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Antibacterial Evaluation of Starch and Chitosan Based Polymeric Blend

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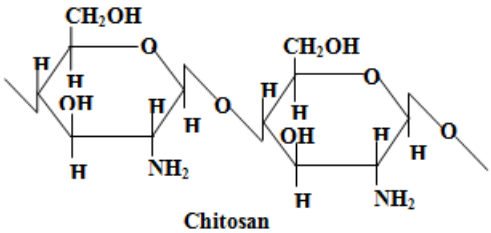
Abstract: *Demand of food safety, quality and environmental concern associated with the handling of plastic waste has emphasized antimicrobial packaging, as one of the promising packaging system. Development of biodegradable and edible films based on natural polymers such as starch has been considered as economical material for antimicrobial packaging. This work aimed at the development of food packaging based on starch blended with chitosan as antimicrobial and gluteraldehyde as crosslinker. Blend of starch (ST) and chitosan (CHI) crosslinked with glutaraldehyde (4ml of 2% aqueous solution) is prepared. The common solvent 1% aqueous solution of acetic acid is used for the preparation of polymeric blend of chitosan and starch in the ratio of 5.5. agent. Chitosan has been extensively used as an antimicrobial agent either alone or blended with other natural polymers [1,2], because of its biodegradable, nontoxic and antimicrobial nature [3]. To extend chitosan's antimicrobial applicability, blend of starch (ST) and chitosan (CHI) are used in food packaging and biomedical applications [2, 3], but barriers still exist to its broader use due to its physical and chemical limitations [3, 4, 5, 6]. The antibacterial property of the blended film is observed along with their controls against two Gram-negative pathogenic and non pathogenic bacteria. The pathogenic bacterium is Salmonella enterica and the non pathogenic bacterium is Escherichia coli. The blend is found effective against pathogenic bacterium as well as non pathogenic bacterium i.e. Salmonella enterica & Escherichia coli both.*

Keywords: *Blend, antibacterial property, chitosan, starch, crosslinking.*

I. Introduction

Antimicrobial packaging system prevents the spoilage and contamination of food by killing or inhibiting the growth of the pathogenic microorganism. Antimicrobial packaging improves the quality of food by extending the shelf-life. The use of agricultural biopolymers that are easily biodegradable is a cleaner and ecofriendly approach which protects the environment from denaturing effects associated with thermal processing processes of plastics and solves the problem of waste disposal from undegraded synthetic polymer.

Starch, a most abundant natural and hydrophilic is regenerated from carbon dioxide and water by photosynthesis in plants [7]. Starch stores in a plant in a granular form. It occurs in variety of sources such as potatoes, wheat, corn and tapioca. It has received great attention in food packaging industry because of its biodegradable[8, 9], edible, low cost, easy to use ,wide availability, thermoprocessability and can be obtain from different left over of harvesting and raw material industrialization[10]. Starch is a semi-crystalline polymer that composed of two repeating unit of 1,4-α-D-glucopyranosyl:amylose and amylopectin. Starch has different proportions of amylose and amylopectin ranging from about 10–20% amylose and 80–90% amylopectin depending on the source [11].The presence of amylase in the starch gives rise to stronger films. However disadvantages associated with starch based biodegradable films are attributed to the water solubility, brittle nature [12] and poor mechanical properties [13] of the starch films. In order to overcome these disadvantages starch can be modified by many methods such as blending with synthetic[12,14,15] and natural polymers [16,17].In the present work starch is blended with chitosan because of the several reasons which include: Chitosan is a natural, cationic, unique linear polysaccharide formed during the deacetylation of chitin in alkaline condition. Chitin is deacetylated in 40% sodium hydroxide at 120^0C for 1–3 h. This treatment produces 70% deacetylated chitosan. It comprises an unbranched chain consisting of β-(1→4) -2-amino-2-deoxy-D-glucopyranose [18].



Chitosan

Chitosan has excellent film-forming ability and antimicrobial properties. Chitosan is suitable for obtaining antimicrobial films[19]. Chitosan has attracted much attention in the field of biomaterials, because of its biological properties, biodegradability, bioactivity and biocompatibility [20, 21, 22]. The positive electrical charge of chitosan allows it to combine with all parts of the skin and hair with negative electrical charge, counts at its application in pharmaceutical, medical, and cosmetic industries [23]. It is used in shampoos, hair gels, as bacteria-inhibiting in tooth pastes and mouthwashes as well as a cationic film forming agent in skin care products [24] and in food coating and packaging [19,25].Bacteriostatic and bactericidal effect of chitosan is due to the binding of its positively charged amino ($-NH_3^+$) group to negatively charged carboxylate ($-COO^-$) group on the surface of bacteria cell wall [26]. Membrane disruption and leakage of cellular protein may be responsible for the antibacterial activity of the chitosan [27]. Film forming ability and antimicrobial property of chitosan is an important feature for food packaging. To prevent the loss of moisture from food material and reduce dripping from meats and fishes, coating of films on raw food/food product is a general way of food packaging. Antioxidant and antimicrobial coating of chitosan film can also restricts the flavor loss and foreign odor pick up [28, 29].

Starch and chitosan are considered to be an efficient food packaging material owing to their biodegradable, biocompatible, inexpensive, hydrophilic and non-toxic nature [30]. Complimentary characteristics of starch and chitosan not only attracted considerable attention in the area of food ingredients/packaging also attracted considerable attention in the area of biotechnology, biomedicine and cosmetics [31, 32]. Hydrogels of chitosan and starch are widely used for various purposes such as drug delivery and tissue engineering systems [33].

It is not wise to use chitosan and starch directly for hydrogel, native starch is poor in processability, also poor in the dimensional stability and mechanical properties for its end products [34], correspondingly the physico-mechanical properties of chitosan are moreover weak [35]. The mechanical properties of starch/chitosan films were improved by incorporating the other polysaccharides through blending [35] or by cross linking agents. Mixing of two or more polymers to produce blends is a well established strategy for achieving specific combination of physical properties [36] moreover it is an effective way to prepare compatible materials. Starch composite films with different polymers are extensively explored and the films obtained from chitosan and starch, was recommended for food packaging and medical applications [37]. Starch blend with chitosan construct mechanical stability with suppress crystallinity [38,39]. The presence of polysaccharide makes it biocompatible and biodegradable [35].

In present study, chitosan and starch were blended with glutaraldehyde as a crosslinking agent to form mechanically strong polymeric blend. Glutaraldehyde has been used more commonly than any other cross-linking reagent, because of its less expensive, readily available, and high solublility in aqueous solution [40] nature. The highly reactive aldehyde groups of glutaraldehyde readily form imine bonds (Schiff's base) with amino groups and acetal bonds with hydroxyl groups [41]. This bonding is responsible for efficient cross-linking of glutaraldehyde.

After blending of chitosan and starch with glutaraldehyde the antibacterial property of hydrogels was observed against two Gram-negative bacteria i.e. pathogenic bacteria and non pathogenic bacteria. The pathogenic bacterium is Salmonella enterica and the non pathogenic bacterium is Escherichia coli.

Salmonella enterica is a rod shaped, flagellated, gram-negative bacterium belongs to the genus Salmonella. Salmonellae are universally available. Salmonella is commonly responsible for food poisoning. All species of Salmonella can infect humans. Infections are usually contracted from sources such as:
Poultry, pork, beef and fish (seafood), if the meat is not properly vooked or is infected with the bacteria after preparation, infected eggs, egg products, tainted fruits and vegetables.

E.coli, Escherichia coli a germ, is a rod shaped, gram- negative bacterium commonly found in the lower intestine of warm blooded organisms. E. coli strains in majority are harmless, but some of them can cause food poisoning. It lives in the digestive tracts of humans and animals.

There are many types of E. coli, and most of them are harmless, but some (strain O157:H7) can cause bloody diarrhea, severe anemia, urinary tract infections or kidney failure, which can lead to death. E. coli infection can happen by coming into contact with the feces, or stool, of humans or animals or by drinking water or eating food that has been contaminated by feces.

II. Experimental

2.1 Polymers and reagents:

Chitosan from HIMEDIA; RM 9358, Acetic acid glacial, free aldehyde.A.R. from HIMEDIA, Glutaraldehyde from HIMEDIA,RM5927, Starch, extra pure from TITAN BIOTECH LTD. Parafilm "M" from Pechiney Plastic Packaging Chicago.

2.2 Preparation of chitosan/starch and glutaraldehyde solutions/blend:

Stock of 1% (v/v) acetic acid solution (aqueous) and 2% (v/v) glutaraldehyde solution (aqueous) were prepared by dissolving acetic acid and glutaraldehyde in distill water, simultaneously 2% (w/v) chitosan solution and 2% (w/v) starch solution were prepared by dissolving chitosan and starch in 1% acetic acid solution (aqueous) with the help of magnetic stirrer. 100ml chitosan and starch blend with 5:5 ratios (50ml of each) was prepared as reported earlier [42].The resultant polymeric blend were characterized by FTIR analysis.

2.3 Characterization of polymeric blend:

FTIR spectrum of the starch and starch-chitosan blend are shown in Fig.5 & 6. All characteristic peaks are present in the FTIR spectrum. Fig.7 shows the changes in FTIR of starch after blending with chitosan.

FTIR of Starch shows peak at 3282 for O-H stretching while at 2930 for C-H stretching and at 1412, 1340 for Bending of C-H and at 1076 & 994 for C-O stretching band.

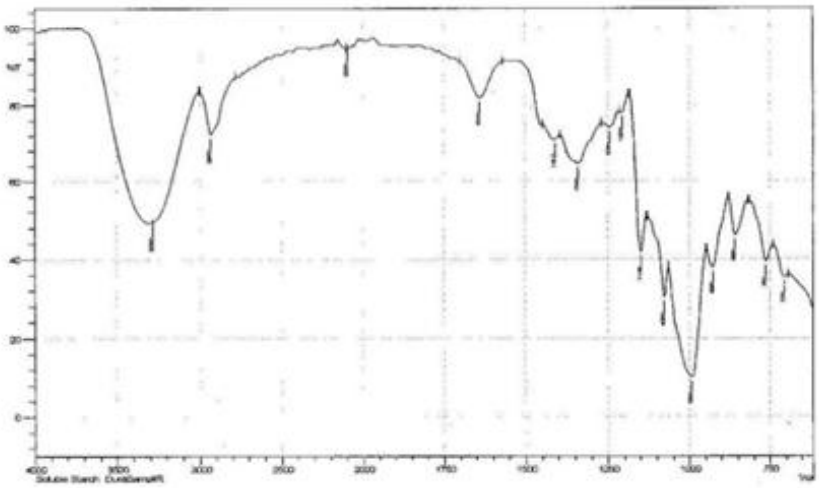


Fig.5 FTIR of soluble starch

When starch was blended with chitosan some significant new packs are arises at 1641.41for C=O stretching of carbonyl (I) and at 1546.91 for asymmetrical stretching of amide and some vital new bands are arises at 1344.38 and 1332.81 for Bending of C-H and band at 1246.02, 1205.51, and 1149.57 illustrate C-O stretching.

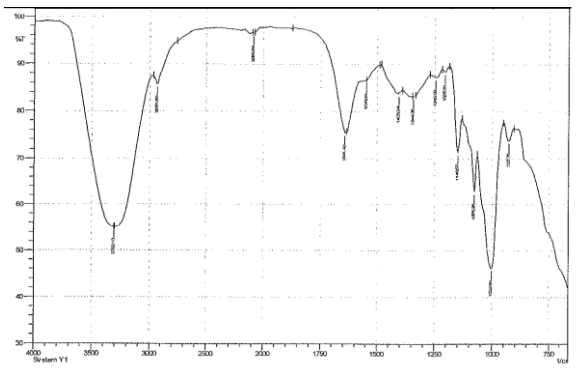


Fig.6 FTIR ofstarch and chitosan blend

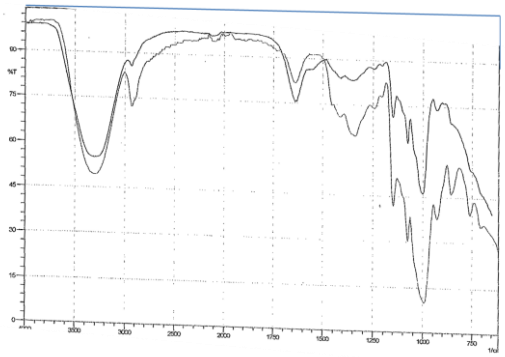


Fig.7 FTIR ofstarch and starch-chitosan blend

FTIR spectrum of the blend is not able to explains accurate relation between starch and chitosan, but the new peacks and bands arises in the spectrum (Fig.6, Fig.7) illustrate physical interaction between starch and chitosan.

2.4 Assay of antibacterial activity:

Disc diffusion method is used to check the antibacterial property of the blend. LB agar petriplates were prepared and test strain Salmonella enterica of 10^{-4} dilution were spread on two (A & B) petriplates of size 90 x 15 mm. 15 µl of each control i.e. 1% acetic acid solution, 2% glutaraldehyde solution, 2% chitosan solution, 2%

starch solution were poured on the discs in petriplate A. While in petriplate B polymeric blend along with its control i.e.1% acetic acid solution, 2% glutaraldehyde solution, 2% chitosan solution (except 2% starch solution) were placed on the disc in B. Petriplates were placed at 37^0C in incubater and zone of inhibition were observed after 24 hours. The effect of glutaraldehyde was minimized by drying and repeated washing of the blend.Same experiment was repeated with same dilution and same quantity of controls/blend with Escherichia coli bacteria and petriplates were named as C and D. To maintain the accuracy and precision all the experiments were performed in triplicate every after two or three day.

III. Result And Discussion

3.1 Antibacterial activity of polymeric blend(fig.8 to fig.12)

In petriplate A (controls containing petriplate i.e. 1% acetic acid solution, 2% glutaraldehyde solution, 2% starch solution and 2% chitosan solution with the strain Salmonella enterica) zone of inhibition was absent around discs containing 1% acetic acid solution and 2% starch solution while zone of inhibition was present around the discs containing 2% glutaraldehyde solution and 2% chitosan solution. Zone of inhibition ranges were 0.0-6.0 and 7.0-10.0mm in diameter respectively.

In petriplate B (strain of Salmonella enterica along with controls: 1% acetic acid solution, 2% glutaraldehyde solution, 2% chitosan solution and polymeric blend except 2% starch solution containing petriplate) zone of inhibition was absent around discs containing 1% acetic acid solution while zone of inhibition was present around the discs containing 2% glutaraldehyde solution, 2% chitosan solution and polymeric blend. Zone of inhibition ranges were 0.0-6.0, 7.0-10.0 and 8.0-9.0 mm in diameter respectively.

In petriplate C (controls contining petriplate i.e. 1% acetic acid solution, 2% glutaraldehyde solution, 2% starch solution and 2% chitosan solution with the strain Escherichia coli) Zone of inhibition was absent around discs containing 1% acetic acid solution and 2% starch solution while zone of inhibition was present around the discs containing 2% glutaraldehyde solution and 2% chitosan solution. Zone of inhibition ranges were 7.0-8.0 and 8.0-9.0 mm in diameter respectively.

In petriplate D (strain of E.coli along with controls: 1% acetic acid solution, 2% glutaraldehyde solution, 2% chitosan solution and polymeric blend except 2% starch solution contining petriplate) zone of inhibition was absent around discs containing 1% acetic acid solution while zone of inhibition was present around the discs containing 2% glutaraldehyde solution, 2% chitosan solution and polymeric blend. Zone of inhibition ranges were 7.0-8.0, 8.0-9.0 and 8.0-9.0 mm in diameter respectively.

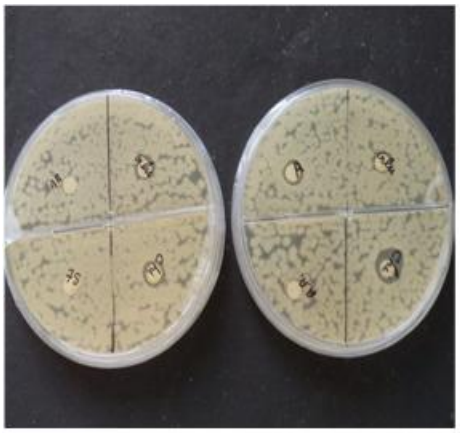


Fig 8: Petriplates A and B showing zone of inhibition for *S. enterica*

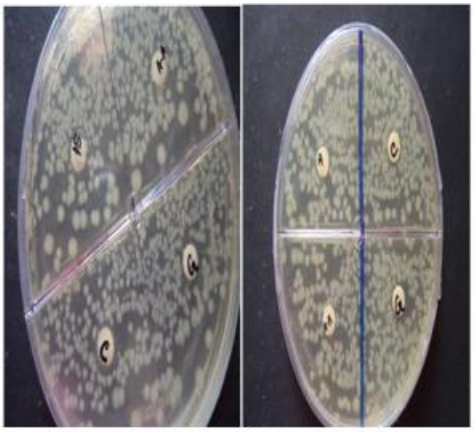


Fig 9: Petriplates C and D showing zone of inhibition for *E.coli*

Where: A = Blend
Chi/C = 2 % Chitosan Solution
Glu/G = 2 % Glutaraldehyde Solution
A.A = 1% Acetic Acid Solution
St/S = 2% Starch Solution

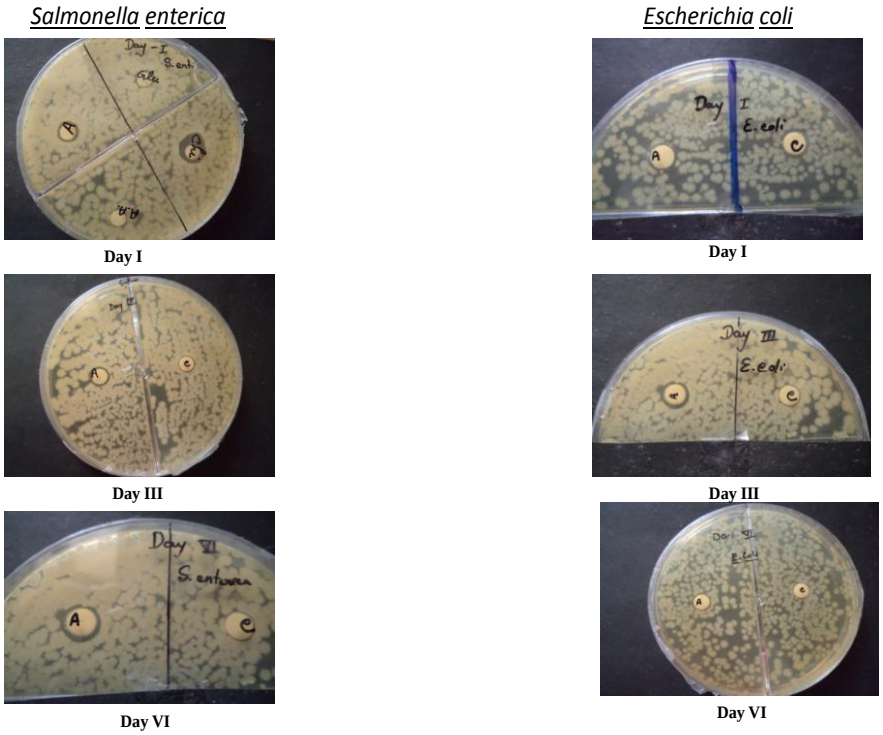


Fig.10: Efficacy Test of Blend and Chitosan Solution every after two or three day

Table 1: Zone of inhibition for Salmonella enterica and Escherichia coli.

Organisms	Diameter of zone of inhibition in mm.					
	Trials	1%A.A.	2%Glu	2%Chi	2%St	A (Blend)
Pathogenic bacteria						
Salmonella enterica	I	-	6.0	10.0	-	8.0
	II	-	6.0	8.0	-	8.0
	III	-	-	7.0	-	9.0
Non- pathogenic bacteria						
Escherichia coli	I	-	8.0	9.0	-	8.0
	II	-	7.0	8.0	-	9.0
	III	-	7.0	8.0	-	8.0

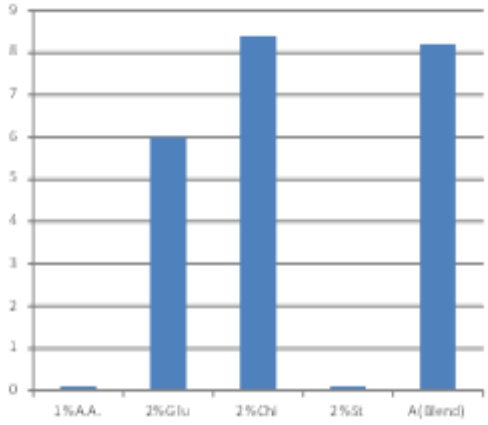


Fig 11: Petriplate A& B Showing Zone of Inhibition for *S. enterica*

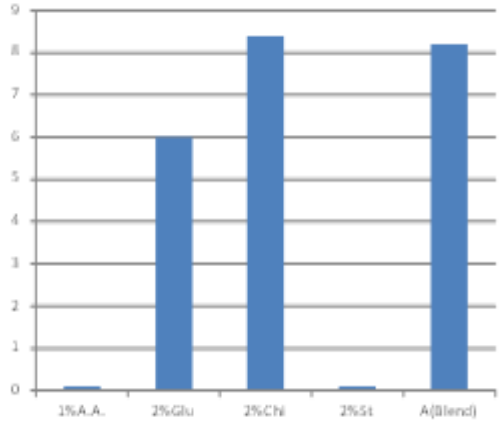


Fig 12: Petriplate C & D Showing Zone of Inhibition for *E.co*

Following observation can be concluded from the above experimental data

i. Acetic acid and starch solution does not show any activity against both the strains (Salmonella enterica and Escherichia coli).

ii. ii.Chitosan solution (2% with 1% acetic acid) shows the highest antimicrobial property (zone of inhibition range is 8.0-9.0 mm in diameter) for both the strains.

iii. iii.Blend/hydrogel also shows appreciable antibacterial property (zone of inhibition range is further same for both strains i.e. 8.0- 9.0 mm in diameter).
iv. Glutaraldehyde solution (2 % with 1% acetic acid) taken as a crosslinking agent in the blend also shows antimicrobial behavior (zone of inhibition range is 0.0-6.0mm in diameter for S. enterica and 7.0-8.0mm in diameter for E.coli).

As the effect of excess glutaraldehyde was minimized by repeated washing of the blend, therefore the antibacterial activity of the blend might be due to chitosan only.

On the basis of the above experimental data and results it can be concluded that antibacterial activity of the blend might be due to chitosan only. Results also predict that the antibacterial property of chitosan reduces day by day. It is due to the biodegradable nature of chitosan. It degrades day by day and reduces the antimicrobial property but, after blending/crosslinking with glutaraldehyde its antibacterial property remains unaffected.

IV. Conclusion

An antibacterial blend of starch and chitosan was prepared using glutarldehyde as crosslinking agent. Blending starch with chitosan in presence of gluteraldehyde as crosslinking agent preserves the biodegradibility of the starch and improves its poor mechanical properties The presence of high density amino and hydroxyl groups with inter and intra molecular hydrogen bonding gives a good film forming property to this antibacterial starch based blend.Incorporation of chitosan showed a good inhibitory property against the bacteria E.Coli and Salmonella Enterica. Starch blended with chitosan can be considered as an ideal candidate for food application specially in view of recent out breaks of contamination associated with food products and the negative environment impact of packaging materials currently in use.The food and drug administration (FDA) has approved the use of chitosan for certain food applications (edible films to protect foods) because of its environment friendly, biodegradability, antimicrobial activity, non-toxic and versatile chemical and physical properties.Antimicrobial packaging will become a potential packaging system which will kill or inhibit the pathogenic microorganism and thus will prevent the food from contamination and spoilage and hence extend the food shelf life leading to improvement of food quality.

References

[1]. Dutta, P. K., Tripathi**,** S., Mehrotra, G. K. and Dutta, J. (2009). Perspectives for chitosan based antimicrobial films in food applications. Food Chemistry, vol. 114 (4), 1173-1182.
[2]. Zhong, Q. P. and Xia, W. S. (2008). Physicochemical properties of Edible and Preservative Films from Chitosan/Cassava Starch/Gelatin Blend Plasticized with Glycerol. Food Technology and Biotechnology, vol. 46(3), 262–269.
[3]. Singla, A. K. and Chawla, M. (2001). Chitosan: Some pharmaceutical and biological aspects – an update. Journal of Pharmacy and Pharmacology, vol. 53, 1047–1067.
[4]. Ming, K., Chen, X. G., Xing, K. and Park, H. J. (2010). Antimicrobial properties of chitosan and mode of action: a state of the art review"International Journal of Food Microbiology, vol. 144, 51-63.
[5]. Xu, J., McCarthy, S. P. and Gross, R.A. (1996). Chitosan film acetylation and effects on biodegradability. Macromolecules, vol. 29, 3436-3440.
[6]. Araújo, M. A., Cunha, A. and Mota, M. (2004). Enzymatic degradation of starch-based thermoplastic compounds used in protheses: Identification of the degradation products in solution. Biomaterials, vol. 25, 2687–2693.
[7]. Teramoto, N., Motoyama, T., Yosomiya, R. and Shibata, M. (2003). Synthesis, thermal properties, and biodegradability of propyl-etherified starch. European Polymer Journal, vol. 39, pp. 255–261.
[8]. Zhang, J. F. and Sun, X. Z. (2004). Mechanical properties of PLA/starch composites compatibilized by maleic anhydride. Biomacromolecules, vol. 5, pp. 1446–1451.
[9]. Araújo, M. A., Cunha, A. and Mota, M. (2004). Enzymatic degradation of starch-based thermoplastic compounds used in protheses: Identification of the degradation products in solution. Biomaterials, vol. 25, pp. 2687–2693.
[10]. Fama, L., Rojas, A. M., Goyanes, S. and Gerschenson, L. (2005). Mechanical properties of tapioca-starch edible films containing sorbates. LWT –Food Science and Technology, vol. 38 (6), pp. 631–639.
[11]. Ramesh, M., Mitchell, J. R. and Harding, S. E. (1999). Amylose content of rice starch. Starch, vol. 51, pp. 311–313.
[12]. Wu, Q. X. and Zhang, L. N. (2001). Structure and properties of casting films blended with starch and waterborne polyurethane. Journal of Applied Polymer Science, vol. 79, pp. 2006–2013.
[13]. Mathew, S., Brahmakumar, M. and Abraham, T. E. (2006). Microstructural imaging and characterization of the mechanical, chemical, thermal, and swelling properties of starch-chitosan blend films. Biopolymers, vol. 82(2), pp. 176-87.
[14]. Arvanitoyannis. I., Biliaderis, C. G., Ogawa, H. and Kawasaki, N. (1998). Biodegradable Films made from Low Density Polythene (LDPE) rice star and potato starch for food packaging application. Carbohydrate polymers, vol. 36, pp. 89-104.
[15]. Bhattacharya, M. (1998). Stress relaxation of starch/synthetic polymer blends. Journal of Material Science, vol. 33(16), pp. 4131-4139.
[16]. Coffin, D. R., Fishman, M. L. and Cooke, P. H. (1995). Mechanical and Microstructural properties of pectin/starch films. Journal of Applied polymer Science, vol. 57(6), pp. 663-670.
[17]. Xu, Y. X., Kim, K. M., Hanna, M. A. and Nag, D. (2005). Chitosan-starch composite film: preparation and characterization. Industrial Crops and Products, vol. 21(2), pp. 185-192.
[18]. Bahrami, S. B., Kordestani, S. S., Mirzadeh, H. and Mansoori, P. (2003). Poly (vinyl alcohol) - Chitosan Blends:Preparation, Mechanical and Physical Properties Iranian Polymer Journal, vol. 12 (2), pp. 139-146
[19]. Dutta, P. K., Tripathi, S., Mehrotra, G. K. and Dutta, J. (2009). Perspectives for chitosan based antimicrobial films in food applications. Food Chemistry, vol. 114 (4), pp. 1173-1182.
[20]. Xu, J., McCarthy, S. P. and Gross, R. A. (1996). Chitosan film acetylation and effects on biodegradability. Macromolecules, vol. 29, pp. 3436-3440.

[21]. Li, Q., Grandmaison, E. W., Goosen, M. F. A. and Dunn, E. T. (1992). Applications and properties of chitosan. J Bioact Compat Polym., vol. 7(4), pp. 370-97.
[22]. Foda, N. H., El-Laithy, H. M. and Tadros, M. I. (2007). Implantable biodegradable sponges: effect of interpolymer complex formation of chitosan with gelatin on the release behavior of tramadol hydrochloride. Drug Dev Ind Pharm., vol. 33(1), pp. 7-17.
[23]. Chenite, A., Buschmann, M. D., Wang, D., Chaput, C. and Kandani, N. (2001). Rheological characterization of thermogelling chitosan/glycerol-phosphate solutions. Carbohydrate Polymers, vol. 46, pp. 39–47.
[24]. Kordestani, S. S. and Bahrami, S. B. (1997) Chitin polymer for medical applications, Proc. of the Int. Sem. On Polym. Sci. & Technology, ISPST-97, Iran Polymer Institute, Vol II, 742-747.
[25]. Niamsa, N. and Baimark, Y. (2009). Preparation and Characterization of Highly Flexible Chitosan Films for Use as Food Packaging.American Journal of Food Technology, vol.4, pp.162-169.
[26]. Friedman, M., and Juneja, V. K. (2010). Review of Antimicrobial and Antioxidative Activities of Chitosans in Food. Journal of Food Protection, vol. 73(9), pp. 1737-1761.
[27]. Qi, L. F., Xu, Z. R., Jiang, X., Hu, C. H., and Zou, X. F. (2004). Preparation and antibacterial activity of chitosan nanoparticles. Carbohydrate Research, vol. 339(16), pp. 2693-2700.
[28]. Gennadios, A., Hanna, M. A., and Kurth, L. B. (1997). Application of edible coatings on meats, poultry and seafoods: A review. Food Science and Technology –Lebensmittel -Wissenschaft & Technologie, vol. 30 (4), pp. 337-350.
[29]. Gomez-Estaca, J., Bravo, L., Gomez-Guillen, M. C., Aleman, A. and Montero, P. (2009). Antioxidant properties of tuna skin and bovine hide gelatin films induced by the addition of oregano and rosemary extracts. Food Chemistry, vol. 112(1), pp. 18-25.
[30]. Senay Akkus. Çetinus, Ebru S. and Saraydin, D. (2009). Preparation of Cu(II) adsorbed chitosan beads for catalase immobilization. Food Chemistry, vol. 114, pp. 962–969.
[31]. Singla, A. K. and Chawla, M. (2001). Chitosan: Some pharmaceutical and biological aspects – an update. Journal of Pharmacy and Pharmacology, vol. 53, pp. 1047–1067.
[32]. Kumari, K. and Rani, U. (2011). Controlled Release of Metformin hydrochloride through crosslinked blends of chitosan-starch. Pelagia Research Library Advances in Applied Science Research, vol. 2 (2), pp. 48-54.
[33]. Giri, T. K., Thakur, A., Alexander, A., Ajazuddin., Badwaik, H. and Tripathi, D. K. (2012). Modified chitosan hydrogels as drug delivery and tissue engineering systems: present status and applications. Acta Pharmaceutica Sinica B, vol. 2 (5), pp. 439-449.
[34]. Choi, E. J., Kim, C. H. and Park, J. K. (1999). Synthesis and characterization of starch-g-polycaprolactone copolymer. Macromolecules, vol. 32, pp. 7402–7408.
[35]. Svetlana, Z. R. and Galina, A. V. (2006). Polysaccharide-based polymer blends: Methods of their production. Glycoconj Journal, vol. 23, pp. 611–618.
[36]. Saravanan, D., Hemalatha, R. and Sudha, P. N. (2011). Synthesis and characterization of cross linked chitin/bentonite polymer blend and adsorption studies of Cu (II) and Cr (VI) on chitin. Scholars Research Library Der Pharma Chemica, vol. 3 (6), pp. 406-424.
[37]. Zhong, Q. P. and Xia, W. S. (2008). Physicochemical properties of Edible and Preservative Films from Chitosan/Cassava Starch/Gelatin Blend Plasticized with Glycerol. Food Technology and Biotechnology, vol. 46(3), pp. 262–269.
[38]. Bourtoom, T. and Chinnan, M.S. (2008). Preparation and properties of rice starchchitosan blend biodegradable film. LWT - Food Science Technology, vol. 41, pp. 1633-1641.
[39]. Zhai, M., Zhao, L., Yoshii, F. and Kume, T. (2004). Study on antibacterial starch/chitosan blend film formed under the action of irradiation. Carbohydrate Polymer, vol. 57, pp. 83–88.
[40]. Jayakrishnan, A. and Jameela, S. R. (1996). Glutaraldehyde as a fixative in bioprostheses and drug delivery matrices. Biomaterials, vol. 17, pp.471–484
[41]. Wang. T., Turhan, M. and Gunasekaran, S. (2004). Selected properties of pH-sensitive, biodegradable chitosan–poly(vinyl alcohol) hydrogel. Polymer Internatioal, vol. 53, pp.911–918.
[42]. Jaya, M. and Neena, S. (2014). Swelling behavior of starch chitosan polymeric blend. Advances in Polymer Science and Technology: An International Journal, ISSN 2277 – 7164, vol. 4(2).