

Physicochemical Properties of Edible and Preservative Films from Chitosan/Cassava Starch/Gelatin Blend Plasticized with Glycerol

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Summary

Edible films from chitosan, cassava starch, and gelatin plasticized with glycerol have been developed by casting method, and the effects of cassava starch (50, 100 and 150 g per 100 g of chitosan), gelatin (0, 25 and 50 g per 100 g of chitosan) and glycerol (21, 42 and 63 g per 100 g of chitosan) from the film solution on various properties of chitosan-based films have been studied using response surface methodology (RSM). The possible interactions between the major components were evaluated by X-ray diffraction (XRD) and Fourier transform infrared spectroscopy (FTIR). The properties of the resulting chitosan-based blends for films were greatly influenced by the incorporation of cassava starch, gelatin and glycerol. The introduction of gelatin and gelatinized cassava starch suppressed the semi-crystalline peaks of chitosan films. The amino peak of gelatin shifted from 1542 to 1559 cm^{-1} , and the NH and/or OH peak of gelatin, cassava starch and chitosan films shifted from 3384, 3414 and 3421, respectively, to 3422 cm^{-1} in the composite film. These results indicate that there was an interaction and molecular miscibility among the major components. The growth inhibition of phytopathogen on mango fruit surface indicated the efficiency of these coatings and they can be applied for the conservation of fresh or minimally processed fruits and vegetables.

Key words: chitosan, cassava starch, gelatin, glycerol, blended film, mango fruit

Introduction

The development of edible films or coatings based on biopolymers has attracted attention mainly due to their friendliness to the environment and their potential substitution for some petrochemicals in the food packaging industry. Edible films and coatings generally formed from renewable natural biopolymers, such as polysaccharides, proteins, lipids or the combination of these components, have been widely used as lipid, water vapour,

gas and flavour barrier for fresh fruits and vegetables, confectioneries, frozen foods and meat products (1–5).

Chitosan is a seafood by-product obtained by deacetylation of chitin (6). It is a cationic polysaccharide with high molecular mass, excellent film-forming ability and antimicrobial activity. Systematic investigations have been made of the effects of factors such as acid types and concentrations, molecular mass of chitosan, and the degree of deacetylation of chitosan on the mechanical properties and barrier characteristics of chitosan films (7–9). The findings show that chitosan films are brittle and not

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suitable for use in the dry state. These properties of chitosan films are ameliorated by incorporating other polysaccharides and hydrophobic materials such as pectin, cellulose, methylcellulose, starch and fatty acids (10–13).

Cassava starch, an important and abundant agricultural commodity, has been used to produce food packaging films which are environmentally safer. These products have excellent properties due to their transparency, grease and oil resistance and heat sealability (14). However, the application of pure cassava starch films is still limited because of its brittleness and solubility in both hot and cold water (15).

Proteins are widely used to form edible films (16–18). Protein-based edible films show satisfactory gas barrier or mechanical properties (5). Guilbert *et al.* (19), Park (20) and Cuq *et al.* (21) indicated that proteins (*e.g.* zein, wheat gluten, albumin, keratin and gelatin) are better gas barriers than polysaccharides (*e.g.* pectin, methylcellulose, hydroxypropylcellulose and starch) due to their unique structure and high intermolecular binding potentiality.

The association among the polymers can be achieved through blending, laminating or coating with other polymers with desirable properties. Blending is an easier and more effective way to prepare compatible multiphase polymeric materials. Thus, the objective of this study is to investigate the influence of cassava starch, gelatin and glycerol on mechanical and barrier properties, fungistatic activity of chitosan-based films or coatings, as well as to evaluate their compatibility.

Materials and Methods

Materials

Chitosan (molecular mass of 1100 kDa and 90 % deacetylation degree) was purchased from Yuhuan Ocean Biochemistry Co. Ltd. (Zhejiang, China). Cassava starch (96 % starch, amylose content 16.8 %) was supplied by Qiongzong Starch Factory, Hainan, China. Gelatin and glycerol were supplied by China Oleochemicals Company. Mango fruit (cv. Kent) samples were picked at ripe stages from a local orchard in Ledong (Hainan, China). Then they were transported in open boxes to South China University of Tropical Agriculture. Fruits were selected for their uniformity of shape, size and colour, and the physically damaged or diseased ones were removed.

Edible chitosan/cassava starch/gelatin blend for film formation

Chitosan solutions of 2 % (by mass per volume) were prepared by dissolving 1.2 g of chitosan in 50 mL of acetic acid solution (1 %, by mass per volume). The pH of these solutions was adjusted to 5.0 and then the solutions were made up to 60 mL. Cassava starch solutions were prepared by dispersing different quantities of cassava starch (50, 100 and 150 g per 100 g of chitosan) in 60 mL of distilled water at 80 °C. They were then stirred at constant heating rate (4 °C/min) until gelatinization. Gelatin solutions were prepared by dissolving different quantities (0, 25 and 50 g per 100 g of chitosan) of gelatin in 10 mL of distilled water at 60 °C. Then, 2 %

chitosan solutions were mixed in a beaker with cassava starch and gelatin at different concentrations at (80±1) °C, as shown in Table 1. The mixed solutions were kept at (80±1) °C for 10 min in a controlled temperature water bath after the addition of glycerol. Chitosan mixtures were filtered through 2 layers of cheesecloth to remove the undissolved impurities and cast onto plexiglas plates (approx. 20×20 cm) after degassing under vacuum. Film thickness was controlled by measuring the volume of film-forming solution. The cast films were dried at (60±1) °C for 16–20 h. They were peeled off and stored in desiccator at 25 °C and 50 % relative humidity (RH) for 48 h after evaporation of the solvent.

Table 1. Mass of chitosan/cassava starch/gelatin blends in g per 100 g of chitosan

Blends	Composition		
	Cassava starch	Gelatin	Glycerol
1	50	0	42
2	50	50	42
3	150	0	42
4	150	50	42
5	100	0	21
6	100	0	63
7	100	50	21
8	100	50	63
9	50	25	21
10	150	25	21
11	50	25	63
12	150	25	63
13	100	25	42
14	100	25	42
15	100	25	42

Tests of mechanical properties

Mechanical properties (tensile strength, TS, and percentage of elongation at break, E) were measured according to ASTM D822 Standard test (ASTM) (22) with an RG 3010 Material Tester. Five film specimens (100×25 mm) of each formulation were clamped between tensile grips. Tensile strength (TS) and elongation (E) were recorded during extension at 50 mm/min, with an initial distance between the grips of 50 mm.

Water vapour permeability (WVP) measurement

ASTM E96–80 Standard test method (23) was applied with some modifications (16) to measure WVP. The film sample was mounted firmly on a circular glass cup (3.5×4 cm) containing 10 g of distilled water. Then, the glass cup was placed in an environmental chamber set at 25 °C and 20 % RH. The chamber was equipped with a fan to eliminate stagnant air above the test cup (24). The cup was weighed periodically on a Mettler analytical balance to evaluate stationary-state water vapour transfer. The water vapour permeability rate (WVPR, g H₂O/(s·m²)) and the WVP (g H₂O/(m·s·Pa)) were calculated.

culated according to the method of Kaya and Kaya (3) using the following equations:

$$WVPR = \frac{k}{A} \quad /1/$$

and

$$WVP = \frac{WVPR \times d}{p_1 - p_2} \quad /2/$$

where k is the constant of linear regression from the values of mass loss against time during a constant period, A is the permeability area, d is the average thickness of the film and $p_1 - p_2$ is real vapour partial pressure difference (Pa) across the film. The real vapour partial pressure at the film inner surface (p_1) was corrected for the stagnant air gap inside the test cup according to the method of Gennadios *et al.* (25). After this correction, the difference in relative humidity was approx. 20–86 % instead of theoretical 20–100 % (24).

Measurement of O₂ and CO₂ permeability

Gas (O₂ and CO₂) permeability at 25 °C was measured in a designed stainless cell using a gas testing instrument, model CYES-2 (Shenyang, China) following the method described by García *et al.* (26). The gas permeability (GP) was calculated according to the following equation:

$$GP = \frac{d}{A} \times \frac{\Delta V}{\Delta t} \times \frac{1}{\Delta P} \quad /3/$$

where ΔP is gas pressure difference between the two sides of the film, and $\Delta V/\Delta t$ is the constant rate of the gas diffusing through the film.

Film thickness measurement

Thickness of the films was measured using a hand-held micrometre (Mitutoyo, Japan) at at least 10 random positions on the film.

X-ray diffraction

X-ray patterns of chitosan, cassava starch, gelatin, and their composite films were analyzed using an X-ray diffractometer (Rigaku D/Max-III A, Tokyo, Japan) with Cu K- α radiation at a voltage of 30 kV and 20 mA. The samples were scanned between $2\theta=3-60^\circ$ with a scanning speed of $2^\circ/\text{min}$. Prior to testing, all the film samples were stored in a desiccator.

Fourier transform infrared spectroscopy (FTIR)

FTIR spectra of the films were recorded using an attenuated total reflection (ATR) method in IR spectrometer (Spectrum One, PerkinElmer, USA). The thin films were applied directly onto the ZeSe ATR cell.

In situ evaluation of the fungistatic activity of chitosan/cassava starch/gelatin blend solution

Botryodiplodia theobromae Pat. isolated from infected mango fruits was maintained on Petri dishes containing potato dextrose agar (PDA). Conidial suspension at a concentration of $2 \cdot 10^5$ conidia per mL was prepared in sterile water containing 0.1 % (by volume) Tween 80 to

maintain uniform conidia distribution. The conidial suspension was mixed with sterile water and 2 % (by mass per volume) of chitosan (pH=5.0) or chitosan/cassava starch/gelatin blend solution (13 different blends) with a final conidial concentration of 10^5 per mL. The selected mango fruits were dipped in the above solutions for 1 min, dried with a fan and then stored at room temperature for disease assay. Fruits dipped in the sterile water or chitosan solution containing conidial concentration of 10^5 per mL were used as control. The mango fruits were evaluated every other day for disease symptoms by observing visible *Botryodiplodia theobromae* Pat. growth on each fruit surface. Spoiled fruits were discarded to avoid secondary infection. More than 30 fruits were used in each treatment. The reported value is the mean of three replications.

Statistical analysis

Response surface methodology (RSM) is a collection of statistical and mathematical techniques useful for designing experiments, building models through regression and evaluating the effects of multiple parameters and their interactions for responses (27). The analysis of variance (ANOVA) and response surface regression procedure of SAS statistical software (v. 8.02) were used to analyze the average data from triplicate experiments at a confidence interval of 95 %. Experimental data were fitted to the following second-order polynomial equation to all dependent Y variables (tensile strength, elongation, WVP, oxygen permeability, and carbon dioxide permeability):

$$Y = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \beta_3 x_3 + \beta_{11} x_1^2 + \beta_{22} x_2^2 + \beta_{33} x_3^2 + \beta_{12} x_1 x_2 + \beta_{13} x_1 x_3 + \beta_{23} x_2 x_3 \quad /4/$$

where β_0 is offset term, β_1 , β_2 and β_3 are the regression coefficients for linear effect terms, β_{11} , β_{22} and β_{33} are quadratic effects and β_{12} , β_{13} and β_{23} are interaction effects. In this model, x_1 , x_2 and x_3 are the independent variables, namely cassava starch, gelatin and glycerol (Table 2).

Results and Discussion

Transparent, homogeneous, thin and flexible films were obtained from chitosan blended with cassava starch, gelatin and glycerol. Visually, all the blended films had a slightly yellow appearance. As chitosan and gelatin concentration of the film-forming solution increased, the colour of the films became more intensively yellow. The average thickness of chitosan-based films was (0.100 ± 0.017) mm.

Mechanical properties of the films

The tensile strength and elongation of chitosan-based films are shown in Table 3. ANOVA indicated that the addition of cassava starch, gelatin and glycerol caused significant differences in both tensile strength and elongation of chitosan-based films ($p < 0.05$). The effect of cassava starch and gelatin on the tensile strength is shown in Fig. 1, where it can be seen that when the cassava starch mass fraction ranges from 50–100 g per 100 g of chitosan, tensile strength decreases as cassava starch in the film solutions increases. However, the ten-

Table 2. Regression coefficients and significant regression models for tensile strength, elongation, water vapour permeability, oxygen permeability and carbon dioxide permeability

Coefficient	Tensile strength	Elongation	WVP	O ₂ permeability	CO ₂ permeability
β_0	33.37	100.40	7.43	0.87	0.40
Linear					
β_1 (cassava starch)	2.98*	-19.00*	0.79*	0.42*	0.21*
β_2 (gelatin)	-5.89*	-8.46*	0.15	-0.24*	-0.14*
β_3 (glycerol)	-6.94*	35.09*	-0.76*	0.03	0.04
Quadratic					
β_{11} (cassava starch×cassava starch)	-0.63	-15.66*	1.52*	0.70*	0.31*
β_{22} (gelatin×gelatin)	0.20	-20.64*	-0.19	0.06	0.04
β_{33} (glycerol×glycerol)	-4.14	-25.64*	-1.03	-0.01	0.01
Crossproduct					
β_{12} (cassava starch×gelatin)	10.71*	3.65	-0.12	-0.03	-0.03
β_{13} (cassava starch×glycerol)	1.57	6.75	0.26	-0.03	0.02
β_{23} (gelatin×glycerol)	-0.37	-11.98*	-0.36	0.02	0
R-square	0.97	0.99	0.88	0.90	0.98
CV	7.70	9.99	9.54	22.50	10.71

CV=coefficient of variance
*Significant at 5 %

Table 3. Responses of tensile strength, elongation, water vapour permeability, oxygen permeability and carbon dioxide permeability

Blends	Tensile strength	Elongation	WVP	O ₂ permeability	CO ₂ permeability
	MPa	%	10 ⁻¹⁰ g/(m·s·Pa)	10 ⁻⁸ cm ³ /(m·s·Pa)	10 ⁻⁸ cm ³ /(m·s·Pa)
1	49.40±1.95	100.40±16.90	9.22±0.31	1.42±0.09	0.67±0.03
2	13.63±1.49	70.60±2.30	9.06±0.31	0.75±0.05	0.36±0.03
3	33.36±1.64	50.30±3.40	10.12±0.36	2.58±0.03	1.17±0.04
4	40.41±1.67	35.10±2.60	9.47±0.34	1.78±0.06	0.75±0.03
5	39.23±1.70	8.90±1.00	7.03±0.20	0.89±0.04	0.50±0.01
6	28.83±0.53	110.70±6.60	5.78±0.11	1.17±0.04	0.58±0.05
7	30.76±2.28	21.50±1.90	8.75±0.22	0.64±0.05	0.31±0.01
8	18.90±0.89	75.40±2.90	6.08±0.14	1.00±0.04	0.39±0.02
9	36.46±0.95	51.20±3.20	8.17±0.36	1.34±0.07	0.50±0.02
10	39.89±3.24	4.51±0.70	10.17±0.45	1.97±0.04	0.86±0.04
11	16.71±0.66	100.20±2.40	6.58±0.22	1.22±0.09	0.53±0.02
12	26.40±3.01	80.50±2.60	9.61±0.25	1.72±0.07	0.97±0.03
13	33.36±1.63	100.30±8.60	8.06±0.15	0.86±0.06	0.39±0.05
14	33.89±0.95	100.80±4.00	8.17±0.22	0.83±0.04	0.44±0.03
15	32.85±1.18	100.10±4.90	8.17±0.20	0.92±0.03	0.36±0.03

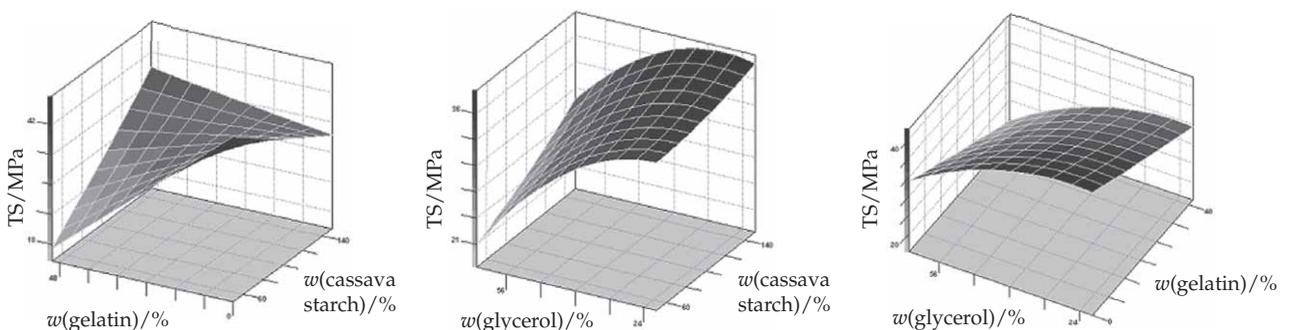


Fig. 1. Response surface plots showing the effect of cassava starch, gelatin and glycerol on tensile strength (TS)

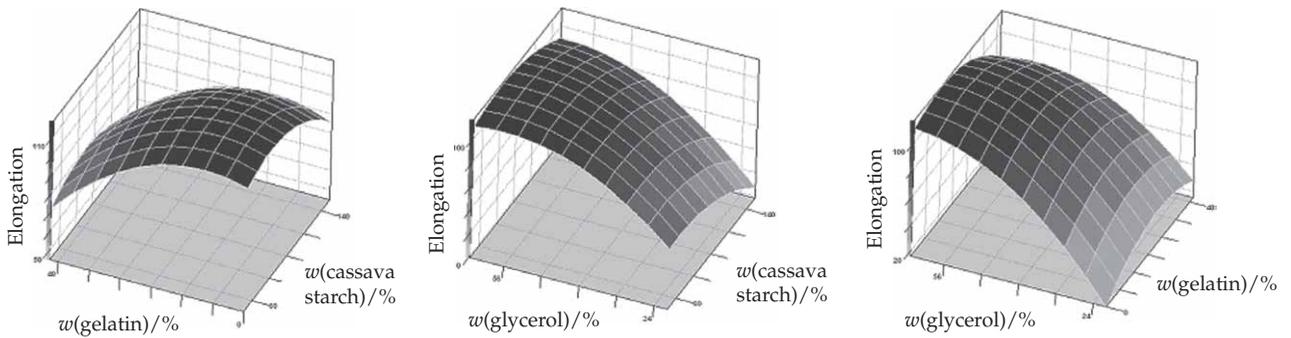


Fig. 2. Response surface plots showing the effect of cassava starch, gelatin and glycerol on elongation

sile strength then increases with further increase of the cassava starch mass fraction up to 100 g per 100 g of chitosan. The increasing tensile strength values of the blended films, with the cassava starch mass fraction increasing from 100 to 150 g per 100 g of chitosan, are attributable to the formation of intermolecular hydrogen bonds between NH_4^+ of the chitosan backbone and OH^- of the cassava starch (13). There was a negative correlation between the tensile strength and gelatin. The effect of glycerol on elongation is shown in Fig. 2. Glycerol, as a plasticizer, is added to the film to modify its mechanical properties, making the film more flexible, since the plasticizer reduces the intermolecular bonds between the polymer chains. The elongation increased with increasing glycerol and reached a maximum value at a level of 63 g per 100 g of chitosan. Therefore, 63 g of glycerol per 100 g of chitosan plasticized the films. Similar behaviour was observed in other hydrophilic films composed of polysaccharides and proteins (28,29).

Water vapour permeability

Since one of the main functions of edible films is at least to decrease moisture transfer between the food and the surrounding atmosphere, water vapour permeability should be as low as possible (30).

Table 3 shows WVP values of chitosan-based films. The ANOVA indicated that only cassava starch and glycerol have a significant effect on WVP at a confidence interval of 95 %. As can be observed in Fig. 3, the addition of cassava starch from 50 to 100 g per 100 g of chitosan decreased WVP in blended films. However, when cassava starch exceeded 100 g per 100 g of chitosan, the WVP increased with the increase of cassava starch and reached the maximum with cassava starch at 150 g per 100 g of chitosan. This phenomenon could be related to the significant hydrogen bonding interaction with water, favouring water vapour transmission through the films (30). The WVP increased initially and then decreased with the increase of glycerol. The blended films produced with 63 g of glycerol per 100 g of chitosan exhibited lower WVP. Similar results were reported regarding the effect of glycerol on WVP of starch films (31). This behaviour could be related to structural modifications of chitosan-cassava starch-gelatin network that occurs when 63 g of glycerol per 100 g of chitosan are added. Initially, chitosan-based films formulated with lower mass fraction of glycerol form a loose structure, which facilitates the water vapour permeation, then, when glycerol reached

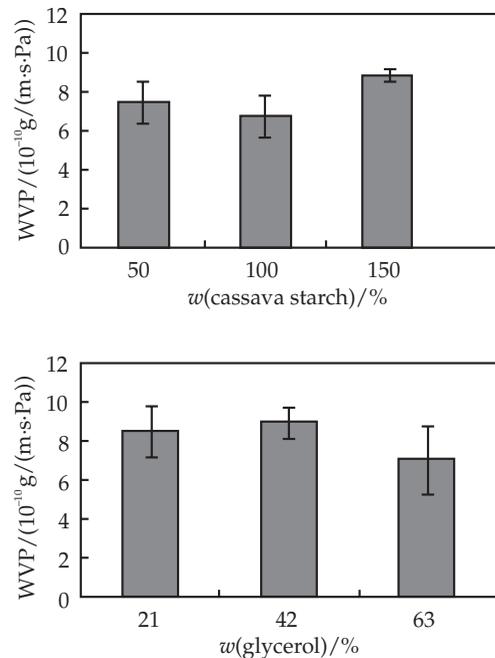


Fig. 3. Water vapour permeability of chitosan-based films as a function of cassava starch and glycerol

63 g of glycerol per 100 g of chitosan, the formation of loose structure was avoided, a more compact structure was formed, and this probably resulted in lower WVP values.

Oxygen and carbon dioxide permeability

ANOVA results indicated that there was a significant effect of cassava starch and gelatin on oxygen and carbon dioxide permeability ($p < 0.05$). As can be observed in Fig. 4, there was an improvement of gas barrier properties of the blended films with cassava starch below 100 g per 100 g of chitosan. When the quantity of cassava starch was increased above 100 g per 100 g of chitosan, the blended films with higher mass fraction of cassava starch had a higher gas (O_2 and CO_2) permeability values compared to those with lower mass fraction of cassava starch. Gelatin improved the barrier properties of the blended films. The permeability of the blended films to oxygen and carbon dioxide decreased with the increase of gelatin. Gas permeability strongly depends on the interaction between the polymer matrix

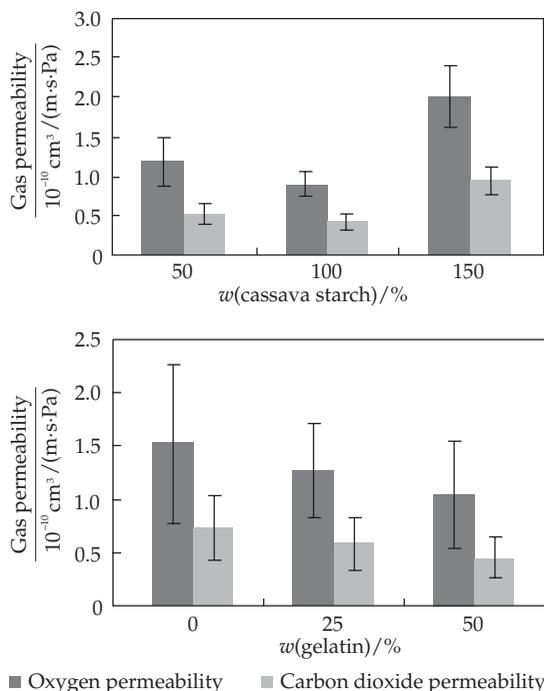


Fig. 4. Oxygen and carbon dioxide permeability of chitosan-based films as a function of cassava starch and gelatin

and the permeating gas (26). The improvement of gas barrier properties of the blended films increased with the increase of cassava starch and gelatin due to the formation of intermolecular hydrogen bonds between NH_4^+ of chitosan and gelatin backbone and OH^- of cassava starch. The increased molecular interaction resulted in a film with compact structure and low permeability (32). Gas permeability increased with the increase of cassava starch from 100 to 150 g per 100 g of chitosan because cassava starch intramolecular hydrogen bonds rather than intermolecular hydrogen bonds are formed, resulting in a phase separation among the main components where the film could not be formed well, facilitating the gas permeation (13). Table 3 shows that CO_2 permeabilities were much lower than those of O_2 , indicating a selective action of these blended films on gas permeability. These effects could be attributed to a lower diffusion and solubility of CO_2 in the blended films.

X-ray diffraction

X-ray diffractograms of chitosan/cassava starch/gelatin composite films are shown in Fig. 5. As observed, the chitosan-based film was in a semicrystalline state with four main diffraction peaks ($2\theta=8.2, 11.28, 15.6$ and 21.98°) present in its X-ray diffraction pattern, whereas an amorphous state was observed in the cassava starch and gelatin films. When incorporating 50 g of cassava starch per 100 g of chitosan, two chitosan peaks ($2\theta=11.2$ and 15.6°) were still observed in film blend 1, indicating that chitosan structure was influenced slightly by the addition of a small mass fraction of cassava starch. However, the semicrystalline peaks of chitosan were suppressed in film blend 5 when incorporating 100 g of cassava starch per 100 g of chitosan. In its place, a broad amorphous peak was observed, demonstrating an inter-

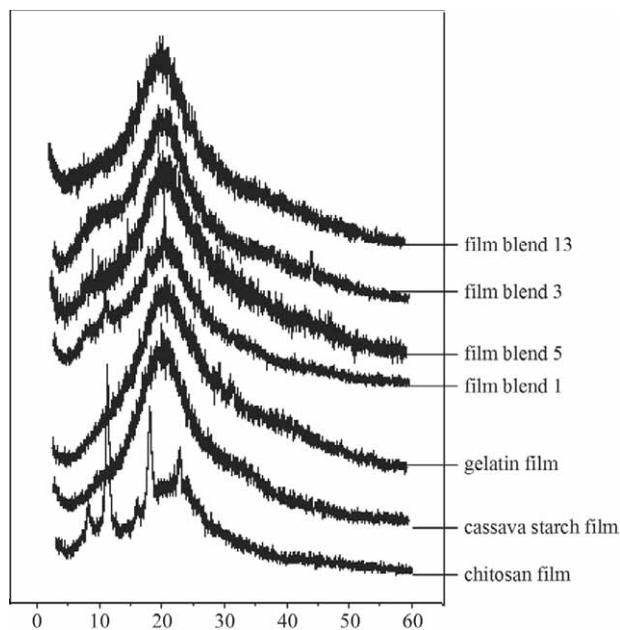


Fig. 5. X-ray patterns of chitosan, cassava starch, gelatin films and their blends

action between these two components. Interestingly, the intensities of the peak at around $2\theta=10^\circ$ were enhanced in film blend 3 when incorporating 150 g of cassava starch per 100 g of chitosan. This suggests that there was a slight phase separation between chitosan and cassava starch. In film blend 13, a new broad amorphous peak was present, indicating that there was a molecular miscibility and an interaction among these three components. This was consistent with the results of film blend 13, exhibiting lower water vapour permeability, good mechanical and improved gas barrier properties.

FTIR spectroscopy

FTIR spectroscopy was used to characterize the interactions between chitosan, cassava starch and gelatin. The infrared spectra of chitosan, cassava starch, gelatin, chitosan/cassava starch, and chitosan/cassava starch/gelatin composite films are shown in Fig. 6. The gelatin, cassava starch and chitosan spectra were similar to previous reports (13,33). In the spectrum, the broad band from $3384\text{--}3422 \text{ cm}^{-1}$ was the OH and/or NH stretching. The band at $2930\text{--}2939 \text{ cm}^{-1}$ was C–H stretching. The bands from $1630\text{--}1660$ and $1540\text{--}1570 \text{ cm}^{-1}$ were the C=O stretching (amide I) and NH bending (amide II), respectively. The peak near 1740 cm^{-1} suggested the presence of a carbonyl group in the starch and chitosan films.

The chemical interactions are reflected by changes in the peaks of characteristic spectra after physical blending of two or more substances. In the spectrum of chitosan/cassava starch/gelatin composite film, the amino peak of gelatin shifted from 1542 to 1559 cm^{-1} with the addition of gelatin, and the OH and/or NH of gelatin, cassava starch and chitosan shifted from $3384, 3414$ and 3421 to 3422 cm^{-1} , respectively. This result indicated that interactions were present between the hydroxyl groups of cassava starch and the amino groups of gelatin and chitosan.

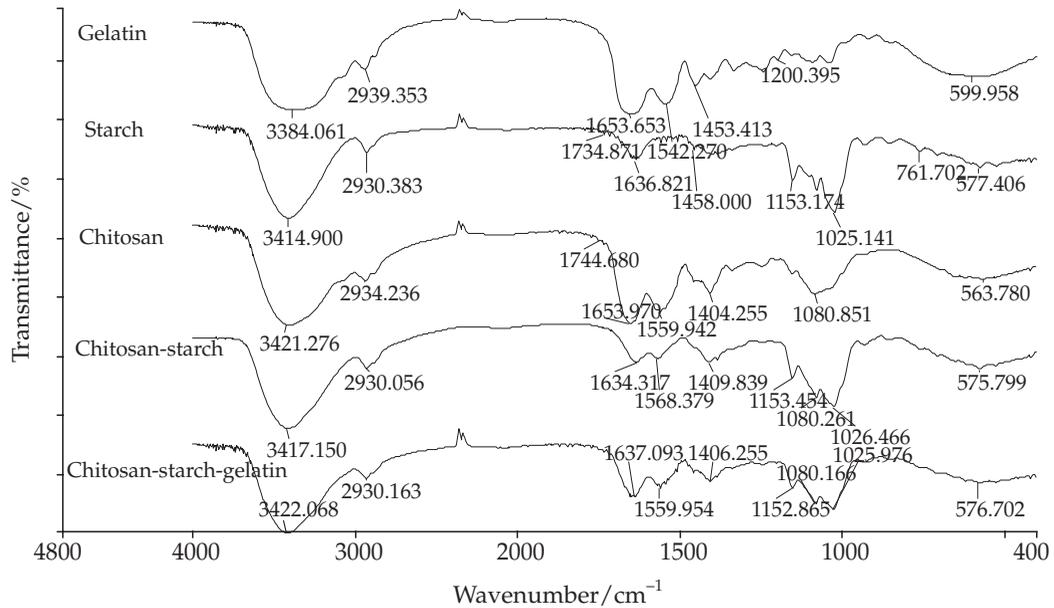


Fig. 6. FTIR spectra of gelatin, cassava starch, chitosan films and their blends

Fungistatic properties of blended coatings

From Table 4, it can be seen that the significantly lower stem-end rot incidence in the fruits treated with chitosan solution (or chitosan-based solution) was comparable with that of samples treated with sterile water after 2 days of storage. No significant difference was observed between the fruits treated with chitosan only and

Table 4. Stem-end rot incidence (*Botryodiplodia theobromae* Pat.) in mango fruits treated with sterile water, 2 % chitosan solution and a blended solution containing *B. theobromae* Pat. of conidial concentration of 10⁵ per millilitre

Storage time day	Disease incidence/%		
	Fruits treated with sterile water	Fruits treated with 2 % of chitosan solution	Fruits treated with blended solution
0	(0.0±0.0) ^a	(0.0±0.0) ^a	(0.0±0.0) ^a
2	(5.2±1.2) ^a	(1.8±0.9) ^b	(2.4±1.1) ^b
4	(27.8±3.6) ^a	(6.5±1.2) ^b	(9.6±1.6) ^b
6	(68.6±5.8) ^a	(27.6±4.3) ^b	(35.7±5.3) ^b
8	(89.4±4.3) ^a	(54.3±3.8) ^b	(62.2±3.4) ^b
10	(100.0±0.0) ^a	(76.2±5.7) ^b	(85.1±6.2) ^b

Means±standard errors for three replicates with different superscript within a row with the same storage time indicate significant difference (p<0.05)

chitosan-based solution (p>0.05). It was demonstrated that the incorporation of cassava starch and gelatin into chitosan solution did not significantly affect the fungicidal activities of the blended solution. Thus, chitosan/cassava starch/gelatin film blend showed fungistatic activities.

Conclusions

The effects and interactions of cassava starch, gelatin and glycerol on the properties of chitosan-based films were studied using RSM. Results showed that all these ingredients greatly influenced the properties of the resulting film blends. For the X-ray diffraction pattern, the semicrystalline structure of chitosan was depressed with the addition of the gelatinized cassava starch and a broad amorphous peak appeared. These results and the FTIR spectra indicated that interactions and molecular miscibility were present between the major components. The measurement of fungistatic activities indicated that incorporation of cassava starch and gelatin into chitosan solution did not significantly affect the antifungal activities of chitosan.

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