

Original Research Article

Characterization of chitosan-based edible films for food packaging applications

ABSTRACT

Aims: To study the optimization and characterization of chitosan-based edible films for food packaging applications

Study design: This study was experimental and conducted in a lab.

Place and Duration of Study: The study was conducted at the Department of Dairy Technology, Dairy Science College, Hebbal, Bengaluru, Karnataka, India, between October 2022 and July 2023.

Methodology: The edible film was developed with 9 different combinations by 3 concentrations of chitosan 1.50, 2.0 and 2.5% along with 3 concentrations of glycerol 0.5, 0.75 and 1%. Various characteristics like moisture content, water solubility, thickness, density, GSM, colour, transmittance, water vapor transmission rate (WVTR), tensile strength, elongation are assessed. The film was optimised on the basis of water vapor transmission rate, tensile strength and elongation. ANOVA was the method used for statistical analysis, and the significance between the control and sample was assessed using a critical difference (CD) at a 5% significance level.

Results: Increase in chitosan concentration the film thickness, GSM, solubility and density increases whereas moisture content decreases. Increase in glycerol concentration the film thickness, GSM, moisture, solubility increases but there was decrease in film density. The film colour parameters lightness L*, redness a* and yellowness b* increase as chitosan content increases, whereas transmittance decreases. Increased glycerol content increased the film's colour parameters redness a* and transmittance, but had no influence on the film's yellowness whereas as the lightness value decreases. The film made with 2.5% chitosan and 0.5% glycerol has the lowest water vapour transmission rate, 10.70 g/m²h. The tensile strength of film made with 2.5% chitosan and 0.5% glycerol has the highest value of 18.56 MPa. Film produced using 1.5% chitosan and 1% glycerol has the highest value of 51.73% elongation.

Conclusion: Increasing the concentration of chitosan improves tensile strength but reduces elongation. On the other hand, adding more glycerol lowers tensile strength but increases elongation. A film containing 2% chitosan and 0.75% glycerol is considered the best choice, offering a balanced water vapor transfer rate, tensile strength, and elongation.

Keywords: Chitosan, edible film, glycerol, physical property, colour characteristic, water vapor transmission rate, tensile strength, elongation

1. INTRODUCTION

Edible biopolymers made of proteins, lipids and carbohydrates can be created as plastic substitutes. The use of these biodegradable and environmentally friendly compounds in packaging can help to reduce the quantity of plastic trash that pollutes the environment. The primary benefit of edible films over typical packaging materials is that they may be consumed along with food packed in it (Dhumal and Sarkar,2018).

Chitosan, a linear polysaccharide consisting of (1,4) linked 2-amino-deoxy- β -D-glucan, is a deacylated derivatives of chitin, which is the second most abundant polysaccharide found in nature after cellulose. Chitosan is a substance with antioxidant and antimicrobial properties and it is also non-toxic, biodegradable, bio-functional and biocompatible. Chitosan-based edible films have good mechanical strength, an excellent oxygen barrier and carry natural antioxidants and antimicrobial substances (Vilela *et al.*,2017).

Compared with synthetic plastic films, an important limitation of chitosan-based films is their mechanical properties, especially their capacity to elongation. One of the most common means to improve the mechanical properties of the biopolymer films is to add a plasticizer into the film formulation. Plasticizers are additives used to make films with higher percentage of elongation at break and more flexible, necessary to facilitate the polymer processing and application (Prateepchanachai *et al.*, 2017).

The content of glycerol affected film properties such as water sorption, water vapour permeability (WVP), mechanical properties and glass transition temperature. Leceta *et al.*, (2013) reported that chitosan-based films exhibited excellent barrier properties against water vapour and oxygen with the addition of glycerol. Increasing the concentration of glycerol from 15 to 45%, there was a minor decrement in film density (1.425–1.316 g/cm³). Higher concentration of chitosan has increase of the tensile strength and a decrease of percentage of elongation values. Tensile strength of the film increases, with increase in storage time.

Packaging material should have property to protect the foods from the effects of light, especially the UV radiation. Films with good barrier properties against the UV-light help to slow the oxidation process of the lipids, increasing the shelf life of the food product keeping the food quality and adequate organoleptic properties. The transparency that

has a direct impact on the consumer acceptability. Consumers prefer transparent packaging to can see the product content and to observe the visual quality. Therefore, it is important to formulate films with good protective barriers against UV radiation while maintaining adequate transparency (Ezatie *et al.*, 2023). The UV-visible spectra of pure chitosan films show a transmittance of 88–93% in the visible range (400–700 nm), being optically transparent films (Vilela *et al.*, 2017).

These films are particularly effective in preserving the quality and extending the shelf life of various food products. For instance, when applied to perishable items like fruits, vegetables, meats and dairy products (Mostafavi *et al.*, 2020). The study focusses to develop and characterize chitosan-based edible films and to assess their physical, mechanical, and optical properties to ensure suitability for food preservation by investigate the effects of varying chitosan and glycerol.

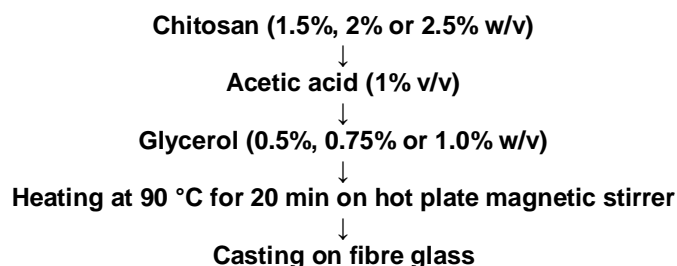
2. MATERIAL AND METHODS

2.1 Materials

The chitosan powder above 90% deacetylation was procured from Meron – Marine hydrocolloids, Kochi, Kerala. Acetic acid 1% (food grade) was sourced from Umang Industries, Shahdara, New Delhi. Food grade glycerol used were manufactured by Bioven Ingredients, Greater Noida, G.B. Nagar, Uttar Pradesh.

2.2 Preparation of chitosan based edible film

Chitosan-based edible film was made by dissolving chitosan powder in 1% v/v acetic acid solution and plasticizer glycerol and heating for 20 minutes on a hot plate magnetic stirrer at 90°C with regular stirring. The solution was uniformly cast on fibre glass plates and dried for 48 hours at 40 °. The dried films were peeled off and kept in a chamber with a humidity of 50% and a temperature of 25°C.



↓
Drying at 40 °c for 48 h in an incubator
↓
Peeling of dried film manually
↓
Store in a humidity chamber at 50% Rh and 25 °C

The film will be optimised on the basis of water vapor transmission rate (WVTR) which shows the barrier property of the film, tensile strength and elongation, which shows the mechanical property of the film. The film with lesser water vapor transmission rate (WVTR) and higher tensile strength and elongation consider to have better quality. The edible film was developed by combining 3 concentrations of chitosan (1.50, 2.0 and 2.5%) along with 3 concentrations of glycerol (0.5, 0.75 and 1%), in 9 different combinations.

2.3 Analysis for different characteristics of edible film

2.3.1 Thickness

Film thickness was measured with a vernier caliper. The thickness was measured at five different places randomly along the length of the film strip preferable one at center and four around the perimeter and mean value was calculated.

2.3.2 GSM

The GSM of the film was measured by loading of 5x5cm film in GSM tester manufactured by Ubique enterprises. The average of 5 film was taken for single analysis.

2.3.3 Moisture content

The amount of moisture of films was measured using the gravimetric method. The films weighing around 1 g were dried at $105\pm 1^{\circ}\text{C}$ for 24 hours (until equilibrium weight was obtained). The sample's weight loss was measured, and moisture content was determined as the proportion of water lost from the system.

2.3.4 Water solubility

The amount of dry matter that dissolved following a 24-hour immersion in water was used to calculate the water solubility of the films. For twenty-four hours, the 1 mg films were submerged in 50 mL of water at $23\pm 1^{\circ}\text{C}$, with periodic stirring. The film pieces were

then removed and dried in an oven set to $105\pm 1^\circ\text{C}$ until they reached a consistent weight. For every sample, three measurements were taken. Solubility of films in water was determined as the percentage of soluble material by the following formula.

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

2.3.5 Density

The density of the film was evaluated using the flotation method at 25°C with CCl_4 (1.5935 g/ml) and hexane (0.659 g/ml) as solvents. The $1.5 \times 1.5 \text{ cm}^2$ film was submerged in 5 ml of isopropanol in a small beaker. CCl_4 was taken in a burette and introduced drop by drop to the beaker until the film floats in the middle of the solution, after which the density of the film was estimated using the formula.

$$\text{Density (g/ml)} = \frac{(V_1d_1 + V_2d_2)}{(V_1 + V_2)}$$

Where, V_1 – volume of hexane in ml,
 V_2 – volume of CCl_4 in ml,
 d_1 – density of hexane in g/ml,
 d_2 – density of CCl_4 in g/ml

2.3.6 Transmittance

Transmittance of chitosan films was determined by placing the film strips ($3\text{cm} \times 1 \text{ cm}$) in the cuvette containing water and was measured the percentage transmittance at fixed wavelength of 660 nm using UV-VIS spectrophotometer.

2.3.7 Colour characteristics

The colour characteristics of chitosan film was determined using colorimeter. YS6060 benchtop colorimeter which was developed by 3nh with reflective d/8 and transmissive d/0 geometry was used with calibration for each trial. Film colour parameters was analysed on five different points on single film.

2.3.8 Water vapor transmission rate (WVTR)

The water vapour transmission rate was determined using a modified ASTM 96-00 method (ASTM 2000). The film was sealed onto a modified test cell holding 15 mL of distilled water. The test cell was then stored in a desiccator containing pre-dehydrated silica gel. To conduct these measurements, silica gels were dried at 180°C for 3 hours.

The assembly was stored at 25°C, and the weight loss of the test cell was determined after 24 hours of storage.

WVTR of the film was calculated according to the equation

$$WVTR = \frac{\Delta W}{(\Delta t \times A)}$$

Where, ΔW is the weight loss of test cell,
 Δt is the time of storage,
 A is the area of exposed film.

2.3.9 Tensile strength

Tensile strength tests were determined using Shimadzu universal testing instrument. The sample of 2 cm width and 10 cm length was clamp with a testing machine. The start button on the computer is pressed and then the tool will pull the sample at a speed of 5 mm/minutes with force of 100N until the sample breaks. Tensile strength of edible film is calculated by trapezium software of the universal testing instrument.

2.3.10 Elongation

Elongation was determined using Shimadzu universal testing instrument. The sample of 2 cm width and 10 cm length was clamp with a testing machine. The start button on the computer is pressed and then the tool will pull the sample at a speed of 5 mm/minutes with force of 100N until the sample breaks. Elongation is calculated with help of external extensometer connected to the instrument. Elongation is calculated using the formula

$$\text{Elongation (\%)} = \frac{\text{Strain isolate (mm)}}{\text{Initial length (mm)}} \times 100$$

2.4 Statistical Analysis

The data was analysed using IBM SPSS Statistics-29 software for statistical computing. Data on the response variables was collected as three replications for each of the treatments. ANOVA tables was prepared to analyse the data and where the F value was significant, the critical difference was calculated ($P=0.05$) and used to identify whether significant differences existed and indicated in the table using superscripts.

The formula for the critical difference (CD) is

$$\frac{\sqrt{2 \times MSS(E)} \times t_{\alpha}}{r}$$

Where,

MSS (E) = Mean Sum of squares of the error

r = number of replications

t_{α} = table t value of the α level of significance at 0.95

3. RESULTS AND DISCUSSION

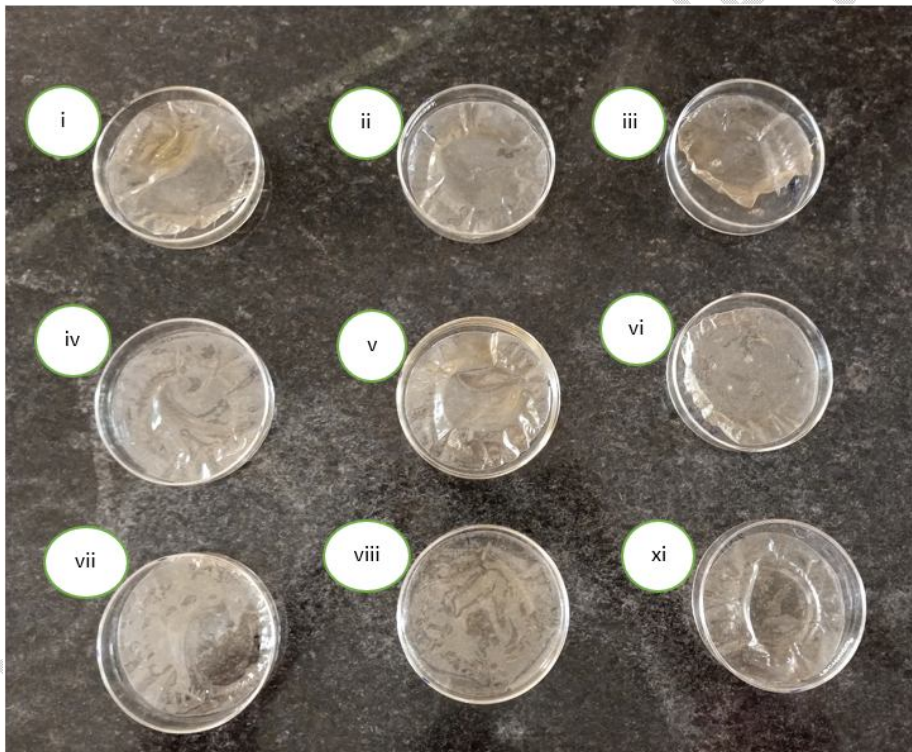


Fig:1- Film prepared with different levels of chitosan(C) and glycerol (G)
(i) C-1.5%, G-0.5% (ii) C-2.0%, G- 0.5% (iii) C-2.5%, G- 0.5%
(iv) C-1.5%, G-0.75% (v) C-2.0%, G- 0.75% (vi) C-2.5%, G- 0.75%
(vii) C-1.5%, G-1.0% (viii) C-2.0%, G- 1.0% (ix) C-2.5%, G- 1.0%

3.1 Effect of different levels of chitosan and glycerol in physical properties of chitosan based edible film

Different levels of chitosan and glycerol had significant ($P < 0.05$) effect on the thickness of edible film. The highest value of 243.83 μm thickness is for film made with 2.5%

chitosan and 1% glycerol. The lowest value of 141.84 μm thickness is for film made with 1.5% chitosan and 0.5% glycerol. The thickness of film increases with the chitosan concentration. With increase in glycerol concentration also thickness of the film increases proportionally. The results in this study are similar and within range with work of Saputra *et al.* (2021) on characteristics of edible film from chitosan as biodegradable packaging.

The GSM of edible film was significantly ($P < 0.05$) affected by varying the levels of glycerol and chitosan. The film made with 2.5% chitosan and 1% glycerol has the highest value, 282.38 g/m^2 GSM. The film made with 1.5% chitosan and 0.5% glycerol has the lowest value, 169.19 g/m^2 GSM. With an increase in chitosan concentration, the GSM of the film rises. The GSM of the film also rises proportionally with an increase in glycerol concentration. The findings of this study are comparable to and within the bounds of those of Rao *et al.* (2021) work on the creation of edible active packaging films which has grammature ranged from 227.40 ± 3.42 to 255.23 ± 3.51 g/m^2 .

The moisture of edible film was significantly ($P < 0.05$) impacted by the glycerol and chitosan concentrations. The film made with 1.5% chitosan and 1% glycerol has the highest value of moisture, 45.47%. Film made with 2.5% chitosan and 0.5% glycerol has the lowest moisture value, 27.52%. With increasing chitosan concentration, the moisture of the film decreases. The moisture content of the film rises proportionally with an increase in glycerol concentration. The findings of this study are consistent and reasonable with work of Cazón *et al.* (2020) where moisture content values ranging from 25 to 45 per cent depending on the chitosan ratio.

On the solubility of edible film, different concentrations of glycerol and chitosan had a big impact. The film made with 2.5% chitosan and 1% glycerol has the highest value of solubility, 42.15%. For a film made with 1.5% chitosan and 0.5% glycerol, the lowest value of 17.67% solubility is recorded. As chitosan concentration rises, film solubility rises as well. Glycerol solubility increases proportionally with an increase in glycerol concentration. The chitosan film's water solubility is influenced by the functional group in the chitosan and water holding capacity of plasticizer. The study was parallel with

findings of Min *et al.*, (2021) where he found chitosan films has solubility range from 27.29% to 41.68%.

Different levels of chitosan and glycerol had significant ($P < 0.05$) impact on the density of edible film. The highest value of 1.442g/ml density is for film made with 2.5% chitosan and 0.5% glycerol. The lowest value of 1.371g/ml density is for film made with 1.5% chitosan and 1% glycerol. The density of film increases with the chitosan concentration. With increase in glycerol concentration density of the film decreases. The results in this study is similar and within range with work of Tarique *et al.*, (2021) on effect of glycerol plasticizer loading on the physical, mechanical, thermal and barrier properties of arrowroot (*Maranta arundinacea*) starch biopolymers. The decrease in density could be associated with the increased thickness (and volume) as a result of increased plasticizer content.

Table 1: Effect of different levels of chitosan and glycerol in physical properties of chitosan based edible film

Sample No	Chitosan	Glycerol	Thickness	GSM	Moisture	Solubility	Density
	% (w/v)	% (v/v)	μm	g/m^2	%	%	g/ml
1	1.50	0.50	141.84 ^a	169.19 ^a	32.36 ^{ab}	17.67 ^a	1.426 ^{ab}
2	2.00	0.50	168.30 ^b	180.08 ^a	30.66 ^a	21.63 ^b	1.433 ^b
3	2.50	0.50	199.02 ^c	217.69 ^b	27.52 ^c	23.83 ^b	1.442 ^c
4	1.50	0.75	193.37 ^c	211.37 ^b	42.58 ^d	29.15 ^c	1.405 ^d
5	2.00	0.75	213.98 ^d	227.62 ^{bc}	39.05 ^e	31.26 ^{cd}	1.411 ^d
6	2.50	0.75	228.37 ^e	257.74 ^e	32.97 ^b	32.32 ^d	1.419 ^a
7	1.50	1.00	203.32 ^{cd}	237.24 ^{cd}	45.47 ^f	37.58 ^e	1.371 ^e
8	2.00	1.00	228.48 ^e	252.74 ^{de}	42.53 ^d	39.57 ^e	1.382 ^f
9	2.50	1.00	243.83 ^f	282.38 ^f	34.17 ^b	42.15 ^f	1.390 ^g
CD (P=0.05)			12.67	17.23	2.12	2.25	0.0073

Note: CD – Critical difference, All the values are average of three trials, Similar superscripts indicate non - significance at the corresponding critical difference

3.2 Effect of different levels of chitosan and glycerol on colour characteristics and transmittance of chitosan based edible film

The film made with 2.5% chitosan and 0.5% glycerol has the lightness value with the highest maximum of 39.31. The film made with 1.5% chitosan and 1% glycerol has the

lowest value of 38.07 lightness. The amount of chitosan in the film makes it lighter. The film's lightness decreases as glycerol concentration rises. The findings of this study are consistent and reasonable with those of Rachtanapunet *et al.*, (2021) where lightness ranged from 41.60 to 39.17 L*.

The film made with 2.5% chitosan and 1% glycerol has the highest redness value, which is -0.51. Film made with 1.5% chitosan and 0.5% glycerol has the lowest redness value, which is -1.10. The concentration of chitosan causes the film to shift to more redness. The redness of the film increases proportionally with an increase in glycerol concentration. The findings of this study are similar with those of López-Mata *et al.*, (2013) where chitosan films had redness a* in the range of -1.30 to -1.37.

The highest value of 4.54 yellowness is for film made with 2.5% chitosan and 1% glycerol. The lowest value of 2.10 yellowness is for film made with 1.5% chitosan and 0.5% glycerol. The yellowness of film slightly increases with the chitosan concentration. With increase in glycerol concentration also yellowness of the film increases proportionally. The results in this study similar and within range with work of Kaczmarek-Szczepańska *et al.* (2022) where chitosan film prepared with 1% chitosan and 1% of different phenolic acid such as ferulic acid, caffeic acid, tannic acid and gallic acid had yellowness b* value which varies from 2.03 to 10.40.

The film made with 1.5% chitosan and 1% glycerol has the highest transmittance value at 94.60%. The film made with 2.5% chitosan and 0.5% glycerol has the lowest value of transmittance, which is 82.80%. As chitosan concentration increases, film transmittance decreases. Transmittance of the film increases as glycerol concentration rises. The chitosan film is very transparent in the visible range (400 to 700nm) with a light transmittance of 90%, but it is low transmittance in the ultraviolet C region (250 to 280nm) due to light absorption of amino acids in chitin that are not deacetylated to chitosan (Nguyen *et al.*, 2022).

Table 2: Effect of different levels of chitosan and glycerol in colour characteristics and transmittance of chitosan based edible film

Sampl	Chitosa	Glycero	Lightnes	Rednes	Yellownes	Transmittanc
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e No	n	l	s	s	s	e
	% (w/v)	% (v/v)	L*	a*	b*	%
1	1.50	0.50	38.81 ^a	-1.10 ^a	2.10 ^a	90.90 ^{ab}
2	2.00	0.50	39.06 ^b	-0.66 ^{bc}	2.47 ^b	88.20 ^{ad}
3	2.50	0.50	39.31 ^c	-0.61 ^{cd}	3.95 ^c	82.80 ^e
4	1.50	0.75	38.67 ^d	-0.78 ^b	2.64 ^b	93.50 ^{bc}
5	2.00	0.75	38.82 ^a	-0.68 ^{bc}	3.25 ^d	90.30 ^{ab}
6	2.50	0.75	38.96 ^b	-0.62 ^{cd}	3.58 ^e	85.40 ^{ed}
7	1.50	1.00	38.07 ^e	-0.72 ^{bc}	2.96 ^f	94.60 ^c
8	2.00	1.00	38.11 ^e	-0.65 ^{bc}	3.78 ^c	92.16 ^{bc}
9	2.50	1.00	38.69 ^d	-0.51 ^d	4.54 ^g	87.80 ^{ad}
CD (P=0.05)			0.109	0.121	0.171	3.19

Note: CD – Critical difference, All the values are average of three trials, Similar superscripts indicate non - significance at the corresponding critical difference

3.3 Effect of different levels of chitosan and glycerol in moisture barrier and mechanical properties of chitosan based edible film

The water vapour transmission rate of edible film was significantly ($P < 0.05$) affected by varying the levels of glycerol and chitosan. Film made with 1.5% chitosan and 1% glycerol has the highest value of 83.15 g/m²h water vapour transmission rate. The film made with 2.5% chitosan and 0.5% glycerol has the lowest water vapour transmission rate, 10.70 g/m²h. With increasing chitosan concentration, a film's water vapour transmission rate declines. The rate at which water vapour transmission through the film increases as glycerol concentration rises. The findings of this study agree with the research conducted by Rodríguez-Núñez *et al.* (2014) on chitosan/hydrophilic plasticizer-based films: preparation, physicochemical and antimicrobial properties. The water vapour permeability values depend on properties of films such as the molecular weight, degree of deacetylation and content of chitosan. Besides, these values are affected by various external factors such as the measuring method, measuring conditions (relative humidity and temperature), correction of air gap effect and the storage time and conditions. The water vapour permeability increases to increase in the molecular weight and degree of deacetylation of chitosan used.

The tensile strength of edible film was significantly ($P < 0.05$) influenced by the

concentrations of glycerol and chitosan. The tensile strength of film made with 2.5% chitosan and 0.5% glycerol has the highest value of 18.56 MPa. The film made with 1.5% chitosan and 1% glycerol has the lowest value of 4.53 MPa tensile strength. With an increase in chitosan concentration, the film's tensile strength rises. The tensile strength of the film also decreases as glycerol concentration rises. The findings of this study line up with the preparation and characterization of glycerol plasticized starch-chitosan films done by Liu *et al.* (2013).

The elongation of edible film was significantly ($P < 0.05$) impacted by varying the chitosan and glycerol levels. Film produced using 1.5% chitosan and 1% glycerol has the highest value of 51.73% elongation. Film made with 2.5% chitosan and 0.5% glycerol has the lowest value for elongation, 11.51%. With increasing chitosan concentration, film elongation decreases. Additionally, the film's elongation increases proportionally as glycerol concentration rises. The results in this study is similar and within range with work of Dong *et al.* (2019) on preparation of glycerol plasticized chitosan films using $\text{AlCl}_3 \cdot 6\text{H}_2\text{O}$ as the solvent: optical, crystalline, mechanical and barrier properties. High molecular weight of chitosan increases the tensile strength and decreases the percentage of elongation values. Tensile strength of the film increases, with increase in storage time.

Table 3: Effect of different levels of chitosan and glycerol in moisture barrier and mechanical properties of chitosan based edible film

Sample No	Chitosan	Glycerol	Water vapour transmission rate	Tensile Strength	Elongation
	% (w/v)	% (v/v)	$\text{g/m}^2\text{h}$	MPa	%
1	1.50	0.50	76.60 ^a	7.63 ^a	23.37 ^a
2	2.00	0.50	52.91 ^b	10.27 ^b	14.67 ^b
3	2.50	0.50	10.70 ^c	18.56 ^c	11.51 ^b
4	1.50	0.75	80.19 ^d	6.23 ^d	48.42 ^{cd}
5	2.00	0.75	55.72 ^e	7.41 ^a	35.64 ^e
6	2.50	0.75	29.65 ^f	13.36 ^e	31.35 ^f
7	1.50	1.00	83.15 ^g	4.53 ^f	51.73 ^d

8	2.00	1.00	68.12 ⁿ	7.01 ^g	46.30 ^c
9	2.50	1.00	43.28 ⁱ	9.16 ⁿ	40.08 ^g
CD (P=0.05)			2.53	0.365	3.68

Note: CD – Critical difference, All the values are average of three trials, Similar superscripts indicate non - significance at the corresponding critical difference

2.4 CONCLUSION

With increase in chitosan concentration the film thickness, GSM, solubility and density increases whereas moisture content decreases. Increase in glycerol concentration the film thickness, GSM, moisture, solubility increases but there was decrease in film density.

The film colour parameters lightness L*, redness a* and yellowness b* increase as chitosan content increases, whereas transmittance decreases. Increased glycerol content increased the film's colour parameters redness a* and transmittance, but had no influence on the film's yellowness whereas as the lightness value decreases.

With increasing chitosan concentration, film tensile strength increases while elongation and water vapour transmission rate decreases. The film's water vapour transfer rate and elongation increased as glycerol concentration increased, but there was reduction in tensile strength.

The film made with 2.5% chitosan and 0.5% glycerol has the lowest water vapour transmission rate, 10.70 g/m²h. The tensile strength of film made with 2.5% chitosan and 0.5% glycerol has the highest value of 18.56 MPa. Film produced using 1.5% chitosan and 1% glycerol has the highest value of 51.73% elongation.

The chitosan film with varying chitosan and glycerol concentrations is optimised based on characteristics such as water vapour transfer rate, tensile strength and elongation.

The concentration of chitosan increases tensile strength but decreases elongation. As increasing the glycerol content decreases tensile strength but increases elongation, a film with 2% chitosan and 0.75% glycerol is selected as the best film with moderate water vapour transfer rate, tensile strength and elongation.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Authors hereby declares that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

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