as compared to COS), a previous

step to induce resistance of *Arabidopsis* to the necrotrophic fungus *Botrytis cinerea* [26]. In this sense, these QCOSs can be considered better protectors against *B. cinerea* attack on *Arabidopsis* than the original COS-C.

Thus, it becomes clear that the chemical modification of the wide variety of existing COS [20], through the infinite range of agents and methods of modification and application of the resulting product (see **Table 3** for a few examples), is a virgin field that could generate derivatives with, for example, greater eliciting properties than the starting compounds [83]. In that sense, computer simulation studies have also been reported to optimize the performance of COS-based systems [84].

Table 3. A few studies on the preparation of derivatized COSs and their bioactivities.

Proposed structure of the derivatized repetitive unit	COS properties	Observations
 <p>1-Aminoethyl-O-COS</p>	<p>MW_{COS} = 800–3000 Da MW_{DCOS} = 800–4765 Da n = NR SD = NR</p>	<p>Aminoethyl chitoooligosaccharides inhibit the activity of the angiotensin-converting enzyme. IC₅₀ = 0.8017 µg/mL [85].</p>
 <p>Glycidyltrimethylammonium chloride-N-COS</p>	<p>MW_{COS} = 2000 Da MW_{DCOS} = NR n = NR SD > 90%</p>	<p>QCOS-induced <i>Arabidopsis</i> resistance to the necrotrophic fungus <i>Botrytis cinerea</i>. It possesses better elicitor properties than the original COS to stimulate plant protection against <i>B. cinerea</i> attack [26].</p>
 <p>1,3-Dithio cyclobutane-N-COS</p>	<p>MW_{COS} = 1500 Da MW_{DCOS} = NR n = NR DD = 46.19% SD = 21.79%</p>	<p>This COS showed notorious nematocidal activities (LC_{50/72h} = 1.23 mg/mL) against <i>Meloidogyne incognita</i> second-stage juveniles as well as egg-hatching inhibitory activity [70].</p>
 <p>Aminourea-COS</p>	<p>MW_{COS} = 2200 Da MW_{DCOS} = NR n = NR DD = NR SD = 23.09%</p>	<p>The COS-derivative had remarkable inhibitory efficiencies against three plant pathogen fungi than initial COS, exhibiting inhibition rates of 60.12%, 82.95%, and 85.23% against <i>Fusarium solani</i>, <i>Verticillium albo-atrum</i>, and <i>Phytophthora capsici</i>, respectively [86].</p>
 <p>Sulfated-COS</p>	<p>MW_{DCOS} = 1600 Da Sulfate content of is 48.5%.</p>	<p>Sulfated-COS alleviates the damage of salt stress on wheat seedlings by adjusting the antioxidant enzyme activities of plant. Its effect on photochemical efficiency was closely related with the sulfate group [87].</p>

SD = Substitution degree; n = average number of repetitive units;
MWCOS = MW of the initial COS; MWDCOS = MW of the derivatized COS.

3.2. COS as a growth stimulator

The bio-stimulation of plant growth by COSs has been well documented [26,46,88], with different approaches having been considered to explain their beneficial effects, i.e., increasing photosynthesis, auxin, and gibberellin content, C and N assimilation, etc. [89]; notwithstanding, the molecular mechanism is still unknown [27], perhaps in part due to the multiple variety of sources and methods used to produce COSs as well as the difficulty in standardizing the characterization of such dissimilar samples. Some COSs perhaps mimic the natural lipo-oligosaccharides used

by arbuscular mycorrhizal fungi [90] and *Rhizobium* bacteria [91] to initiate the root colonization and symbiotic relationship with plants, respectively, by triggering some processes that favor the plant growth and development. In this regard, some COSs have been reported to induce genes belonging to functional categories of the plant developmental processes [27] and, through metabolomic and proteomic studies, the regulation of different key genes in the signaling pathway of plant growth by hetero-COS have also been observed [92].

3.3. COS against phytopathogen

In addition to their ability to induce plant protection mechanisms against pathogens by activating the metabolic pathways of salicylic acid or jasmonic acid/ethylene [89], the antifungal activity of COS has been known for a long time, i.e., inhibition of *Fusarium solani* by non-acetylated heptamers [93]. This activity has also been related to DP [94], among other factors. Thus, COS prepared by enzymatic hydrolysis (DP between 3–9) showed greater antifungal activity than the starting chitosan for various phytopathogens, but with more noticeable effects on mycelial growth, and on other growth stages, of *Phytophthora capsici*; interestingly, the proposed mechanism of action of COS was not solely related to the cationic character of COS [95].

Another line of action currently in focus, because of the utilization of naturally occurring chemicals, is the derivatization of COSs using essential oils with their biocidal activity, seeking to achieve synergistic advantages from the chemical union of them, as it has been observed for physical mixtures of COS and ethylenediaminetetraacetic acid on the pathogen *Fusarium fujikuroi*, causing the rice bakanae disease [96]. Some studies have shown that such derivatives have the potential to control certain pathogens, such as the COS derivatives containing cinnamyl moieties which demonstrated enhanced antibacterial activities [97]. As can be inferred, the topic of biocidal activity of COSs is quite broad, being of interest to different sectors such as agriculture, food, health, environment, etc., and is closely related to the chemical structures of these materials [98,99]. Regarding agricultural applications, the appropriate use of different COS could help to establish an era of sustainable agriculture and avoid the use of chemical or synthetic pesticides, as advanced by Karamchandani et al. [100] during the control studies of pokkah boeng diseases on sugar cane (also caused by *Fusarium fujikuroi*).

3.4. Soil amending and bioremediation

The ability of LMWC and COS to chelate metal ions, i.e., Fe^{2+} , has been demonstrated to be highly dependent on their molecular weights (MWs), with greater chelating activity observed for low MWs [101]. Therefore, soil amendment with COS could favor the bioavailability of some metals, which facilitates their uptake by plants [102]. On the other hand, phytoremediation can take advantage of the COS's chelating ability to mobilize soil heavy metals, as it has been reported for *Hylotelephium spectabile* in Pb-contaminated fields [103]. Further, COS could also stimulate the resistance of the phytoremediation plant to the stress conditions generated by contaminated soils, as it has been observed for diverse plants [104]; however, in the

phytoremediation of metal-contaminated soils assisted by exogenous agents, chitinous materials included, it is also important to consider the potential risks of the spread of resistant genes, as it has already been observed [105].

3.5. Other potential agricultural-related applications of COSs

In addition to their bioactivities directly related to the agricultural sector, COS can be used in other applications close to or overlapping with it, such as the food and nutraceutical sectors. Thus, COSs have shown antioxidant activities whereby they could be incorporated in foods as a functional ingredient to promote consumers' health and to improve the shelf life of food products by retarding lipid oxidation; furthermore, they would also help to improve the shelf life of food products by inhibiting some pathogens [101]. Similarly, some COS derivatives have been reported to inhibit adipogenesis and lipid accumulation, i.e., sulfated (*N, O*)-COS (MW < 1 kDa), which confer high potential to be utilized as a bioactive agent in the nutraceutical and food industries, among others [106].

On the other hand, trials with COS for the treatment of pesticide poisoning in laboratory animals have shown that some of these materials alleviate and remove the toxicological effects, as has been observed for chlorpyrifos, an organophosphorus pesticide. COS showed significant biological effects in removing and mitigating blood biochemistry, antioxidants, inflammation, apoptosis, gut barrier structure, and metabolic function changes induced by this pesticide [107].

4. Concluding remarks

The preparation and application of COS is a very active field of research due to the variety of sources and preparation methods available, as well as the multiple modification possibilities that these materials offer for the preparation of bioactive derivatives. A clear relationship has been established between the low molecular weights of these chitinous materials and their striking bioactivities in fields such as agriculture, food, health, environment, etc. However, in addition to achieving a consensus definition of when chitoooligomers can be referred to as COS, much more research needs to be carried out on this point, to optimize the sustainable production of COS with better-defined and characterized structures and where the effects of the PA begin to be disclosed [29,108,109].

Likewise, research on the chemical modification of the wide variety of existing COS, through the infinite range of agents and methods of modification and application of the resulting products, is a virgin field that could generate products with powerful elicitor properties, including computer simulation studies, which have also been reported to optimize the performance of COS based systems. Similarly, the biocidal activities of COSs, advantaged with their greater water solubility than chitin and chitosan, are remarkably attractive due to the possibility of replacing, partial or completely, synthetic bioactive products in use, a fact of current importance in different sectors such as agriculture, food, health, environment, etc.

In a sense, chitin, chitosan, and COS are usually mixtures of similar molecules, containing species of different molecular sizes, DD, and PA, capable of activating specific processes; unfortunately, the use of these mixtures has prevented clarifying

the importance that some of the species, or a combination of them, may have on certain biological processes. Therefore, obtaining COS with well-defined structures can help discover, among others, the specific up-regulating mechanisms that each of them can activate (or repress), i.e., defense mechanisms in plants, which makes research in this field more exciting every day. As has already been proposed [48], the next development of these materials may be based on taking the *in vitro* biorefinery approaches to the *in vivo* cell factory levels for the biotechnological production of well-defined COS, using recombinant microbial strains capable of expressing oligomer-specific chitin synthases and chitin deacetylases. Additionally, the bioactivities of these COSs could be further improved by derivatization.

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