iJRASET
International Journal For Research in
Applied Science and Engineering Technology

INTERNATIONAL JOURNAL FOR RESEARCH

IN APPLIED SCIENCE & ENGINEERING TECHNOLOGY

Volume: 7 **Issue:** X **Month of publication:** October 2019

DOI: http://doi.org/10.22214/ijraset.2019.10103

Food Packaging Formulation using Chitosan and Bacteriocin as an Antimicrobial Agent

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***Abstract:** Fruits are the major source of nutrients that are consumed as raw by most of the people across the country. Being nutritive in its value, most of the fruits have a high amount of water content which makes them vulnerable to microbial spoilage. Both bacterial and fungal contaminants are more common in fruit spoilage. Need to preserve them in its true form is necessary without the loss of nutritive values of fruits. Packaging of fruits with antimicrobial agents gives promising results. Chitosan-based packaging with certain bacteriocin has given satisfactory results for the preservation of fruits like papaya. Papaya packaged with chitosan-bacteriocin coated LDPE kept under refrigerated conditions was preserved for more than 3 days.*
***Keywords:** Chitosan, Bacteriocin, Preservation, LDPE, Spoilage.*

I. INTRODUCTION

Animal as well as Plant origin food both contribute a good source of nutrients to the human diet. The carbohydrates, proteins, vitamins, minerals, as well as essential amino acids, provide high biological values for human consumption. However, these foods are very much susceptible to chemical deterioration and microbiological spoilage due to which they possess a high risk to consumer's health along with the producer's losses. So for the prevention of such food, these need to be packaged with such material that can provide both protection from contaminants as well as to preserve food for a longer period. In recent times a new type of packaging has developed that combines food packaging materials along with antimicrobial substances to control and prevent surface contamination of food by microbes so that the food product safety is ensured in terms of, extended shelf-life [1], [3].

Many antimicrobial agents have been suggested and used as packaging materials like many natural antimicrobial agents examples, bacteriocins (nisin), enzymes (lysozyme, naringinase), phytochemicals, essentials oils (thymus essential oils, cumin, fennel, laurel, mint, oregano, etc.), microbial chitosan. These antimicrobials are being incorporated into packaging films and developing packaging materials. But nowadays trend has been a shift from synthetic polymers to biopolymers. Biopolymers are defined as such polymers which are originated from living beings which may have a combination of many molecules. Such biopolymers are chitin, chitosan, alginates, collagen, etc [1].

Of many suggested bio-polymer, Chitin is the second most widely used biopolymer found in nature and chitosan-based biopolymer is being used widely nowadays. Chitosan, a modified and natural carbohydrate polymer derived from chitin by the process of deacetylation of chitin [2]. Chitin is generally obtained from shells of crustaceans such as crab, shrimps but mostly obtained from microbial cell walls mainly fungi.

Chitosan is water-insoluble and weakly soluble in organic acid solution. It has attracted notable interest and understanding in edible food packaging of food materials due to its biological activities as an antimicrobial agent. Many functional properties are associated with chitosan like polyoxysalt formation, the formation of a film, chelating metal and optical structural characteristics [2], [4].

Chitosan also offers other benefits making it suitable for coating like anti-tumor and hypocholesterolemic functions. Due to the antimicrobial activity of chitosan, it is used against a range of foodborne fungi, yeast, bacteria. Apart from its antimicrobial property, it also improves the storage ability of perishable food just modifying food internal atmosphere as well as decreasing the transpiration losses. One major factor for choosing chitosan is for the growing consumer for food safety and its preservation without chemical preservations. Chitosan received US FDA approval for GRAS status as a food additive and also its application in the food system [3].

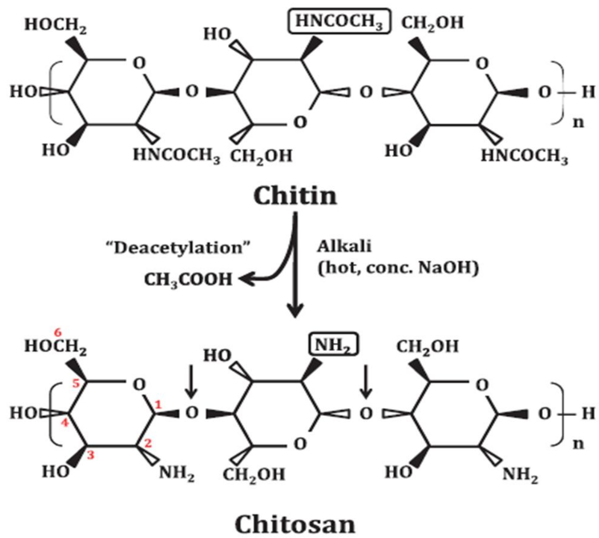
Yet another antimicrobial substance can use for food packaging materials. Bacteriocins obtain from various lactic acid-producing bacteria (LAB). By nature, bacteriocins are proteinaceous, short inhibitory peptides. The role is to inhibit the growth of related bacteria. These bacteriocins behave as antibiotic-like compounds. The names given to bacteriocins depend on bacteria that produce them like Colicin which is produced by Escherichia coli, pyocin by Pseudomonas pyocynea, Megacin from Bacillus megaterium, Dipthericins from Corynebacterium diphtheria [5].

International Journal for Research in Applied Science & Engineering Technology (IJRASET)
ISSN: 2321-9653; IC Value: 45.98; SJ Impact Factor: 7.177
Volume 7 Issue X, Oct 2019- Available at www.ijraset.com

II. LITERATURE SURVEY

A. *Chitosan: a Polysaccharide*

In recent years chitosan application in the food sector and medical sector has gained a huge success due to its biodegradability, biocompatibility, non-toxicity and wide spectrum of anti-microbial activity [2], [3], [7], [6]. Chitosan a polysaccharide made from repeating units of N-acetyl-D-glucosamine and D-glucosamine residues which are linked by β-1,4 glycosidic bonds [1], [3], [4], [7]. Many functional properties are associated with chitosan like polyoxysalt formation, the formation of a film, chelating metal and optical structural characteristics [6]. Chitosan producing strains *Aspergillus niger*, *Rhizopus oryzae*, *Zygosacharomyces rouxii* and many more.



Structure of chitosan [8]

B. *Chemical Properties of Chitosan*

1) A linear polyamine [9].
2) It has a reactive amino group ($-NH_2$) [9].
3) Availability of reactive hydroxyl groups (-OH) [9].
4) The chelating ability for many transitional metal ions [9].
5) Water-insoluble and little soluble in weak acids like acetic acid [2].
6) Solubility of chitosan depends on the degree of deacetylation, molecular weight, distribution of acetyl groups and nature of acids used for protonation [10].

C. *Biological Properties of Chitosan*

1) It is biocompatible due to [9]:
   a) Being a natural polymer
   b) Non-toxic to human and safe
   c) Bio-degradation property with the normal body constituents
2) It binds aggressively with mammalian and microbial cells [9].
3) Regenerative capacity of chitosan on connective gum tissue [9].
4) It helps in accelerating the formation of the osteoblast which is responsible for bone formation [9].
5) It helps in hemostasis [9].
6) Act as a fungistatic agent [9].
7) It helps in maintaining birth control [9].
8) Act as an antitumor or anticancer agent [9].
9) Act as an anticholesteremic agent [9].
10) It also slows down brain activity [9].
11) Given as immunoadjuvant [9].
12) It is an antimicrobial agent against foodborne pathogens like Escherichia coli, Bacillus cereus, Listeria monocytogenes, Staphylococcus aureus [10].

D. Chitosan Producing Strains

Sr. No.	Strain used	Medium used	Inoculum size	Incubation condition	Production of chitosan	References
1	Serratia sp	yeast extract, ammonium sulfate, potassium dihydrogen phosphate, chitin	1 mL 0.1 OD_{600} suspension	Liquid state fermentation at 25˚C, pH: 8.0 in rotatory incubator for 2 days.	0.1 g L^{-1}	Kaur, Dattajirao, Shrivastava, Bhardwaj (2012) [3]
2	Bacillus sp	yeast extract, ammonium sulfate, potassium dihydrogen phosphate, chitin	1 mL 0.1 OD_{600} suspension	Liquid state fermentation at 25˚C, pH: 8.0 in rotatory incubator for 2 days.	0.16 g L^{-1}	Kaur, Dattajirao, Shrivastava, Bhardwaj (2012) [3].
3	Aspergillus niger TISTR3245	Potato Dextrose Broth	1 mL spore suspension (10^7 spores m^{-1})	Liquid state fermentation at 30˚C, 180 rev min^{-1} for 15-21 days.	107 mg g^{-1}	Pochanavanich, Suntornsuk (2002) [3].
4	Rhizopus oryzae TISTR3189	Potato Dextrose Broth	1 mL spore suspension (10^7 spores m^{-1})	Liquid state fermentation at 30˚C, 180 rev min^{-1} for 15-21 days.	138 mg g^{-1}	Pochanavanich, Suntornsuk (2002) [7].
5	Lentinus edodes no. 1	Potato Dextrose Broth	1 mL spore suspension (10^7 spores m^{-1})	Liquid state fermentation at 30˚C, 180 rev min^{-1} for 15-21 days.	33 mg g^{-1}	Pochanavanich, Suntornsuk (2002) [7].
6	Pleurotus sajo-caju no. 2	Potato Dextrose Broth	1 mL spore suspension (10^7 spores m^{-1})	Liquid state fermentation at 30˚C, 180 rev min^{-1} for 15-21 days.	12 mg g^{-1}	Pochanavanich, Suntornsuk (2002) [7].
7	Zygosaccharomyces rouxii TISTR5058	Yeast Malt extract Broth	1 mL spore suspension (10^7 spores m^{-1})	Liquid state fermentation at 30˚C, 180 rev min^{-1} for 6 days.	36 mg g^{-1}	Pochanavanich, Suntornsuk (2002) [7].
8	Candida albicans TISTR5239	Yeast Malt extract Broth	1 mL spore suspension (10^7 spores m^{-1})	Liquid state fermentation at 30˚C, 180 rev min^{-1} for 6 days.	44 mg g^{-1}	Pochanavanich, Suntornsuk (2002) [7].
9	Aspergillus niger	Soya beans	Solid-state fermentation (SSF) and Submerged fermentation (SMF): 1 mL spore suspension (3 x10^6 spores/mL).	SSF: incubated at 30˚C for 16 days. SMF: incubated at 30˚C for 16 days.	SSF: 6.18/kg SMF: 0.16 g/L	S., D., M.R. Gandhi (2014) [11].
10	Rhizopus arrhizus	Corn steep liquor and honey			29.3 mg g^{-1}	Cardoso et al. (2012) [12]
11	Cunninghamella bertholletiae	Sugar cane juice and molasses			128 mg g^{-1}	Cardoso et al. (2012) [12]
12	Aspergillus niger	soybean meal and canola oil			120.5 mg g^{-1}	Cardoso et al. (2012) [12]
13	Absidia corymbifera	corn steep liquor with heavy metals		Sub-merged fermentation.	67.29 mg g^{-1}	Cardoso et al. (2012) [12]

		Cu^{+} and Zn^{+}				
14	Mucor rouxii RCMB 015002	Yeast Peptone Glucose	106 spores/mL	Incubated at 28 ˚C ± 2, pH 5.0 and 125 rpm of shaking for 7 days	35 mg/mL	Gharieb, El-Sabbagh, Shalaby, Darwesh (2015) [13].
15	Cunninghamella elegans RCMB 012002	Yeast Peptone Glucose	106 spores/mL	Incubated at 28 ˚C ± 2, pH 5.0 and 125 rpm of shaking for 7 days	29 mg/mL	Gharieb, El-Sabbagh, Shalaby, Darwesh (2015) [13].
16	Rhizopus sp	Yeast Peptone Glucose	106 spores/mL	Incubated at 28 ˚C ± 2, pH 5.0 and 125 rpm of shaking for 7 days	13 mg/ 50 mL	Gharieb, El-Sabbagh, Shalaby, Darwesh (2015) [13].
17	Aspergillus niger MTCC 872	Potato Dextrose Broth, D-Glucose, L-Asparagine, Thiamine.	1 mL of 10^{-7} spores/mL	Incubate in an orbital shaker incubator at 30°C with 180 rpm for 120 hours	23.1 %	Kumaresapillai, Basha, Sathish (2011) [14].
18	Aspergillus niger MTCC 1785	Potato Dextrose Broth, D-Glucose, L-Asparagine, Thiamine.	1 mL of 10^{-7} spores/mL	Incubate in an orbital shaker incubator at 30°C with 180 rpm for 120 hours	25.2 %	Kumaresapillai, Basha, Sathish (2011) [14].
19	Aspergillus niger MTCC 2208	Potato Dextrose Broth, D-Glucose, L-Asparagine, Thiamine.	1 mL of 10^{-7} spores/mL	Incubate in an orbital shaker incubator at 30°C with 180 rpm for 120 hours	26.1 %	Kumaresapillai, Basha, Sathish (2011) [14].

E. Application of chitosan-based films

Chitosan with Bacteriocin like nisin as antimicrobial agent provides a protective barrier by reducing the respiration rates and control the growth of the microbes. It preserves the natural color of the food even on the storage and helps in prolonging the shelf life of many fruits like table grapes, pear, peppers, tomatoes, lotus roots, mushrooms and red pitayas [15]-[17].

Chien *et al* [18] applied the chitosan coating of low molecular weight to keep the quality and to extend the shelf life cut red pitayas. This was due to reduce sensory effects that maintain the content of soluble solids, titratable acidity and the ascorbic acid content in the fruits. The coating also reduced the growth of microorganisms.

The demonstration of the combination of chitosan-ascorbic acid improved the quality of the litchi fruit on preservation.

Treatment of chitosan to guava fruit could significantly control and delay the loss of firmness and MDA content and chlorophyll change was suggested by Hong et al. [19].

Reports from Gao et al [20] that complex of chitosan and glucose was effective in inhibiting the aging and diseases of table grapes. The complex could decrease the respiration rate, POD and SOD activities in fruits.

The application of chitosan coating along with Origanum vulgare L. oil was effective for inhibiting the fungal growth on the grapes.

Control of food spoilage, as well as pathogenic microorganisms, can be achieved by incorporating Bacteriocin in packaging films. The nisin can be incorporated in proteins to make biodegradable films and another polyethylene plastic film incorporated with nisin can be based used as a packaging film for the preservation purpose [21].

The preservation of pineapple fruit was demonstrated by Ibramin et al [22]. The pineapple was treated with chitosan and on storage no fungal growth was observed.

Dipping of orange in chitosan solution decreases the weight loss and green tea and chitosan used in combination was useful in the reduction of fruit decay, chilling injury and its POD activity [23].

Chitosan inhibits degrading enzymes against the cell wall of carambola fruits after 12 days of storage [24].

Chitosan-Pichia inhibits the infection of fungi Colletotrichum gloeosporioides on citrus fruits by controlling the mycelial and spore germination growth [25].

Composite coating of chitosan with LAE, cinnamon oil or EDTA can control the growth of foodborne pathogens on cantaloupes during storage [26].

Pushkala et al [27] investigated the chitosan effect on controlling the spoilage of shredded radish and increasing the shelf life.

F. Bacteriocin

Natural peptides that compete with the bad microbes and to eliminate them are bacteriocin. The structure of bacteriocins is quite complicated and these are synthesized by ribosomes and some of these peptides are highly modified after the post transcription process [28].

G. Functions of Bacteriocins

1) *Function as Colonizing Peptides:* A high number of bacteriocin producing bacteria is associated with the GI tract results nearby of a cell to cell contact with the same or the different species of microbes just promoting co-operative or antagonistic interaction. Production of some antimicrobials provides a mechanism by which the producers get to win the competition and kills the other microbes which tend to cause disease [29].
2) *Function as Killing Peptides:* The peptides produce against the closely related microbes tend to get change concerning the type of microbes. Gram-positive bacteria producing the bacteriocin will not be able to show its effect well on gram-negative bacteria, whereas, gram-negative producers tend to kill the pathogens faster than the gram-positive producers [29].
3) *Function As Signaling Peptides:* Communication is done with the help pf signaling molecules like histidine protein kinase (HPK) located on the cell membrane of gram-negative. Communication is extracellular and allows bacteria to synchronize the group behavior and helps to coordinate for multicellular function [29]

H. Bacteriocin Producing Strain

Lactobacillus lacti [30], Lactobacillus acidophilus [30], Lactobacillus sporogenes [30], Lactobacillus casei [30], Lactobacillus brevis [30], Lactobacillus delbruckii [30], Lactobacillus lactis [30], Streptococcus faecalis [31], Streptococcus cremoris [31], Streptococcus lactis [31], Streptococcus thermophilis [31], Streptococcus intermedius [31], Streptococcus lactis [31], Leuconostoc mesenteroids [31].

I. Nisin- A Bacteriocin

Bacteriocins are considered to be a safe natural peptide that is used as bio-preservative. The property of being bio-preservative is because of being sensitive to proteases that are found in the gastrointestinal tract. So these are effective in killing and controlling the food-borne pathogens.

Among all the bacteriocin, nisin is regarded as safe by the FDA for preservation purposes [32]. Nisin, a bacteriocin produced by Lactococcus lactis subsp. Lactis has a molecular weight of 3.5 kDa. The mode of action by which nisin acts is by creating pores in the membrane of the food-borne pathogens [25].

http://www.scielo.br/img/revistas/babt/v50n3/18f1.gif

Diagram 2: Structure of nisin

J. Different Strategies for Making a chitosan-bacteriocin Film

As used for packaging food, many methods were used for the preparation of the film. These were direct casting, coating, spread coating, spray coating, dipping or immersing, layer-by-layer assembly, extrusion.

1) *Direct Coating:* It is a simple method where chitosan was dissolved in acid solution and then blending, composting or cross-linking with other functional material like bacteriocin at different concentrations. The mixture was stirred for many hours and then cast on polystyrene tray or Teflon plates. The plates were kept in a hot air oven, convection oven, and vacuum oven for drying purposes. An additional step was done to keep the film for long dryings like the use of a humidity chamber or desiccator [32]. The technique showed overall enhanced properties of the film when done with the programmed formulation. This technique showed a promising feature for the packaging of food to ensure its safety.
2) *Coating:* A method where chitosan is mixed with acid solution and then functional material like bacteriocin or any other antimicrobial agent is used and then applied as thin film on the food material. The coating solution prepared can be applied to low-density polyethylene (LDPE) and food is wrapped with it [32].
3) *Dipping: or Immersing:* In this, the solution of chitosan mixed with an acid solution is blended with functional material and the solution is cleared by filtration using a muslin cloth or by use of centrifugation and then food is dipped in the solution and then air-dried. The process is simple with less equipment and is not very sophisticated [32].
4) *Layer-By-Layer Assembly:* The chitosan solution was prepared and then food material is dipped and air-dried and again dipped in the solution and again air-dried and so on for 3-4 times [32].

K. Chitosan-based Packaging Effect on Different Fruits and Food Materials

Sr. No.	Food material	Chitosan effect	Chitosan combination	References
1	Bread	Showed retardation in starch retrograding, hardness and better shelf life of about 36 hours.	1% chitosan treated with baguette	No H.K., Meyers S.P., Z. Ku (2007) [2]
2	Egg	An offered protective barrier for moisture, gas transfer from the albumin through eggshell which helps in extending the life of the egg. 2% of chitosan found to be useful in reducing weight loss and obtaining more albumin content up to 5 weeks at 25˚C.	2 % chitosan	No H.K., Meyers S.P., Z. Ku (2007) [2]
3	Fruits and vegetables	Modify internal atmosphere without anaerobic respiration as chitosan coating is permeable to O_2 than to CO_2 just act as fungicide and have prolonged shelf life.	Chitosan	No H.K., Meyers S.P., Z. Ku (2007) [2]
4	Fruit juices	Act as clarifying agent	Chitosan hydrolyzed with 7% acetic acid	No H.K., Meyers S.P., Z. Ku (2007) [2]
5	Kimchi	Delay softening of kimchi tissue during fermentation and increase storage up to 10 days when 0.1-0.5% used.	0.1% or 0.15% chitosan	No H.K., Meyers S.P., Z. Ku (2007) [2]
6	Meat	Chitosan along with nitrite and *Lactobacillus plantarum* has a desirable development of red color.	0.5% to 1% chitosan	No H.K., Meyers S.P., Z. Ku (2007) [2]
7	Seafood and its product	The antioxidant property of chitosan enhances the storage period.	Chitosan	No H.K., Meyers S.P., Z. Ku (2007) [2]
8	Fruits and vegetables	Prevention of enzymatic browning due to oxidation	Chitosan with phytic acid	Jianglian, Shaoying. (2013) [33]
9	Fruits and vegetables	Reduces fungal contamination and maintaining firmness	Chitosan with metal ions (Ca^{2+}, Zn^{2+}, Ce)	Jianglian, Shaoying. (2013) [33]
10	Strawberries	Antifungal activity during cold storages	Chitosan with essential oils like terpenes, terpenoids.	Jianglian, Shaoying. (2013) [33]
11	Fruits	Delay decay incidence, weight loss, respiration rate	Chitosan with inorganic nanomaterials (nano-ZnO, nano-silica, nano-$CaCO_3$)	Jianglian, Shaoying. (2013) [33]
12	Brinjal	Delay signs of aging, the formation of wrinkles, softening of the skin texture, reduction in weight and changes in color	Chitosan	Prabha A., Sivakumar K. (2015). [4]
13	Capsicum	Delay signs of aging, the formation of wrinkles, softening of the skin texture, reduction in weight and changes in color	Chitosan	Prabha A., Sivakumar K. (2015) [4]
14	Tomatoes	Delay ripening and reduced decay incidence	Chitosan	Prabha A., Sivakumar

International Journal for Research in Applied Science & Engineering Technology (IJRASET)
ISSN: 2321-9653; IC Value: 45.98; SJ Impact Factor: 7.177
Volume 7 Issue X, Oct 2019- Available at www.ijraset.com

				K. (2015) [4]
15	Roast beef	Inhibit *Listeria monocytogenes*	Chitosan (high or low molecular weight) coatings dissolved in lactic or acetic acid	Sanchez-Ortega *et al* (2014) [1]
16	Codfish fillets	Some food pathogens and spoilage bacteria can be reduced effectively	Gelatin-chitosan films with clove, fennel, cypress, lavender, thyme, herb of the cross pine, rosemary	Sanchez-Ortega *et al* (2014) [1]
17	Rainbow trout	The extended shelf life of trouts about 16 days at 4˚C	Chitosan films (2 % w/v) with cinnamon ess. Oil (1.5 % v/v)	Sanchez-Ortega *et al* (2014) [1]
18	Carrot sticks	Extended shelf life about 12 days at 4˚ C	Chitosan edible films (0.05 mL/mL) under MAP conditions	Sanchez-Ortega *et al* (2014) [1]
19	Fresh cut cantaloupe	Extended the shelf life up to 9 days	Chitosan edible films with trans-cinnamaldehyde and pectin	Sanchez-Ortega *et al* (2014) [1]
20	Strawberries	High anti-Botyrtis effect	Chitosan (1% w/w) with lemon ess oil 3% (v/w)	Sanchez-Ortega *et al* (2014) [1]
21	Sliced bread	Showed anti-fungal effect	Chitosan composite films with grapefruit seed extract (0.5-1.5% w/w)	Sanchez-Ortega *et al* (2014) [1]
22	Agar media	Decreased viable cell counts for *Staphylococcus aureus* and *Bacillus cereus* significantly	Alginate or Chitosan films with garlic ess. Oil	Sanchez-Ortega *et al* (2014) [1]
23	Agar media	Shows antibacterial activity against gram-positive food pathogens	Chitosan-PVA films with mint extract/pomegranate peel extract	Sanchez-Ortega *et al* (2014) [1]
24	Pork meat hamburgers	Reduction of mesophilic microorganisms as well as coliforms	High molecular weight chitosan (1% w/v), acetic acid (1% w/v), lactic acid (1% w/v) films and sunflower oil	Sanchez-Ortega *et al* (2014) [1]
25	Pork sausages	Inhibit yeast and molds	Chitosan films with green tea extract 20% (w/v)	Sanchez-Ortega *et al* (2014) [1]
26	Roasted turkey	Reduction/inhibition of *Listeria monocytogenes* during storage	Chitosan, starch, alginate, or pectin coating, sodium lactate, sodium diacetate	Sanchez-Ortega *et al* (2014) [1]
27	Chicken breast fillets	Extended the shelf life of chicken fillet up to 6-21 days	Chitosan coating, deacetylation degree of 75-85% with oregano oil 0.25% v/w (OO)	Sanchez-Ortega *et al* (2014) [1]
28	Chitosan breast fillets	Reduction and maintaining low microbial fillets of mesophiles, psychrophiles, yeast, molds, *Pseudomonas,* coliforms, LAB, and hydrogen sulfide-producing bacteria	Chitosan films, Chitosan-LAE	Sanchez-Ortega *et al* (2014) [1]
29	Sliced turkey deli meat	Inhibit *Listeria innocua*	Chitosan (2-5% w/w) films with 2% solution of either acetic, lactic or levulinic acids, lauric arginate, nisin	Sanchez-Ortega *et al* (2014) [1]
30	Atlantic cod and herring	Reduces the number of psychrotrophic microorganisms	Chitosan films	Sanchez-Ortega *et al* (2014) [1]
31	Sea bass	Reduces total mesophilic bacteria and psychrotrophic aerobic bacteria	Chitosan films	Sanchez-Ortega *et al* (2014) [1]
32	Cod	Reduces total bacterial count, H_2S-producers organisms, luminescent organisms, *Pseudomonas,* Enterobacteriaceae and lactic acid bacteria	Gelatin with combination of chitosan, clove essential oil	Sanchez-Ortega *et al* (2014) [1]
33	Indian oil sardine	Reduced mesophilic microorganisms	Chitosan (1 and 2% w/v) coating	Sanchez-Ortega *et al* (2014) [1]

L. Mode of action of Chitosan and Bacteriocin

Chitosan-based coating with bacteriocin of food material inhibits or kills spoilage/pathogenic microorganisms by various mechanisms such as [34]

1) Damage of cell wall due to electrostatic interaction of chitosan-bacteriocin with the cell wall of the target cell
2) Changing the permeability of the cytoplasmic membrane of the target cell
3) In the cytoplasm, the generation of ROS takes place with lead to toxicity of target cell
4) These antimicrobial agents enter the nucleus and damage DNA
5) Proteins also get damage
6) The collapse of the outer membrane of the cell wall

Due to all the above changes in the target cell, ultimately it leads to the death of the cell.

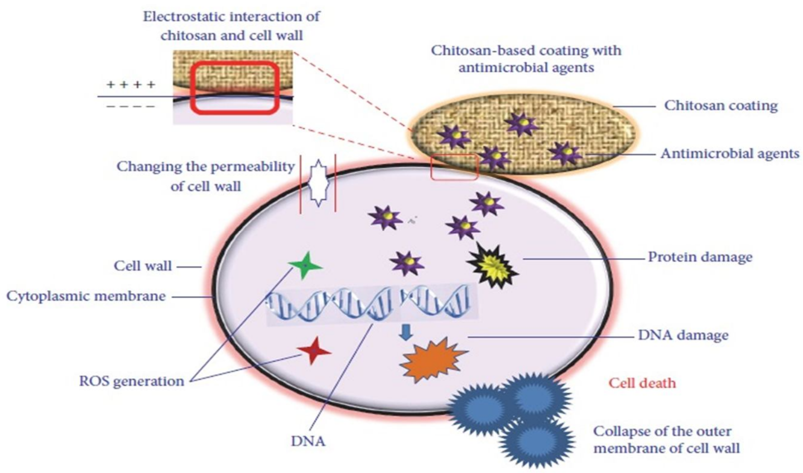


Figure-3: Mode of action of chitosan-bacteriocin

III. CONCLUSION

In recent years chitosan application in the food sector and medical sector has gained a huge success due to its biodegradability, biocompatibility, non-toxicity and wide spectrum of anti-microbial activity. Chitosan a polysaccharide made from repeating units of N-acetyl-D-glucosamine and D-glucosamine residues which are linked by β-1,4 glycosidic bonds. Many functional properties are associated with chitosan like polyoxysalt formation, the formation of a film, chelating metal, and optical structural characteristics.

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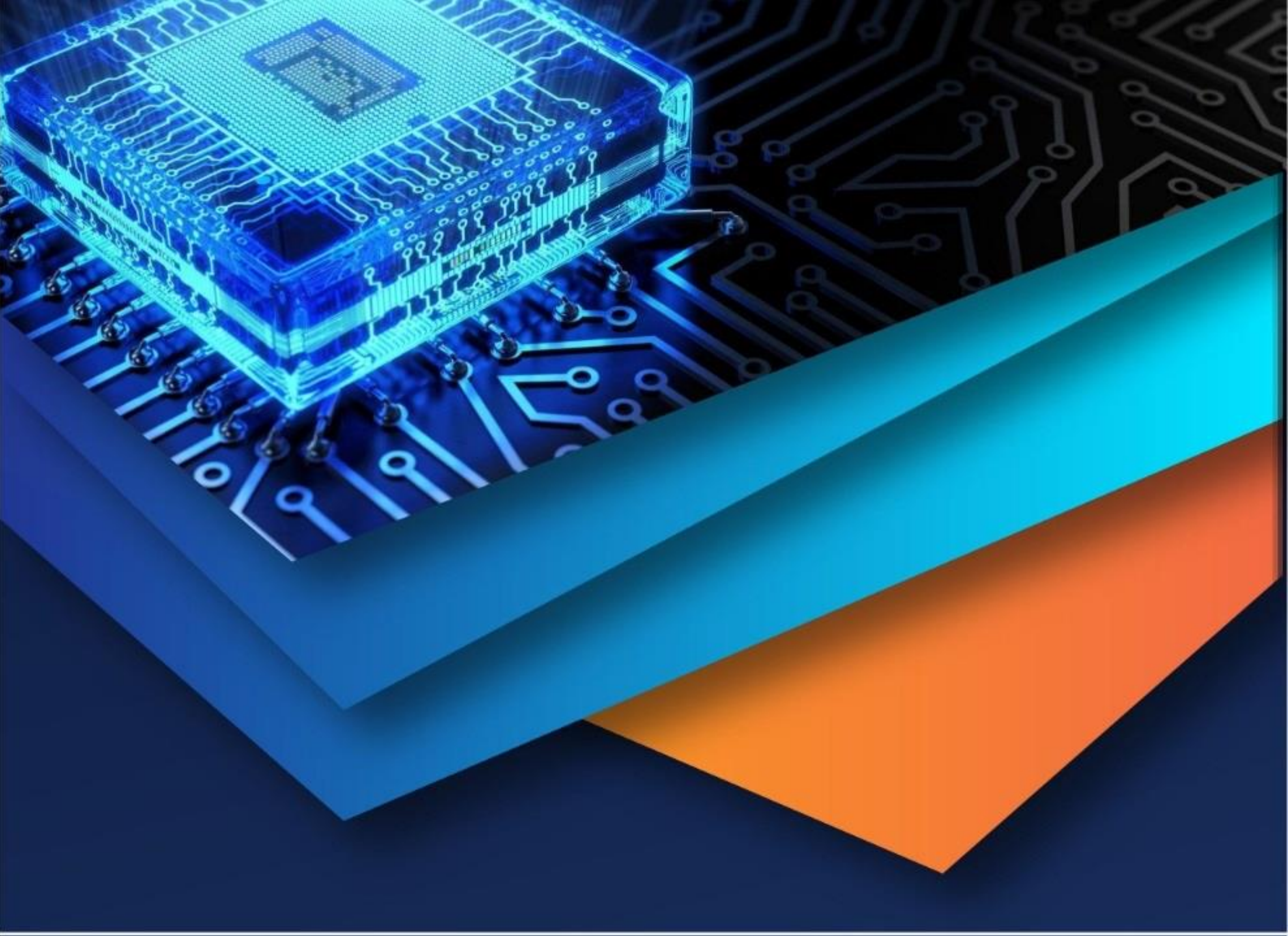

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