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Chitosan and guar gum composite films: Preparation, physical, mechanical and antimicrobial properties

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ABSTRACT

Films were prepared by the casting method using chitosan (CH) and guar gum (GG) in different ratios. The concentration of GG ranged from 0% to50% (v/v). The optical properties such as transparency, opacity and color were measured. Water vapor transmission rate and oxygen permeability of the films were also investigated. Films were evaluated for mechanical and antibacterial properties. Addition of GG in varied proportions to CH solution led to changes in transparency and opacity of films. The water vapor transmission rate did not change significantly upon addition of GG. Films containing 15% (v/v) GG showed very low oxygen permeability, good tensile and puncture strength. The antimicrobial activity of films containing 15% (v/v) GG was comparable to CH films against *Escherichia coli* and *Staphylococcus aureus*. Composite films obtained from CH and GG may reduce environmental problems associated with synthetic packaging.

1. Introduction

Shelf life of food is governed by its numerous interactions with the surroundings and can be extended using proper packaging material. The deterioration of packaged foodstuffs largely depends on the transfers that may occur between the internal environment of the packaged food and the external environment. Films based on biopolymers can be used to reduce water vapor, oxygen, lipid, and flavor migration between components of multi-component food products, and between food and the surroundings. The film properties depend on the type of material used and the process conditions employed which in turn determine their applications (Krochta & De Mulder-Johnston, 1997). Biological materials used to prepare packaging material include polysaccharides, proteins, lipids and their derivatives. Specifically, within polysaccharides, cellulose derivatives, chitosan (CH), starch, alginate, carrageenan and pectin are preferred because of their high film forming ability. Polymer blending is one of the useful methods to obtain new materials with desired functional properties and there has been great scientific and commercial progress made in the area of food applications.

Chitin and its deacetylated product, chitosan (CH), have received much interest for their application in agriculture, biomedicine, biotechnology and food industry due to their biocompatibility, biodegradability and bioactivity (Kumar, 2000; Tharanathan & Kittur, 2003). Due to its antimicrobial activity (Chen, Yeh, & Chiang, 1996), CH film is a promising packaging material that can be included in the active film category (Vermeiren, Devlieghere, Van Beest, de Kruijf, & Debevere, 1999). However, CH is expensive and functional properties of CH films can be improved by combining it with other hydrocolloids (Park, Lee, Jung, & Park, 2001). Thus importance is attached to the research on the combination of CH with other macromolecules.

Guar gum (GG), a galactomannan obtained from the Indian cluster bean *Cyamopsis tetragonoloba* (L.) Taub., is a water soluble polysaccharide. The backbone of GG is a linear chain of β 1,4-linked mannose residues to which galactose residues are 1,6-linked at every second mannose, forming short side-branches (Dea & Morrison, 1975). Galactomannans are often used in different forms for human consumption. Featuring different physicochemical properties, GG is a versatile material used for many applications. It is an excellent stiffener and the absence of toxicity allows its use in the textile, pharmaceutical, biomedical, cosmetic and food industries (Srivastava & Kapoor, 2005). In addition, GG is found to exhibit surface, interfacial and emulsification activities (Garti, Madar, Aserin, & Sternheim, 1997). Synergism of GG with other materials, including xanthan gum, agar, carageenan, starch, etc., is well studied (Cui, Eskin, Wu, & Ding, 2006). The objective of the present work was to develop composite films based on CH and GG, and to evaluate optical, mechanical, barrier and antimicrobial properties of these films.

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2. Material and methods

2.1. Materials

Commercial CH from shrimp shells with a minimum deacetylation degree of 90% was purchased from Mahatani Chitosan Pvt Ltd. (Veraval, India). Guar gum used was of HiMedia, India. Microbiological media was also procured from HiMedia (Mumbai, India). All other reagents used were of analytical grade and procured from S. D. Fine Chemicals (Mumbai, India).

2.2. Methods

2.2.1. Film preparation

CH (1%, w/v) was solubilized in 1% (v/v) acetic acid solution while GG (1%, w/v) was dissolved in distilled water. The polymer films were prepared by casting method. A series of blends were prepared with varying concentration of CH and GG. The concentration of GG (v/v) in film forming mixture was 0% (film A), 15% (film B), 25% (film C), 40% (film D) and 50% (film E). Film forming solutions were homogenized using Polytron (Kinematica, Switzerland) and the solutions were degassed. To prepare the films, 150 ml of film forming solutions were poured on teflon plates (15 cm × 15 cm) resting on a level surface. These plates were then dried at 50 °C in a ventilated oven at 50% relative humidity (RH). The dried films were peeled from the casting surface. Films were equilibrated at 23 °C and a RH of 50% in a controlled temperature and humidity chamber for measuring barrier and mechanical properties.

2.2.2. Thickness measurement

Thickness of the films was determined using a dial thickness gauge micrometer (Mitutoyo Manufacturing Co. Ltd., Japan) at five random positions on the film and the mean thickness was calculated. Samples with air bubbles, nicks or tears and having mean thickness variation of greater than 5% were excluded from analysis.

2.2.3. Optical properties

Each film specimen was cut into a rectangular piece and placed directly in a spectrophotometer test cell, and measurements were performed using air as the reference. The light transmittance of the films was scanned from wavelength of 190 to 800 nm using a Jasco V-630 spectrophotometer (Japan). The measurement was done in triplicate and the average of three spectra was calculated. The transparency at 600 nm (*T*600) was obtained from the following equation (Han & Floros, 1997):

$$T600 = \frac{\log \%T}{b}$$

where $\%T$ is percentage transmittance and b is the film thickness (mm).

The opacity of the films was calculated by the following equation according to the method described by Gontard and Guilbert (1994):

$$\text{Opacity} = \text{absorbance at } 500\,\text{nm} \times \text{film thickness}$$

The color of the film was assessed using a colorimeter (Minolta, CM-3600d, Tokyo, Japan). A white standard color plate ($L = 97.75$, $a = 0.49$, $b = 1.96$) for the instrument calibration was used as a background for color measurements of the films. The system provides the values of three color components; L^* (black-white component, luminosity), and the chromaticness coordinates, a^* (+red to −green component) and b^* (+yellow to −blue component). Hunter L^*, a^*, and b^* values were averaged from six readings across for each film, and then the total color difference (ΔE) was calculated using the following equation (Ghorpade, Gennadios, & Hanna, 1995): $\Delta E = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{0.5}$ where $\Delta L^* = L^* - L_0^*$, $\Delta a^* = a^* - a_0^*$, $\Delta b^* = b^* - b_0^*$, where L_0^*, a_0^* and b_0^* are color values for CH films and L^*, a^* and b^* are color values for CH films containing GG.

2.2.4. Mechanical properties

Tensile strength (TS) was measured by Texture Analyzer TA-HD plus (Stable Micro Systems, Surrey, UK) following the guidelines of ASTM D 882-91 (1991). The film samples were cut into strips that were 100 mm in length by 25 mm in width and fit to the tensile grips. The initial grip separation was set at 50 mm and the crosshead speed was set at 5 mm/min. The film specimens were mounted in the film-extension grips of the testing machine and stretched at a rate of 50 mm/min until breaking. TS was expressed in kPa and was calculated by dividing the maximum load (N) by the initial cross-sectional area (m^2) of the specimen. Puncture strength was evaluated using a needle probe of 2 mm in diameter at a constant rate of 1 mm/s and the results were expressed in terms of N. For both, tensile or puncture tests, each reported value corresponded to at least five determinations.

2.2.5. Water vapor transmission rate (WVTR) and oxygen permeability (OP)

WVTR tests were carried out using an automatic water vapor permeability testing machine L80-5000 (PBI Dansensor, Denmark) at 37 °C and 10/15% RH. WVTR of films was measured using aluminium sample cards (reduction to 5% area). Oxygen permeability of the film was estimated with automated oxygen permeability testing machine OPT-5000 (PBI Dansensor, Denmark) at 23 °C and 0% RH. Sample was placed in sample holder having an exposed testing area of 50 cm^2. Oxygen permeability of each sample was averaged from three separate tests.

2.2.6. Antimicrobial tests

The test organisms used were *Escherichia coli* JM109 and *Staphylococcus aureus* ATCC 6538P. The organisms were grown in nutrient broth for 18 h at 37 °C. The cells were washed and suspended in phosphate buffer saline (pH 7.2). The cells were further diluted to get concentration of 10^4–10^5 cfu/ml. Films (diameter of 1.5 cm) were put in tubes and aliquot of samples were immediately removed, diluted with sterile saline and plated on plate count agar. The tubes were incubated at 37 °C for 24 h and samples were again withdrawn and plated as described above. The plates were incubated at 37 °C for 24 h and the colonies were counted.

2.2.7. Statistical analysis

All results given in the figures are mean ± standard deviation. Differences between the variables were tested for significance by one-way ANOVA with Tukey's post-test using GraphPad InStat version 3.05 for window 95, GraphPad Software, San Diego, CA, USA; http://www.graphpad.com/. Differences at $p < 0.05$ were considered to be significant.

3. Results and discussion

3.1. Film thickness and appearance

Homogeneous, thin and flexible films were obtained from CH and GG mixture solutions. Films formed could be easily removed from the teflon plates and had an average thickness of 35 μm. The films were free standing as they did not roll over or break. Visually, all the films were colorless and translucent.

3.2. Optical and color properties

The light transmittance of a package affects the rate of oxidation of lipids, and thereby food quality. All the films blocked the

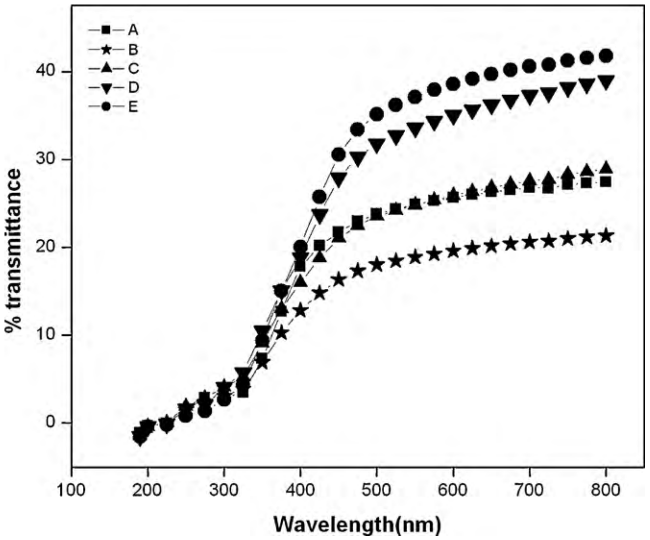

Fig. 1. Transmittance of CH films as affected by the addition of GG. A, B, C, D and E correspond to CH films with 0%, 15%, 25%, 40% and 50% (v/v) GG.

ultraviolet light transmission in 190–300 nm (Fig. 1), which can be beneficial for product preservation in some food applications as the most pronounced deleterious effects of light on food are caused by ultraviolet light (Bekbölet, 1990). These results suggest that CH–GG films can potentially retard lipid oxidation induced by UV light in food systems.

Films made from CH (100%) transmitted 27% of incident visible light. Use of GG in a ratio of 15% (v/v) in the blend decreased the light transmittance in visible region to 20% (Fig. 1). However, further increase in GG concentration led to increase in transmittance in visible region. Films with 25% (v/v) GG showed similar transmittance as CH films. Highest transmittance in visible region was found for CH films containing 50% (v/v) GG.

Addition of GG to CH films also led to changes in transparency and opacity of films (Table 1). It can be seen that, opacity and transparency are inversely correlated. CH films with 50% (v/v) GG films showed highest transparency followed by films with 40% and 25% (v/v) GG. CH films with 15% (v/v) GG were least transparent. The interaction with GG and water molecules modifies the refractive index of CH thus affecting the film transparency. The opacities of films also varied with GG concentration. CH films with 15% (v/v) GG were most opaque, followed by CH films without GG, 25%, 40% and 50% (v/v) GG (Table 2). These findings are important since film transparency or opacity are critical properties in various film applications, particularly if the film will be used as a surface food coating or for improving product appearance.

Color attributes are of prime importance because they directly influence consumer acceptability. The results of evaluation of color of the CH–GG films are shown in Table 2. It can be seen that there was no significant change in L^* value in CH Films with 0%, 15%, 25% (v/v) GG whereas b^* value increased significantly upon addition of GG. These findings suggest a net increase in yellow-brown color due to incorporation of GG up to 25% (v/v) level. But, further increase in concentration of GG in the film resulted significant decrease in L^* and b^* values. Negative values of a^* suggest the films had a green tint. The color index (ΔE) is influenced by L^*, a^* and b^* values and could describe how far apart two colors are in the color space. It was seen that ΔE values changed upon addition of GG due to alterations in L^*, a^* and b^* values of film. The ΔE indices observed in the present study were higher to that in CH-gelatin films (Rivero, García, & Pinotti, 2009) but were comparable to CH-hydroxy propyl methyl cellulose films (Rotta et al., 2009).

Table 2
Color parameters of CH–GG films.

Film sample[a]	L^*	a^*	b^*	ΔE
A	47.90 ± 0.77	−2.37 ± 1.15	4.91 ± 0.32	–
B	48.31 ± 0.58	−3.96 ± 2.03	7.01 ± 0.42	2.59 ± 0.37
C	49.18 ± 0.94	−4.72 ± 2.41	7.62 ± 0.38	3.17 ± 0.41
D	43.83 ± 0.51	−2.97 ± 1.55	6.86 ± 0.46	5.94 ± 0.41
E	43.83 ± 0.23	−3.62 ± 1.84	5.74 ± 0.12	5.33 ± 0.23

[a] A, B, C, D and E correspond to CH films with 0%, 15%, 25%, 40% and 50% (v/v) GG.

3.3. *Water vapor transmission rate and oxygen permeability of films*

The water vapor transmission rate of films plays an important role in deteriorative reactions of food; therefore, it is the most extensively studied property of films. Water vapor permeability is assumed to be independent of the water vapor pressure gradient applied across the films. However, hydrophilic materials, such as polysaccharide films, deviate from this ideal behavior due to interactions of permeating water molecules with polar groups in the film's structure (Hagenmaeir & Shaw, 1990). The WVTR values ranged from 4003 to 4141 g/m^2/day for the CH–GG films and did not change significantly with varying concentrations of GG (Table 3). Butler, Vergano, Testin, Bunn and Wiles (1996) reported that the chitosan films have relatively poor water vapor barrier characteristics, which result from their hydrophilicity. Glycerol, through its plasticizing action, changes the polymer network creating mobile regions with larger interchain distances, promoting water clustering by competing with water at active sites of the polymer matrix and the formation of micro cavities in the polymer network structure. Water sorption by biopolymers often results in swelling and conformational changes. The absorbed water plasticizes the film matrix, leading to a less dense structure where chain ends are more mobile, thus increasing transmission rate (Diab, Biliaderis, Gerasopoulos, & Sfakiotakis, 2001).

Oxygen is the key factor for oxidation, which is responsible for changes in food odor, color, and flavor and nutrients deterioration. Therefore, films that provide a proper oxygen barrier can help in improving food quality and extending food shelf life. Generally, hydrophilic biopolymer films show good oxygen barrier property. The oxygen permeability (OP) of films with different concentrations of CH and GG were compared (Table 3). Compared with GG, CH is more hydrophilic. Consequently, incorporation of GG to the polymer matrix at lower concentrations can reduce the oxygen bar-

Table 1
Transparency and opacity of CH–GG films.

Film sample[a]	Transparency	Opacity
A	46.96 ± 1.23	22.68 ± 0.62
B	43.06 ± 0.90	25.86 ± 1.09
C	47.06 ± 0.78	21.94 ± 0.85
D	51.47 ± 0.81	17.46 ± 0.49
E	53.87 ± 0.56	15.91 ± 0.52

[a] A, B, C, D and E correspond to CH films with 0%, 15%, 25%, 40% and 50% (v/v) GG.

Table 3
WVTR and OP of CH–GG films.

Film sample[a]	WVTR (g/m^2/day)	OP (ml/m^2/day)
A	4110 ± 2.62%	1846 ± 0.4%
B	4141 ± 1.41%	0
C	4071 ± 1.63%	1.15 ± 3.9%
D	4113 ± 1.97%	162 ± 3.0%
E	4003 ± 0.66%	481 ± 1.2%

[a] A, B, C, D and E correspond to CH films with 0%, 15%, 25%, 40% and 50% (v/v) GG.

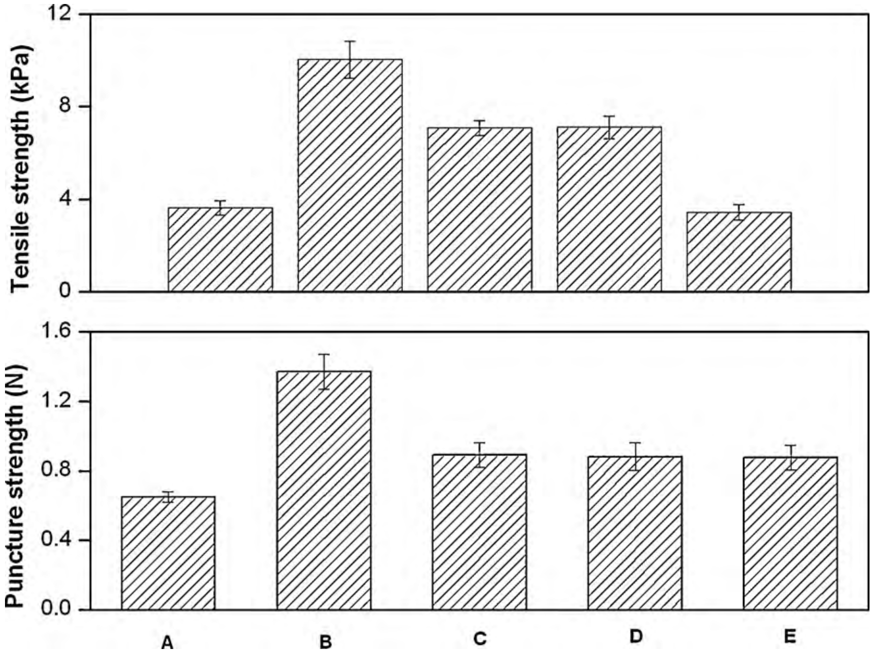

Fig. 2. Puncture strength (a) and tensile strength (b) of CH–GG films. A, B, C, D and E correspond to CH films with 0%, 15%, 25%, 40% and 50% (v/v) GG.

rier property of the films. When the volume ratio between CH and GG was 85:15, the blend film showed the lowest oxygen permeability. This result may be attributed to reducing compatibility and enhancing electrostatic repulsion between CH and GG. But further increase in GG ratio in blend led to increase in oxygen permeability of films. It is known that the addition of GG contributes to the decrease of oxygen permeability, while it is normally accepted that a higher concentration of GG increases oxygen permeability (Kester & Fennema, 1986; Caner, Vergano, & Wiles, 1998). CH films with 15% (v/v) GG films are more suitable for foods where oxidation is major problem. This result indicates the potential of CH–GG films to be used as a natural packaging to protect food from oxidation reactions. The films with low oxygen permeability would be suitable for confectionery products, baked foods, nuts and other foods that are susceptible to oxidation (McHugh, Huxsoll, & Krochta, 1996).

3.4. Mechanical properties

Adequate mechanical strength and extensibility are generally required for a packaging film to withstand external stress and maintain its integrity as well as barrier properties during applications in packaging. Blends of polysaccharides with different ratios of polymers can be useful to improve tensile properties. The tensile strength (TS) of the different films was measured and is shown in Fig. 2. It can be seen from Fig. 2 that the TS increased with the incorporation of GG in CH films. Comparing the TS values between films of different ratio of CH–GG, it can be seen that there was a two-fold increase in tensile strength of CH films with a concentration of 15% (v/v) GG. The high TS values of these films could be attributed to the formation of intermolecular hydrogen bonding between NH_3^+ of the CH backbone and OH^- of the GG. The amino groups (NH_2) of CH are protonated to NH_3^+ in the acetic acid solution, whereas the ordered structures of GG molecules are destroyed with the solubilization process, resulting in the OH^- groups being exposed to readily form hydrogen bonds with NH_3^+ of the CH. For pure CH films, TS values reported in literature were higher than those obtained in the present work, and this may be attributed to CH composition, presence of plasticizer and method of film preparation (Butler et al., 1996; Caner et al., 1998; Chen & Lin, 1994). However, further increase in concentration of GG (25–50%, v/v) in blend, the tensile strength of films decreased significantly. This could be attributed to thixotropic behavior of GG solution which resulted in reduction in viscosities of film forming solution containing GG (25–50%, v/v) in blend (results not shown). The puncture force of films showed similar trend as that of tensile strength. On addition of GG to CH films at concentration of 15% doubled the puncture force. But further addition of GG led to decrease in the force (Fig. 2). These results indicate CH films with 15% (v/v) GG had better mechanical properties as compared to other blends.

3.5. Antimicrobial activity

The development of complementary methods to inhibit the growth of pathogenic bacteria such as packaging material-associated antimicrobial agents is an active area of research. A number of studies on the antimicrobial characteristics of films made from chitosan have been carried out earlier (Chen et al., 1996; Coma, Martial-Gros, Garreau, Copinet, & Deschamps, 2002; Ouattara, Simard, Piette, Begin, & Holley, 2000). Antibacterial activity of CH–GG films against *E. coli* and *S. aureus* is shown in Fig. 3. It can be seen from the figures that CH films were effective against both the organisms. Although, the exact mechanism of the antimicrobial action of CH, and its derivatives is still imperfectly known, but different mechanisms for Gram-positive and Gram-negative bacteria have been proposed (Zheng & Zhu, 2003). One of the reasons for the antimicrobial character of CH is its positively charged amino group which interacts with negatively charged microbial cell membranes, leading to the leakage of proteinaceous and other intracellular constituents of the microorganisms (Shahidi, Arachchi, & Jeon, 1999). Addition of GG to CH film at 15% (v/v) led to further decrease in log cfu/ml of *E. coli*. But in case of *S. aureus*, no significant difference was observed in log cfu/ml reduction values of CH films and CH films containing 15% (v/v) GG. Further increase in concentration of GG in CH films led to decrease in the antibacterial activity, which could be attributed to the formation of intermolecular hydrogen bonding between NH_3^+ of the CH backbone and OH^- of the GG. Similar findings have been reported where antibacte-

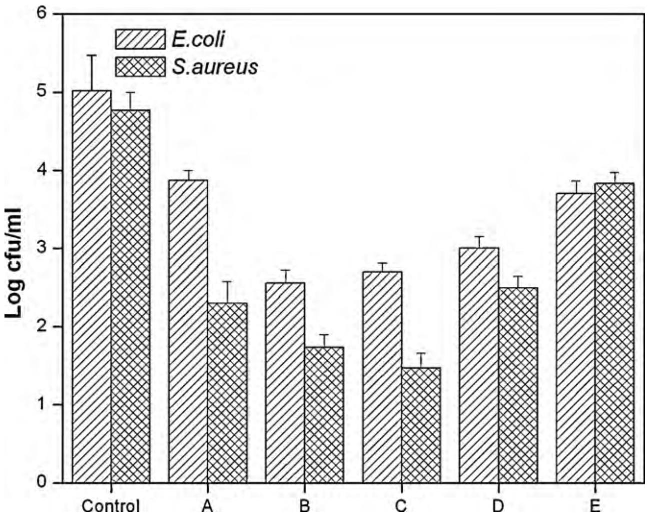

Fig. 3. Antibacterial activity of CH–GG films against *E. coli* and *S. aureus*. In control no films were added. A, B, C, D and E correspond to CH films with 0%, 15%, 25%, 40% and 50% (v/v) GG. Data represented in this figure is log cfu/ml after 24 h of growth. Original log cfu/ml (0 h) is same in all the treatments and is therefore not shown in the figure.

rial activity of chitosan is diminished by chitosan–tapioca starch and chitosan–potassium sorbate interactions, which was due to the reduced availability of chitosan NH_3^+ position to interact with cell membrane (Coma, Martial-Gros, Garreau, Copinet, & Deschamps, 2002; No, Meyers, Prinyawiwatkui, & Xu, 2007).

4. Conclusions

Chitosan and guargum can be used to make a composite film having desirable packaging characteristics. The concentration of the two polysaccharides in the film affected the optical, mechanical and antimicrobial properties of the films to various extents. The film prepared with the blend containing 85% CH and 15% GG (v/v) was found to be the best as it had lower oxygen permeability, better mechanical properties while retaining the similar antibacterial properties of chitosan, when compared with CH film not containing GG. Further studies would be required to determine the use of these films in commercial food systems.

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