Journal of Bacteriology and Mycology

Open Access

Austin Publishing Group

Research Article

Modification of Bio-polymer and It's Application

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Received: November 14, 2018; **Accepted:** January 22, 2019; **Published:** January 29, 2019

Abstract

Polymers contain monomeric units that are covalently bonded to form larger structures. Further biopolymers are polymers from biological origin. Some examples of biopolymers are; cellulose, starch, collagen, chitin etc. Chitin is waste products of sea shells, shrimps and prawns when the internal molluscs have been eaten or are dead and these sea shells are mostly scattered along many of the sea coasts creating waste. In crustaceans or more specifically shellfish, chitin is found as a constituent of a complex network with proteins onto which calcium carbonate deposits to form the rigid shell. Chitosan is commanding a new research initiative partly due to its physicochemical properties. Chitosan is obtained from chitin by deacetylation to a higher greater degree of 70-90 %. One of the benefits of chitosan is that it can form physical and chemical barriers against invading pathogens. The aim of this work is to obtain chitosan from chitin and thereby modifying and to study the quality improvement effects via its application on the bio produce and rubber compounds.

Keywords: Chitosan; Chitin; Bio Produce; Biopolymer

Introduction

Chitosan is a natural polymer which is biodegradable and synthesized from marine waste. Chitosan, partially deacetylated chitin. The biopolymer is characterized as either chitin or chitosan according to the degree of deacetylation (DD) which is determined by the proportion of D-glucosamine and N-acetyl D-glucosamine. Structurally, chitosan is a straight-chain copolymer composed of D-glucosamine and N-acetyl-D-glucosamine being obtained by the partial deacetylation of chitin.

History of chitosan

Chitosan (Kite-O-San) can be easily traced back to 1811 when the French scientist Braconnot discover the -chitin‖, from which chitosan is derived. Braconnot was a professor of natural history in France. Braconnot was conducting research on mushrooms, from which he isolate substance which was later to be called as chitin.

Chitosan is a substance which made from the chitin, a polysaccharide present in the exoskeletons of the various crustaceans. It is isolated from the shells of shellfish like lobster, shrimp and crab. Chitosan is popular as the -fat magnet in the scientific world, because of the reason that it inhibits the fat, which is beneficial for those who want to get rid of their body fat.

After 20 years, there was scientist Lassaigne who authored an article on the insects where he found that identical substance was present in the structure of insects and the structure of plants. He called this amazing substance-chitin. This name chitin is derived from Greek language, meaning-tunic or -outer shell or -envelope. This same concept was further studied in 1843 when Lassaigne evaluated the presence of nitrogen in chitin structure.

After the discovery of substance chitin, the name-chitosan emerged on. It was first discovered by scientist Rouget while experimenting with chitin. Rouget found that the compound of chitin could be easily derivatized by chemical and temperature treatments which made it soluble. In 1878 when Ledderhose report that chitin is combination of glucosamine and acetic acid molecules. In 1894 the scientists Hoppe-Seyler named the derivatized chitin, as the chitosan.

After 1920, chitosan became the favorite issue of research by most of the researchers. They utilized sources of the chitin like crab shells and fungi. Rammelberg in the 1930 worked hard which result to the confirmation that chitosan is obtained from these natural sources. It was also found that by hydrolyzing chitin in a number of ways, the chitin is a polysaccharide of glucosamine.

In the 1950, the use of x-ray analysis method had improved the study of the presence of chitin or chitosan in the fungi. It is only the most advanced technique that proved to be most reliable in proving the presence of chitin, as well as cellulose, in the cell walls of plants. After 140 years of the initial observation of Braconnot, first book on chitosan was published in the year 1951.

Chitosan is the most abundant basic biopolymer and is structurally similar to cellulose, which is composed of only one

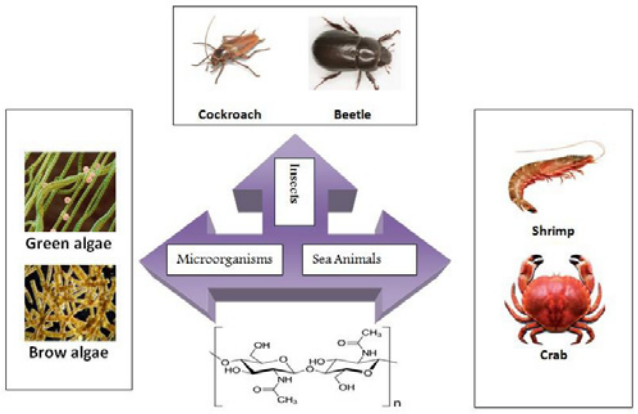


Figure 1: Sources of chitin.

Citation: Limbani A, Bishnoi A and Shah M. Modification of Bio-polymer and It's Application. J Bacteriol Mycol. 2019; 6(1): 1095.

Figure 2 and Figure 3: Deacetylation of chitosan.

Figure 4 and Figure 5: Purification of chitosan.

monomer of glucose. Chitosan solubility, biodegradability, reactivity, and adsorption of many substrates depend on the amount of protonated amino groups in the polymeric chain, therefore on the proportion of acetylated and non-acetylated D-glucosamine units. The amino groups are completely protonated in acids with making chitosan soluble [5-7].

Chitosan is insoluble in water, organic solvents and aqueous bases and it is soluble after stirring in acids such as acetic, nitric, hydrochloric, and phosphoric. The fungicidal and bactericidal action of chitosan appears to be mediated by the electrostatic forces between the protonated NH_2 groups in chitosan. The number of protonated NH_2 groups present in chitosan increases with increased degree of deacetylation (DD). Therefore, the DD for chitosan influences antimicrobial activity [1].

Chitosan is considered one of the most valuable polymer for biomedical and pharmaceutical applications due to its biodegradability, biocompatibility, antimicrobial, non-toxicity, and anti-tumor properties. Nanoparticles, microspheres, hydrogels, films, and fibers are typical chitosan based forms for biomedical and pharmaceutical applications. Examples of such applications include nasal, ocular, oral, and parenteral and transversal drug delivery.

Properties like non-toxicity, excellent antimicrobial and antifungal activity chitosan provides wide range of applications. It is a biodegradable it is used in edible coating on fresh fruits.

Chitin and chitosan: Properties and applications

Chitin is the second most essential characteristic polymer on the planet. The primary sources of which are marine shellfish, shrimp and crabs. Chitosan, which is soluble in acidic fluid media, is utilized as a part of numerous applications (food, cosmetics, biomedical and pharmaceutical applications). Chemical modifications of chitosan an area in which a variety of synthesis have been proposed tentatively. It can also be modified, it desired derivatives. Chitosan is a weak base

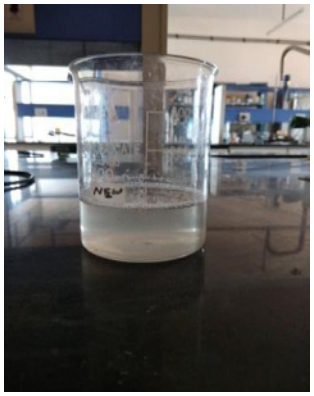


Figure 6: Solution of chitosan based coating.

Figure 7: Guava were dipped into solution for 1 min.

and is insoluble in water and organic solvent. However, it is soluble in dilute aqueous acidic solution (pH<6.5) [6].

Materials and Methods

Sodium hydroxide, Tap water, Hydrochloric acid was available in the lab and used. Exoskeletons of the prawn waste (Local Seafood Market, India) were used.

Isolation and extraction of chitosan

The exoskeletons of the prawn waste (shell) were removed separately and was rinsed thrice with tap water and then twice with distilled water. Then they were dried in a hot air oven for about 22hrs at 60°C. The sample obtained was soaked in boiling 4% sodium hydroxide using 1000ml beaker for 1hr. The sample was removed and then allowed to cool at room temperature for 28 minutes. They were then crushed further to small pieces of about 0.5-3.0 mm (Scheme 1) (Figure 1).

Demineralization

Solution was then filtered and the solid particles were soaked in 1% HCl for 24 hours. After filtering the solution, the residue was washed with demineralised water and the process was followed by deacetylation (Figures 2-3).

Deacetylation

The residue was boiled in 50% NaOH solution at 100°C-110°C.

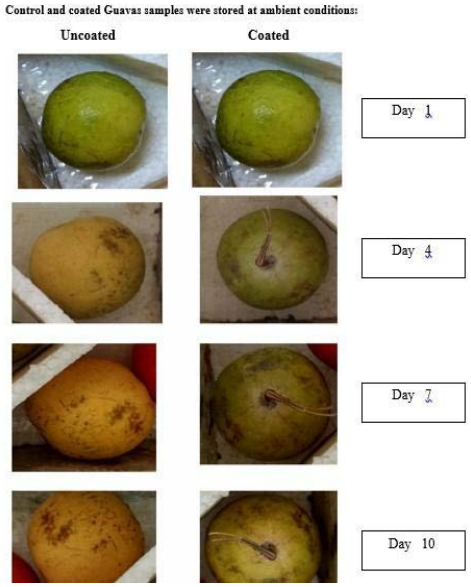


Figure 8: Control and coated guava samples at ambient condition.

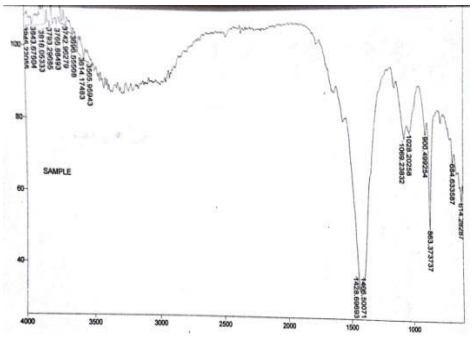


Figure 9: IR Spectrophotometer.

With the help of heating metal for 140 minutes. Then the sample was placed under the hood and allowed to cool for 1 hour. Then the sample was washed continuously with 50% NaOH solution. After which it was oven dried at 90°C for 12 hours. The chitosan obtained by above method will be of creamy white colour (Figures 2-3).

Purification of chitosan

The obtained chitosan has to be purified to make it suitable for use. The purification process was designed in three steps-removal of insoluble with filtration, recrystalization of chitosan with 1N sodium hydroxide, demetallization of retrieved chitosan (Figures 4-5).

Moisture content

Moisture content of the prepared chitosan was determined by the gravimetric method. The water mass was determined by drying the sample to constant weight and measuring the sample after and before drying. The water mass was the difference between the weights of the wet and oven dry samples.

$$\text{Percentage of Moisture Content} = \frac{(Wet\ Weight - Dry\ Weight)}{Wet\ Weight} \times 100$$

Ash value

The ash value of chitosan was determined by taking the prepared

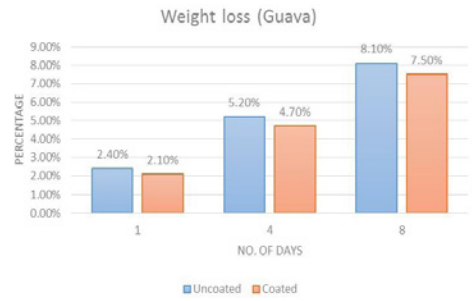


Figure 10: Weight loss.

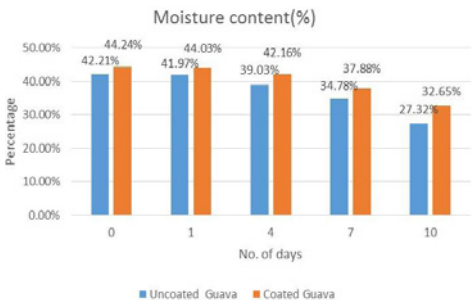


Figure 11: Moisture content.

chitosan sample which was previously ignited, cooled, and tarred crucible. The samples were heated in a muffle furnace preheated to 650°C for 4hrs. The crucibles were then allowed to cool in the furnace to >200°C and then were placed into desiccators with a vented top.

$$\text{Percentage of Ash} = \frac{\text{Weight of Residue}}{\text{Sample Weight}} \times 100$$

Loss on drying

Loss on drying of the prepared chitosan was determined by the gravimetric method. The water mass loss was determined by drying the sample to constant weight and measuring the sample after and before drying. The water mass (or weight) obtained showed the difference between the weights of the wet and oven dry samples.

$$\text{Percentage of Loss on Drying} = \frac{(Wet\ Weight - Dry\ Weight)}{Dry\ Weight} \times 100$$

Characterization of Chitosan

FT-IR (Fourier Transform Infrared) spectroscopy

In this method, the FTIR (Fourier transform infrared) is used to analyze the chitosan sample. Fourier transform infrared (FTIR) spectrum is very reliable method to analyze any type of compound. The chitosan samples are prepared as KBr pellet and scanned against a blank KBr pellet background at wave number range 4000-400 cm^{-1} with resolution of 4.0cm^{-1}. The KBr spectrum is taken as the background and chitosan-KBr complex is considered as the main sample.

Acid-base titration

Chitosan (0.5g) was dissolved in 30ml 0.1M HCl at 25°C with stirring in a 250ml flask and then two drops of methyl orange indicator was added. 0.1M NaOH was used to titrate the solution. At the final point of titration, the colour changes from pink to orange yellow. The method was augmented to include the use of a pH meter to make the

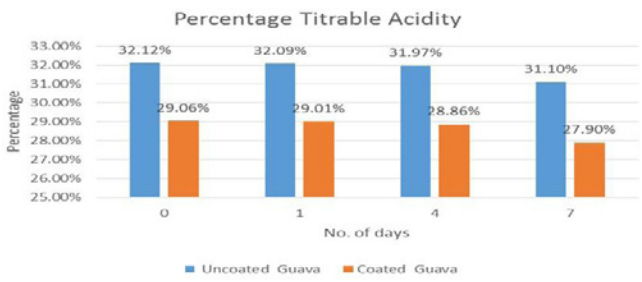


Figure 12: Percentage titrable acidity.

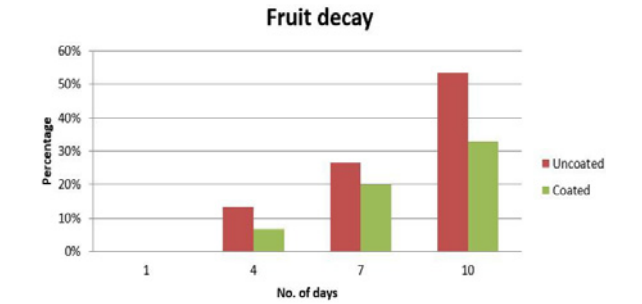


Figure 13: Fruit decay.

final titration point determination more precise. To calculate water content, 0.5g chitosan was heated at 105˚C until a constant weight was reached. Three parallel samples were used. The percent of free NH_2 groups in chitosan was calculated as follows:

$$NH_2\% = \frac{[(C1V1 - C2V2) \times 0.016]}{[G(100 - W)]} \times 100$$

Free $NH_2\%$ = $NH_2\%/9.94\% \times 100\%$

Chitosan theoretic NH_2 content% = $(16/161) \times 100\% = 9.94\%$

C1: Concentration of HCl (M); C2: Concentration of NaOH (M); V1: the volume of HCl added (ml); V2: the volume of NaOH added by titration (ml); G: Sample weight (g); W: sample water content (%); 0.016: equal to NH_2 content (g) in 1ml of 1M HCl.

Antimicrobial tests

One percent chitosan solution in 0.1N HCl was prepared as the stock solution by adding 1g of chitosan to 50ml of deionized water, followed by sterilization at 121˚C for 15min and then the addition of 50ml of sterile 0.2N HCl. To 50ml flasks containing 10ml NB plus 3% NaCl various volumes of chitosan stock solution were added and the pH value was adjusted to 6.0.

A volume of 100ml of each tested bacterium was inoculated into the flasks. After incubation with shaking (120r.p.m.) at 37˚C for 2 days, duplicate samples of 0.1ml of decimal dilutions were spread on nutrient agar (NA) plates with NA plus 3% NaCl. After incubation at 37˚C for 2 days, the colonies on the plates were counted. These processes remain same for other two temperatures like 35˚C & 40˚C.

Solubility

The solubility of chitosan was demonstrated in various solvents like distilled water, acetone, ethanol, acetic acid and lactic acid. The chitosan obtained here was dissolved completely in acetic acid.

Synthesis of Chitosan Based Coating

Materials and Methods

Guavas of green mature stage were purchased from a local wholesale market. Chitosan (medium molecular weight) as main edible component and glycerol (87%) as plasticizer and Tween 80 as surfactant and glacial acetic acid.

Coating and storage of guavas

Edible coating emulsions were prepared by dissolving chitosan (2% w/v) in 100mL of 0.5% glacial acetic acid in distilled water. Then, glycerol (1% w/v) and Tween 80 (0.1% w/v) were added and the solutions were agitated overnight. The pH of the solutions was adjusted to pH 5.6 with 0.1M sodium hydroxide (Figure 6). Guavas were dipped into the prepared coating emulsions for 1min and then drained (Figure 7). Uncoated Guava as control samples were immersed in a water at same duration of time. The treated and control Guava samples were dried in ambient conditions (26±2˚C and 40-50% relative humidity) for 2 hours. After setting a thin layer of edible coating on the surface of treated samples, control and coated Guava samples were stored at ambient conditions in the laboratory for 10 days and 9 days (Table 1).

Testing

Weight loss

Water loss usually occurs from the vapor phase in fresh horticultural crops. Chitosan coatings act as barriers, thereby restricting water transfer and protecting fruit skin from mechanical injuries, as well as sealing small wounds and thus delaying dehydration. In the present study, Chitosan coating also helped retain moisture and weight loss of fruits increased during storage period which was significantly higher in control than coated fruits.

Weight loss determination (%) The weight loss during storage was determined by the weight differences at days 4, 7 and 10, which were then compared with day 0, and expressed in percentage (fresh weight basis) (Figure 8). Fruit was weighted using a weighing scale. Fruits weight was recorded, then the percentage of weight loss were calculated according to the following equation.

$$\text{Fruit weight loss (\%)} = \frac{(Wi - Ws)}{Wi} \times 100$$

Wi: Fruit weight at initial period,

Ws: Fruit weight at sampling period

Moisture content

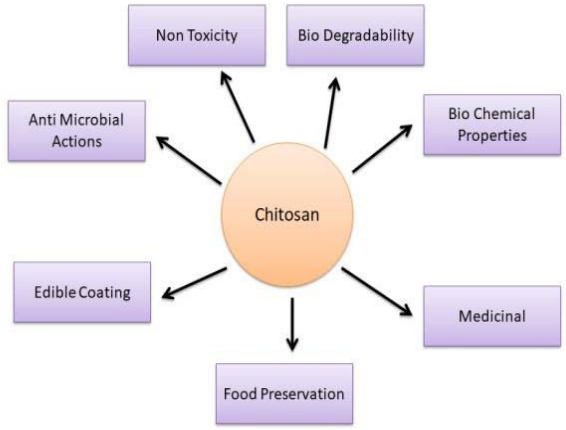
Moist sample was weighed immediately and record as "wet weight of sample" Dry the weight sample again to a constant weight, at a temperature not exceeding 90˚C using the suitable drying equipment. Allow the sample to cool. And weight to know again, and recorded as the "dry weight of sample". The moisture content of the five randomly selected samples was calculated using the following equation.

$$\text{Moisture content (\%)} = \frac{A - B}{A} \times 100$$

M.C (%): Percentage of moisture in the sample.

A: Weight of wet sample (gram),

B: weight of dry sample (gram).



Scheme 1: Application & Properties of Chitosan.

Percent titratable acidity

Titratable Acidity of product is the acidity in terms of the predominant acid present in the juice i.e. citric acid. The % titratable acidity was determined by taking 5ml of sample, adding 4 to 5 drops of 1% phenolphthalein indicator and titrating with 0.1N NaOH. The following formula was used to calculate the total acid %, (Ranganna, 2001).

$$\text{Total Acid (\%)} = \frac{(\text{Titrate} \times \text{Equivalent weight of acid} \times 100)}{(\text{Volume of sample taken} \times 1000)}$$

Fruit decay (%)

The number of decayed fruits due to fungus or any micro-organisms infection was recorded at days 0, 1, 4, 7 and 10, which were compared with a day 0, and calculated as a percentage of the total number of fruits using the following equation.

$$\text{Decay Percentage} = \frac{\textit{Total number of decayed fruits}}{\textit{Initial number of stored fruits}} \times 100$$

Total soluble solids (˚Brix)

Total soluble solids (TSS˚Brix) were determined at a room temperature of 21˚C, with a hand refractometer using 2 to 3 drops of juice obtained by squeezing the fruits.

Ripening Index (RI)

Ripening Index (RI) was determined as a ratio of total soluble solids (TSS) and Titratable acidity (TA). 10g of fruit pulp was homogenized using a blender with 40mL of distilled water; followed by filtration with muslin cloth. The TA was determined by titrating 10mL juice with 0.1N NaOH using phenolphthalein till the solution turned light pink in colour (pH=8.1). The results were expressed as % of citric acid. TSS of the sample was determined using a digital refractometer.

Results and Discussion

Characterization of chitosan

Chitosan was extracted from chitin got from prawn shell and further purified and confirmed by FTIR (Figure 9).

The major absorption band is observed between 1220cm^{-1} and 1020cm^{-1} which represents the free amino group (-NH_2) at C_2 position of glucosamine, a major group present in chitosan. Further the sample showed the absorption bands at the various peaks 684, 863, 900.49, 1028.20, 1069.23, 1406.50, 1428.69, and 3565.95 which are similar to standard chitosan. This shows the conformation of chitosan.

Table 1: Formulations of chitosan based coating solution.

Sr. No	Chemical Name	Weight (gm or ml)
1	Chitosan	20gm
2	Tween 80	1gm
3	Glacial Acetic Acid	5ml
4	Glycerol	10ml
5	Distil. Water	q.s 1000ml

Table 2: Physiochemical properties.

Parameters	Chitosan
Moisture content	7%
Ash value	1.73%
PH	6.7
Solubility	Acetic acid (93%)
	HCL (70%)
Degree of deacetylation	84%
Loss on drying	1.60%

Table 3: Antimicrobial test for chitosan.

No.	Temperature	Growth observed
1	35˚C	Increase
2	37˚C	Increase (maximum)
3	40˚C	Increase

Results

An effort had been made to explore the physicochemical properties and antimicrobial activity as well as structural properties of prawn waste (shell) collected from market, Bharuch. The results of physicochemical and functional properties of the prepared chitosan are given in the (Table 2).

The prepared Chitosan from chitin was confirmed as reported data. Chitosan from shrimp shell contains moisture in the range 1.0-1.30 % depending upon the season, relative humidity and intensity of sunlight. There is no significant difference in the percentage of moisture content between the reported data elsewhere 1-1.30 %. The moisture content of chitosan powder should be <10%. The moisture content obtained was in the range of 7%.

Chitosan has very low ash value, 1.73%; indicates the efficiency of demineralization step followed in the preparation of the chitosan sample by removing the minerals. Commercial chitosan is reported to have ash value about 1.18%.

The solubility of chitosan was checked with five different solvents such as water, ethanol, NaOH, acetic acid and lactic acid. It was not soluble in alkaline or neutral solution, but was soluble in acidic condition, whereas you compare with lactic acid, it was more soluble in acetic 90-95 % solubility was seen. The pH value of chitosan varies from 6.2 to 7.5.

Table 4: Antimicrobial test for coated guava.

Sr. No.	Organisms Name	Antimicrobial Activity (4 day)	Antimicrobial Activity (7 Day)	Antimicrobial Activity (10 Day)
1	*Bacillus subtillis*	Ex. Positive	Ex. Negative	Ex. Positive
2	*Pseudomonas putida*	Ex. Positive	Ex. Positive	Ex. Positive
3	*Pseudomonas stutzeri*	Moderately Positive	Ex. Positive	Ex. Positive
4	*Bacillus megaterium*	Ex. Positive	Ex. Positive	Ex. Positive
5	*Alcaligens faecalis*	Moderately / Negative	Ex. Positive	Ex. Positive
6	*Pseudomonas acidovorans*	Ex. Negative	Ex. Positive	Ex. Positive
7	*Pseudomonas maltophila*	Ex. Positive	Ex. Positive	Ex. Negative
8	*Pseudomonas denitrificans*	Ex. Positive	Ex. Positive	Ex. Negative
9	*Pseudomonas piclorum*	Ex. Positive	Ex. Positive	Moderately / Positive

Meaning of Extremely Positive: Chemical Compound has very sound (Positive) effect against bacterial culture.
Meaning of Extremely Negative: Chemical Compound has very weak (Negative) effect against bacterial culture.
Meaning of Moderately Positive: Chemical Compound seems to be effective against bacterial culture, but not 100% sound effect.

Weight loss

Guava fruits store for different days were significantly affected weight loss (%), Loss of water from the surface of fruits, cell wall degradation, and rapid respiration results to weight loss of fruits. Storage of guava fruits period increase with increased in Cumulative.

Physiological Loss in Weight (CPLW).It might be due to increase in respiration and loss of moisture from the surface of fruits cause significant loss in fruit weight of guavas.

The graph of No. of days *vs.* Weight loss percentage of coated and uncoated guava is plotted and result is shown below (Figure 10).

Moisture content (%)

Data on moisture content were significantly affected by varieties of guava and storage intervals. Weight loss decrease regularly having direct affect on moisture content. Increase in weight loss, reduced metabolic activity and moisture loss from skin of the fruits. Moisture levels totally depend on storage temperature and water pressure gradient between the fruit tissue and the surrounding atmosphere. The graph of No. of days *vs.* Moisture content percentage of coated and uncoated guava is plotted and result is shown below (Figure 11).

Percent titratable acidity

Figure no 12 showed that the acidity of the coated guava was decreased as the ripening time prolonged. This phenomenon probably similar to the uncoated guava acidity changes. Coated guava had a certain trend of pH development during ripening process related to organic acid content. Coated and uncoated guava at mature stage would have a pH ascending and organic acid descending. The acidity of the coated guava in this research was ranged from 29.06% - 27.9%. The graph of No. of days *vs.* Titrable Acidity in percentage of coated and uncoated guava is plotted and result is shown below [8-16] (Figure 12).

Fruit decay (%)

The number of decayed fruits due to fungus or any micro-organisms infection was recorded at days 1, 4, 7 and 10 which were compared with a day 0. The graph of No. of days *vs*. Fruit decay in percentage of coated and uncoated guava is plotted and result is shown below (Figure 13).

Antimicrobial test

In Antimicrobial test throw the bacterial growth observed at all different temperature at 35, 37, 40 degree. As a reference table no 3.2 In general the lower the MW and the DA, the higher will be the effectiveness on reducing microorganism growth and multiplication (Table 3).

The chitosan-based coating incorporated with antimicrobials could extend the shelf life and improve the storage quality of fruits and vegetables. The chitosan coating enriched with antimicrobials exhibited the excellent inhibition on the growth of bacteria, yeast, and molds. Postma displayed that the combined application of chitosan and Lysobacter enzymogen could express better control effects on the development of Pythium aphanidermatum in cucumber. Based on result of antimicrobial activity, we firmly confirmed that chitosan has a strong capability to inhibit the growth of various microbes such as *Bacillus, Pseudomonas, Staphylococcus* and *Streptococcus* (Table 4).

Conclusion

This project work focused on developing chitosan from chitin, which is a marine waste. Chitin was processed & by surface modification, chitosan was synthesized. To confirm the chitosan formation, tests such as moisture content, pH, loss on drying, solubility, degree of deacetylation & Ash value were studied, which confirmed the formation of chitosan also IR spectroscopy was employed to study & be more affirmative.

Further, chitosan & other chemicals majorly Tween 80 was used to formulate a coating, which was employed on fruit & vegetable subjects. This coating is edible & each be consumed. Further the tests such as physical appearance, color and texture were employed. Majorly antimicrobial activity was carried out, which showed positive effect on the subject, thus, the developed coating may help in extension of fruit life.

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J Bacteriol Mycol - Volume 6 Issue 1 - 2019
ISSN : 2471-0172 | www.austinpublishinggroup.com

Citation: Limbani A, Bishnoi A and Shah M. Modification of Bio-polymer and It's Application. J Bacteriol Mycol. 2019; 6(1): 1095.