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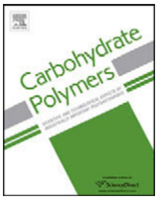

Properties of pullulan-based blend films as affected by alginate content and relative humidity

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ABSTRACT

Pullulan–sodium alginate blend films were prepared and characterized as a function of water activity (a_w). At low a_w, the incorporation of alginate into pullulan film increased the tensile strength and elastic modulus, but decreased the elongation at break of the composite films; the opposite trends were observed at elevated a_w. Above 0.43 a_w, water exerted a typical plasticization effect upon the biopolymer blends. As a_w increased from 0.23 to 0.43, an anti-plasticization effect was observed as tensile strength and elastic modulus increased. The glass transition temperature of all samples decreased substantially as aw increased from 0.23 to 0.84 due to the plasticization effect of water. Within this a_w range, one transition temperature was observed for all film specimens. The stretching vibration band of O–H was investigated using attenuated total reflection Fourier transform infrared spectroscopy to identify the various species of water interacting with the polysaccharide films.

1. Introduction

Pullulan is an extracellular and water-soluble microbial polysaccharide produced by *Aureobasidium pullulans* in starch and sugar cultures. The linear polymer mainly consists of maltotriose units interconnected to each other by α-(1,6) glycosidic bonds. This unique linkage pattern endows pullulan with distinctive physical properties to form film that is strong, transparent, and water soluble and with low permeability to oil and oxygen (Singh, Saini, & Kennedy, 2008; Yuen, 1974). Although pullulan polymer has many desirable characteristics, its use is currently limited due to high cost. One approach used to overcome this problem is blending pullulan with other abundant polysaccharides such as alginate. Alginate is a polysaccharide composed of variable proportions of β-D-mannuronic acid and α-L-guluronic acid linked by 1–4 glycosidic bonds (Hay, Rehman, Ghafoor, & Rehm, 2010). The incorporation of alginate into pullulan not only reduces the cost of pullulan films, but also potentially enhances the material properties of the blend films (Tong, Xiao, & Lim, 2008).

The performance of edible films depends on their composition and end-use condition. As hydrophilic polymers, pullulan and alginate have a tendency to absorb water from the surrounding environment or from contact with food materials during storage. Therefore, study of the moisture sorption behavior of pullulan-based films is important to characterize their material properties. Moisture sorption isotherms are typically modeled using empirical and semi-empirical models, such as Brunauer–Emmett–Teller (BET), Guggenheim–Anderson–deBoer (GAB), Henderson and Oswin equations (Schuchmann, Roy, & Peleg, 1990). In particular, the GAB equation has been successfully applied to predict water sorption isotherm of many biopolymer films such as konjac glucomannan, pure pullulan, pullulan–starch, cassava starch, and chitosan-polyvinyl alcohol blend films (Biliaderis, Lazaridou, & Arvanitoyannis, 1999; Cheng, Karim, & Seow, 2006; Diab, Biliaderis, Gerasopoul, & Sfakiotakis, 2001; Muller, Yamashita, & Laurindo, 2008; Srinivasa, Rameshb, Kumarc, & Tharanathan, 2003). Although the moisture sorption properties of many edible films have been reported, such information for pullulan–alginate blend films is currently not available.

In hydrophilic biopolymers, water can act as a plasticizer that disrupts hydrogen bonds between polymer chains. The resulting increase in chain mobility depresses glass transition temperature (T_g) and imparts material flexibility (Gontard & Ring, 1996; Lourdin, Coignard, Bizot, & Colonna, 1997; Seow, Cheah, & Chang,

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Abbreviations: ATR-FTIR, attenuated total reflection Fourier transform infrared spectroscopy; DSC, differential scanning calorimetry; GAB, Guggenheim–Anderson–deBoer; BET, Brunauer–Emmett–Teller; a_w, water activity; T_g, glass transition temperature; RH, relative humidity; TS, tensile strength; EAB, percentage elongation at break; EM, elastic modulus; M, water content; K, multilayer heat of sorption; C, monolayer heat of sorption; SEE, standard error of estimate; P, mean relative deviation modulus.

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1999; Stading, Rindlav-Westling, & Gatenholm, 2001). Physical and barrier properties of moisture-sensitive polymers are largely affected by water existing in different states (Herrera-Gomez, Velazquez-Cruz, & Martin-Polo, 2001). In general, the sorbed water can exist in three different states: (1) tightly bound monolayer water; (2) less tightly bound multilayer water; and (3) bulk water.

One technique which has been successfully applied for probing the interaction of water in different polymers is attenuated total reflection Fourier transform infrared spectroscopy (ATR-FTIR) (Kitano, Ichikawa, Ide, & Fukuda, 2001; Lasagabaster, Abad, Barral, & Ares, 2006; Sammon, Mura, Yarwood, Everall, Swart, & Hodge, 1998; Sutandar, Ahn, & Franses, 1994). With ATR-FTIR, it is possible to discern different types of O–H stretching vibration in 3700–3000 cm^{-1} range, including water molecules that are weakly H-bonded, moderately weak H-bonded, moderately strong H-bonded, and strongly H-bonded to the polymer (Sammon et al., 1998).

In the present study, the first objective was to investigate the effect of alginate content and water activity (a_w) on moisture sorption, mechanical and thermal properties of pullulan–alginate blend films. The second objective was to elucidate the different species of water that interact with the polysaccharide films using ATR-FTIR spectroscopy.

2. Materials and methods

2.1. Materials

Pullulan PI20 (MW 200,000 Da) was donated by Hayashibara Biochemical Laboratories (Okayama, Japan). Sodium alginate was obtained from Sigma–Aldrich (Oakville, ON, Canada). Inorganic salts (LiCl, CH_3COOK, $MgCl_2$, K_2CO_3, $NaNO_2$, NaCl and KCl) used for relative humidity (RH) control were purchased from Fisher Scientific (Ottawa, ON, Canada).

2.2. Film preparation

Pullulan and sodium alginate powders were mixed at four pullulan:alginate weight ratios (100:0, 60:40, 40:60, and 0:100), dispersed in distilled water, and mixed for 3 h under shear to form a homogeneous film-forming solution. The film-forming solutions were then casted on leveled glass plates and allowed to dry in an environmental chamber maintained at 52 °C and 55% RH. The resulting films were peeled off from the glass plates and further conditioned at 23 ± 1 °C and 64 ± 1% RH prior to testing.

2.3. Mechanical properties measurement

Before mechanical properties testing, film samples were equilibrated for one week at 23 °C at 11, 23, 65, or 84% RH. Tensile strength (TS), elongation at break (EAB), and elastic modulus (EM) of film specimens were determined using an Instron Universal Testing Machine (model 1011, Instron Engineering Corp., Canton, MA). TS, EAB and EM were determined from the stress–strain curves according to ASTM standard method D882-02 (ASTM, 2002). TS was calculated on the basis of the original cross-sectional area of the test specimen using the equation $TS = F/A$, where TS is the tensile strength in MPa, F is the force (N) at maximum load, and A is the initial cross-sectional area (m^2) of the film specimen. The EAB was calculated by dividing the extension-at-break of the specimen by the initial gauge length and multiplying by 100. Elastic modulus was calculated from the initial slope of the stress–strain curve.

2.4. Water sorption isotherm

The pure pullulan, pullulan–alginate, and pure alginate films were dried under vacuum at 60 °C for 48 h to reach a constant weight. Dried films were then placed on weighing dishes and equilibrated within glass bottles containing various saturated salt solutions to provide the equilibrium RH at different levels. Saturated salt solutions of LiCl, CH_3COOK, $MgCl_2$, K_2CO_3, $NaNO_2$, NaCl and KCl were used to provide 0.11, 0.23, 0.32, 0.43, 0.65, 0.75, and 0.84 equilibrium a_w, respectively. The bottles were stored in an environmental chamber maintained at 23 °C for at least one week until equilibrium weight was attained. Samples were then taken out and weighed quickly to determine their equilibrium moisture contents.

The resulting moisture sorption isotherm data were fitted to the GAB sorption isotherm model (Eq. (1); Van den Berg, 1985) using SAS software (Statistical Analysis System Inst. Inc., Cary, NC, USA):

$$M = \frac{M_0 C K a_w}{(1 - K a_w)(1 - K a_w + C K a_w)} \tag{1}$$

where M_0 is the monomolecular layer moisture content, and C and K are temperature-dependent parameters related to heats of sorption for monolayer and multilayer water, respectively. Standard error of estimate (SEE) defined by Eq. (2), was used as the indicator for the accuracy of the fit. The mean relative percentage deviation (P) defined by Eq. (3) was used to define the model accuracy.

$$\text{SEE} = \sqrt{\frac{\sum (M_i - M_{Ei})^2}{n}} \tag{2}$$

$$P = \frac{100}{n} \sum \frac{|M_i - M_{Ei}|}{M_i} \tag{3}$$

where M_i is the experimental moisture content for experiment i, M_{Ei} is the predicted moisture content, and n is the number of data points. According to Lomauro, Bakshi, and Labuza (1985), a P value of less than 5% (95% confidence limit) is considered as a good fit, 5–10% (<90% confidence) a reasonable fit, and above 10% (90% confidence) a poor fit.

2.5. Film thickness measurement

Film thickness was measured with a digital micrometer (Testing Machines Inc. New Castle, DE, USA). For each film type, measurements were taken at five different positions on one film sample and the average value was calculated.

2.6. Differential scanning calorimetry (DSC)

The thermal properties of the films were evaluated by a differential scanning calorimeter (DuPont 2910 DSC, TA Instruments, Mississauga, ON, Canada). Samples were cut into small pieces and placed onto aluminum pans (TA Instruments), and then conditioned at various RH as outlined in Section 2.4. Samples were sealed quickly with a lid after equilibrating for at least one week. All samples were scanned from −50 to 150 °C with a heating rate of 40 °C/min, to determine the T_g.

2.7. ATR-FTIR analysis

Infrared spectra of film specimens were determined using a FTIR spectrometer (IRPrestige-21, Shimadzu Corporation, Tokyo, Japan) equipped with a total attenuated reflection press (MIRacle$^{\text{TM}}$, Pike Technologies, Madison, WI). All spectra, including anhydrous films as outlined in Section 2.4, were collected at 21 ± 2 °C from 4000 to 700 cm^{-1} with a resolution of 4 cm^{-1} from 30 averaged scans. Baseline correction was done using Grams-32 spectral analysis software

(Galactic Industries Corp., Salem, NH, USA). The band at around 2930 cm^{-1}, which is due to C–H stretching vibration, was taken as an internal standard to normalize the spectrum of film samples. Spectra for the hydrated specimens were subtracted by the spectrum for the anhydrous specimen. This treatment removed the signal due to O–H stretching from the biopolymers, thereby revealing the underlying O–H stretching bands attributed to the sorbed water. To determine the relative proportion of the different species of water involved, the O–H stretching region (3700–3000 cm^{-1}) of the spectra was fitted using Grams-32 spectral analysis software. The O–H band was deconvoluted into three peaks when the films were equilibrated at $0.23 \le a_w \le 0.65$. At 0.84 a_w, one additional band for the unassociated water (around 3600 cm^{-1}) was used for fitting the O–H band. The approximate wavenumbers for the underlying peaks were determined from the second derivative of the spectra. These wavenumbers were used as initial "guesses" for nonlinear regression analyses, assuming a Gaussian shape for all peaks. All results were performed in triplicate; standard deviations of less than 10% were obtained.

2.8. *Statistical analysis*

All experiments were conducted in triplicate. Nonlinear regression procedure (PROC NLIN) and analysis of variance (ANOVA) procedures were adopted to analyze the data, using SAS software.

3. Results

3.1. *Moisture sorption isotherm*

Moisture sorption isotherms of pullulan–alginate films are presented in Fig. 1. As shown, water content increased linearly as a_w increased up to about 0.5. Above this a_w level, upward curvature trends were observed. Similar isotherm shapes have been reported in the literature for other hydrophilic biopolymers (Biliaderis, Lazaridou, & Arvanitoyannis, 1999; Mali, Grossmann, Garcia, Martino, & Zaritzky, 2002; Stading, Rindlav-Westling, & Gatenholm, 2001). Among the samples tested, pure pullulan films exhibited the lowest moisture content and pure alginate films had the highest. The moisture content for pure alginate films at 0.84 a_w was approximately two times higher than that for pure pullulan films, indicating that the former was more hydrophilic. The greater moisture content for alginate can be attributed to the formation of water hydration spheres around the substituted hydrophilic –COO$^-$Na$^+$ of the uronic acid monomers (Fringant, Desbrieres, Milas, Rinaudo, & Esgoubes, 1996). In contrast, such ionic interaction is absent in pullulan since it is a neutral polysaccharide.

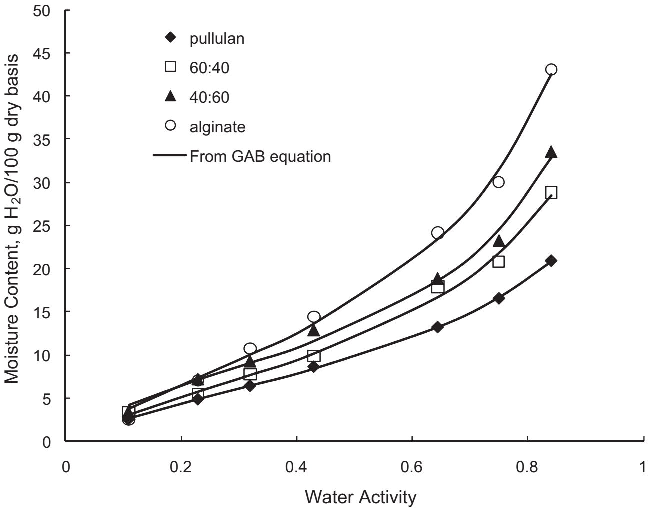

Fig. 1. Moisture sorption isotherms at 23 °C of pullulan–alginate blend films with different alginate concentrations (solid lines represent the GAB model fit to the data).

Table 1

GAB equation parameters for pure pullulan, alginate, and blend films at 23 °C.

Film	M_0 (g H_2O/g dry basis)	*C*	*K*	SEE	*P*%
Pullulan	0.0802	4.5145	0.7793	0.001	1.33
Pul:alg (60:40)[a]	0.0919	4.2767	0.8409	0.007	4.44
Pul:alg (40:60)[b]	0.0892	7.1046	0.8829	0.008	6.35
Alginate	0.1258	3.5238	0.8714	0.009	9.82

[a] Blend film with pullulan/alginate ratio of 60:40.

[b] Blend film with pullulan/alginate ratio of 40:60.

The predicted sorption isotherms, based on the GAB equation, are shown as continuous lines in Fig. 1. The estimated GAB parameters (M_0, *C*, and *K*) are presented in Table 1. Overall, the GAB parameters estimated for pure pullulan films are comparable to values reported in the literature (Biliaderis, Lazaridou, & Arvanitoyanni, 1999; Diab et al., 2001). The *P* values of prediction for all samples were less than 10, indicating that the GAB equation fitted the experimental data well (Lomauro et al., 1985). The predicted M_0 value of the pure pullulan films (0.0802 g H_2O/g dry basis) was lower than that of the pure alginate films (0.1258 g H_2O/g dry basis). For pullulan/alginate blend films, M_0 value tended to increase with increasing alginate content, implying that the addition of alginate might have induced a more open film morphology that increased the number of active sites. The parameter *C* in the GAB model is related to the monolayer heat of sorption (Galin & Galin, 1992); the greater the *C* value, the stronger the bonds between water molecules and hydrophilic sites of polysaccharides. The *C* values (Table 1) did not show a clear trend with the change in alginate content. However, the *C* value for the pure alginate films was the lowest among the four types of films, suggesting that the monolayer moisture interaction was the weakest for the pure alginate films. All *K* values were less than 1, implying that the multilayer layer water (above the monolayer) is less structured than the pure water (Timmermann, 2003). The magnitudes of the *K* values are comparable with GAB fits for biopolymers commonly encountered in foods (Al-Muhtaseb, McMinn, & Magee, 2002; Enrione, Hill, & Mitchell, 2007; Kouhila, Kechaou, Otmani, Fliyou, & Lahsasni, 2002). It is noteworthy that the *K* value for pure pullulan film was the lowest among the four films tested. Furthermore, the *K* value increased with increasing alginate contents. This is consistent with the shape of the moisture sorption isotherms as shown in Fig. 1; it has been reported that the higher the *K* value, the more pronounced the upswing of the isotherm beyond the intermediate a_w range (Timmermann, Chirife, & Iglesias, 2001).

3.2. *Mechanical properties*

The effects of alginate content and RH on the mechanical properties of pullulan-based films are summarized in Fig. 2. At low a_w (0.23), TS values were not significantly different between samples (Fig. 2a). However, from 0.23 to 0.65 a_w, TS values for pure alginate films were significantly higher than for pure pullulan films. Moreover, an anti-plasticization effect of water was observed for the pure alginate films, as indicated by the increasing TS when a_w increased from 0.23 to 0.43. Within the 0.23 to 0.65 a_w range, increasing the alginate content strengthened the blended film, but above 0.65 a_w, alginate had a film-weakening effect. The elastic modulus (EM), which is indicative of the stiffness of the film, showed similar trends (Fig. 2b). Here, EM increased with alginate content at low to intermediate a_w range, suggesting that the incorporation of alginate increase the stiffness of the blended films. The anti-plasticization

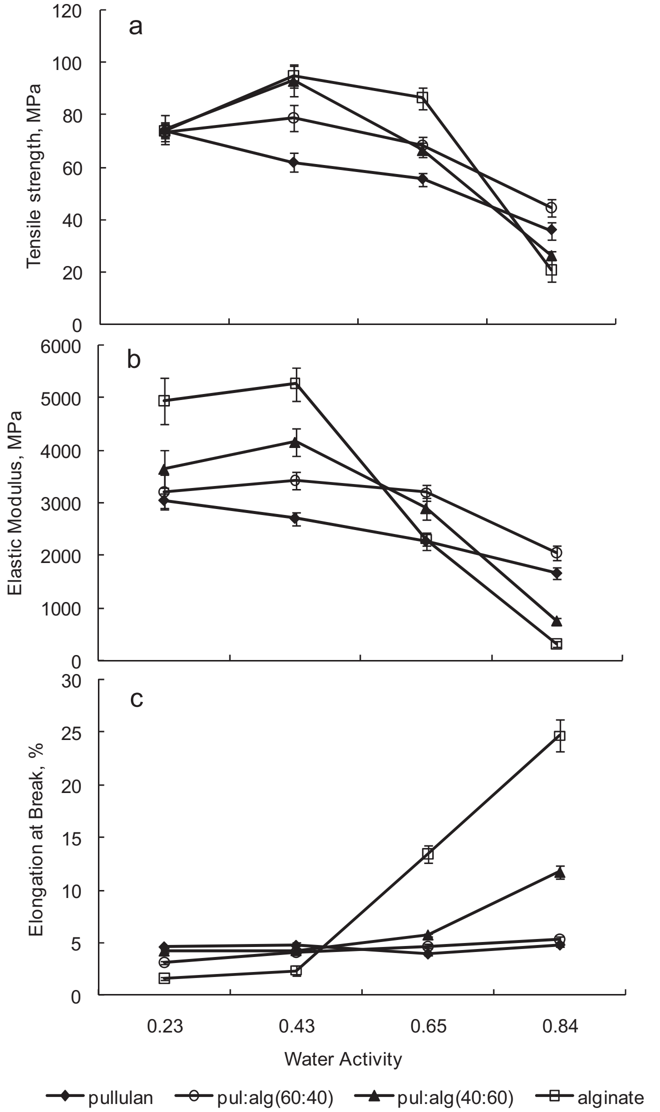

Fig. 2. Mechanical properties of pure pullulan, alginate, and blend films as a function of relative humidity: (a) tensile strength; (b) elastic modulus; (c) elongation at break.

effect of water was also observed from the EM data for alginate and blend films, i.e., increasing a_w up to 0.43 did not cause significant changes in EM. Similar to the TS data, at 0.84 a_w, 60:40 pullulan:alginate film was the strongest and stiffest among all the tested films. In terms of film stretchability, EAB of pure pullulan and 60:40 pullulan:alginate films was not affected significantly by a_w (Fig. 2c). However, with the increased alginate content in the 40:60 pullulan:alginate blend, significant film plasticization was observed at 0.84 a_w. The pure alginate film had the highest EAB values above 0.43 a_w, indicating that it was the most flexible among the four films tested. Nevertheless, it was also the most brittle (lowest EAB) when equilibrated under dry conditions (0.23–0.43 a_w). These observations indicated that alginate is more sensitive to moisture than pullulan.

The anti-plasticization effect observed can be attributed to the association of water molecules with hydrophilic side groups, causing a decrease in free volume in the polymer matrix and suppressing polymer chain motion. The effects are known to hinder secondary relaxation mechanisms, thereby increasing the tensile strength and elastic modulus of the polymer (Sears & Darby, 1982; Seow, Cheah, & Chang, 1999). As a_w increases, water molecules continue to interact with monolayer sorption sites and ultimately reach saturation. Further increases in a_w results in the formation of water clusters, causing the polymer to swell. Consequently, the anti-plasticization effect reverts to the plasticization effect, thereby increasing the flexibility of the polymer, weakening both its tensile strength and elastic modulus (Fairley, Monahan, German, & Krochta, 1996; Guo, 1993; Jackson & Caldwell, 1967). Similar effects have been reported for gluten and tapioca starch films (Chang, Cheah, & Seow, 2000; Chang, Karim, & Seow, 2006; Gontard, Guilbert, & Cuq, 1993). In the present study, the anti-plasticization effect was not observed for pure pullulan films as both TS and EM values decreased continuously with increasing a_w.

3.3. DSC analysis

T_g of a polymer blend can reveal useful information about miscibility between its constituant components (Kalichevsky & Blanshard, 1992). Fig. 3 summarizes the effects of a_w on the thermal properties of pure pullulan, alginate, and blend films. For all samples, one T_g was detected on the thermograms. This observation does not necessarily imply complete miscibility between pullulan and alginate at a molecular level, since the T_g values for the two polymers are similar in magnitude (Biliaderis, Lazaridou, & Arvanitoyannis, 1999; Lazaridou & Biliaderis, 2002). The plasticization effect of water is apparent, as indicated by the decreasing T_g value as a_w increased from 0.23 to 0.84. This can be attributed to the disruption of chain-chain interactions by the sorbed water, which enhanced segmental movement. From the previous mechanical testing results, small amounts of water had an anti-plasticization effect on the blend films and pure alginate films. However, the T_g of those films continuously decreased with increasing water content at the anti-plasticization range ($0.23 < a_w < 0.65$). Similar results have been reported for tapioca starch films and cellulose acetate films (Chang, Cheah, & Seow, 2000; Guo, 1993).

3.4. FTIR analysis

The subtracted FTIR spectra for pullulan, alginate and blended films around the O–H stretching region are summarized in Fig. 4. As a_w increased from 0.23 to 0.84, the overall absorbance increased, which is consistent with the increasing moisture content of the films. As a_w increased from 0.43 to 0.65 (Fig. 4b and c), two bands appeared at around 3420 and 3233 cm^{-1}. The former band was attributed to water molecules hydrogen bonded with water and polar groups of polysaccharides, or with other water molecules. The latter band was assigned to water molecules hydrogen bonded to specific binding sites of the polysaccharides (Kitano et al., 2001; Lasagabaster et al., 2006; Sammon et al., 1998). As the alginate content increased, the absorbance around 3420 cm^{-1} progressively became stronger. Moreover, at 0.84 a_w, the shape of subtraction spectra became more similar to the spectrum of pure water (Fig. 5a) as the alginate content increased. To determine the different species of water involved, the O–H vibration band was deconvoluted into four components. The four peaks shown in Fig. 5a can be assigned to O–H stretching vibration of monomeric non-hydrogen bonded water (peak 1, around 3608 cm^{-1}), partially hydrogen-bonded water (peak 2, around 3520 cm^{-1}), fully hydrogen-bonded water (peak 3, around 3411 cm^{-1}), and associated chains in water (peak 4, around 3248 cm^{-1}) (Ide, Yoshikawa, Maeda, & Kitano, 1999; Kitano et al., 2001; Lasagabaster et al., 2006; Sammon et al., 1998; Sutandar, Ahn, & Franses, 1994).

The typical spectra of film samples (equilibrated at 0.84 a_w), along with their fitted curves, are shown in Fig. 5b–e. According to their positions from high to low wavenumber, the hydrogen bonding strength involved for peaks 1, 2, 3, and 4 can be qualitatively assigned as very weak, weak, medium, and strong, respectively. Peak 1 has been attributed to the asymmetric O–H stretching

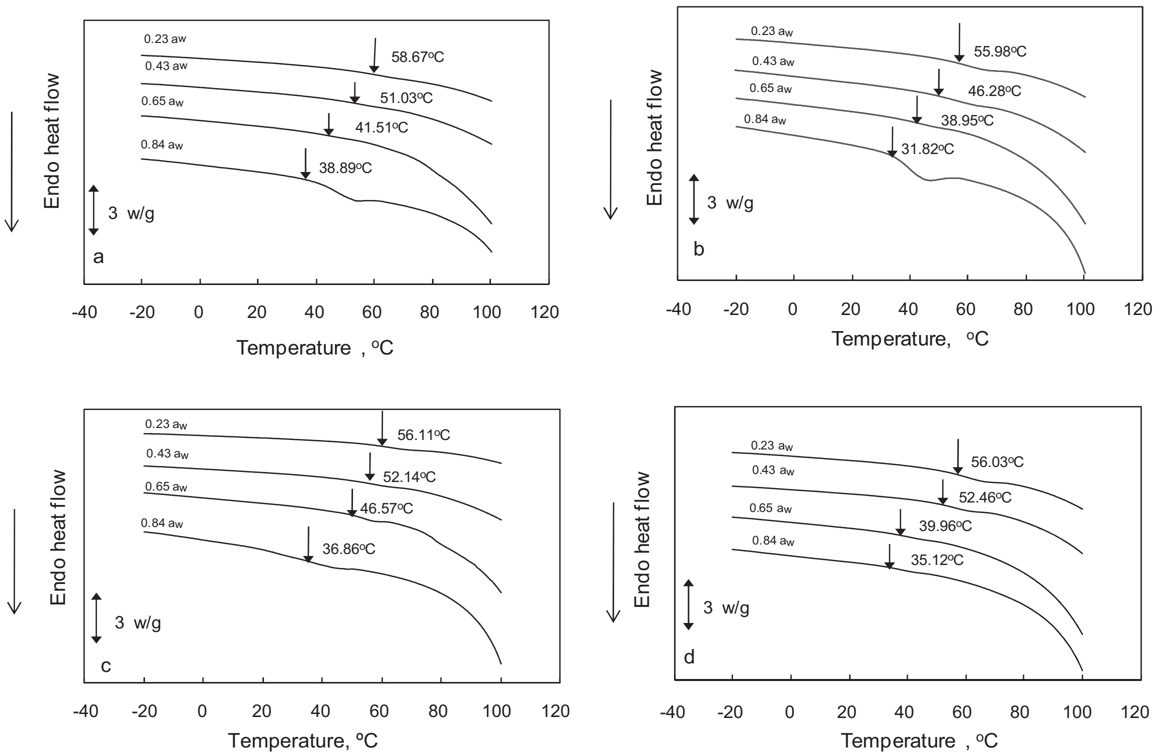

Fig. 3. Glass transition temperatures of pure pullulan, alginate, and blend films as influenced by relative humidity: (a) pullulan; (b) pullulan:alginate (60:40); (c) pullulan:alginate (40:60); (d) alginate. Arrows indicate the position of the glass transition temperature.

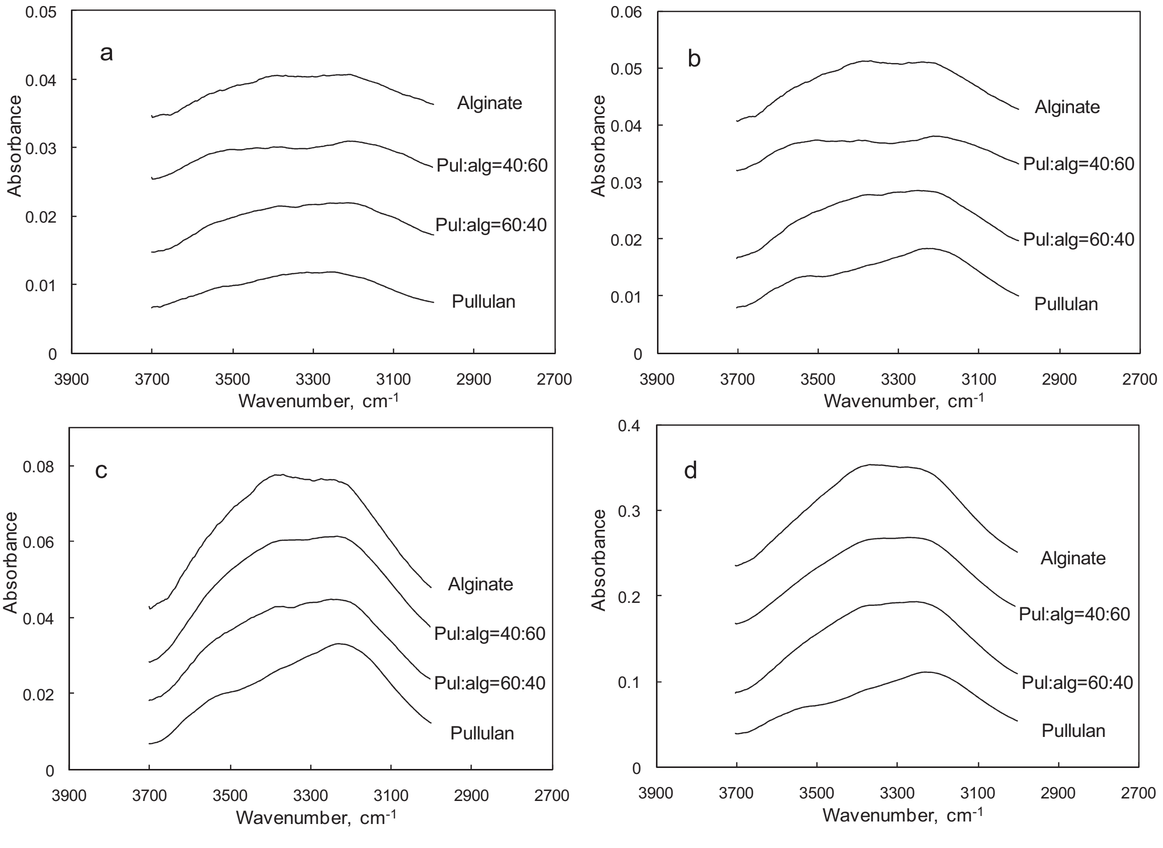

Fig. 4. ATR-FTIR subtraction spectra in the 3700–3000 cm^{-1} range of pure pullulan, alginate, and blend films: (a) 0.23 a_w; (b) 0.43 a_w; (c) 0.65 a_w; (d) 0.84 a_w.

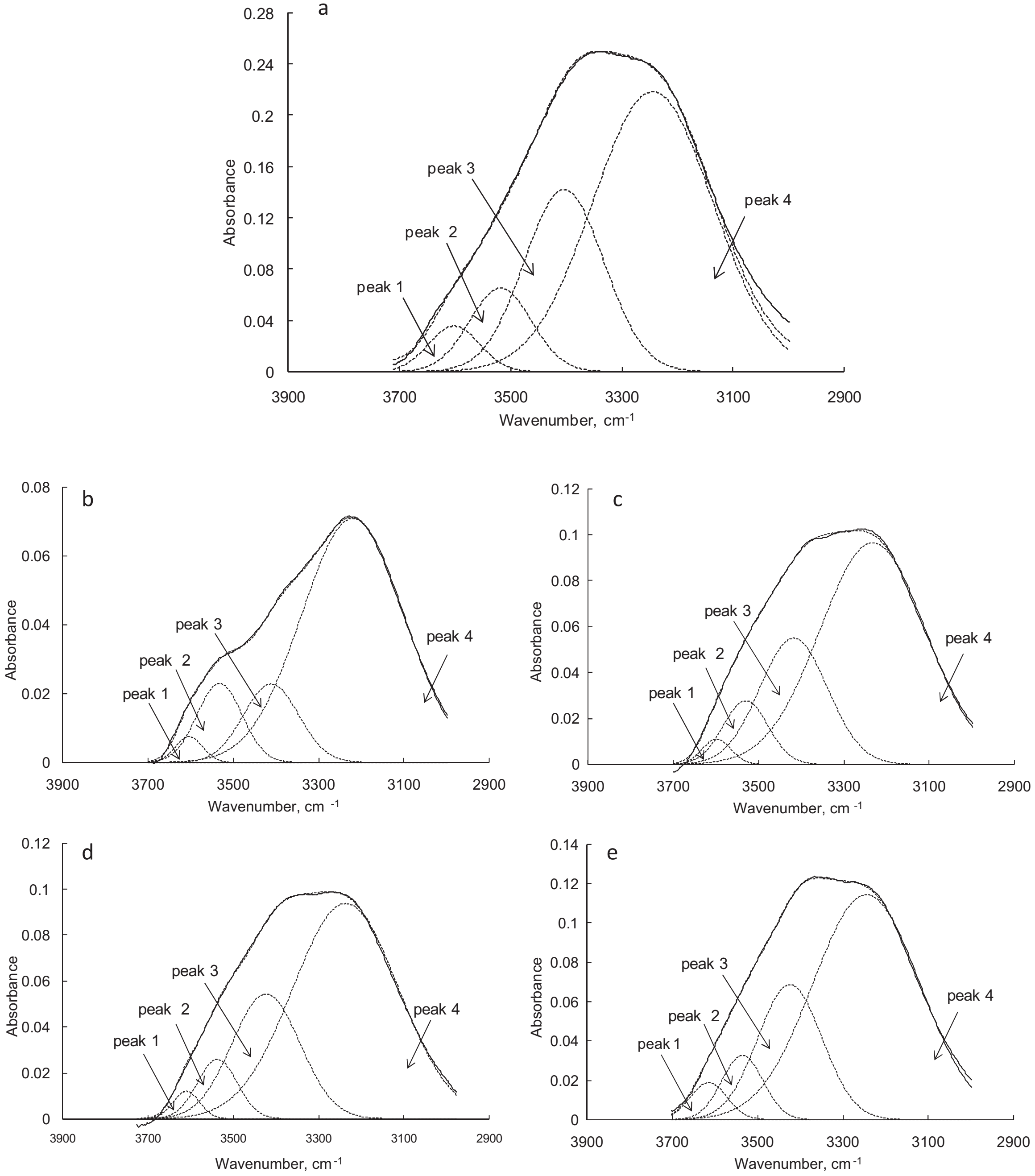

Fig. 5. Curve fitting analysis of subtracted spectra corresponding to water absorbed at equilibrium ($a_w = 0.84$) in pure and blend films: (a) pure water; (b) pullulan; (c) pullulan:alginate (60:40); (d) pullulan:alginate (40:60); (e) alginate (solid line: experimental; dotted line: curve fitted).

vibration of unassociated water which is confined to excess free volume (microvoids) (Cotugno, Larobina, Mensitieri, Musto, & Ragosta, 2001; Lasagabaster et al., 2006). This peak only appeared in samples equilibrated to high moisture content at 0.84 a_w but was absent for samples tested at all other a_w levels. This species of water is highly mobile and might not play a prominent role in plasticizing the biopolymers. At 0.84 a_w, the overall O–H bands had similar shapes to those of pure water (Fig. 5a), although frequencies of the

Table 2
Summary of band positions (wavenumber, cm^{-1}) for O–H stretching vibration of pure water, pullulan, alginate, and blend films (at $a_w = 0.84$).

Band	Pure water	Pullulan	Pul:alg (60:40)[a]	Pul:alg (40:60)[b]	Alginate
Peak 1	3608.8 ± 4.5	3604.7 ± 3.2	3599.4 ± 6.1	3610.4 ± 3.4	3615.0 ± 2.0
Peak 2	3520.1 ± 2.4	3533.0 ± 4.1	3531.1 ± 4.6	3537.7 ± 5.6	3535.9 ± 4.0
Peak 3	3411.2 ± 6.2	3412.3 ± 3.5	3418.6 ± 6.9	3423.5 ± 4.9	3424.5 ± 5.0
Peak 4	3248.6 ± 3.3	3220.8 ± 2.8	3233.4 ± 3.4	3237.0 ± 3.0	3245.4 ± 3.0

[a] Blend film with pullulan/alginate ratio of 60:40.
[b] Blend film with pullulan/alginate ratio of 40:60.

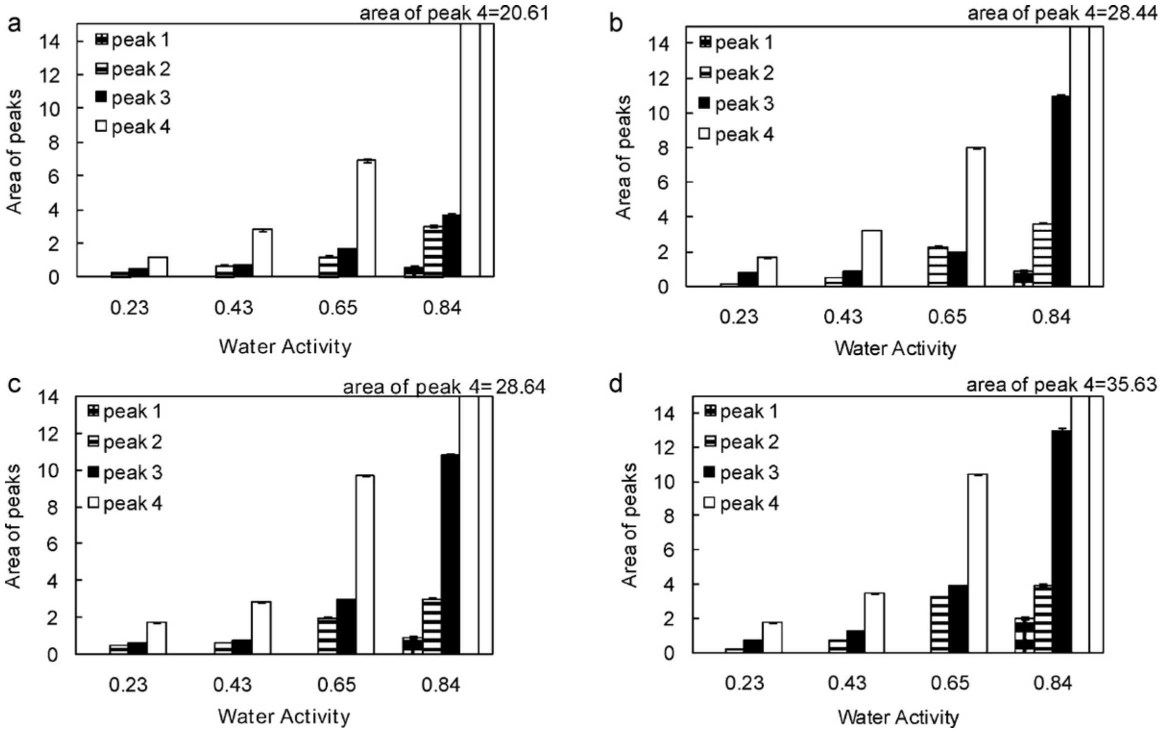

Fig. 6. Absorbance area of the different peaks of the spectra corresponding to pure and blend films equilibrated at different relative humidities: (a) pullulan; (b) pullulan:alginate (60:40); (c) pullulan:alginate (40:60); (d) alginate.

underlying bands were shifted due to interaction with the polymers (Table 2). The exception was peak 1, in which the frequency values for water and film specimens were not significantly different. This observation supports the hypothesis that peak 1 is related to unassociated water molecules that were not hydrogen bonded.

From Fig. 5, it can be seen that the O–H vibration profile for pure pullulan is markedly different than that of other alginate-containing films, primarily due to the smaller area of peak 3 for the pullulan. Also, peak 3 became larger as the alginate content increased, suggesting that peak 3 is related to the alginate polymer. Pullulan is made up of maltotriose repeating units that are non-ionic. By contrast, alginate is made up of mannuronic and guluronic monomers with polar COO^- groups, which might have contributed to the increased absorbance of peak 3 due to the enhanced hydrogen bonding of water with these polar sites.

Peak 4, the largest component that appeared at the lowest wavenumber, was assigned to the O–H stretching band for water molecules hydrogen bonded to two polar groups on polysaccharide chains. This water is expected to be the least mobile. In addition to representing water molecules that are strongly associated with the active sites on the polymer chains, this peak contains absorbance from the self-associated water molecules. The formation of water clusters of more than two water molecules, which has been reported in other polymer systems, also cannot be entirely ruled out (Hernandez & Gavara, 1994; Lim, Britt, & Tung, 1999; Orofino, Hopfenberg, & Stannett, 1969). The broad profile exhibited by this band highlighted the existence of multiple modes of interaction for this species of water (Cotugno et al., 2001; Lasagabaster et al., 2006).

Changes in peak area for the four underlying bands, as affected by a_w, are summarized in Fig. 6. Since the molar absorptivity changes with interaction strength (Cotugno et al., 2001), quantitative interpretation on the amount of each type of water molecule is difficult. Nevertheless, these data provide an indication of the properties of the water–polymer interaction involved. As shown in Fig. 6, minimal changes in peaks 2 and 3 were observed as a_w increased from 0.23 to 0.43. In contrast, peak 4 increased substantially, suggesting that most of the water sorbed in this a_w range were hydrogen bonded to active sites of the polysaccharides. Consistent with the moisture sorption isotherm (Fig. 1), above 0.43 a_w, a considerable increase in area was observed for all water species due to the swelling of polymer matrix as moisture content increased. The effect of alginate on the sorption modes of water is also evident from Fig. 6. Overall, peak 4 increased by the largest extent as the alginate content increased, showing that the proportion of water molecules that were strongly hydrogen-bonded was more abundant in the alginate than the pullulan films. The lower proportion of this species of water in pullulan could be caused by its more compact morphology (i.e., smaller free volume) than the alginate polymer, thereby limiting the interaction of water with pullulan polymer chains. This is consistent with the electron scanning microscopy findings from our previous study, where film cross-sections for pullulan films exhibited compact morphologies, whereas alginate films were rough and porous (Tong, Xiao, & Lim, 2008). These observations may also explain the lower a_w-sensitivity of the mechanical properties for the pullulan films (Fig. 2) and the lowest M_0 value predicted by the GAB equation (Table 1).

4. Conclusions

In this study, the interaction of water with pullulan, alginate, and pullulan–alginate blend films was elucidated by studying their moisture sorption characteristic, mechanical and thermal properties, as well as ATR-FTIR spectra. At low to intermediate a_w levels, incorporation of alginate into pullulan-based films significantly improved the TS and EM, but decreased the EAB of the films. However, at elevated a_w (0.84), the blend films weakened considerably as alginate content increased. The T_g values for all the films tested decreased as a_w increased from 0.23 to 0.84, indicating that water is an effective plasticizer. Pure pullulan films exhibited the lowest equilibrium moisture contents, whereas the pure alginate films had the highest. These data indicate that alginate-containing films were more moisture sensitive than pure pullulan, and thus the pullulan:alginate ratio will affect the material properties of the blend films. The hydrogen bonding characteristics of different species of water in the various films can be analyzed readily by ATR-FTIR through deconvoluting the O–H stretching region (3700–3000 cm^{-1}) of the IR spectrum.

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