Active film and coating from chitosan as a functional food

Moslem sabaghi
M. Sc. Student of Food Science & Technology
University of Agricultural Sciences & Natural Resources
Gorgan, iran
m_sabaghi2@yahoo.co.uk

Dr.Yahya maghsoudlou
Associate Professor of Food Science & Technology
University of Agricultural Sciences &Natural Resources
Gorgan, iran
ymaghsoudlou@yahoo.com

Abstract— *In recent times, increasing attention has been paid to develop and test films with functional properties in order to improve food safety and shelf life. Active biomolecules such as chitosan have a significant role in food application for maintenance quality a wide variety of food products. Chitosan properties is mainly due to its biodegradability, biocompatibility, antimicrobial and antioxidant activity, non-toxicity , drug delivery, therapeutic aspects and versatile chemical and physical properties.*

Keywords-film; functional; Chitosan; antimicrobial; antioxidant

I. *INTRODUCTION*

Active packaging is the packaging system possessing attributes beyond basic barrier properties that are achieved by adding active ingredients in the packaging system and /or using functionally active polymers [1]. The primary goals of a conventional packaging system such as safety assurance, quality maintenance and shelf-life extension[2]. Chitin is a naturally abundant mucopolysaccharide distributed in the shell of crustaceans, in the cuticle of insects, and also in the cell wall of some fungi and microorganisms. Chitosan, a linear polysaccharide consisting of (1,4)-linked 2- amino-deoxy-b-D-glucan, is a deacetylated derivative of chitin, which is the second most abundant polysaccharide found in nature after cellulose.

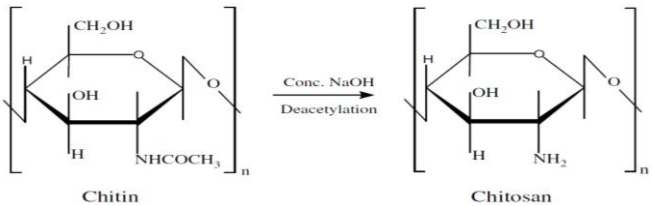

Figure1. Structure of chitin and chitosan [20].

Chitosan has been found to be nontoxic, biodegradable, biofunctional, biocompatiblein addition to having antimicrobial characteristics [3]. partially acetylated chitosan having about 50% d-glucosamine units is only able to dissolve in water [4]. chitosan films have been used as a packaging material for the quality preservation of a variety of food. [5] Nowadays, commercially chitosan are produced from biowastes obtained from aquatic organisms [6]. The present review focus on the latest studies and summarize application of chitosan as a film and coating for food preservation and therapeutic aspects.

II. FUNCTIOAL ABILITY OF CHITOSAN

A. Antioxidant activity

The oxidative changes that occur in the food items reduce the shelf life of foods, making them unsuitable for human consumption. Living organisms are constantly exposed to reactive oxygen species formed as by-products of normal respiration [7]. However, use of synthetic antioxidants in food products is under strict regulation due to the potential health hazards caused by such compounds [8]. Therefore, search for natural antioxidants as alternatives to synthetic ones is of great interest among researchers [9].
The effect of the molecular weight of chitosan in the prevention of the lipid oxidation of salmon has been investigated [10] and, according to this study, 30 kDa chitosan has exhibited the highest reduction of lipid oxidation in salmon. With the increase in the molecular weight of chitosan, the effectiveness against the lipid oxidation reduces reflecting the unsuitability of higher molecular weight chitosan as the food preservative.
Oxygen permeability is highly dependent on the relative humidity As relative humidity increases, more water molecules interact with the material and the film becomes more plasticized. In these conditions, the mobility and the extensive mass transfer across the film are favoured. For this reason, the antioxidant ability of edible films should always be tested under controlled relative humidity conditions [11]
The antioxidant efficiency of edible films has been tested using different approaches. chitosan films can be dissolved in distilled water. Testing the activity of an antioxidant by more than one assay is desirable, because different methods approach this measurement in different ways Very often, radical trapping methods have been applied. These methods 2,2-diphenyl-1-picryhydrazyl radical (DPPH), N-diethyl-p-phenylenediamine (DPD) radical scavenging assay, ferric-reducing antioxidant power (FRAP), 2,20-azinobis(3-ethylbenzothiazoline-6-sulphonate) (ABTS) assay, amongst

others measure the ability of an antioxidant agent to intercept free radicals [12]. Yen et al. (2008) reported that various crab chitosan were prepared by alkaline *N*-deacetylation of crab chitin for 60 (C60), 90 (C90), and 120 (C120) min and antioxidative activity of the prepared chitosans exhibited antioxidative effects of 58.3%–70.2% at 1.0 mg/mL concentration.

TABLE I. EXAMPLE FOR APPLICATION OF CHITOSAN AS A ANTIOXIDANT

Film or coating	Antioxidant Compound	Application	Analyses	Ref.
Chitosan Coating	Oleoresins: rosemary, onion	Butternut squash	Peroxidase	[13]
Chitosan coating	Fish oil, vitamin E	Lingcod fillets	TBA	[14]
Chitosan film	Green Tea Extract	Edible film	DPPH	[15]

The C120 with more amino groups on C-2 position showed the highest antioxidative activity. Generally, the degree of deacetylation was correlated with *N*-acetylation times[16]. These results suggest that chitosan eliminate various free radicals by the action of free amino group at C-2 position contribute to their antioxidant activity as those groups have abstractable hydrogen atoms [17].

B. *Antimicrobial activity*

Both Gram-positive and Gram-negative bacteria are involved in the spoilage of food. The type of bacteria varies with the type of food as the optimum conditions needed for the bacterial growth differ with their species. The chitosan have the property of antibacterial activity against the wide range of food spoilage and food pathogenic bacteria which reduce the shelf life of food. chitosan based films have proven to be very effective in food preservation. There are various intrinsic and extrinsic factors that affect the antimicrobial activity of chitosan. It has been demonstrated that lower molecular weight chitosan (of less than 10 kDa) has a greater antimicrobial activity than native chitosans. Furthermore, a degree of polymerization of at least 7 is required; lower molecular weight fractions have little or no activity [18]. Highly deacetylated chitosans are more antimicrobial than those with a higher proportion of acetylated amino groups due to increased solubility and higher charge density [19].Lower pH increases the antimicrobial activity of chitosan for much the same reasons, in addition to the 'hurdle effect' of inflicting acid stress on the target organisms [20].

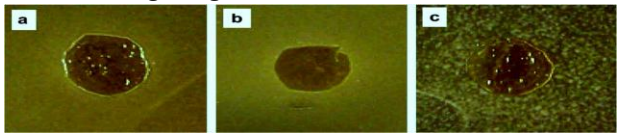

Figure2.Inhibitory effect of chitosan-starch film against (a)E.coli, (b)s.aureus, (c)B.subtilis [20].

One of the reasons for the antimicrobial character of chitosan is its positively charged amino group which interacts with negatively charged microbial cell membranes, leading to the leakage of proteinaceous and other intracellular constituents of the microorganisms [21]. Chitosan also acts as a chelating agent that selectively binds trace metals and thereby inhibits the production of toxins and microbial growth [22].

TABLE II. SELECTED EXAMPLES OF THE ANTIMICROBIAL ACTIVITY

Microorganisms	Molecular Weight	Degree Deacetylation	Ref.
S.aureus	Chitosan(21-27 KDa)	84.9-95%	[23]
	Chitosan(140-190 KDa)	83-84.9%	
E.Coli	Chitosan(21-27 KDa)	84.9-95%	
	Chitosan Chitosan(140-190 KDa)	83-84.9%	
Candida albicans	Chitosan(<18 KDa)	96.9%	[24]

III. TECHNOLOGICAL EFFECT ON THE STRUCTURE CHITOSAN FILM

A. *Effect of electric fields on the sructure*

presence of a moderate electric field during the preparation of chitosan coating solutions influence their transport properties. [25] XRD analyses indicated that electrically treated chitosan films exhibited a more ordered structure and a clearly higher crystallinity when compared with non-treated films, thus displaying significant effects on the value of the crystallinity index. SEM micrographs evidenced that the surface morphology of chitosan film was influenced by the electric field. In fact, the electric field treatment led to a structure with more regular layers as can be seen in the cross-sections of the films observed under SEM [26].

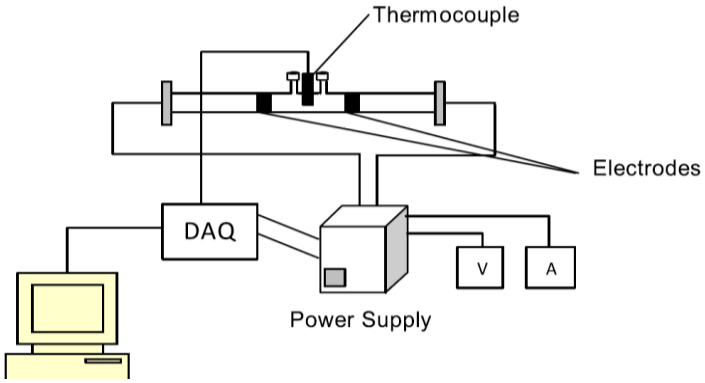

Figure3. Ohmic heater and data acquisition system [25].

The AFM results show that the surface of chitosan films is much more uniformwhen an electric field is applied. In general, a positive correlation being found between the water vapor, oxygen and carbon dioxide permeability coefficients and field strength., which may be related with a more uniform gel structure leading to the differences observed in terms of transport properties [25].

As shown in Figure (3), The chitosan solution samples were treated in an ohmic heater using different field strengths and consisted of a cylindrical glass tube; two electrodes were placed at each end of the tube. Temperatures were monitored using a thermocouple, placed at the geometrical centre of the chamber. A datalogger was employed to record continuously and simultaneously,current intensity, voltage and temperature [26].

B. *Effect of drying methods on some mechanical properties*

It is well recognized that mechanical properties, including tensile strength and percent elongation, of synthetic packaging films are significantly better than those of edible films [27]. Among many steps needed to prepare edible films, drying is considered one of the most important [28]. The antimicrobial activity, and intermolecular interaction were all affected by the tested drying methods and conditions [29]. the best drying method that should be used by low-pressure superheated steam drying [30].

IV. THERAPUTIC ASPECT

A. *Immune effect and anti-inflammatory activity*

Inflammation is a natural biological and immune-related response from a host to certain stimuli such as infections, other foreign agents, and physical or psychological distress. The ability of chitosan to modulate immune responses seems to be related to the presence of N-acetyl-D-glucosamine and its ability to trigger different membrane surface receptors and pathways of immune cells [31]. The immunologic-stimulating effects of chitosan may be expected to yield exciting and promising applications in the vaccine industry, as they were proved to enhance antigen-specific antibody titers when incorporated in vaccines as adjuvants [32].

B. *Anti cancer activity*

Although chitosan have been mainly used for the design of drug carriers for cancer treatment [33]. In vitro and in vivo experimental data indicate that low-MW chitosan, but not high-MW chitosan, may present intrinsic antitumor activity [34]. In general, this activity seems to be mostly derived from their immune-stimulating effects and not by direct damage to tumor cells [35].

C. *Blood coagulation effectt*

Chitosan have the potential to interact with blood cells and interfere with coagulation in various ways. Chitosan is able to induce coagulation or shorten clotting time by inducing the aggregation of red blood cells and platelets in a dose-dependent manner [36]. This effect is not reliant on the blood coagulation cascade, being generally attributed to the ionic interaction between the positively charged polymer and the negatively charged cell membrane [37]. The coagulation activity of chitosan is variable according to its molecular weight and degree deacetylation, since these features can significantly influence the final cationic properties of the polymer [38]. In general, higher molecular weight favors coagulation, while the effect of degree deacetylation seems to be more uncertain [39].

D. *Anti diabetic activity*

Chitosan is able to reduce hyperglycemia associated with non-insulin-dependent diabetes, as shown by different animal studies. Further studies in mice indicate that low-molecular weight chitosan (0.2–0.8% in drinking water) may be of particular value, not necessarily in the treatment, but in preventing or decreasing progression to non-insulin-dependent diabetes [40]. Also, low-molecular weight chitosan may be useful as an adjuvant in alleviating hyperglycemia in insulin-dependent diabetes [41]. Recent studies suggest that both low- and high-MW chitosan are able to decrease hepatic gluconeogenesis and increase glucose use in skeletal muscle, thus justifying its hypoglycemic activity [42]. In the specific case of high-molecular weight chitosan, reduction of the activity of intestinal disaccharidases may also be partially responsible for reduced glucose absorption [43].

E. *Obesity*

Chitosan-based preparations are available worldwide as over-the-counter dietary supplements for weight loss. Daily oral intake of 2000–2400 mg, divided in two doses, has been recommended, although daily doses of up to 6000 mg have also been tested [44]. The rationale behind the use of chitosan for the treatment of overweight and obese patients is thought to be related to the ability of the polymer to form fat-entrapping gels upon pH neutralization in the intestine, thus reducing the absorption of fat [45]. The ionic interaction between amine groups of chitosan and fatty acids seems to be important in fat retention [44].

F. *Ionization activity*

Chitosan has been shown able to bind selectively to different metallic ions such as Molybdate polyoxyanions, Cu^{2+}, Zn^{2+}, Cd^{2+}, and Ni^{2+} [46]. The underlying mechanisms are thought to be related with adsorption, ion exchange, and chelation phenomena, with the amine groups of chitosan considered particularly important in the establishment of polymer–ion interactions. This biological effect has a variety of potential applications, but, in the particular case

of human consumption, concerns were raised due to dietary implications in the event of long-term use. Animal data suggest that chitosan intake is not able to deplete the levels of important trace metals such as iron, zinc, and copper [47].

V. TECHNOLOGICAL APPLICATIONS

Chitosan widely used in the postharvest coating of intact and fresh-cut fruits and vegetables [48]. These coatings present generally good gas (mostly oxygen) barriers due to their hydrogen-bonded network structure [49], and adhere well to the hydrophilic cut surfaces of fruits and vegetables. However, they are poor water barriers [50], which may increase product desiccation and weight loss. As a result of chitosan coating, a reduction in the respiration rate and ethylene production, control of decay, and retention of firmness have been reported for fruits such as apple [51].

Chitosan coating is likely to modify the internal atmosphere without causing anaerobic respiration, because chitosan films are more selectively permeable to O_2 than to CO_2 [49]. There is ample evidence that chitosan coatings have the potential to prolong storage life and control decay of Fruits [52].

VI. CONCLUSION AND FUTURES PERSPECTIVES

Chitosan offered as versatile and promising biodegradable polymers owing to its antimicrobial, antioxidant activity and nontoxicity leading to their extensive use over a wide range of applications. Among various applications chitosan is the very promising system for the future improvement of food quality and preservation during processing and storage. Chitosan can also be helpful in extending the food shelf life. Further confirmatory studies are required in order to attest the real biological effects of chitosan and derivatives. In particular, additional elucidation on the involved molecular mechanisms is needed. Also, the lack of a standard chitosan material introduces substantial variability in the interpretation of available clinical data, a fact that can limit the translation of chitosan into the clinical practice. The increase in the film water content reduces the oxygen barrier effect, but can enhance the chemical action of the antioxidants. For this reason, the moisture content of the foodstuff and the relative humidity in the ambient should be taken into account in order to develop effective films and coatings with antioxidant activity.

REFERENCES

[1] J. H. Han, "Antimicrobial food packaging," Food Technology, vol. 54, Mar. 2000, pp. 56–65.

[2] T. Jin, and H. Zhang, "Biodegradable polylactic acid polymer with nisin for use in antimicrobial food packaging," Food Science, vol. 73, Mar. 2008, pp. 127-134.

[3] S. Tokura, and H. Tamura, "Chitin and chitosan. In Comprehensive Glycoscience," Elsevier Ltd., Amsterdam, the Netherlands, 2007, pp. 449–475.

[4] S. Aiba,. 1989. "Studies on chitosan: 2. Solution stability and reactivity of partially N-acetylated chitosan derivatives in aqueous media, " Biol. Macromol,Nov. 1989, pp. 249–252.

[5] T. Wu, S. Zivanovic, F. A. Draughon, W. S. Conway and C. E. Sams, "Physicochemical properties and bioactivity of fungal chitin and chitosan, " Agricultural and Food Chemistry, Vol. 53, Oct 2005, pp. 3888–3894.

[6] N. Nwe, and W. F. Stevens, "Production of chitin and chitosan and their applications in the medical and biological sector, " In Recent Research in Biomedical Aspects of Chitin and Chitosan, ed. H. Tamura, 2008, pp. 161–176.

[7] B. Halliwell, and J. Gutteridge, "Free Radicals in Biology and Medicine, " Clarendon Press, Oxford, 2007.

[8] S. Hettiarachchy, K. C. Glenn, R. Gnanasambandam, and M. G. Johnson, 1996. "Natural antioxidant extract from fenugreek for ground beef patties, " Food Science, vol. 61, 1996, pp. 516–519.

[9] D. D.Debashis, B. M. Bhattacharjee, and R. K. Banerjee, "Hydroxyl radicals is the major causative factor in stress-induced gastric ulceration, " Biol. Med, vol. 23,1997, pp. 8–18.

[10] K. W. Kim, and R. L. Thomas, "Antioxidative activity of chitosans with varying molecular weights, " Food Chemistry, vol. 101, 2007, pp. 308–313.

[11] L. Atares, J. Bonilla, A. Chiralt, "Characterization of sodium caseinate-basededible films incorporated with cinnamon or ginger essential oils, " FoodEngineering, vol. 100, 2010, pp. 678–687.

[12] J. Bonilla, L. Atares, L.Vargas, and A. Chiralt, "Edible films and coatings to prevent the detrimental effect of oxygen on food quality: Possibilities and limitations," Food Engineering, vol. 110, 2012, pp. 208–213, doi:10.1016/j.jfoodeng.2011.05.034.

[13] A.Ponce, S.I. Roura, C.E. del Valle and M.R. Moreira, "Antimicrobial and antioxidant activities of edible coatings enriched with natural plant extracts: in vitro and in vivo studies," Postharvest Biology and Technology, vol. 49,2008, pp. 294–300.

[14] J. Duan, G. Cherian and Y. Zhao, , 2010. "Quality enhancement in fresh and frozen lingcod (Ophiodon elongates) fillets by employment of fish oil incorporated chitosan coatings," Food Chemistry, vol. 119, 2010, pp. 524–532.

[15] U. Siripatrawn, and B. Harte, "Physical properties and antioxidant activity of an active film from chitosan incorporated with green tea extract," Food Hydrocolloids, vol. 24, 2010, pp. 770-775, doi:10.1016/j.foodhyd.2010.04.003.

[16] M. T. Yen, J. H.Yang and J. L. Mau, "Antioxidant properties of chitosan from crab shells," Carbohydr. Polym, vol. 74, 2008, pp. 840–844.

[17] P. J.Park, J. Y. Je and S. K. Kim, "Free radical scavenging activities of differently deacetylated chitosans using an ESR spectrometer," Carbohydrate Polymers, vol. 55, 2004, pp. 17–22.

[18] Y. Uchida, M. Izume and A. Ohtakara "Preparation of chitosan oligomers with purified chitosanase and its application," Elsevier Applied Science, 1989, pp. 373–382.

[19] S. Sekiguchi, Y. Miura, H. Kaneko, "Molecular weight dependency of antimicrobial activity by chitosan oligomers," Food Hydrocolloids, 1994, pp. 71–76.

[20] P. K. Dutta, s, Tripathi, g.k. Mehrotra and Dutta, "Perspectives for chitosan based antimicrobial films in food applications," Food Chemistry, vol. 114, 2009, pp. 1173–1182.

[21] F. Shahidi, J. K. V. Arachchi and Y. J. Jeon, "Food application of chitin and chitosans," Trends in Food Science and Technology, vol. 10, Feb 1999, pp. 37–51.

[22] R. G. Cuero, G. Osuji and A. Washington, "N-carboxymethyl chitosan inhibition of aflatoxin production: Role of zinc," Biotechnology Letters, vol. 13, Jun 1991, pp. 441-444.

[23] A.A.Tayel, S. Moussa, K. Opwis, "Inhibition of microbial pathogens by fungal chitosan," Biol. Macromol, vol. 47, 2010, pp. 10–14.

[24] F. Tajdini, M.A. Amini, N. Nafissi-Varchehand and M. A. Faramarzi, "Production, physiochemical and antimicrobial properties of fungal

chitosan from Rhizomucor miehei and Mucor racemosus," Biol. Macromol, vol. 47, 2010, pp. 180–183.

[25] B. W. S. Souza, M.A. Cerqueira, A. Casariego, A.M.P. Lima, J.A. Teixeira and A.A. Vicente, "Effect of moderate electric fields in the permeation properties of chitosan coatings," Food Hydrocolloids, vol. 23, 2009, pp. 2110–2115, doi:10.1016/j.foodhyd.2009.03.021.

[26] B. W. S. Souza, M. A. Cerqueira, J.T. Martins, A. Casariego, J.A. Teixeira and Vicente, A.A. "Influence of electric fields on the structure of chitosan edible coatings," Food Hydrocolloids, vol. 24, 2010, pp. 330–335, doi:10.1016/j.foodhyd.2009.10.011.

[27] S. Mathew, T. E. Abraham, "Characterisation of ferulic acid incorporated starch–chitosan blend films. Food Hydrocolloids," vol. 22, 2008, pp. 826–835.

[28] I. S. Arvanitoyannis, A. Nakayama, S. Aiba, , 1998. "Chitosan and gelatin based ediblefilms: state diagrams, mechanical and permeation propertiesm, " Carbohydrate Polymers, vol 37, 1998, pp. 371–382.

[29] p. Mayachiew, s. Devahastin, B.M. Mackey and k. Niranjan, "Effects of drying methods and conditions on antimicrobial activity of edible chitosan films enriched with galangal extract," Food Research International, vol 43, 2010, pp. 125-132, doi:10.1016/j.foodres.2009.09.006.

[30] W. Thakhiew, s. Devahastin and s. Soponronnarit, "Effects of drying methods and plasticizer concentration on some physical and mechanical properties of edible chitosan films," Food Engineering, vol 99, 2010, pp. 216-224, doi:10.1016/j.jfoodeng.2010.02.025.

[31] J. Feng, L. Zhao, and Q. Yu, (2004) "Receptor-mediated stimulatory effect of oligochitosan in macrophages," Biochem. Biophys. Res. Commun, vol. 317 pp. 414–420.

[32] D. A. Zaharoff, C.J. Rogers, K. W. Hance, "Chitosan solution enhances both humoral and cell-mediated immune responses to subcutaneous vaccination, " Vaccine, vol. 25, 2007, pp. 2085–2094.

[33] C. R. Dass and P. F. Choong, "The use of chitosan formulations in cancer therapy, " Microencapsul, vol. 25, 2008, pp. 275–279.

[34] C. Qin, Y. Du, L. Xiao, (2002) "Enzymic preparation of water-soluble chitosan and their antitumor activity," Biol. Macromol, vol. 31, 2002, pp. 111–117.

[35] Y. Maeda, and Y. Kimura, "Antitumor effects of various low-molecular-weight chitosans are due to increased natural killer activity of intestinal intraepithelial lymphocytes in sarcoma,"nutr, vol. 134, 2004, pp. 945–950.

[36] Y. Okamoto, R. Yano, K. Miyatake, (2003) "Effects of chitin and chitosan on blood coagulation, " Carbohydr.Polym, vol 53, pp. 337–342.

[37] H. S. Whang, W. Kirsch, Y. H. Zhu, "Hemostatic agents derived from chitin and chitosan, " Polym. Rev, vol. 45, 2005, pp. 309–323.

[38] J. Yang, F. Tian, Z. Wang "Effect of chitosan molecular weight and deacetylation degree on hemostasis," Biomed. Mater. Res. Part B Appl. Biomater, vol. 84, 2008, pp. 131–137.

[39] J. Zhang, W. Xia, P. Liu, (2010) "Chitosan modification and pharmaceutical/biomedical applications, " Mar. Drugs, vol 8, pp. 1962–1987.

[40] Y. Kondo, A. Nakatani, K. Hayashi and M. Ito, "Low molecular weight chitosan prevents the progression of low dose streptozotocin-induced slowly progressive diabetes mellitus in mice," Biol. Pharm. Bull, vol. 23, 2000, pp. 1458–1464.

[41] K. Hayashi and M. Ito, "Antidiabetic action of low molecular weight chitosan in genetically obese diabetic KK-Ay mice," Biol. Pharm. Bull, vol. 25, 2002, pp. 188–192.

[42] S. H. Liu, Y. H. Chang, and M. T. Chiang, (2010) "Chitosan reduces gluconeogenesis and increases glucose uptake in skeletal muscle in streptozotocin-induced diabetic rats," Agric. Food Chem, vol. 58, 2010, pp. 5795–5800.

[43] H. T. Yao, S. Y. Huang and M. T. Chiang, (2008) "A comparative study on hypoglycemic and hypocholesterolemic effects of high and low molecular weight chitosan in streptozotocin-induced diabetic rats, " Food Chem Toxicol, vol. 46, 2008, pp. 1525–1534

[44] K. M. Shields, N. Smock, C. E. McQueen and P.J. Bryant, "Chitosan for weight loss and cholesterol management," Health. Syst. Pharm, vol. 60, 2003, pp. 1310– 1312, 1315-1316.

[45] L. K. Han, Y. Kimura, and H. Okuda, "Reduction in fat storage during chitin-chitosan treatment in mice fed a high-fat diet," Obes. Relat. Metab. Disord, vol 23, 1999, pp. 174–179.

[46] I. M. N. Vold, K. M. Varum, E. Guibal, and O. Smidsrod, "Binding of ions to chitosan-selectivity studies," Carbohydr. Res, vol. 54, 2003, pp. 471–477.

[47] L. Zeng, C. Qin, G. He, (2008) "Effect of dietary chitosans on trace iron, copper and zinc in mice, " Carbohydr. Polym, vol. 74, 2008, pp. 279–282.

[48] H. K. No, S.P. Meyers, W. Prinyawiwatkul and Z. Xu, 2007. "Applications of chitosan for improvement of quality and shelf life of foods: A review, " Food Sci, vol. 72, 2007 pp. 87–101.

[49] T. McHugh and J. Krochta. 1994. "Water vapor permeability properties of edible whey proteinlipid emulsion films," Amer Oil Chem Soc, vol. 71,1994, pp. 307–312.

[50] E. A. Baldwin, M. Nisperos-Carriedo, P.E. Shaw and J.K. Burns, 1995. "Effect of coatings and prolonged storage conditions on fresh orange flavor volatiles, degrees brix, and ascorbic acid levels," *Agric* Food Chem, vol. 43, 1995, pp. 1321–1331.

[51] Y. Hwang, Y. Kim and J. Lee, 1998. "Effect of postharvest application of chitosan and wax, and ethylene scrubbing on the quality changes in stored 'Tsugaru' apples," Korea Soc Hort Sci, vol. 39, pp. 579–582.

[52] G. Romanazzi, F. Nigro and A. Ippolito, "Short hypobaric treatments potentiate the effect of chitosan in reducing storage decay of sweet cherries, " Postharvest Biol Technol, vol. 29, 2003, pp. 73–80.