J Food Processing & Beverages
July 2013 Vol.:1, Issue:1

Open Access Review Article

Journal of Food Processing & Beverages

Recent Advances of Chitosan and Its Derivatives for Novel Applications in Food Science

Yangchao Luo, Qin Wang*
Department of Nutrition and Food Science, University of Maryland, USA

Address for Correspondence
Qin Wang, Department of Nutrition and Food Science, 0112 Skinner Building, University of Maryland, College Park, MD 20742, Tel: (301)-405-8421; Fax: (301)-314-3313; E-mail: wagnqin@umd.edu

Submission: 09 May 2013
Accepted: 08 July 2013
Published: 12 July 2013

Abstract

Chitosan is produced by deacetylation of chitin, the second most abundant polymer in nature next to cellulose. Being a unique cationic polysaccharide, chitosan possesses many functional properties and bioactivities, such as antioxidant property, lipid-lowering activity, antimicrobial capacity, film-forming and gelling property, encapsulation potentials, and so on. Since last decade, it has been extensively studied in food, pharmaceutical, biomedicine, and chemical industries. In recent years, many advanced applications of chitosan and its derivatives have been developed in food science, including novel chitosan derivatives with enhanced antioxidant and antimicrobial activities, chitosan-based active films for food packaging to extend shelf life, as well as chitosan-based encapsulation and delivery systems for nutrients. This review focuses on the advances in recent five years in the development of chitosan and its derivatives for their novel applications related to food science.

Introduction

Chitosan is a natural-based biopolymer derived from chitin, the second most abundant polymer in nature next to cellulose. Chitin can be found from many sources in nature, including exoskeletons of crustaceans, insects, as well as mollusks and fungi. Cellulose, chitin, and chitosan share very similar backbone structures, as shown in (Figure 1). The difference among these three molecules is the functional group at C-2 position. In molecular chain of chitin, it consists of linear structures of 2-acetamido-2-deoxy-β-D-glucose through β (1 → 4) linkage, by replacing hydroxyl group at C-2 position in cellulose molecular chain with acetamido group. Chitosan is the chitin derivative produced by the *N*-deacetylation process, resulting in the amino group at C-2 position on its backbone. Like cellulose, both chitin and chitosan are considered as naturally-occurring polysaccharides, and they are of particular commercial interest because of great nitrogen content (6.89%) [1]. Apparently, both cellulose and chitin are water-insoluble polymers with very low chemical reactivity. When the deacetylation degree reaches about 50%, chitosan becomes water-soluble in acidic pH (lower than its pKa~6.2), in which condition the protonation of $-NH_2$ groups occurs resulting in solubilization. After protonation, chitosan carries positive surface charges on its D-glucosamine repeat unit and therefore becomes the only pseudo natural cationic biopolymer. Chitosan is also well-known for its biocompatibility, biodegradability and low-toxicity. Due to these unique properties, chitosan is considered as a versatile biopolymer that can be developed into different forms, such as gels, films, nano/micro-particles, beads, etc., and find numerous applications in various fields, including food, pharmaceutical, and cosmetic sciences.

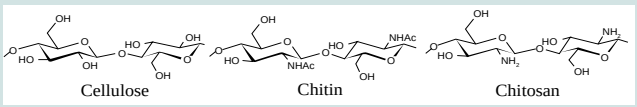

Figure 1: Molecular structure of cellulose, chitin, and chitosan.

Due to the hydroxyl and amino groups on its backbones, chitosan is an amenable molecule that can be easily modified by various methods. Modifications of chitosan are aimed to improve physicochemical properties of chitosan and thus expand its applications in different situations. Several modification methods have been well documented for chitosan, including chemical, physical, and enzymatic approaches. For instance, because the high molecular weight and high viscosity of chitosan may limit its applications in certain biological conditions, depolymerization is often applied to chitosan to obtain the oligosaccharides and/or monomers [2]. The depolymerization is often achieved via enzymatic modifications using chitonase. Many chemical means are also reported to modify chitosan, such as quaternization, *N*-alkylation, hydroxylalkylation, carboxyalkylation, thiolation, glycation, etc, [2]. The physical modifications include electromagnetic radiation and sonication. The modified chitosan with enriched properties, such as excellent solubility in aqueous solutions at different pHs, modulated surface charges, higher absorption efficiency, new crosslinking sites, find novel applications and can be tailored for particular purpose.

Since last decade, chitosan has received increasing attention and its applications have been extensively explored in all aspects of science. In food science, chitosan is of particular interest over synthetic polymers, because it is considered as GRAS (Generally Recognized as Safe) by Food and Drug Administration (FDA). The present review focuses on the most recent advancement of applications of chitosan and its derivatives in food sciences, including the nutritional properties, antimicrobial activities, edible coating, food packaging, emulsions, encapsulation, as well as enzyme immobilization and water purification.

Biological Activities of Chitosan and its Derivatives

Antioxidant properties

Antioxidant activities of chitosan have been extensively studied both *in vitro* and *in vivo* using different methodologies. The antioxidant properties of chitosan are reported to be correlated to its structural characteristics, including Molecular Weight (MW), Degree

ISSN: 2332-4104

of Deacetylation (DD) as well as the sources of chitosan. The MW and DD may also present some synergistic effects on the biological activities of chitosan. Different results may be obtained for different free radical generating systems. The recent advances on antioxidant properties of various chitosan-based products are summarized in Table 1.

Antioxidant properties of native chitosan and its oligomers

Yen and coworkers investigated the antioxidant properties of chitosan prepared from crab shells [3] and shiitake stipes [25]. Both studies pointed out that the antioxidant activities of chitosan increased with increase of DD during preparation. The longer *N*-deacetylation time results in more amino groups on C-2 positions which contribute significantly to the antioxidant activities. Chitosan from both sources showed the greatest antioxidant activities in hydroxyl radicals scavenging, conjugated diene formation, and reducing power assays, showing the EC50 lower than 1.5 mg/ml, whereas less satisfactory activities in (1, 1-diphenyl-2-picrylhydrazyl) DPPH radicals and ferrous ion chelating ability tests, showing the EC50 as high as 16.3 mg/ml. EC50 is defined as the effective concentration at which the antioxidant activity was 50%. The MW of chitosan was reported to have major effect on its antioxidant activities. Chitosan with lower MW have more pronounced scavenging effects on superoxide and hydroxyl radicals than the one with higher MW [26]. Another study investigated the antioxidant properties of chitosan oligomers obtained by H_2O_2 degradation, and confirmed that the MW is negatively related to the antioxidant activities of chitosan [27]. The chitosan oligomer with MW as small as 2300 Da has the greatest antioxidant activities against superoxide anion and hydroxyl radicals. Chitosan with higher MW was considered to be more compact in structure due to the stronger intramolecular hydrogen bonds, such as N_2–O_6 and O_3–O_5, than the one with lower MW. Therefore, the hydroxyl and amino groups in chitosan with lower MW are more flexible to react with free radicals, and hence exhibited higher free radical scavenging effects.

Antioxidant properties of modified chitosan

In order to further improve the antioxidant properties of chitosan, various modifications have been applied on chitosan molecules to overcome its solubility limitation. Sulfation represents a very important family of chitosan derivatives with enhanced biological activities, especially antioxidant properties. It has been shown that sulfated chitosan and sulfanilamide chitosan have significantly better free radical scavenging activities than native chitosan [26,28] mainly

Table 1: Antioxidant properties of chitosan and its derivatives (2008-2013)*

Chitosan and derivatives	MW / DD	*In vitro* studies – free radicals scavenging and metal chelation assays	Ref.
Chitosan from crab	Unknown / 83.3-93.3%	DPPH, reducing power, hydroxyl radicals, Fe^{2+} chelation	[3]
Chitosan from the larvae of housefly, *Musca domestica*	426 kDa / 90%	DPPH, hydroxyl and superoxide anion radicals, reducing power, Fe^{2+} chelation	[4,5]
Phosphorylated Chitosan from squid gladius	Unknown	DPPH, ABTS, Fe^{2+} chelation, hydroxyl radicals	[6]
Chitosan supplement (Chitosamin®)	100 kDa / 90%	DPPH, ABTS	[7]
Water soluble chitosan (Chitosan-up®)	20 kDa / 95%	Human serum albumin, DPPH, ABTS	[8]
N-acyl chitosan oligosaccharide	7310 kDa / -	hydroxyl and superoxide anion radicals, reducing power	[9]
N-(2-hydroxy-3-trimethylammonium) propyl chitosan chloride	9641 (weight average molar mass) / 80%	β-carotenen linoleic acid activity, DPPH, reducing power, hydrogen peroxide radical	[10]
Quaternized chitosan conjugated with gallic acid or caffeic acid	70 kDa / 97%	Hydroxyl radical, superoxide radical, DPPH	[11]
2-[phenylhydrazine (or hydrazine)-thiosemicarbazone]-chitosan	200, 8 kDa / 96%	hydroxyl and superoxide anion radicals, reducing power, Fe^{2+} chelation	[12]
Gallic acid grafted chitosan	307 kDa / 80%; 950 kDa / 92%	DPPH, reducing power, horseradish peroxidase, carbon-centered alkyl radicals, hydroxyl radicals	[13,14]
Ferulic acid grafted chitosan	200 kDa / 90%	DPPH	[15]
Caffeic acid-grafted chitosan	47, 198, 544 kDa / unknown	DPPH, reducing power	[16]
Chitosan-glucose conjugate	Unknown / 78-82%; 105 kDa / 90%;	DPPH, reducing power, superoxide radical, β-carotenen linoleic acid activity	[17-19]
Chitosan-xylan conjugate	810 kDa, 90% 118 kDa / 95%	Lipid peroxidation	[20]
		***In vivo* studies – Oxidative stress**	
Chitosan	750 kDa / 85 – 87%	Age-associated dyslipidemic rats – attenuated oxidative stress in heart tissue of aged rats	[21]
Chitosan	Unknown	Hyperlipidemic rats - elevated serum levels of antioxidative enzymes	[22,23]
Chitosan supplement (Chitosamin®)	100 kDa / 90%	Healthy human subjects and nephrectomized rats – reduced albumin oxidation and increased plasma antioxidant activity;	[7,24]
Water soluble chitosan (Chitosan-up®)	20 kDa / 95%	Healthy human subjects – reduced albumin oxidation and increased plasma antioxidant activity	[8]

*__Abbreviations__: M.W: Molecular Weight; DD: Degree of Deacetylation; DPPH: 1, 1-diphenyl-2-picrylhydrazyl; ABTS: 2, 2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid).

ISSN: 2332-4104

due to the enhanced water solubility and hence increased accessibility of chitosan to free radicals. Many other modifications have also been reported to be able to improve free radical scavenging activities of chitosan, such as Schiff bases reaction [29], quaternization [30, 31] carboxymethylation [29,32] and acylation [33]. In addition, conjugation of small antioxidant molecules with chitosan has been developed as a new approach in recent years to improve antioxidant properties of chitosan. In this modification, it is aimed to upgrade chitosan functionality with incorporation of natural plant antioxidant to its polysaccharide backbone. The chemical cross-linking methods are generally adopted to graft antioxidants to chitosan molecules, including essential oils [34,35] and phenolics [14,36] The antioxidant properties of grafted chitosan are significantly improved, especially for DPPH scavenging and metal ions chelating activities, in which systems native chitosan has very little activity. However, because the toxic and irritating reagents are needed in the chemical modification process, this modification becomes less popular. Another novel and attractive method has been recently developed and received increasing interest as an alternative to chemical cross-linking modification, i.e. enzymatic modification. Using this method, several bioactives have been reported to successfully graft to chitosan molecules, including flavonoids [37,38] and phenolics [39,40]. Some other physical modifications have also been reported to improve antioxidant activities of chitosan, such as ionizing radiation [41] and irradiation [42].

In vivo studies of antioxidant properties of chitosan

Antioxidant properties of chitosan has not only been shown by *in vitro* antioxidant assays, but also evidenced in many *in vivo* studies using various animal models as well as clinical trials. Anraku and coworkers investigated the effect of high MW chitosan on antioxidant stress and chronic renal failure using nephrectomized rats [24]. The study showed that ingestion of chitosan over a 4-week period not only resulted in a significant decreased ratio of oxidized to reduced albumin and an increase in biological antioxidant potential, but also alleviated renal failure. Another recent study also revealed the antiaging effect of high MW chitosan in glutathione-dependent antioxidant system in rats [21]. The oral administration of chitosan was reported to signicantly attenuate oxidative stress in heart tissue of aged rats by maintaining antioxidant enzymes. The antioxidant effect of high MW chitosan has been recently evidenced in a clinical trial [7]. Besides the high MW chitosan, the antioxidant properties of low MW chitosan, especially oligosaccharides have also been widely studied in many *in vivo* experiments. For instance, chitosan oligosaccharides have been shown to protect mice from liposaccharide-induced sepsis by attenuating organ dysfunction and improving antioxidative enzymes' levels and preventing redox imbalance [43]. Similar effect of chitosan oligosaccharides have also been observed in diabetic rats induced by streptozotocin [44]. As discussed above, low MW chitosan is believed to possess greater antioxidant activities than high MW chitosan, as observed by *in vitro* assays; however, the comparison of MW-dependent antioxidant activities has not been well understood yet. Furthermore, the *in vivo* evaluation of chitosan derivatives has not been well-established so far, because some toxic chemicals are usually involved in the modification process which may pose potential toxicity to animals or humans.

Lipid-lowering effects

The lipid-lowering effects of chitosan have been observed since 1980s [45,46] in animal models, and this effect was reported for the first time in humans without any side effects as early as 1993 [47]. Then, increasing evidence of potent lipid-lowering effects of chitosan has been consistently shown in numerous literatures. Chitosan has been well-documented to possess pronounced capabilities to lower plasma and hepatic triacylglycerol, total cholesterol levels, as observed in a large number of animal studies. While nowadays, more and more attention has been drawn to explore the underlying mechanisms of hypolipidemic activity of chitosan. Several mechanisms have been proposed recently. First, chitosan possesses strong binding capabilities to fat, cholesterol, and bile salt. This binding effect is contributed by the electrostatic attraction between positively charged amino groups of chitosan and negatively charged carboxyl groups of fatty acid and bile salts; by the entrapment of fat droplets in stomach and later precipitation in small intestine, delaying the digestion of fat [48]; and by the hydrophobic interactions as well as hydrogen bonding between lipid and chitosan [49]. Second, chitosan can help body maintain the antioxidant enzymes, such as superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GSH-PX), which play important roles in lipid peroxidation [22,23]. Third, chitosan has profound impact on plasma adipocytokines, which significantly reduce adiposity index, and therefore chitosan can regulate the level of circulating triglycerides and counteract some inflammatory disorders and metabolic alterations [50].

Different chitosans exert different lipid-lowering effects *in vivo*, because the physicochemical properties of chitosan affect its efficacy significantly. Different DD of chitosan results in different amount of free amino groups on its backbones. The more free amino groups convey more positive surface charges of chitosan, which will strengthen the electrostatic binding capacity with fatty acid and bile salts. The MW also plays an important role in lipid-lowering efficacy as well. The higher MW chitosan has higher viscosity, which can help binding and entrapment of fat droplets in gastrointestinal tract and thus prevent their absorption. This phenomenon has been verified in a recent study, showing that high MW chitosan exhibited more pronounced effect on the increase of fecal fat and cholesterol in mice, while lower MW chitosan seemed to be more effective in elevating lipoprotein lipase activities in both plasma and liver [51]. Additionally, the particle size of chitosan powders has great influence on lipid-lowering effects. The nanopowdered chitosan prepared by ultrafine milling has been reported to be more effective than ordinary chitosan, due to the larger surface area to interact with lipids resulting in increased excretion of lipids in feces [52]. Several studies also suggested that different modification methods result in different lipid-lowering mechanisms [53-55].

Other biological activities

Chitosan has many other biological activities and health benefits that are well-documented [56-58], including prevention of renal failure, wound healing, reduction of gastric ulcers (anti-inflammatory), antigenotoxic effects, anti-cancers, etc. As novel modification methods have been developed in recent years, more and more studies focus on the biological activities of chitosan derivatives, especially those with low molecular weight. For instance, the chitosan oligosaccharides prepared from enzymatic degradation/depolymerization exhibited many new biological functions, such as immune-modulating and hemostasis effects [59].

ISSN: 2332-4104

Chitosan and its Derivatives for Food Applications

Antimicrobial applications

Chitosan is widely recognized for its potent antimicrobial activity with, broad spectrum, and high killing rate but low toxicity toward mammalian cells. Although the mode of antimicrobial action of chitosan is not completely understood, it is well established that the molecular structure of chitosan is prerequisite for its antimicrobial activity [60,61]. The polycationic characteristic of chitosan in acidic medium is the main factor contributing to the antimicrobial activity. Due to the positive surface charges at acidic condition, chitosan interacts with anionic components on bacteria surface, such as negatively charged lipopolysaccharide in outer membrane of Gram-negative bacteria and peptidoglycan and teichoic acid in cell wall of Gram-positive bacteria. This electrostatic interaction causes release of major proportion of proteinaceous materials from the cells. In a recent study, chitosan was suggested to possess profound effect on the negative charges of cryotococcal cellular membrane of fungus, and consequently interfering with surface colonization and cell-cell interactions during biofilm formation [62]. As revealed in Figure 2, chitosan significantly reduced capsule size and sheared capsular polysaccharide of crytococcal cells, as well as clipped exopolymeric matrix from biofilms, which in combination explained the underlying mechanisms of chitosan on preventing the formation of fungal biofilms. This potent effect was ascribed to the electrostatic interactions between chitosan molecules and microbial cell membranes, leading to the leakage of proteinaceous and consequently increased chitosan penetration to nucleus and binding to DNA, which in turn inhibited mRNA synthesis.

Many factors can affect its antimicrobial efficacy. The intrinsic factors include DD, MW, chemical modifications, and the extrinsic factors consist of environmental conditions, especially the pH and ionic strength of the medium and bacterial species. Because different DD results in different number of amino groups on the chitosan backbones, which determines the charge density and thus the electrostatic interactions with microbial cell membranes; while the MW reflects the compactness of the chitosan molecular structure, which may affect the flexibility of the functional groups that can react with microbial cells. The chitosan modifications that increase amino groups or decrease MW are known to change the molecular structure and hence increase its antimicrobial activity. For instance, the asparagines *N*-conjugated chitosan oligosaccharide that has two positively charged sites and small MW exhibit stronger interaction with carboxyl-negative charges on the bacteria cell wall [63] not only due to the greater number of amino groups but also more flexibility of the chitosan structure after MW decreased. Another possible mode is the hydrophobic interactions and metal chelating effects, particularly in the case of water soluble chitosan [64] or when the pH of the medium is above pKa of chitosan [60].

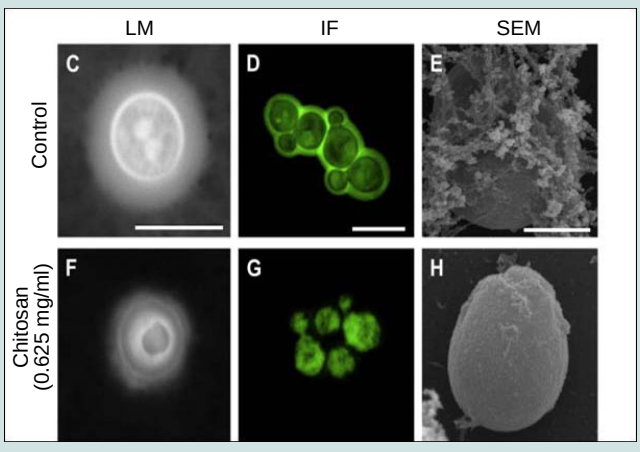

Figure 2: The effects of chitosan on capsule size (E and F) and capsular polysaccharide (D and G) of *Cryptococcus neoformans* cells, and exopolymeric matrix of biofilms (E and H). Scale bars in all pictures represent 2 μm. Reprinted with permission from Ref [62], copyright (2010), Elsevier.

Due to the biodegradability, nontoxicity and its intrinsic antibacterial effect, chitosan has been widely used as an antimicrobial agent in food science area to improve food quality and extend shelf life. To exert its antimicrobial effects, chitosan and its derivatives can be used alone or blended with other ingredients in food industry. For instance, chitosan and its combination with biocontrol yeast and/or calcium chloride has been applied to control blue mold in pear fruit [65]. The synergistic effects were found among chitosan, biocontrol yeast and calcium chloride that the combination demonstrated a more effective and stable reduction in the fungal decay compared with the treatment with either chitosan or with biocontrol yeast alone. In addition to bacterial and antifungal activity, chitosan has been recently tested for its efficacy toward foodborne viruses, including human norovirus and enteric virus surrogates [66]. In some cases, modification of chitosan is needed to further improve its antimicrobial efficacy in certain food systems. For example, to better protect mushroom from microbial degradation and improve the postharvest quality, chitosan-glucose complex coating was found to be the most effective treatment, compared with chitosan or glucose coating treatment alone [67]. The complex coating not only maintained tissue firmness of mushroom, inhibited increase of respiration rate, microorganism counts (pseudomonads, yeast, and moulds tested), but also delayed changes of ascorbic acid and maintained overall sensory quality. Water soluble chitosan has also been well characterized for their antimicrobial activities recently [68, 69]. A recent study has developed chitosan- and carboxymethyl chitosan-zinc complexes to compare their antimicrobial activity [70]. The study revealed that carboxymethyl chitosan-zinc complex exhibited much better antimicrobial activity against both Gram-positive and Gram-negative bacteria than chitosan-zinc complex. It was deduced that the carboxymethyl groups greatly improved water solubility of chitosan and thus higher concentration was available at the site action due to enhanced diffusivity of complex.

Edible film/coating and food packaging applications

Since the last decade, increasing interest in food industry has been drawn to development of novel active films/coatings as food packaging materials, which not only have natural origins for food applications, but also can enhance food safety and quality during storage. Among the biomaterial candidates, chitosan is one of the most promising biopolymers, thanks to the combination of its excellent film-forming property and antimicrobial activity.

The biopolymer-based edible films for food packaging are generally characterized by several parameters, including mechanical properties (elongation, tensile strength and breaking force), thickness, water vapor and oxygen permeability, as well as moisture content and color evaluations. Chitosan film is usually prepared by

ISSN: 2332-4104

casting chitosan solution on a certain plate. Since chitosan is only soluble in acidic conditions, the type of organic acid used is crucial to the mechanical properties of chitosan films. Although acetic acid has been considered as a common solvent for chitosan, it was reported to have adverse effects on chitosan films, compared with many other organic acids, such as malic, lactic and citric acid [71]. Acetic acid resulted in the toughest films and lowest elongation rate, making it too brittle for its coating applications. Therefore, the plasticizer is necessary to prepare adequate chitosan film. A recent study showed that both plasticizer concentration and drying methods significantly affected the mechanical properties of chitosan film [72], due to the changes of glass transition temperature of chitosan under different conditions.

However, due to the hydrophilic nature of chitosan, the films prepared from chitosan alone are unable to exhibit adequate water vapor/oxygen barrier properties and antimicrobial activities during long term storage. The new trend to develop chitosan films or coatings is the preparation of active films/coatings by combination of natural antimicrobials or incorporation of various bioactive compounds. The chitosan-based active films/coatings have been applied in various food products, especially fruits, fresh produce and meat. For instance, incorporation of green tea extract into chitosan active film has been reported to improve both mechanical and water vapor barrier properties, mainly due to the enhanced intermolecular interactions between phenolic compounds and chitosan molecules [73]. This green tea extract incorporated chitosan film has been further applied as active packaging for shelf life extension of pork sausages by improving antioxidant, minimizing color change, and reducing microbial growth during storage at 4°C [74]. Our group developed edible coatings by incorporation of sodium chloride into both chitosan and carboxymethyl chitosan coatings and investigated their synergistic effects on the quality of fresh-cut d'Anjou pears [75]. Our findings revealed that the combination of sodium chloride and chitosan adversely affected the quality of pear slices by accelerating discoloration of cut surfaces and increasing polyphenol oxidase activity, while the combination of sodium chloride and carboxymethyl chitosan showed desirable effects. In addition of incorporation of bioactive small molecules into chitosan active films, an emerging trend is to develop composite films using chitosan and other natural polymers, including both proteins and polysaccharides [76]. The objective of using composite films as food packaging material is to combine the advantages from different polymers, including mechanical property, water vapor permeability, solubility, thermal property, appearance, antimicrobial activity, etc. Chitosan-gelatin composite film has been recently studied [77]. It was shown that by incorporation of gelatin in chitosan film, it was able to produce more flexible films with lower tensile strength and higher elongation rate. The more gelatin ratio was included in the film, the more translucent film was obtained due to the reduced light transmission. The translucent appearance of the film will greatly expand chitosan-based active film applications as edible coating.

Chitosan-based emulsions for food quality preservation

Among food grade hydrocolloids, chitosan is considered as an excellent emulsifier that can be used to stabilize oil in water (O/W) emulsion without addition of any other surfactant. Because in acidic conditions, the amino groups are protonated and chitosan carries positive surface charge, which make chitosan become an amphiphilic substance that can adsorb at oil/water interfaces and facilitate formation of emulsion by lowering interfacial tension. The emulsifying capability of chitosan is highly dependent on its MW and DD [78,79]. The low MW chitosan was reported to exhibit better emulsifying properties than high MW chitosan, while chitosan with low (60%) or high DD (86%) showed better emulsifying properties to produce unimodal oil droplet size, compared to chitosan with intermediate DD (65-77%). The emulsion stabilization effects of chitosan is also concentration-dependent, especially for the chitosan with intermediate DD [80] The hydrophile-lipophile balance for chitosan was reported as 36.7, suggesting the high hydrophilic property of chitosan [81]. Although the surface activity of chitosan is limited, using higher concentration of chitosan result in higher viscosity of emulsion, and consequently provide stabilization of oil droplet by forming a denser and thicker polyelectrolitic brush at the water side of interface [82].

Chitosan-based emulsions have been widely reported as a novel approach to improve food quality, in the form of edible coating or film. Mineral oil-chitosan emulsion has been studied recently as a coating material on chicken eggs to maintain quality and prolong shelf-life during storage. The coating minimized weight loss and significantly preserved albumen and yolk quality of eggs for at least 3 weeks longer than those without coating stored at room temperature, and this effect was more pronounced at refrigerated temperature [83]. The sensory discrimination indicated that mineral oil-chitosan emulsion coating on eggs did not affect the overall appearance and the purchase intent was the same as uncoated eggs [84]. Soybean oil-chitosan emulsion coating on eggs has also been recently shown to have similar beneficial effects [85]. Lemon essential oil-chitosan emulsion coatings has been studied on the preservation of strawberry quality [86]. The coating maintained high quality of strawberry during cold storage at 5°C by slowing down the respiration rate and enhancing anti-fungal activity. Various essential oils-chitosan emulsions have been characterized and developed so far, and the type of essential oils greatly affects the emulsion film forming property and antimicrobial activities [87,88]. Besides essential oils, some other lipids-chitosan emulsions have also been studied for their applications in food industry. For instance, a novel application of oleic acid-chitosan emulsion in osmotic dehydration during food processing has been studied by Garcia et al. [89]. They evaluated the effects of chitosan emulsion coatings in the osmotic dehydration of scalded-cut papaya in two different ripening stages, i.e. green and ripped. Immersion of papaya cubic cuts in the oleic acid-chitosan emulsion significantly improved the efficiency of osmotic dehydration process for papaya in both ripening stages, by increasing the water loss and decreasing the solids gain, compared with uncoated samples. Additionally, to further improve quality maintaining capability, chitosan nanoemulsion has been prepared by ultrasonication, and the nanosized chitosan emulsion exhibited better efficiency as a biofungicide for controlling anthracnose of tropical fresh fruits, compared with conventional chitosan emulsion [90].

Encapsulation and Nutrient Delivery Applications

Encapsulation technology for food science

Encapsulation is defined as a process to entrap one substance within a certain matrix. This technology has been widely applied in pharmaceutics and biomedicine, with aims to protect drugs from gastric condition and hence improve absorption in intestine as well as provide targeted delivery of encapsulated drugs after it enters blood circulation. The biodegradable polymeric nanoparticles are of

ISSN: 2332-4104

particular interest in this area [91]. As more and more biodegradable, natural polymers have been studied for encapsulation and delivery of drugs, food scientists are beginning to explore this emerging technology in food industry, where encapsulation can be applied for a variety of reasons [92]. First, encapsulation can be used to protect food labile bioactives from harsh processing conditions, e.g. heat, oxygen, high pressure, etc. Second, some nutraceuticals are susceptible to low pH condition and form isomers or oligomers in stomach, thus encapsulation can provide a matrix to protect these compounds. Third, the fat-soluble bioactives, such as lipophilic vitamins and flavonoids, have limited applications in food industry due to low solubility. By encapsulating them into a hydrophilic polymeric matrix, they become soluble in water and their applications are greatly expanded. Forth, encapsulation can also be adopted to mask undesirable flavors or smells of certain active compounds and hence improve the overall acceptance as food products. Fifth, like many drugs, the food derived bioactive compounds also need targeted delivery and prolonged blood circulation time to exert their functions. Sixth, encapsulation of probiotic living cells in microcapsules is currently receiving considerable interest in food industry, in order to provide proper protection of cells from adverse environmental conditions.

Chitosan-based delivery systems for food applications

Among various food biopolymers investigated in encapsulation and delivery systems, chitosan is one of the most popular polymers, and it is considered as a versatile polymer in drug delivery [93]. In food industry, chitosan has also been extensively investigated for its encapsulation and delivery potentials for various nutrients. The cationic characteristic of chitosan in acidic conditions provides simplicity to fabricate encapsulation and delivery systems in different forms, including nano/micro-particles, hydrogel beads, emulsions, fibers, films and membranes. Chitosan-based polymeric delivery systems for biomedical applications can be fabricated by a variety of approaches [93]. The commonly adopted fabrication methods and their applications in food science are briefly introduced in the following sections. The recent advances of chitosan-based particulate systems for encapsulation of nutrients are summarized in Table 2. In these approaches, electrostatic interactions between chitosan and anionic molecules are the main driving forces, and hydrogen bonding and hydrophobic interactions are also involved in some cases to assemble chitosan delivery systems.

Nano/micro-particles

Chitosan nano/micro-particles can be easily prepared using sodium TriPolyPhosphate (TPP) which carries five negative charges per molecule. In this method, TPP solution is added to chitosan solution in a dropwise manner, and the particles are spontaneously formed when chitosan and TPP are mixed together, and then

Table 2: Chitosan-based particulate systems for encapsulation of nutrients (2008 – 2013)*.

Chitosan and derivatives	Preparation methods	Particulate systems	Nutrients encapsulated	Beneficial effects	Ref.
Chitosan	Emulsification, ionic gelation with TPP	Nanoparticles, 280 – 400 nm	Essential oil	Controlled release	[94]
Chitosan	Ionic gelation with peptides	Nanoparticles, 150 nm	EGCG	Enhanced bioavailability, lower toxicity	[95,96]
Chitosan	Ionic gelation with TPP	Nanoparticles, 125– 300 nm	Selenium	Controlled release, enhanced bioactivity, improved bioavailability	[97,98]
Chitosan	Ionic gelation with TPP	Nanoparticles, 180– 580 nm	Vitamin C	Prolonged shelf life, enhanced *in vivo* delivery	[99,100]
Chitosan	Coating on zein nanoparticles	Nanoparticles, 200 – 800 nm	Vitamin E	Controlled release in GI track	[101]
Carboxymethyl chitosan	Coating on zein nanoparticles and ionic gelation with calcium	Nanoparticles, 80 – 250 nm	Indole, Vitamin D3	Controlled release, improved stability	[102,103]
Carboxymethyl chitosan	Ionic gelation with calcium and soy protein	Nanoparticles, 160 – 240 nm	Vitamin D3	Controlled release	[104]
Linoleic acid-modified chitosan	Ionic gelation with β-lactoglobulin	Nanoparticles, 170 – 350 nm	Quercetin	–	[105]
Chitosan	Spray drying with tween	Nanoparticles, 320 – 360 nm	Curcumin	Solubilization, controlled release	[106]
Chitosan	Emulsification, ultrasonication, and freeze drying	Microparticles, 0.8– 1.4 *μm*	Fish oil	–	[107]
Chitosan	Emulsification, lay-by-lay deposition with caseinate and pectin	Microparticles, 0.2 – 2 μm	Corn oil	Controlled release, delayed digestion in GI track	[108]
Chitosan	Spray drying	Microparticles, 3 μm	Betalains	Improved stability, retained bioactivity	[109]
Chitosan hydrochloride	Ionic gelation with TPP and spray drying	Microparticles, 5 μm	Antioxidant extract	Controlled release	[110]
Chitosan	Ionic gelation with alginate and calcium	Hydrogel beads, 10 – > 100 μm	Lipid	Controlled digestion in GI track	[111]
Chitosan	Emulsification and crosslinking with vanillin	Microparticles, 53– 311 μm	Resveratrol	Controlled release, improved stability	[112]
Chitosan	Ionic gelation with alginate and calcium	Hydrogel beads, 780 μm – 1 mm	Plant extracts	Controlled release	[113]
Carboxymethyl chitosan	Ionic gelation with calcium in alcohol solution	Hydrogel beads 1 mm	Vitamin D3	High loading capacity, controlled release	[114]

*__Abbreviations__: TPP: Sodium TriPolyPhosphate; EGCG: (-)-EpiGalloCatechin-3-Gallate

ISSN: 2332-4104

collected by centrifugation or drying methods (freeze-drying or spray drying). As shown in (Figure 3), pure chitosan exhibited rough membrane-like morphology; however, upon dropwise addition of TPP to chitosan solution spherical nanoparticles were formed spontaneously. Particle sizes can range from 100 nm to several μm, by controlling the concentration of chitosan and TPP, their mass ratios, and drying methods. Due to the simplicity of this method, chitosan/TPP particles have been widely adopted to encapsulate both hydrophilic and hydrophobic nutraceuticals. To encapsulate, the bioactive compound (dissolved in water or ethanol) is either added to chitosan or TPP solution before they are mixed together. Several chitosan derivatives are also able to form nano/micro-particles with oppositely charged molecules. For instance, glycol chitosan [110] and chitosan hydrochloride [115] are positively charged chitosan derivatives, and both can form nano/micro-particles with TPP, while the negative charged derivatives, e.g. carboxymethyl chitosan [116], can form nano/micro-particles with calcium.

Alishahi and coworkers investigated the encapsulation of vitamin C into chitosan/TPP nanoparticles to extend its shelf life and achieve controlled release [117]. The particle size and encapsulation efficiency were greatly affected by MW of chitosan used. The nanoparticles prepared with lower MW chitosan had more uniform and smaller particle size, while higher encapsulation efficiency was achieved by using higher MW chitosan. The shelf life of vitamin C was greatly improved, compared with unencapsulated samples. The controlled release profile of vitamin C was found as pH responsive, quicker release in pH 7.4 but slower release in acidic medium, however, the effect of digestive enzyme was not tested. Encapsulation of sodium selenite, a nutraceutical supplement of trace element selenium, in chitosan/TPP nanoparticles has been studied by Luo et al. [98]. The particle size, zeta potential and encapsulation efficiency were greatly influenced by the concentration of chitosan and TPP, as well as the loading concentration of selenite. The encapsulation of selenite in chitosan nanoparticles enhanced its antioxidant properties, which was attributed to the antioxidant activities of chitosan. Although a fast release of selenite was observed in both PBS and simulated gastrointestinal fluids containing digestive enzymes, the release profile was significantly improved after chitosan/TPP nanoparticles were coated with zein, a water insoluble protein. The selenite-encapsulated chitosan nanoparticles were also demonstrated by cellular evaluation to not only improve selenium cellular uptake but also protect cells from selenium-induced DNA damage response [97]. Encapsulation of nutraceuticals in zein nanoparticles coated with carboxymethyl chitosan/calcium has been recently reported by our group [102,103]. Carboxymethyl chitosan/calcium coating on zein nanoparticles not only increased encapsulation efficiency, retarded controlled release, but also improved thermal and photo-stabilities of the encapsulated compounds, i.e. vitamin D3, indole-3-carbinal, and diindolylmethane.

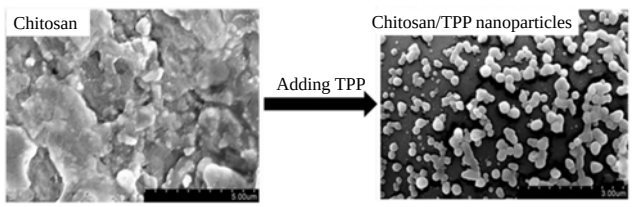

Figure 3: Scanning Electron Microscope (SEM) photographs of chitosan and chitosan/TPP nanoparticles. Adapted with permission from Ref [98], copyright (2010), Elsevier.

Chitosan nanoparticles can also be prepared by crosslinking with oppositely charged biopolymers. The nanoparticles prepared by this method are usually considered as polyelectrolyte complex. This trend has received increasing attention in recent years, since more and more research provides evidence that nanoparticles prepared with two or more polymers are able to protect encapsulated compounds better against environmental conditions [118]. A lot of biopolymers have been reported to form polyelectrolyte complex nanoparticles with chitosan, including alginate, hyaluronic acid, carrageenan, gum Arabic, carboxymethyl cellulose, etc. On the other hand, complex nanoparticles can be prepared by crosslinking negatively charged chitosan derivatives and biopolymer together with cationic ions, such as calcium. Our group developed carboxymethyl chitosan-soy protein complex nanoparticles for nutrient delivery [104]. In this method, carboxymethyl chitosan and soy protein complex was crosslinked by calcium. The complex nanoparticles exhibited remarkable encapsulation efficiency and improved release profile of vitamin D3 in simulated gastrointestinal conditions, compared with nanoparticles prepared with single ingredients. Another promising development is to prepare nanoparticles using chitosan and negatively charged polypeptides, which are nontoxic and edible. Chitosan-poly(r-glutamic acid) nanoparticles have been studied for encapsulation of tea catechins [119]. Both negatively charged or positively charged nanoparticles can be prepared depending on the ratio of chitosan to polypeptide. The antioxidant activity of tea catechins were greatly retained in simulated gastric conditions, due to the encapsulation in nanoparticles. It was shown that the positively charged nanoparticles could transiently open the tight junction between Caco-2 cells and thus increase the paracellular transport of tea catechins. Chitosan-caseinophosphopeptide nanoparticles were also developed to improve bioavailability of epigallocatechin gallate [95,96].

Chitosan microparticles are prepared with similar procedures as nanoparticles, but dried through spray drying method. The typical particle size ranges from 2 to 20 μm, depending on the crosslinker type and chitosan concentration. Unlike chitosan nanoparticles, microparticles are widely studied in pharmaceutical industries to encapsulate drugs, but not nutrient for food applications [120]. Vitamin C encapsulation in spray dried chitosan/TPP microparticles has been systematically studied by Park group [121-123], including the chitosan concentration, chitosan/TPP mass ratio, chitosan molecular weight, as well as spray drying parameters, all of which affected vitamin C encapsulation efficiency and release profile. A recent study investigated the encapsulation of orange oil in chitosan emulsion microparticles by spray drying [124]. The prepared chitosan microparticles were aimed to help orange oil retain in fabrics after washing in detergent solution. By choosing the proper formulation, small microparticles with uniform particle size were successfully prepared to effectively deposit orange oil in cotton fabrics, and the encapsulated orange oil was very stable during storage due to the slow release. This may find new applications of chitosan microparticles for textile applications.

Hydrogel beads

Hydrogels are defined as the hydrophilic polymer networks that can absorb a significant amount of water, from 10% to thousands

ISSN: 2332-4104

of times of their dry weight [125], because they swell but do not dissolve in water. Hydrogels are formed when a three-dimensionally polymeric network is crosslinked loosely, either chemically or physically. Among various hydrogels, hydrogel bead is one of the most investigated forms, due to its simplicity of preparation. Hydrogel beads are usually formed spontaneously by dropping polymer solution in high concentration into a crosslinking solution containing oppositely charged ions or polyelectrolyte polymers, and no further cutting or shaping procedure is needed.

Among various polymers, chitosan and its derivations are the most studied polyelectrolytes, due to its abundance, non-toxicity, and biodegradability. To prepare hydrogel beads, concentrated chitosan solution (as high as 3%) is added into TPP solution through syringe needle to ensure the small particle size. Chitosan/TPP hydrogel beads have been proven to possess pH-sensitive swelling behavior, and the encapsulation efficiency and release profile are dependent on the preparation parameters [126]. Chitosan hydrogel beads can also be prepared by crosslinking chitosan with negative charged polymers, such as alginate [127] and pectin [128]. Chitosan-based hydrogel beads not only have a variety of applications in biomedicine and pharmaceutics [129], but also hold several promising potentials in food science [130,131].

Encapsulation and delivery of nutrients

Chitosan-based hydrogel beads are believed to be a useful tool to encapsulate and deliver nutrients. Due to the large size of hydrogel beads, several advantages are considered over nano/micro-particles delivery systems, such as higher loading capacity and encapsulation efficiency, better controlled release, simpler and easier preparation and collection procedures. Chitosan-alginate beads prepared with ionic gelation were tested for encapsulation of polyphenolic antioxidants from different plant extracts, including raspberry leaf, hawthorn, ground ivy, yarrow, nettle and olive leaf [113]. It was found that the encapsulation efficiency and kinetic release profile were greatly affected by constituents of plant extracts. However, the hydrogel beads did not provide proper protection against rapid degradation and loss of antioxidative stability of encapsulated nutrients, which was possibly due to the hydrogel beads in the study were not dried during storage so that the excess water caused deterioration of bioactivities. Chitosan/alginate hydrogel beads were also studied for controlling lipid digestion [11]. It was shown that the hydrogel beads with larger diameter (>100 μm) were more effective than smaller hydrogel beads in delaying lipid digestion tested by an *in vitro* digestion model with lipase.

Carboxymethyl chitosan, a water soluble chitosan derivative with negative charge, is reported as unable to form hydrogel beads on its own due to the chain rigidity, while alginate is normally used as an adjuvant polymer to form hydrogel beads with carboxymethyl chitosan/calcium [132]. Recently, a novel method to prepare carboxymethyl chitosan hydrogel beads has been successfully developed in our group [134]. In this method, calcium dissolved in aqueous-alcohol was used a cross-linking solution to prepare carboxymethyl chitosan hydrogel beads. As shown in Figure 4, the formation of hydrogel beads was dependent on the concentrations of alcohol-aqueous solvent, in which calcium chloride was dissolved to physically crosslink carboxymethyl chitosan. It was found that 30% alcohol concentration was the optimal solvent to form hydrogel beads with the most spherical shape, and that drying methods had

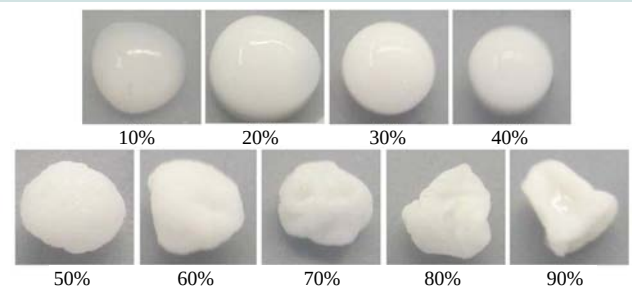

Figure 4: Digital photos of carboxymethyl chitosan hydrogel beads prepared in different alcohol-aqueous binary solvents. The percentage below each photo represents the alcohol concentration for preparation of each bead. Reprinted with permission from Ref [114]. Copy right 2013, Elsevier.

significant impact on surface morphology and swelling behavior. Since the beads were prepared in aqueous-alcohol solvent, they were the ideal encapsulation and delivery system for hydrophobic nutrients, such as vitamin D3. The encapsulation efficiency was as high as 97%, which was much higher than other delivery systems for hydrophobic nutrients.

Encapsulation and delivery of probiotics

Probiotics are defined as a group of bacteria that can confer health benefits to the host when they are administered at adequate amounts. The benefits of probiotics to human host include production of nutrients and cofactors, competition with pathogens and hence inhibition of their growth, maintaining beneficial gut microflora, stimulation of immune response, as well as treatment of bowel-associated diseases [133]. These microorganisms are used in production of functional foods and pharmaceutical products. However, it is important to protect their viability from harsh conditions and preserve their health benefits. Plus, probiotics are extremely susceptible to gastric environment where the greatest viability will be lost due to high levels of acid. Therefore, immobilization of probiotics in a polymer matrix could protect them from gastric condition and then release them in small intestine to confer their healthy benefits. Encapsulation of probiotics has received increasing attention in recent years, as an emerging technology that can effectively enhance their viability [134].

Chitosan-based hydrogel beads find many applications in this area; particularly, chitosan is often used as a coating material on alginate beads to enhance protective effects of probiotics in harsh conditions. For instance, chitosan coated alginate hydrogel beads were reported to be much more effective than alginate/calcium beads in protecting viability of probiotics in simulated gastric solution, as well as prolonging controlled release of viable probiotics in simulated intestinal solution [135]. A similar system has been further tested in pomegranate juice to explore the protective effects of chitosan/alginate beads, showing that chitosan coating significantly increased the cell viability by 5.5 log CFU/ml, compared with uncoated alginate beads [136]. Besides chitosan, carboxymethyl chitosan was also proved as an effective coating on alginate beads to help increase the survival rate in gastric and bile conditions [137]. It is worth mentioning that before coating chitosan on alginate beads it is crucial to adjust pH of chitosan solution to 6, since the original pH of chitosan solution is lower than 5 which is harmful for probiotics. It is recently reported that another benefit to coat alginate beads with

ISSN: 2332-4104

chitosan is to enhance mucoadhesive properties [138]. Although alginate itself is a mucoadhesive polymer, the crosslinking with calcium ions to produce hydrogel beads significantly reduced its mucoadhesive property. By coating alginate beads with chitosan or thiolated chitosan, the mucoadhesion performance was substantially enhanced, and thus the coated beads were able to deliver markedly higher amount of probiotics to the *in vitro* model of colonic mucosa than that of uncoated alginate beads.

Other Applications of Chitosan in Food Science

Immobilization of enzymes on chitosan-based biomaterials is an important application of chitosan in food science. By immobilizing onto chitosan supports, enzymes are more robust and more resistant to environmental changes; especially, the heterogeneity of the immobilized enzyme systems allows easier recovery, multiple reuse, and more rapid termination of reactions, and many other benefits [139]. Chitosan-based biomaterials in different geometrical configurations have all been studied for enzyme immobilization applications, including powders, flakes, beads, films, and membranes. Enzyme-immobilized chitosan biomaterials have been developed into various biosensors for their novel applications in food industry, such as glucose biosensor [140], choline biosensor [141], food-borne pathogens biosensor [142], and polyphenol detection kit [143].

Another application of chitosan is the purification of waste water in food industry. Recently, purification of waste water has attracted great scientific and industrial interest, because water pollution is one of the largest environmental problems affecting quality of life adversely. The purification process involves many steps, such as removal of heavy metal ions, dyes, phenol compounds, sludge conditioning, and so on. The use of polyelectrolyte polymer in production of drinking water and treatment of waste water is the commonly adopted method in water industry [144]. Due to the abundant amino groups, chitosan is a very effective adsorbent for removing water impurities and is mostly used in the form of hydrogel beads and microspheres. Chitosan has been widely investigated in the process of phenol compounds bioconversion [145], dyes removal [146], Cu^{2+} and Zn^{2+} removal [147]. In order to achieve more efficient process, various modifications have been applied to chitosan, such as functionalized chitosan membrane with carbon nanotubes [148] and chitosan beads impregnated by ion imprinting for metal removal [149], water soluble chitosan for clay flocculation [150], and conjugated chitosan for phenols removal [151]. In addition to waste water, chitosan is also currently being studied to improve the quality of drinking water, such as removal of trace element [152], defluoridation [153], and microtoxin removal [154].

Future Directions: Chitosan-based Nanotechnology for Food Applications

Nowadays, there is no doubt that chitosan-based nanotechnologies are becoming more and more popular and important. The current research efforts are mainly focused in biomedical fields, but very limited in food applications. Although chitosan has been considered as a GRAS material for its use as food additives by FDA, the use of chitosan at nanoscale in food/medical products has not been approved yet. Especially, chitosan composite nanomaterials have been developed for their potential applications in food industries, for instance, chitosan-TiO_2 nanotubes films [155] for food packaging and chitosan-silver nanoparticles composite for drinking water filtration applications [156]. But the safety of these materials when they have direct contact with food products is still unknown. In addition to chitosan, a lot of chitosan derivatives are also being shown in literature to have numerous benefits and sometimes more advantages than native chitosan in food applications. However, compared with chitosan, little information on toxicity and safety of chitosan derivatives is available, and so far none of chitosan derivatives have been approved to be used in food products as a GRAS material, due to the difficulties to remove toxic solvent residues from modification process. Therefore, in addition to develop chitosan-based nanomaterials, there is an urgent need to evaluate their toxicity and safety aspect in real food products, in order to facilitate their approval for applications in food industry.

Conclusions

Being a deacetylated product of chitin, chitosan is a versatile food biopolymer that finds a variety of applications in all areas of food science. As a functional biopolymer, chitosan has many intrinsic nutritional values, such as antioxidant properties, health-promoting bioactivities against many chronic diseases, including hypercholesterolemia, hypertension, inflammation, immune diseases, etc. Chitosan possesses promising antimicrobial activities with broad spectrum, and hence it has been widely studied as a food preservative to improve food quality and extend shelf life of perishable food products. Chitosan also has excellent emulsifying properties and is used to stabilize various oil-in-water emulsions in food industry to avoid the use of synthetic surfactants. All of these intrinsic properties vary with MW and DD, both of which are the most important characteristics of chitosan. Due to the abundant amino groups, chitosan carries many positive charges in acidic medium, and becomes a popular biopolymer to develop encapsulation and delivery systems for food industry, such as nano/micro-particles, hydrogel beads, as well as nanocomposites. With proper modification of chitosan, its functional properties and biological activities can be further enhanced and more applications are being developed.

References

1. Ravi Kumar MN (2000) A review of chitin and chitosan applications. React Funct Polym 46: 1-27.
2. Mourya V, Inamdar NN (2008) Chitosan - modifications and applications opportunities galore. React Funct Polym 68: 1013-1051.
3. Yen MT, Yang JH, Mau JL (2008) Antioxidant properties of chitosan from crab shells. Carbohydr Polym 74: 840-844.
4. Ai H, Wang F, Xia Y, Chen X, Lei C (2012) Antioxidant, antifungal and antiviral activities of chitosan from the larvae of housefly *Musca domestica* L. Food Chem 132: 493-498.
5. Ai H, Wang F, Yang Q, Zhu F, Lei C (2008) Preparation and biological activities of chitosan from the larvae of housefly,*Musca domestica*. Carbohydr Polym 72: 419-423.
6. Subhapradha N, Ramasamy P, Sudharsan S, Seedevi P, Moovendhan M, et al. (1830) Preparation of phosphorylated chitosan from gladius of the squid Sepioteuthis lessoniana (Lesson) and its *in vitro* antioxidant activity. Bioact Carbohydr Diet Fibre.
7. Anraku M, Fujii T, Kondo Y, Kojima E, Hata T, et al. (2011) Antioxidant properties of high molecular weight dietary chitosan *in vitro* and in vivo. Carbohydr Polym 83: 501-505.
8. Anraku M, Fujii T, Furutani N, Kadowaki D, Maruyama T, et al.(2009) Antioxidant effects of a dietary supplement: Reduction of indices of oxidative

ISSN: 2332-4104

stress in normal subjects by water-soluble chitosan. Food Chem Toxicol 47: 104-109.

9. Sun T, Zhu Y, Xie J, Yin X (2011) Antioxidant activity of N-acyl chitosan oligosaccharide with same substituting degree. Bioorg Med Chem Lett 21: 798-800.

10. Zhang X, Geng X, Jiang H, Li J, Huang J (2012) Synthesis and characteristics of chitin and chitosan with the (2-hydroxy-3-trimethylammonium)propyl functionality, and evaluation of their antioxidant activity *in vitro*. Carbohydr Polym 89: 486-491.

11. Ren J, Li Q, Dong F, Feng Y, Guo Z (2013) Phenolic antioxidants-functionalized quaternized chitosan: Synthesis and antioxidant properties. Int J Biol Macromol 53:77-81.

12. Zhong Z, Zhong Z, Xing R, Li P, Mo G (2010) The preparation and antioxidant activity of 2-[phenylhydrazine (or hydrazine)-thiosemicarbazone]-chitosan. Int J Biol Macromol 47: 93-97.

13. Schreiber SB, Bozell JJ, Hayes DG, Zivanovic S(2013) Introduction of primary antioxidant activity to chitosan for application as a multifunctional food packaging material. Food Hydrocolloid 33: 207-214.

14. Pasanphan W, Buettner GR, Chirachanchai S (2010) Chitosan gallate as a novel potential polysaccharide antioxidant: an EPR study. Carbohydr Polym 345: 132-140.

15. Woranuch S, Yoksan R (2013) Preparation, characterization and antioxidant property of water-soluble ferulic acid grafted chitosan. Carbohydr Polym 2: 495-502

16. Aytekin AO, Morimura S, Kida K (2011) Synthesis of chitosan–caffeic acid derivatives and evaluation of their antioxidant activities. J Biosci Bioeng 111: 212-216.

17. Rao MS, Chawla SP, Chander R, Sharma A (2011) Antioxidant potential of Maillard reaction products formed by irradiation of chitosan–glucose solution. Carbohydr Polym 83: 714-719.

18. Ying GQ, Xiong WY, Wang H, Sun Y, Liu HZ (2011) Preparation, water solubility and antioxidant activity of branched-chain chitosan derivatives. Carbohydr Polym 83: 1787-1796.

19. Kosaraju SL, Weerakkody R, Augustin MA (2010) Chitosan−glucose conjugates: Influence of extent of Maillard reaction on antioxidant properties. J Agric Food Chem 58: 12449-12455.

20. Li X, Shi X, Jin Y, Ding F, Du Y(2013) Controllable antioxidative xylan–chitosan Maillard reaction products used for lipid food storage. Carbohydr Polym 91: 428-433.

21. Anandan R, Ganesan B, Obulesu T, Mathew S, Asha KK et al. (2013) Antiaging effect of dietary chitosan supplementation on glutathione-dependent antioxidant system in young and aged rats. Cell Stress Chaperones 18: 121-125.

22. Xia WS, Liu P, Zhang JL, Chen J (2011) Biological activities of chitosan and chitooligosaccharides. Food Hydrocolloid 25: 170-179.

23. Osman M, Fayed S, Ghada IM, Romeilah R (2010) Protective effects of chitosan, ascorbic acid and Gymnema sylvestre against hypercholesterolemia in male rats. Aust J Basic Appl Sci 4: 89-98.

24. Anraku M, Tomida H, Michihara A, Tsuchiya D, Iohara D et al. (2012) Antioxidant and renoprotective activity of chitosan in nephrectomized rats. Carbohydr Polym 89: 302-304.

25. Yen MT, Tseng YH, Li RC, Mau JL (2007) Antioxidant properties of fungal chitosan from shiitake stipes. LWT-Food Sci Technol 40: 255-261.

26. Xing R, Liu S, Guo ZY, Yu H, Wang P, et al. (2005) Relevance of molecular weight of chitosan and its derivatives and their antioxidant activities *in vitro*. Bioorg Med Chem 13: 1573-1577.

27. Sun T, Zhou DX, Xie JL, Mao F (2007) Preparation of chitosan oligomers and their antioxidant activity. Eur Food Res Technol 225: 451-456.

28. Zhong Z, Ji X, Xing R, Liu S, Guo Z, et al. (2007) The preparation and antioxidant activity of the sulfanilamide derivatives of chitosan and chitosan sulfates. Bioorg Med Chem 15: 3775-3782.

29. Guo Z, Xing R, Liu S, Yu H, Wang P, et al. (2005) The synthesis and antioxidant activity of the Schiff bases of chitosan and carboxymethyl chitosan. Bioorg Med Chem Lett 15: 4600-4603.

30. Guo ZY, Liu HY, Chen XL, Ji X, Li P (2006) Hydroxyl radicals scavenging activity of N-substituted chitosan and quaternized chitosan. Bioorg Med Chem Lett 16: 6348-6350.

31. Xing R, Liu S, Guo Z, Yu H, Zhong Z, et al. (2008) Relevance of molecular weight of chitosan-N-2-hydroxypropyl trimethyl ammonium chloride and their antioxidant activities. Eur J Med Chem 43: 336-340.

32. Sun T, Yao Q, Zhou DX, Mao F (2008) Antioxidant activity of N-carboxymethyl chitosan oligosaccharides. Bioorg Med Chem Lett 18: 5774-5776.

33. Sun T, Zhu Y, Xie J, Yin XH (2011) Antioxidant activity of N-acyl chitosan oligosaccharide with same substituting degree. Bioorg Med Chem Lett 21: 798-800.

34. Chen F, Shi Z, Neoh KG, Kang ET (2009) Antioxidant and antibacterial activities of eugenol and carvacrol-grafted chitosan nanoparticles. Biotechnol Bioeng 104: 30-39.

35. Jung BO, Chung SJ, Lee SB (2006) Preparation and characterization of eugenol-grafted chitosan hydrogels and their antioxidant activities. J Appl Polym Sci 99: 3500-3506.

36. Cho YS, Kim SK, Je JY (2011) Chitosan gallate as potential antioxidant biomaterial. Bioorg Med Chem Lett 21: 3070-3073.

37. Sousa F, Guebitz GM, Kokol V (2009) Antimicrobial and antioxidant properties of chitosan enzymatically functionalized with flavonoids. Process Biochem 44: 749-756.

38. Fras-Zemljic L, Kokol V, Cakara D (2011) Antimicrobial and antioxidant properties of chitosan-based viscose fibres enzymatically functionalized with flavonoids. Text Res J 81: 1532-1540.

39. Aytekin AO, Morimura S, Kida K (2011) Synthesis of chitosan-caffeic acid derivatives and evaluation of their antioxidant activities. J Biosci Bioeng 111: 212-216.

40. Torres E, Marin V, Aburto J, Beltran HI, Shirai K, et al. (2012) Enzymatic modification of chitosan with quercetin and its application as antioxidant edible films. Appl Biochem Microbiol 48: 151-158.

41. Abd El-Rehim HA, El-Sawy NM, Hegazy el-SA, Soliman el-SA, Elbarbary AM (2012) Improvement of antioxidant activity of chitosan by chemical treatment and ionizing radiation. Int J Biol Macromol 50: 403-413.

42. Feng T, Du YM, Li J, Hu Y, Kennedy JF (2008) Enhancement of antioxidant activity of chitosan by irradiation. Carbohydr Polym 73: 126-132.

43. QiaoY, Bai XF, Du YG (2011) Chitosan oligosaccharides protect mice from LPS challenge by attenuation of inflammation and oxidative stress. Int Immunopharmacol 11: 121-127.

44. Yuan WP, Liu B, Liu CH, Wang XJ, Zhang MS, et al. (2009) Antioxidant activity of chito - oligosaccharides on pancreatic islet cells in streptozotocin-induced diabetes in rats. World JGastroenterol 15: 1339-1345.

45. Sugano M, Fujikawa T, Hiratsuji Y, Nakashima K, Fukuda N, et al. (1980) A novel use of chitosan as a hypocholesterolemic agent in rats. Am J Clin Nutr 33: 787-793.

46. Sugano M, Watanabe S, Kishi A, Izume M, Ohtakara A (1988) Hypocholesterolemic action of chitosans with eifferent viscosity in rats. Lipids 23: 187-191.

47. Maezaki Y, Tsuji K, NakagawaY, Kawai Y, AkimotoM, et al. (1993) Hypocholesterolemic effect of chitosan in adult males. Biosci Biotechnol Biochem 57: 1439-1444.

48. Zhou K, Xia W, Zhang C, Yu L (2006) *In vitro* binding of bile acids and triglycerides by selected chitosan preparations and their physico-chemical properties. LWT-Food Sci Technol 39: 1087-1092.

49. Wydro P, Krajewska B, Hac-Wydro K, et al. (2007) Chitosan as a lipid binder: a langmuir monolayer study of chitosan-lipid interactions. Biomacromolecules 8: 2611-2617.

50. Neyrinck AM, Bindels LB, De Backer F, Pachikian BD, Cani PD, et al. (2009) Dietary supplementation with chitosan derived from mushrooms changes adipocytokine profile in diet-induced obese mice, a phenomenon linked to its lipid-lowering action. Int Immunopharmacol 9: 767-773.

51. Zhang JL, Zhang W, Mamadouba B, Xia WS (2012) A comparative study on

ISSN: 2332-4104

hypolipidemic activities of high and low molecular weight chitosan in rats. Int J Biol Macromol 51: 504-508.

52. Zhang W, Zhang J, Jiang Q, Xia W (2013) The hypolipidemic activity of chitosan nanopowder prepared by ultrafine milling. Carbohydr Polym 95: 487-491.

53. Liu X, Zeng A, Song T, Li L, Yang F, et al. (2012) Hypocholesterolemic effects of N-[(2-Hydroxy-3-N, N-Dimethylhexadecyl Ammonium) propyl] chitosan chloride in high-fat-diet-induced rats. J Biomat Sci-Polym E 23: 1107-1114.

54. Liu X, Zhi X, Liu Y, Wu B, Sun Z, et al. (2012) Effect of chitosan, O-carboxymethyl chitosan, and N-[(2-Hydroxy-3-N, N-dimethylhexadecyl ammonium) propyl] chitosan chloride on overweight and insulin resistance in a murine diet-Induced obesity. J Agr Food Chem 60: 3471-3476.

55. Liu X, Yang F, Song T, Zeng A, Wang Q, et al. (2011) Effects of chitosan, O-carboxymethyl chitosan and N-[(2-hydroxy-3-N, N-dimethylhexadecyl ammonium) propyl] chitosan chloride on lipid metabolism enzymes and low-density-lipoprotein receptor in a murine diet-induced obesity. Carbohydr Polym 85: 334-340.

56. Singla AK, Chawla M (2001) Chitosan some pharmaceutical and biological aspects - an update. J Pharm Pharmacol 53: 1047-1067.

57. Xia W, Liu P, Zhang J, Chen J (2011) Biological activities of chitosan and chitooligosaccharides. Food Hydrocolloid 25: 170-179.

58. Kim SK, Rajapakse N (2005) Enzymatic production and biological activities of chitosan oligosaccharides (COS): A review. Carbohydr Polym 62: 357-368.

59. Zhang J, Xia W, Liu P, Cheng Q, Tahi T, et al. (2010) Chitosan modification and pharmaceutical/biomedical applications. Mar Drugs 8: 1962-1987.

60. Kong M, Chen XG, Xing K, Park HJ (2010) Antimicrobial properties of chitosan and mode of action: A state of the art review. Int J Food Microbiol 144: 51-63.

61. Rabea EI, Badawy ME, Stevens CV, Smagghe G, Steurbaut W (2003) Chitosan as antimicrobial agent: Applications and mode of action. Biomacromolecules 2003 4: 1457-1465.

62. Martinez LR, Mihu MR, Han G, Frases S, Cordero RJ, et al. (2010) The use of chitosan to damage Cryptococcus neoformans biofilms. Biomaterials 31: 669-679.

63. Kim SK (2001) Effect of antimicrobial activity by chitosan oligosaccharide N-conjugated with asparagine. J Microbiol Biotechnol 11: 281-286.

64. Chanthateyanonth R, Ruchirawat S, Srisitthiratkul C (2010) Preparation of new water - soluble chitosan containing hyperbranched vinylsulfonic acid sodium salt and their antimicrobial activities and chelation with metals. J Appl Polym Sci 116: 2074-2082.

65. Yu T, Yu C, Chen F, Sheng K, Zhou T, et al. (2012) Integrated control of blue mold in pear fruit by combined application of chitosan, a biocontrol yeast and calcium chloride. Postharvest Biol Technol 69: 49-53.

66. Davis R, Zivanovic S, D'Souza DH, Davidson PM et al. (2012) Effectiveness of chitosan on the inactivation of enteric viral surrogates. Food Microbiol 32: 57-62.

67. Jiang TJ, Feng LF, Li JR (2012) Changes in microbial and postharvest quality of shiitake mushroom (Lentinus edodes) treated with chitosan-glucose complex coating under cold storage. Food Chem 131: 780-786.

68. da Silva LP, de Britto D, Seleghim MHR, Assis OBG (2010) *In vitro* activity of water-soluble quaternary chitosan chloride salt against E-coli. World J Microbiol Biotechnol 26: 2089-2092.

69. Feng YW, Xia WS (2011) Preparation, characterization and antibacterial activity of water-soluble O-fumaryl-chitosan. Carbohydr Polym 83:1169-1173.

70. Patale RL, Patravale VB (2011) O,N-carboxymethyl chitosan-zinc complex: A novel chitosan complex with enhanced antimicrobial activity. Carbohydr Polym 85: 105-110.

71. Park S, Marsh K, Rhim J (2002) Characteristics of different molecular weight chitosan films affected by the type of organic solvents. J Food Sci 67: 194-197.

72. Thakhiew W, Devahastin S, Soponronnarit S (2010) Effects of drying methods and plasticizer concentration on some physical and mechanical properties of edible chitosan films. J Food Eng 99: 216-224.

73. Siripatrawan U, Harte BR (2010) Physical properties and antioxidant activity of an active film from chitosan incorporated with green tea extract. Food Hydrocolloid 24: 770-775.

74. Siripatrawan U, Noipha S (2012) Active film from chitosan incorporating green tea extract for shelf life extension of pork sausages. Food Hydrocolloid 27: 102-108.

75. Xiao Z, Luo Y, Luo Y, Wang Q (2011) Combined effects of sodium chlorite dip treatment and chitosan coatings on the quality of fresh-cut d'Anjou pears. Postharvest Biol Technol 62: 319-326.

76. Aider M (2010) Chitosan application for active bio-based films production and potential in the food industry: Review. LWT-Food Sci Technol 43:837-842.

77. Hosseini SF, Rezaei M, Zandi M, Ghavi FF (2013) Preparation and functional properties of fish gelatin-chitosan blend edible films. Food Chem 136: 1495-1500

78. Del Blanco LF, Rodriguez MS, Schulz PC, Agullo E (1999) Influence of the deacetylation degree on chitosan emulsification properties. Colloid Polym Sci 277: 1087-1092.

79. Li X, Xia W (2011) Effects of concentration, degree of deacetylation and molecular weight on emulsifying properties of chitosan. Int J Biol Macromol 48: 768-772.

80. Rodrıguez M, Albertengo L, Agullo E (2002) Emulsification capacity of chitosan. Carbohydr Polym 48: 271-276.

81. Schulz P, Rodriguez M, Del Blanco L, Pistonesi M, Agullo E (1998) Emulsification properties of chitosan. Colloid Polym Sci 276: 1159-1165.

82. Payet L, Terentjev EM (2008) Emulsification and stabilization mechanisms of O/W emulsions in the presence of chitosan. Langmuir 24: 12247-12252.

83. Torrico DD, No HK, Prinyawiwatkul W, Janes M, Corredor JA, et al (2011) Mineral oil–chitosan emulsion coatings affect quality and shelf - life of coated eggs during refrigerated and room temperature storage. J Food Sci 76: S262-S268.

84. Torrico DD, No HK, Sriwattana S, Ingram D, Prinyawiwatkul W (2011) Effects of initial albumen quality and mineral oil–chitosan emulsion coating on internal quality and shelf life of eggs during room temperature storage. Int J Food Sci Technol 46: 1783-1792.

85. Wardy W, Torrico DD, Jirangrat W, No HK, Saalia FK et al. (2011) Chitosan-soybean oil emulsion coating affects physico-functional and sensory quality of eggs during storage. LWT-Food Sci Technol 44: 2349-2355.

86. Perdones A, Sánchez-González L, Chiralt A, Vargas M (2012) Effect of chitosan - lemon essential oil coatings on storage-keeping quality of strawberry. Postharvest Biol Technol 70: 32-41.

87. Bonilla J, Atarés L, Vargas M, Chiralt A (2012) Effect of essential oils and homogenization conditions on properties of chitosan-based films. Food Hydrocolloid 26: 9-16.

88. Wang L, Liu F, Jiang Y, Chai Z, Li P, et al.(2011) Synergistic antimicrobial activities of natural essential oils with chitosan films. J Agr Food Chem 59: 12411-12419.

89. García M, Díaz R, Martínez Y, Casariego A (2010) Effects of chitosan coating on mass transfer during osmotic dehydration of papaya. Food Res Int 43: 1656-1660.

90. Zahid N, Ali A, Manickam S, Siddiqui Y, Maqbool M (2012) Potential of chitosan - loaded nanoemulsions to control different Colletotrichum spp. and maintain quality of tropical fruits during cold storage. J Appl Microbiol 113: 925-939.

91. Kumari A, Yadav SK, Yadav SC (2010) Biodegradable polymeric nanoparticles based drug delivery systems. Colloid Surf B Biointerfaces 75: 1-18.

92. Nedovic V, Kalusevic A, Manojlovic V, Levic S, Bugarski B (2011) An overview of encapsulation technologies for food applications. Proced Food Sci 1: 1806-1815.

93. Dash M, Chiellini F, Ottenbrite R, Chiellini E (2011) Chitosan - A versatile semi-synthetic polymer in biomedical applications. Prog Polym Sci 36: 981-1014.

94. Hosseini SF, Zandi M, Rezaei M, Farahmandghavi F (2013) Two-step

ISSN: 2332-4104

method for encapsulation of oregano essential oil in chitosan nanoparticles: Preparation, characterization and *in vitro* release study. Carbohydr Polym 95: 50-56.

95. Hu B, Ting Y, Yang X, Tang W, Zeng X, et al. (2012) Nanochemoprevention by encapsulation of (−)-epigallocatechin-3-gallate with bioactive peptides/ chitosan nanoparticles for enhancement of its bioavailability. Chem Commun 48: 2421-2423.

96. Hu B, Ting Y, Zeng X, Huang Q (2012) Cellular uptake and cytotoxicity of chitosan-caseinophosphopeptides nanocomplexes loaded with epigallocatechin gallate. Carbohydr Polym 86: 362-370.

97. Zhang S, Luo Y, Zeng H, Wang Q, Tian F, et al. (2011) Encapsulation of selenium in chitosan nanoparticles improves selenium availability and protects cells from selenium-induced DNA damage response. J Nutr Biochem 22: 1137-1142.

98. Luo Y, Zhang B, Cheng WH, Wang Q Preparation, characterization and evaluation of selenite-loaded chitosan/TPP nanoparticles with or without zein coating. Carbohydr Polym 2010 82: 942-951.

99. Alishahi A, Mirvaghefi A, Tehrani MR, Farahmand H, Shojaosadati SA, et al. (2011) Shelf life and delivery enhancement of vitamin C using chitosan nanoparticles. Food Chem 126: 935-940.

100. Alishahi A, Mirvaghefi A, Tehrani MR, Farahmand H, Koshio S, et al. (2011) Chitosan nanoparticle to carry vitamin C through the gastrointestinal tract and induce the non-specific immunity system of rainbow trout (*Oncorhynchus mykiss*). Carbohydr Polym 86: 142-146.

101. Luo Y, Zhang B, Whent M, Yu L, Wang Q (2011) Preparation and characterization of zein/chitosan complex for encapsulation of α-tocopherol, and its *in vitro* controlled release study. Colloid Surf B Biointerfaces 85: 145-152.

102. Luo Y, Teng Z, Wang Q (2012) Development of zein nanoparticles coated with carboxymethyl chitosan for encapsulation and controlled release of vitamin D3. J Agric Food Chem 60: 836-843.

103. Luo Y, Wang T, Teng Z, Chen P, Sun J, et al (2013) Encapsulation of indole-3-carbinol and 3,3′-diindolylmethane in zein/carboxymethyl chitosan nanoparticles with controlled release property and improved stability. Food Chem 139: 224-230.

104. Teng Z, Luo Y, Wang Q (2013) Carboxymethyl chitosan-soy protein complex nanoparticles for the encapsulation and controlled release of vitamin D3. Food Chem 141: 524-532.

105. Ha HK, Kim JW, Lee MR, Lee WJ (2013) Formation and characterization of quercetin-loaded chitosan oligosaccharide/β-lactoglobulin nanoparticle. Food Res Int 52: 82-90.

106. O'Toole MG, Henderson RM, Soucy PA, Fasciotto BH, Hoblitzell PJ, et al. (2012) Curcumin encapsulation in submicrometer spray-dried chitosan/ tween 20 particles. Biomacromolecules 13: 2309-2314.

107. Klaypradit W, Huang YW (2008) Fish oil encapsulation with chitosan using ultrasonic atomizer. LWT-Food Sci Technol 41: 1133-1139.

108. Hu M, Li Y, Decker EA, Xiao H, McClements DJ (2011) Impact of layer structure on physical stability and lipase digestibility of lipid droplets coated by biopolymer nanolaminated coatings. Food Biophys 6: 37-48.

109. Gandía-Herrero F, Cabanes J, Escribano J, García-Carmona F, Jiménez-Atiénzar M (2013) Encapsulation of the most potent antioxidant betalains in edible matrixes as powders of different colors. J Agr Food Chem 61: 4294-4302.

110. Harris R, Lecumberri E, Mateos-Aparicio I, Mengíbar M, Heras A (2011) Chitosan nanoparticles and microspheres for the encapsulation of natural antioxidants extracted from Ilex paraguariensis. Carbohydr Polym 84: 803-806.

111. Li Y, McClements DJ (2011) Controlling lipid digestion by encapsulation of protein-stabilized lipid droplets within alginate–chitosan complex coacervates. Food Hydrocoll 25: 1025-1033.

112. Peng H, Xiong H, Li J, Xie M, Liu Y, et al. (2010) Vanillin cross-linked chitosan microspheres for controlled release of resveratrol. Food Chem 121: 23-28.

113. Belščak-Cvitanović A, Stojanović R, Manojlović V, Komes D, Cindrić I J, et al. (2011) Encapsulation of polyphenolic antioxidants from medicinal plant extracts in alginate–chitosan system enhanced with ascorbic acid by electrostatic extrusion. Food Res Int 44: 1094-1101.

114. Luo Y, Teng Z, Wang X, Wang Q (2013) Development of carboxymethyl chitosan hydrogel beads in alcohol-aqueous binary solvent for nutrient delivery applications. Food Hydrocoll 31: 332-339.

115. Trapani A, Sitterberg J, Bakowsky U, Kissel T (2009) The potential of glycol chitosan nanoparticles as carrier for low water soluble drugs. Int J Pharm 375: 97-106.

116. Jayakumar R, Prabaharan M, Nair S, Tokura S, Tamura H, et al. (2010) Novel carboxymethyl derivatives of chitin and chitosan materials and their biomedical applications. Prog Mater Sci 55: 675-709.

117. Alishahi A, Mirvaghefi A, Tehrani MR, Farahmand H, Shojaosadati SA, et al. (2011) Shelf life and delivery enhancement of vitamin C using chitosan nanoparticles. Food Chem 126: 935-940.

118. Hamman JH (2010) Chitosan based polyelectrolyte complexes as potential carrier materials in drug delivery systems. Mar Drugs 8: 1305-1322.

119. Tang DW, Yu SH, Ho YC, Huang BQ, Tsai GJ, et al. (2013) Characterization of tea catechins-loaded nanoparticles prepared from chitosan and an edible polypeptide. Food Hydrocoll 30: 33-41.

120. Estevinho BN, Rocha F, Santos L, Alves A (2013) Microencapsulation with chitosan by spray drying for industry applications – a review. Trends Food Sci Technol 31: 138-155.

121. Desai K, Park H (2005) Encapsulation of vitamin C in tripolyphosphate cross-linked chitosan microspheres by spray drying. J Microencapul 22: 179-192.

122. Desai KG, Park HJ (2006) Effect of manufacturing parameters on the characteristics of vitamin C encapsulated tripolyphosphate-chitosan microspheres prepared by spray-drying. J Microencapul 23: 91-103.

123. Desai KG, Liu C, Park HJ (2006) Characteristics of vitamin C encapsulated tripolyphosphate-chitosan microspheres as affected by chitosan molecular weight. J Microencapul 23: 79-90.

124. Li Y, Ai L, Yokoyama W, Shoemaker CF, Wei D, et al. (2013) Properties of chitosan-microencapsulated orange oil prepared by spray-drying and its stability to detergents. J Agric Food Chem 61: 3311-3319.

125. Hoffman AS (2002) Hydrogels for biomedical applications. Adv Drug Deliv Rev 54: 3-12.

126. Sun P, Li P, Li YM, Wei Q, Tian LH (2011) A pH - sensitive chitosan - tripolyphosphate hydrogel beads for controlled glipizide delivery. J Biomed Mater Res B Appl Biomater 97: 175-183.

127. Takka S, Gürel A (2010) Evaluation of chitosan/alginate beads using experimental design: formulation and *in vitro* characterization. AAPS PharmSciTech 11: 460-466.

128. Maestrelli F, Cirri M, Mennini N, Bragagni M, Zerrouk N, et al. (2012) Influence of cross-linking agent type and chitosan content on the performance of pectinate-chitosan beads aimed for colon-specific drug delivery. Drug Dev Ind Pharm 38: 1142-1151.

129. Bhattarai N, Gunn J, Zhang M (2010) Chitosan-based hydrogels for controlled, localized drug delivery. Adv Drug Deliv Rev 62: 83-99.

130. Muzzarelli R, Muzzarelli C, Phillips G, Williams P (2009) Chitin and chitosan hydrogels. Handbook Hydrocolloid 849-888.

131. Liu LS, Kost J, Yan F, Spiro RC (2012) Hydrogels from biopolymer hybrid for biomedical, food, and functional food applications. Polymers 4: 997-1011.

132. Lin YH, Liang HF, Chung CK, Chen MC, Sung HW (2005) Physically crosslinked alginate/N, O-carboxymethyl chitosan hydrogels with calcium for oral delivery of protein drugs. Biomaterials 26: 2105-2113.

133. Cook MT, Tzortzis G, Charalampopoulos D, Khutoryanskiy VV (2012) Microencapsulation of probiotics for gastrointestinal delivery. J Control Release 162: 56-67.

134. Manojlović V, Nedović VA, Kailasapathy K, Zuidam NJ (2010) Encapsulation of probiotics for use in food products. In Encapsulation technologies for active food ingredients and food processing. Springer 269-302.

135. Cook MT, Tzortzis G, Charalampopoulos D, Khutoryanskiy VV (2011) Production and evaluation of dry alginate-chitosan microcapsules as an

ISSN: 2332-4104

enteric delivery vehicle for probiotic bacteria. Biomacromolecules 12: 2834-2840.

136. Nualkaekul S, Lenton D, Cook MT, Khutoryanskiy VV, Charalampopoulos D (2012) Chitosan coated alginate beads for the survival of microencapsulated Lactobacillus plantarum in pomegranate juice. Carbohydr Polym 90: 1281-1287.

137. Li XY, Chen XG, Sun ZW, Park HJ, Cha DS (2011) Preparation of alginate/chitosan/carboxymethyl chitosan complex microcapsules and application in *Lactobacillus casei* ATCC 393. Carbohydr Polym 83: 1479-1485.

138. Chen S, Cao Y, Ferguson LR, Shu Q, Garg S (2013) Evaluation of mucoadhesive coatings of chitosan and thiolated chitosan for the colonic delivery of microencapsulated probiotic bacteria. J Microencapul 30: 103-115.

139. Krajewska B (2004) Application of chitin- and chitosan-based materials for enzyme immobilizations: a review. Enzyme Microb Technol 35: 126-139.

140. Shi W, Ma Z (2010) Amperometric glucose biosensor based on a triangular silver nanoprisms/chitosan composite film as immobilization matrix. Biosens Bioelectron 26: 1098-1103.

141. Dai H, Chi Y, Wu X, Wang Y, Wei M, et al. (2010) Biocompatible electrochemiluminescent biosensor for choline based on enzyme/titanate nanotubes/chitosan composite modified electrode. Biosens Bioelectron 25: 1414-1419.

142. Lin H, Lu Q, Ge S, Cai Q, Grimes CA (2010) Detection of pathogen *Escherichia coli* O157: H7 with a wireless magnetoelastic-sensing device amplified by using chitosan-modified magnetic Fe3O4 nanoparticles. Sens Actuators B Chem 147: 343-349.

143. Diaconu M, Litescu SC, Radu GL (2010) Laccase-MWCNT-chitosan biosensor - A new tool for total polyphenolic content evaluation from *in vitro* cultivated plants. Sens Actuators B Chem 145: 800-806.

144. Bolto B, Gregory J (2007) Organic polyelectrolytes in water treatment. Water Res 41: 2301-2324.

145. Yamada K, Akiba Y, Shibuya T, Kashiwada A, Matsuda K, et al. (2005) Water purification through bioconversion of phenol compounds by tyrosinase and chemical adsorption by chitosan beads. Biotechnol Prog 21: 823-829.

146. Shen C, Song S, Zang L, Kang X, Wen Y, et al. (2010) Efficient removal of dyes in water using chitosan microsphere supported cobalt (II) tetrasulfophthalocyanine with H2O2. J Hazard Mater 177: 560-566.

147. Zhang C, Jia Z (2010) Preparation of porous chitosan microsphere absorbent and research on Its absorption ability for Cu2+ and Zn2+. Int J Chem 2: P113.

148. Salehi E, Madaeni S, Rajabi L, Vatanpour V, Derakhshan A, et al. (2012) Novel chitosan/poly (vinyl) alcohol thin adsorptive membranes modified with amino functionalized multi-walled carbon nanotubes for Cu (II) removal from water: Preparation, characterization, adsorption kinetics and thermodynamics. Sep Purif Technol 89: 309-319.

149. Liu Y, Kang Y, Huang D, Wang A (2012) Cu^{2+} removal from aqueous solution by modified chitosan hydrogels. J Chem Technol Biotechnol 87: 1010-1016.

150. Rojas-Reyna R, Schwarz S, Heinrich G, Petzold G, Schütze S et al. (2010) Flocculation efficiency of modified water soluble chitosan versus commonly used commercial polyelectrolytes. Carbohydr Polym 81: 317-322.

151. Saitoh T, Asano K, Hiraide M (2011) Removal of phenols in water using chitosan-conjugated thermo-responsive polymers. J Hazard Mater 185: 1369-1373.

152. Bleiman N, Mishael YG (2010) Selenium removal from drinking water by adsorption to chitosan–clay composites and oxides: Batch and columns tests. J Hazard Mater 183: 590-595.

153. Jagtap S, Yenkie M, Labhsetwar N, Rayalu S (2011) Defluoridation of drinking water using chitosan based mesoporous alumina. Micropor Mesopor Mat 142: 454-463.

154. Zhang H, Zhu G, Jia X, Ding Y, Zhang M, et al. (2011) Removal of microcystin-LR from drinking water using a bamboo-based charcoal adsorbent modified with chitosan. J Environ Sci (China) 23: 1983-1988.

155. Díaz-Visurraga J, Meléndrez MF, García A, Paulraj M, Cárdenas G (2010) Semitransparent chitosan - TiO2 nanotubes composite film for food package applications. J Appl Polym Sci 116: 3503-3515.

156. Saifuddin N, Nian C, Zhan L, Ning K (2011) Chitosan-silver nanoparticles composite as point-of-use drinking water filtration system for household to remove pesticides in water. Asian J Biochem 6: 142-159.