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Chitooligosaccharide: An evaluation of physicochemical and biological properties with the proposition for determination of thermal degradation products

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ABSTRACT

Being the most versatile biopolymer, chitooligosaccharide/chitosan oligosaccharide (COS) has been extensively studied for a range of exceptional biological activities and potential developments of novel medical devices and systems in biomedical and pharmaceutical fields. While possessing intrinsic biocompatibility, mucoadhesiveness, and non-toxicity it gained more interests in the biomedical development of novel systems, devices, and pharmaceutical formulations. The bioactive relativity of chitosan and COS are of highly significant and thus explored in this paper while highlighting its multiple biological activities and promising biomedical applications. More emphasis is on the molecular weight, degree of acetylation/deacetylation, degree of polymerization and reactive groups in relation to chitin and chitosan. Despite COS wide acceptance and utilization, the associated viscosity and instability are crucial factors that posed a great challenge to researchers. The apparent reason attributed to instability and viscosity could be the presence intrinsic variable oligomers within COS. Due to lack of data on safety and impurity analysis of thermal exposure of COS, we hypothesized that different molecules could be generated with thermal treatment of COS, thus finally suggested a prospective determination of thermal degradation product(s)in COS. Hence the aim of this paper is to highlight COS physicochemical and biological significance with reference to its recent developments and propose a further chemical analysis thermal treated COS. This could trigger future researchers for possible isolation and characterization of distinct biomolecules from COS.

1. Introduction

Numerous studies have highlighted the initiation and progress of researchers on chitin, chitosan and their derivatives due to their multiple intrinsic biomedical properties. Significantly, the biopolymers are biodegradable, biocompatible, nontoxic, and are versatile. They were recently further revealed to have great potentials for a wide range of applications including agriculture [1,2] biomedical [3,4] and pharmaceutical formulation developments [5,6], functional food productions [1,7–9] and cosmetics industries [10,11], environmental protection [12,13], and wastewater management [14,15].

Chitin isolation involves deproteinization, demineralization and finally removal of lipids and discoloration [10,16]. For the generation and purification of chitosan, the acetyl moiety is removed through deacetylation with chemical/enzymatic hydrolysis [17,18]. Yet chitosan's insolubility, high viscosity and low permeability due to the presence of *N*-acetyl groups, long chain of glycosidic bonded saccharides and high molecular weight (Mw) [19,20] led to further deacetylation and depolymerization [21,22], yielding COS. In relation to its parent biopolymers, COS is endowed with Mw of less than 2 kDa with almost 100% absorbability, thus can entirely dissolve both in water and physiological conditions that recently captured numerous researchers' attention.

Yet remarkably both chitosan and COS are non-toxic [23], non-allergenic [24], mucoadhesive [25], with substantial exhibition of antioxidative, antimicrobial [26,27], anti-inflammatory [28], immuno-stimulating and anticancer activities [29]. They are suitably implicated in drug and gene delivery biopolymers [30–32] thus received substantial considerations in biomedical and pharmaceutical applications [33] and even in commercial ventures.

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However, in recent times COS is favorably employed with the ambition to achieve higher tissue and membrane permeation and cellular transduction [34,35] due to its lower Mw, low viscosity, complete solubility and absorbability [36,37]. Its bioactivities have attracted many researchers for its various applications for human health benefits, nutritional supplementations [38] and environmental safety and protection [12]. Of particular significance in this paper, are COS physicochemical and biological fate with reference to pharmaceutical and biomedical applications. With excellent therapeutic benefits, superior physiological and pharmacological effects [39,40], and complete absorbability [41], while retaining intrinsic biocompatibility [42,43], mucoadhesiveness [44] and non-toxicity [45] COS gain fervent interest in the biomedical development of novel systems, devices [46], and pharmaceutical excipients [47]. Of particular significance, COS was substantially implicated in both common bacterial and fungal infections [21,48,49], inflammatory degenerative disorders [50–52], multi-drug resistant (MDR) in chemotherapy [45,53], hypertension [29,54] and in metabolic syndromes like diabetes [5,6]. In addition, few fascinating cases about anti-HIV-1 [55] and anti-Alzheimer's effects of COS were reported.

For in-depth researches into COS biological activities for further biomedical applications, it is imperative to note thechemical composition of COS, particularly its oligomers, like *N*-acetyl glucosamine, chitobiose, chitotriose, chitotetraose, chitopentaose and chitohexaose [56]. In addition, structure and functional group relationship with the physicochemical and biological properties [57,58] in relation to chitosan and chitin is as well crucial when consideration chemical modifications to the basic COS structure [21]. Chitin (*N*-acetylated) and chitosan (*N*-deacetylated) [59] both have a functional structural relationship with COS as these units form the backbone of the biopolymers thus intrinsically vital for their physicochemical properties and biological activities of COS as well as the Mw and DD [21,60].

Despite COS wide acceptance and utilization in the biomedical application [3,4] and functional food processing [1,7–9], instability and viscosity [61,62] still posed a considerable challenge to researchers these days [56,63]. There have been chemical modifications in developing COS derivatives and conjugates to overcome the issue of stability and viscosity [58,64], yet the safety and impurity profiling of its derivatives are rarely established. Particularly noted here is COS safety and purity evaluations of thermal exposure are noted to be crucial, yet limited data is available. Alluded to the fact that COS is composed of multiple fragments of oligosaccharides as viscosity and instability could be attributed to these constituents. This poses the hypothetical probability of generation of thermal degradation products from COS, therefore this is potential research option. With the fact about lack of thermal analysis of COS, this paper could be the initial proposal for determination of thermal degradation of COS. This could involve heating of COS at high temperature for 8–12 h, HPLC analysis and purification then characterization with LC–MS, NMR, and IR including other feasible techniques.

2. COS physicochemical relativity with chitosan and chitin

Chitin is described as cellulose with one hydroxyl group on each monomer replaced with an acetyl amine group (−NHCOCH3) on C2 [65], which primarily allows for increased hydrogen bonding between adjacent polymers, giving the chitin-polymer matrix improved strength [66]. It is primarily composed of β(1 → 4)-linked 2-acetamido-2-deoxy-β-D-glucose (*N*-acetylglucosamine) [16]. Chitin's physicochemical and biological properties are very much dependent on *N*-acetylglucosamine and exposure of hydroxyl groups yet somewhat inert [67], that subsequently lead to the generation of chitosan first then COS. The structure of *N*-acetylglucosamine unit (Fig. 1) is a continuous repeat to form long chains in β-(1 → 4)-linkage in chitin that is similar in chitosan and COS even after deacetylation and depolymerization processes [68]. The only notable differences are the varying repeated amino-D-glucosamine and *N*-acetyl-D-glucosamine chain, low Mw, a low degree of acetylation (DA) [69] in chitosan, and much lower Mw [70], almost completely deacetylated and a higher degree of polymerization (DP) in COS [71].

Physically in its unmodified form chitin is translucent, pliable, resilient, and quite hard yet biodegradable and relatively inert [72]. It cannot dissolve in water and most regular solvents and cannot be readily fabricated into fibers or membranes [73]. Being the parent biomaterial, chitin and its derivatives were initially revealed to be potential biopolymer for multiple functional substrates [29] for many biomedical and pharmaceutical systems and devices thus preceded the phase for practical researches in biomedical, pharmaceutical, food and environmental industries [74]. Due to its natural abundance, it is easily isolated from crustacean shells and certain fungi species involving demineralization, deproteinization and depigmentation [17,18], Fig. 2. Further deacetylation of chitin yields chitosan. It is important to note that the DA or DD is the differentiating factors between chitin and chitosan (hydrolyzed form).

Relative to chitin, chitosan, partially deacetylated form is much lighter and was preferred due to its higher portion of deacetylated glucosamine moieties.

Thus, it can be cross-linked with other suitable substrates to form hydrogels and other feasible chemical modifications as well. Chitosan with higher DD has improved biological activities [75], especially absorbability, cellular transduction, permeation, and DNA/siRNA delivery [76] compared to chitin. From irradiation of chitin, chitosan is generated with 95.19% DD exhibited anti-oxidant activities of 47.71–72.31% while exhibiting scavenging ability ranged from 43.03% to 90.48% [77] compared to DD below 50%. In addition, it markedly inhibited the growth of tested bacteria, although inhibitory effects differed with Mw of chitosan and the species of bacteria. Chitin is generally had Mw more than 1000 kDa with poor absorbability, hardly dissolves in water, acid and alkali solvents [78].

The properties such as DD and Mw are closely related to chitosan's biological performance [18,77] thus much attention is demanded chitosan production both for commercial and researchers. Determination of the desired DD values helps in the polymer's characterization [68]. Chitin is soluble in *N*,*N*-dimethylacetamide, *N*-methyl-2-pyrollidone and their mixed solvents in the presence of 5% Lithium chloride [72]. It was utilized in combination with salicylic acid for the damping off of pepper against Pseudomonas spp. [79,80] and for a control against bacterial root infection in pepper [79].

Several degradation studies were done on chitin and chitosan involving chemical, enzymatic and thermal degradation approaches. Ke at al. initiated a degradation of chitosan with low concentrations of hydrogen peroxide, which revealed induced random degradation of partially deacetylated chitin and chitosan [81]. Depending on their previous studies with ultrasonic and enzymatic degradations, chain-end scission was resulted in severe degradation of chitosan and obtained a significant amount of oligosaccharides (low Mw) at temperatures ≥80 °C [28,82]. The presence of trace transition metal ions was noted in addition to amino groups that is critical to the breakdown of β-1,4 glycosidic linkages and also for chitosan safety and purity [83,84]. The postulated HPLC analysis of degraded fragments revealed glucosamine and COS in correlation to logarithmic values of retention time with higher DP. Despite the presence of inorganic ions, they concluded that formation of glucosamine and COS depended on the concentration of H_2O_2, temperature, and the physicochemical property of the substrate (chitin/chitosan). Three stages of degradation behavior were reported from an evaluation of chitosan copolymer (chitosan-*g*-poly-acrylic acid) under 200 °C [85]. The analysis was conducted in a lysozyme solution and an active slurry solution, where higher degradation rate was reported in lysozyme solution and attributed the composition of the copolymers. Another thermal study on chitosan polymers (poly[azo] amino-chitosan) [86] described the thermal behavior of chitosan suggested the potential of developing chitosan polymers with thermal sensor. In a recent study by Saadet al., *in-vitro* and *in-vivo* investigation