*THINK INDIA JOURNAL*

ISSN:0971-1260
Vol-22- Issue-12-November-2019

# Chitosan-Nisin Composite Edible Film Preparation, Optimization By RSM And Analysis Of Different Assays Of The Optimized Edible Film.

**Sweety Kaur[a,ł], Richa Arora[a*]**

[a]School of Bioengineering and Biosciences, Lovely Professional University, Phagwara, Punjab, India

[ł]Present address: Nestle India Limited, Rajarhat, Kolkata, India

[*]Corresponding author; E-mail: richa.22440@lpu.co.in

## INTRODUCTION:

Depending on the dynamic changes in the consumer's demand and market trends, the area of edible film with enormously different types of combinatory of edible polymers has resulted in performing active packaging of the food materials [1]. Active packaging of food by introducing edible films including fresh fruits and vegetables, poultry and sea foods, milk products and meat products which is becoming significant, especially in this modern success-racer world where nobody have the time to think even about their health.

Food contamination is one of the common phenomenon of deterioration of food due to its exposure to unhealthy environment that permits the growth of the pathogenic microbes. There are some traditional preservation techniques to preserve the food and to increase the shelf life durability of the food but recontamination occurrence can spoil the food, making it unappetizing for the consumers to consume it. Introduction of antimicrobial edible film or edible coating packaging system is a novel development in this field that significantly emphasizes on the incorporation of antimicrobial agents into the polymer matrix or film that suppresses the activity of the targeted microorganisms that are duly responsible for the contamination of food products [2].

An edible film is specified by U.S. Food and Drug Administration and Generally regarded as safe (GRAS) derived thin membranous sheet made up of edible biopolymers which can be ingested directly, layered on the food peripheral surface or stowed as hurdle between the food and the surrounding atmosphere bearing parameters such as moisture, O2 and CO2 gases, fats and oils, vapors and hence shield the food product, increase its shelf life and enhance food quality too [3]. Most of the edible films are thin layered that can be introduced as a thin substratum positioned on the periphery or within the food materials [4] and can also applied as wrappers or in the form of sachets for the food product. In comparison with conventional packaging, these edible films exhibit extraordinary properties that includes biocompatibility with the food to be packed, barrier to O2, CO2 and vapors, harmless, eco friendly, mechanically coherent and relatively inexpensive and affordable [5]. Furthermore, edible films represent as

ISSN:0971-1260
Vol-22- Issue-12-November-2019

carriers for antioxidants that arrests the oxidation of food, off-flavors issue and loss of nutritionality while the antimicrobial carriers eludes food-borne bacterial spoilage and organoleptic decline by the growth proliferation of microorganisms [6] and these supplements enhances the shelf-life of food along with structural integrity and handling attributes. The main reason of the increment in the usability of edible films and coating recently grasped attention due to its eco friendly pollution free and biodegradable nature [7] and next field of development in edible film technology will be dependent on the safety measures related with addition of natural antimicrobial agents [8]. Edible films and coatings are grouped into three categories depending upon the nature of the components or ingredients employed are: hydrocolloides (polysaccharides, proteins, and alginates, lipids (comprises of fatty acids, glycerides or waxes) and composites (by blending the ingredients from the two groups) [9].

Nowadays, a great emphasis is given to chitosan which is an continuous undeviating polysaccharide constructed of β-(1→4)-linked D-glucosamine (deacetylated unit) and N-aectylD-glucosamine (acetylated unit) because of its less toxic, biodegradable, strengthness, comparable inexpensive source, renewable substance from the industry with exceptional antimicrobial properties [10]. Chitosan exhibits twofold helical crystalline structure which can be adapted to a dehydrated form and similarly into the hydrated form with molecular packing [11]. Chitosan is the deacetylated form of chitin which is having source of cell wall of fungi, insect's cuticle and crustaceans shells providing structural support to these species. Chitosan films are efficiently prepared and applied in the food packaging system such as meats, poultry, fruits, and vegetables [12]. Chitosan film are clear, elastic films with recognized mechanical features with selective permeability to vapors [13] and inferior sensitivity towards water with respect to hydroxypropyl methylcellulose (HPMC) films [14]. Though in case of certain foods the antimicrobial potential ceases upto particular limit and not achieve the antiseptic packing range [15] so to improve the effect of chitosan film against food-borne contaminants incorporation of antimicrobial agents such as nisin, organic acids like sodium benzoate and potassium sorbate are inserted that increases the durability and shelf-life period of those food products [16].

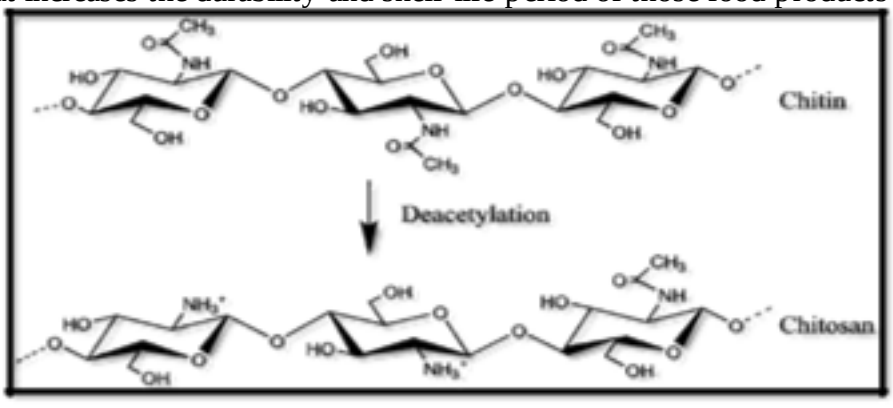



**Figure 1: Structure of chitosan after deacetylation of chitosan extracted from natural waste with buffer.**

ISSN:0971-1260
1-22- Issue-12-November-2019

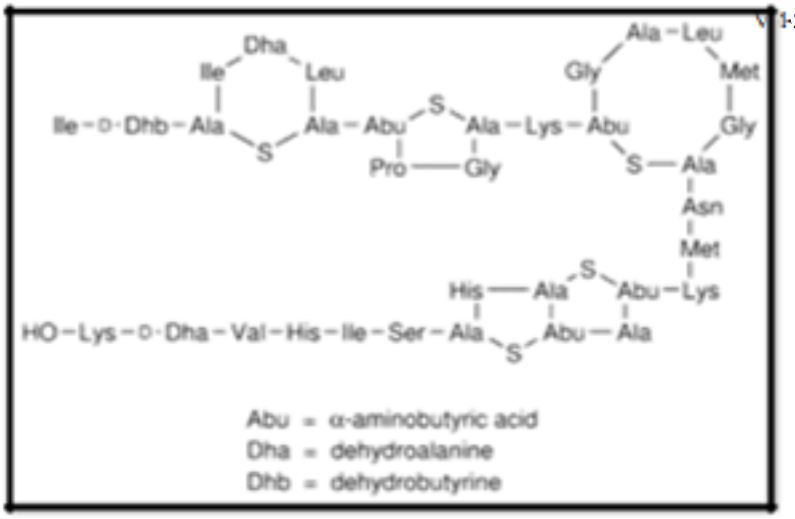



**Figure 2: Structure of Nisin derived from *Lactococcus lactis***

Nisin is an antimicrobial agent that improves the preservation period of the food products with combination with biopolymers incorporated for the preparation of edible films. It is a heatresistant polycyclic antibacterial polypeptide composed of 34 amino acid derivatives synthesized by the bacterium *Lactococcus lactis* that kills the responsive contaminating pathogens by acting upon their cell membrane, leading to the leakage of intracellular entity causing death of the causal microorganism [17]. The capacity of nisin as an antimicrobial is bounded upto raw or minimally processed seafoods due to some parameter that includes proteolytic action of raw or minimally processed foods which leads to the rapid degradation of incorporated nisin and decline in the antimicrobial activity [18].

The challenges in the area of edible film technology with antimicrobial incorporation is the gap between the laboratory analyzed results and their realistic concurrent applications. In earlier years, serious concern was the quality, safety and preservation of the food product, but there is yet growing trouble upon the food spoilage along with its components that are causing problematic issues among consumers [19]. Hence these issues demands requirement for much more potent regulation systems for the protection, shelf-life increment and transportation of food products to customers in healthy way. The currently employed conventional methods for the preserving the quality of food materials across time period not raised to fulfill the consumer needs for the food extracted from natural sources and moreover there is necessity in the food based industries to look into more options to currently using chemical reagents. Among the list of antimicrobial and antioxidant additives, there are many that have verified in laboratory scale

ISSN:0971-1260
Vol-22- Issue-12-November-2019

but their influence in real time potential is till date challenged by particular property of food product and their medium for uses.

The main objective of our study is to design an chitosan edible film with incorporation of nisin as an antimicrobial agent, to check the antimicrobial activity of the edible film with optimized concentration after optimization with RSM against the food-borne pathogenic contaminants and structural and physicochemical characterization study of the optimized edible film including transparency, FT-IR analysis, and moisture content. The study also focuses on the preservation period exhibited by the films prepared by their incorporation on the food products outer surface.

## 1. Materials and Methods:

### 1.1 Procurement of microbial cultures, edible film materials and fruit samples:

*Escherichia coli MTCC 1687* and *Listeria monocytogenes MTCC 1143* were obtained from the culture collection centre of IM-TECH Chandigarh, India. Both cultures obtained were revived and grown in nutrient broth at 37˚C for 24 hours in incubator aerobically for future use.

Fresh grapes fruit were brought from the local vendors of LPU campus to check the preservation assay of fruit by the edible film incorporation and its comparison with polyethene wrapped grapes along with unwrapped grapes.

Chitosan biopolymer applied for edible film preparation was obtained from HiMedia Pvt. Ltd. Nisin as an antimicrobial agent in the edible film was obtained from Sigma Aldrich Ltd. Glycerol, acetic acid and tween 80 was obtained from Loba Cheme Pvt. Ltd.

### 1.2 Preparation of antimicrobial chitosan edible film:

Chitosan film was prepared by dissolving 2.5 % (w/v) chitosan flakes in 100ml distilled water containing 2.5 % glacial acetic acid (v/v) and agitated uniformly using magnetic stirrer (REMI 2MLH) for 2 hrs at room temperature. Nisin was added as an antimicrobial agent into the film solution at concentration of 0.05 % (5000 IU/ml or 5 mg). Glycerol was added as an plasticizer at concentrations of 0.25 % (w/v) and tween 80 was also mixed into the film suspension as an emulsifier at the concentrations of 0.2 % (w/v) The pH adjusted to 6.0 using 1N NaOH buffer and stirred for 30 minutes to attain proper dissolution. The solution was then filtered using muslin cloth to remove the undissolved matter and to maintain the thickness of the film, the

ISSN:0971-1260
Vol-22- Issue-12-November-2019

amount of solution poured on to the plates were same as 15 ml for all films that was casted in Teflon coated petriplate which was dried at 37 ˚C for 24-48 h.

### 1.3 Optimization of antimicrobial chitosan edible film using Response Surface Methodology:

The chitosan edible film was optimized using Response surface methodology (RSM) which is a compilation of statistical and mathematical procedures that are functional for designing experimental setups, building models by regression and estimating the effects of different variable parameters along with their interactions for responses. According to Design Expert (v.11 Stat-Ease, Inc., Minneapolis, USA) software, the varying concentration of chitosan, acetic acid, glycerol and nisin were considered as variables whereas zone of inhibition of Escherichia coli and Listeria monocytogenes were considered as response against these variables.

**Table 1: Representation of low coded and high coded values of processing variables**

| Factor | Name | Units | Maximum | Minimum | Coded Low (-1) | Coded High (+1) | Mean | Std. Dev |
|---|---|---|---|---|---|---|---|---|
| A | Chitosan concentration | % | 0.5000 | 2.5000 | 0.50 | 2.50 | 1.50 | 0.8330 |
| B | Glycerol concentration | % | 0.2500 | 1.0000 | 0.25 | 1.00 | 0.6250 | 0.3124 |
| C | Acetic acid concentration | % | 0.5000 | 2.5000 | 0.50 | 2.50 | 1.50 | 0.8330 |
| D | Nisin concentration | % | 0.0500 | 0.7500 | 0.05 | 0.75 | 0.4000 | 0.2915 |
| E | pH | | 4.00 | 6.00 | 4.00 | 6.00 | 5.00 | 0.8330 |

### 1.4 Comparative antimicrobial analysis of nisin, chitosan and nisin-chitosan against food borne pathogens:

A comparative analysis of antimicrobial potential of nisin (NS) as an antimicrobial agent into the film and its comparison with the effectiveness of chitosan (CH) alone and together nisin-chitosan (NS-CH) combination was performed. Muller Hinton plates were prepared and both the cultures Escherichia coli and Listeria monocytogenes were spreaded evenly under sterile conditions. After the inoculation, total of three wells were made in each plate of both the

ISSN:0971-1260
Vol-22- Issue-12-November-2019

cultures for the three different samples and were labeled. All the three samples were taken in same concentration of 100µl and were filled into the wells carefully. The plates were incubated at 37˚C for 24 h to check the effectiveness of all the three samples with their comparison study.

## 1.5 Physical properties of the optimized chitosan film:

### 1.5.1 Film transparency:

The transparency of the chitosan film was determined by cutting film strips of 3 cm × 1 cm in triplicates and positioning them inside the quartz cuvette containing water longitudinally without any folding and dissimilarity to pass the UV light from it. The percentage transmittance (% T) was measured at a fixed wavelength of 660 nm using UV-Visible Spectrophotometer.

### 1.5.2 Moisture Content:

The moisture content of the chitosan film was estimated after drying the film at 105 ˚C in hot air oven under agitated air flow for 24 h. The small section of edible film was cut and weighed 0.200gm for all the three plates and kept for drying placing in the dried pertiplate for 24 h at 105 ˚C. The dried plates containing dried films were kept in dessicator for an hour and then the weight loss was measured. The weight lost by the film sample was calculated after 24 h drying incubation and the moisture content of the film was estimated at all the three intervals and an average moisture content was estimated using the equation:

$$\text{Moisture Content (\%)} = \frac{(\text{I.M} - \text{F.M})}{\text{I.M}} * 100$$

(Here, I.M = Initial Moisture and F.M = Final Moisture)

### 1.5.3 Film Solubility:

The films were cut into 2cm×2cm small strips to determine the solubility of the optimized edible films. The film strips were dried at 105 ˚C to attain the constant weight for 1-2 hrs and then are further weighed which was the initial dry weight (M1) of the chitosan film. After that, these dried film strips were immersed into the 100 ml beaker containing 50 ml distilled water and covered with cellophane wraps upon the mouth of the beaker. The film containing solution immersed in water was stored at 25 ˚C for 24 h with stirring at constant intervals. Next, the films pieces that were undissolved were taken out, dried upon filter paper and then again dried at 105 ˚C to attain the constant weight and to obtain the final dry weight (M2). Then, the film solubility was determined using the equation [20]:

$$\text{Solubility (\%)} = [(\text{Wt. of initial dry film} - \text{Wt. of undissolved film})/\text{Wt. of initial dry film}] \times 100$$

ISSN:0971-1260
Vol-22- Issue-12-November-2019

### 1.5.4 Film Swelling Degree:

The films were cut into 2 cm × 2 cm small strips to determine the swelling degree of the optimized edible film. The film strips were weighed to get the initial dry weight of the film before swelling (M1). Then, the films were immersed into the 50 mL beaker containing 30 mL of distillled water at 25 ˚C for 24 h. The water immersed films were then dried apparently using Whattmann filter papers, further followed by the weighing of the immersed wet films to get the final weight of the film (M2). The swelling degree of the optimized film was estimated by the equation [20]:

$$\text{Film swelling degree (\%)} = \frac{(M_2 - M_1)}{M_1} \times 100\%$$

(Here $M_1$= initial dry weight of film and $M_2$= final swelling weight of the film after 24 hrs immersion)

### 4.6 Bioactivity Assay

#### 4.6.1 Resolution of antimicrobial activity of the optimized film:

For the culture revival of E.coli and L. monocytogenes, Brain heart infusion agar medium and MacConkey Agar medium were used along with their revival into the Nutrient Broth medium with incubation of 24 h at 37 ˚C under aerobic incubator and shaker incubator.

The antimicrobial property of the film was analyzed against both the bacterial strains by the method of paper disk diffusion assay. Both the bacterial strains were subjected to serial dilution upto $10^{-6}$ CFU/ml dilution and then, 100µl of the dilution containing both the test strains were spread evenly on the solidified agar surface plate. After that, small circular filter disk were cut with the diameter of 8 mm uniformly. The 8 mm circular filter disk was immersed into the optimized chitosan film solution and placed at the centre of the culture inoculated plates. These plates were further subjected to 24 h of incubation at 37 ˚C and then the diameters (mm) of zone of inhibition were measured using scale.

### 4.7 Characterisation of Optimized film:

#### 4.7.1 FTIR analysis:

ISSN:0971-1260
Vol-22- Issue-12-November-2019

The preliminary structures and uniformity of the optimized chitosan film containing nisin as an antimicrobial agent was characterized through Fourier Transform Infrared (FTIR) spectrometry analysis using SHIMADZU JAPAN Model Spectrum. The FTIR spectral measurements were done in the transmittance state. The measurements were done in the mid-infrared range from 4000 to 500 cm-1 with air as the background reference [21].

**4.7.2 SEM analysis:**

The morphology and the cross-section of the optimized chitosan film was determined by Scanning Electron Microscopy (SEM) using MODEL JSM-IT300LV (JEOL) in IIIM, Jammu. The cross-section of the optimized film was obtained by cutting the film with scissor or sterile blade. The morphology of the film was observed at two magnifications: 250X and 500X. Before SEM measurement, the sample optimized film was coated with metal solution to increase the conductivity effectiveness on the film surface.

**4.8 Preservation assay:**

**4.8.1 Comparative study of grapes spoilage assay at room temperature (37˚C) and at refrigerated temperature (4˚C):**

The fresh fruit grapes were brought from the vendor and were subjected to different parameters that are responsible for the analysis of preservation and spoilage assay of grapes of 3 types i.e., chitosan edible film wrapped grapes, polyethene wrapped grapes and control grapes without any wrapping and their comparison at 2 different temperatures of 4˚C and 37˚C. The parameters that are involved includes physical weight loss analysis and microbiological analysis after the sanitization and preparation of grapes for the assay.

**4.8.1.1 Sanitization and Preparation of grapes:**

Fresh grapes fruit were subjected to sanitization with chlorine solution of concentration 0.2 gl-1 for 10-15 minutes by dipping and again rinsing the grapes with 3 mgl-1 of chlorine solution. The rinsed grapes were further kept in trays and left for drying at room temperature for few minutes.

Fresh grapes after sanitization were subjected to wrapping with polyethene and edible films. Total of complete 6 grapes were taken, out of which two grapes were wrapped with optimized chitosan edible film, two wrapped with low density polyethene sheet and two were left unwrapped as an control. One set of all the three types were kept at room temperature 37˚C in

ISSN:0971-1260
Vol-22- Issue-12-November-2019

open air named as Set 1 whereas the other set of all the three types were preserved in refrigerated condition at 4˚C named as Set 2. Both the sets were subjected to different preservation parameters to check the durability and spoilage of fruits.

**4.8.1.2 Weight loss analysis of grapes:**

The weight loss analysis of fruit was estimated by the method depicted by [22] using a digital weight balancing machine. All the six representative samples were weighed after preparation to get the initial weight on the initial day of preservation and then weighed consecutive days till the day of spoilage attained by grapes of both the sets. Weight loss during the preservation and storage was expressed as percentage (%) and can be estimated by the equation:

$$\textbf{Weight loss (\%)} = \frac{\textbf{Final weight loss per day}}{\textbf{Initial fresh weight}} \times 100\%$$

**4.8.1.3 Microbiological assay:**

The microbiological analysis methodology was demonstrated by [23] to depict the total microbial load and spoilage of grapes during preservation that was conducted at the final day of preservation to demonstrate the performance of film in extension of shelf life of fruit.

The grape fruits of both sets were immersed into 50 ml peptone water which is considered as the stock solution for dilutions separately. From this stock solution, 1 ml was taken using micropipette and transferred into a test tube containing 9 ml of peptone water and mixed well to make 10-1 dilution and serially diluted to obtain 10-2 and 10-3 dilutions. Now, after serial dilution, all the six samples were plated on nutrient agar medium using the dilutions of 10-2 and 10-3 for each of six samples to check the microbial load of aerobic mesophillic and psychrotrophic bacteria at two different temperatures. The inoculated plates were incubated at

37 ˚C for 24-48 h and the results were expressed in CFU/ml as:

$$\textbf{CFU/ml} = \frac{\text{No. of colonies}}{\text{Dilution plated} \times \text{Vol. of culture plated}}$$

**4.9 Evaluation of Hemolytic activity on Human erythrocytes against Chitosan edible film:**

ISSN:0971-1260
Vol-22- Issue-12-November-2019

The hemolytic activity of chitosan edible film was determined using the ex-vivo hemolysis assay by the quantification of hemolysis percent using equation with substitution of absorbance values estimated using [24] and [25]. In brief, 2% human erythrocyte suspension was prepared by extracting blood from healthy human body and separating erythrocytes from plasma by centrifugation. Then, the erythrocytes were treated with chitosan film methanolic extract solution at different concentrations (25 µg/mL, 50 µg/mL, and 100 µg/mL), incubated for 2 hrs at 37°C with shaking which will be considered as test samples. The human erythrocyte suspension was incubated with phosphate buffer saline (PBS) with same procedure which will be regarded as negative control. The human RBC suspension was also incubated with 10% v/v Triton X-100 to attain the complete hydrolysis. After two hours of incubation, all the three test samples with different concentrations were volume makeup with PBS to attain 1 ml of total suspension. All the three suspensions were centrifuged at 1000×g for 10-15 minutes. Supernatents are collected and subjected to estimate the absorbance at 540nm to check the amount of hemoglobin released which is calculated by the equation:

$$\text{Hemolysis}\,(\%) = (\text{Abs} - \text{Abs}_0)/(\text{Abs}_{100} - \text{Abs}_0) \times 100$$

(Here, Abs= absorbance of 3 different test samples ; Abs0= absorbance of RBC treated with PBS; Abs100= absorbance of RBC treated with Triton X-100)

## 2. Results and Discussion:

### 5.1 Revival of MTCC microbial cultures:

The microbial cultures Escherichia coli MTCC 1687 and Listeria monocytogenes MTCC 1143 obtained from IMTECH Culture collection were at first revived in nutrient broth and then again revived to their specific MacConkey agar medium and Brain Heart Infusion (BHI) agar medium to attain the pure isolated colonies of the cultures after incubation for 24 h at 37 ˚C and the results are depicted in figure 3 (a) and (b).

### 5.2 Study of optimized chitosan edible film using Response Surface Methodology:

Response surface methodology (RSM) using central composite rotatable design (CCRD) with a quadratic model and linear model was employed. The level of 5 factors (processing variables) and 2 experimental responses are presented in Table5 that were performed in order to attain the optimized variables and responses. The data was analysed employing multiple regression technique to develop a response surface model. The analysis of variance (ANOVA) for two responses showed that all models developed were statistically significant ($P \leq 0.0001$) with no significant lack of fit, which suggested that they adequately described the true functions. The

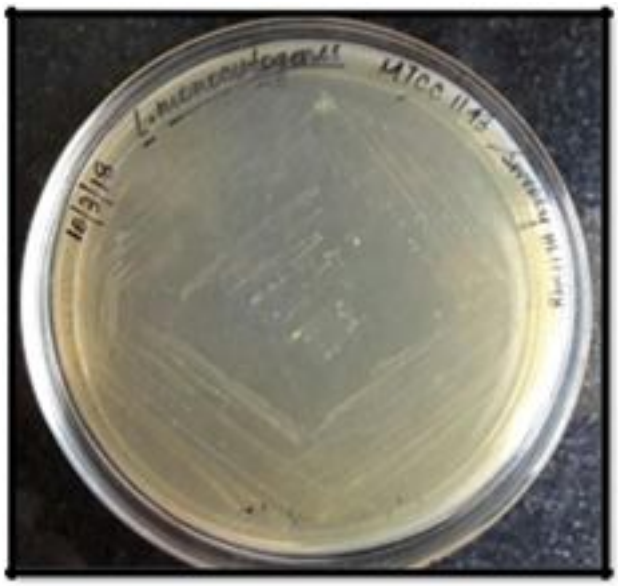
 

ISSN:0971-1260
Vol-22- Issue-12-November-2019

ANNOVA of the quadratic model of both the responses i.e., ZOI *E.coli* and ZOI *L.monocytogenes* were depicted in table 6 and table 7. The values of the both the responses were plotted by the software showing the predicted vs. actual results of the responses. Figure 5 and 6 depicts the surface 2-dimensional plot of the effect of the concentration of glycerol and chitosan against ZOI of *E.coli* and *L.monocytogenes*.

**Figure 3: Revival of cultures in their specific medium**

***Escherichia coli* MTCC 1687 in MacConkey agar medium**



***Listeria monocytogenes* MTCC 1143 in BHI agar medium.**

ISSN:0971-1260
Vol-22- Issue-12-November-2019

**Table 2: Design matrix used to evaluate the effects of process variables and value of experimental responses for chitosan based edible film**

| | | Factor 1 | Factor 2 | Factor 3 | Factor 4 | Factor 5 | Response 1 | Response 2 |
|---|---|---|---|---|---|---|---|---|
| Std | Run | A:chitosan concentration | B:glycerol concentration | C:acetic acid concentration | D:nisin concentration | E:pH | ZOI E.coli | ZOI L. monocytogenes |
| | | (%) | (%) | (%) | (%) | | (mm) | (mm) |
| 1 | 30 | 0.5 | 0.25 | 0.5 | 0.05 | 4 | 9 | 30.5 |
| 2 | 44 | 2.5 | 0.25 | 0.5 | 0.05 | 4 | 13.5 | 14 |
| 3 | 28 | 0.5 | 1 | 0.5 | 0.05 | 4 | 12 | 18 |
| 4 | 49 | 2.5 | 1 | 0.5 | 0.05 | 4 | 9.5 | 13.5 |
| 5 | 34 | 0.5 | 0.25 | 2.5 | 0.05 | 4 | 18 | 22 |
| 6 | 48 | 2.5 | 0.25 | 2.5 | 0.05 | 4 | 11.25 | 21 |
| 7 | 21 | 0.5 | 1 | 2.5 | 0.05 | 4 | 9.5 | 20 |
| 8 | 3 | 2.5 | 1 | 2.5 | 0.05 | 4 | 9.25 | 18.5 |
| 9 | 7 | 0.5 | 0.25 | 0.5 | 0.75 | 4 | 12.5 | 19.5 |
| 10 | 6 | 2.5 | 0.25 | 0.5 | 0.75 | 4 | 12.5 | 13.5 |
| 11 | 45 | 0.5 | 1 | 0.5 | 0.75 | 4 | 10.5 | 13 |
| 12 | 40 | 2.5 | 1 | 0.5 | 0.75 | 4 | 15.5 | 13.5 |
| 13 | 13 | 0.5 | 0.25 | 2.5 | 0.75 | 4 | 11.25 | 15 |
| 14 | 35 | 2.5 | 0.25 | 2.5 | 0.75 | 4 | 10.5 | 19.5 |
| 15 | 20 | 0.5 | 1 | 2.5 | 0.75 | 4 | 11.75 | 27.5 |
| 16 | 50 | 2.5 | 1 | 2.5 | 0.75 | 4 | 7.512 | 20 |
| 17 | 43 | 0.5 | 0.25 | 0.5 | 0.05 | 6 | 10 | 0 |
| 18 | 8 | 2.5 | 0.25 | 0.5 | 0.05 | 6 | 0 | 18 |
| 19 | 16 | 0.5 | 1 | 0.5 | 0.05 | 6 | 0 | 15 |
| 20 | 25 | 2.5 | 1 | 0.5 | 0.05 | 6 | 8.75 | 14 |
| 21 | 23 | 0.5 | 0.25 | 2.5 | 0.05 | 6 | 14.6 | 20.6 |
| 22 | 24 | 2.5 | 0.25 | 2.5 | 0.05 | 6 | 20.6 | 25 |
| 23 | 4 | 0.5 | 1 | 2.5 | 0.05 | 6 | 18 | 18 |
| 24 | 42 | 2.5 | 1 | 2.5 | 0.05 | 6 | 23 | 23 |
| 25 | 17 | 0.5 | 0.25 | 0.5 | 0.75 | 6 | 0 | 0 |
| 26 | 14 | 2.5 | 0.25 | 0.5 | 0.75 | 6 | 0 | 0 |
| 27 | 10 | 0.5 | 1 | 0.5 | 0.75 | 6 | 0 | 0 |
| 28 | 29 | 2.5 | 1 | 0.5 | 0.75 | 6 | 0 | 0 |
| 29 | 2 | 0.5 | 0.25 | 2.5 | 0.75 | 6 | 0 | 0 |

ISSN:0971-1260
Vol-22- Issue-12-November-2019

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 30 | 1 | 2.5 | 0.25 | 2.5 | 0.75 | 6 | 10 | 22 |
| 31 | 39 | 0.5 | 1 | 2.5 | 0.75 | 6 | 0 | 0 |
| 32 | 41 | 2.5 | 1 | 2.5 | 0.75 | 6 | 0 | 0 |
| 33 | 5 | 0.5 | 0.625 | 1.5 | 0.4 | 5 | 0 | 0 |
| 34 | 15 | 2.5 | 0.625 | 1.5 | 0.4 | 5 | 0 | 0 |
| 35 | 32 | 1.5 | 0.25 | 1.5 | 0.4 | 5 | 0 | 0 |
| 36 | 31 | 1.5 | 1 | 1.5 | 0.4 | 5 | 0 | 0 |

**Table 3: ANOVA for quadratic model of Escherichia coli**

| Source | Sum of Squares | Df | Mean Square | F-value | p-value | |
|---|---|---|---|---|---|---|
| **Model** | **1981.72** | **20** | **99.09** | **8.70** | **<0.0001** | **significant** |
| A-chitosan concentration | 6.41 | 1 | 6.41 | 0.5628 | 0.4592 | |
| B-glycerol concentration | 10.00 | 1 | 10.00 | 0.8780 | 0.3565 | |
| C-acetic acid concentration | 156.57 | 1 | 156.57 | 13.75 | 0.0009 | |
| D-nisin concentration | 212.19 | 1 | 212.19 | 18.63 | 0.0002 | |
| E-pH | 183.85 | 1 | 183.85 | 16.14 | 0.0004 | |
| AB | 2.40 | 1 | 2.40 | 0.2107 | 0.6497 | |
| AC | 0.3325 | 1 | 0.3325 | 0.0292 | 0.8655 | |
| AD | 0.8653 | 1 | 0.8653 | 0.0760 | 0.7848 | |
| AE | 19.12 | 1 | 19.12 | 1.68 | 0.2052 | |
| BC | 7.94 | 1 | 7.94 | 0.6971 | 0.4106 | |
| BD | 0.6435 | 1 | 0.6435 | 0.0565 | 0.8138 | |
| BE | 1.78 | 1 | 1.78 | 0.1559 | 0.6958 | |
| CD | 117.96 | 1 | 117.96 | 10.36 | 0.0032 | |
| CE | 168.54 | 1 | 168.54 | 14.80 | 0.0006 | |
| DE | 225.58 | 1 | 225.58 | 19.81 | 0.0001 | |
| $A^2$ | 1.30 | 1 | 1.30 | 0.1138 | 0.7383 | |
| $B^2$ | 1.30 | 1 | 1.30 | 0.1138 | 0.7383 | |
| $C^2$ | 103.66 | 1 | 103.66 | 9.10 | 0.0053 | |
| $D^2$ | 1.30 | 1 | 1.30 | 0.1138 | 0.7383 | |
| $E^2$ | 1.30 | 1 | 1.30 | 0.1138 | 0.7383 | |
| **Residual** | 330.25 | 29 | 11.39 | | | |
| Lack of Fit | 330.25 | 22 | 15.01 | | | |
| Pure Error | 0.0000 | 7 | 0.0000 | | | |
| **Cor Total** | 2311.96 | 49 | | | | |

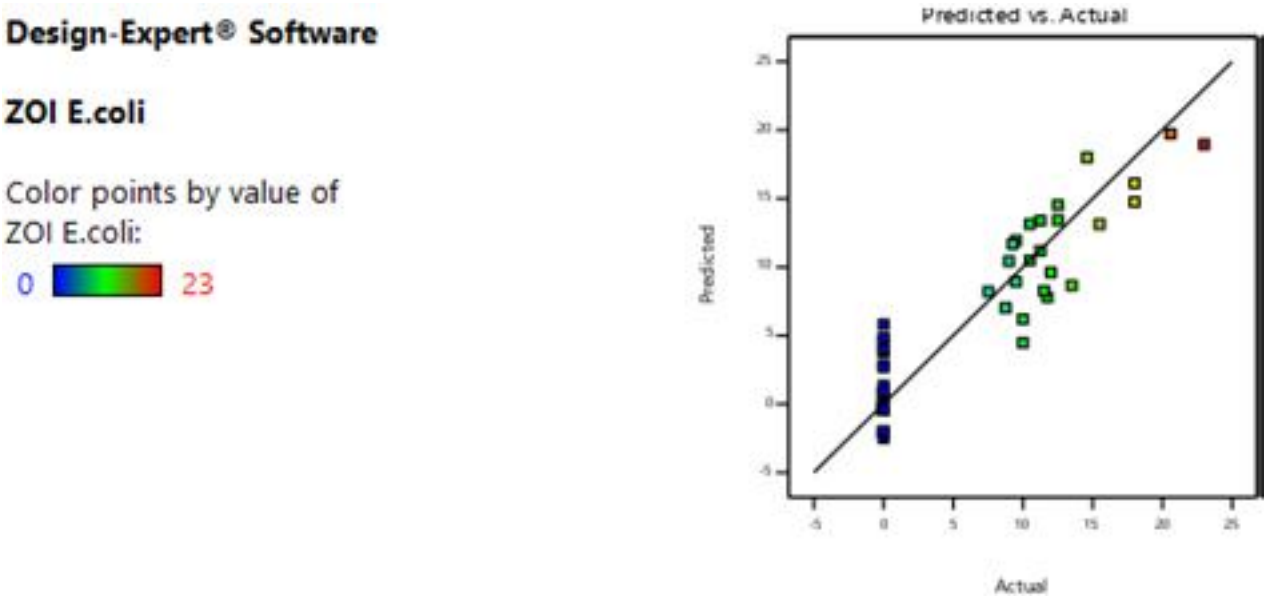
ISSN:0971-1260
Vol-22- Issue-12-November-2019

**Table 4: ANNOVA for quadratic model of *Listeria monoctyogenes***

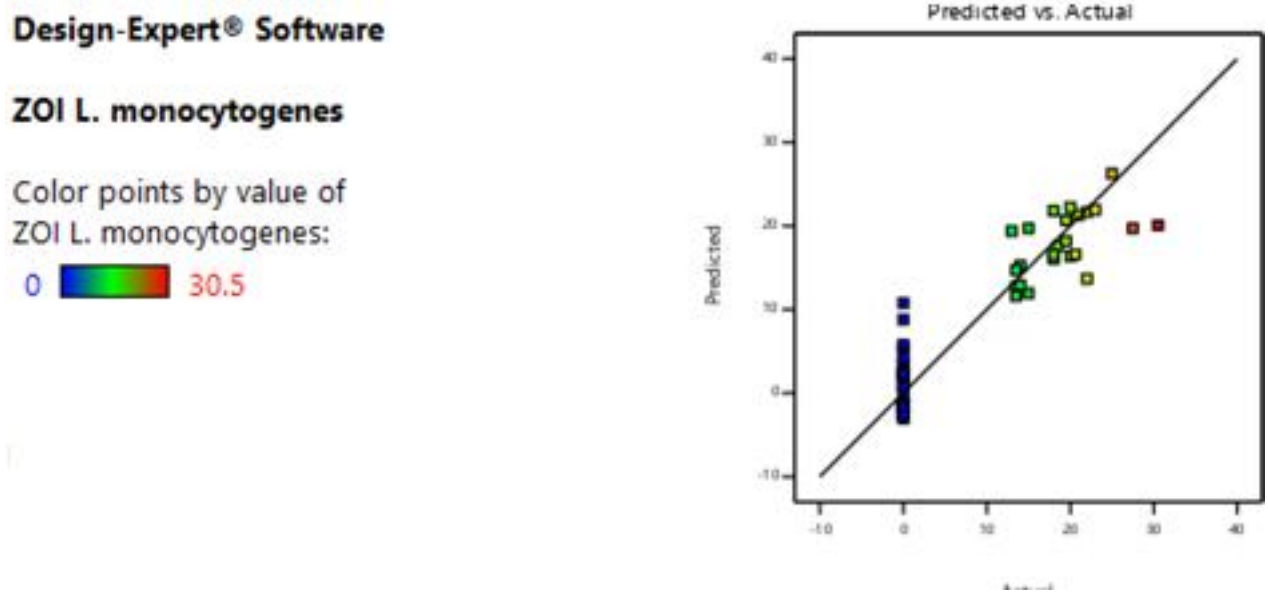
| Source | Sum of Squares | df | Mean Square | F-value | p-value | |
|---|---|---|---|---|---|---|
| **Model** | 4235.63 | 20 | 211.78 | 8.33 | <0.0001 | significant |
| A-chitosan concentration | 7.91 | 1 | 7.91 | 0.3111 | 0.5813 | |
| B-glycerol concentration | 20.81 | 1 | 20.81 | 0.8183 | 0.3731 | |
| C-acetic acid concentration | 236.12 | 1 | 236.12 | 9.28 | 0.0049 | |
| D-nisin concentration | 478.88 | 1 | 478.88 | 18.83 | 0.0002 | |
| E-pH | 604.81 | 1 | 604.81 | 23.78 | <0.0001 | |
| AB | 36.98 | 1 | 36.98 | 1.45 | 0.2376 | |
| AC | 39.16 | 1 | 39.16 | 1.54 | 0.2246 | |
| AD | 3.51 | 1 | 3.51 | 0.1381 | 0.7129 | |
| AE | 202.01 | 1 | 202.01 | 7.94 | 0.0086 | |
| BC | 2.88 | 1 | 2.88 | 0.1132 | 0.7389 | |
| BD | 0.6050 | 1 | 0.6050 | 0.0238 | 0.8785 | |
| BE | 0.6613 | 1 | 0.6613 | 0.0260 | 0.8730 | |
| CD | 0.0113 | 1 | 0.0113 | 0.0004 | 0.9834 | |
| CE | 35.28 | 1 | 35.28 | 1.39 | 0.2484 | |
| DE | 285.61 | 1 | 285.61 | 11.23 | 0.0022 | |
| $A^2$ | 24.31 | 1 | 24.31 | 0.9560 | 0.3363 | |
| $B^2$ | 24.31 | 1 | 24.31 | 0.9560 | 0.3363 | |
| $C^2$ | 24.31 | 1 | 24.31 | 0.9560 | 0.3363 | |
| $D^2$ | 24.31 | 1 | 24.31 | 0.9560 | 0.3363 | |
| $E^2$ | 24.31 | 1 | 24.31 | 0.9560 | 0.3363 | |
| **Residual** | 737.51 | 29 | 25.43 | | | |
| Lack of Fit | 737.51 | 22 | 33.52 | | | |
| Pure Error | 0.0000 | 7 | 0.0000 | | | |
| **Cor Total** | 4973.14 | 49 | | | | |

ISSN:0971-1260
Vol-22- Issue-12-November-2019

**Figure 4: Graphical predicted vs. actual values representation of (a) *E.coli* responses and (b) *L.monocytogenes* responses**




***(a) Escherichia coli***



ISSN:0971-1260
Vol-22- Issue-12-November-2019

*(b) Listeria monocytogenes*

**Figure 5: Surface plot (2-D) for zone of inhibition (ZOI) *E.coli* (mm) showing the effect of glycerol and chitosan against ZOI *E.coli*.**

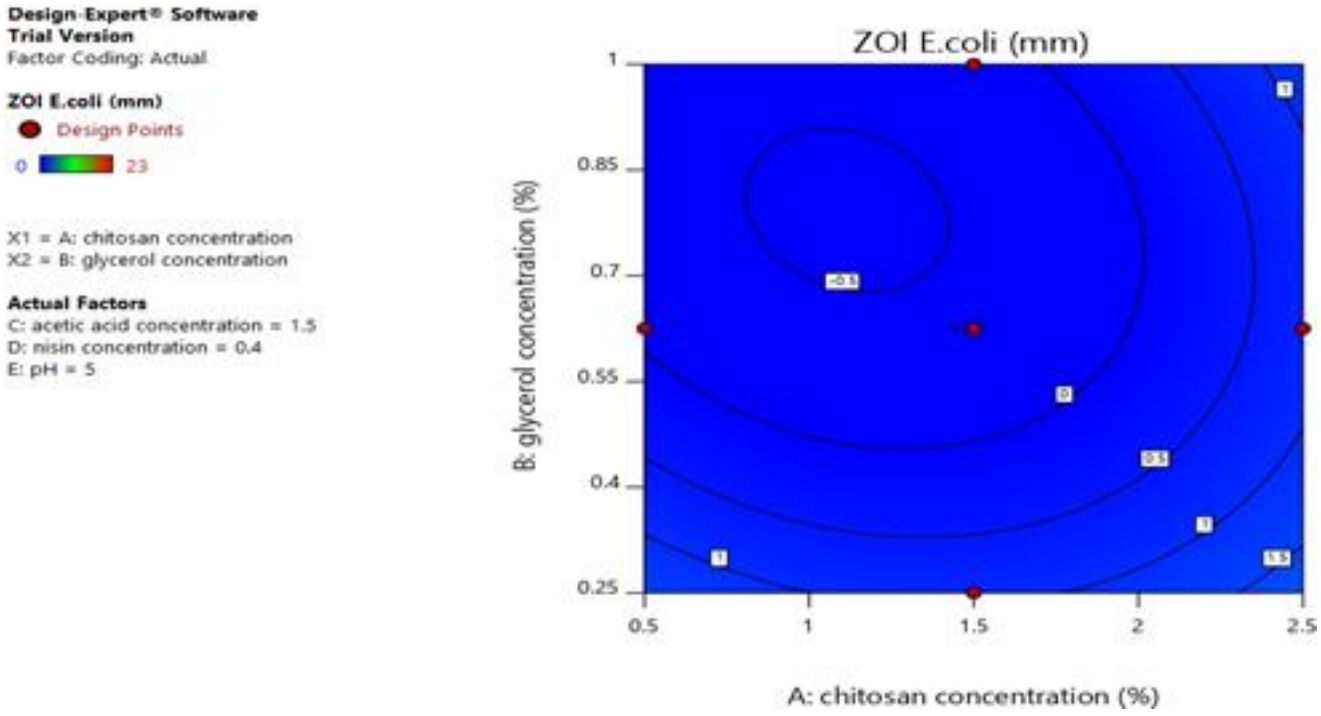
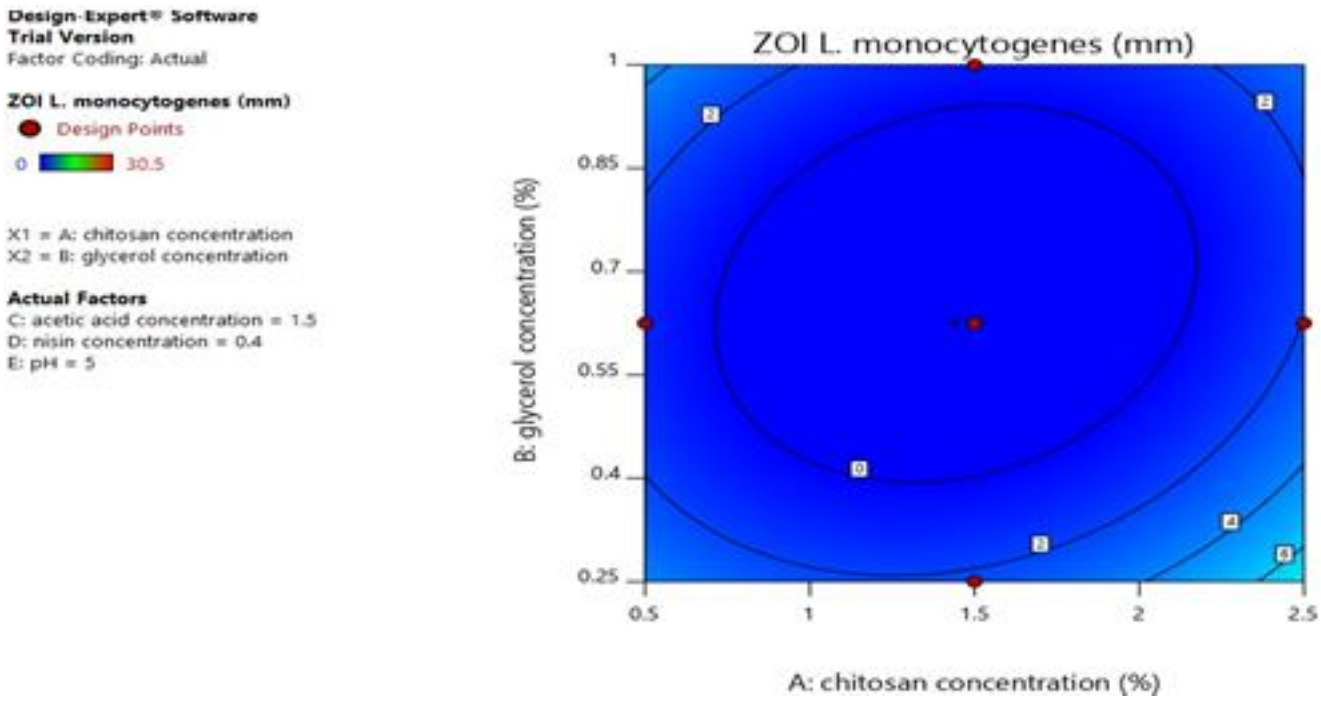



**Figure 6: Surface plot (2-D) for zone of inhibition (ZOI) *L.monocytogenes* (mm) showing the effect of glycerol and chitosan against ZOI *L.monocytogenes*.**

ISSN:0971-1260
Vol-22- Issue-12-November-2019




## 5.3 Bioactive assay:

### 5.3.1 Antimicrobial potential activity of optimized film:

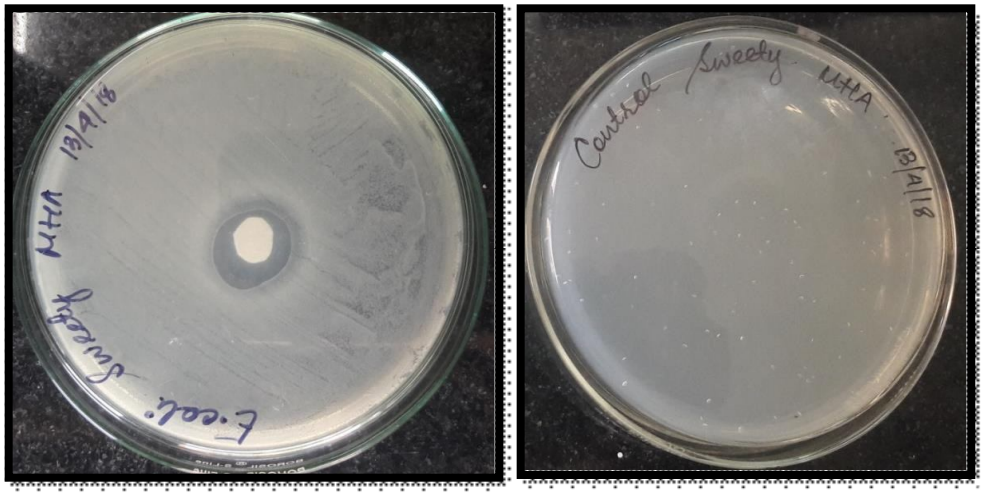
The antimicrobial activity of chitosan film was checked out using paper disc diffusion assay against two food-borne pathogens (*Escherichia coli* MTCC 1687 and *Listeria monocytogenes* MTCC 1143). According to the RSM software, the optimized value that were suggested of different variables and responses after the preparation of total 50 runs with varying concentration along with the antimicrobial activity of each run as responses are highlighted as:

**Table 5: Representation of the optimized concentrations of processed variables and their responses**

| S.no | chitosan conc | glycerol conc | acetic acid conc | nisin conc | pH | ZOI E.coli | ZOI L.monocytogenes | Desirability | |
|---|---|---|---|---|---|---|---|---|---|
| **1** | **2.500** | **0.250** | **2.500** | **0.050** | **6.000** | **19.730** | **26.269** | **0.860** | **Selected** |
| 2 | 2.488 | 0.275 | 2.500 | 0.050 | 5.926 | 19.244 | 24.953 | 0.827 | |
| 3 | 2.500 | 0.254 | 2.443 | 0.051 | 5.991 | 18.527 | 25.424 | 0.819 | |

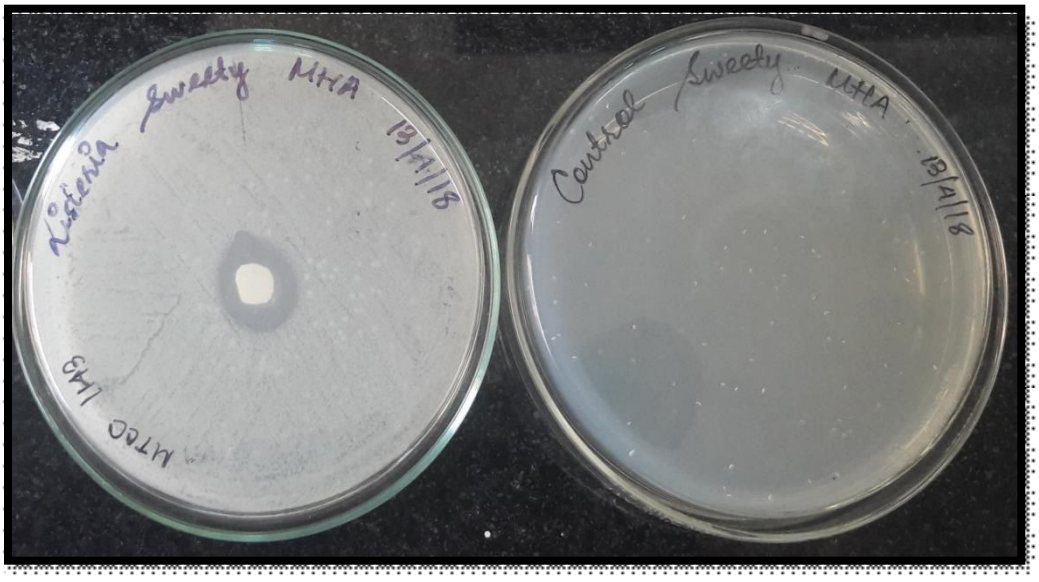
ISSN:0971-1260
Vol-22- Issue-12-November-2019

After getting the optimized value with zone of inhibition for *E.coli* and *L.monocytogenes*, the confirmatory antimicrobial activity was performed against both the cultures. The inhibitory effects of optimized chitosan film on the growth of both the cultures are depicted in the figure.




(a) (b)

**Figure 7:** (a) **zone of inhibition of E.coli of 18.8 mm** against the chitosan film solution dipped 8mm filter disc and (b) control plate of muller hinton without any inoculation



ISSN:0971-1260
Vol-22- Issue-12-November-2019

**Figure 8: zone of inhibition of L.monocytogenes of 20 mm** against chitosan film solution dipped 8mm filter disc (left) and control plate of muller hinton without any inoculation(right).

In comparison to the suggested value by RSM as depicted in above table, the results of zone of inhibition of *E.coli* was 18.8 mm (suggested value 19.73 mm) and *L.monocytogenes* was 20 mm (suggested value 26.263 mm) which was similar to the suggested results. As shown in results, nisin incorporated as an antimicrobial agent was more effective against *L.monocytogenes* than *E.coli*. Previous studies on the effect of nisin against gram negative *E.coli* and gram positive *L.monocytogenes* have depicted that nisin could inhibit the growth of gram positive bacteria more effectively than gram negative bacteria. The antimicrobial mechanism of nisin has been studied by [26]. Nisin bacteriocin derived from *Lactococcus lactis spp. lactis* binds to the outer cell membrane by ionic interactions of C-terminus resulting in formation of pores in cell membrane by the infiltration of hydrophobic N-terminus which leads to the disintegration of proton motive force and release of intra-cellular components [27]. Nisin is less effective against gram negative bacterium because of its failure to penetrate into the cell wall, hence preventing entry to inner membrane but the effectiveness can be increased by combining nisin with chelators like EDTA against gram negative bacteria [28].

### 5.4 Study of physical properties of chitosan film:

#### 5.4.1 Film transparency:

Transmittance characterization demonstrates about the light barrier property of chitosan films which is an important feature to check the prevention of lipid oxidation due to UV light penetration in the food products and to check the efficiency of the packaging edible films for different food products [29].

| Observation | % Transmittance at 660nm |
|---|---|
| **Average %T of film strip** | **94.1%** |

According to the above table as depicted, the chitosan optimized film that was prepared have the average transparency rate of 94.1% at 660 nm. The appearance of the films is mainly rely upon

ISSN:0971-1260
Vol-22- Issue-12-November-2019

the capacity of film to deviate and to absorb light waves exhibited in the visible range of the UV spectrum. Deviation and absorption of the light upon the film demonstrates the transparency, opacity and the color including lightness, yellowness, redness, greenness, and blueness of the film. The interior and exterior microstructure of the film also plays a very important role in the opacity of the film. Film transparency is very important parameter to be checked as it can effect the outer appearance of the packed food products. More transparent a film is, more it will allow the light to pass across it with least deviation or reflection and absorbance [30].

**5.4.2 Film Moisture Content:**

Edible films are mainly used for the for the food packaging in order to extend the shelf life and to prevent the food product from contamination. Moisture content of the film is an important parameter to be estimated because it have to be packed further upon the food product. These packaging films must have the capacity to maintain the level of moisture within the film incorporated food product. It was noted that the film moisture content is 13.3 % of the optimized film. The increase in the moisture content in chitosan film as compared to other films is due to increase in glycerol concentration which is because of the hygroscopic nature of glycerol which further leads to the retardation of forces between the nearby adjacent molecules [31] and vice-versa with increase in chitosan level.

**Table 6: Moisture content of optimized chitosan edible films**

| Film Sample | Temperature (˚C) | Initial weight (g) | Drying Time (hrs) | Final weight (g) | Weight loss (g) | Moisture content (%) |
|---|---|---|---|---|---|---|
| | | | | | | Moisture Content (%) = $\frac{(I.M - F.M)}{I.M} * 100$ |
| Mean value of 3 film samples | 105˚C | 0.2 | 24 | 0.173 | 0.026 | 13.3% |

**5.4.3 Film swelling degree and film solubility:**

ISSN:0971-1260
Vol-22- Issue-12-November-2019

The swelling degree and the water solubility of the film are the two important physical parameters that have to be characterized, because they are affecting directly the water resistant property of the optimized chitosan film. It was noted that the film swelling degree was calculated after 24 h of dissolution in distilled water is 40 % whereas the film solubility of the film after constant stirring at room temperature for 24 hrs is 60 %. The higher values of swelling degree and water solubility of the chitosan film may be attained due to the hydrophilic groups present in the glycerol and other components that can attract easily with the water molecules when in contact with each other.

$$\textbf{Film swelling degree (\%)} = \frac{(M_2 - M_1)}{M_1} \times 100\%$$

$$= \frac{0.07 - 0.05}{0.07} \times 100\% = \textbf{40\%}$$

(Here $M_1$= initial dry weight of film = **0.05gm**
$M_2$= final swelling weight of the film after 24 hrs immersion= **0.07 gm**)

$$\textbf{Solubility(\%)} = [(\text{Wt. of initial dry film} - \text{Wt. of undissolved film}) / \text{Wt. of initial dry film}] \times 100$$

$$= \frac{0.05 - 0.02}{0.05} \times 100\% = \textbf{60\%}$$

### 5.5 Study of characterization of chitosan film:

#### 5.5.1 FTIR spectroscopy analysis:

FTIR spectroscopy was mainly performed to determine the functional groups and to know the intermolecular interactions between the chitosan biopolymer with the antimicrobial agent incorporated which is linked with physical and chemical properties of the film. The FTIR spectroscopy spectrum displayed below is the characteristic interaction of the chitosan film as shown in figure below from 500 to 4000 $cm^{-1}$.

ISSN:0971-1260
Vol-22- Issue-12-November-2019

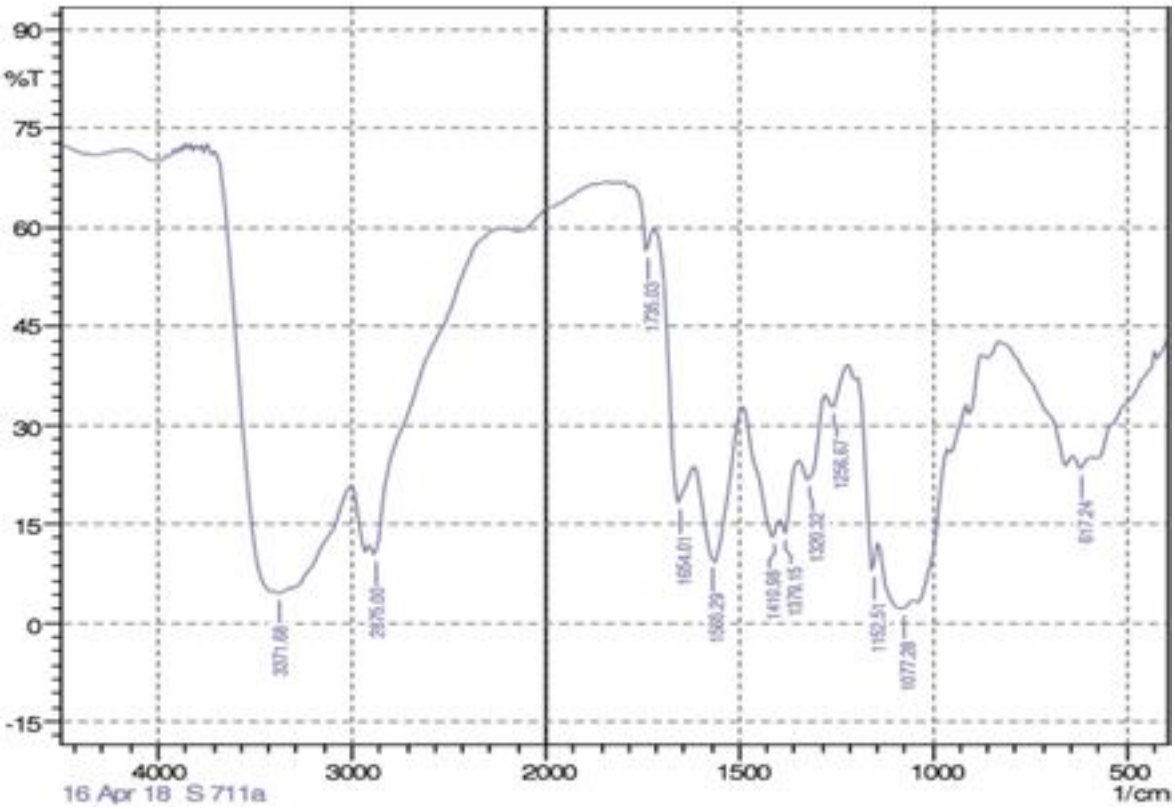



**Figure 9: FTIR spectra of optimized chitosan edible film**

### 5.5.2 SEM analysis:

The morphology of the film was determined using Scanning electron microscopy (SEM) MODEL JSM-IT300LV (JEOL) in IIIM, Jammu. The films were observed at two different magnifications of 250X and 500X. The SEM images were in the range of 50 µm to 100 µm. The SEM images are denoted below with their different magnifications:

ISSN:0971-1260
Vol-22- Issue-12-November-2019

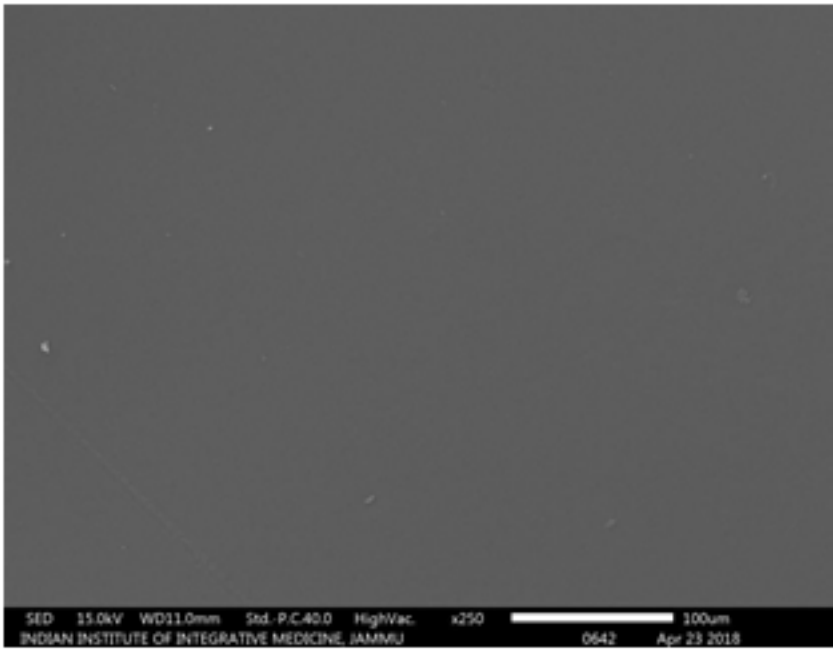



**Figure 10: SEM image of chitosan film at 250 X magnification**

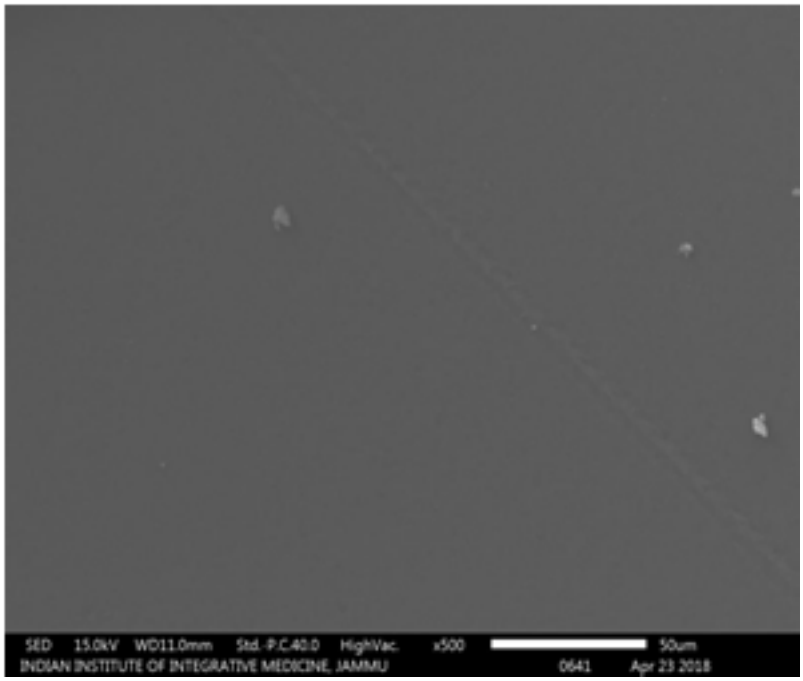


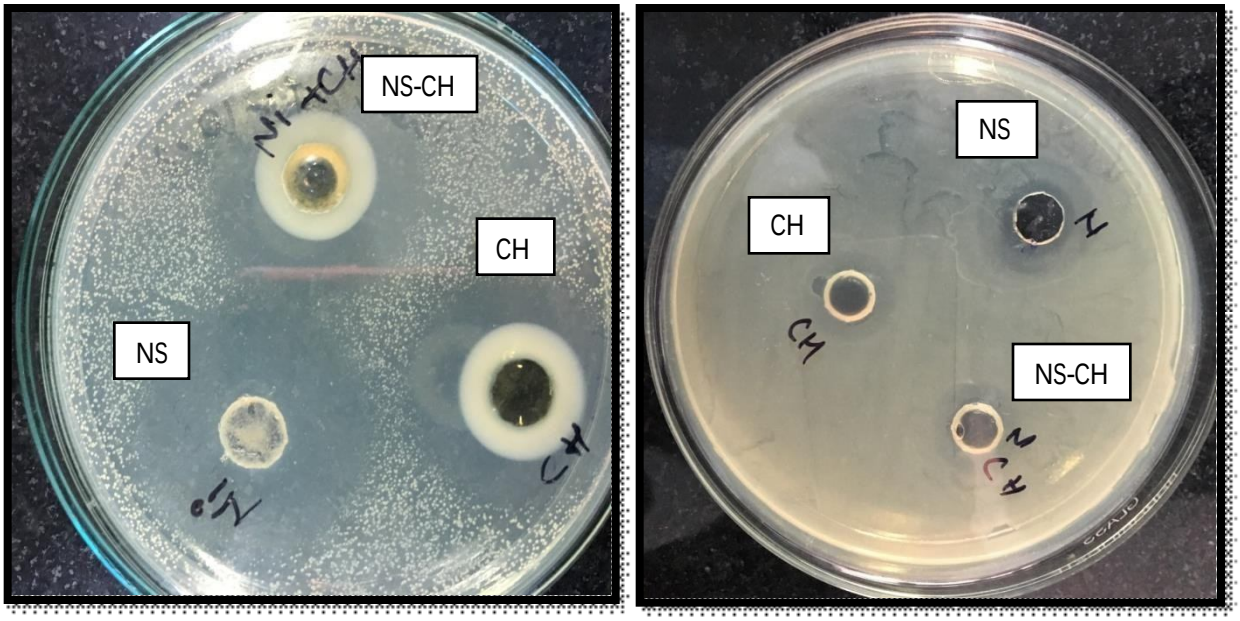

**Figure 11: SEM image of chitosan film at 500X magnification**

### 5.6 Study of Comparative antimicrobial potential of nisin, chitosan and nisin-chitosan:

ISSN:0971-1260
Vol-22- Issue-12-November-2019

According to the study estimated after the incubation of 24 h, nisin alone has exhibited highest zone of inhibition of 40 mm, chitosan alone 32 mm and combinatory nisin-chitosan exhibited 37.5 mm zone of inhibition against gram positive *Listeria monocytogenes*. All the three sampes were also tested against gram negative *Escherichia coli* and the results are nisin alone 22 mm, chitosan alone 17 mm and nisin-chitosan with 18.3 mm which in comparison to the *Listeria monocytogenes* is very less effective.

The reason behind this less effectiveness against gram negative bacterium is because of the failure of nisin to penetrate into the cell wall, hence preventing entry to inner membrane but the effectiveness can be increased by combining nisin with chelators like EDTA against gram negative bacteria. By concluding the overall effect among the three samples against both the pathogens, nisin shows highest effectiveness as compared to other two samples which depicts that nisin is the effective antimicrobial agent incorporated into the edible film and below is the figure depicting the results as follows:




*(a) Listeria monocytogenes* (b) *Escherichia coli*

**Figure 12: Comparative ZOI analysis of nisin, chitosan and nisin-chitosan against both the cultures**

ISSN:0971-1260
Vol-22- Issue-12-November-2019

### 5.7 Study of preservation assay:

#### 5.7.1 Weight loss study comparison at 37˚C and 4˚C:

The weight loss study was conducted of grapes at two different temperatures i.e., 37˚C (set 1) and 4˚C (set 2) to perform the comparative study of total three samples of grapes at both the temperatures after the sanitization of fruits. In both the sets, one sample of grape were wrapped with polyethene, one sample of grape were wrapped with optimized chitosan edible film and the third left sample grape of both the sets were unwrapped. The weight loss of the grapes at 37˚C and 4˚C are depicted below:

**Figure 13: figure depicting the weight loss of grapes at 37˚C**

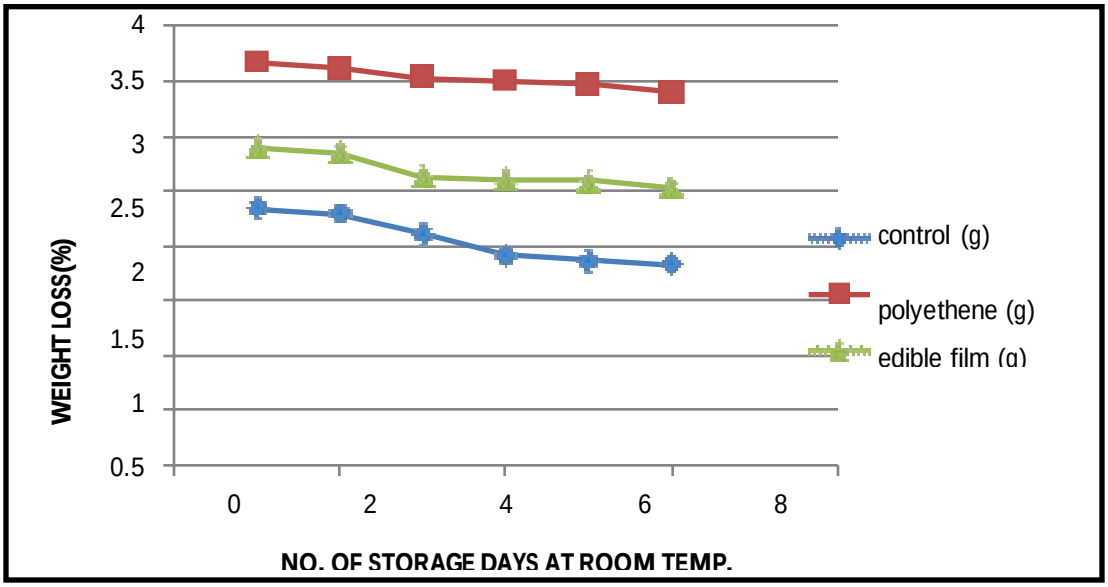
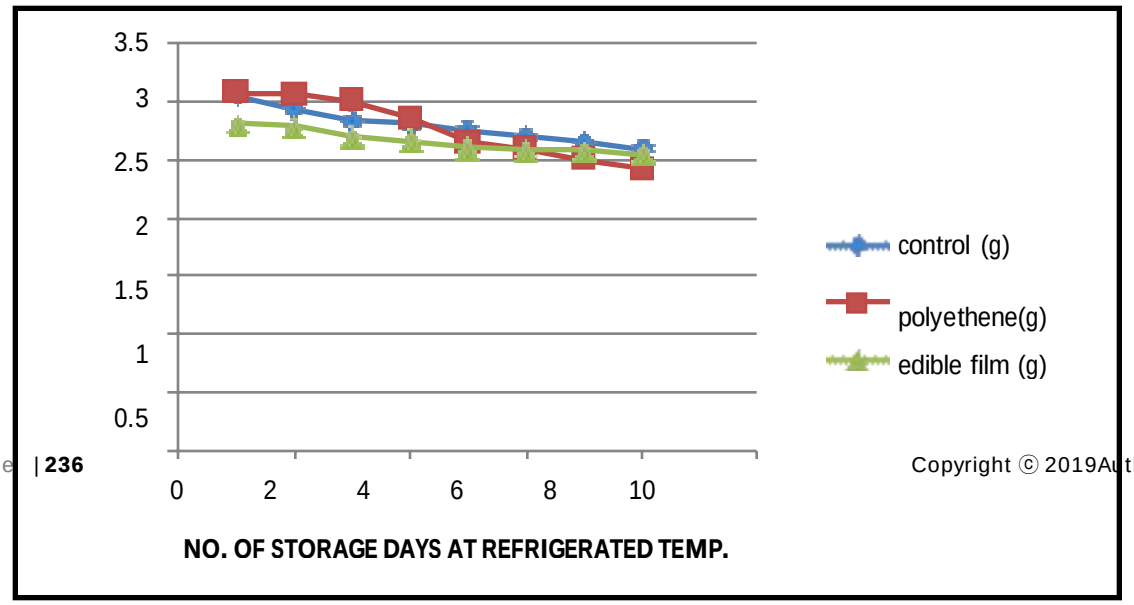



**Figure 14: figure depicting the weight loss of grapes at 4˚C**



ISSN:0971-1260
Vol-22- Issue-12-November-2019

### 5.7.2 Microbiological analysis study:

The main purpose to conduct this study was to evaluate the microbial load content in the grapes after the spoilage of the grapes at two different temperatures. The analysis was conducted using SPC method using the dilutions of $10^{-2}$ and $10^{-3}$ for each of six samples from the stock solution. The microbial load of aerobic mesophilles and psychrotrophes was compared at 37˚C and 4˚C temperatures. The inoculated plates were incubated at 37 ˚C for 24-48 h and the results were expressed in CFU/ml.

Now, after serial dilution, all the six samples were plated on nutrient agar medium using the dilutions of $10^{-2}$ and $10^{-3}$ for each of six samples to check the microbial load of aerobic mesophillic and psychrotrophic bacteria at two different temperatures. The inoculated plates were incubated at 37 ˚C for 24-48 h and the results were expressed in CFU/ml and the calculations are depicted below:

### 5.8 Quantitative estimation of hemolytic activity rate (%) with human erythrocytes:

The hemolysis rate and polymer interaction was analyzed using erythrocyte lysis test. The methanolic film extract was checked that whether it has caused RBC membrane disruption after incubation at three different concentrations (25 µg/mL, 50 µg/mL, and 100 µg/mL) along with positive and negative control. If ruptured, it means RBC lysis and leading to the release of hemoglobin that was estimated quantitatively using spectrophotometer. As shown the graph, it can be concluded that the hemolysis rate of the human erythrocytes was <10% at all the three concentrations compared to positive control i.e., RBC treated with 10% triton X- 100 exhibited 100% hemolysis rate. When the human erythrocytes were treated with film extract at 25 µg/mL, 50 µg/mL, and 100 µg/mL for 2 hrs, the hemolysis rate was 1.9%, 4.54% and 9.74% respectively. All the hemolysis rate caused by the chitosan film methanolic extract at the three different concentration were <10%, indicating that the chitosan edible film is non-hemolytic in nature, according to Standard Practice for Assessment of Hemolytic Properties of Materials F756-93 standards.

ISSN:0971-1260
Vol-22- Issue-12-November-2019

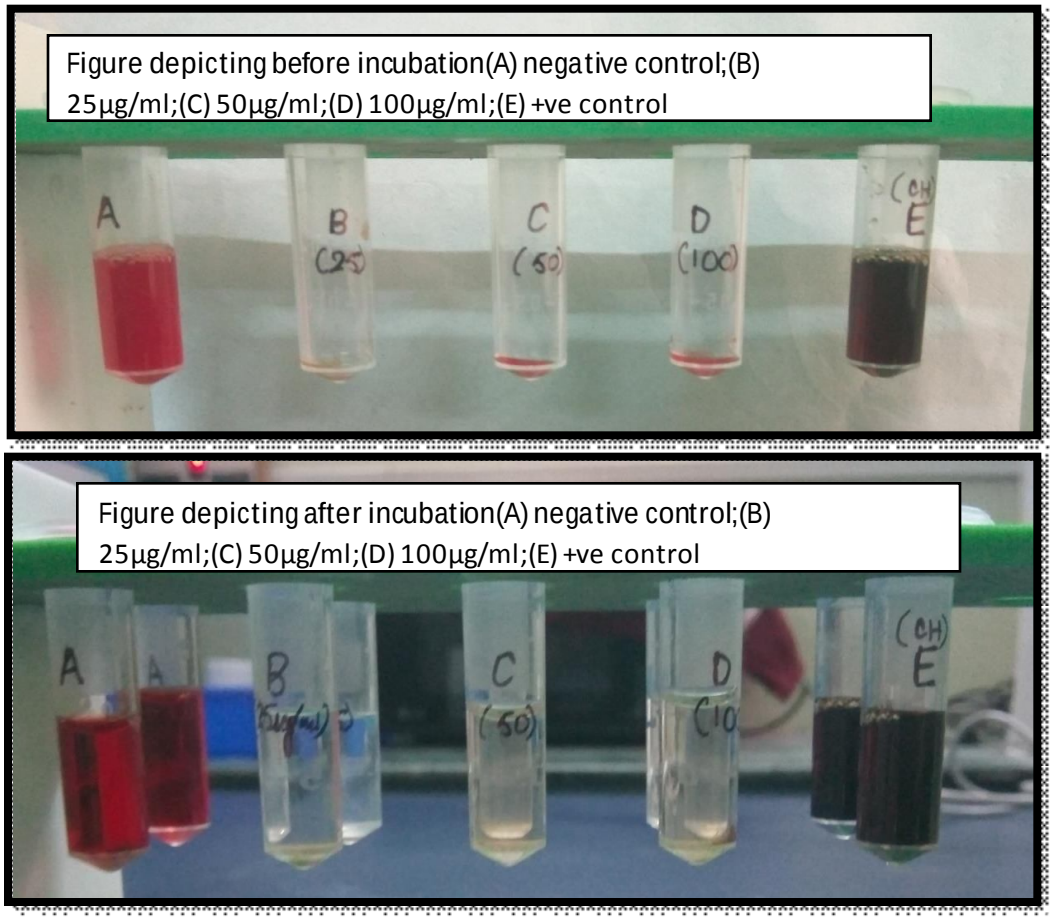
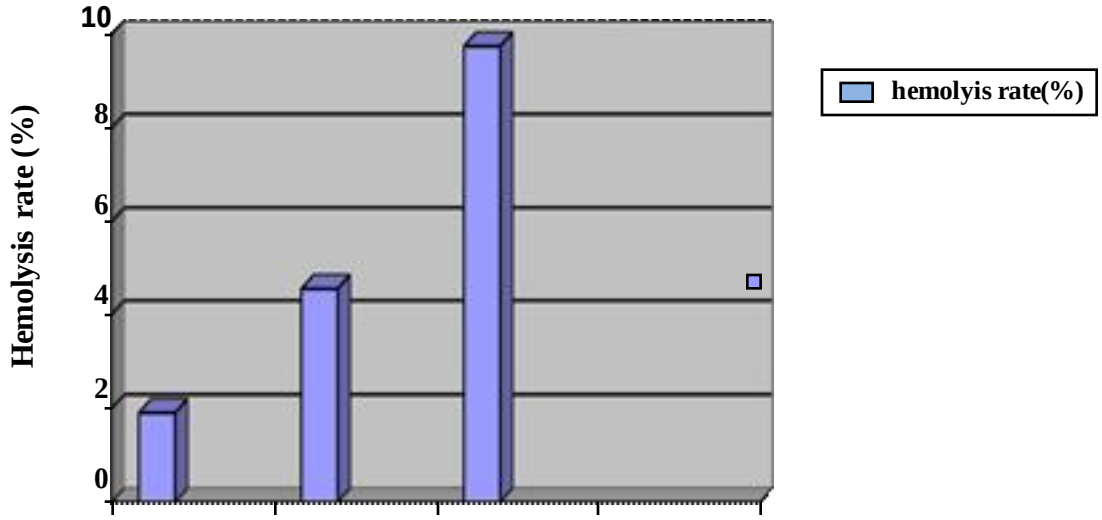



**Figure 16: Effect of different concentration of the film extract on the hemolysis of human erythrocytes**



ISSN:0971-1260
Vol-22- Issue-12-November-2019

**25(ug/ml) 50(ug/ml) 100(ug/ml)**

**Concentration of film extract(µg/ml)**

**Table 7: Table depicting the count of colonies in CFU/ml**

| Sample | Dilution | Temperature(˚C) | Days | No.of colonies | CFU/ml |
|---|---|---|---|---|---|
| **Control grapes** | $10^{-2}$ | 37˚C | 5 | Lawn | Lawn |
| | $10^{-3}$ | 37˚C | 5 | 5 | $5\times10^{5}$ CFU/ml |
| **Edible film+ grapes** | $10^{-2}$ | 37˚C | 5 | 10 | $1\times10^{5}$ CFU/ml |
| | $10^{-3}$ | 37˚C | 5 | 5 | $5\times10^{5}$ CFU/ml |
| **Polyethene+grapes** | $10^{-2}$ | 37˚C | 5 | 30 | $3\times10^{5}$ CFU/ml |
| | $10^{-3}$ | 37˚C | 5 | 5 | $5\times10^{5}$ CFU/ml |
| **Control grapes** | $10^{-2}$ | 4˚C | 7 | 28 | $2.8\times10^{5}$ CFU/ml |
| | $10^{-3}$ | 4˚C | 7 | 23 | $2.3\times10^{5}$ CFU/ml |
| **Edible film + grapes** | $10^{-2}$ | 4˚C | 7 | 2 | $2\times10^{3}$ CFU/ml |
| | $10^{-3}$ | 4˚C | 7 | 2 | $2\times10^{4}$ CFU/ml |
| **Polyethene+grapes** | $10^{-2}$ | 4˚C | 7 | 216 | $2.16\times10^{5}$ CFU/ml |
| | $10^{-3}$ | 4˚C | 7 | 60 | $6\times10^{6}$ CFU/ml |

## 3. Conclusion and future perspectives:

From the study, it can be concluded that chitosan edible film incorporated with nisin as an antimicrobial agent derived from a natural microorganism source can influence the storage period of various food products. The developed film was optimized using RSM along with its antimicrobial potential and effectiveness. Hence, these films can retard the spoilage assay and

ISSN:0971-1260
Vol-22- Issue-12-November-2019

contamination upto much extent as analyzed by microbiological analysis. Furthermore, the chitosan film, developed in this study exhibits the property of best alternative to the present day commercializing coatings because of its biodegradable nature.

This study can be extended in future by the analysis of toxicology study and edibility test of the optimized and characterized chitosan edible film. Furthermore, the packaging criteria can be given more attention while wrapping the film upon the food products with various modifications.

## References:

1. P. Suppakul, J. Miltz, K. Sonneveld, SW. Bigger, "Active packaging technologies with an emphasis on antimicrobial packaging and its applications", Journal of food science, pp. 408-20, Mar, 2003.
2. SY. Sung, LT. Sin, TT. Tee, ST. Bee, AR. Rahmat, WA. Rahman, AC. Tan, M. Vikhraman, "Antimicrobial agents for food packaging applications",Trends in Food Science & Technology, pp. 110-23, Oct, 2013.
3. N. Suvatma, L. Tighzert, A. Copinet, V.Coma, "Effects of hydrophilic plasticizers on mechanical, thermal, and surface properties of chitosan films", *Journal of Agricultural and Food Chemistry*, pp. 3950-3957, 2005.
4. S. Galus, J. Kadzińska, "Food applications of emulsion-based edible films and coatings" ,Trends in Food Science & Technology, pp. 273-83, Oct, 2015.
5. A. Acevedo-Fani, L. Salvia-Trujillo, MA. Rojas-Graü, O. Martín-Bellos, "Edible films from essential-oil-loaded nanoemulsions", Physicochemical characterization and antimicrobial properties. Food Hydrocolloids, pp. 168-77, May, 2015.
6. M. López-Mata, S. Ruiz-Cruz, N. Silva-Beltrán, J. Ornelas-Paz, P. Zamudio-Flores, S. Burruel-Ibarra, "Physicochemical, antimicrobial and antioxidant properties of chitosan films incorporated with carvacrol", Molecules, pp. 13735-53, 2013.
7. RN. Tharanathan, "Biodegradable films and composite coatings: past, present and future, Trends in food science & technology, pp. 71-78, Mar, 2003.
8. DS. Cha, MS. Chinnan, "Biopolymer-based antimicrobial packaging: a review", Critical reviews in food science and nutrition, pp. 223-37, Jul, 2004.
9. IG. Donhowe, O. Fennema, "The effects of plasticizers on crystallinity, permeability, and mechanical properties of methylcellulose films", Journal of Food Processing and Preservation, pp. 247-57, Aug,1993.
10. AM. Durango, NF. Soares, S. Benevides, J. Teixeira, M. Carvalho, C. Wobeto, NJ. Andrade, "Development and evaluation of an edible antimicrobial film based on yam starch and chitosan", Packaging Technology and Science: An International Journal, pp. 55-59, Jan, 2006.

ISSN:0971-1260
Vol-22- Issue-12-November-2019

11. K. Ogawa, T. Yui, K. Okuyama, "Three D structures of chitosan", International Journal of Biological Macromolecules, pp. 1-8, Apr, 2004.
12. MD. Moreira, M. Pereda, NE. Marcovich, SI. Roura, "Antimicrobial effectiveness of bioactive packaging materials from edible chitosan and casein polymers: assessment on carrot, cheese, and salami", Journal of Food Science, pp. 54-63, Jan, 2011.
13. M. Pereda, G. Amica, NE. Marcovich, "Development and characterization of edible chitosan/olive oil emulsion films", Carbohydrate polymers, pp. 1318-25, Jan, 2012.
14. I. Sebti, E. Chollet, P. Degraeve, C. Noel, E. Peyrol, "Water sensitivity, antimicrobial, and physicochemical analyses of edible films based on HPMC and/or chitosan", Journal of agricultural and food chemistry, pp. 693-99, Feb,2007.
15. M. Ye, H. Neetoo, H. Chen, "Control of Listeria monocytogenes on ham steaks by antimicrobials incorporated into chitosan-coated plastic films", Food Microbiology, pp. 260-68, Feb, 2008.
16. MD. Moreira, M. Pereda, NE. Marcovich, SI. Roura, "Antimicrobial effectiveness of bioactive packaging materials from edible chitosan and casein polymers: assessment on carrot, cheese, and salami", Journal of Food Science, pp. 54-63, Jan, 2011.
17. K. Winkowski, RD. Ludescher, TJ. Montville, "Physiochemical characterization of the nisin-membrane interaction with liposomes derived from Listeria monocytogenes", Appl. Environ. Microbiology, pp. 323-27, Feb, 1996.
18. M. Al-Holy, M. Lin, B. Rasco, "Destruction of Listeria monocytogenes in sturgeon (Acipenser transmontanus) caviar by a combination of nisin with chemical antimicrobials or moderate heat", Journal of food protection, pp. 512-20, Mar, 2005
19. S. Burt, "Essential oils: their antibacterial properties and potential applications in foods—a review", International journal of food microbiology, pp.223-253, 2004.
20. S. Sun, P. Liu, N. Ji, H. Hou, H. Dong, "Effects of various cross-linking agents on the physicochemical properties of starch/PHA composite films produced by extrusion blowing", Food Hydrocolloids,pp.964-75, Apr, 2018.
21. J. Wu, S. Ge, H. Liu, S. Wang, S. Chen, J. Wang, J. Li, Q. Zhang, "Properties and antimicrobial activity of silver carp (Hypophthalmichthys molitrix) skin gelatin-chitosan films incorporated with oregano essential oil for fish preservation",Food Packaging and Shelf Life, pp. 7-16, Sep, 2014.
22. L. Scanavaca, N. Fonseca, ME. Pereira, "Use the cassava starch in the surprise of mango postharvest", Brazilian Journal of Fruit, pp. 67-71, Apr, 2007.
23. LC. Maistro, NT. Miya, AS. Sant'Ana, JL. Pereira, "Microbiological quality and safety of minimally processed vegetables marketed in Campinas, SP–Brazil, as assessed by traditional and alternative methods", Food Control, pp. 258-64, Dec, 2012.

ISSN:0971-1260
Vol-22- Issue-12-November-2019

24. Y. Zhou, J. Li, F. Lu, J. Deng, J. Zhang, P. Fang, X. Peng, SF. Zhou, "A study on the hemocompatibility of dendronized chitosan derivatives in red blood cells", Drug design, development and therapy,2015.
25. M. López-Mata, S. Ruiz-Cruz, N. Silva-Beltrán, J. Ornelas-Paz, P. Zamudio-Flores, S. Burruel-Ibarra, "Physicochemical, antimicrobial and antioxidant properties of chitosan films incorporated with carvacrol", Molecules, pp. 13735-53, 2013.
26. K. Winkowski, RD. Ludescher, TJ. Montville, "Physiochemical characterization of the nisin-membrane interaction with liposomes derived from Listeria monocytogenes", Appl. Environ. Microbiology, pp. 323-27, Feb, 1996.
27. E. Breukink, C. van Kraaij, A. van Dalen, RA. Demel, RJ. Siezen, B. de Kruijff, OP. Kuipers," The orientation of nisin in membranes. Biochemistry, pp. 8153-62, Jun, 1998
28. JK. Branen, PM. Davidson, "Enhancement of nisin, lysozyme, and monolaurin antimicrobial activities by ethylenediaminetetraacetic acid and lactoferrin", International journal of food microbiology, pp. 63-74, Jan, 2004.
29. OL. Ramos, I. Reinas, SI. Silva, JC. Fernandes, MA. Cerqueira, RN. Pereira, AA. Vicente, MF. Poças, ME. Pintado, FX. Malcata, "Effect of whey protein purity and glycerol content upon physical properties of edible films manufactured therefrom", Food Hydrocolloids, pp. 110-2, Jan, 2013.
30. M. Abdollahi, M. Rezaei, G. Farzi, "Improvement of active chitosan film properties with rosemary essential oil for food packaging", International Journal of Food Science & Technology, pp.847-53, Apr, 2012.
31. PD. Sobral, FC. Menegalli, MD. Hubinger, MA. Roques, "Mechanical, water vapor barrier and thermal properties of gelatin based edible films", Food Hydrocolloids, pp. 423-32. Jul, 2001.