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Extraction and Optimization of chitosan from edible mushroom using for food coating and water treatment

Shivashankar. M
Department of Life Science, Bangalore University, India
Corresponding Author: Shivashankar. M

Abstract: *Chitosan being the second most abundant polysaccharide available on earth. In the present investigation extraction and optimization on Chitosan from mushroom as a source was carried out. The confirmation for the Chitosan was done using TBA Assay, DNS test and confirmed by FTIR analysis. Food coating was carried out and efficacy was tested comparing with that of non coated one. The firmness of control fruit during the red stage on the 20th day was 31.65N where as for the coated fruit on the same day it was 46.79N. Coating was the most effective treatment for retardation of softening of harvested fruits and vegetables. The titratable acidity of control at the time of red stage on the 20th day was 0.611% whereas for coated fruit on the same day it was 0.559%. Biopolymer as an edible coating with 2% solid percentage remains a viable alternative to delay the ripening of tomato. By optimizing the solid percentage, it could be applicable to other fruits and vegetables.*

Keywords: *Mushroom, Chitosan, TBA Assay, DNS Assay, FTIR analysis, Food coating*

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I. Introduction

Chitin is a white, hard, inelastic, nitrogenous polysaccharide found in the exoskeleton of invertebrates as well as in the cell wall of fungi. The principle derivative of chitin is chitosan. Chitosan is a linear polymer of α (1-4) linked 2-amino-2-deoxy-β-D-glucopyranose and is easily derived by N-deacetylation and is a copolymer of N-acetylglucosamine and glucosamine. Most of the naturally occurring polysaccharides are acidic in nature, whereas chitin and chitosan are basic in nature. Their properties include solubility in various media, solution, viscosity, polyelectrolyte behavior, poly-oxy salt formation, ability to form films, metal chelations, optical, and structural characteristics.

Chitin is a natural antimicrobial compound and are found in crustacean shells (crabs, shrimp and crayfishes) either by chemical or microbiological processes and on the other hand it can be produced by some fungi (*Aspergillusniger, Mucorrouxii, Penecilliumnotatum*). The antimicrobial activity of chitosan will depend factors such as the kind of chitosan (deacetylation degree, molecular weight) used, the pH of the medium, the temperature, the presence of several food components, etc. The most feasible hypothesis is a change in cell permeability due to interactions between the polycationic chitosan and the electronegative charges on the cell surfaces. This interaction leads to the leakage of intracellular electrolytes and proteinaceous constituents.

In the food industry, edible active bio-based films and coatings offer manyadvantages because or their edibility, biocompatibility with human tissues, aesthetic appearance, barrier properties against pathogenic micro-organisms, non-toxicity, non-polluting and low cost[1].

Chitosan intrinsic factors affect its antimicrobial and antifungal activity. It is found that the average molecular weight is less than 10 kDa have greater antimicrobial activity than native chitosan and that the degree of polymerisation of at least seven monomeric units is required to get significant antibacterial effect[2].

The chitosan–Zn complexes showed wide spectrum of effective antimicrobial activities, which were from 2 to 8 and 4 to 16 times higher than those of chitosan alone and zinc sulphate, respectively. The antibacterial effect has been shown to increase by increasing zinc ions content in the complex. However, under the studied conditions, it was reported that the antibacterial activity of chitosan– Zn complex was better than its antifungal activity.

The sorption and bactericide properties of chitosan films with various humidity and preparation temperatures were studied. Chitosan-based film samples have been found to possess high transport properties to water and hydrochloric acid vapours and to show bactericide activity. The temperature of film preparation practically does not influence the sorption properties. The swelling degree, sorption rate, and bactericide effect increase by increasing chitosan film humidity[3].

Chitosan can be used to inhibit fibroplasias in wound healing and promote tissue growth and differentiation in tissue culture.Apart from their application in medical field, chitin and chitosan have potential

applications in wastewater treatment, where the removal of heavy metal ions by chitosan is done through the process of chelation.

The use of edible films and coatings has several aims, most importantly: the restriction of moisture loss, control of gas permeability, control of microbial activity (e.g., chitosan, which acts against microbes), preservation of the structural integrity of the product and the gradual release of enrobed flavors or antioxidants into the food. The current study focus on the optimization of the extraction process of chitosan from two species of edible mushrooms – *Agaricusbisporus* and *Pleurotusostratus*and their subsequent application on food coating and water treatment.

II. Material And Methods

Button mushroom, oyster mushroom, sodium hydroxide, hydrochloric acid, hydrogen peroxide, acetic acid, sodium nitrate, thiobarbituric acid, potassium iodide, sulphuric acid, Glucose, 5% phenol, DNS, Rochelle salt, nutrient agar, saline, spectrophotometer, pH meter, tomato, carrot, filter paper, filter cloth.

Extraction of chitosan from button mushroom: Mushroom was collected from local market, Cleaned and was optimized with NaOH of different concentrations. Then the sample was subjected to Deprotenization with NaOH and autoclaved at 121^0C. Sample is deminarilized by treating with HCl followed by neutralization with water. Decolourization was done with 30% of hydrogen peroxide. Dry and make it as powder at neutral pH.

TBA Assay – Thiobarbituric Acid Assay: The most prominent and currently used assay as an index for lipid peroxidation products is the thiobarbituric acid assay. It is based on the reactivity of an end product of lipid peroxidation, malondialdehyde with TBA to produce a red adduct is measured at 555nm.

Estimation of glucose by DNS method: Reducing sugar have the property to reduce many of the reagents. One such reagent is 3,5-dinitrosalicyclic acid (DNS). 3,5-DNS in alkaline solution is reduced to 3 amino 5 nitro salicylic acid, which strongly absorbs light at 575nm.

Food coating: The extracted chitosan 1% biopolymer solution is prepared by taking 10 ml of biopolymer liquid and 990ml distil water.The fruits and vegetables are socked in 2% biopolymer solution before socking it should be washed with distil water, sock the vegetable for 15min.After socking for 15min it is dried and kept in the room temperature.Vegetables and fruits are examined in the regular interval of time.

Water Treatment: Initially collect the waste water.1 % standard solution is prepared from the extracted chitosan.The filter cloth is socked in 1% biopolymer solution for 15-20min.After socking for 20min it is dried in the autoclave or in room temperature, till all the wetness in the cloth is completely dried. Collected waste water is serial diluted (10^{-1},10^{-2},10^{-3}) and treated with chitosan socked filter cloth.1µl solution is taken from both before treatment and after treatment with filter cloth, spread on nutrient agar medium using the sterilized glass rod.Petri plate are incubated, the microbial load is observed after 24hours.Microbial count of waste water is observed for before and after treatment with filter cloth

III. Result & Discussion

Extraction of chitosan from oyster mushroom

Extraction and optimization of chitosan from oyster mushroom is summarized in **table 1**. Optimization is done by treating oyster mushroom with different normality's (2N, 4N, 6N, 8N, 10N) of sodium hydroxide, 2N HCl and 20% hydrogen peroxide. The obtained final product is sent for FTIR analysis to know the purity of chitosan that is obtained from oyster mushroom.

Table No 1: Standardization of NaOH for oyster mushroom

SL.NO	Weight of the sample	Normality of NaOH	Volume of NaOH (ml)	Volume of 2N HCl(ml)	Volume of 20% H2O2 (ml)	Weight of final product (g)
1.	200g	2N	100	100 (2N HCl)	50	6.2
2.	200g	4N	100		50	6.9
3.	200g	6N	100		50	6.2
4.	200g	8N	100		50	5.8
5.	200g	10N	100		50	5.1

In table 2, shows optimization is done by treating the oyster mushroom with different normality's (2N, 4N, 6N, 8N, 10N) of hydrochloric acid, 2N sodium hydroxide and 20% hydrogen peroxide. The obtained final product is sent for FTIR analysis to check the purity of Chitosan that is obtained from oyster mushroom.

Table 2: Standardization of HCl for oyster mushroom

SL. NO	Weight of the sample (g)	Volume of NaOH(2N) (ml)	Normality of HCl	Vol of HCl (ml)	Volume of 20% Hydrogen peroxide(ml)	Weight of final product (g)
1.	200	100ml	2N	100ml of 2N	50	7.8
2.	200		4N		50	9.3
3.	200		6N		50	10.5
4.	200		8N		50	9.6
5.	200		10N		50	9.2

Extraction of chitosan from button mushroom

Extraction and optimization of chitosan from button mushroom is summarized in **table 3**. Optimization is done by treating oyster mushroom with different normality's (2N, 4N, 6N, 8N, 10N) of sodium hydroxide ,2NHCl and 20% hydrogen peroxide. The obtained final product is sent for FTIR analysis to know the purity of chitosan that is obtained from button mushroom.

Table 3 : Standardization of NaOH

SL.NO	Weight of the sample (g)	Normality of NaOH	Volume of NaOH (ml)	Volume of 2N HCl(ml)	Volume of 20% H2O2 (ml)	Weight of final product (g)
1.	200	2N	100	100 (2N HCl)	50	3.8
2.	200	4N	100		50	4.6
3.	200	6N	100		50	4.0
4.	200	8N	100		50	3.6
5.	200	10N	100		50	4.4

Table 4 indicates the optimization done by treating the button mushroom with different normality's (2N, 4N, 6N, 8N, 10N) of hydrochloric acid, 2N sodium hydroxide and 20% hydrogen peroxide. The obtained final product is sent for FTIR analysis to check the purity of Chitosan that is obtained from button mushroom.

Table 4: Standardization of HCl

SL.NO	Weight of the sample (g)	Normality of NaOH	Volume of NaOH (ml)	Volume of 2N HCl(ml)	Volume of 20% H2O2 (ml)	Weight of final product (g)
1.	200	2N	100	100 (2N HCl)	50	4.8
2.	200	4N	100		50	6.2
3.	200	6N	100		50	5.0
4.	200	8N	100		50	8.5
5.	200	10N	100		50	9.9

FTIR Analysis

Fouier-transform infrared spectroscopy is a technique used to obtain an infrared spectrum of absorption or emission of a solid, liquid or gas. An FTIR spectrometer simultaneously collects high-spectral-resolution data over a wide spectral range. This confers a significant advantage over a dispersive spectrometer, which measures intensity over a narrow range of wavelengths at a time. FTIR analysis is done to determine the presence of chitosan in the sample.

Button mushroom FTIR result for sample

The sample extracted from button mushroom were determined for its purity by Fouier-transform infrared spectroscopy.The peak was observed at 2921.46 wavenumber cm^{-1}.

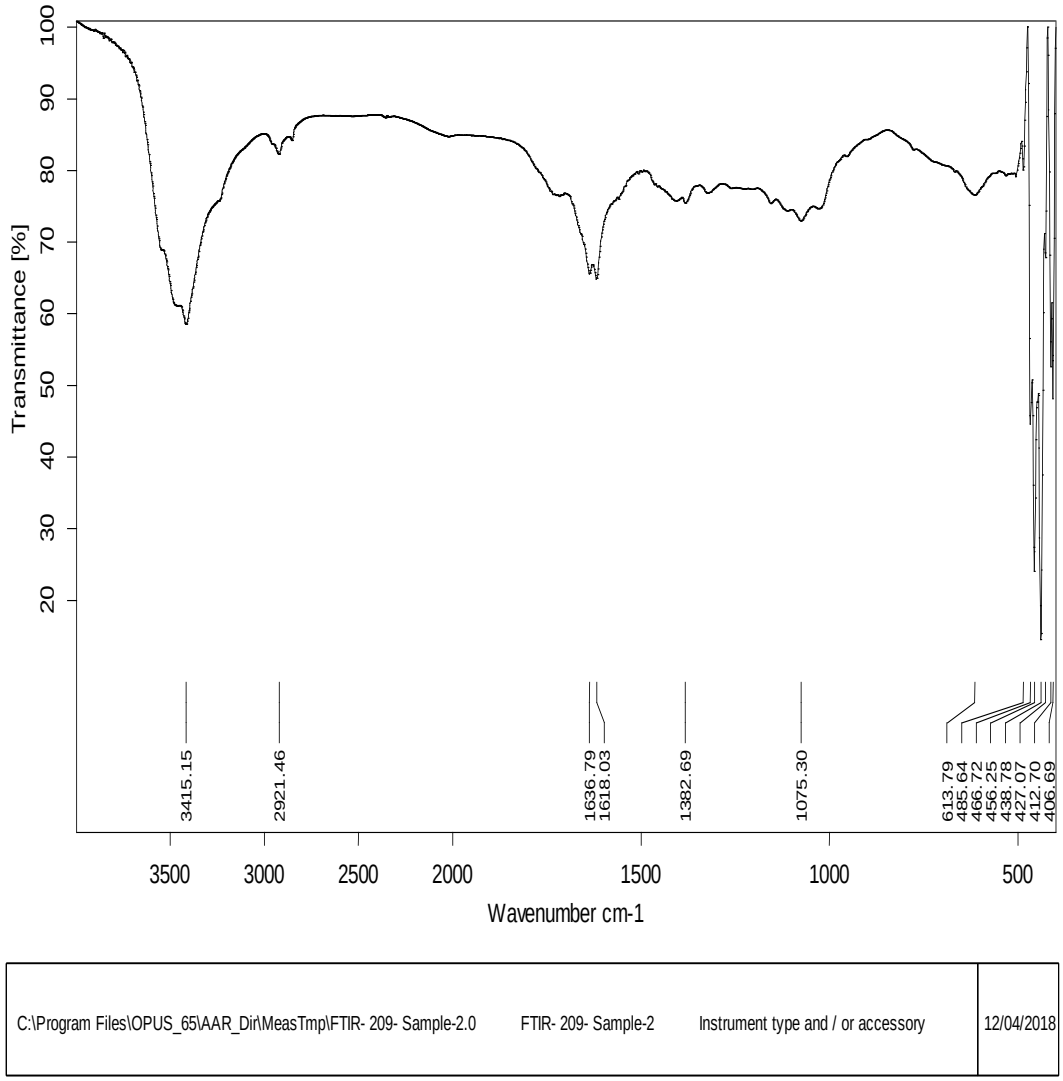

Figure 1: FTIR analysis for the extracted button mushroom sample

Oyster mushroom FTIR result for the extracted sample

The sample extracted from oyster mushroom were determined for its purity by Fouier-transform infrared spectroscopy.The peak was observed at 2922.37 wavenumber cm^{-1}.

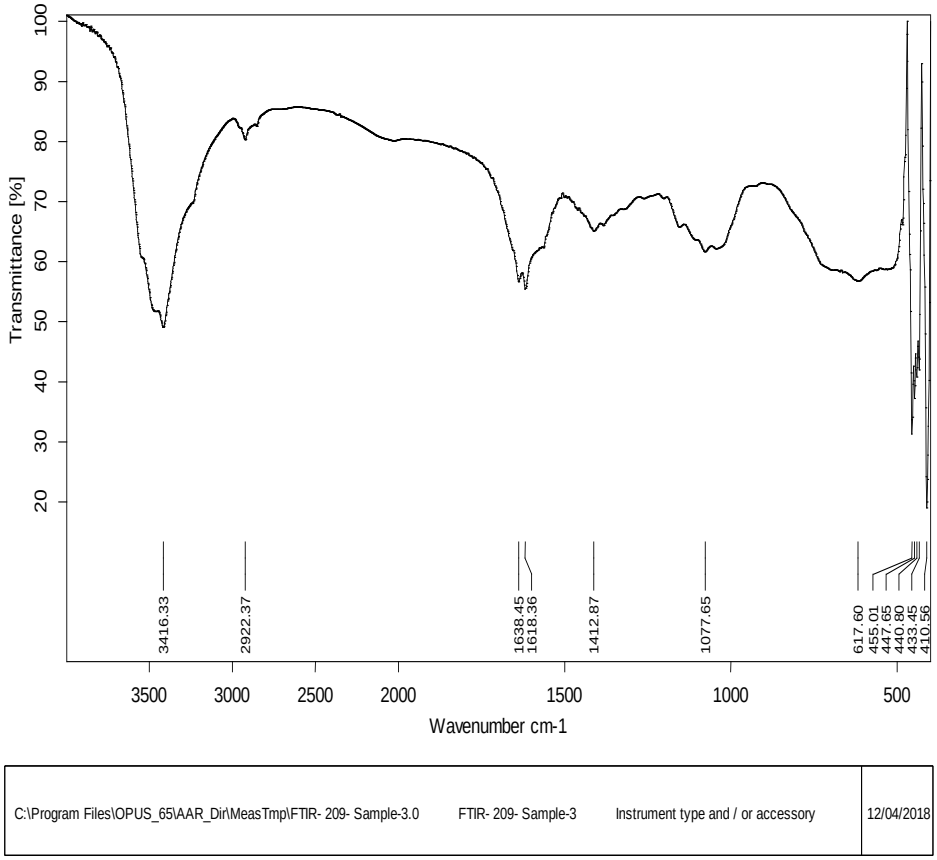

Figure 2: FTIR analysis for extracted oyster mushroom sample

Food coating

Tomatoes both from control and chitosan coated showed mass loss throughout the storage period. Tomato not coated with chitosan solution had statistically higher mass loss compared to fruit coated with chitosan solution. The experiment was conducted using 1, 2, and 3 % concentration of biopolymer. The loss in mass of coated and control tomatoes was monitored continuously to optimise biopolymer percentage. Study indicated the effect of the biopolymer concentration of coating on mass loss of tomatoes. 3% biopolymer coated tomato showed a drastic increase in mass loss of 23.09 % while compared to the control, and coatingwith1% biopolymer followed a curve very near to the control itself; it showed a decrease in mass loss at initial period of storage while at the 15th day the values were nearer to the control. Out of this, the coating with 2% biopolymer showed a decrease in mass loss of 8.32% while compared to control 11.23%. Hence, further coatings studies were conducted using 2% biopolymer on tomatoes.

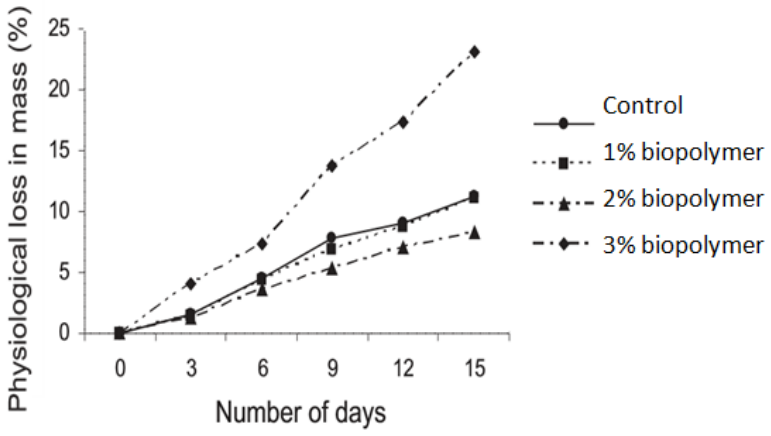

Figure 3: Effect of coating with different concentrations on physiological loss in mass of tomato

Tomatoes, both control and coated, registered some changes in *L**, *a** and *b** values during thestorage period. Table 5 shows the effect of coating on *L**, *a**, *b** and firmness values of tomatoes.*L** means lightness (from white to black). *L**values did not change until the turning stage,indicating that there was no change in lightness when the green color was still predominant. Theinitial *L** values for the control and coated tomatoes were 43.57 and 43.66 (slight difference in theinitial *L**value is due to coating) . When red color pigments started to synthesis, there was a declinein *L** value[15]. Though there was a decrease in *L** value in both coated and control tomatoes, coating showed a significant difference in *L**value when compared to control on 20th day. *L** value of control during its red stage on the 20thday was 26.74, whereas for the coated fruit on the same day It was 40.87. *L** value of coated fruit decreased during its ripening. *L** value of coated fruit during its red stage on 40th day was 27.05.*a** values change from negative (green color) to positive (red color). *a** valueof control during its red stage on the 20th day was 22.50, whereas for the coated fruit on the same day it was 8.38. The increase in *a** value was, however, slower for the tomatoes treated with biopolymer compared to control, resulting in significant differences among the treatments. *a** value of tomato increased during its ripening, and for the coated fruit during its red stage on the 40th day it was 22.70.The initial *b** (blue to yellow) values for control and coated tomatoes were 21.45 and 21.60, afterwards the values gradually decreased to 12.43 for control tomatoes and to 20.43 for coated tomatoes on 20th day. *b** value of coated tomato during its red stage on the 40th day was 12.71.

Table No 5: Effect of color L*value, a*value, b*value, chroma value and Firmness

Days	Color L* value		Color a* value		Color b* value		Chroma value		Firmness (N)	
	Control	Coated	Control	Coated	Control	Coated	Control	**Coated**	**Control**	**Coated**
0	43.57 ±0.36a	43.66 ±0.12c	-7.77 ±0.15d	-7.58 ±0.08i	21.45 ±0.23a	21.60 ±0.09b	22.18 ±0.08a	22.89 ±0.19c	57.34 ±0.66a	58.67 ±0.53b
5	43.73 ±0.21a	44.29 ±0.24a	-4.87 ±0.06c	-6.37 ±0.32h	21.45 ±0.23a	21.60 ±0.09b	21.99 ±0.21a	22.51 ±0.23c	51.28 ±0.57a	66.22 ±0.55c
10	36.74 ±0.19b	43.97 ±0.07i	-4.40 ±0.10e	-5.27 ±0.25g	21.72 ±0.20a	21.83 ±0.09a	22.16 ±0.09a	22.45 ±0.07c	48.55 ±0.35c	54.57 ±0.45b
15	30.82 ±0.20c	44.04	18.83 ±0.76b	-4.30 ±0.10f	15.59 ±0.23b	21.90 ±0.02a	24.44 ±0.1b	22.31 ±0.09c	34.67 ±0.18d	50.70 ±0.82e
20	26.74 ±0.17d	40.87 0.16b	22.50 ±0.20a	8.38 ±0.08e	12.43 ±0.12c	20.43 ±0.14c	25.62 ±0.13d	21.51 ±0.09c	31.65 ±0.22e	46.79 ±0.19f
25	–	35.8 0.10e	–	13.93 ±0.15d	–	18.45 ±0.06d	–	23.11 ±0.08b	–	41.56 ±0.39g
30	–	33.69 ±0.17f	–	16.70 ±0.20c	–	16.51 ±0.03e	–	23.48 ±0.13b	–	37.97 ±0.27h
35	–	30.50 ±0.06g	–	20.83 ±0.21b	–	14.27 ±0.12f	–	25.24 ±0.06d	–	34.64 ±0.24i
40	–	27.05 ±0.16h	–	22.70 ±0.20a	–	12.71 ±0.11g	–	25.69 ±0.14e	–	32.51 ±0.37j

The chroma value (*C*) depends on *a** and *b** values. The chroma value indicates the color intensity (saturation) of the sample. There was a slight increase in the Chroma value from the initial value. But there were significant differences in chroma value of coated tomatoes when compared to control tomato during its ripening on the 20th day, whereas for the coated fruit it was 21.51 on the same day. *C* value increased during ripening of tomato. *C* value of coated fruit during its red stage on the 40th day was 25.69.

Fruit firmness for both control and coated tomatoes gradually decreased during the storage period. Initial values of firmness for control and coated tomatoes were 57.34 and 58.67 N. The firmness of control during its red stage on the 20th day was 31.65 N, whereas for the coated fruit on the same day it was 46.79 N. Coating showed statistical difference in firmness when compared to control on 20th day. Firmness gradually decreased for coated tomatoes after 20th day. The firmness of coated fruit during the red stage on the 40th day was 32.51 N. Coating was the most effective treatment for retardation of softening of harvested fruits and vegetables compared to the control[15]

Table 6 shows the effect of coating on pH, acidity, ascorbic acid and lycopene of tomato. pH value of control at the time of red stage on the 20th day was 4.15, whereas for coated fruit it was 4.07. The increase in pH value may be due to break-up of acids with respiration during storage. The pH value of coated fruit was increased to 4.25 during red stage on the 40th day.

Table No 6: Effect of coating on pH, Acidity, Ascorbic acid, Lycopene

Days	pH		Acidity (%)		Ascorbic acid (mg 100 g^{-1})		Lycopene (g g^{-1})	
	Control	Coated	Control	Coated	Control	Coated	Control	Coated
0	3.81±0.01d	3.93±0.03d	0.685±0.006a	0.694±0.004a	8.67±0.01a	8.74±0.03a	0.63±0.03d	0.64±0.03d
5	3.98±0.01c	3.43±0.03e	0.579±0.003d	0.681±0.002b	8.52±0.02b	8.74±0.03a	0.93±0.01e	0.77±0.01g
10	4.04±0.01b	3.97±0.02d	0.545±0.005e	0.657±0.002d	8.19±0.01c	8.57±0.03b	7.68±0.02c	0.93±0.01f
15	3.99±0.01c	4.13±0.03b	0.648±0.003b	0.565±0.004f	7.96±0.01d	8.64±0.04c	33.64±0.55b	0.93±0.01f
20	4.15±0.01a	4.07±0.06c	0.611±0.003c	0.559±0.003f	8.18±0.01c	8.52±0.02d	42.09±0.24a	1.39±0.01e
25	-	4.07±0.06c	-	0.537±0.002g	-	8.57±0.03d	-	2.46±0.01d
30	-	4.06±0.01c	-	0.521±0.005h	-	8.56±0.05d	-	7.67±0.01c
35	-	4.08±0.01bc	-	0.667±0.003c	-	6.52±0.03e	-	37.20±0.08b
40	-	4.25±0.05a	-	0.623±0.007e	-	8.28±0.02f	-	43.27±0.02a

The acidity of tomato plays a major role and imparts taste to the fruit. The predominant acids in ripened tomato fruit are citric acid and malic acid. Acidity does not for malinear curve, one author said that malic acid concentration falls during ripening and citric acid increases up to turning stage, whereas another reported that malic acid increased steadily throughout maturation (Humle, 1971). The titratable acidity of control at the time of red stage on the 20th day was 0.611%, whereas for coated fruit on the same day it was 0.559%. Titratable acidity of the coated fruit during its red stage on the 40th day was 0.623%. At the red stage, both for control and coated fruit, acidity remained the same in which it showed the decline at the turning stage.

Ascorbic acid content during maturity stage was continuously increasing with a slight fall during the light red stage. The ascorbic acid content of control during its red stage on 20th day was 8.18 mg 100 g^{-1}, whereas for coated fruit on the same day it was 8.52 mg 100 g^{-1}. The ascorbic acid content of the coated fruit during its red stage on 40th day was 8.28 mg 100 g^{-1}. Whereas, the coated sample a slight increase in the ascorbic acid level was observed on 40th day, because the ascorbic acid level depends upon the relative exposure to sunlight.

Physio- Chemical Parameters:

Chitosan can effectively remove these contaminations that severely affect the water quality[16]. To measure the impact on the water quality, sample were collected, treated and analyzed in the laboratory. Corresponding results related to physico-chemical parameters are shown in table 7.

Table No 7: Physico- Chemical parameter study using chitosan filter

Parameters (Unit)	Sample ID	Before treatment	Chitosan-Filter		
			After treatment	Efficiency (%)	∑
pH	S1	7.8	7.1	8.97	8.92
	S2	7.8	7.1	8.97	
	S3	7.9	7.2	8.86	
Turbidity (NTU)	S1	116	7	93.97	94.01
	S2	103	6	94.18	
	S3	107	6	94.39	
Total Dissolved solid (mg/l)	S1	184	27	85.33	85.33
	S2	184	25	86.41	
	S3	189	29	84.66	
Electric conductivity (µS/cm)	S1	374	56	85.03	84.91
	S2	372	52	86.02	
	S3	386	61	84.20	

Table 8 indicated that Removal rate of metal is excellent and up to 99.00 % efficient for Pb, Cr, Zn and Ni by Chitosan filter. The unique properties of Chitosan together with availability, makes Chitosan an exciting and promising agent for the purification of surface water for household drinking purpose[19].

Table No 8: Removal of heavy metals using chitosan filter

Metals	Sample ID	Metal Conc. Before Treatment (ppm)	Chitosan-Filter		
			Metal Conc. After Treatment (ppm)	Efficiency (%)	∑
Lead (Pb)	S1	8.9368	0.0056	99.94	99.76
	S2	8.5680	0.0032	99.96	
	S3	10.345	0.0342	99.67	
Chromium (Cr)	S1	71.653	0.0475	99.93	99.89
	S2	73.576	0.0740	99.90	
	S3	70.320	0.0394	99.94	
Zinc (Zn)	S1	17.322	0.0253	99.85	99.89
	S2	16.595	0.0034	99.98	
	S3	16.983	0.0074	99.96	
Nickel (Ni)	S1	6.2515	0.0038	99.94	99.87
	S2	6.3502	0.0276	99.57	
	S3	6.0998	0.0028	99.95	

IV. Conclusion

Chitosan, being the second most abundant polysaccharide available on earth it has wide range of application both in food and water treatment. Currently chitin, chitosan are being extracted from crustaceans for various applications. Our study manifested the extraction and optimization of chitosan from mushroom source, were the purity and commercial availability of mushroom based chitosan can increase over time.

Our study, comparing the extraction process of two different mushroom species opens up lots of new areas of research. We had extracted mushroom based chitosan efficiently 5kg of button mushroom chitin. This method is found to be of greater economic efficiency, in contrast with the enzymatic method of extraction.

Further, the preliminary confirmation for the given product to be chitosan was done using TBA assay and DNS Test and the final confirmation was done using FTIR analysis. Food coating was carried out and efficacy was tested comparing with that of the non-coated one. The firmness of control fruit during red stage on the20th day was 31.65 N, whereas for the coated fruit on the same day it was 46.79 N. Coating was the most effective treatment for retardation of softening of harvested fruits and vegetables. The titratable acidity of control at the time of red stage on the 20th day was 0.611% whereas for coated fruit on the same day it was 0.559%. *Biopolymer* as an edible coating with 2% solid percentage remains a viable alternative to delay the ripening of tomato. By optimizing the solid percentage, it could be applicable to other fruits and vegetables too.

Study also indicated it can be effectively used for the water purification as it indicated good efficiency with respect to physic- chemical and heavy metal study.

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