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Chitosan Gelatin Multi-layered Biodegradable Films For Wrapping Food Materials

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Abstract: : *The development of edible films based on polysacharides and proteins has brought a significant increse in their applications and in extending shelf life of fruit and vegetables.The composite film of such biopolymers is effective for improving the physical properties of edible films; however, film performance can be reduced if there is a lack of careful consideration of the compatibility of these blended components. The present work addresses to the experimental study on multilayer casting of chitosan and gelatin films and investigate comparative study of chitosan, Gelatin, chitosan-gelatin blends and multi-layered edible films. Film samples are analysed for thickness, functional group presence and water solubility. In order to evaluate the potential application of the prepared films in food packaging, the shelf life of the milk bread wrapped with films was studied. Based on the observations, result and discussion it can be concluded that, the present work involving multilayer casting of chitosan and gelatin have successfully resulted in films with better moisture and water solubility resistance along with good strength and show antimicrobial performance against E. coli , L. mono-cytogenes.*

Key Words: *Biodegradable, Chitosan-gelatin films, Edible, Food packaging, Shelf life, Water solubility.*

1. INTRODUCTION:

The environmental impact of non-biodégradable packaging waste material is of increasing global concern & it is a necessity of the hour to develop eco friendly packing materials.[1] The development of edible films based on polysacharides and proteins is one such initiative & has brought a significant increse in their applications by extending shelf life of fruit and vegetables. Edible coatings and films can provide a protective layer around food product. Film-forming polysaccharide materials include starch &its derivatives, cellulose derivatives, alginate, carrageenan, pectin, chitosan etc. Proteins that have received great attention for their capability of forming edible films and coatings include corn zein, wheat gluten, soy protein, whey protein, casein, collagen/gelatin, pea protein, rice bran protein, cottonseed protein, peanut protein, and keratin [2]. Plasticizers used for edible films and coatings include sucrose, glycerol, sorbitol, propylene glycol, polyethylene glycol, fatty acids, and monoglycerides. [3] Edible films containing proteins and polysaccharides as the film matrix materials typically utilize the distinct functional characteristics of each film forming component. The composite film of such biopolymers is effective for improving the physical properties of edible films; however, film performance can be reduced if there is a lack of careful consideration of the compatibility of these blended components. The incorporation of polysaccharides into globular protein matrices may extend the functional properties of these ingredients.[4][5] Among these biopolymers, chitosan and gelatin have attracted incresed attention since derived films and coating improve the food quality & shelf life of the protected food due to their adequate mechanical & excellent gas barrier properties.[6] The advantages such as biodegradability, non-toxicity and improved shelf life have caused edible packaging used widely in the food industry.Chitosan is one of the versatile functional polysaccharides comprising β -1,4-linked polymer of glucosamine (2-amino-2-deoxy- β -D-glucose[7] Gelatin has good film forming property. However, the poor mechanical, thermal & barrier properties in comparison to conventional plastics are the major disadvantages to use gelatin as food packaging material. Edible films of protein show satisfactory gas barrier & mechanical properties. In general, blending of these two different biopolymers may optimize the physico-chemical and barrier properties of the gelatin based films for food applications.[8] Edible films produced from denatured proteins have the potential for decreased moisture and gas permeability and solubility and improved mechanical properties, thereby enhancing their potential use as packaging materials.[9]

2. LITERATURE REVIEW:

Gelatin films, with high puncture strength, low puncture deformation, and high water vapor permeability, prepared from bovine and porcine skin have been reported.[10][11] Simon-Lukasik and Ludescher studied oxygen permeability though gelatin films, also the functional and thermal properties of edible films made from blends of gelatin and starches have been studied.[12] It can form films and coating with good optical properties and act as bio-packaging materials. [13] Composite films of chitosan and gelatin have been reported to have improved mechanical properties compared with those of single component based- films.[14] The antimicrobial activity of composite films is reported by Lopez- caballero et al. by plate diffusion assay over four micro-organisms in which there was no microbial activity observed in gelatin or in composite film of chitosan and gelatin.[15] Gomez- Guillen et al. have developed a coating from a chitosan and gelatin blend to applied to patties of cod. The coating prevented spoilage of the cod patties. Due to presence of chitosan gram negative bacteria was not observed on cod patties.[16] Highly polar polymers containing hydroxyl groups, such as proteins and polysaccharides, generally present a good barrier to oxygen at low relative humidity due to their tightly packed, ordered hydrogen-bonded network structure and low solubility.[17]

The present work addresses to the experimental study on multilayer casting of chitosan and gelatin films and investigate comparative study of chitosan, Gelatin, chitosan-gelatin blends and multi-layered edible films.

3.MATERIALS & METHOD:

3.1 Objective

The main objective of the present work is to prepare edible, biodegradable and eco-friendly multi-layered packaging films. The present work puts emphasis on the use of natural polysaccharide that make them biodegradable.

3.2 Methodology

The present work is divided into four parts:
I)Preparation of biodegradable films: The films were prepared using individual chitosan and gelatin components by direct casting and multi-layered films of these two components by layer by layer casting method.

II)Analysis of prepared films: Several experimental runs have been conducted and film samples were analysed using Fourier-transform infrared spectroscopy (FTIR) & Water solubility tests.

III)Antimicrobial analysis: The microbial assays were performed for *Escherichia coli*, Coliform and L. monocytogenes under accelerated conditions at 37°C and yeast & mold at 25°C by pour plate method.

IV)Assessment of the films: Newly developed biodegradable films from chitosan, Gelatin, composite film of chitosan gelatin and multilayered films were wrapped on milk bread for shelf life study.

The general methodology is depicted in flow chart as shown in Figure 3.1

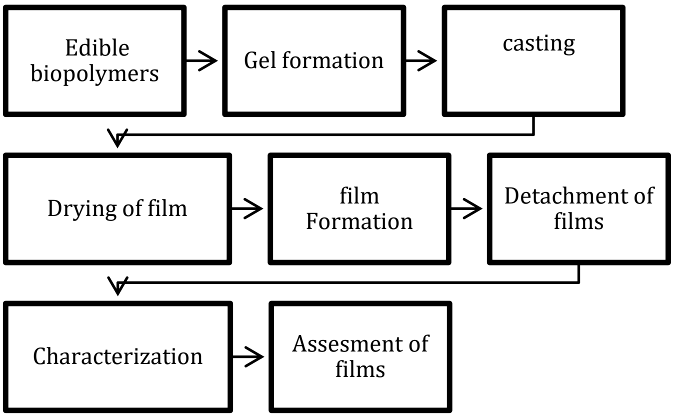

Fig.3.1 Methodology of present work

3.3 Materials

Commercial grade chitosan (high MW, having more than 90% degree of deacetylation) from HiMedia Laboratories Pvt. Ltd, Gelatin, Glycerol, laboratory grade glacial acetic acid.

3.4 Chitosan-Gelatin based biodegradable film preparation (CG1):

Biodegradable films from chitosan were prepared according to the casting technique described by Shekhar Pandharipande, Rajashri Turak with slight modification. [18] Chitosan solution was prepared by dissolving commercial grade chitosan (90% deacetylate) in 2 v/v % acetic acid and gelatin solution was prepared by dissolving gelatin in distilled water. Glycerol was added to mixture of both solutions with continues stirring for 20 minutes. Film forming solution is casted in petri dish and dried at room temperature. Then, aliquots of 20ml of film forming solution was poured in petri dish and dried at ambient temperature. Fig.3.2 gives the actual photograph of process steps in preparation of the CG1 sample.

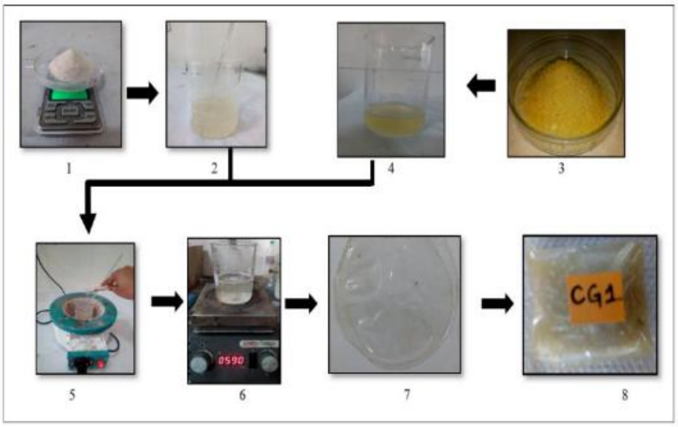

Fig 3.2 Photographs of process steps for CG1 sample
1.Chitosan 2. Chitosan in Acetic acid 3.Gelatin 4. Gelatin solution
5. Heating 6. Magnetic Stirring 7. Composite film 8. Food packaging

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3.5 Multi-layered chitosan gelatin based film (CGM):

Multi-layered films were synthesized by alternately casting solutions of chitosan and gelatin as mentioned in the previous section.

- ➢ Sterile aqueous solution was prepared by dissolving the chitosan powder in glacial acetic acid.
- ➢ The solution was stirred at room temperature for 10 minutes.
- ➢ Glycerol was added in the film forming solution while continuous stirring.
- ➢ Gelatin was dissolved in distilled water and heated up to 70˚ C.
- ➢ Film forming solution was cooled down with the help of ice bath.
- ➢ Second layer was given followed by chitosan layer and let it dry.
- ➢ Further layers were coated alternatively followed by drying of previous layer. The detailed of process parameters for experimental runs are given in table 3.1 and photographs of film samples are shown in figure 3.4

3.6 Observation table:

Table 3.1 : Details of process parameters

Sample name	Qty of CH(g)	Qty of acetic acid (2v/v%) (ml)	Qty of G(g)	Qty of glycerol (ml)
C1	0.5	30	---	0.2
G1	---	---	1.07	0.6
CG1	0.1	10	0.7	0.4
CGM	0.6	40	1.2	1.2

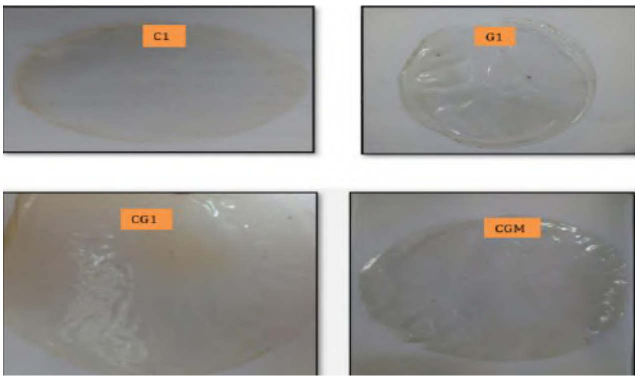

Fig 3.4 photographs of film samples C1, G1, CG1 and CGM.

3.7 Assessment of the films as packaging material

The efficiency of newly developed C1, G1, CG1, CGM was assessed for shelf life study of wrapped milk bread with initial moisture content of 35.64 %. Milk bread was purchased from dairy store. The bread samples were cut in four pieces of 2.5 x 2.5 cm^2.Films were wrapped around each of the piece, and taped as shown in figure 3.5 One of the bread samples was wrapped with polyethylene film as a control. The wrapped bread samples were kept at room temperature and microbial test was done after 10 days.

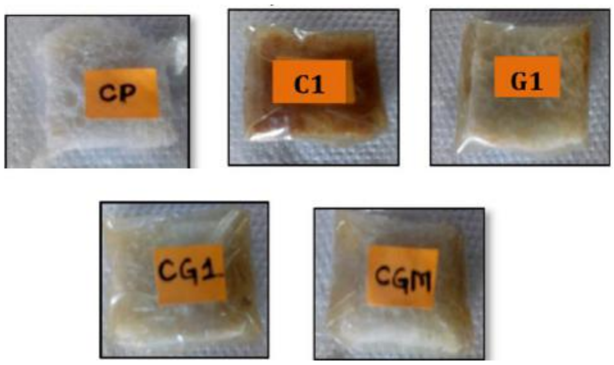

Fig 3.5 Photographic representation of wrapped bread with edible packaging

4. RESULTS & DISCUSSION:

4.1 Characterization of films:

4.1.1 FTIR

Figure 4.1 shows FTIR spectra of chitosan, gelatin, chitosan -gelatin and multilayer films. The position of relevant peaks in the spectrum of chitosan film(C1) film was similar to those described by other authors.[19]The characteristic bands appeared at 1644 cm^{-1} (amide I band of acetyl group), 1552 cm^{-1} (NH3+ absorption band, partially over imposed with amide II band) and the broad absorption band at 3100-3500 cm^{-1} due to O-H stretching. Peaks at 923 cm^{-1} and 1154 cm^{-1} were assigned to pyranose rings and amino groups. The spectrum of gelatin film showed relevant peaks arise from C=C stretching at 1644 cm-1, N- stretching at 1552 cm^{-1}(amide II) and C-N and N-H stretching at 1240cm^{-1} (amide III). Moreover, the spectra of the composite and multilayer film exhibited the characteristic peaks of chitosan and gelatin with a slight difference in the regions ranging from 1240 to 1132 cm^{-1} and 1750 to 1630 cm^{-1} and 2879 to 3100 cm^{-1}.

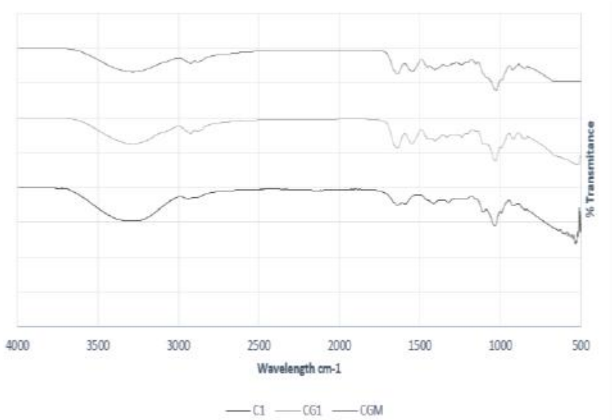

Fig 4.1: FTIR spectra of film samples

4.1.2 Thickness

Thickness of the films were calculated using Micrometre having least count of 0.01μm. Thickness and water solubility of films are given in table 4.1

4.1.3 Water solubility

Water solubility of the films was carried out according to the method of Gontard et al. [20] Three pieces (2.5 x 2.5 cm^2) of the films were weighed to determine their initial dry weight (Wi). Afterwards, the sample were immersed in 20 ml of distilled water for 24 h at room temperature. The samples were then passed through a filter paper. The filter paper together with insolubilize fraction was dried in air oven at 110°C and weighed (Wf). The film solubility (fS %) was calculated using the following equation

$$fS\,\% = \frac{Wi - Wf}{Wi} \times 100$$

$$Wi = initial\ dry\ weight$$

$$Wf = final\ dry\ weight$$

Table 4.1: Thickness and water solubility of films

Sample	Thickness(μm) [Error ±4]	Weight of film	Area of formed film (m^2)	Initial dry wt. of sample	Film solubility in water (%)
C1	40	0.516	0.0038	o.16	75
G1	48	1	0.0038	0.11	36.36
CG1	42	1.97	0.0153	0.08	62.5
CGM	46	2.93	0.0530	0.09	33.3

4.2 Self-life study of milk bread wrapped with Films (Food preservation application):

In order to evaluate the potential application of the prepared films in food packaging, the shelf life of the milk bread wrapped with films was studied keeping plastic wrap as control. The microbial assays were performed for *Escherichia coli*, Coliform and L. mono-cytogenes under accelerated conditions at 37°C and yeast & mold at 25°C by pour plate method. The microbial count for wrapped films samples are given in table 4.2 and petri plates are shown in fig. 4.2 for CG1 and CGM film samples.

Table 4.2: Microbial count in film samples

Film samples	*Escherichia coli (Gram -)*	Coliform	L. mono-cytogenes (Gram +)	Yeast & mold
Control	---	--	---	0/0
CP	---	--	---	0/1
C1	---	---	---	0/0
G1	---	1	---	0/1
CG1	---	--	---	0/2
CGM	---	1	---	0/0

The antimicrobial activity of chitosan has been widely reported due to presence of the positively charged amino groups which interact with negatively charged microbial cell membranes. Table 4.2 showes that coliform was sensitive to both G1 and CGM films while CG1 presented antimicrobial performance against coliform.

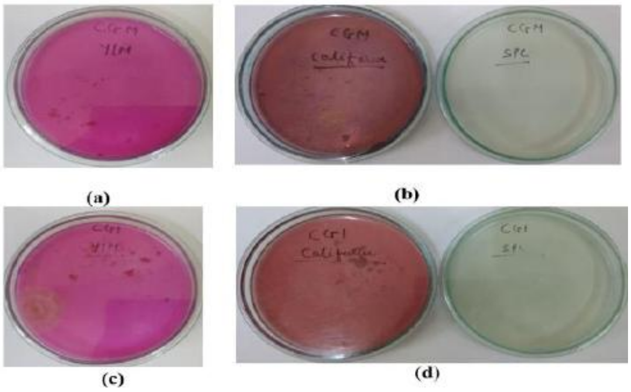

Fig 4.2 Microbial test results of the film samples against yeast & mold and E. coli:(a) CGM (C) CG1 (b) CGM (d) CG1

5. CONCLUSION:

Multilayer films from chitosan and gelatin were obtained by layer by layer solution casting method. Chitosan-gelatin multi-layered films resulted effective alternatives to reduce water solubility. Strength of chitosan film was affected by the presence of gelatin; composite films resulted softer and more flexible than chitosan based one.

Based on comparative studies following inferences can be drawn:

- Biopolymers used in composite films should have alternate charges to improve the film properties.Chitosan is positively charged polysachharide while gelatine has a negative charged.
- The spectra of the composite and multilayer film exhibited the characteristic peaks of chitosan and gelatin with a slight difference in the regions ranging from 1240 to 1132 cm^{-1} and 1750 to 1630 cm^{-1} and 2879 to 3100 cm^{-1}.
- Individual Chitosan based films are transparent, flexible with low strength, when compared with composite films of chitosan and gelatin. The antimicrobial activity of chitosan has been widely reported due to presence of the positively charged amino groups which interact with negatively charged microbial cell membranes.Chitosan films has least water soluble resistance with 75% water soluble after 1 day.
- Individual Gelatin based films are opaque, more flexible having strength but highly *Escherichia coli* sensitive therefore cannot be used alone for packaging of food material. Gelatin based films get swelled when kept in water for long time. Apart

from this it shows less water solubility in cold water while in hot water films are completely soluble.

- Chitosan and gelatin composite films are transparent, labile and 62.5 % water soluable after 24 hr in water at ambient conditions. Incarporating gelatin decreases water solubility of composite films. This films shows antimicrobial performance against coliform but sensitive to mold.
- Multilayer films of chitosan and gelatin are clear with more strength than composite or single component films. It shows 33% water solubility after 24 hr in water at ambient conditions. Multi-layered films showed antimicrobial performance against Gram+ and Gram- (*Escherichia coli, L. mono-cytogenes).* The growth of yeast and mold was not found after 4 days.

The present work has demonstrated the potential in multi-layered casting technique in making of Chitosan-gelatin films can be alternative for the water-soluble films of chitosan and gelatin components. However, it is felt necessary to conduct more experimental runs followed by rigorous tests to validate the claims further.

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