Effects of Vanillin and Plasticizer on Properties of Chitosan-Methyl Cellulose Based Film

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ABSTRACT: Chitosan-methyl cellulose based films which incorporatate vanillin as an antimicrobial agent and polyethylene glycol 400 (PEG) as a plasticizer were developed in this study. The effects of vanillin and plasticizer concentration on mechanical, barrier, optical, and thermal properties of chitosan-methyl cellulose film were evaluated. When the vanillin concentration was increased at a given PEG level, film flexibility decreased while tensile strength increased slightly. Vanillin increased the barrier to oxygen but not water vapor. Increasing vanillin content resulted in less transparency and a more yellowish tint. The bulky nature of vanillin reduced film crystallization. When PEG concentration was increased at a given vanillin level, it resulted in greater film flexibility but reduced film strength. Water vapor permeability (WVP) and oxygen permeability (OP) increased with increase in PEG content. PEG contributed less to the opacity, yellowness, and crystallization of the film than did vanillin. © 2008 Wiley Periodicals, Inc. J Appl Polym Sci 109: 3540–3545, 2008

Key words: chitosan; vanillin; polyethylene glycol; biodegradable; mechanical and barrier properties

INTRODUCTION

Recent food-borne microbial outbreaks have stimulated interest in searching for innovative ways to inhibit microbial growth in foods. At the same time, consumers are demanding foods that do not contain synthetic chemical preservatives, so natural preservatives are receiving more attention. However, antimicrobials, directly incorporated into food may be rapidly lost because of evaporation into the atmosphere or thorough reaction with food components.[1,2] One option is to use packaging materials that have antimicrobial properties to help assure food safety and quality. Antimicrobials can be designed so as to be gradually released from the packaging material so as to remain on the food surface as long as possible. Research and development of antimicrobial materials for food applications such as packaging and other food contact surfaces is expected to grow in the next decade with the advent of new polymer materials and antimicrobials. The next generation of food packaging may include materials with antimicrobial properties.[3] These packaging technologies could help extend the shelf-life of foods while reducing the risk from pathogens. 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.

Some researchers have incorporated essential plant oils in biodegradable packaging as antimicrobial active agents, including anise, basil, coriander, oregano, and garlic oil.[4,5] Antimicrobial chitosan edible films incorporating garlic oil up to levels at least 100 μL/g were found to have antimicrobial activity against *S. aureus*, *L. monocytogenes*, and *B. cereus*. Films with 1 and 2% oregano essential oil decreased the number of *L. monocytogenes* by 3.6 to 4 logs and *Escherichia coli* 0157:H7 by 3 logs. Garlic oil components did not affect the physical and mechanical properties of chitosan films. Addition of oregano essential oil to chitosan films decreased water vapor permeability, puncture and tensile strength while increasing the elasticity of the film. However, they still faced a sensory problem when these films were used with food since essential oils have strong odors.

Vanillin (4-hydroxy-3-methoxybenzaldehyde) is the major flavor constituent of vanilla beans and a principle flavor compound used in numerous foods. Recent reports have shown that vanillin can be effective

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in inhibiting bacteria, yeasts, and molds.[6–9] Vanillin is hydrophobic and structurally similar to eugenol. Rupasinghe et al.[10] examined the antimicrobial effect of vanillin on four pathogenic or indicator organisms; *Escherichia coli*, *Pseudomonas aeruginosa*, *Enterobacter aerogenes*, and *Salmonella enterica* and four spoilage organisms: *Candida albicans*, *Lactobacillus casei*, *Penicillum expansum*, and *Saccharomyces cerevisiae*. They found that the minimum inhibitory concentration of vanillin was dependent on the microorganism (6–18 m*M*). Microbial growth was inversely proportional to vanillin concentration. Oral LD50 values for vanillin in animals were reported to be 3.0 g/kg for rabbits, 1.58–2.8 g/kg for rats, and 1.4 g/kg for guinea pigs, which indicates that these animals have low oral toxicity.[11] They also found that vanillin was found to be a moderate skin allergen in animals. Vanillin is not responsible for most cases of natural vanilla sensitivity.

Direct incorporation of vanillin as an antimicrobial agent into chitosan-methyl cellulose mixtures is an economical method for producing antimicrobial packaging. However, the interaction between vanillin and film may affect the film properties. Therefore, effects of vanillin and plasticizer content (PEG) in chitosan-methyl cellulose based film on mechanical (tensile strength and percent elongation), physicochemical (water vapor permeability and oxygen permeability), optical, and thermal properties were investigated.

MATERIALS AND METHODS

Film preparation

Chitosan with 90% deacetylation and purity of more than 99.75% (Bannawach Bio-line Co., Chonburi, Thailand) was prepared by dissolving 1.5 g of chitosan in 100 mL of a 1% acetic acid solution (MERCK, Darmstadt, Germany). One half gram of methyl cellulose (M-043, BENECEL®, Wilmington, DE) was dissolved in 50% ethanol (MERCK). Polyethylene glycol 400 (PEG) was used as a plasticizer according to Table I (low plasticized film) and (high plasticized film). Solutions of chitosan and methyl cellulose were mixed and heated to 72°C. Stearic acid was added (15% of cellulose derivatives) to improve the water barrier property. Vanillin (Vn), purchased from Sigma, St. Louis, was incorporated into the chitosan-methyl cellulose solution after the temperature of solution reached 83°C. The film-forming solution was then filtered to remove particles, degassed, poured onto glass plates, and dried at 40°C for 42 h. Dried films obtained were peeled off and conditioned at (25 ± 2)°C, (50 ± 5)% RH for at least 48 h before testing. Film thickness was measured using a gauge micrometer (GT-313-A, Gotech testing machines, Taichung City, Taiwan) with an accuracy of 0.01 mm. The reported thickness values are the average of at least 30 measurements.

TABLE I
Film Formulation

Film	No vanillin	Low vanillin	Medium vanillin	High vanillin
Low plasticized film with different vanillin concentrations				
%PEG[a]	0.17	0.17	0.17	0.17
%Vn[a]	0	0.15	0.30	0.45
High plasticized film with different vanillin concentrations				
%PEG[a]	0.50	0.50	0.50	0.50
%Vn[a]	0	0.15	0.30	0.45

[a] % in the table expressed % w/v of film forming solution.

Determination of mechanical properties

Tensile strength (TS) and percent elongation (%*E*) were tested by using the Instron Universal Testing Instrument (Model 5565, Instron, Canton, MA) following ASTM D638M (ASTM, 1993). Film specimens were cut into rectangular strips, 1 × 10 cm^2. Initial grip separation was 5 cm and cross-head speed was 25 mm/min. TS was calculated by dividing the peak load by the cross-sectional area (average thickness × 1 cm) of the initial specimen. %*E* was defined as the percentage of a change in the length (ΔL) of the specimen to the original length (L) between the grips (5 cm). Data of TS and %*E* were obtained from 8 replications of samples.

Determination of water vapor permeability and oxygen permeability

Water vapor permeability (WVP) was determined according to ASTM E96-00 (ASTM, 2000). Film specimens with approximately 8 cm diameter were mounted on the aluminum cups containing 10 mL of distilled water. Paraffin was used to fix a film specimen with the wide rim of aluminum cup. They were weighed and then placed in a desiccator containing saturated magnesium nitrate salt. The relative humidity of the chamber was kept at 53% and 23°C throughout the experiment. Weight loss of the aluminum cups covered with films was recorded daily for 5 days. The test was performed in triplicate. WVP was calculated by using the following equation.

$$\mathrm{WVP} = \left(\frac{W}{t}\right)\left(\frac{x}{A\Delta P}\right)$$

where

W/t = the slope of the plot between weight loss and time,

x = the average thickness of the films,

A = the permeation area, and

ΔP = the partial pressure difference of distilled water in the cup and atmosphere in desiccator.

Oxygen permeability (OP) was tested by using Gas Permeability Tester VAC-V1 (M and E Instruments, Jinan, China) according to ASTM D1434-82 (ASTM, 2003). Film specimen with 10 cm diameter was fixed between upper and lower chamber. Oxygen in both chambers was removed by vacuum for 8 h. After 8 h, oxygen was filled in the upper chamber. The amount of oxygen that permeated through the film in the lower chamber was determined. The test was done in duplicate at 23°C.

Determination of opacity and color

Opacity and color of film were measured by Hunterlab color meter (ColorQuest® XE, HunterLab, Reston, VA). Absolute measurements were displayed as tristimulus color values which closely represents human sensitivity. All data were obtained from 8 replications of samples.

Thermal properties

The determination of the thermal properties of films was accomplished by differential scanning calorimetry (TA Instruments, New Castle, DE), using a DSCQ100 (TA Instrument). Approximately 10 mg film (±0.001 mg) was weighed in a precision balance model xs205 (METTLER TOLEDO, Menlo Park, CA). Aluminum pan containing film was heated up at 10°C/min from −50 to 350°C in inert atmosphere (50 mL/min of N_2).

RESULTS AND DISCUSSION

The inhibition effect of two experimental films (chitosan-methyl cellulose film and chitosan-methyl cellulose film with high vanillin concentration) on *Escherichia coli* and *Saccharomyces cerevisiae* was tested by wrapping the films around inoculated fresh-cut cantaloupe and pineapple. Both films provided an inhibitory effect against *Escherichia coli* on fresh-cut cantaloupe. The chitosan-methyl cellulose film rapidly reduced the number of *Saccharomyces cerevisiae* yeast inoculated on cantaloupe and pineapple. Chitosan-methyl cellulose film with vanillin was more efficient than chitosan-methyl cellulose alone in reducing the number of yeast, which decreased by 4 logs in fresh-cut pineapple on day 6.[12]

Chitosan-methyl cellulose film was apparently more hydrophilic than films with vanillin when applied on fresh-cut fruit. After absorbing some water from fruit wedges, chitosan-methyl cellulose film swelled and no longer had a smooth surface. Therefore, chitosan-methyl cellulose film should only be used with low moisture products because it has poor wet strength. Film containing vanillin maintained its strength when wet much better than film without vanillin. It can be used as a wrap with high moisture foods such as fresh-cut fruits.[12]

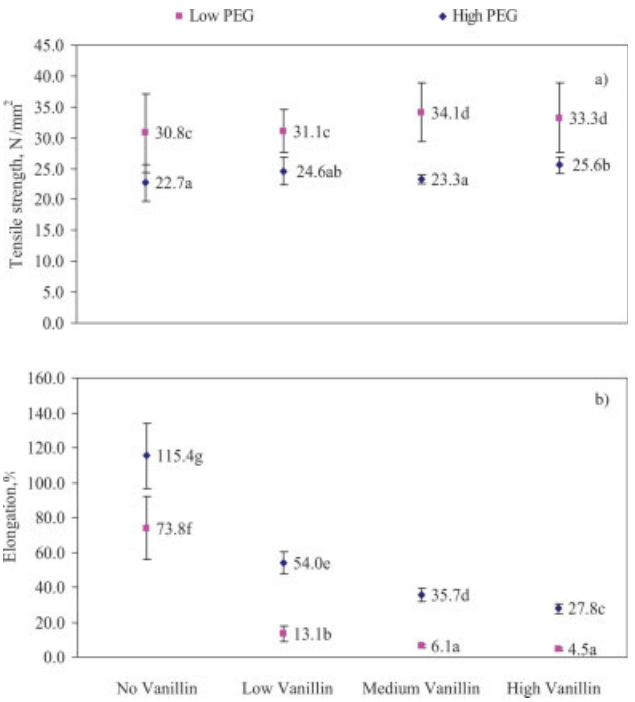

Figure 1 (a) Tensile strength and (b) percent elongation of chitosan-methyl cellulose based films containing different combination levels of vanillin and plasticizer (PEG). [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

Mechanical properties

Thickness of films containing low PEG (0.17%) and high PEG (0.5%) varied from 30 to 42 and 37–47 micron, respectively. At a given vanillin level, films with higher amount of PEG were thicker than those containing low PEG. At a given plasticizer (PEG) level, film thickness increased with increasing amount of vanillin in formula. Therefore, both PEG and vanillin contributed to film thickness.

The tensile strength of the chitosan-methyl cellulose film was affected by the concentration of vanillin, the antimicrobial agent, and PEG, the plasticizer [Fig. 1(a)]. The tensile strength of film containing low PEG was greater than those containing high PEG regardless of the vanillin content. The tensile strength of film with low PEG was not affected by low vanillin content, but increased when the vanillin concentration was increased to medium or high level. With film containing high level of PEG, addition of vanillin had an effect on the tensile strength, but was not consistent with the level of vanillin.

The percent elongation, indicator of flexibility, was affected by the various combinations of vanillin and PEG [Fig. 1(b)]. The percent elongation of film containing low PEG was less than those containing high PEG at a given vanillin content. The percent elongation of low PEG film decreased substantially when low vanillin content was added and decreased more with additional amount of vanillin in the film. Addition of vanillin to high PEG film also decreased the percent elongation and the decrease continued as the vanillin level increased. High vanillin film which had the very low %*E* cracked instantly when it was folded, thus these film required more PEG to remain flexible.

Incorporation of the antimicrobial vanillin into the film changed the functional characteristics of the packaging materials. Molecular structure of vanillin is composed of an aromatic benzene ring like styrene monomer. The bulky structure of vanillin made the film more rigid and contributed to the loss of its segmental mobility.[13] TS of all films were comparable with those of commercial HDPE films (20–37.2 N/mm^2).[14] The high TS values were attributed to the numerous hydrogen bonds between methyl cellulose chains. These bonds contribute to the cohesiveness and low flexibility of unplasticized films.[15] Our results were similar to that of Zivanovic et al.[5] who found that addition of oregano essential oil into the chitosan films decreased puncture and TS, but increased elasticity of the films. Pranoto et al.[4] found a greater reduction of TS and increment of %*E* of chitosan film when incorporating potassium sorbate and nisin but TS and %*E* of chitosan film with garlic oil did not alter because garlic oil components have no interaction with the functional groups of chitosan. However, Chen et al.[16] demonstrated that addition of 4% benzoate and sorbate into chitosan/methyl cellulose film resulted in higher TS and %*E*. The effect of plasticizer on mechanical properties of film agreed with Park et al.[17]

Water vapor permeability and oxygen permeability

The presence of vanillin was expected to increase the water vapor barrier property due to hydrophobicity of vanillin entities. However, vanillin did not significantly alter the water barrier property of films [Fig. 2(a)]. Plasticizer had dominant effect on water barrier property. Higher PEG content in film significantly allowed water to pass through. Butler et al.[18] reported that higher plasticizer concentration in chitosan film yielded higher water vapor permeability value. Pranoto et al.[4] incorporated garlic oil which was hydrophobic like vanillin into chitosan films. They found that garlic oil did not affect WVP of film as well. Even though the WVP of the films did not differ significantly, film with vanillin was superior

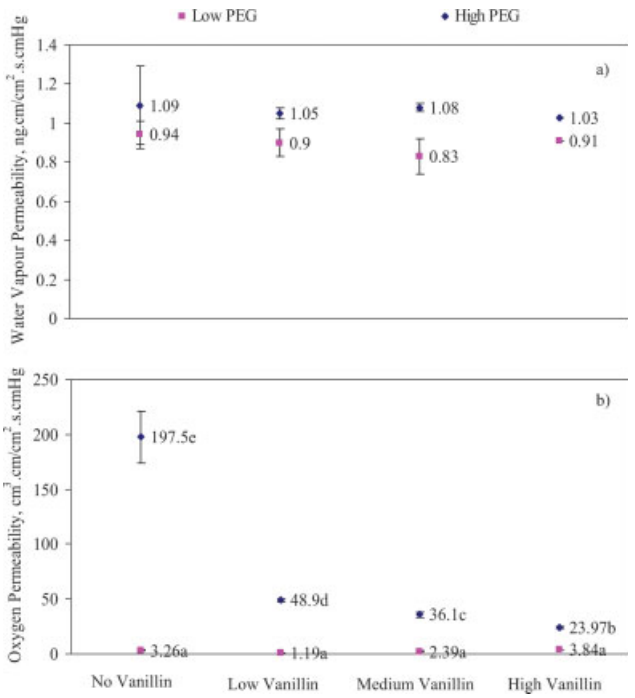

Figure 2 (a) Water vapor permeability and (b) oxygen permeability of chitosan-methyl cellulose based films containing different combination levels of vanillin and plasticizer (PEG). [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

in maintaining wet strength than film without vanillin. Chitosan-methyl cellulose film without vanillin was easy to tear apart when wet.

In films containing low PEG, OP did not change with increase in vanillin concentration [Fig. 1(b)]. In contrast, OP was reduced significantly with addition of vanillin to high PEG film and the decrease was significant with each incremental increase in vanillin. The molecular structure of vanillin contains a hydroxyl group which might increase polarity and hydrogen bond formation of film. Park et al.[17] reported that OP and WVP values of methyl cellulose containing PEG generally were not affected by plasticizer concentration.

At a given vanillin content, OP of chitosan-methyl cellulose film increased as concentration of PEG was increased. Park and Chinnan[19] also reported that OP and WVP increased as the concentration of plasticizer increased. Permeability (P) is equal to the product of the diffusion coefficient (D), which represents the mobility of permeant molecules in the polymer, and the solubility coefficient (S), which represents the permeant concentration in the film in balance with the external pressure: $P = DS$. Increased permeability could thus be related to an increase in the diffusion coefficient, due to structural changes in the polymer matrix, and also to an increase in the oxygen solubility in the film because of the increased

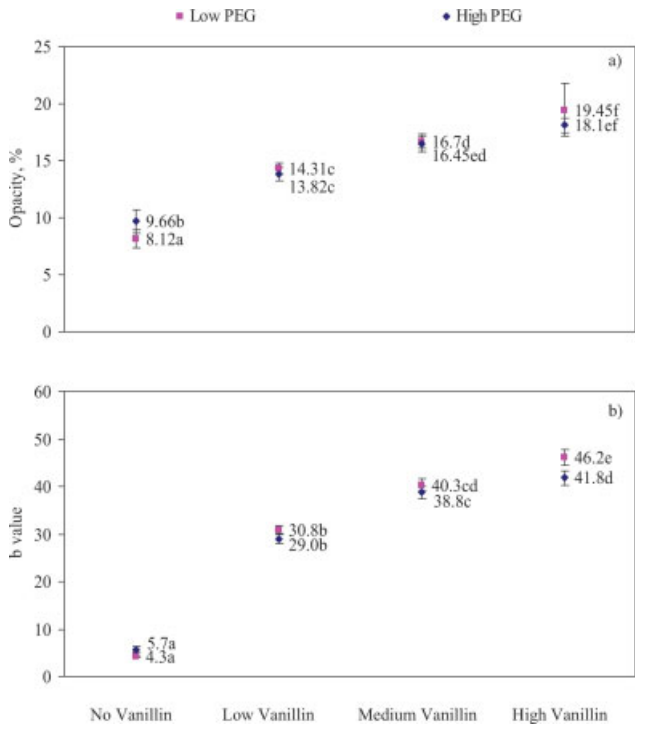

Figure 3 (a) Opacity and (b) *b* value of chitosan-methyl cellulose based films containing different combination levels of vanillin and plasticizer (PEG). [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

water content in the high PEG film (oxygen solubility in water = 1.25 m*M* at 25°C and 1 atm). Water molecules in the polymer interact with hydrophilic groups and thus may disrupt hydrogen bonding, creating additional sites for the dissolution of oxygen and increase mobility of oxygen molecules within the polymer.[20]

Chitosan-methyl celluose film provided an excellent oxygen barrier compared with commercial plastics. All films in this study had better oxygen barrier compared with PETP, PA, PCTFE, OPP, HDPE, PS, PC, and LDPE which is commonly used as food packaging.

Opacity and color

Opacity and *b* value of films are shown in Figure 3. Opacity is a reflection of the transparency of films while the *b* value represents the yellowish color of the films. The increase in the *b* value indicates that the color of the film became more yellow. Without vanillin in the formula, high PEG film was more opaque and yellow than low PEG film. Migration of PEG to film surface was observed in high PEG film with no vanillin which caused the greasy appearance. The opacity and yellowness of film increased significantly with increasing percent of vanillin in the film-forming solution. At a given level of vanillin, both films containing high- and low-level of PEG had almost the same color and opacity. Therefore, PEG had less effect on film color and opacity than vanillin. Zivanovic et al.[5] found that addition of oregano essential oil into the chitosan films resulted in opaque film.

Thermal properties

DSC thermograms of highly plasticized films with different vanillin concentrations are shown in Figure 4. A temperature scan from −50 to 350°C at 10°C/min with no preheat treatment was used to observe the thermal properties of the film, including the miscibility of vanillin in chitosan-methyl cellulose film and relative crystallinity of film. Chitosan-methyl cellulose film had an endothermic peak around 100°C which is associated with the loss of absorbed water from the film matrix. Thermograms of chitosan-methyl cellulose film containing high vanillin concentration observed in this study had only one peak, which indicated well miscibility of film component. Thermogram area expressed the heat of fusion of film. At a given PEG level, thermogram area decreased with increase in vanillin concentration [Fig. 5(a)]. The reduction of area should be the effect of lower crystallization due to vanillin has an aromatic benzene structure. As the result, the bulky benzene structure interrupted the rearrangement of polymer chain.

At a given level of vanillin, both high- and low-PEG film had insignificant different heat of fusion.

Effects of vanillin and plasticizer on melting temperature were not obvious [Fig. 5(b)]. Nothing was observed in the second scan at the cooling rate of 2°C/min. This cooling rate should be too fast to allow the rearrangement of polymer chains.

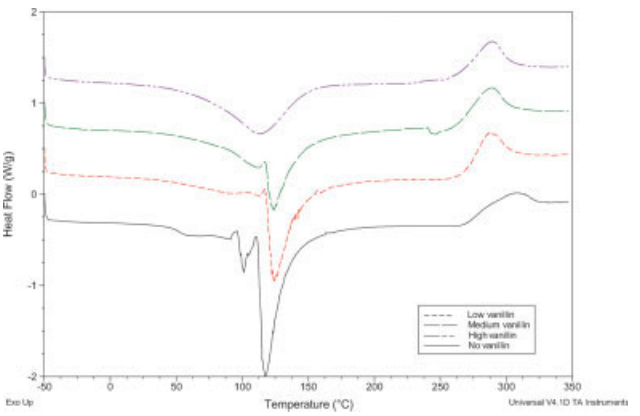

Figure 4 DSC thermograms of high plasticized chitosan-methyl cellulose films containing different levels of vanillin. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

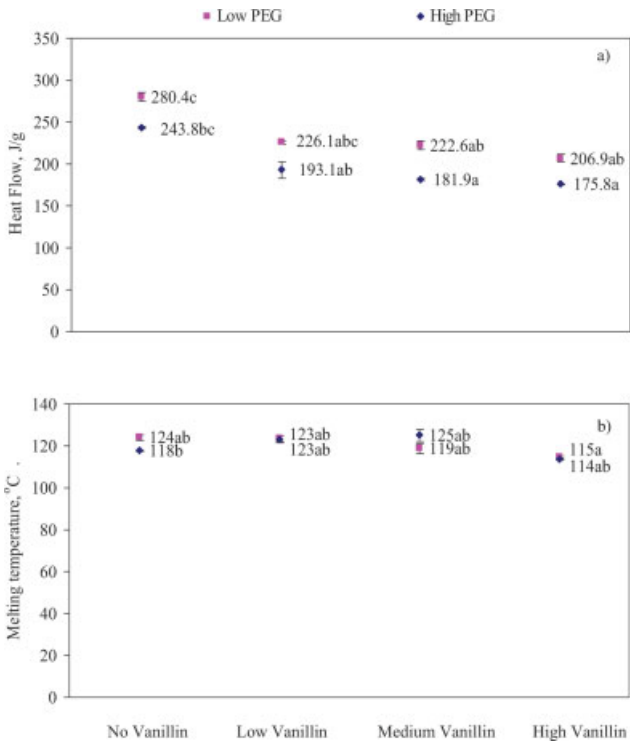

Figure 5 (a) Heat flow and (b) melting temperature of chitosan-methyl cellulose based films containing different combination levels of vanillin and plasticizer (PEG). [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

CONCLUSIONS

The concentration of vanillin and PEG affected mechanical, barrier, optical, and thermal properties of chitosan-methyl cellulose based film. Vanillin reduced and PEG increased film flexibility. Addition of vanillin content in film slightly improved film strength while PEG decreased film strength. Vanillin did not improve water barrier of film but improve oxygen barrier. Higher plasticized film provided the negative effect on both water and oxygen barrier. Vanillin affected film opacity and yellowness more than PEG. Both vanillin and PEG reduced crystalline formation of film.

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