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DEVELOPMENT OF CHITOSAN BASED ACTIVE FILM TO EXTEND THE SHELF LIFE OF MINIMALLY PROCESSED FISH

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ABSTRACT

Chitin and its deacetylated form, chitosan, have major interest in the field of food package because of biodegradability, long lasting, eco friendly, flexible, tough, very difficult to tear and its antimicrobial activity. In the crustacean processing unit, the dumping of large quantities of discards into the environment lead to a major problem of accumulation of discards due to the very slow biodegradation of chitin. Instead of simply dumping the waste, the shrimp and crab wastes have been widely used for the isolation of chitin, followed by its usage in food packaging. In this study, the prawn wastes were collected from the Muttom and the chitin and chitosan were isolated using simple chemical method. The yield of chitin and chitosan was 31% and 58% respectively. The maximum percentage of degree of deacetylation (90.7%) was achieved through this experiment. The high degree of deacetylation makes the chitosan as water soluble and bio adhesive. So, the chitosan based food packaging film was developed without addition of antimicrobial agents. In the antimicrobial study, the chitosan based film without antimicrobial agent was shown 3.6cm and 2.9cm in diameter for *Listeria monocytogene* and *Pseudomonas putida* respectively. Then the biodegradability test of this packaging material was shown that 93% of reduction in weight in 20 days of incubation with *B.subtilis.* In the final study, the chitosan based film was used to pack 5g of fish fillets and the freshness of the fish was calculated using quality index method.

KEYWORDS: Action of Chitosan, Films and Coatings, Chitin Extraction

INTRODUCTION

Chitin is a long chain of polymer of a N-Acetyl glucosamine, a derivative of glucose. The chitin is mainly found in cell wall of fungi, the exoskeletons of arthropods such as crustaceans and insects, radulas of mollusks and the beaks and internal shells of cephalopods such as squid and octopuses. Chitin has many derivatives, of which chitosan plays a main role in food package and other industrial applications.

Chitin and chitosan the naturally abundant and renewable polymers have excellent properties such as biodegradability, biocompatibility, non toxicity and adsorption [1]. Due to their film-forming properties, chitin [2] and chitosan [3] have been successfully used as food wraps. Among two of these (Chitin and Chitosan), Chitosan is widely used in the edible film industry. Due to its ability to form semi-permeable film, chitosan coating can be expected to modify the internal atmosphere as well as decrease the transpiration loss [4].

Now days, the antimicrobial activity of chitin and its derivatives against different group of microorganisms such as bacteria, yeast and fungi have important attention. The exact mechanism of the antimicrobial action of chitin, chitosan and their derivatives is still unknown, but different mechanisms have been proposed. Interaction between positively charged chitosan molecules and negatively charged microbial cell membranes leads to the leakage of proteinaceous and other intracellular constituents [5, 6-8, 10]. Chitosan also acts as a chelating agent that selectively binds trace metals and thereby inhibits the production of toxins and microbial growth [11]. It also activates several defense processes in the host tissue [9], acts as a water binding agent and inhibits various enzymes [10]. Binding of chitosan with DNA and inhibition of

mRNA synthesis occurs via chitosan penetrating the nuclei of the microorganisms and interfering with the synthesis of mRNA and proteins [6, 12].

The edible film developed from chitin and its derivatives are mainly used in food industry in order to improve the food quality, long shelf life. These outer layers/films can provide supplementary and sometimes essential means of controlling physiological, morphological and physicochemical changes in food products [13]. High density polyethylene film, a common packaging material used to protect foods [16], has disadvantages like fermentation due to the depletion of oxygen [17] and condensation of water due to fluctuation of storage temperature, which promotes fungal growth [18]. There are many mechanisms involved in extending shelf life of food by coating films.

These include controlled moisture transfer between food and surrounding environment, controlled release of chemical agents like antimicrobial substances, antioxidants, reduction of oxygen partial pressure in the package that results in a decreased rate of metabolism, controlled rate of respiration, high impermeability to certain substances like fats and oils, temperature control, structural reinforcement of food and coat flavor compounds and leavening agents in the form of microcapsules [14, 15].

In this present study, the chitosan based thin film was developed without addition of any antimicrobial agents in order to increase the shelf life of the fish products and also the tensile strength, gas and water vapor permeability of the thin packaging material were studied.

MATERIALS AND METHODS

Sample Collection and Chitin Extraction

Prawns were collected from the Muttom, Kanyakumari District. 20g of prawn shell (powdered) was weighed and chitin was extracted from the prawn shells by using Takiguchi's method [19].

Chitin Deacetylation and Degree of Deacetylation (%DD)

Linear potentiometric titration method was used in order to calculate %DD. The chitosan yield was calculated by comparing the weight measurements of the raw material to the chitosan obtained after treatment.

Characterization of Chitosan

Proximate Analysis was conducted to determine the ash content, moisture content, protein content of chitosan, carbohydrate content and other characterizations such as fat binding capacity (FBC) and water binding capacity (WBC). Turbidity of the chitosan was determined by turbidimeter. Thermogravimetric analysis or thermal gravimetric analysis (TGA) and Fourier transform infrared spectroscopy (FTIR) were carried out in KCCEC lab

Preparation of Chitosan Film

Chitosan edible films were prepared by dissolving 2% (w/v) of chitosan in a 1% lactic acid solution and stirred, at room temperature, until chitosan was completely dissolved. Glycerol at 1% (w/v) and Tween 80 at 0.2% (w/v) were added as a plasticizer and as a surfactant, respectively. The addition of glycerol and Tween 80 was based in reported chitosan-based films formulations (Casariego *et al.*, 2008; Cerqueira *et al.*, 2009). The pH of the film forming solution was 4.5. In order to obtain films with a similar thickness, a constant amount (20ml) of the Chitosan solutions was casted in 8.5 cm diameter Petri dish and dried in an oven at 35°C, over night. The films were stored at 20°C until further use.

Characterization of Chitosan Based Film

The chitosan based film was characterized in order to evaluate its ability to pack the food products.

Film thickness, Film Solubility, Moisture content of the film, Tensile Strength (TS), Water vapor permeability measurement, Oxygen and carbon dioxide permeability measurement, Transparency were analysed

Antimicrobial activity was determined by the agar well diffusion method. The pathogenic organisms used were *Listeria monocytogene* and *Pseudomonas putida.* and zones of inhibition were measured and compared with the standard.

Biodegradability Test of Film

Biodegradability test of the chitosan based film was carried out with the culture of *Bacillus subtilis*. A set of control experiment was performed in the conical flask containing only chitosan based film in nutrient broth devoid of bacterial inoculums. The degradability of the film was calculated using weight loss calculation.

Application of Chitosan Based Film in Fish Packaging

The three fish pieces (weight 1g each) were taken and washed with distilled water. Then the two pieces of fish were packed with chitosan based film and normal plastic covers respectively. The remaining one was unpacked. The packed sample was regularly monitored and quality was checked.

RESULTS AND DISCUSSIONS

Extraction of Chitin and Deacetylation

The dried shells were then used directly for extraction of chitin. Chitin was extracted from the shells of prawn by Takaguchi method. The fine powder of chitin was obtained at the end of the extraction. The final chitin product of prawn shells was shown in figure 1.

Figure 1: Final Chitin Product of Prawns

The percentage of deacetylation of chitin was calculated to be 90.7% and it was measured using titration method. The percentage yield of chitin and chitosan from Prawn shells was found to be 31% of chitin and 58% of chitosan.

Characterization of Chitosan

The deproteinized and demineralized prawn shells chitosan was assayed for moisture content, ash content, Fat binding capacity and water binding capacity, Protein and Carbohydrate content and the results were tabulated in table 1.

Table 1: Characterization of Chitosan

Characterization	Values
Moisture	2.2%
Ash	0.7%
Fat binding capacity	290%
Water binding capacity	450%
Turbidity	20.2 NTU
Protein	0.83%
Carbohydrate	82%

Solubility	97.7%

The FT-IR spectrum of chitin and chitosan extracted from prawn shells was obtained and compared with that of standard chitosan. The FT-IR spectrum of standard chitosan showed 10 major peaks lying between 455.30 cm^{-1} and 3332.25 cm^{-1} which used to evaluate possible chemical interactions present in the polymeric film. Chitosan based film containing the polysaccharide was shown the lowest weight loss, which means that the higher stability polysaccharide polysaccharides were produced at 19% loss at 440°C. This stability was probably due to the increased hydrogen bonding interaction between glycerol and chitosan.

Preparation of Chitosan Based Film

The chitosan slurry containing glycerol and tween was poured in the petridish and dried at oven to get a flexible film. The dried chitosan based film was shown in figure 2.

Figure 2: Chitosan Based Film

Characterization of Chitosan Based Film

The isolated chitosan sample (1g) was characterized. The result of the chitosan characterization was given in the table 2.

Table 2: Characterization of Chitosan Film

Film Properties	Obtained Values
Film Thickness	0.12 mm
Film Solubility	39.9%
Moisture content of the film	12.72%
Tensile strength	10.9 MPa
Water vapor permeability measurement	1.52×10^{-10} g Pa^{-1} s^{-1} m^{-1}
Oxygen permeability measurement	4.11×10^{-14} g m Pa^{-1} s^{-1} m^{-2}
carbon dioxide permeability measurement	5.41×10^{-13} g m Pa^{-1} s^{-1} m^{-2}
Transparency	Clear and transparent

Antimicrobial Activity of the Film

The diameter of the inhibition zone for *Listeria monocytogene* was about 3.6cm while *Pseudomonas putida* was about 2.9cm. when compare to *Pseudomonas putida* inhibition, the rate of inhibition was very high in *Listeria monocytogene.* The major problematic organisms in fish processing unit is *Listeria monocytogene* can be effectively controlled by chitosan based film without addition of the antimicrobial agents.

Biodegradability Test of Film (Weight Loss Measurement)

The biodegradability of the chitosan based film was calculated based on the percentage of the weight loss after treated with the *B.subtilis* culture. The 1 g of film was taken and incubated for 20 days. Every two days interval, sample

was taken and air dried. The final weight was calculated in order to measure percentage loss of weight of film. At the final day (20th day), the percentage loss of the film was noted as 93%. At the second day itself, 11% reduction of weight was observed. The results were shown graphically in figure 3.

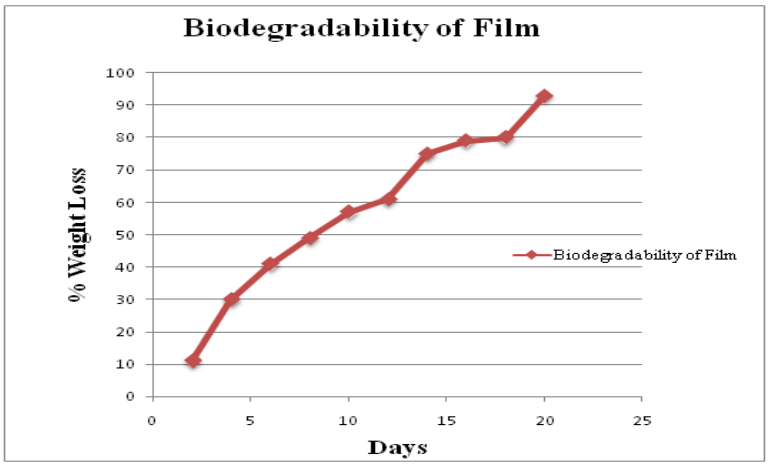

Figure 3: Biodegradability of Chitosan Based Film

The biodegradability of the polymer films may vary based on the source from which it developed. The starch based film will be degraded easily by commonly available microbes in the environment.

Application of Chitosan Based Film in Fish Packaging

The three pieces of the fish cubes (5g each) were washed and marked as A, B, and C. The fish piece A was wrapped with chitosan based film, Fish piece B was wrapped with plastic cover and fish piece C was without any wrappers. All were incubated at Room temperature and the physical changes were observed. The fish piece wrapped with plastic cover and without wrapper were shown the thin layer of yellow color molds on the surface while the fish piece covered with chitosan based film was shown no changes.

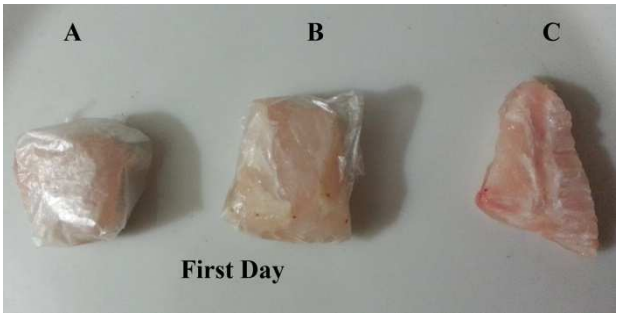

Figure 4: The Fish Fillets were Covered with Different Wrappers

Figure 4A: Represented that Fish Fillet was Wrapped with Chitosan Based Film, 4B Represented that

Fish Fillet was Wrapped with Plastic Cover and 4C – Fish Fillet was without Wrappers

CONCLUSIONS

Even though chitin, chitosan and their derivatives have been considered as versatile biopolymers in food applications their potential uses as functional food ingredients have to be studied with broader emphasis. In that sense, research and development should have great potential in finding novel uses in product development, microbiology, edible film industry, water purification, purification of waste discharge from food processing waste and nutritional aspects related to chitin, chitosan and their derivatives. Most physiological activities and functional properties of chitin and chitosan oligomers clearly depend upon their molecular weights and that a chain length of at least five residues is required. In that sense, further detailed physiological and sensory studies are required to determine the mechanisms of these effects and, ultimately, to come to a better understanding of how they may be manipulated in the creation of better quality foods. The film produced with the present value of WVP, GP and TS can be used as active packaging materials, and further studies must be developed in order to access bioactivity and behavior along shelf life.

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