EXTRACTION OF CHITOSAN FROM SEAFOOD WASTE BY BIOLOGICAL METHOD AND ITS APPLICATION IN ENHANCEMENT OF SHELF LIFE AND QUALITY OF DAIRY PRODUCTS - A REVIEW

Shruti S. Gawde[1], Anupama Tomar[2]

[1]Student, [2]Professor

Department of Microbiology and Biotechnology,

Chikitsak Samuha's S.S & L.S Patkar- Varde College, Goregaon, Mumbai, India.

ABSTRACT- Now-a-days, seafood processing industries produce large amount of waste like skin, tail, shells, etc. These waste products may often encompass many important bioactive and industrially important products. If this waste is not managed properly it leads to negative impact on earth's environment. Chitin is a linear polysaccharide of the amino sugar N-acetyl glucosamine. It is present in the extracellular matrix of a variety of invertebrates including sponges, molluscs, nematodes and arthropods and fungi. Chitosan is a modified, natural biopolymer derived by deacetylation of chitin, a major component of the shells of crustacean. Recently, chitosan has made its prominent place in pharmaceutical, medical, biomaterial and food industry. Use of chitosan in food industry is readily seen due to its several distinctive biological activities and functional properties. The antimicrobial activity and film-forming property of chitosan make it a potential source of food preservative or coating material of natural origin. This review focuses on the extraction of chitin from seafood waste produced by seafood processing industry using microorganisms, converting chitin to chitosan, and using chitosan as a preservative agent to improve shelf life and quality of dairy products.

KEYWORDS- seafood waste, chitin, chitosan, dairy products, shelf-life, quality.

INTRODUCTION:

Seafood waste often contains a huge amount of chitin, a polysaccharide that exhibits exceptional inherent characteristics including biocompatibility, biodegradability, antimicrobial, antitumor and antioxidant activities. Chitin is a best candidate as food preservative as it is an odourless, tasteless, non-toxic and eco-friendly natural product exhibiting antimicrobial action.

With growing population, waste generation is also increasing, and major proportion of by-products generated by contemporary food remains underutilized which may often contain high-value substances. Crucial problem faced by industries and society during food processing is disposal of food waste. Around 10^{12}–10^{14} tons of chitin are produced annually by living organisms in ocean, out of which 2.8×10^{10} kg

is generated by arthropods in freshwater and 1.3×10^{12} kg in marine environment. This huge quantity of chitin would provide enough raw material, if commercial procedures were developed for extraction of commercially competent polymers. Habitually, seafood waste is burned, land filled, dumped at sea or left to get spoiled. If not processed properly, it may have a negative impact on human health, biodiversity and environment. **(Monika Yadav, 2019)**

Following cellulose, chitin is the second most abundant polysaccharide. Chitin is converted into its deacetylated form, i.e., chitosan on a commercial scale. Chitin and chitosan both have enormous economic value because of their flexible biological properties. Crystallinity and insolubility of chitin demote its commercial applications. Conversion of chitin into derivatives viz. chitosan, chito-oligosaccharides and glucosamine augment its biological properties and applications in agriculture, food, textile, medical and cosmetic industries.

Chitin is the most abundant polysaccharide in the marine ecosystem and second in nature after cellulose. Various sources of chitin in marine have been summarized in Fig. 1. Chitin, in nature, is present in three different types of crystalline forms α, β and γ and varies in degree of deacetylation. The primary sources of chitin are the crustacean shells obtained from the shellfish processing businesses. These crustaceans include crabs, shrimps, lobsters and krill. **(Leen Bastiaens, 2020)**

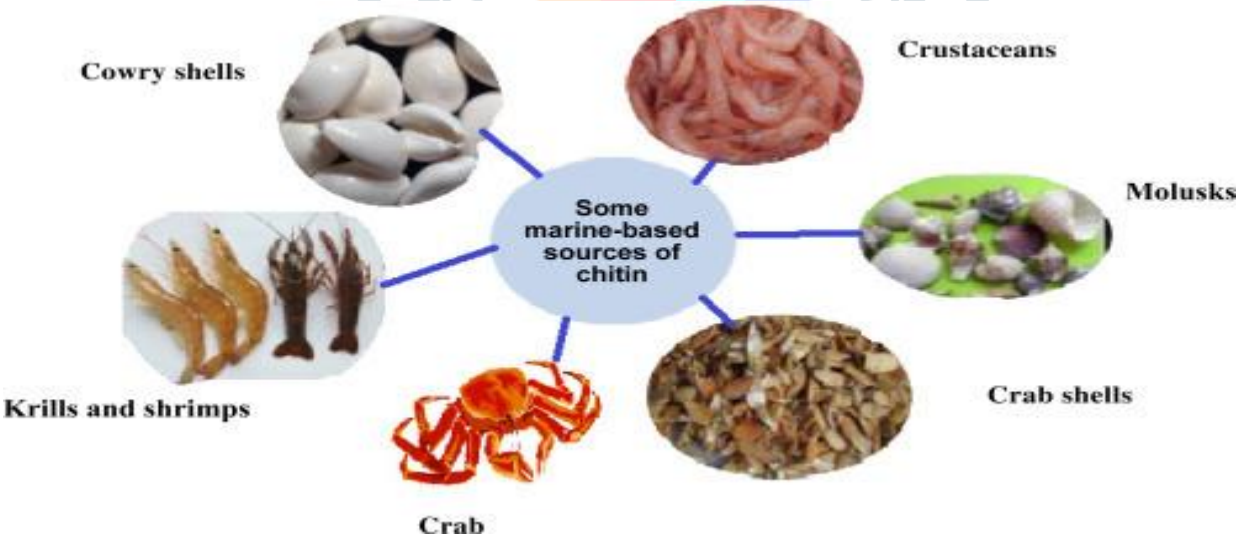

Fig 1- Various marine sources of chitin **(Leen Bastiaens, 2020)**

It is estimated that a large portion of chitin produced in the biosphere is present in the oceans. It can be found in aquatic species belonging to phyla such as Cnidaria (corals), Ectoprocta, Phoronid (horseshoe worms), Ectoprocta, Brachiopods (lamp shells), Bryozoa, Porifera (sponges), and Mollusca (squid, cuttlefish, and clams). Further, chitin has also been detected in fungi (mushrooms and yeasts), algae (diatoms, coralline algae, green algae), Onychophoran (velvet worms), and protozoa. The most easily accessible sources of chitin, however, are the exoskeletons of Arthropoda, which includes insects, arachnids (spiders and scorpions), myriapods (millipedes and centipedes), as well as Crustaceans (shrimp, krill, crab, and lobster). The shells of crustaceans mainly contain chitin (20–30%), proteins (20–

40%), minerals (30–60%), pigments, and sometimes also lipids (0–14%). Crabs and shrimp are mostly used at an industrial level and contain 10–72% chitin. Chitosan is mainly known as a partially deacetylated derivative of chitin but has also been found to be naturally present in some types of biomass. Some fungi contain chitosan as an important constituent of their cell wall at various stages their life cycle. **(Leen Bastiaens, 2020)**

Chitin, a linear polysaccharide composed of (1-4)-linked 2-acetamido-2-deoxy b-D-glucopyranose units, is the second prevalent form of polymerized carbon in nature. It is categorized as a cellulose derivative. Every year, molluscs, crustaceans, insects, fungus, algae, and related organisms approximately produce 10 billion tons of chitin. Chitin is bio-renewable, environmentally friendly, biocompatible, biodegradable and bio-functional, and is beneficial as a chelating agent, water treatment additive, drug carrier, biodegradable pressure-sensitive adhesive tape, wound-healing agents, in membranes and has other advantages for several important applications. Because of these advantages, much attention is paid to this characteristic biomaterial. Because of its weak solubility, it has unique applications. Chitin is insoluble in common organic solvents and diluted aqueous solvents because it is highly hydrophobic due to the highly expanded hydrogen-bonded semi-crystalline structure of chitin. Its derivative, chitosan, is prepared by deacetylation and depolymerization of native chitin, deacetylation of chitin in the solid state under alkaline conditions, or enzymatic hydrolysis in the presence of a chitin deacetylase. Chitin is a white, inelastic, rigid, nitrogenous polysaccharide that is present in the exoskeleton and internal structure of invertebrates. The wastes of these natural polymers cause surface pollution in coastal regions. The waste of the food industry, particularly if it contains the recovery of carotenoids, is a suitable source for production of chitosan from crustacean shells and economically feasible.

Chitosan is a natural, cationic amino polysaccharide copolymer of glucosamine and N-acetylglucosamine, obtained by the alkaline, partial deacetylation of chitin, which originates from shells of crustaceans such as crabs and prawns. Chitosan is a non-toxic, hydrophilic, biodegradable, biocompatible, mucoadhesive, and anti-bacterial, biopolymer which has led to a very diverse range of its applications. It has attracted tremendous attention as novel functional materials and potentially important renewable agricultural resource, and has been widely applied in the fields of agriculture, medicine, pharmaceuticals, cosmetic, and food industries, environmental protection, and biotechnology. The presence of a large number of amine groups on the chitosan chain increases the adsorption capacity of chitosan. Physical modifications may increase the sorption properties: gel formation decreases the crystallinity of the sorbent and involves an expansion of the porous network. Chemical modifications also offer a wide spectrum of tools to enhance the sorption properties of chitosan for metals. Both hydroxyl and amine groups of chitosan can be chemically modified. They may increase the chemical stability of the sorbent in acid media and, especially, decrease the solubility in most mineral and organic acids. They also increase its resistance to biochemical and microbiological degradation.

CS (Chitosan) is a linear polysaccharide composed of randomly distributed β- (1–4)-linked D-glucosamine and N-acetyl-D-glucosamine. CS is a cationic biopolymer produced from CN (Chitin),

which is one of the most abundant biopolymers in nature after cellulose. Because of its low solubility at physiological pH (above 6.0), the use of CS as an enhancer of solubility and rate release of drugs is limited. Furthermore, another limitation of CS in the preparation of sustained release systems is its rapid adsorption of water and a higher swelling degree in aqueous environments, which can lead to rapid drug release. To overcome these limitations, mainly the pH-dependent solubility of CS, chemical modifications can be used to control the polymer-drug interaction, enhance the loading capability, improve the bulk properties of drug delivery systems, and control the drug release rate of the matrix. For chemical modification, CS chains have three attractive reactive sites consisting of two hydroxyl groups (primary or secondary) and one primary amine group, and the modification of these groups will change the bulk properties of CS derivatives. CN and CS (and its derivatives) have attracted attention as materials for use in pharmaceutical drug delivery systems. The use of CS is limited by its low solubility in water, but this polymer is extensively used in drug delivery systems targeting major routes of administration. CS can be formulated as derivatives with improved water solubility, thereby expanding its applications to the development of novel dosage forms or biomaterials. This natural polysaccharide, owing to its versatility, can be used as a flocculating agent for the treatment of wastewater, as an adsorbent for clarification of oils, and especially for the production of chitosan. CS derivatives have been actively studied because of their physical, chemical, and biological properties such as high viscosity, low toxicity, superior bio/mucoadhesive properties, biocompatibility, and ability to inactivate pathogens. In addition, their suitability for the manufacture of tablets, films, and nanotechnology-based systems, as well as biomaterials for tissue engineering combined with their practicality, and the feasibility of production from commercial CS, and industrial scale-up have also made them a focus of research studies. **(Monika Yadav, 2019)**

Chitin naturally exists as ordered crystalline microfibrils forming structural components in the exoskeleton of arthropods or in the cell walls of the kingdom of fungi like yeast. Chitin is a homopolymer of 2-acetamido-2-deoxy-b- D-glucopyranose, although some of the glucopyranose residues are in the deacetylated form as 2-amino-2-deoxy-b-D-glucopyranose. Chitosan is the polymer with a majority of the glucopyranose residues in the deacetylated form. Chitin is infrequently found entirely in the N-acetyl or acetamido form, nor is chitosan completely deacetylated except under rigorous conditions. Neither is found in the pure state in nature but in conjunction with other polysaccharides, proteins, and perhaps minerals. Similar to cellulose, chitin can be found in three various polymorphic forms (alpha, beta, gamma). Recent investigations have declared that the g-form is a different form of a family. The polymorphic forms of chitin are different in the packing and polarities of near chains in successive sheets; in the b-form, all chains are arranged in a parallel mode, which is not the case in α-chitin. All types of chitin are comprised of piles of chains attached together by CO-NH bonds originating from the N-acetyl side groups of glucosamine residues. **(Rinaudo, 2015)**

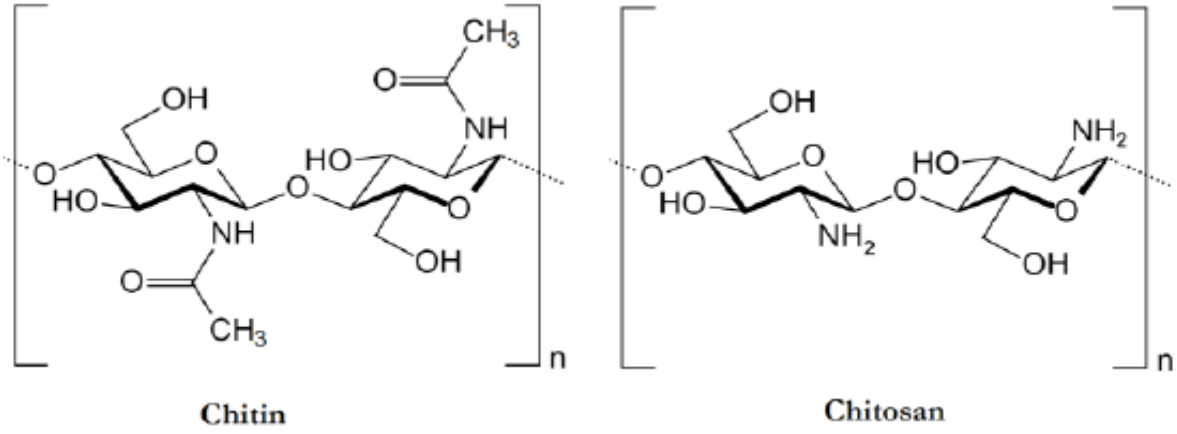

Fig 2- Structure of Chitin and Chitosan. **(Rinaudo, 2015)**

Biological extraction of chitin from seafood waste:

Extraction of chitin from shrimp shells can be done by two methods: Chemical method using chemicals or by Biological method using micro-organisms.

Chemical methods were the first approach used. A wide range of chemicals have been tested as deproteinization reagents including NaOH, Na_2CO_3, $NaHCO_3$, KOH, K_2CO_3, Ca $(OH)_2$, Na_2SO_3, $NaHSO_3$, $CaHSO_3$, Na_3PO_4 and Na_2S. Reactions conditions vary considerably in each study. In addition of deproteinization, the use of NaOH invariably results in partial deacetylation of chitin and hydrolysis of the biopolymer lowering its molecular weight. Demineralization consists in the removal of minerals, primarily calcium carbonate. Demineralization is generally performed by acid treatment using HCl, HNO_3, H_2SO_4, CH_3COOH and HCOOH. Among these acids, the preferential reagent is dilute hydrochloric acid.

The extraction by chemical treatments has many drawbacks: (i) it harms the physio-chemical properties of chitin and leads to MW and DA (Degree of acetylation) decrease that negatively affects the intrinsic properties of the purified chitin; (ii) it affects wastewater effluent that contains some chemicals (iii) it increases the cost of chitin purification processes. Furthermore, the development of the green extraction techniques based on the concept of 'Green chemistry' is gaining greater attention, favouring the application of enzymes and microorganisms for chitin extraction. Chitin extraction requires the use of proteases. Proteolytic enzymes are mainly derived from plant, microbial and animal sources. Many proteases such as alcalase, pepsin, papain, pancreatin, devolvase and trypsin remove proteins from crustacean shells and minimize the deacetylation and depolymerization during chitin isolation. The cost of using enzymes can be decreased by performing deproteinization by fermentation process, which can be achieved by endogenous microorganisms (called auto-fermentation) or by adding selected strains of microorganisms. This latter can be achieved by single-stage fermentation, two-stage fermentation, co-fermentation or successive fermentation. Fermentation of crustacean shells can be performed by selected Lactobacillus sp. strain as inoculum which produces lactic acid and proteases. Lactic acid is obtained by

conversion of glucose resulting in lower pH condition of silage suppressing the growth of spoilage microorganisms.

Taking shrimp into consideration, the average dry matter content of the samples of shrimp shells was 22 ± 2%, with no significant seasonal variation. The protein content was found to vary between 33% and 40% of the dry weight, the chitin content varied between 17% and 20% and the ash content of dried shrimp shells was found to be relatively constant with an average value of 34 ± 2% of the dry weight and consisting mainly of calcium carbonate ($CaCO_3$). No clear seasonal variations were found for the content of these three main components of shrimp shells (protein, chitin and ash). **(Leen Bastiaens, 2020)**

Fermentation of shrimp shell in jaggery broth using Bacillus subtilis to produce chitin and chitosan was investigated by (**Theruvathil K. Sini 2007)** . It was found that B. subtilis produced enough acid to remove the minerals from the shell and to prevent spoilage organisms. The protease enzyme in Bacillus species was responsible for the deproteinization of the shell. The pH, proteolytic activity, extent of demineralization and deproteinization were studied during fermentation. About 84% of the protein and 72% of the minerals were removed from the shrimp shell after fermentation. Mild acid and alkali treatments were given to produce characteristic chitin and their concentrations were standardized. Chitin was converted to chitosan by N-deacetylation and the properties of chitin and chitosan were studied. FTIR spectral analysis of chitin and chitosan prepared by the process was carried out and compared with spectra of commercially available samples. Jaggery was used for the preparation of media for Bacillus growth and was obtained from the local market at Cochin. From this study it was found that B. subtilis was found to be an efficient starter culture for fermentation of shrimp shells. About 84% of the protein and 72% of the minerals were removed from the fermented residue at the end of fermentation. Chitin and chitosan were prepared from the fermented residue and the physiochemical properties of these products were found to be like commercial grades of these materials. Also, jaggery is a cheap and easily available source for fermentation medium. Hence, reducing the cost of fermentation and reducing use of synthetic chemicals.

Other ways of fermenting include two- step fermentation using two different organisms, producing different enzymes, which would help in extraction of chitin from shrimp shell waste. Production of chitin from shrimp shell powders using Serratia marcescens B742 and Lactobacillus plantarum ATCC 8014 successive two-step fermentation was studied by **(Hongcai Zhang 2012)** . Shrimp shell powders (SSPs) were fermented by successive two-step fermentation of Serratia marcescens B742 and Lactobacillus plantarum ATCC 8014 to extract chitin. Taguchi experimental design with orthogonal array was employed to investigate the most contributing factors on each of the one-step fermentation first. The identified optimal fermentation conditions for extracting chitin from SSPs using S. marcescens B742 were 2% SSP, 2 hr of sonication time, 10% incubation level, and 4 days of culture time, while that of using L. plantarum ATCC 8014 fermentation was 2% SSP, 15% glucose, 10% incubation level, and 2 days of culture time. Successive two-step fermentation using identified optimal fermentation conditions resulted in chitin yield of 18.9% with the final deproteinization (DP) and demineralization (DM) rate of

94.5% and 93.0%, respectively. The obtained chitin was compared with the commercial chitin from SSP using scanning electron microscopy (SEM), Fourier transform infrared spectrometer (FT-IR) and X-ray diffraction (XRD). Results showed that the chitin prepared by the successive two-step fermentation exhibited similar physicochemical and structural properties to those of the commercial one, while significantly less use of chemical reagents. This study found that fermentation using S. marascens B742 or L. plantarum ATCC 8014 alone did not give chitin with satisfactory DM (demineralization) and DP (deproteinization) efficacy, but the successive two-step fermentation using these two bacteria improved the extraction efficacy with chitin yield of 18.9%, and the resultant chitin has similar physio-chemical and structural properties to commercial chitin. The microbial fermentation is a relatively simple and environment friendly alternative to the chemical method. However, it should be noted that this study at shaking flask level under laboratory conditions may not be suitable for large scale operations. Hence, modifications and adjustments on the specific treatment conditions and processing procedures might be necessary. Moreover, the application of ultrasonic treatment appeared to change the morphology and crystallinity index of chitin, which should be considered in the future studies.

APPLICATION OF CHITOSAN IN ENHANCEMENT OF SHELF LIFE AND QUALITY OF DAIRY PRODUCTS:

Chitosan has attracted attention as a potential food preservative of natural origin due to its antimicrobial activity against a wide range of foodborne filamentous fungi, yeast, and bacteria. The mechanism of the antimicrobial activity of chitosan has not yet been fully elucidated, but several hypotheses have been proposed. The most feasible hypothesis is a change in cell permeability due to interactions between the positively charged chitosan molecules and the negatively charged microbial cell membranes. This interaction leads to the leakage of proteinaceous and other intracellular; Other mechanisms are the interaction of diffused hydrolysis products with microbial DNA, which leads to the inhibition of the mRNA and protein synthesis and the chelation of metals, spore elements, and essential nutrients.

The low price and elevated nutritional value of milk make it a pivotal raw material in the production of numerous foods, worldwide. Nevertheless, milk is a fertile environment ideal for the growth of many pathogenic microbes inducing quick spread foodborne diseases (FBDs). The most common milk-borne pathogens (MBPs) are coliforms such as enterohemorrhagic E. coli (EHEC). The coliforms colonization in milk is due to the unsanitary milking environments. Among coliforms, E. coli O157:H7 is considered one of the major sources for several serious public health problems in developing countries. Dairy animals are major reservoirs for many milk-borne pathogens (MBPs) such as Staphylococcus aureus (S. aureus) and Escherichia coli (E. coli O157:H7). Thus, dairy industries dedicate most of their processes to eliminate or minimize microbial contamination. Although pasteurization may offer an ideal solution for microbial decontamination; nevertheless, it may negatively impact organoleptic and nutritive values of dairy products. In this context, this work done by **(Mohammad Y. Alfaifi 2020)** aimed to develop an innovative strategy, to tackle this challenge, based on the chemical preservation of milk. In this endeavour, they have succeeded to design a new safe multifunctional bio-preservative based on natural

antimicrobials (water-soluble chitosan (WSC) and 2-azidopropanoic acid (APA), (WSC-APA) conjugate). Interestingly, the minimum inhibitory concentrations (MICs) of WSC-APA have the capacity to completely suppress the proliferation of E. coli O157:H7 and S. aureus cells (100% bacterial reduction) in refrigerated milk samples during 20 and 24 hours, respectively. Moreover, no Staphylococci species could be detected in refrigerated milk samples remediated with WSC-APA (0.25 mg/mL) after 6 storage days. Meanwhile, the coliform count has reduced by 99.7% in conjugate-treated milk samples during 10 storage days. Thus, new bio-preservative (WSA-APA) can be safely used to increase the shelf-life of milk without sacrificing its organoleptic and nutritive values. This study reported a simple protocol for the utilization of primary natural-based antimicrobials such as water-soluble chitosan (WSC), isolated from shrimp shells, and lactic acid derivative (2-azidopropanoic acid, APA) to fabricate safe, green, and smart antimicrobial (WSC-APA conjugate). This conjugate was applied as a bio-preserving agent to enhance the safety of fresh cow's milk for prolonged shelf life through the eradication of milk-borne pathogens (MBPs).

Milk is synthesized in alveoli of the mammary gland and is virtually sterile when secreted into alveoli of the udder. Once raw milk leaves the udder, it becomes susceptible for microbial contamination from different sources such as the environmental conditions around the udder and from the surface of milk handling and storage equipment's. It is well known that the microbial alternation is responsible for the great losses of food and hence, over the years, various chemical and physical processes have been developed to extend the shelf-life of foods. So, to meet the growing consumers demand for natural preservatives without chemical preservatives, there is an urgent need to find new antimicrobials to compact these problems so, many antimicrobial substances recently developed like chitosan and lysozyme. Milk preservation is closely related with its microbiological quality. The spoilage may occur at any stage from production, during processing and till consumption. Many potent antimicrobials were discovered recently but, it is important to test their efficacy against undesirable bacteria inside the food materials that help to improve food safety and validity. Research done by **(Dina 2020)** focused on comparative antibacterial activities between chitosan, lysozyme and their different mixtures against different undesirable bacterial strains by agar well diffusion assay. Then apply the selected antibacterial substances in raw cow milk contaminated with food borne pathogens and spoilage bacteria then monitoring the bacterial growth or inhibition. Our preliminary investigation showed that, chitosan 0.5% exhibited the largest inhibition zones diameter followed by lysozyme hydrolysates with chitosan complex against Salmonella enteritidis and Bacillus subtilis in-vitro by agar well diffusion method. During application in raw cow milk, lysozyme hydrolysates and chitosan complex exhibited powerful bactericidal effect followed by chitosan especially against Clostridium perfringens, Staphylococcus aureus and Listeria monocytogenes after 24h from cooling storage of raw cow milk. The bactericidal activity of lysozyme and lysozyme hydrolysates were greatly enhanced upon their combination with chitosan. But the bactericidal activity of lysozyme hydrolysates with chitosan complex exhibited great killing power than the conjugation between lysozyme with chitosan complex at same concentrations. This may be attributed to chitosan oligomers and lysozyme peptides acting in a synergistic manner in

penetrating and killing the undesirable bacteria. Although chitosan was effective in inhibiting the growth of spoilage microorganisms, but it induced changes in raw milk pH. Accordingly, they suggest lysozyme hydrolysates with chitosan complex will be a promising antibacterial additive to produce a highly safe raw milk with recommendation further future studies to explore its antibacterial mechanism. Milk spoilage may occur at any stage from milk production till its consumption. There are many synthetic and natural antimicrobials were discovered recently like chitosan and lysozyme but, it is important to test their efficacy against undesirable bacteria inside the food to improve food safety and validity. So, their target was focused on testing the antibacterial activities of chitosan, lysozyme and or their mixtures against different undesirable bacterial strains in raw milk. The results revealed the bactericidal activity of native LZ and LZH could be broaden upon their conjugation with chitosan. But LZHC complex exhibited a great killing power than the LZC complex. This may be attributed to the synergistic effect of chitosan oligomers and lysozyme peptide. Although chitosan was effective in inhibiting the growth of spoilage microorganisms but, it is induced changes in the food matrix due to its low solubility in water. Accordingly, they nominate the novel LZHC complex to be a promising additive in dairy sector. Also, they recommend chitosan to be applied in fermented dairy products especially kareish cheese (the most popular soft cheese in Egypt) to produce highly safe and good quality product.

Bio-preservation is an important field in the food industry. Natural molecules as bacteriocins are a good alternative to extend the shelf life of the products and to avoid the growth of foodborne pathogens. Bacteriocins of lactic acid bacteria are secure and easy to find in nature using bioprospecting techniques. Edible coatings activated with bacteriocins have the properties to protect the microbiology quality of food and generating a natural product. Bio-preservation is the extension of shelf life and enhanced safety of food by natural antimicrobial agents such as bacteriocins. **(Adriana Jutinico-Shubach 2020)** studied the antimicrobial effect of edible coating incorporated with bacteriocins produced by Pediococcus pentosaceus 147 was evaluated. First, the in vitro antimicrobial activity of the partial purified cell-free supernatant (CFS) of P. pentosaceus 147 against Listeria monocytogenes was measured (8,533.3 AU/ml). Also, the lowest antimicrobial concentration of the CFS (5.72 µg/ml) was found. Characterization of physicochemical properties of chitosan edible coatings incorporated with the CFS was carried out. Coatings on fresh cheese were evaluated. Five treatments were tested on fresh cheese: T1 negative control, T2 positive control, T3 only CFS, T4 chitosan coating without CFS, and T5 chitosan coating with CFS. The results showed that the chitosan coatings plus CFS (5.72 µg/ml) of P. pentosaceus 147 can inhibit the growth of L. monocytogenes during the storage of cheese contaminated after production. Further purification of pediocin produced by P. pentosaceus 147 could be more advantageous in order to evaluate its specific antimicrobial activity against foodborne pathogens and evaluate other potential applications in food products. Finally, this work has shown the action of bacteriocins presented in CFS produced by P. pentosaceus 147 against L. monocytogenes growth on fresh cheese. The reduction of the microbial population was evident when the CFS was applied directly and through chitosan edible coating. The results also showed a possible synergistic effect between the CFS and chitosan, so that further research should focus on the effect of both components. As a future work, we propose the purification of

CFS and further physicochemical analysis in order to obtain specific knowledge about the bioactive molecules inside the CF.

Packaging of cheese as the main past processing has key role on quality and shelf life of the produced cheese. In the field of food packaging, several polymers such as polystyrene and polyethylene are extensively used which those are synthetic and non-degradable in nature, and cause severe concerns related to the human and environment, which those highlight potential application of edible films and coatings. Natamycin, as a natural fungicidal agent, is fermentative produced using Streptomyces natelensis and has been used in several countries to prepare different types of cheese including hard, semi-hard and semi-soft cheeses, and their surface treatment. Edible coatings can be applied on the surface of fruits and food pieces to improve their nutritional and sensory qualities. Study done by **(Sahar Nottagh & Javad Hesari 2019)** focuses on the effects of applied edible coating formulation containing 1.6% W/V chitosan and 18.5 ppm Natamycin, on the surface of Iranian Ultra-filtrated (UF) cheese and evaluates biological, physio-chemical and organoleptic attributes of the coated cheeses as compared to those of the control samples, during 6 weeks of storage at temperature of 4 ± 2 °C. Coated and control samples were analyzed for bacterial total count, molds and yeasts population, coliform count and starter populations, as biological characteristics. pH, acidity, firmness, and salt, moisture, dry mater, fat and fat in dry mater contents were evaluated for both samples, as their physio-chemical properties. Appearance, odour and flavour, taste, texture and overall acceptability were evaluated for both coated and control cheese samples at weeks 3 and 6 of storage, as their sensorial attributes. Sensorial analysis indicated that, however there were insignificant differences between the values of sensory parameters for both samples at week 6 but provided coating could significantly maintained sensory attributes of the coated cheese as compared to those of the control samples, at week 6 of storage. Effect of edible coating based on chitosan and Natamycin, as antibacterial and antifungal agents, on the quality of the Iranian UF cheese was assessed. Obtained results indicated that the prepared coating significantly inhibited the growth of the cheese spoilage microorganisms without negative effects starters which those play important role in cheese ripening and quality. However, prepared edible coating had moderate effects on some physio-chemical attributes of the coated cheeses, but it had significant effects on organoleptic attributes of the coated cheese samples as compared to those of the control cheese samples. Developed edible coating formulation can be commercially used to coat of other type of cheeses and increased their shelf-life extension.

CONCLUSION:

Chitosan is a modified, natural carbohydrate polymer derived by deacetylation of chitin, a major component of the shells of crustacea such as crab, shrimp, and crawfish. The antimicrobial activity of chitosan against a wide range of foodborne filamentous fungi, yeast, and bacteria has made it a potential food preservative.

Chitosan also possesses film-forming and barrier properties, thus making it a potential raw material for edible films or coatings. Inherent antibacterial/antifungal properties and film forming ability of chitosan make it an ideal for use as biodegradable antimicrobial packaging material that can be used to improve the storability of perishable foods. Numerous research have clearly demonstrated that chitosan can be used as an effective preservative or coating material for improvement of quality and shelf life of various foods. Chitosan has been approved as a food additive in Korea and Japan since 1995 and 1983, respectively. In the United States, upon receiving the US FDA approval for GRAS status, chitosan as a food additive and its applications in food systems will certainly be in more demand in the near future.

Although chitin is widely used in various fields, but still not applied on large scale in food industry as a preservative agent. As it is non-toxic and edible, it makes up a very good preservative agent. Milk products like yogurt, ice cream, cheese, milk sweets, etc can use chitin and chitosan as preservative agents. As chitin does not change the taste and consistency of the food product it is an appropriate and good preservative. Other food products like meat, poultry and ready to eat foods can employ chitin as preservative, which will remain non-toxic and is a naturally derived preservative. Instead of use of chemicals for chitin production, which causes environmental pollution and hazard, biological method is the best alternative. Biological method employs cost effective ways using bacteria and avoids harsh chemical treatments. Substitutes to fermentation media like usage of fruit and vegetable wastes can be employed, as to reduce fruit and vegetable waste too. Thereby, reducing seafood as well as fruit and vegetable waste.

Lastly, numerous research conducted on food applications of chitosan's have been done at a small or laboratory scale. Further research on quality and shelf life of foods containing or coated with chitosan should be conducted on a scale-up or commercial trial under a large volume typical of commercial conditions. This would provide us more realistic and practical information needed for actual commercialization of food products containing or coated with chitosan's.

BIBLIOGRAPHY:

1) **Monika Yadav, Priynshi Goswami, Kunwar Paritosh, Manish Kumar, Nidhi Pareek and Vivekanand Vivekanand**. Seafood waste: a source for preparation of commercially employable chitin/chitosan materials. *Bioresour. Bioprocess*. **(2019)**.
2) **Leen Bastiaens Lise Soetemans Els D'Hondt Kathy Elst**, Sources of Chitin and Chitosan and their Isolation, *2020 John Wiley & Sons Ltd*, 1-34. **(2020)**.
3) **Islem Younes and Marguerite Rinaudo**, Chitin and Chitosan Preparation from Marine Sources. *Structure, Properties and Applications. Mar. Drugs* 2015, 13, 1133-1174. **(2015)**.

4) **Adriana Jutinico-Shubach, Carolina Gutiérrez-Cortés, et.al**, 2020. "Antilisterial activity of chitosan-based edible coating incorporating cell-free supernatant from Pediococcus pentosaceus 147 on the preservation of fresh cheese." *J Food Process Preserv. 2020, 2020 Wiley Periodicals LLC* 1-12. **(2020)**.

5) **Hongcai Zhang, Yafang Jin,et.al.**, 2012. "Production of chitin from shrimp shell powders using Serratia marcescens B742 and Lactobacillus plantarum ATCC 8014 successive two-step fermentation." *Carbohydrate Research 362 (2012), Elsevier Ltd* 13–20. **(2012)**.

6) **Theruvathil K. Sini, Sethumadhavan Santhosh and Paruthapara T. Mathew**., 2007. "Study on the production of chitin and chitosan from shrimp shell by using Bacillus subtilis fermentation." *Carbohydrate Research 342 Elsevier Ltd* 2423–2429. **(2007)**.

7) **Sahar Nottagh & Javad Hesari, et.al**., 2019. "Effectiveness of edible coating based on chitosan and Natamyci on biological, physico-chemical and organoleptic attributes of Iranian ultra-filtrated cheese." *Biologia, Springer* 1-8. **(2019)**.

8) **Mohammad Y. Alfaifi, J. Alkabli, et.al**., 2020. "Suppressing of milk-borne pathogenic using new water-soluble chitosan-azidopropanoic acid conjugate: Targeting milk-preservation quality improvement." *International Journal of Biological Macromolecules 164 (2020), 2020 Elsevier B.V* 1519–1526. **(2020)**.

9) **9) Dina, A.B. Awad, Sobhy, et.al**., 2020. "Evaluation the Efficacy of Lysozyme Hydrolysates-Loaded Chitosan Against Food Borne Pathogens in Raw Milk During Cooling Storage." *Alexandria Journal of Veterinary Sciences, AJVS. Vol. 64 (1)* 86-96. **(2020)**.